

感染症定期報告に関する今後の対応について

平成16年度第5回

運営委員会確認事項

(平成16年9月17日)

1 基本的な方針

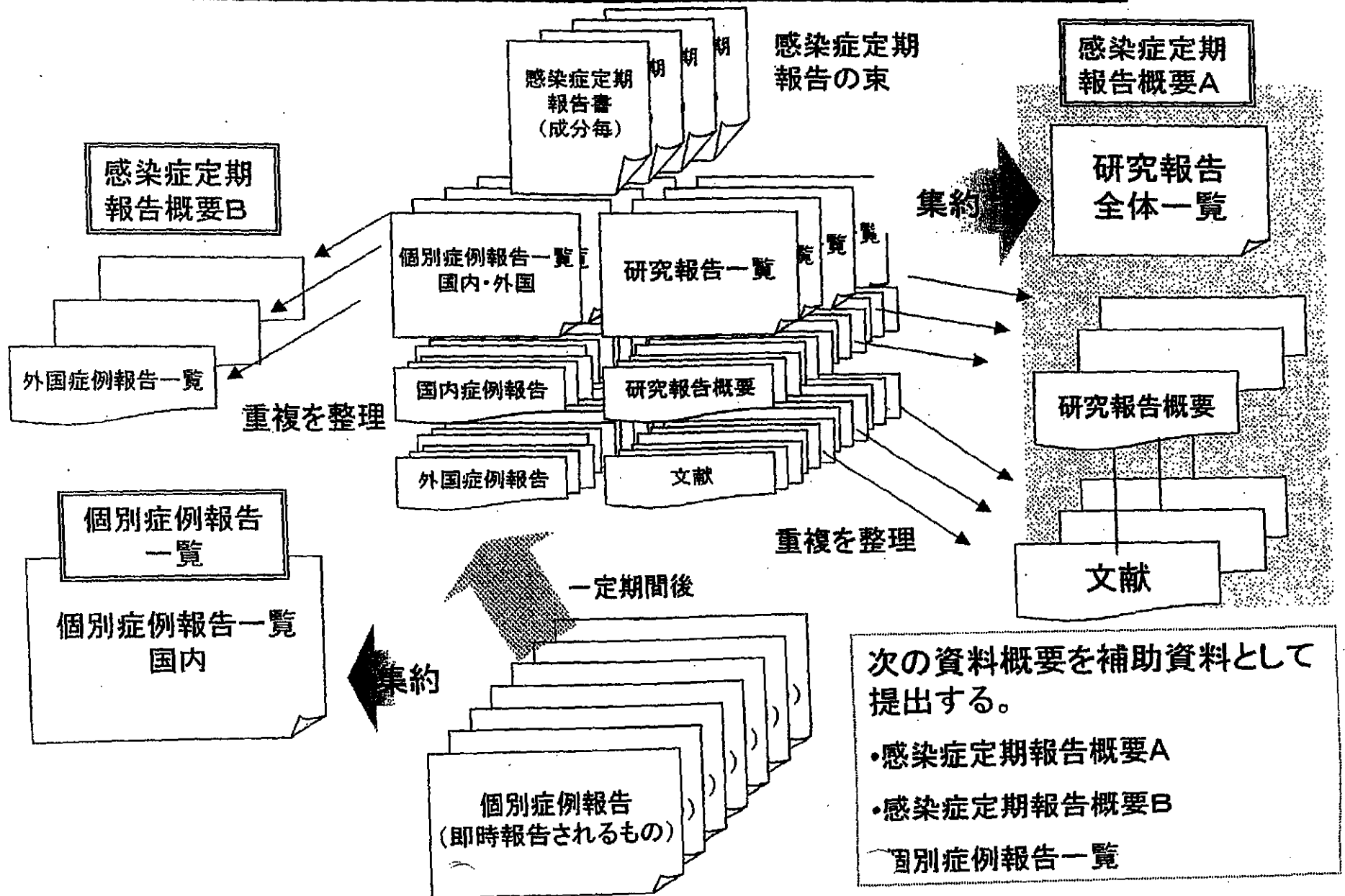
運営委員会に報告する資料においては、

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

2 具体的な方法

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

感染症定期報告・感染症個別症例報告の取り扱い



感染症定期報告概要

(平成23年9月28日)

平成23年3月1日受理分以降

- A 研究報告概要
- B 個別症例報告概要

A 研究報告概要

- 一覧表（感染症種類毎）
- 感染症毎の主要研究報告概要
- 研究報告写

研究報告のまとめ方について

- 1 平成23年3月1日以降に報告された感染症定期報告に含まれる研究報告（論文等）について、重複している分を除いた報告概要一覧表を作成した。
- 2 一覧表においては、前回の運営委員会において報告したもの以降の研究報告について、一覧表の後に当該感染症の主要研究報告の内容を添付した。

感染症定期報告の報告状況(2011/3/1～2011/6/30)

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No
100329	2011/4/20	110078	B型肝炎	Vaccine. 28(2010)7723-7730	オランダにおける公的な予防接種プログラムに関する報告。現在オランダではB型肝炎ウイルス(HBV)感染防御のため特定のリスク群の小児(母親がHBVキャリア等)を対象に予防接種をする方針がとられている一方で、WHOでは全小児に対する接種を奨励している。そこでオランダにおける対象を限定したB型肝炎予防接種プログラムを評価した結果、対象となっているリスク群に予防接種を受けていない者がいることが明らかとなった。このことから、現在の方針では感染防御として不十分であり、ユニバーサルワクチン接種を全小児に行いさらにリスク群へ適切な予防接種プログラムを行う事で、公衆衛生上の利益が増す可能性が示唆された。	1
100359	2011/4/20	110078	B型肝炎	Clin Infect Dis. 52(2011)624-632	オカルトB型肝炎(OHB)供血者によるHBVの伝播性に関する報告。ヒト肝細胞を移植したキメラマウス4匹に対し、2名のOHB供血者の血清サンプルを接種し、血清および肝臓HBV-DNAレベルを測定したところ、1匹で接種から5および7週間後に血清HBV-DNAが検出された。また、OHB供血者10名(うち9名はHBsAb陽性)から選んで特定した受血者49名についてHBV感染の検査を行ったところ、陽性であったのは4名であった。そのうち3名では供血者とのHBVゲノム相同性から輸血による伝播が否定され、1名が輸血によるHBV感染と判定された。これらの結果より、OHB供血者由来の血液で感染する可能性があることが示唆されたが、ヒトにおいてHBVの伝播リスクは依然として低かった。	2
100369	2011/4/20	110078	B型肝炎	J Med Virol. 83(2011)791-795	B型肝炎ウイルスの母児感染に関する報告。HBs抗原陽性の母親から生まれた214人の新生児において、出生時または子宮内でのHBV感染がHBIG及びHBワクチンにより効果的に予防できるかどうかを判断するために、HBs抗原を測定した。母児感染は約4.7%(10/214)で、内訳は出生時感染は3.7%(8/214)、子宮内感染は0.9%(2/214)であった。母児感染のリスクは、母親のHBs抗原又はHBV DNAレベルに応じて上昇した。36カ月の追跡調査後、出生時感染症例のすべてがHBs抗原陰性になり、子宮内感染症例のすべてがキャリア化した。これらの結果により、HBIG及びHBワクチンは、出生時感染の予防には効果があつたが、子宮内で感染した新生児には十分でないことが示された。	3
100307	2011/3/22	101060	C型肝炎	J.Hepatol. 2010 Dec 8.	C型肝炎ウイルス(HCV)遺伝子型4の管理:国際的専門家委員会の推奨について。HCVは6つの主要な遺伝子型と一連の亜型に分類される。HCV遺伝子型は、そのヌクレオチド配列、地理的分布と治療への反応に関してそれぞれ特徴を持つ。遺伝子型4は、HCV感染の80%以上を占め、分布はエジプトとアフリカの一部に限られていたが、現在はヨーロッパ諸国に拡大している。HCV-4は慢性肝炎、肝硬変、肝臓、肝移植の主な原因と考えられている。現在世界中の慢性C型肝炎のうち約20%がHCV-4によるものである。HCV-4は広範囲な研究の対象ではなかったが、今回、国際的専門家委員会によって疫学・自然史・臨床・病理学的特徴と治療に関する概説が示された。	4
100307	2011/3/22	101060	HIV	Euro Surveill. 2010 Dec 2;15(48)	欧州連合(EU)と欧州経済領域(EEA)の28カ国から、2009年に新たにHIV感染と診断された25,917症例について報告された。新規感染者の割合はエストニア、ラトビア、イギリス、ベルギーの順で高かった。主要な感染経路は男性間の性的接触(35%)であり、ついで異性間性的接触(24%)であった。全体として2009年のHIV症例数は増加したが、AIDS診断の報告は減少している。HIV感染と診断された時にCD4細胞数が350 cells/ μ L未満の感染後期初診患者が多いことは、適切な時期に治療を受けられなかったことを示唆しており、考慮すべきである。	5

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100307	2011/3/22	101060	HIV	Transfusion. 2010 Dec;50(12):2707-12	2005-2007年のHIV-1非B型及び抗レトロウイルス薬剤耐性株に関する米国の供血者における広範な評価について報告された。1999-2005年までの米国のHIV感染供血者66人では、4.7%がHIV-1非B型、6.5%が抗レトロウイルス薬剤耐性HIV-1であった。今回、2005年1月-2007年12月に収集した感染確定例197例と最近感染した症例11例を対象として調査を行った。HIV感染供血者の検出は、FDAが認可した検査により行った。プロテアーゼ及び逆転写酵素遺伝子の薬剤耐性プロファイルは、遺伝子タイピングシステム(ViroSeq, Celera Diagnostics)により決定した。サブタイプは、これらの配列を系統発生解析することにより得た。計208検体のうち203検体について、薬剤耐性プロファイルが得られ、そのうち9.9%が薬剤耐性変異を持っていた。10例が1つの薬剤に耐性を示し(9例が非核酸系逆転写酵素阻害剤(NNRTIs)に、1例が核酸系逆転写酵素阻害剤(NRTIs)に耐性)、8例が2つの薬剤に耐性を示し(5例がNNRTIとNRTIに、2例がNRTIとプロテアーゼ阻害剤(PI)に、1例がNNRTIとPIに耐性)、2例が3つの薬剤に耐性を示した。非B型株はHIV感染供血者の2.5%で同定され、サブタイプA1、D、CRF02_AG、CRF43-02G、およびURF_BFから構成されていた。本研究と従来の研究から、抗レトロウイルス薬剤耐性HIV-1が1999-2007年のHIV感染供血者の9.1%に存在(感染確定例の9.3%、最近感染した例の6.9%)することが示された。また、米国の供血者において、HIV-1非B型はHIV感染症の3.0%を占めた。	6
100315	2011/3/28	101079	E型肝炎	Vector-Borne and Zoonotic Diseases 2010;10(8765):765-770	中国の家畜におけるE型肝炎ウイルス(HEV)の血清学的感染率とHEV遺伝子型4型の多様性について報告された。中国の家畜のHEV感染率を評価するために血清3579検体(豚1967匹、ヤギ700匹、牛912頭)を26省から収集され、酵素免疫測定法にてHEV抗体及び抗原の検出を行った。その結果、豚検体の82.2%が抗HEV抗体陽性であったが、ウシ検体では10.4%、ヤギ検体では28.2%であった。HEV抗原陽性は、豚1.9%、ヤギ1.6%、牛0.8%であった。HEV抗原陽性を含む検体のいくつかでHEV RNAの検出を試みたところ、豚15検体が陽性であり、ヤギ及び牛からは検出されなかった。遺伝子及び系統学的解析から、すべての豚のHEVがHEV遺伝子型4型であった。	7
100307	2011/3/22	101060	E型肝炎	Vox Sanguinis 2010;99(4)、307-313	日本の献血適格者におけるE型肝炎ウイルス抗体(HEV IgG)陽性率に関する全国調査について報告された。日本のほぼ全域を代表する7つの血液センターにおける献血適格者(HBV、HCV、HIV-1/2、HTLV-1、梅毒、ヒトパルボウイルスB19 が全て陰性)から各1,800検体、計12,600検体を選択し、HEV IgG ELISAを実施した。その結果、431検体(3.4%)が陽性で、HEV IgG陽性率は西日本(1.8%)より東日本(5.6%)で高く、年齢依存性であり、女性(2.9%)より男性(3.9%)で高かった。	8
100312	2011/3/22	101060	パルボウイルス	Virology Journal 2010; 7:272	PARV4(parvovirus 4)は既知のヒトパルボウイルスと関連のないパルボウイルス属科の新種である。ウイルス血症はPARV4感染症の特徴を示し、ウイルスDNAの残存が数種の組織で示されている。現在まで、PARV4はいかなる疾病にも関連せず、ヒトでの感染率も明確ではなかった。本研究では、PARV4の組織分布と持続感染能力をパルボウイルスB19(B19V)と比較し、評価した。急性ウイルス感染の疑いのない様々なヒト組織を対象に、ORF2とORF1をターゲットとしたリアルタイムPCRとnested PCRにより、PARV4とB19VのDNAを検出した。低量のPARV4 DNAが成人の心臓と肝臓において高頻度(40%以上)に検出され、肺と腎臓(23.4%、18%)、骨髄と皮膚と滑膜検体中(5.5%、4%、5%)でも検出された。肝臓と心臓へのPARV4の特徴的な親和性はウイルス複製の標的の可能性や、肝疾患や心疾患におけるPARV4感染の役割の可能性についての今後の研究に焦点を当てるべきことを示している。	9
100315	2011/3/28	101079	パルボウイルス	Virus Genes. 2010;41:305-308.	パルボウイルス亜科とデンスウイルス亜科の2つの亜科に分類されるパルボウイルス科は脊椎動物と昆虫に感染し、昆虫、動物及びヒトにおいて広範囲にわたる疾病に関連する。いくつかの新しいパルボウイルスが中国南東部で集められたブタ血清で特定された。塩基配列分析は中国南東部で検出されたパルボウイルスがパルボウイルス亜科で異なる亜系であることを示した。これらの結果に基づき、これらのパルボウイルスを記述するために新しいパルボウイルス亜系「Cuvirus」を提案する。	10

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100361	2011/4/20	110078	パルボウイルス	Transfusion. 2011 Feb 18.	輸血用血液製剤による症候性パルボウイルスB19感染に関する報告。1999年から2008年までの期間に、日本赤十字社のヘモビジランスシステムを通じて、輸血によるヒトパルボウイルスB19感染(TT-B19V)の可能性のある臨床報告を収集したところ、成分輸血に由来するTT-B19V患者8人が同定された。4人が特続性の貧血と赤芽球病を発症し、1人が汎血球減少症を発症した。これら5人の患者の基礎疾患は、悪性血液疾患又は溶血性疾患のいずれかであった。これらの症例について原因製剤中のウイルス量は $10^3 \sim 10^8$ IU/mLの範囲であった。血液疾患に罹患しておらず外科的治療を受けた患者2人は、マイルドな症状を示しただけであった。これらの結果より、重篤なB19関連疾患を発症するかどうかは、輸血した製剤中のウイルス量ではなく、患者の基礎疾患に依存することが示された。	11
100375	2011/4/20	110078	パルボウイルス	Vox Sanguinis. 100(2011)351-358	血漿由来凝固因子製剤に含まれるノンエンベローブウイルスについての報告。10種類の血漿由来凝固因子製剤及び3種類の組換え凝固因子製剤において、それぞれ3つ以上のバッチを用い5種類のノンエンベローブウイルスの核酸の存在を定量PCRによって調べたところ、1社の第Ⅷ因子/フォン・ウィルブラント因子製剤の2バッチでパルボウイルスB19の遺伝子型1、2及び3のDNAが検出された。この結果より、凝固因子濃縮製剤中に含まれるノンエンベローブウイルス由来の核酸が検出可能レベルで見つからない可能性が示唆された。	12
100307	2011/3/22	101060	レトロウイルス(XMRV)	American Red Cross Latest Press Releases and Statements. December 03, 2010.	米国赤十字社からXenotropic Murine Leukemia Virus-Related Virus (XMRV)と慢性疲労症候群(CFS)に関して報告された。2010年12月3日現在、XMRV感染に関連するとされているCFSやその他の疾患を呈する者による供血を見送ることに伴って、特定の連邦政府勧告は出されていない。しかし、患者と供血者の安全のため、米国赤十字社は問診でCFSと診断されたことがあると答えた供血者による供血を無期限で延期する。	13
100307	2011/3/22	101060	レトロウイルス(XMRV)	第58回日本ウイルス学会学術集会; 2010 Nov. 7-9	Xenotropic Murine Leukemia Virus-Related Virus(XMRV)抗体特異性の解析について報告された。これまでに同定されたXMRV抗体陽性例が真のXMRV感染によるものかどうかを知るためのひとつのアプローチとして、これら抗体陽性血清の反応特異性を精査した。抗体スクリーニングには、100倍希釈血清を用いて、XMRVのウイルス粒子を抗原としたウエスタンブロッティング(WB)を行った。スクリーニング対象は一般感染症検査に合格した献血検体から無作為抽出したもの500検体、2007年4月-2010年3月受診の前立腺がん患者67名、2010年4月-2010年6月受診の慢性疲労症候群(CFS)患者87名の計654名とした。WBスクリーニングで陽性は供血者8名(1.6%)、前立腺がん患者2名(3.0%)、CFS患者2名(2.3%)であり、グループ間の抗体保有率に有意差はなかった。これら陽性検体のGag p30抗体には3パターンへの反応特異性が存在した。XMRV p30のみに結合するもの、Mo-MuLV p30に弱いながらも結合するもの、Mo-MuLV p30にもXMRVと同程度の強さで結合するものである。反応パターンと対象グループとの間に有意な関連性は認められなかった。Envに対する抗体反応は通常のWBでもNative gelを用いたWBでも認められなかった。XMRV GagとMo-MuLV Gagのアミノ酸レベルでの相同性は約87%である。XMRV抗体陽性者の一部がMo-MuLVと交差反応し、反応強度にもばらつきがあることから、現在までに同定されたXMRV Gag抗体陽性例の必ずしも全てがXMRV既感染を示すものではない可能性が示唆される。	14
100307	2011/3/22	101060	レトロウイルス(XMRV)	第58回日本ウイルス学会学術集会; 2010 Nov. 7-9	慢性疲労症候群(CFS)患者血液におけるXenotropic Murine Leukemia Virus-Related Virus(XMRV)の核酸調査について報告された。ヒト胎児腎由来株化細胞(HEK 293T)にXMRV持続感染前立腺癌細胞である22Rv1細胞の上清を接種し、この感染細胞から抽出したDNAを鋳型にしてXMRVのgag及びenv遺伝子領域を標的としたプライマー及びXMRV特異的なプライマーを用いてPCRの検出感度を調べた。次に2010年4月-2010年6月のCFS患者87名の血液細胞からゲノムDNAを抽出し、2種類のプライマーを用いてPCRを行った。また、gag及びenvのプライマーではコンタミでマウス白血病ウイルスを検出してしまう可能性があるため、XMRV特異的プライマーも用いた。今回調査したCFS患者87名においてPCR解析で陽性は見られなかった。	15

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100307	2011/3/22	101060	レトロウイルス(XMRV)	Retrovirology 2010,7:110	市販のRT-PCRキット内の内在性マウス白血病ウイルス(MLV)ゲノム汚染は、Xenotropic Murine Leukemia Virus-Related Virus(XMRV)のための標準プライマーの使用で増幅されることが報告された。日本で慢性疲労症候群(CFS)患者からの血清でXMRV感染症のウイルスRNAの存在を調査するパイロットスタディにおいて、XMRVの部分的gag領域を検出するRT-PCRキットを使用すると、陰性コントロール(水)でも陽性のバンドが頻繁に認められた。そのため、キット自体がMLVまたはXMRVで汚染されていることを疑い、2つの独立した研究所で、4種のRT-PCRキット(Invitrogen、TaKaRa、Promega、QIAGEN)の品質を評価した。Invitrogen社のキット使用により、増幅DNAの部分的gag領域のヌクレオチド配列は、7番染色体のpolytropic内在性MLV(PmERV)とほとんど一致(99.4%)、最近特定されたCFS患者に由来するMLV様のウイルスと非常に類似していた(96.9~97.6%一致)。また部分的env領域のヌクレオチド配列がPmERVとほとんど一致(99.6%)した。結果からInvitrogen社のキットの酵素混合物が、PmERVに由来するRNAで汚染されていることが明らかとなった。	16
100307	2011/3/22	101060	レトロウイルス(XMRV)	Retrovirology. 2010 Dec 20;7:112	マウス白血病ウイルス(MLV)をコードしている核酸による臨床検体汚染:XMRV(Xenotropic Murine Leukemia Virus-Related Virus)と他のヒトレトロウイルス検体との関連について報告された。前立腺がん患者と慢性疲労症候群(CFS)患者でXMRVの感染を評価する研究は、臨床検体のPCR検査に頼り、大きく異なる結果を与えた。今般、Retrovirology誌において報告された4つの研究結果から、PCR分析において、DNAまたはRNAの汚染を除外するために必要な処置が示された。また、以前報告されたXMRV配列が、一般的に用いられる前立腺がん細胞株(22Rv1)に由来することを示唆している系統発生解析が提示された。これらの報告は、前立腺がん患者とCFS患者におけるMLV関連ウイルスの存在を裏付けるのに用いられているPCRの結果に重大な疑問を投げかけた。	17
100312	2011/3/22	101060	レトロウイルス(XMRV)	Virology Journal 2010; 7:224	Xenotropic Murine Leukemia Virus-Related Virus(XMRV)が慢性疲労症候群(CSF)の病因に寄与するかどうかの最近の論争に対して、中国において調査を行った。CSF患者65人と供血者コントロール85人が登録され、multiplex real-time PCRまたはRT-PCRを行った。その結果、XMRVは、65人のCSF患者と健康成人65/85において検出されなかった。本研究で、中国のCSF患者の末梢血単核細胞と血漿中にXMRVは検出されなかったことから、XMRVとCFS発症との関連性を支持していない。	18
100307	2011/3/22	101060	ウイルス感染	Emerging Infectious Diseases 16(12) 1979- 1982 (CDC Emerging Infectious Diseases,16,12,2010の 出典源)	2010年、エジプト渡航者におけるAlkhurma出血熱について報告された。Alkhurma virus(ALKV)はFlavivirus属のダニ媒介性出血熱の一種として最近報告された。1990年代後半に分離され、現在89%の塩基配列相同性を共有し、Kysanur Forest病ウイルスの亜型と考えられている。この病原体は、発熱、頭痛、関節痛、筋肉痛、嘔吐、血小板減少症などの症状を発現させ、重症の場合は、出血症状、脳炎を示し、死に至る(25%の致死率が報告されている)。ラクダ、ヒツジが宿主と考えられている。最近、サウジアラビアのJeddah近くで収集されたOrnithodoros savignyiというダニにおいてALKV RNAが検出された。アラビア半島では、これらのダニの生育場所とラクダとは関連があり、ヒトALKV感染例が報告された場所でこれらのダニが認められている。蚊が宿主であるとする仮説について、2つの研究報告がある。ALKVは経皮から、あるいは低温殺菌していない汚染したミルクを通して経口からヒトに感染していると考えられている。ALKVはサウジアラビアでのみ検出されているが、関連したKysanur Forest病ウイルスがインドや中国に広まっている。2010年エジプトからイタリアに戻った2人の旅行者においてAlkhurma出血熱が認められた。	19

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No
100361	2011/4/20	110078	ウイルス感染	J Clin Virol. 50(2011)130-135	帯状疱疹を呈する患者における水痘帯状疱疹ウイルス(VZV)によるウイルス血症に関する報告。帯状疱疹が消退した後のVZVウイルス血症の動態とウイルス量、及び帯状疱疹後神経痛(PHN)発症との関連性について調査された。血液サンプルは活動性の帯状疱疹を呈する患者63人を対象として前向き調査のベースライン時である1カ月、3カ月、6カ月時に採取された。急性期には全ての患者の血液においてVZV DNAが検出できた。6カ月の患者血液でも、レベルは有意に低下したものの、91%でVZV DNAが検出できた。前駆症状の既往歴やベースライン時に強い痛みがあった患者は、抗ウイルス薬を服用している患者や免疫不全の患者と同様に、PHNから回復するまでの時間が長かった。ウイルスDNA量は一貫してPHNのリスク因子を有する患者で多かった。また、ウイルスDNA量が多いほど、回復までの時間が長かった。これらの結果より、VZVの増殖が急性の帯状疱疹を持続させること及び高ウイルスDNA量がPHNのリスク因子となることが示唆された。	20
	2011/4/20	110078	トリパノソーマ症	ABC Newsletter #46. Dec 17; 2010	米国におけるT.cruzi感染率についての報告。米国赤十字社ではT.cruzi抗体検査について、高有病率地域では全供血を検査対象とし、その他の地域では1回でも陰性結果を受けた供血者は追加検査の対象としない体制がとられている。346万人の供血者を観察した結果、陽転化した供血者は1例も同定されず、95%信頼区間の上限信頼期間は0.08例/10万人年であった。この結果は設定された許容リスク基準よりも低く、現在の体制は受血者の安全性を損なうことなく、必要な検査の数を低減していると示唆された。	21
100329	2011/4/20	110078	細菌感染	病原体微生物検出月報. Vol.31 No.12	日本の路上生活者におけるBartonella quintana検出に関する報告。東京と大阪の路上生活者(それぞれ491例、10例)から採取したコロモジラミからゲノムDNAを抽出しB.quintanaの同定を行ったところ、それぞれ17例、6例において遺伝子が検出された。また、東京の路上生活者151例に対して血液検査を行ったところ、一般人(200例)に比べB.quintana抗体価が有意に高かった。これらの結果より、蜱媒熱が特殊な集団において蔓延している可能性が懸念された。	22
100329	2011/4/20	110078	細菌感染	AABB Annual Meeting and TXPO, 2010 Oct.9-12	供血者におけるBartonella henselae菌血症に関する報告。ブラジルにおいて、供血者500例に対しBartonella菌の感染や曝露を調査するために問診及び血液培養を行ったところ、Bartonella henselaeによる感染が2例確認された。2例ともイヌに接触しダニに咬まれており、1例はネコおよびコロモジラミとも接触していた。	23
100361	2011/4/20	110078	細菌感染	Transfusion. 51(2011) 531-538	アフレーシス血小板細菌汚染を防止するための皮膚消毒に関する報告。血小板採取時の細菌汚染を防止するための皮膚消毒について、2つの方法の効果を評価した。試験群として3つの血液センターでワンステップ2%クロルヘキシジン/70%イソプロピルアルコール(2% CHX/IPA)皮膚消毒法を評価し、対照群として32の血液センターでツーステップのポビドンヨード(P-I)法を用いた。細菌培養の結果について比較したところ、試験群は、細菌培養陽性率と培養偽陽性(汚染)率の双方で低い傾向にあった。2%CHX/IPAシングルスステップ皮膚スワブは、ツーステップP-I 皮膚消毒法より効果があり、敗血症性輸血副作用のリスクを低下させる可能性があることが示唆された。	24
100361	2011/4/20	110078	真菌感染	Clin Infect Dis. 52(2011)e94-98	臓器移植によるCryptococcus neoformansの伝播に関する症例報告。クリプトコッカス髄膜炎であった1人のドナーから臓器移植を受け、クリプトコッカス症と診断を受けた3人のレシピエントについて報告された。肝臓を移植された患者と片方の腎臓を移植された患者はクリプトコッカス敗血症と肺炎を発症した。もう片方の腎臓を移植された患者はクリプトコッカス敗血症と髄膜炎を発症した。腎臓を移植された2人の患者は、抗真菌治療により回復した。多遺伝子配列タイピングによりレシピエント分離株4つを検査したところ、それらが同じ対立遺伝子を有する事を認めた。このドナーは移植時、未解明の神経病態であり後の剖検によりクリプトコッカス髄膜炎であったと診断されていた。	25

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No
100307	2011/3/22	101060	クロイツフェルト・ヤコブ病	ProMED-mail 20110110.0119	2011年プリオン疾患最新情報が更新された。①英国:2011年1月10日現在、2010年では疑い例146例、弧発性CJD69例、vCJD3例、医原性CJD2例、家族性CJD5例、GSS1例であった。vCJD確定例または疑い例の死亡者総数は2010年までで170名である。②フランス:2011年1月4日現在、1992年以来、確定例及び疑い例は計25例である。2011年では、疑い例1610例、弧発性CJD98例、vCJD6例であった。③米国:2010年1月1日から10月31日までに疑い例333例、弧発性CJD158例、家族性CJD33例であった。④台湾:2010年12月8日、36歳男性がvCJD疑いで死亡したと報告された。症例は1989年から1997年まで英国に居住していた。	26
100359	2011/4/20	110078	クロイツフェルト・ヤコブ病	Nat Med. 17(2011)175-178	異常プリオン(PrP ^{sc})の検出法に関する報告。QUIC法を用いたPrP ^{sc} 増幅技術は超音波処理の必要がないためamyloid seedingアッセイ法より簡便であるが、CJD検体の脳脊髄液(CSF)から高感度にPrP ^{sc} を検出することはできない。そのため、QUIC法を改良したreal-time quaking-induced conversion: RT-QUIC法を開発したところ、CJD脳ホモジネートにおいて1fg以上のPrP ^{sc} を48時間以内に検出することが可能であった。また、日本の18検体及びオーストラリアの30検体のCSFを用いた盲検試験を行ったところ、感度は80%以上、特異性は100%であった。これらの結果から、RT-QUIC法を用いたCSFの検査は早期の迅速かつ特異的なCJDの検出を可能とし得ることが示唆された。	27
100375	2011/4/20	110078	クロイツフェルト・ヤコブ病	PLoS ONE 2011;6:e17815	尿性ゴナドトロピン製剤中のプリオン蛋白質に関する報告。古典的なプロテオミクスのアプローチにより、尿性ヒト絨毛性ゴナドトロピン(u-hCG)製剤中に33種類、高純度尿性ヒト閉経期ゴナドトロピン(hMG-hp)製剤中に34種類のゴナドトロピン以外の蛋白質が特定された。高度なプロテオミクスの手法を用いた結果、hCG、hMG、hMG-hp製剤中にヒトプリオン蛋白質のペプチドを検出したが、遺伝子組換え製剤中には検出しなかった。	28
100361	2011/4/20	110078	異型クロイツフェルト・ヤコブ病	Lancet. 377(2011)487-493	異型クロイツフェルト・ヤコブ病におけるプリオン感染の検出(血液検査)に関する報告。vCJDプリオン感染を検出するための血液ベースアッセイについて、その感度と特異性を確定するために、疾患に関連するプリオン蛋白を捉えて濃縮するための固体結合マトリックスを開発し、この方法を表面結合物質の直接免疫検出法と合わせた。また、内因性vCJDを検出するためのアッセイの感度と特異性を確定するために、vCJD患者21人、弧発性CJD患者27人、その他の神経性疾患患者42人、正常対照群100人からの全血190検体を盲検化したパネルを分析した。その結果、10 ⁻¹⁰ 倍に希釈した外因性vCJDプリオン感染脳と、10 ⁻⁶ 倍に希釈した正常脳を識別することが出来た。アッセイの感度は、過去に報告されていたものよりも桁違いに高かった。また、盲検化したパネルのうち15検体が陽性であった。15検体はすべてvCJD患者からのものであり、vCJDのアッセイ感度が71.4%、特異性が100%であることが示された。これらの研究によって症候性の患者におけるvCJD診断のための血液検査の基本を確立し、無症候性のvCJDプリオン感染のための大規模なスクリーニング検査の開発を見込むことができた。	29
100352	2011/4/20	110078	その他	http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000388.jsp&mid=WC0b01ac0580032ec8&muri=menus/regulations/regulations.jsp&jsenabled=true	EMAより発出された、血漿由来製剤の添付文書の感染性因子に関する警告の記載についてのガイドライン。血漿分画製剤における注意として記載すべき事項として、次のことをあげている。①供血者のスクリーニングを実施していること、②製造工程中に不活化/除去工程があること、③既知又は未知のウイルス、他の感染因子の伝播の可能性が否定できないこと、特に脂質膜を持たないウイルス(HAV、パルボウイルスB19)の潜在的危険性、④投与した際には氏名と製造番号を記録することを推奨すること。	30

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 2010. 12. 17	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	新鮮凍結人血漿	研究報告の公表状況	Houweling H, Wittevrongel CF, Verweij M, Ruitenberg EJ; National Immunisation Programme Review Committee of the Health Council of the Netherlands. Vaccine. 2010 Nov 16;28(49):7723-30. Epub 2010 Oct 14.	公表国 オランダ	
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社)				
研究報告の概要	○オランダのB型肝炎に対する公的な予防接種プログラム:対象を限定したアプローチまたは全小児を対象とするアプローチの適切性の評価 現在オランダではB型肝炎ウイルス(HBV)感染防御のため、全小児を対象とするよりむしろ特定のリスク群(母親がHBVキャリアの小児・片親または両親がB型肝炎流行地出身の小児・HBV感染リスク行動がある者)に予防接種をする方針がとられている。一方、WHOは全小児にユニバーサルワクチン接種することを推奨している。 Health Council of the Netherlands (HCN)は、特定の予防接種を公的プログラムに含めるべきか否かを評価するための基本的な枠組みを提案し、これをB型肝炎の予防接種に適用し、評価した。 その結果、対象を限定したアプローチではリスク群(HBV感染リスク行動がある者)の中に予防接種が行われていない者がおり、ハイリスク群の多くはアプローチが到達していないことが明らかとなった。これは、対象を定めたプログラムを通して得た公衆衛生上の利益が、全小児へのユニバーサルワクチン接種とすることによりかなり増える可能性があることを示している。結果として、ユニバーサルワクチン接種を全小児対象とし、さらにハイリスク群への適切な予防接種プログラムを行うことで、全人口を保護することが出来るとHCNは評価した。				使用上の注意記載状況・ その他参考事項等 新鮮凍結血漿「日赤」 新鮮凍結血漿-LR「日赤」 新鮮凍結血漿-LR「日赤」成分採血 血液を介するウイルス、細菌、原虫等の感染 vCJD等の伝播のリスク
	報告企業の意見	今後の対応 現在オランダで行われている対象を限定したB型肝炎予防接種プログラムを評価した結果、対象となっているリスク群に予防接種を受けていない者がいることが明らかとなり、ユニバーサルワクチン接種を全小児に行い、さらにリスク群へ適切な予防接種プログラムを行う事で、公衆衛生上の利益が増す可能性が示唆されたとの報告である。 なお、現在日本では、HBVキャリアの母親からの母児感染対策のためHBV免疫グロブリン及びワクチン接種と、医療従事者など希望者に対するワクチン接種が行われているが、多くの国で行われている全小児へのユニバーサルワクチン接種は行われていない。今後、垂直感染だけでなく水平感染なども防止するため、日本のHBV感染予防対策を再検討する時期かも知れない。			





Public vaccination programmes against hepatitis B in The Netherlands: Assessing whether a targeted or a universal approach is appropriate

Hans Houweling^{a,*}, Christiaan F.W. Wittevrongel^a, Marcel Verweij^{a,b}, E. Joost Ruitenberga^{a,c}, on behalf of the National Immunisation Programme Review Committee of the Health Council of the Netherlands

^a Health Council of the Netherlands, The Hague, The Netherlands

^b Ethics Institute, Utrecht University, The Netherlands

^c Athena Institute, VU University Amsterdam, The Netherlands

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Public vaccination programme

ABSTRACT

To date, the policy to control hepatitis B in the Netherlands is to vaccinate specific risk groups, rather than all children. Low incidence of the disease has fueled debate whether such a targeted vaccination strategy or rather a universal strategy, as recommended by the World Health Organization, is appropriate. The standard framework for assessing whether a particular vaccination should be included in a public programme, as recently proposed by the Health Council of the Netherlands (HCN), was applied to the various options for hepatitis B vaccination. This framework includes seven selection criteria, grouped under five thematic headings: seriousness and extent of the disease burden, effectiveness and safety of the vaccination, acceptability of the vaccination, efficiency of the vaccination, and priority of the vaccination. From about 1990 the disease burden has stayed more or less the same over time and careful assessment has made it clear that the targeted approach has failed to reach a significant part of the risk groups. Models suggest that the public health benefits obtained through targeted programmes could be augmented considerably by universal vaccination. Based on the assessment that universal vaccination means better protection for high-risk groups as well as the whole population, the HCN calls for universal immunisation, even though hepatitis B to a large extent is limited to specific high-risk groups. Should the Netherlands adopt universal vaccination, several immunisation programmes targeted to high-risk groups will, however, remain of crucial importance for years to come.

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1. Introduction

Hepatitis B virus (HBV) infection causes a high burden of disease. Worldwide, an estimated 2 billion people were infected at some time in the past, and 4–5 million new infections occur every year. Over 350 million people are estimated to be chronically infected and every year about 600,000 people die from HBV-related liver disease or hepatocellular carcinoma (HCC) [1]. The burden of disease is distributed unevenly across the globe, with relatively high prevalence in sub-Saharan Africa, south-east Asia and parts of South America, but low incidence in North America, Australia and north-west Europe.

By 1992 the World Health Organization (WHO) had called for the worldwide introduction of universal vaccination of children against hepatitis B. However, the low incidence of the disease north-west Europe has fueled debate whether targeted or universal vaccina-

tion strategies are the way to go [2,3]. To date, the policy in the Netherlands has been to vaccinate specific risk groups, rather than all children. So far, the controversy regarding universal vaccination has not been resolved.

To further the discussion, the standard framework for assessing whether particular vaccinations should be included in a public programme, as recently proposed by the Health Council of the Netherlands (HCN), was applied to hepatitis B [4]. That framework includes seven selection criteria and provides a framework for the systematic examination of arguments for and against the inclusion and prioritisation of particular vaccinations within public programmes.

2. Hepatitis B in the Netherlands

2.1. Disease burden

In the Netherlands each year between 200 and 300 cases of acute hepatitis B (1.4 and 2.0 per 100,000 persons) and about 1500 cases of chronic hepatitis B are reported, as are a number of deaths from acute disease and an average of 23 deaths from

* Corresponding author at: Health Council of the Netherlands, PO Box 16052, 2500 BB, The Hague, The Netherlands. Tel.: +31 70 3406625; fax: +31 70 3407520.
E-mail address: hans.houweling@gr.nl (H. Houweling).

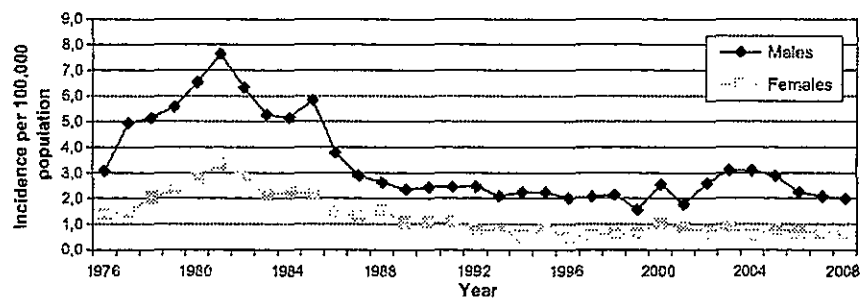


Fig. 1. Reports of acute hepatitis B among males and females in the Netherlands, 1976–2008, per 100,000 people per year.

Source: Koedijk FDH, personal communication.

chronic hepatitis B. The number of reported cases has always included more males than females (Fig. 1). In the early 1980s the total number of cases fell, which may have been linked to changes in sexual risk behaviour—whether or not in connection with the AIDS epidemic—and to preventive measures, including vaccination. Since about 1988, however, the number has remained more or less constant, with small fluctuations.

In the period from 2003 to 2008 inclusive, unsafe sexual contact was the single most important transmission mode for acute hepatitis B in the Netherlands (Table 1): almost two-thirds of the cases were transmitted through sexual contact. In at least half of these cases transmission took place in homosexual contact between men. In about a quarter of cases no mode of transmission is reported. By molecular analysis of the virus HBV genotype A is found in about half of these cases suggesting transmission among men who have sex with men (MSM), but in about a quarter genotype D is concerned, which is linked to heterosexual contact and the Mediterranean [5].

The number of cases of chronic hepatitis B is largely determined by the immigration of carriers from countries where hepatitis B is prevalent. Naturally, vaccination in the Netherlands would not confer protection to these people. However, about 15 percent of all carriers turn out to have been infected in the Netherlands [6].

The available data on the disease burden brought about by hepatitis B are subject to under-reporting, because in its early stages chronic infection often runs an asymptomatic course.

2.2. Current, targeted vaccination programmes

Public programmes in the Netherlands currently focus on three groups for hepatitis B vaccination (Table 2): (1) children whose mothers carry the virus, (2) children with at least one parent from a country where hepatitis B is relatively prevalent, and (3) people at elevated risk of infection through behavioural risks. The first two programmes are provided through the National Immunisation Programme (NIP), the third programme through municipal health services in specific outreaching activities. All programmes are provided free of charge.

Children whose mother carry the virus are exposed to HBV during pregnancy or during delivery. Without curative vaccination, such children have a 10–90 percent chance to develop chronic infection depending on absence or presence of the hepatitis B e antigen (HBeAg) [7,8]. In 1989 a national programme was set up to screen all pregnant women for HBV carrier status during the twelfth week of pregnancy. The midwife or gynaecologist supervising the birth administer the child's first vaccine dose as soon as possible

Table 1

Mode of transmission in acute hepatitis B patients in the Netherlands, reported 2003–2008, absolute numbers and percentages.

Transmission route	2003	2004	2005	2006	2007	2008	Total	Percent
Sexual contact								
MSM	101	101	101	75	77	67	522	32.5
Heterosexual	81	68	87	79	69	65	449	28.0
Not specified	12	8	3	6	2	5	36	2.2
Drug injection	7	3	0	1	1	0	12	0.7
Occupational incidents	7	7	4	4	3	3	28	1.7
Perinatal	2	2	3	0	0	1	8	0.5
Other	19	31	21	18	22	13	124	7.7
Unknown	90	76	86	59	53	65	426	26.5
Total	319	296	302	242	227	219	1605	100

Source: Koedijk FDH, personal communication.

MSM, men who have sex with men.

Table 2

Vaccinations against hepatitis B currently provided through public programmes, the Netherlands, 2009.

Target group	Year programme started	Purpose of vaccination	Vaccination schedule
Children of mothers carrying the virus	1989	Secondary prevention: post-exposure treatment	Paediatric (half) doses at 0,2,3,4,11 months
Children with at least one parent from a relatively high-prevalence country	2003	Primary prevention: infection prevention	Paediatric doses at 2,3,4,11 months
People at elevated risk of infection (men who have sex with men, injecting drug users, commercial sex workers, and heterosexuals with a variety of sexual partners)	1996, intensified 2002	Primary prevention: infection prevention	Three full doses at intervals of 1 and 5 months respectively

after birth, at the same moment that hepatitis B immunoglobulin is given to confer immediate protection.

Since 2003, children with at least one parent from a country where hepatitis B is relatively prevalent (hepatitis B surface antigen (HBsAg) prevalence more than 2 percent) are targeted for vaccination, covering about 15 percent of all neonates in the Netherlands. The vaccination provided is intended to prevent horizontal, non-sexual transmission of the virus from carriers to young children, in the context of intensive domestic contact.

In 1996 the HCN concluded that higher vaccination coverage levels and improved protection were needed for people who are at elevated risk of infection through behavioural risks: men who have sex with men (MSM), problematic drug users (DU), commercial sex workers (CSW), and heterosexuals with a variety of sexual partners (HVSP) [9]. In 2002 separate outreaching vaccination programmes were launched to target these so-called 'behavioural' high-risk groups. On the basis of these programmes MSM, DU, CSW, and HVSP all qualified for free vaccination. A number of outreach programmes were set up on behalf of these groups; for instance, MSM were approached via places of entertainment and meeting spots and outpatients' clinics for sexually transmitted infections (STI), CSW and HVSP via STI clinics.

The programmes targeted towards behavioural risk groups were evaluated in 2007. From molecular epidemiological research it became clear that in the Netherlands, heterosexual contacts were not responsible for an infection chain of any importance [5,6]. For that reason, the vaccination of heterosexual men and women with a variety of sexual contacts was stopped on 1 November 2007 [10].

3. The HCN assessment framework to support informed decision-making and prioritisation for public vaccination programmes

In the HCN framework the factors that determine a vaccine's suitability for inclusion in a public programme were translated into seven selection criteria, grouped under five thematic headings: seriousness and extent of the disease burden, effectiveness and safety of the vaccination, acceptability of the vaccination, efficiency of the vaccination, and priority of the vaccination (Table 3). The criteria are based on two ethical principles: (1) that the best possible protection should be afforded to the population as a whole and (2) that benefit should be fairly distributed across population groups, with protection provided on the basis of need. A more detailed explanation of each criterion and discussion about its use is available elsewhere [4].

4. Assessment of current targeted hepatitis B vaccination programmes in the Netherlands

4.1. Infants born to mothers carrying the virus

The vaccination of children against hepatitis B born to mothers carrying the virus satisfies criteria 1–5 and criterion 7. It was estimated that the programme prevents around 200 acute and 180 chronic infections every year [11]. The screening programme of pregnant women and the related programme of vaccination of infant children of HBsAg-positive mothers has been subjected to operational evaluation on a number of occasions [11–14]. Each time the conclusions have been that vaccination coverage and timeliness of the various vaccine dose administrations were suboptimal. Figures for the years 2006–2008 (children born in 2003–2005) show that the degree of vaccination in the children had risen from 90.3 percent to 97.4 percent [15]. Screening actually takes place among about 90 percent of the country's pregnant women [16]. No analysis of the vaccination's cost-effectiveness (criterion 6) was made at

Table 3

Criteria for inclusion of vaccinations in public programmes.

Seriousness and extent of the disease burden
1. The infectious disease causes considerable disease burden within the population
The infectious disease is serious for individuals, and:
The infectious disease affects or has the potential to affect a large number of people
Effectiveness and safety of the vaccination
2. Vaccination may be expected to considerably reduce the disease burden within the population
The vaccine is effective for the prevention of disease or the reduction of symptoms
The necessary vaccination rate is attainable (if eradication/elimination or the creation of herd immunity is sought)
3. Any adverse effects associated with vaccination are not sufficient to substantially diminish the public health benefit
Acceptability of the vaccination
4. The inconvenience or discomfort that an individual may be expected to experience in connection with his/her personal vaccination is not disproportionate in relation to the health benefit for the individual concerned and the population as a whole
5. The inconvenience or discomfort that an individual may be expected to experience in connection with the vaccination programme as a whole is not disproportionate in relation to the health benefit for the individual concerned and the population as a whole
Efficiency of the vaccination
6. The balance between the cost of vaccination and the associated health benefit compares favourably to that associated with other means of reducing the relevant disease burden
Priority of the vaccination
7. Relative to other vaccinations that might also be selected for inclusion, provision of this vaccination serves an urgent public health need at reasonable individual and societal costs

the time of its introduction in the Netherlands. A cost-effectiveness analysis was made, however, prior to the introduction of vaccination for children from high-prevalence countries. In view of the findings of that analysis, the HCN has taken the view that the cost-effectiveness ratio is likely to be favourable [14].

4.2. Children with at least one parent from a high-prevalence country

The vaccination of children who have at least one parent from a high-prevalence country meets all seven criteria [17]. Early models indicated that, without vaccination, there would be between 1150 and 2550 infections a year in the population as a whole, 90–220 of which would become chronic [11]. In the recent comparative model of the National Institute for Public Health and the Environment (RIVM) it is estimated that over a period of 50 years 2490 deaths would be prevented by targeted vaccination of this group [18]. Vaccination, implemented in 2003, probably has largely eliminated infection within this target group [14]. A comparison of incidence of notified acute infection among children aged 0–4 years in post-vaccination as opposed to prevaccination birth cohorts (0.3 vs. 2.9 per 1,000,000 person years) was consistent with effective vaccination, but was hampered by a change in case definition for notification [19].

4.3. Behavioural high-risk groups

The effectiveness of the vaccination of people from behavioural high-risk groups has been evaluated since the start of an enhanced programme in 2002, using epidemiological data, blood samples and genotyping [6,20]. During the research period (2003–2007) the reported incidence of acute hepatitis B did fall—among males from 3.1 to 2.1 per 100,000 per year and among females from 0.9 to 0.6 per 100,000—but in the period immediately preceding this study

the incidence had risen, so the fall during the research period may have been part of a normal fluctuation.

Indications for an effect also emerge from the analysis of the data per high-risk group. The number of cases of acute hepatitis B in the largest high-risk group, MSM, fell from above 100 per year in the period 2003–2005 to about 80 in the period 2006–2007. The median age rose from 38 in 2004 to 42 in 2007; no similar rise in median age appeared in the other high-risk groups. In a study in Amsterdam, which compared the period 1991–1997 (before the introduction of targeted vaccination of behavioural high-risk groups) with the period 1998–2003, a reduction in the number of cases of acute hepatitis B among DU and their heterosexual partners was observed. However, there was also a fall in the size of the group itself, so this reduction was probably not caused by the vaccination programme. In Amsterdam, no fall in incidence levels was observed among MSM; median age of MSM with hepatitis B rose from 31 years in the first period to 35 in the second [21].

Genotyping made it clear that different HBV types are found in different high-risk groups. Overall, six genotypes were found. A single genotype A strain circulates among MSM, indicating that hepatitis B in the Netherlands in this group is effectively spreading in one large sexual network. Among heterosexuals, there was no clear ongoing transmission of a single strain and new introductions occur with greater frequency, including via carriers from countries where HBV has relatively high prevalence [5,6].

It is not entirely clear how the findings among MSM are to be interpreted. The researchers in Amsterdam are of the opinion that since the incidence of acute hepatitis B remained stable despite indications for increased sexual risk behaviour, vaccination may well have prevented a rise in this incidence [21]. This interpretation is in line with modelling studies and a coinciding observed decline in genetic diversity [22].

An important finding of these studies is that intensive campaigns nevertheless failed to reach a large part of the behavioural high-risk groups. Estimates of the vaccination coverage varied from 12 percent (MSM) to 42 percent (HSVP) in Amsterdam, and even lower levels in the rest of the country [20,21]. A substantial number of those in the high-risk groups is already infected and therefore would no longer benefit from vaccination, but even allowing for this fact the researchers estimated that 48–72 percent of the members of behavioural high-risk groups in Amsterdam still run the risk of contracting hepatitis B [21]. Other research into MSM in Amsterdam reached similar conclusions [23].

In mathematical models the effectiveness (criterion 2) and the efficiency (criterion 6) of vaccination of people in behavioural risk groups were evaluated. It was estimated that over a period of 50 years 3226 deaths could be prevented by vaccination of people from behavioural high-risk groups. However, it also appeared that, despite large-scale intensification, programmes targeted towards MSM especially still have an incomplete reach and are insufficient to further reduce disease incidence [18].

The cost-effectiveness ratio of the high-risk group approach is favourable: for the vaccination of the infant children of parents from intermediate or high-endemic countries it lies, depending on the prevalence scenario, between 100 and 700 Euro per QALY gained (the vaccination only of children born to mothers carrying the virus was used as reference scenario). For the vaccination of people from behavioural high-risk groups it amounts to 1800–3500 Euro per QALY gained [18]. These figures are well below the maximum acceptable cost for a preventive measure, which in the Netherlands is, quite arbitrarily, often set at 20,000 Euro per life year of QALY gained.

In conclusion, the current targeted strategies yield considerable public health benefits at reasonable cost. However, these strategies fail to reach a significant proportion of those in the risk groups involved, and given the already extensive efforts it seems that the

potential for further improvement is limited. After a recent assessment in 2009 the HCN recommended retention of the targeted vaccination programmes. However, in the opinion of the Council the suboptimal perfect reach of the targeted programmes made reconsideration of universal vaccination expedient [24].

5. Assessment of potential future universal hepatitis B vaccination programmes in the Netherlands

In 2001, the HCN concluded that insufficient data were available to assess infection risk in the population at large and the effectiveness of universal vaccination. In the view of the Council such an assessment and a comparison with the current targeted strategy could only be made using mathematical modelling and cost-utility analysis [11]. The existing epidemiological models, however, were not deemed suitable and RIVM was commissioned to refine them [18,25,26]. A report of RIVM's comparative modelling research was made available to the Council in late 2007, supplementary results were supplied in 2008. The HCN is of the opinion that the RIVM model, in its current version, accurately represents the nature of HBV infection, its spread in the population, and the effects of targeted and universal vaccination programmes. The model has been described in detail elsewhere [18]. In March 2009 the HCN published an assessment of universal vaccination of either infants or preadolescents (Table 4) against the seven criteria [24]. A summary of that report is presented here.

5.1. Criterion 1: seriousness and extent of disease burden

In a 2007 advisory report the HCN evaluated hepatitis B as a serious disease and the current disease burden caused by it in the Dutch population as 'substantial'. The arguments for this were that acute hepatitis B can have serious consequences; that chronic infection carries a considerable risk of complications and death; and that carrier status forms a source for the further spread of the disease in the population. Taken together, the various subpopulations facing these risks form a substantially large group [17]. In its 2009 advisory report the Council has further substantiated the evaluation of hepatitis B as a public health issue [24]. In conclusion, despite extensive targeted vaccination programmes hepatitis B in the Netherlands continues to pose a public health problem.

5.2. Criterion 2: effectiveness

For the prevention of hepatitis B efficacious vaccines are available. A course of vaccination, depending on age and purpose comprising two to four doses, induces protective levels of antibodies in over 95 percent of healthy infants, children, and adolescents, and in more than 90 percent of healthy adults younger than 40 years [27].

In several countries universal vaccination has reduced disease incidence substantially [28–30]. In the Netherlands, a marked reduction of disease incidence has been achieved by behavioural change and targeted vaccination of specific risk groups.

Table 4
Potential future public hepatitis B vaccination programmes in the Netherlands.

Target group	Purpose of vaccination	Vaccination schedule
All infants	Primary prevention: infection prevention	Three pediatric (half) doses at intervals of 1 and 5 months
All preadolescents	Primary prevention: infection prevention	Three half doses at 1 and 5 months intervals or two full doses at 6 months interval

The potential for further reduction by universal vaccination was explored by RIVM in the forementioned comparative mathematical model [18,24]. The models confirm that the targeted approaches leave room for improvement: over a period of 50 years about 5000 extra deaths would be prevented by adding universal vaccination of either infants or prepubertals. In the case of universal infant vaccination it would also be worthwhile to consider including an 11-year catch-up vaccination campaign for 12-year olds, in order to reach the favourable effects of vaccination sooner. It is estimated that by doing so about 650 extra deaths would be prevented over a period of 50 years.

5.3. Criterion 3: safety

After more than 25 years of extensive use, the available data show that vaccination against hepatitis B is indeed safe. The use of hepatitis B vaccines is associated with generally mild and transient reactogenicity [31,32]. Clinical research and post-marketing research have shown that severe adverse effects—defined as death, serious neurological symptoms, or permanent bodily impairment—are very rare [33–36].

In a study by Hernán vaccination against hepatitis B was associated with increased risk of multiple sclerosis [37]. This finding is at variance with that of a number of other studies and WHO concluded that the Hernán study provides no convincing support for the hypothesis that immunisation with recombinant hepatitis B vaccine is associated with an increased risk of multiple sclerosis [38]. However, the careful design of the study prompted the HCN to review all available data. On the basis of that analysis the HCN concluded that a causal association between vaccination with recombinant hepatitis B vaccine and MS is most unlikely [24,39,40].

For sure when used in high-risk groups, the side effects of vaccination against hepatitis B do not detract from the health benefits obtained. And even though the balance between favourable effects (reduction of disease burden) and adverse effects—that can never be totally excluded—may be more critical for use in the general population, the HCN concluded that the favourable safety profile forms no impediment to the eventual vaccination of all infant or prepubertal children.

5.4. Criteria 4 and 5: acceptability

5.4.1. In connection with the individual vaccination

The discomfort associated with hepatitis B vaccination is limited and for each of the target groups important health benefits are to be gained. For all risk groups involved the discomfort associated with vaccination is in reasonable proportion to the health benefit. This also holds true for the general population, since universal vaccination enables an important additional health benefit to be achieved in the population as a whole.

Universal vaccination against hepatitis B can be achieved at infant age by making use of a combined vaccine. This makes it possible to introduce it without subjecting those participating in the NIP to any additional injections. Universal vaccination of prepubertal children does require extra injections and new injection moments. However, the burden posed by these is limited and, in the judgement of the HCN, acceptable.

5.4.2. In connection with the NIP as a whole

In universal vaccination of infant children, the injection burden can stay the same—two injections per session—if use is made of a DTP-polio-Hib-HepB combined vaccine, and there is no question of potential conflicts with other NIP vaccinations. Universal vaccination of prepubertal children does require extra injections and new injection moments. But, here too, the HCN sees no impediment to the introduction of possible universal vaccination from

the perspective of its acceptability within the NIP. If it is decided to introduce the universal vaccination of prepubertal children, the Council advises that, for girls, this be administered simultaneously with the vaccination against cervical cancer.

The fourth and fifth criteria are not meant to assess public support. Yet, as discussed elsewhere, in a programme that builds upon public trust, it is important to take people's views seriously, and to take these into account when discussing inclusion of vaccinations in public programmes. Public support is a prerequisite for any public programme and should be secured before implementation of any vaccination in such a programme [4].

5.5. Criterion 6: efficiency

In the case of hepatitis B prevention there are no good alternatives to vaccination, but several strategies could be followed. In the RIVM mathematical model the cost-effectiveness ratios of the various targeted and universal vaccination strategies were compared, starting from the vaccination only of children born to mothers carrying the virus [18].

For the universal vaccination of infant children, the cost-effectiveness ratio was estimated, depending on the prevalence scenario, between 2300 and 4800 euro per QALY gained (reference scenario: vaccination of children born to mothers carrying the virus and behavioural risk groups). An 11-year catch-up vaccination campaign among 12-year-olds could add a marked health benefit at reasonable cost, about 5000–10,000 euro per QALY gained. For the universal vaccination of prepubertal children, cost-effectiveness ratios similar to those for infants were calculated. All these figures are well below the threshold of 20,000 Euro per life year of QALY gained.

In conclusion, universal vaccination of either infants or prepubertal children in addition to targeted programmes for children born to mothers carrying the virus and members of behavioural risk groups provides an efficient way of preventing hepatitis B, even in a low prevalence country like the Netherlands.

5.6. Criterion 7: priority

The final criterion concerns assessment of the priority of hepatitis B vaccination compared to other vaccinations that might also be selected for inclusion in the NIP. Priority should be given to the vaccination that serves the most urgent public health need, measured as the greatest public health benefit at reasonable individual and societal costs. A discussion how this criterion is used and the possibilities and limitations of extending priority setting to other areas of public health is given elsewhere [4].

The 2007 HCN advisory report *The future of the National Immunisation Programme: towards a programme for all age groups* assessed 23 'candidate vaccinations' for the NIP and listed four which qualified for further assessment in the short to medium term: vaccination against cervical cancer, hepatitis B, rotavirus infection-induced diarrhoea, and shingles/chickenpox [17].

In its 2008 advisory report on vaccination against cervical cancer the HCN concluded that, despite a greatly reduced disease burden and mortality brought about by existing screening programmes, that disease continues to pose a significant public health problem [41]. It was estimated that vaccination against cervical cancer in addition to screening could prevent about 300 extra illnesses and 100 deaths per year. It was recommended that vaccination for girls aged 12 years be included in the NIP and subsequently the Minister of Health decided to do so.

Like cervical cancer, the HCN considers the disease burden posed by hepatitis B in the Netherlands as being substantial, despite concerted targeted vaccination programmes. On the basis of illness reporting data, the number of cases of disease that could be

prevented by universal vaccination against hepatitis B is of the same order of magnitude as that for cervical cancer, but the number of deaths prevented is lower. However, the actual numbers are supposedly higher because of under-reporting.

Other diseases for which vaccination might be considered for inclusion in the NIP in the short to medium run do not pose a public health problem of comparable nature and size as hepatitis B. Chickenpox does occur frequently in the Netherlands but usually runs a benign course. Hospital admissions and complications are reported less frequently than in neighbouring countries, possibly because of a relatively early median age at infection [42]. Shingles is a fairly common and quite serious illness among the elderly in particular, for which vaccination has recently become possible. The HCN considers vaccination against shingles to be potentially valuable, but the vaccination will not easily be integrated within the NIP and is costly [17]. Each year rotaviruses cause an estimated 58,000 infections, more than 3000 hospital admissions, and 1–3 deaths in children aged 0–4 years of age [43]. Vaccination is attractive because it prevents a large number of infections and hospital admissions, and because it can be given orally. However, use of the vaccine in a public programme is restrained because of a cost-effectiveness ratio which is higher than what is usually considered reasonable in the Netherlands [44].

The HCN concluded that universal vaccination against hepatitis B addresses an important public health problem, and that compared to other candidate vaccines, it deserves to be given priority.

6. Discussion

In the Netherlands, extensive targeted hepatitis B vaccination programmes have been in place for several years. In addition to the programmes already presented in this paper children with Down syndrome are vaccinated via the NIP, and people at risk through their work are targeted through occupational health services. The targeted programmes have resulted in the alleviation of the considerable disease burden associated with acute and chronic infection.

When sufficient, a targeted approach to vaccination is to be preferred over a universal approach. However, after an initial fall since the introduction of the targeted programmes the disease burden has stayed more or less the same over time and despite enhancement of the programmes from 2002 hepatitis B continues to pose a public health problem. Careful assessment has made it clear that the targeted approach has failed to reach a significant part of the risk groups.

The WHO recommends vaccinating all children, even in the low incidence countries of north-west Europe. Efficacious vaccines are available and in several countries universal vaccination has reduced disease incidence substantially. Extensive, worldwide use has provided a large safety database. Allegations that vaccination could cause multiple sclerosis can be conclusively refuted.

Over the years reliable models of hepatitis B and its prevention have been constructed. These models suggest that the public health benefits obtained through targeted programmes could be augmented considerably by universal vaccination and these findings constitute a strong argument for universal vaccination. However, one could wonder whether it is fair to call for universal immunisation for a disease which to a large extent is limited to specific high-risk groups. Does universal vaccination constitute a fair distribution of the benefits and drawbacks of vaccination?

In a public programme like the NIP the emphasis is on collective protection. In the case of vaccination against hepatitis B, the protection of the individual is a less prominent issue because of the relatively low risk, in general, of being infected. Although hepati-

tis B to a large extent is limited to specific high-risk groups, cases do occur with considerable frequency outside these groups. Those cases could not be prevented by a high-risk group based policy. Therefore, universal vaccination serves a collective interest. Yet, it is worth noting that universal vaccination protects not only those who fall outside the well-known high-risk groups, but also those within an important high-risk group which considerable efforts have so far failed to yield an adequate reach: MSM. Protection here, too, serves a collective interest, because it is impossible to know in advance which people are going to belong to this high-risk group in the future. Universal vaccination means better protection for consecutively targeted age cohorts, including future high-risk groups.

Based on the analysis presented in this paper the HCN has recommended to implement universal vaccination. Both vaccination of infant or of prepubertal children are reasonable choices, but infant vaccination is expected to deliver the greater health benefit because of a vaccination coverage that is likely to be higher. Infant vaccination would also be easier to incorporate into the existing NIP and, since combination vaccines are available and no additional injections are involved, would not increase the vaccination burden for participants. The ministerial decision upon the advice is pending.

Our analysis shows that universal vaccination can replace the current targeted programmes only in the long run. Should the Netherlands elect to adopt universal vaccination, screening of pregnant women and the related immunisation programme of infant children of HBsAg-positive mothers as well as vaccination of people from behavioural high-risk groups will remain of crucial importance for years to come.

In recent years the content of the NIP has become the subject of considerable public debate. Use of standardised criteria furthers a trustworthy, transparent and accountable process of decision-making about inclusion of new vaccinations and may help to retain public confidence in public vaccination programmes. However, such a decision-making process may not be sufficient to gain public confidence when target diseases and target groups beyond childhood are concerned. The example of vaccination against cervical cancer may be illustrative in this regard. In several countries, including the Netherlands, untoward discussion in the lay and scientific media has resulted in so far insufficient uptake of that vaccination. A probable cause is that initial campaigning was too much based upon experiences with routine childhood vaccinations; too little implementation research was done into the specific needs and customs of young girls and their parents. A restart of the campaign was planned for April 2010. Similarly, the HCN has recommended to do implementation research to guide the introduction of hepatitis B into the NIP. That a negative attitude towards universal vaccination may indeed be an obstacle is suggested by a small survey—that is, however, hampered by a small size and low response rate—into parental attitudes towards hepatitis B vaccination. In this survey about ten percent of both parents of children currently eligible for targeted vaccination and parents of children not currently eligible had a negative attitude towards universal vaccination [45].

Note added: On July 22, 2010 the Minister of Health decided that he will follow the advice to implement universal vaccination against hepatitis B in the NIP, at infant age. The targeted programmes will be continued until the groups at risk will be protected through universal vaccination. The minister decided not to follow the advice for an eleven-year catch-up vaccination for 12-year olds [46].

The full report including assessment of hepatitis B vaccination strategies for the Netherlands against each individual criterion is available at <http://www.gezondheidsraad.nl/en/publications/general-vaccination-against-hepatitis-b-revisited>.

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医薬品 研究報告 調査報告書

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一般の名称		—	研究報告の 公表状況	Clinical Infectious Diseases, March 1, 2011, volume 52, issue 5, 624-632	公表国	
販売名(企業名)		—			中国	
研究報告の概要	輸血によるオカルト B 型肝炎 (OHB) における B 型肝炎ウイルス (HBV) の伝播性に関する研究は少ない。本稿は、動物およびヒトを対象とした試験において、輸血による OHB 供血者の HBV 伝播性を明らかにすることを目的とした。供血者 217, 595 名のうち、OHB 供血者 67 名を特定した。 ヒト肝細胞を移植したキメラマウス 4 匹に対し、2 名の供血者の血清サンプルを接種し、血清および肝臓 HBV DNA レベルを測定したところ、血清 HBV DNA は、接種から 5 および 7 週間後に、マウス 1 匹で検出可能であった (10^4 copy/mL)。 また、OHB 供血者 10 名 [うち 9 名は抗 HBs 抗体陽性] から辿って特定した輸血受血者 49 名を対象に、HBV 感染の検査を行い、供血者と受血者の HBV ゲノム配列間の相同および系統発生解析を行ったところ、受血者 49 名中 45 名 (91.8%) は HBV に感染しておらず、検査結果が陽性であった受血者は 4 名であった。そのうち 3 名では、その供血者の HBV との 83~86% の相同性や系統発生的関係の遠さから、輸血による伝播が否定され、残り 1 名の HBV は供血者の HBV と 95% の配列相同性を示し、輸血による HBV 感染と判定した。 今回の動物とヒトを対象とした試験では、OHB 供血者由来の血液で感染する可能性があることが分かったが、ヒトでは抗 HBs 抗体陽性 OHB 供血者から提供された血液の輸血による慢性 B 型肝炎の伝播リスクは依然として低かった。					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			
オカルト HBV 感染者からの輸血による B 型肝炎感染に関する報告である。 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、採血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、原血漿 (プール血漿)、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。				

②

MAJOR ARTICLE

Transmissibility of Hepatitis B Virus (HBV) Infection through Blood Transfusion from Blood Donors with Occult HBV Infection

Man-Fung Yuen,^{1,*} Danny Ka-Ho Wong,^{1,*} Cheuk-Kwong Lee,² Yasuhito Tanaka,³ Jean-Pierre Allain,⁶ James Fung,¹ Jennifer Leung,² Che-Kit Lin,² Masaya Sugiyama,³ Fuminaka Sugauchi,⁴ Masashi Mizokami,⁵ and Ching-Lung Lai¹

¹Department of Medicine, The University of Hong Kong, Queen Mary Hospital, and ²Hong Kong Red Cross Blood Transfusion Service, Hospital Authority, Hong Kong; ³Department of Virology and Liver Unit, Nagoya City University Graduate School of Medical Sciences, Kawasumi, Mizuho, Nagoya, ⁴Department of Gastroenterology and Metabolism, Nagoya City University Graduate School of Medical Sciences, Nagoya; ⁵Research Center for Hepatitis and Immunology, International Medical Center of Japan Konodai Hospital, Ichikawa, Japan, and ⁶Department of Haematology, University of Cambridge, Cambridge, United Kingdom

Background. Studies of the transmissibility of hepatitis B virus (HBV) in occult hepatitis B (OHB) through blood transfusion are scarce. We aimed to determine the transmissibility of HBV in blood donors with OHB through transfusion in animal and human studies.

Methods. Among 217,595 blood donors, 67 donors with OHB were identified. Four chimeric mice populated with human hepatocytes were inoculated with 2 donor serum samples. Serial serum and liver HBV DNA levels were measured. Forty-nine recipients of blood transfusions traced from 10 donors with OHB (9 of whom were positive for antibody to hepatitis B surface antigen [anti-HBs]) were tested for HBV infection. Homology and phylogenetic analyses between the HBV genomic sequences of donors and recipients were performed.

Results. Serum HBV DNA was detectable (10^4 copies/mL) in 1 mouse at weeks 5 and 7 after inoculation. Total HBV DNA and HBV replication template (covalently closed circular DNA) and hepatitis B core antigen were detected in the mouse liver. After transfusion, 45 recipients (91.8%) had no HBV infection (ie, they tested negative for hepatitis B surface antigen and HBV DNA). Four tested positive for HBV DNA. In 3 recipients, 83%–86% homology and distant phylogenetic relatedness with their donor HBV excluded transmission through transfusion. The remaining recipient HBV had 95% sequence homology with her donor HBV, compatible with acquisition of HBV infection from the transfusion. High anti-HBs levels in 7 other recipients suggested recent transfusion-related HBV immune response.

Conclusions. OHB donor blood infectivity was shown in our animal and human studies. However, the risk of HBV transmission in humans was low, especially from blood products obtained from donors with OHB who were anti-HBs positive.

Occult hepatitis B (OHB) virus infection is defined as having detectable hepatitis B virus (HBV) DNA in the serum or livers of subjects who test negative for the hepatitis B surface antigen (HBsAg). Owing to the

extremely low serum HBV DNA level (usually <100 IU/mL) [1], which requires highly sensitive assays to confirm the diagnosis, accurate data on the prevalence and transmissibility of OHB are very limited. The latter issue is of particular importance in improving transfusion safety. Posttransfusion acute hepatitis B has been reported to occur at a rate of 0.9% [2], even after the implementation of serological screening for HBV.

Current information on the transmissibility of OHB through transfusion mainly relies on a few case series in which the possible link has not been clearly established by strict viral sequence comparisons between the HBV strains in the donors and the recipients. OHB is not

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*M.F.Y. and D.K.H.W. contributed equally for the study.

Correspondence: Man-Fung Yuen, MD, Dept of Medicine, The University of Hong Kong, Queen Mary Hospital, Pokfulam Rd, Hong Kong (mfyuen@hkucc.hku.hk).

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a common entity in most countries, with a typical prevalence of <1% [3–6]. Identification of a blood donor with OHB requires considerable time for performance of nucleic acid testing (NAT) followed by extremely sensitive polymerase chain reaction (PCR) assays. These also require a high expenditure on resources. Once the donated blood is identified as OHB positive, it is discarded for obvious ethical reasons. Retrospective tracing studies are difficult to conduct because of the time lag between identification of the OHB products and contact of recipients. In addition, in areas where HBV is highly endemic, a large proportion of the population would have been naturally infected with or vaccinated against HBV.

We conducted the present study to determine the transmissibility of HBV from donors with OHB in 2 parts: (1) a chimeric mice study, to determine whether serum obtained from donors with OHB was infectious; and (2) a recipient look-back study to determine whether OHB-containing blood components were transfusion-transmissible in human recipients.

SUBJECTS AND METHODS

Subjects

The present study recruited all healthy blood donors found to have OHB during the period from 1 April 2007 through 31 January 2009 in the Hong Kong Red Cross Blood Transfusion Service. These subjects were identified by testing their blood samples for HBsAg (Prism HBsAg; Abbott Laboratories) and NAT for HBV DNA (Procleix Trigris system; Chiron; 95% detection limit, 12.2 IU/mL). All HBsAg-negative and HBV DNA-positive samples had HBV DNA levels measured by real-time PCR in the Mx4000 Multiplex Quantitative PCR System (Stratagene), using primers and 6-carboxyfluorescein (FAM)-labeled TaqMan probe targeting to conserved regions in the HBV surface gene, as described previously [7].

Of 217,595 donors, 67 subjects with OHB (0.031%) were identified. They were all negative for antibodies to human immunodeficiency virus and hepatitis C virus. Of these 67 subjects with OHB, 44 consented to participate in this study for the following additional tests: determination of the HBV DNA level, with the Artus HBV RG assay (Qiagen), which has a linear range of detection of 1.1 IU/mL to $>4 \times 10^9$ IU/mL; assessment for antibodies to HBsAg (anti-HBs) and hepatitis B core antigen (anti-HBc; Roche Diagnostics); the chimeric mice study; and the recipient look-back study with HBV genome sequencing.

Chimeric Mice Study

Two donor samples indicating OHB—1 with undetectable anti-HBs and 1 with anti-HBs titer ≥ 10 IU/L (conventionally taken as “protective” against HBV infection)—were used to inoculate 4 chimeric mice (2 mice for each sample). These were severe

combined immunodeficiency (SCID) mice transgenic for the urokinase-type plasminogen activator gene (uPA+/+/SCID+/+ mice), with the livers replaced by human hepatocytes [8] (Phoenix Bio). Human serum albumin produced by human hepatocytes was measured by enzyme-linked immunosorbent assay (Bethyl Laboratories). One hundred microliters of the donor serum, after 10-fold concentration, were intravenously injected into the mice. Serum samples were taken weekly from the mice from week 4 to 9 after inoculation. At week 9, the mice were killed for liver immunostaining and intrahepatic HBV DNA and covalently closed circular DNA (cccDNA) measurements. HBV DNA levels were quantified by real-time PCR targeting a small S region [9], with the sensitivity of 1000 copies/mL (200 IU/mL). The cccDNA, replicative template of HBV was quantified by real-time detection-PCR using a set of conserved primers [10]. Animal protocols were performed in accordance with guidelines for animal experimentation.

Liver sections were blocked with antibody diluent (Dako), incubated with rabbit anti-HBc (Dako) at room temperature for 1 h, washed in phosphate-buffered saline, and then incubated with goat anti-rabbit immunoglobulin G conjugated with Cy3 (Chemicon international) or goat anti-human albumin labeled with fluorescein isothiocyanate (Bethyl Laboratories). Sections were washed with phosphate-buffered saline and observed in a fluorescent microscope (Eclipse E800M; Nikon).

Look-Back Study

Data tracing of all recipients of blood components from the 44 donors with OHB were retrieved from the database of the Hong Kong Red Cross Blood Transfusion Service. Data obtained included sex, age, the date and type of blood products transfused, indication for transfusion, the nature of the illness, and HBsAg status prior to the targeted (look-back) transfusion.

Two genomic regions (pre-S and core) were sequenced from the serum of the 44 donors with OHB. In brief, HBV DNA was extracted from their serum samples using the QIAamp MinElute Virus Vacuum Kit (Qiagen). The pre-S and core regions were amplified by nested PCR, and the 2 resulting amplicons, covering the pre-S and core regions at nt 2814–237 and 1607–2385, respectively, were sequenced bi-directionally. These 2 most variable HBV regions [1, 11] were used for homology and phylogenetic comparisons between paired donors and recipients strains. Thirty-one of the 44 donors with OHB had successful viral sequencing results at either the pre-S or core regions. Recipients of transfusions from these 31 donors with OHB were contacted. HBsAg, anti-HBs, anti-HBc, and HBV DNA levels (determined as described above) were checked in recipients who consented to take part in the study. For recipients with detectable HBV DNA, the pre-S and core regions were also sequenced by PCR. Viral sequence homology of the donor and recipient

strains was performed using the CLUSTAL X program [12]. Genetic distances were estimated by the maximum composite likelihood method, and phylogenetic trees were constructed by using the neighbor-joining method, using the Molecular Evolutionary Genetics Analysis (MEGA) software, version 4 [13]. Bootstrap resampling tests were performed 1000 times to confirm the reliability of the phylogenetic tree, and values >70% were considered to be significant [14, 15]. The presence of the common surface gene mutants (G145R) were determined by PCR sequencing using primers spanning the HBV surface gene at nucleotides 426–600 (amino acid residues, 134–148).

The study was approved by the Institutional Review Board of the University of Hong Kong and the Hospital Authority Hong Kong West Cluster. Written, informed consent was obtained from both the donors and recipients. For those recipients aged <18 years, consent was obtained from parents.

Statistical Analysis

The present study was descriptive in nature. Continuous variables were expressed as mean values with standard deviations and ranges.

RESULTS

Forty-four of the 67 identified OHB donors consented to have additional blood samples obtained for further studies. Thirty-three donors (75%) had anti-HBs levels ≥ 10 IU/L. The donors' demographic characteristics, serologic parameters, and HBV DNA levels are listed in Table 1.

Chimeric Mice Study

Serum samples from 2 randomly selected donors (Rx5 and Rx7) with HBV DNA levels of 9.1 and 11.0 IU/mL, respectively, and anti-HBs levels of 34 IU/L and below the detection limit, respectively, were inoculated into 4 mice (2 mice for each serum sample at the DNA amount of 52.8 and 63.8 copies). Serum HBV DNA was detectable in 1 mouse (PXB 8–22), which was inoculated with serum with undetectable anti-HBs from donor Rx7 at weeks 5 and 7 (2.4×10^4 and 2.0×10^4 copies/mL, respectively). The other 3 mice had no detectable serum HBV DNA up to 9 weeks. Total DNA and cccDNA (0.0458 and 0.0103 copies/cells, respectively) were detected in the liver of the PXB 8–22 mouse, whereas HBV DNA and cccDNA were not detectable in the livers of the other 3 mice. The immunofluorescent microscopy for hepatitis B core antigen (HBcAg) and human albumin in the liver tissues showed that the human hepatocytes expressed HBcAg (Figure 1).

Look-Back Study

Thirty-one donors with OHB had successful viral sequence generation. Two hundred seventy-two recipients who had

Table 1. Demographic Characteristics, Serologic Parameters, and Hepatitis B Virus (HBV) DNA Levels for 44 Donors with Occult Hepatitis B Infection

Characteristic	Value
Ratio of male to female subjects	32:12
Age, years	
Mean $\pm$ SD	46.9 $\pm$ 11.9
Range	16.8–61.2
HBV DNA level	
No. (%) of donors with an HBV DNA level <1.1 IU/mL (%)	26 (59.1)
No. (%) of donors with an HBV DNA level $\geq$ 1.1 IU/mL (%)	18 (40.9)
Mean	15.8
SD	45.5
Range	1.1–196.6
G145R mutations (the vaccine escape mutants), no. (%) of donors	0 (0)
Anti-HBs level	
No. (%) of donors with anti-HBs $\geq$ 10 IU/mL	33 (75%)
Titer	
Mean $\pm$ SD	217.4 $\pm$ 309.9
Range	11.1 to >1000
Anti-HBc	
No. of donors with positive anti-HBc	42 (95.5%)

NOTE. All samples positive for antigen to hepatitis B core antigen (anti-HBc) samples were of immunoglobulin G subtype. Anti-HBs, antibody to hepatitis B surface antigen.

received blood products within 3 years from these 31 donors with OHB were identified. Forty-nine recipients from 10 donors were successfully traced and consented to participate in the study. The remaining 223 recipients were not being able to undergo further investigations because they could not be contacted ($n = 46$), refused to participate in the study ($n = 30$), or died ($n = 147$). The causes of death for these 147 subjects were traced successfully. Only 16 deaths (10.9%) were liver related (3 recipients died of hepatitis C virus-related cirrhosis and/or liver cancer, and 13 recipients were known to have chronic HBV infection before transfusion and died of HBV related cirrhosis and/or liver cancer). None died because of fulminant HBV infection.

Detailed demographic characteristics and clinical, serological, and virological parameters of the 10 donors with OHB and the 49 recipients (ratio of male to female subjects, 24:25) are listed in Table 2. All 10 donors were repeated donors. The sample with which OHB was first diagnosed by NAT is defined as the "index sample"; the sample from the donation received by the 49 recipients is the "look-back sample" (HBV DNA levels were available in some cases); the sample taken when they were recalled for further study is the "sample at the time of further investigations." The median age of the recipients was 49.9 years

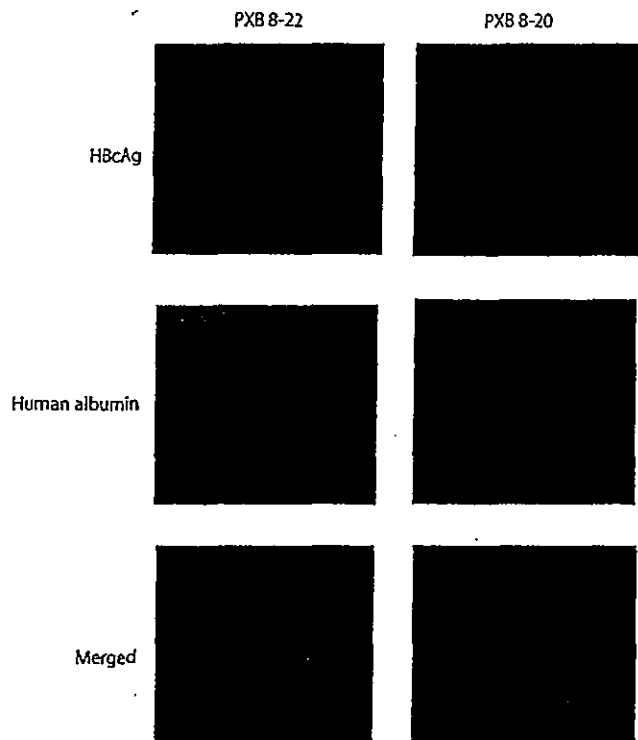


Figure 1. Staining for hepatitis B core antigen (HBcAg). In the PXB 8-22 mouse, staining for HBcAg was confined to areas where mouse liver had been replaced for human hepatocytes (the areas stained for human albumin). Co-localization of HBcAg and human hepatocytes (~40% of total human hepatocytes at this area) was demonstrated by double-staining for HBcAg and human albumin, whereas no merged stain was found in the PXB 8-20 mouse.

(range, 3.1–87.4 years). Fifteen recipients were immunocompromised because they had undergone organ transplantation with immunosuppressive therapy, had a hematologic malignancy, or had end-staged renal failure.

Twenty recipients (40.8%) were positive for immunoglobulin G anti-HBc (15 with anti-HBs) (Table 2). Thirteen (26.5%) had detectable anti-HBs levels but no anti-HBc, suggesting HBV vaccination. Nine of these 13 recipients were aged <35 years. Both anti-HBc and anti-HBs were undetectable in 16 recipients (32.7%), who were considered to be susceptible to HBV infection but noninfected. Only the 15 recipients carrying both anti-HBc and anti-HBs were considered possibly HBV infected by transfusion, and among these, 2 (Rx69 and Rx96) were found to be HBV DNA positive (HBV DNA level, 9.3 and 14 IU/mL, respectively), indicating an OHB carrier state. The viral sequence homology between the recipients and donors HBV was 83% and 95%, respectively, with values confirmed by the phylogenetic analyses (Figure 2). Another 2 recipients (Rx42 and Rx70) tested positive for HBsAg with detectable HBV DNA, indicating chronic HBV carriage (one was immunocompromised [viral load, 7.3×10^7 IU/mL]; the other had a viral load of 33

IU/mL). The viral sequence homology for these 2 recipients' and donors' HBV was only 88% and 86%, respectively, with values confirmed by the phylogenetic analyses (Figure 2). The liver biochemistries of these 4 recipients were all normal.

Of the remaining 45 recipients, 16 had undetectable anti-HBc and anti-HBs; 3 were positive for anti-HBc, but anti-HBs was undetectable; 13 had undetectable anti-HBc but were positive for anti-HBs; and 13 were positive for both anti-HBc and anti-HBs (Table 2). Of the 26 recipients with positive anti-HBs, 8 had moderately high anti-HBs titers (≥ 200 IU/mL). Two (Rx39 and Rx66) of these 8 recipients were negative for anti-HBc, and 6 (Rx40, Rx47, Rx67, Rx71, Rx95, and Rx97) were positive for anti-HBc. One 12-year-old recipient (Rx66) had an anti-HBs titer of 412 IU/L tested 26 months after transfusion. The other 7 recipients (age, 47–75 years) had anti-HBs levels between 299 and >1000 IU/L tested 20–52 months after transfusion. These 7 recipients received blood components carrying HBV DNA in all tested donations (5–27 IU/mL) from 4 donors (Rx36, Rx34, Rx58, and Rx15). Only 6 of the 27 recipients of transfusions from these 4 donors remained susceptible and noninfected (ie, negative for both anti-HBc and anti-HBs).

There were 223 recipients for whom further investigations were not performed. Twenty-four were HBsAg positive and 199 had unknown HBsAg status before transfusions. Of these 199 recipients, 24 underwent HBsAg testing after transfusions. Twenty-three of these 24 recipients were negative for HBsAg. The remaining recipient (who died of lung cancer 4 months after the transfusion) was positive for HBsAg.

DISCUSSION

In the present study, we demonstrated in the chimeric mice that serum obtained from donors with OHB with undetectable anti-HBs could be infectious. Serum HBV DNA was detected in 1 infected mouse, and more importantly, intrahepatic HBV DNA, cccDNA (the viral template in hepatocyte nucleus for viral transcription), and HBcAg (the viral protein) were found in the human hepatocytes of the mouse liver. On the contrary, the sample with positive anti-HBs titer did not infect the mice. Additional animal studies using chimeric mice model should be performed with more samples from donors with OHB, with and without anti-HBs, to confirm our preliminary finding.

It has been shown that anti-HBc-positive blood products can cause acute HBV infection in recipients [16–19]. The infectivity of anti-HBs-positive blood products is still not well defined in humans [20]. Of the 49 recipient follow-up samples, 4 were positive for HBV DNA. Two were HBsAg positive, indicating chronic hepatitis B carriage; 2 were HBsAg negative, indicating OHB. The viral sequence homology of only 83%–86%, confirmed by the distant phylogenetic relatedness between donor and recipient strains, excluded a transfusion origin in 3 of these

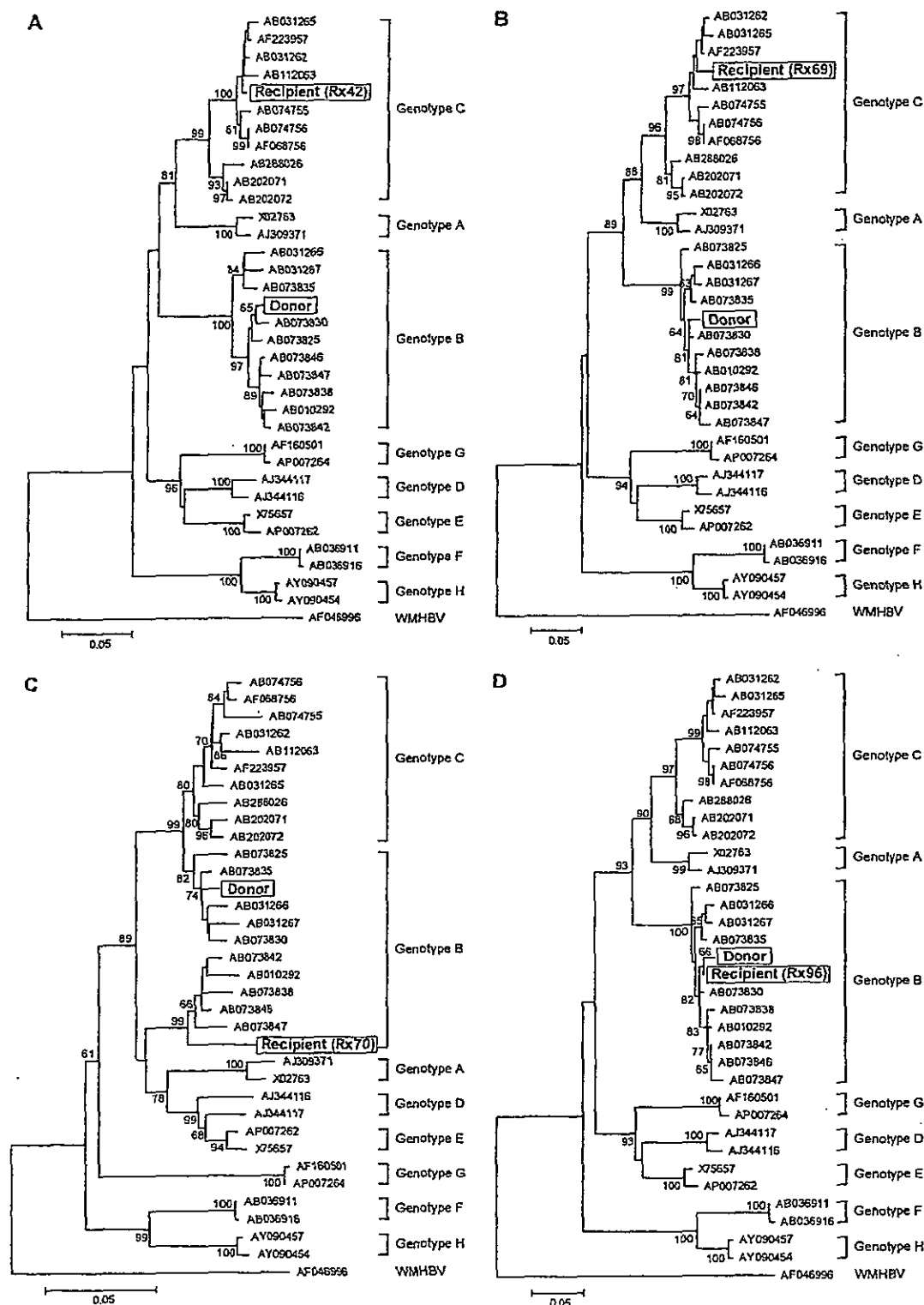


Figure 2. Phylogenetic analysis of the pre-S or core sequences of the 4 recipients with detectable HBV DNA (A, Rx42; B, Rx69; C, Rx70; and D, Rx96) and their corresponding donors. Sequences from the recipients and donors (boxed and in bold-face fonts) were subjected to phylogenetic analysis, along with the same pre-S or core regions from GenBank reference HBV sequences of various HBV genotypes (10 each of genotypes B and C, the 2 most common genotypes in Hong Kong, and 2 each of genotypes A, D, E, F, G, and H) and a reference woolly monkey hepatitis B virus (WMHBV) sequence. Sequences retrieved from the GenBank are designated by their accession numbers. The figures on the nodes indicate the percentage of occurrences by bootstrap analysis of 1000 replicates. Only bootstrap values >70% are shown. The length of the horizontal bars indicates the number of nucleotide substitutions per site.

Table 2. Detailed Demographic Characteristics and Serological and Virological Data for the Donor and Recipient Pairs

Donor	Donor index sample HBV markers	Look-back sample HBV DNA, IU/mL	Donor sample at the time of further investigations	Recipient	Blood component transfused	Recipient HBV markers	Transfusion- follow-up sample collection (months)	HBV status						
Code Sex/ age, years	Anti- HBc (U/L)	Anti- HBs (IU/mL)	HBV DNA (IU/mL)	Code Sex/age, years	Immuno- deficiency	Anti-HBc (U/L)	Anti-HBs (IU/mL)	HBV DNA (IU/mL)						
Rx15 M/59	Pos	Neg ^a	16	Pos	32.7	UD ^b	Rx93 M/70	No	RC ^c	Neg	Neg	UD	35	Susceptible
			19	Pos	80.1	2.79	Rx97 M/72	Yes	Plt	Pos	>1000	UD	33	Recovered HBV ^a
				Pos	80.5	4.79	Rx69 F/76	No	RC	Pos	52	9.3	25	OHB
				Pos	866.5	UD	Rx73 F/77	No	RC	Pos	87	UD	49	Recovered HBV
				Pos	80.1	2.79	Rx77 F/58	Yes	FFP	Neg	Neg	UD	48	Susceptible
				Pos	80.5	4.79	Rx95 F/52	No	Plt	Pos	299	UD	38	Recovered HBV ^a
				Pos	80.1	2.79	Rx82 F/34	No	FFP	Neg	102	UD	32	Vaccinated
				Pos	80.5	4.79	Rx76 F/69	No	Plt	Pos	175	UD	49	Recovered HBV
				Pos	866.5	UD	Rx100 M/6	No	Plt	Neg	Neg	UD	51	Susceptible
				Pos	80.1	2.79	Rx96 F/51	No	RC	Pos	30	14	60	OHB
				Pos	80.5	4.79	Rx83 M/19	No	Plt	Neg	Neg	UD	50	Susceptible
			Pos	866.5	UD	Rx84 F/22	No	RC	Neg	131	UD	46	Vaccinated	
			Pos	80.1	2.79	Rx85 F/59	Yes	Plt	Pos	22	UD	52	Recovered HBV	
Rx28 M/48	Pos	30	UD	Pos	80.1	2.79	Rx49 F/46	Yes	Plt	Neg	Neg	UD	44	Susceptible
Rx34 M/58	Pos	38	UD	Pos	80.5	4.79	Rx47 F/75	No	RC	Pos	387	UD	20	Recovered HBV ^a
			14	Pos	80.1	2.79	Rx46 F/63	No	RC	Pos	Neg	UD	23	Recovered HBV
			14	Pos	80.5	4.79	Rx45 M/4	Yes	Plt	Neg	Neg	UD	24	Susceptible
Rx24 F/48	Pos	430	UD	Pos	866.5	UD	Rx92 M/22	No	RC	Neg	84	UD	34	Vaccinated
				Pos	80.1	2.79	Rx99 M/3	No	FFP	Neg	Neg	UD	36	Susceptible
				Pos	80.5	4.79	Rx91 M/65	No	Plt	Neg	14	UD	47	Vaccinated
				Pos	866.5	UD	Rx75 F/35	No	FFP	Neg	Neg	UD	63	Susceptible
			UD	Pos	80.1	2.79	Rx81 F/60	No	RC	Neg	17	UD	33	Vaccinated
Rx19 M/50	Pos	19	UD	Pos	75.4	UD	Rx102 M/6	No	FFP	Neg	54	UD	23	Vaccinated
				Pos	80.1	2.79	Rx74 M/57	No	FFP	Neg	Neg	UD	40	Susceptible
			26	Pos	80.5	4.79	Rx98 F/31	No	RC	Neg	74	UD	31	Vaccinated
			UD	Pos	866.5	UD	Rx70 F/55	No	RC	Pos	Neg	33	23	OHB
				Pos	80.1	2.79	Rx68 F/87	No	Plt	Pos	137	UD	46	Recovered HBV
Rx23 F/46	Pos	39	10	Pos	85.5	1.81	Rx94 M/60	No	RC	Neg	Neg	UD	25	Susceptible
Rx36 M/61	Pos	18	6.2	Pos	67.2	UD	Rx39 M/53	No	RC	Neg	>1000	UD	48	Vaccinated ^a
			7	Pos	67.2	UD	Rx66 M/12	Yes	Plt	Neg	412	UD	26	Vaccinated
				Pos	85.5	1.81	Rx40 M/47	Yes	FFP	Pos	>1000	UD	39	Recovered HBV ^a
				Pos	67.2	UD	Rx44 M/48	Yes	Plt	Pos	Neg	UD	43	Recovered HBV

Table 2. (Continued)

Donor	Donor index sample HBV markers				Look-back sample HBV DNA, IU/mL	Donor sample at the time of further investigations			Recipient	Blood component transfused		Recipient HBV markers		Transfusion- follow-up sample collection (months)	HBV status			
Rx33 F/59	Pos	Neg	UD	27					Rx50 M/17	No	RC	Neg	175	UD	19	Vaccinated		
Rx58 M/56	Pos	Neg	8.5	UD	Pos	85.2	UD		Rx43 F/42	Yes	FFP	Pos	77	UD	41	Recovered HBV		
Rx63 M/49	Pos	Neg	1.1	UD					Rx38 F/50	Yes	RC	Neg	Neg	UD	15	Susceptible		

NOTE. **Bolded** recipient data indicates those found HBV DNA positive in the follow-up sample. CRYO, cryoprecipitate; FFP, fresh frozen plasma; HBV, hepatitis B virus; Neg, antibody to hepatitis B surface antigen (anti-HBs) titer of <10 IU/mL; OHB, occult hepatitis B; Plt, platelet concentrate; RC, red cell concentrate; UD, samples with HBV DNA detectable by nucleic acid tests but not quantifiable by the real-time polymerase chain reaction assays, either with total absence of signal or below the lower limit of detection.

^a Recipients suspected of having exposure/reexposure to HBV after receiving the blood products from donors with OHB as suggested by a high anti-HBs titer (>200 IU/mL).

4 cases. One of the 3 recipients (Rx42) was likely to have experienced HBV reactivation after receiving chemotherapy for non-Hodgkin lymphoma. For the fourth recipient (Rx96), who was negative for HBsAg, with 95% homology in the highly variable pre-S genomic between the donor and recipient HBV (Figure 2), it is probable that this immunocompetent recipient acquired the HBV infection through the blood transfusion from a donor with OHB who had undetectable anti-HBs titer in the index sample and low titer (32.7 IU/L) in the sample at the time of further investigations. Discounting the 3 recipients who were probably infected with HBV before transfusion, transmission of HBV resulting in chronic HBV disease was possible but uncommon (1 [2.2%] of 46 recipients). This may be an underestimation, because 13 of the recipients were positive for anti-HBs but negative for anti-HBc, possibly because of previous HBV vaccination. The chronic HBV disease rate for the remaining 33 recipients who were susceptible to HBV infection would therefore be 3.0% (1 of 33 recipients).

Moderately high anti-HBs levels (≥ 200 IU/mL) were found in 8 recipients (Table 2). One 12-year-old recipient (Rx66) with an anti-HBs titer of 412 IU/mL might have the high titer because of comparatively recent HBV vaccination during childhood. The high anti-HBs levels in the remaining 7 adult recipients (age, >45 years) in samples collected 20–52 months after transfusion with blood components from 4 donors was suggestive of a relatively recent anti-HBs immune response secondary to exposure or re-exposure to HBV. (Of the 4 donors, 2 had undetectable anti-HBs in the index samples; all 4 were positive for anti-HBs at the time of additional investigations for the present study). The recent anti-HBs immune response is compatible with the transfused blood products carrying either circulating viral particles or very low level of the otherwise undetectable free HBsAg. Although not directly indicative of infectivity, it constitutes supportive evidence of potential infectivity of HBV DNA-positive blood products even in the presence of anti-HBs. Future studies correlating the detection of HBV DNA over time with respect to the timing of increase of anti-HBs levels in recipients are required to verify this hypothesis.

Nevertheless, the relative rarity of HBV transmission from donors with OHB held true for different scenarios. These included recipients who were children or elderly (4 recipients were aged <7 years, 1 was aged 12 years, and 10 were aged >65 years in the present study) (Table 2). Of the 15 immunocompromised recipients, none acquired HBV from transfusion (except 1 who had probably OHB before transfusion, with reappearance of HBsAg after chemotherapy, suggesting reactivation) [21].

This low infectivity may only be applicable to donors with OHB who are positive for anti-HBs (75% of all 44 donors [Table 1] and 90% of the 10 donors in the donor-recipient pairs [Table 2] were positive for anti-HBs). According to another study [22], more than one-half (57.8%) of subjects with OHB

were anti-HBs positive. It has been suggested that the risk of HBV transmission is low for blood products from anti-HBs-positive donors with OHB [23, 24]. A recent study reported a case of HBV transmission from an anti-HBs positive donor to two recipients when the low level of anti-HBs was insufficient to neutralize an increased level of HBV DNA [25].

The present study has 2 limitations. First, 147 recipients had died, making a complete recipient-tracing not possible. However, according to data on the causes of death, it appeared unlikely that the deaths were related to the transfusions. Second, clinical information with regard to the disease status, including liver function tests and liver histology, was not addressed in the present study. However, those were studied in detail in a previous study of ours: subjects with OHB subjects have normal liver function test results and nearly normal liver histology findings [26].

In conclusion, blood taken from donors with OHB was shown to be potentially infectious in our animal and human studies. However, the risk of chronic hepatitis B transmission through transfusion of blood donated by anti-HBs-positive donors with OHB in human remained low.

Acknowledgments

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Potential conflicts of interest. M.-F.Y. has served on the speakers' bureau or has given lectures at GSK and BMS. C.-L.L. has received institutional grant support from LG Life Sciences and FibroGen and has served on the speakers' bureau or given lectures to BMS and Gilead Sciences Europe. All other authors: no conflicts.

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医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日 2011. 4. 8	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称		人全血液		Shao ZJ, Zhang L, Xu JQ, Xu DZ, Men K, Zhang JX, Cui HC, Yan YP. J Med Virol. 2011 May;83(5):791-5. doi: 10.1002/jmv.22043. Epub 2011 Feb 25.	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)			中国	
研究報告の概要	OB型肝炎ウイルスの母児感染: 中国の研究 B型肝炎ウイルス(HBV)に母児感染した新生児は90%がキャリアになるとされている。2002年以降、中国では周産期のHBV母児感染を予防するため、B型肝炎(HB)ワクチン及び抗HBs人免疫グロブリン(HBIG)が使用されている。本研究は、HBs抗原陽性の母親から生まれた214人の新生児において、出生時または子宮内でのHBV感染がHBIG及びHBワクチンにより効果的に予防できるかどうかを判断するために行い、HBs抗原を測定した。母児感染は約4.7%(10/214)で、内訳は出生時感染は3.7%(8/214)、子宮内感染は0.9%(2/214)であった。母児感染のリスクは、母親のHBs抗原又はHBV DNAレベルに応じて上昇した。36カ月の追跡調査後、出生時感染症例のすべてがHBs抗原陰性になり、子宮内感染症例のすべてがキャリア化した。これらの結果により、HBIG及びHBワクチンは、出生時感染の予防には効果があったが、子宮内で感染した新生児には十分でないことが示された。					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			
HBs抗原陽性の母親から生まれた新生児において、出生時または子宮内でのHBV感染がHBIG及びHBワクチンにより効果的に予防できるかどうかを判断するために研究を行ったところ、出生時感染の予防には効果があったが、子宮内感染した場合には十分でないことが示されたとの報告である。		HBV感染に関する新たな知見等について、今後も情報の収集に努める。				



Mother-to-Infant Transmission of Hepatitis B Virus: A Chinese Experience

Zhong-Jun Shao,¹ Lei Zhang,¹ Jian-Qiu Xu,² De-Zhong Xu,¹ Ke Men,¹ Jin-Xia Zhang,¹ Heng-Chun Cui,¹ and Yong-Ping Yan^{1*}

¹Department of Epidemiology, School of Public Health, The Fourth Military Medical University, Xi'an, China

²Department of Obstetrics, Shaanxi Maternal and Neonatal Health Hospital, Xi'an, China

Over 90% of infants infected with hepatitis B virus (HBV) caused by mother-to-infant transmission will evolve to carrier status, and this cannot be prevented until widespread administration of the HB vaccine and hepatitis B immune globulin (HBIG) is implemented. This prospective study of 214 infants born to HBsAg-positive mothers was carried out to determine if either perinatal or intrauterine HBV transmission could be effectively prevented with HBIG and the HB vaccine. Peripheral blood was collected from mothers and from newborns before they received HBIG and the HB vaccine, as well as at 0, 1, 7, 24, and 36 months after birth. Infants born with an ratio of signal to noise (S/N) value of >5 for HBsAg (ABBOTT Diagnostic Kit) were defined as mother-to-infant transmission cases, those with an S/N between 5 and 50 were classified as perinatal transmission cases, and those with an S/N >50 were considered intrauterine transmission cases. Mother-to-infant transmission occurred in approximately 4.7% (10/214) of the infants; the perinatal transmission and intrauterine transmission rates were 3.7% (8/214) and 0.9% (2/214), respectively. The risk of mother-to-infant transmission increased along with maternal HBeAg or HBVDNA levels. After 36 months of follow-up, all perinatal cases became HBsAg-negative, whereas all intrauterine transmission cases evolved into carrier status. These results indicate that infants infected via intrauterine transmission cannot be effectively protected by HBIG and HB vaccine. *J. Med. Virol.* 83:791–795, 2011. © 2011 Wiley-Liss, Inc.

KEY WORDS: perinatal transmission; intrauterine transmission; chronic HBV carrier status; follow-up; infants

INTRODUCTION

Hepatitis B virus (HBV) infections are a global health problem. One-third of the world population has

serological evidence for current or past HBV infection, and 15–25% of the 350 million people with chronic HBV face a risk of early death from HBV-related liver diseases, such as end-stage liver cirrhosis or hepatocellular carcinoma [Chang, 2007; Wilt et al., 2008]. Mother-to-infant HBV transmission contributes significantly to the persistence of the high number of HBV carriers because the risk for newborns to develop a chronic infection is greater than 90%, whereas no more than 5% of the adult population develops chronic HBV infection [Burk et al., 1994; Zhu et al., 1997]. Thus, infected newborns often have poor prognoses. Carrier status during childhood facilitates intrafamilial transmission, particularly to siblings, as well as transmission in preschools and daycare centers.

Since 2002, HBIG and HB vaccine have been used to prevent perinatal HBV transmission in China. The aim of this prospective study was to determine whether mother-to-infant transmission of HBV in China could be effectively prevented with HB vaccine and HBIG.

METHODS

Study Population

HBsAg-positive mothers and their infants were recruited for the study; pregnant women aged 20–40 years who underwent regular checkups for serum HBsAg at the Maternal and Neonatal Hospital of Shaanxi Province, Xi'an, China from September 2002 to October 2005 were screened, and their infants were followed until the end of 2008. Blood specimens were taken from mothers and their newborns at delivery. To

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Shao ZJ and Zhang L contributed equally to the work.

*Correspondence to: Yong-Ping Yan, Department of Epidemiology, School of Public Health, The Fourth Military Medical University, No. 17, Changle West Road, Xi'an 710032, China. E-mail: yanyaping@fmmu.edu.cn

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prepare serum, blood samples were collected from the newborns via the femoral artery and stored at -20°C until use. The parents or legal guardians of the infants were contacted by the Maternal and Neonatal Hospital via telephone, email, or letters and were interviewed by an experienced investigator using a structured questionnaire to collect demographic data at each follow-up visit. Infants were seen at 1, 7, 24, and 36 months, at which time blood samples were collected by venipuncture.

Passive and active immunoprophylaxis was given to infants according to the Chinese HBV vaccination schedule. Within 12 hr of delivery, a 200-IU-dose of hepatitis B immune globulin (HBIG, Shanghai Institute of Biological Products, Shanghai, China) and a 10- μg -dose of recombinant yeast-derived hepatitis B vaccine (manufactured by Kangtai Biological Products Co. Ltd, Shenzhen, China) were given intramuscularly after blood collection. Subsequently, 10- μg doses of hepatitis B vaccine were given to the infants at 1 and 6 months after birth according to the manufacturer's protocol.

The study protocol was approved by the Ethics Committee for Medical Research of the Fourth Military Medical University, and written informed consent was obtained prior to the study.

Determination of HBV Biomarkers

All maternal serum specimens were tested by ELISA for HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc (Kehua Co., Shanghai, China). Maternal HBV DNA analysis was performed using the COBAS AMPLICOR HBV monitor test (Roche Molecular Systems). Viral loads above the linear range were determined by dilution as recommended by the manufacturer. HBV DNA was extracted from the serum of HBsAg-positive infants and their mothers and amplified by polymerase chain reaction (PCR) as described in our previous report [Shao et al., 2007]. Serum specimens were tested by ELISA for HBsAg (Abbott Laboratories, Abbott Park, IL, USA), anti-HBs, HBeAg, and anti-HBe plus anti-HBc [total anti-HBc; if positive, also for anti-HBc IgM (Kehua Co., Shanghai, China)] if enough serum was available.

For HBsAg ELISA (Abbott Laboratories, Abbott Park, IL, USA), the signal to noise ratio (S/N) is used to reflect infant HBsAg titers. An S/N of 5 was used as the cut-off value for HBV diagnosis in infants. An S/N between 5 and 50 was considered HBV perinatal transmission, and cases with an S/N > 50 were designated intrauterine transmission.

Statistical Analysis

Fisher's exact test was used to detect associations between grouped maternal viral load, maternal HBeAg positivity, and transmission rates. Statistical analysis was performed using the Statistical Package for the Social Sciences (version 10.0, SPSS, Chicago, IL, USA). Statistical significance was defined as $P < 0.05$ (two-tailed).

RESULTS

Characteristics of the Included Infants and their Mothers

A total of 212 HBsAg-positive pregnant women and their 214 infants (including two sets of twins) were recruited between September 2002 and October 2005. All enrolled mothers were Xi'an citizens belonging to the Han ethnic group. Of the 214 infants, 140 were delivered by caesarean section, and 110 were male. Five infants had an APGAR score of less than 5 within 5 min after birth, and five infants were born weighing under 2500 g. Of the 212 mothers, 47 (22.1%) were HBeAg-positive, none were anti-HBc-positive, all were anti-HBc-positive, and 5 were IgM anti-HBc-positive. Of the 212 HBsAg-positive pregnant women, 64 (30.2%) had detectable HBV DNA. The viremic mothers were arbitrarily stratified into three groups based on their HBV DNA levels: 21 had levels between 10^3 and 10^5 copies/mL (low viral load), 32 had levels between 10^5 and 10^6 copies/mL (middle viral load), and 11 had levels $>10^6$ copies/mL (high viral load). Of mothers with detectable HBV DNA, 43 (20.1%) were HBeAg-positive. HBeAg positivity was strongly correlated with high viral load ($P < 0.001$), and all patients with HBV DNA levels $>10^6$ copies/mL were HBeAg-positive (Table I).

S/N values ranged from 0.98 to 109 among the 214 infants. Seventy-one infants were born with an S/N > 5 for HBsAg by ABBOTT diagnostic reagent, and ten were diagnosed as mother-to-infant transmission cases. There were no differences in maternal education levels (10.40 ± 3.59 vs. 10.83 ± 3.23 years), maternal age at interview (27.37 ± 3.73 vs. 28.08 ± 3.48 years) or neonatal weight (3250.00 ± 336.45 vs. 3035.64 ± 860.37 g) between newborns with mother-to-infant HBV transmission and those without. Two infants with HBsAg S/Ns of 50.93 and 109.71 were identified as intrauterine HBV transmission cases. Perinatal transmission (HBsAg S/N under 50) was identified in 8 of the 214 infants (3.7%).

Compliance of Mothers and their Infants During Follow-Up

Of the 214 infants, 57 (26.6%), 158 (73.8%), 144 (67.3%), and 38 (17.8%) attended follow-up visits at 1, 7, 24, and 36 months of age, respectively. Thirty-one infants did not attend any of the scheduled follow-up visits. The main reasons for failure to follow-up were fear of venipuncture, concern about cross-infection,

TABLE I. HBeAg Status and Viral Load in 212 HBsAg-Positive Pregnant Women

HBV DNA level	HBeAg-negative mothers (n = 165)	HBeAg-positive mothers (n = 47)
$<10^3$ copies/mL	144 (87.3%)	4 (8.5%)
10^3 – 10^5 copies/mL	11 (6.6%)	10 (21.3%)
10^5 – 10^6 copies/mL	10 (6.1)	22 (46.8%)
$>10^6$ copies/mL	0 (0%)	11 (23.4%)
Totals	165	47

living too far from the hospital, a change in telephone number, and wrong address/relocation. A total of 122 infants entered the cohort from month 7 to month 24 but did not attend the month 36 visit. Among the remaining 48 infants, attendance varied at months 1, 7, 24, or 36. There were no differences in maternal age, maternal educational level, or neonatal weight at birth between infants who attended follow-up visits and those who failed to follow-up at various time points (Table II).

Mother-to-Infant Transmission Cases

Mother-to-infant transmission was detected in 10 infants. In two of these cases, high HBsAg titers were detected in their peripheral serum at birth (50.93 in infant No. 54, 109.71 in infant No. 154), and they developed HBV carrier status during the follow-up period. Moreover, they were continuously positive for HBV DNA, HBsAg, and HBeAg during the follow-up period. The other eight identified perinatal transmission cases became negative for HBV DNA, HBsAg, and HBeAg during the follow-up period: six of these newborns were born to HBeAg-positive women, and four of the six were born to HBeAg-positive mothers. The risk of transmission was significantly higher from HBeAg-positive mothers than HBeAg-negative mothers (6/41 vs. 4/163, odds ratio = 5.96 (1.60–22.12), $P < 0.001$) as well as from mothers with detectable HBV DNA levels compared with mothers with non-detectable HBV DNA levels (7/57 vs. 3/145, odds ratio = 5.94 (1.48–22.75), $P < 0.001$). The risk of HBV mother-to-infant transmission increased with maternal HBV DNA levels (3/145 low viral load group vs. 1/20 middle viral load group vs. 5/27 high viral load group vs. 1/10 very high viral load group, $P < 0.001$).

Dynamic Changes in Other HBV Biomarkers in Infants

Anti-HBs were not detected in any infants at birth. The rate of anti-HB positivity was 82.5% (47/57), 91.7% (145/158), 70.8% (102/144), and 73.7% (28/38) at 1, 7, 24, and 36 months of age, respectively. The trend of anti-HB over time was characterized by a steep rise in the first month followed by a gradual decrease, eventually reaching a plateau at 24 months of age (Fig. 1).

Forty-seven of the 212 mothers were HBeAg-positive, and 35 of their infants (74.5%) were HBeAg-positive at birth. Of the HBeAg-positive infants, all became HBeAg-negative at the first month, except for two infected via intrauterine transmission, and remained so thereafter. No HBeAg was detected in the 167 infants born to mothers who were HBeAg-negative at birth.

Anti-HBe was detected in 152 mothers (152/214, 71.0%), and all infants born to anti-HBe-positive mothers were anti-HBe-positive at birth. However, anti-HBe was not detected in the 62 infants born to the 60 anti-HBe-negative mothers. Anti-HBe was detected in 27/52 (47.4%) infants at month 1 and 52/116 (44.8%) at month 7, but not at month 24 (0/144) or

TABLE II. Baseline Characteristics of Infants Who Attended the Follow-up Visits and Those Did Not

Characteristics	Month 1			Month 7			Month 24			Month 36		
	Follow-up	Failed to follow up	Number	Follow-up	Failed to follow up	Number	Follow-up	Failed to follow up	Number	Follow-up	Failed to follow up	Number
Birth weight (Kg)	3124.2 ± 650.8	3132 ± 550.2	57	3244.6 ± 441.8	3067 ± 550.3	158	3274.4 ± 450.8	3357.7 ± 450.1	144	3489 ± 450.2	3143 ± 550.1	38
Number of male infants	32(56.1%)	77(49.0%)		64(40.5%)	45(80.3%)		78(54.2%)	32(45.7%)		23(60.5%)	85(48.3%)	
Maternal age at delivery	27.5 ± 4.5	27.6 ± 6.9		28.7 ± 5.6	27.9 ± 7.5		27.1 ± 4.1	28.2 ± 5.2		28.6 ± 5.5	26.2 ± 5.6	

TABLE III. Dynamic Changes in Other HBV Biomarkers in the 10 Infants During Follow-up Period

No.	Maternal status ^a	Maternal HBV DNA copies	Neonatal HBsAg titer ^b	Neonatal status at birth	Month 1	Month 7	Month 24	Month 36
9	1.4.5	<10 ³ /mL	5.35	1.4.5	2.4.5	2.4.5	2.5	2
17	1.3.5.6.7	5 × 10 ⁶ /mL	9.42	1.3.5.6	2.5	2.5	2.5	2.5
39	1.3.5.7	2 × 10 ⁷ /mL	7.06	1.5.6	5	2.5	2.5	2
54	1.3.5.7	4 × 10 ⁸ /mL	103.1	1.3.5.7	1.3.5.7	1.3.5	1.3.5	F
97	1.4.5	3 × 10 ⁶ /mL	5.76	1.4.5	2.4.5	F	F	2.5
154	1.3.5.7	5 × 10 ⁸ /mL	50.93	1.3.5.6.7	1.3.5.7	1.3.5.7	1.3.5	1.3.5
178	1.3.5.7	3 × 10 ⁶ /mL	7.54	1.3.5	2.5	F	F	2.5
197	1.4.5	<10 ³ /mL	5.87	1.4.5	4.5	2.4.5	2.4.5	2
200	1.3.5.7	7 × 10 ⁷ /mL	8.83	1.5	F	2.5	2	2
210	1.4.5	<10 ³ /mL	7.34	1.4.5	4.5	2.5	2.5	2

1 = HBsAg, 2 = anti-HBs, 3 = HBeAg, 4 = anti-HBe, 5 = anti-HBc, 6 = IgM anti-HBc, 7 = HBVDNA, F = fail to follow up

^aMaternal bloods were collected during labor before delivery.

^bS/N value.

month 36 (0/38). No infants became anti-HBe-positive during the follow-up period.

Anti-HBc was detected in all infants born to anti-HBc positive mothers both at birth (214/214) and at month 1 (57/57). The anti-HBc positivity rate was 84.8% (134/158) at month 7, 34.0% (49/144) at month 24, and 21.1% (8/38) at month 36. However, two of the infants identified as HBV carriers at birth were positive for IgM anti-HBc, but this disappeared within 7 months after delivery.

DISCUSSION

HBV infection is a leading cause of acute and chronic liver disease in children and adolescents worldwide [Chang, 2007; McMahon, 2009]. In areas of high endemicity in China, especially in northwestern China, the carrier rate for hepatitis B surface antigen (HBsAg) is as high as three times the national average of China (<http://www.chinacdc.cn/n272442/n272530/n294176/n339985/index.html>). Treatment strategies for children with chronic hepatitis B have focused on inducing HBeAg seroconversion and inhibiting viral replication to prevent active liver damage. However, the initiation of antiviral therapy in children is challenging and often controversial [Wilt et al., 2008; Beasley, 2009; McMahon, 2009]. Therefore, prevention is still important for controlling mother-to-infant transmission. HBIG is a plasma product that can prevent hepatitis B if given

to an infected individual within 14 days of exposure [Buchanan and Tran, 2010; Gane, 2010]. HBIG is given in conjunction with a three-dose hepatitis B vaccine series after exposure to the blood or sexual body fluids of an HBV-infected person. Beginning in 2002, similar measures were implemented in China; infants born to HBsAg-positive mothers now receive HBIG and hepatitis B vaccination within 24 hr of birth. In this study, all enrolled infants received prophylaxis within 24 hr of birth according to the hospital records. Eight infants with verified perinatal HBV transmission became negative during the follow-up period. In contrast, both infants diagnosed with intrauterine transmission cases evolved to HBV carrier status. This finding is consistent with previous reports that suggest preventive measures play a limited role in eliminating HBV in infants infected via intrauterine transmission [Zhu et al., 2003; Ni, 2010]. In the present study, the rate of mother-to-infant transmission was 4.7% (10/214), and the 0.9% of infants that remained positive for HBsAg were verified to be intrauterine transmission cases. These rates are lower than the 5–17% reported by some Chinese studies with both larger and smaller sample sizes [Xu et al., 1998; Yan et al., 1998; Dz and Yp, 1999].

HBeAg and HBV DNA in maternal serum are considered reliable markers of potential infectivity and may be associated with a high risk of perinatal HBV transmission [Xu et al., 2002; Shao et al., 2007]. In the present study, the risk of transmission was significantly higher from HBeAg-positive mothers than HBeAg-negative mothers [6/41 vs. 4/163, odds ratio = 5.96 (1.60–22.12)], and the same was true for maternal HBV DNA load [7/57 vs. 3/145, odds ratio = 5.94 (1.48–22.75)], which confirms previous observations [Burk et al., 1994; Zhu et al., 2003; Wang et al., 2005; Shao et al., 2007]. HBV DNA positivity is more accurate than HBeAg to predict infectivity. In the present study, maternal HBV DNA level was closely and positively associated with the incidence of HBV perinatal transmission, which is consistent with previous reports [Burk et al., 1994; Xu et al., 2002; Shao et al., 2007]. The risk of transmission was significantly higher from HBeAg-positive mothers than HBeAg-negative mothers [6/41

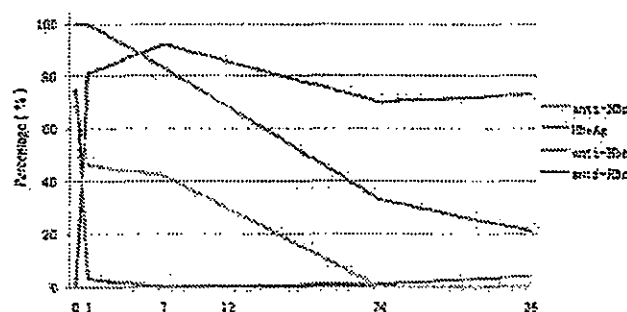


Fig. 1. Dynamic changes in serum anti-HBs, HBeAg, anti-HBe, and anti-HBc after delivery and during the 36 months of follow up.

vs. 4/163, odds ratio = 5.96 (1.60–22.12), $P < 0.001$], and significantly higher in mothers with detectable HBV DNA levels than mothers with undetectable HBV DNA levels [7/57 vs. 3/145, odds ratio = 5.94 (1.48–22.75)]. The risk of mother-to-infant HBV transmission increased with maternal HBV DNA levels (3/145 vs. 1/20 vs. 5/27 vs. 1/10), confirming the importance of HBV DNA in HBV mother-to-infant transmission [Wang et al., 2003; Buchanan and Tran, 2010; Ni, 2010].

In addition to HBsAg, maternal anti-HBc, anti-HBe and HBeAg can be transmitted to a fetus in utero. In the present study, 100% of the anti-HBc- or anti-HBe-positive infants had mothers who were also positive. However, the anti-HBc positivity rate remained high (21%) even after 24 months of age and lasted longer than that of anti-HBe, which is in agreement with the observations of a previous study [Wang et al., 2003]. Although one animal experiment reported that HBeAg could not efficiently cross the murine placenta [Milich et al., 1990], several clinical studies have shown that HBeAg can indeed cross the human placenta [Zhu et al., 1997; Wang and Zhu, 2000]. In the present study, HBeAg was detected in nearly 74% of the infants at birth, consistent with previous report [Wang et al., 2003]. HBeAg disappeared in most infants during the follow-up period, except for those who became HBV carriers, indicating that HBeAg detected in infants at birth is due to transient leakage instead of HBV replication.

Limitations existed in the present study. Of the 214 enrolled infants, 31 did not attend follow-up visits and, thus, could not be analyzed. This limited the study's power to assess the incidence of intrauterine HBV transmission. However, unless the distribution of intrauterine HBV transmission was different in these infants, it would not substantially change the present findings. In this study, there was no significant difference in average maternal age at delivery, maternal educational level (which might influence the mother's behavior), or average neonatal weight between infants who attended follow-up visits and those who did not. Thus, the results obtained here are reliable. Second, the interpretation of these findings was hampered by limitations in the study design, including the small sample size and the lack of a "gold standard" (generally accepted measure) to differentiate intrauterine transmission of HBV from other mother-to-infant transmission routes. Liver biopsy is considered the gold standard for HBV diagnosis but was not used in this study for ethical reasons. Due to these limitations, a multicenter study with a larger sample size is necessary to obtain a comprehensive overview of HBV marker dynamics in this population.

In conclusion, HBV mother-to-infant transmission was observed in approximately 5% of neonates born to HBV-positive mothers. Perinatal transmission can be effectively prevented by combined administration of

HBIG and HB vaccine. However, these measures do not prevent intrauterine transmission.

ACKNOWLEDGMENTS

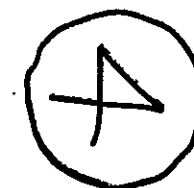
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医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日 2011.1.6	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	人全血液	研究報告の公表状況	Khattab MA, Ferenci P, Hadziyannis SJ, Colombo M, Manns MP, Almasio PL, Esteban R, Abdo AA, Harrison SA, Ibrahim N, Cacoub P, Eslam M, Lee SS. J Hepatol. 2010 Dec 8. [Epub ahead of print]	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)		エジプト		
研究報告の概要	OC型肝炎ウイルス遺伝子型4の管理:国際的専門家委員会の推奨 C型肝炎ウイルス(HCV)は6つの主要な遺伝子型と一連の亜型に分類される。 HCV遺伝子型は、そのヌクレオチド配列、地理的分布と治療への反応に関してそれぞれ特徴を持つ。治療に対して抵抗性を示す遺伝子型の一つである4型(HCV-4)の分布はエジプトとアフリカの一部に限られていたが、現在はヨーロッパ諸国にまで広がった。エジプトでのHCV-4の流行は、大規模な住血吸虫症拡大防止運動での注射器の使用が原因となり、その後、性感染や伝統的医療により中東やアフリカに蔓延した。そして移住や静注薬物の使用により世界中に広まった可能性があり、現在世界中の慢性C型肝炎のうち約20%がHCV-4によるものである。 臨床的特徴や病理学的所見は、他の遺伝子型のものと類似しているが、中東やアフリカの風土病である住血吸虫症により肝線維化が促進され、自然治癒の可能性が低下する。 エジプトにおけるHCV-4と肝細胞癌(HCC)との分布が類似していることから、HCV-4とHCCの間に関連性がある可能性が示唆されている。 HCV-4はこれまで広範囲な研究のテーマではなかったが、今回、国際的専門家委員会によって疫学・自然史・臨床・病理学的特徴と治療に関しての概説が示された。				使用上の注意記載状況・ その他参考事項等
	報告企業の意見 中東やアフリカからヨーロッパにまで拡大しているC型肝炎ウイルス遺伝子型4についての疫学・自然史・臨床・病理学的特徴と治療に関しての概説である。				今後の対応 日本赤十字社では、HCV抗体検査を実施することに加えて、精度を向上させたNATシステムを導入し、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。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。



Management of hepatitis C virus genotype 4: Recommendations of An International Expert Panel

Mahmoud A. Khattab^{1,*}, Peter Ferenci², Stephanos J. Hadziyannis³, Massimo Colombo⁴, Michael P. Manns⁵, Piero L. Almasio⁶, Rafael Esteban⁷, Ayman A. Abdo⁸, Stephen A. Harrison⁹, Nazir Ibrahim¹⁰, Patrice Cacoub¹¹, Mohammed Eslam¹, Samuel S. Lee¹²

¹Department of Internal Medicine, University of Minia, Egypt; ²Department of Internal Medicine III, Medical University, Vienna, Austria; ³Henry Dunant Hospital, Athens, Greece; ⁴First Division of Gastroenterology, Department of Medicine, Fondazione IRCCS Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Università degli Studi di Milano, Milan, Italy; ⁵Department of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Carl-Neuberg-Str. 1, D-30625 Hannover, Germany; ⁶Gastroenterology & Hepatology Unit, Di.Bi.M.I.S., University of Palermo, Palermo, Italy; ⁷Liver Unit, Hospital General Universitario Valle Hebrón, Barcelona, Spain; ⁸Gastroenterology Unit, College of Medicine, King Saud University, Riyadh, Saudi Arabia; ⁹Department of Medicine, Division of Gastroenterology and Hepatology, Brooke Army Medical Center, 3851 Roger Brooke Drive, Fort Sam Houston, TX 78234, USA; ¹⁰Department of Gastroenterology and Hepatology, Al-Kalamoon University, Damascus, Syria; ¹¹Service de Médecine Interne II, AP-HP, Hôpital Pitié-Salpêtrière, Université Pierre et Marie Curie-Paris 6, UMR 7211 (UPMC/CNRS), U 959 (INSERM), Paris F-75013, France; ¹²Liver Unit, University of Calgary, Calgary, Alberta, Canada

HCV has been classified into no fewer than six major genotypes and a series of subtypes. Each HCV genotype is unique with respect to its nucleotide sequence, geographic distribution, and response to therapy. Genotypes 1, 2, and 3 are common throughout North America and Europe. HCV genotype 4 (HCV-4) is common in the Middle East and in Africa, where it is responsible for more than 80% of HCV infections. It has recently spread to several European countries. HCV-4 is considered a major cause of chronic hepatitis, cirrhosis, hepatocellular carcinoma, and liver transplantation in these regions. Although HCV-4 is the cause of approximately 20% of the 170 million cases of chronic hepatitis C in the world, it has not been the subject of widespread research. Therefore, this document, drafted by a panel of international experts, aimed to review current knowledge on the epidemiology, natural history, clinical, histological features, and treatment of HCV-4 infections.

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of subtypes [3]. Each HCV genotype is unique with respect to its nucleotide sequence, geographic distribution, and response to therapy [4]. Genotypes 1, 2, and 3 are common throughout North America and Europe. HCV genotype 4 (HCV-4) is common in the Middle East and in Africa, where it is responsible for more than 80% of HCV infections. It has recently spread to several European countries [5,6]. HCV-4 is considered a major cause of chronic liver disease and cirrhosis, which leads to liver failure and is the root cause of hepatocellular carcinoma. Because of these complications, extended cirrhosis during chronic infection is a primary cause of liver transplantation in these regions. Although HCV-4 is the cause of approximately 20% of the 170 million cases of chronic hepatitis C in the world, it has not been the subject of widespread research. Therefore, this document, drafted by a panel of international experts, aimed to review current knowledge on the epidemiology, natural history, clinical, histological features, and treatment of HCV-4 infections.

Introduction

Hepatitis C virus (HCV), a member of the Flaviviridae family of RNA viruses, is characterized by a high spontaneous mutation rate with an estimated frequency of $1.4\text{--}1.9 \times 10^{-3}$ mutations per nucleotide per year [1,2]. As a result, HCV exists as a heterogeneous group of viruses sharing approximately 70% homology. On the basis of nucleotide sequence homology, HCV has been classified into no fewer than six major genotypes and a series

Epidemiology

Approximately 34 million people are chronically infected with HCV-4. The infection is common in the Middle East and Africa, where it accounts for more than 80% of all hepatitis C cases [5–8]. The risk factors for HCV-4 transmission are determined by the geographical distribution of this genotype.

Egypt has the highest prevalence of HCV worldwide (15%) and the highest prevalence of HCV-4, which is responsible for 90% of infections, with a predominance of subtype 4a (55%) [5–9]. Epidemiological and molecular evolutionary analysis on Egyptian genotype 4a isolates suggest the origin of the HCV-4 epidemic arises from the antischistosomal campaign, which was administered parenterally, and only stopped in the mid-1960s [10,11]. However, other risk factors, mostly related to prevailing social and cultural conditions, are responsible for maintaining the high

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* Corresponding author. Address: Department of Internal Medicine, University of Minia, Minia 61111, Egypt. Tel.: +20 225197818/862378181; fax: +20 86242813. E-mail address: mkhattabmed@hotmail.com (M.A. Khattab).



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rates of HCV-4 transmission even after the treatment campaign was stopped. Currently, the major route of transmission appears to be health-related procedures with inadequately sterilized instruments. Procedures performed by non-medical professionals and traditional healers have been identified as important risk factors for HCV transmission in Egypt [12–14]. Intrafamilial and sexual transmissions also play a role in the high prevalence of HCV-4 in this country [15,16].

The prevalence of HCV in Saudi Arabia is 3–5%, [17] with a predominance of genotype 4 (62%). Unlike the predominance of subtype 4a in Egypt, subtypes 4c/4d are the most prevalent subtypes among Saudis, followed by subtypes 4b, 4e, and 4a, suggesting that the origin and transmission of HCV-4 is different from that in Egypt [17]. Similarly, studies from other parts of the Middle East also suggest a high prevalence of HCV-4. For example, 36–46% of HCV-infected Lebanese patients have HCV-4 [18], 59% of Syrian patients [19] and 27% of HCV-infected Jordanian patients on dialysis have HCV-4 [20].

HCV-4 is also endemic throughout Central and West African countries such as the Congo, Liberia, and Uganda (where it accounts for 100% of HCV infections), as well as Gabon, Tanzania, and Cameroon (97%, 50%, and 36% of HCV infections, respectively) [21–25]. Scarification, circumcision practices, and sexual transmission may contribute to the persistence and propagation of HCV transmission in these countries [21,25].

Recently, HCV-4 has become increasingly prevalent in some southern European countries on the Mediterranean Sea, particularly Italy, France, Greece, and Spain, where prevalence rates of 10–24% have been reported in some areas [26–31]. HCV-4 infection is frequent among intravenous drug users (IVDUs) (European and non-European), HCV/HIV-coinfected patients, and immigrants from North and sub-Saharan Africa [26–31]. HCV-4 was probably introduced into Europe through immigration and the movement of IVDUs across European borders [31].

HCV-4 infections are uncommon in the United States, Canada, South America, and Asia. The prevalence of HCV-4 in the United States is about 1% [32]. Most HCV-4 cases reported from the United States were clustered among IVDUs or immigrants from countries where subtype HCV-4 is known to be most prevalent or among individuals who acquired the infection in these countries [32,33]. There are no reliable data on the prevalence of HCV-4 either in Australia or in South East Asia. However, HCV-4 appears to be rare in these regions.

Natural history

There are few data on the natural history of HCV-4. It is likely that the course of genotype 4 infection is similar to that of other genotypes [25,34].

HCV-4 represents more than 30% of the annually reported acute hepatitis cases in Egypt [35]. Very few studies address the outcome of acute HCV-4 infection. Indeed, prospective studies have shown 20–50% rates of spontaneous resolution in acute HCV-4 infections [36–38]; whereas those rates are reduced in patients with a coinfection with HIV or *Schistosoma mansoni*, as frequently occurs in Egyptians [34,37]. The presence of schistosomiasis is a negative predictor of outcome, being associated with accelerated progression of hepatic fibrosis among HCV-4 patients. In fact, the fibrosis progression rate of 0.1 ± 0.06 fibrosis units/year observed in HCV-4 patients (similar to that of patients

infected with other genotypes) increased up to 0.6 ± 0.13 in patients with associated schistosomiasis [39–43]. An Egyptian origin was independently associated with severe fibrosis in two French studies, however the higher fibrosis scores in these studies might be attributed to concomitant schistosomiasis in these Egyptian patients rather than ethnicity or HCV subtype [39,40]. Insulin resistance was also found to be correlated independently with severity of fibrosis [40]. The known association between hepatocellular carcinoma (HCC) and HCV needs to be weighed against other potential risk factors for HCC like schistosomiasis and exposure to aflatoxins or pesticides [44–47].

Utility of liver biopsy and noninvasive fibrosis tests

The biopsy is assessed for grade and stage of the liver injury, but also provides information on other histological features that might have a bearing on liver disease progression [48]. The two more common non-HCV conditions that might affect disease progression and possibly impede treatment response are steatosis [49,50] and excess of hepatocellular iron [51]. The pathological findings in chronic HCV-4 are in general similar to other types of viral hepatitis C. However, certain features may be prominent in this genotype, one of which is the presence of moderate to severe steatosis [50,52,53] with no associated sinusoidal fibrosis [53]. Host and viral factors contribute to the development of steatosis in hepatitis C, but their relative importance varies with genotype [54–56]. Hepatic steatosis in patients infected with HCV-4 is mainly associated with metabolic factors and follows the same pattern as those infected with genotype 1 [50,54]. Steatosis, in particular moderate-to-severe steatosis was detected in similar proportions of patients with genotype 1 and 4 [50,52,54]. Several studies have shown that steatosis in chronic HCV-4 is macrovesicular [50,52,53] and is seen without any prominent zonal preference [53]. More detailed studies are needed to determine if there is a characteristic histological pattern that might distinguish chronic HCV-4. Efforts are ongoing to seek alternative means focusing on noninvasive blood marker panels. In a recent study, the combination of hyaluronic acid, YKL-40, platelet count and serum aminotransferases provided information about the amount of hepatic inflammation and steatosis in Egyptian patients and achieved this cost-effectively [57].

Hepatocellular carcinoma

HCC is a major cause of cancer death worldwide [58], with evidence that its incidence has sharply increased in many countries as a consequence of the accumulation of patients with chronic liver disease caused by viral hepatitis or alcohol abuse [58,59]. The incidence of HCC in Egypt is also increasing [60–62] and is now the second most frequent cause of cancer and cancer mortality among men [63]. Hospital based studies have reported an increase in the relative frequency of all liver-related cancers in Egypt (>95% as HCC), from ~4.0% in 1993 to 7.3% in 2003 [60–62]. Data from the National Cancer Registry of Egypt, the National Cancer Institute and the Middle East Cancer Consortium recently reported that the incidence rate among males was 7 times greater than the next highest rate (among Israeli Jews) and more than 3 times that reported in the United States Surveillance Epidemiology and End Results summary [63,64].

A possible association has been suggested between HCV-4 and HCC based on the similarity of distribution of HCC and HCV-4 in

Table 1. Summary of Studies of PEG-IFN- α in Patients with Chronic HCV-4.

Sample size	Study design	Treatment and duration	Country	EVR (%)	ETR (%)	SVR (%)	Ref.
12	Open-label, uncontrolled pilot study	PEG-IFN- α -2b (1.5 mcg/kg/wk) + RBV (15 mg/kg/d) x 48 wk	Kuwait	83	83	75	[76]
226	Prospective	PEG-IFN- α -2a (180 mcg/wk) or PEG-IFN- α -2b (1.5 mcg/kg/wk) + RBV (1000 mg or 1200 mg/d if $\leq$ or >75 kg, respectively) x 48 wk	Egyptians 40%, Europeans 35%, Africans 24%	74	64	54	[40]
131	Prospective	PEG-IFN- α -2b (1.5 mcg/kg/wk) + RBV (800 mg for < 50 kg, 1000 mg for 50-65 kg, 1200 mg for 65-80 kg, 1400 mg for > 80 kg) x 48 wk	Egypt	RVR: 34.3 cEVR: 64.8	NA	60.3	[77]
97	Prospective, randomized, PEG-IFN + RBV + pioglitazone in patients with HOMA-IR >2	Arm A: PEG-IFN- α -2b (1.5 mcg/kg/wk) + RBV (1000 mg or 1200 mg/d) if $\leq$ or >75 kg respectively + pioglitazone 30 mg/d Arm B: PEG-IFN- α -2b (1.5 mcg/kg/d) + RBV (1000 mg or 1200 mg/d) if $\leq$ or >75 kg respectively x 48 wk	Egypt	RVR: 271 vs. 6.1 EVR: 68.7 vs. 48.9	66.6 vs. 44.89	60.4 vs. 38.7	[78]
95	Prospective open-label	PEG-IFN α -2a (180 mcg/wk) + RBV ≥ 11 mg/kg/d x 48 wk	Egypt	NA	69.5	61.1	[79]
240 82 (34%) HCV-4	Retrospective	PEG-IFN α -2a (180 mcg/wk) or PEG-IFN- α -2b (1.5 mcg/kg/wk) + RBV (1200 mg/d) x 48 wk	Saudi Arabia	NA	NA	64	[80]
58	Retrospective	PEG-IFN- α -2b (1.5 mg/kg/wk) + RBV (1000 mg or 1200 mg/d) if $\leq$ or >75 kg respectively x 48 wk	Greece	63.8	NA	53.4	[81]
30	Prospective open-label	PEG-IFN- α -2a (180 mcg/wk) + RBV (1000 mg or 1200 mg/d) if $\leq$ or >75 kg respectively x 48 wk	Middle East	83.3%	NA	63.3	[82]
96	Prospective, randomized, PEG-IFN + RBV + nitazoxanide (NTZ)	PEG-IFN- α -2a + RBV x 48wk, n = 40); NTZ monotherapy x 12 wk followed by NTZ + PEG-IFN- α -2a x 36 wk (n = 28); NTZ monotherapy x 12 wk followed by NTZ + PEG-IFN- α -2a + RBV x 36 wk (n = 28)	Egypt	RVR: 38, 54, 64 cEVR: 70, 68, 86	75, 71, 82	50, 61, 79	[83]
66	Prospective, response-guided therapy	PEG α -2a (180 μ g/wk) + RBV 1000 or 1200 mg/d according to virological response at wk: 4 x 24, 48, 72	Austria	RVR: 45	NA	87	[84]
84	Prospective	PEG-IFN- α -2a (180 mcg/wk) + RBV (1000-1200 mg/d) if $\leq$ or >75 kg respectively x 48 wk	Qatar	NA	77	67.9	[85]
308	Prospective, response-guided therapy	PEG-IFN- α -2b (1.5 mcg/kg/wk) + RBV 10.6 mg/kg/d for either a fixed duration of 48 wk (control group) or a variable duration at 24, 36, 48 wk	Egypt	22 RVR, cEVR 26, pEVR 52	90 : 24 W 86 : 36 W 70: 48 W	88 : RVR 87: cEVR 64: pEVR	[53]

Egypt [63–68]. A recent meta-analysis showed that more than 84% of Egyptian patients with HCC are positive for HCV-4 [68].

A significant association seems to exist not only with the most prevalent subtype 4a, but also with subtype 4o [44] even though this association has not been confirmed by others [69]. Other factors related to HCC development may play a role, such as coinfection with schistosomiasis which is known to increase risk of HCC [4,66], exposure to pesticides [47], and dietary aflatoxins [70].

Evolution of treatment

Combination therapy with pegylated interferon alpha and ribavirin represents the current standard of care in chronic HCV-4. Interferon (IFN) based therapies were introduced in chronic hepatitis C in the 1980s and have improved dramatically over the subsequent years. With conventional IFN- α monotherapy the SVR rates were very poor, similar to those achieved in HCV geno-

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Sample size	Study design	Treatment and duration	Country	EVR (%)	ETR (%)	SVR (%)	Ref.
242	Retrospective non randomized study	PEG-IFN- α -2b (1.5 mcg/kg/wk) + RBV (1000-1200 mg/d) x 48 wk	French, Egyptian, African	NA	NA	Egyptian: 48 French: 37 African: 25	[39]
73	Randomized (patients with history of bilharziasis)	PEG-IFN- α -2a (180 μ g/wk) + RBV 1200 mg/d x 48 wk, (n = 38); IFN- α -2b 3 MU/tiw + RBV 1200 mg/d x 48 wk (n = 35)	Qatar	NA	Peg-IFN vs. non-peg-IFN (76.3 vs. 40; $p = <0.002$)	Peg-IFN vs. non-peg-IFN (65.8 vs. 25.7; $p <0.05$)	[86]
180	Prospective non randomized	Peg-IFN- α -2b (100 μ g/wk) + RBV (1000-1200 mg/d) x 48 wk (n = 40); Peg-IFN- α -2b (100 μ g/wk) + RBV 1000-1200 mg/d x 24 wk (n = 70); IFN- α -2b 3 MU + RBV 1000-1200 mg/d + amantadine 200 mg/d, x 24 wk (n = 70)	Egypt	72.5, 72.9, 54.3	65.0, 65.7, 47.1	55.0, 48.6, 28.6 I and II: ($p = 0.517$), I and III: ($p = 0.006$), II and III: ($p = 0.015$)	[87]
260	Prospective, double-blind, randomized	PEG-IFN- α -2 b (1.5 mcg/kg/wk) + RBV (1000-1200 mg/d) for 24, 36, 48 wk	Egypt	69, 68, 69	48, 68, 70	29, 66	[88]
66	Prospective, open-label	PEG-IFN- α -2 b (1.5 mcg/kg/wk) + + RBV (1000-1200 mg/d) x 48 wk	52 % Egyptian; remainder Kuwaiti and Syrian	78	77	68	[89]
100	Open-label	PEG-IFN- α -2a (180 μ g/wk) + RBV 1200 mg/d x 48 wk (n = 51) IFN- α -2a 3 MU + RBV 800-1000 mg/d (n = 49) x 48 wk	Egyptian	NA	NA	69	[90]
98	Post hoc analysis of HCV-4 patients from two large RCTs	Study 1: PEG-IFN- α -2a (180 mcg/wk) + RBV 1000-1200 mg/d x 48 wk (n = 13) Study 2: PEG-IFN- α -2a (180 mcg/wk) + RBV 800-1200 mg/d x 24 or 48 wk High-dose RBV (n = 24) Low-dose RBV (n = 8) High-dose RBV (n = 12) Low-dose RBV (n = 5)	International	NA	NA	79, 63, 67, 0	[91]
59	Randomized, parallel-group	PEG-IFN- α -2b (100 mcg/wk) + RBV 800 mg/d x 48 wk (n = 28) , IFN- α -2b 3 MU+ RBV 800 mg/day x 48 wk (n=31)	Saudi Arabia	NA	67.9 54.8	42.9 32.3	[92]
180	Open-label multicenter	PEG-IFN- α -2a (180 μ g/wk) + RBV (800mg/d) PEG-IFN- α -2a (180 μ g/wk) IFN- α -2a 4.5MIU tiw + RBV (800mg/d) x48 wk	Saudi and Egyptian	77, 60, 43	67, 59, 37	50, 28, 30	[74]
190	prospective, open-label RCT	IFN- α -2b 3 MU tiw or PEG-IFN α -2b - (100 mcg/wk) + RBV (800 or 1000 mg for both groups) x 48 wk	Egypt	NA	NA	55 40	[75]

Abbreviations: Wk: week, d: day, x: for treatment duration, HOMA-IR: homeostatic model assessment index NA: no available data, EVR, early virologic response; RVR: rapid virologic response, ETR: end of treatment response, PEG-IFN, pegylated interferon; RBV, ribavirin; SVR, sustained virologic response.

Table 2. Outcome by virological response at weeks 4 and 12 in genotype four patients treated for 48 weeks with peginterferon alfa-2 plus ribavirin.

Study	N	Week 4		Week 12	
		RVR, n (%)	SVR, n (%)	EVR, n (%)	SVR, n (%)
Kamal [53]	387	77 (19.9)	66 (86) *	275 (71)	170 (64) **
Ferenci <i>et al.</i> [84]	66	30 (13.3)	26 (96) *	28 (56)	13 (46) **

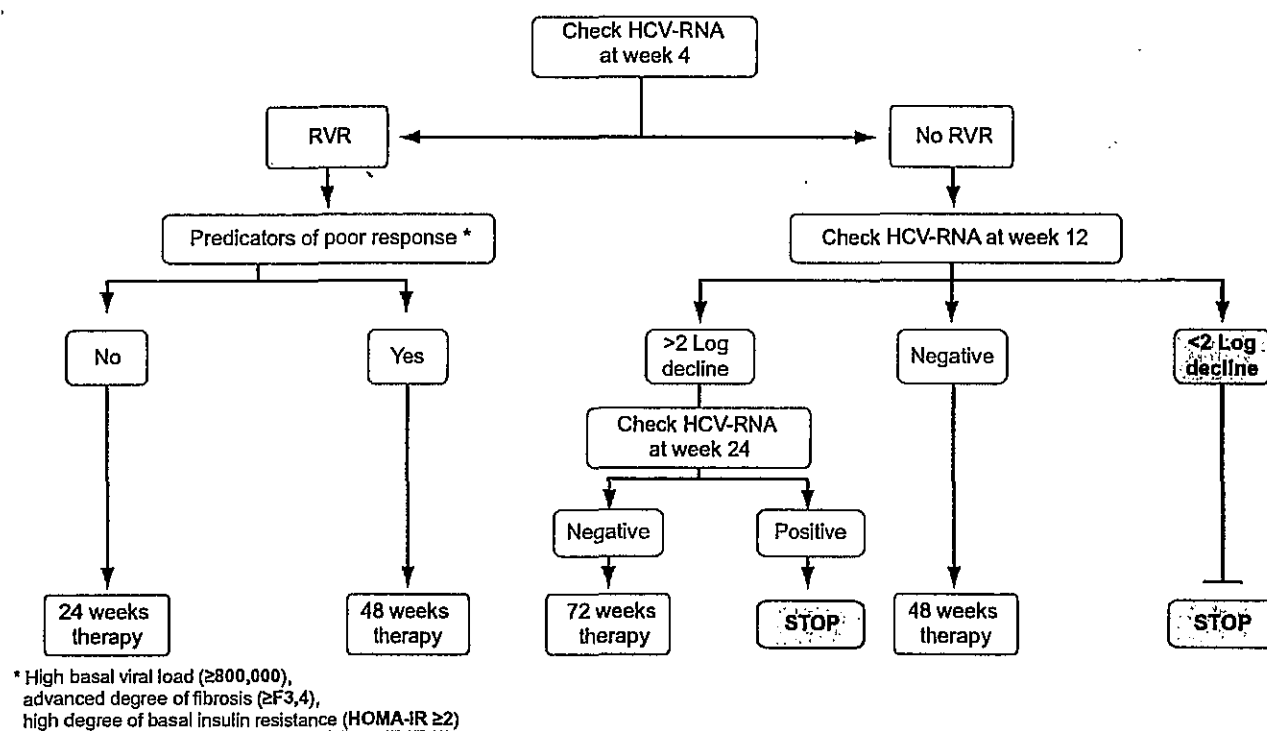


Fig. 1. A proposed algorithm for treating patients with chronic HCV-4 based on the kinetics of viral response (response-guided therapy). RVR, rapid viral response.

type 1 infection, ranging between 5% and 25% [71,72]. However, with the addition of ribavirin to conventional IFN- α , SVR rates increased to 25–42% [73,74], about intermediate between those achieved in HCV-1 and HCV-2 or HCV-3. With the introduction of pegylated IFN compounds in combination with ribavirin, the efficacy of treatment in HCV-4 further improved significantly. In various studies from European and Middle Eastern countries, SVR rates ranging from 43% to 70% were reported [39,40,53,75–88,73,89–91] (Table 1). These results are indeed higher than those (42–46%) achieved in genotype 1 patients but still lower than those (76–82%) reported in chronic hepatitis C genotype 2 and 3.

Studies looking at predictive factors on HCV-4 patients are relatively scarce. Negative predictive factors at baseline include high viral load, presence of cirrhosis and steatosis, insulin resistance, IL-28B polymorphism TT, and HIV coinfection [75,86,73,91–93]; these factors are associated with a lower SVR. A rather unex-

pected finding of the therapeutic trials in HCV-4 has been that under the same treatment regimen, patients from Egypt and the Middle East experienced higher SVR rates than patients from Europe or Africa. In a French retrospective study, conducted in 242 HCV-4 patients from various geographical areas (40% Egyptians, 35% Europeans, and 24% Africans) the respective SVR rates were 54.9%, 40.3%, and 32.4%. Egyptian origin and the absence of severe fibrosis were found to be independently associated with SVR [39]. It remains unclear whether the difference in SVR is related to ethnicity, HCV-G4 subtype, the mode of transmission or IL-28B genotype [93].

Similar results have recently been reported in a prospective study of HCV-4 patients from Europe, France, and Africa with respective SVR rates of 63%, 51%, and 39% and an overall SVR percentage of 54%. Despite the presence of severe fibrosis in a large proportion of the Egyptian patients, Egyptian origin was again independently associated with SVR ($p = 0.001$); OR: 5.87 (95%

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Table 3. Summary of studies of combined therapy in HCV-4 patients with incomplete (F3) or complete (F4) cirrhosis.

First Author, year [Ref.]	n	Therapeutic regimen	Duration (wk)	SVR, n (%)	Liver histology
Hasan, 2004, [89]	20	PEG-IFN- α 2b 1.5 mcg/kg/wk RBV 1000-1200 mg/d	48	30.0	F3-F4
Derbala, 2005, [105]	12	PEG-IFN- α 2b 1.5 mcg/kg/wk RBV 800-1200 mg/d	48	8.3	F4
Derbala, 2006, [86]	13	PEG-IFN- α -2a 180 mcg/wk RBV 1200 mg/d	52	38.5	F3-F4
Males, 2007, [106]	14	PEG-IFN- α -2a 180 mcg/wk RBV $\geq$ 11 mg/kg/d	48	35.7	F4
Kamal, 2007, [53]	27	PEG-IFN- α -2b 1.5 mcg/kg/wk Ribavirin 10.6 mg/kg/d	24-48	0	F4
Roulot, 2007, [39]	76	PEG-IFN- α -2b 1.5 mcg/kg/wk RBV 1000-1200 mg/d	48	31.6	F3-F4
Ferenci, 2008, [84]	4	PEG-IFN- α -2a 180 mcg/wk RBV 1000-1200 mg/d	48	75.0	F3-F4
Gad, 2008, [107]	78	PEG-IFN- α -2a 180 mcg/wk, PEG-IFN- α -2b 1.5 mcg/kg/wk, standard IFN- α -2b 3 MU thrice/wk, RBV 1000-1200 mg/d	48	37.2	F3-F4
El Makhzangy, 2009 [79]	33	PEG-IFN- α -2a 180 mcg/wk RBV $\geq$ 11 mg/kg/d	48	45.5	F3-F4
Giannini, 2009, [108]	5	PEG-IFN- α -2a 180 mcg/wk, PEG-IFN- α -2b 1.5 mcg/kg/wk, RBV 800-1200 mg/d	48	0	F4 and portal hypertension

Abbreviations: Wk: week, d: day, n = number, PEG-IFN, pegylated interferon; RBV, ribavirin; SVR, sustained virologic response.

CI: 2.75–12.55) ($p = 0.001$). SVR was also independently associated with insulin resistance measured by the homeostatic model assessment index (HOMA-IR) < 2 ($p = 0.001$) and by non-severe fibrosis ($p = 0.001$) [40]. Moreover, in another trial from Egypt, HOMA-IR was found to be a predictor not only of SVR but also of rapid virologic response (RVR) ($p = 0.002$ and 0.0041 ; respectively) [77]. The use of insulin-sensitizing agents such as pioglitazone in conjunction with HCV-4 treatment increases both SVR and RVR rates [78]. HCV kinetics under therapy has been found to be extremely useful to predict SVR [53,88]. Other factors during treatment are patients' compliance and no dosage reductions or dose fulfilled with 80/80/80 [94].

IL28B polymorphism

A genome wide association study in patients infected with HCV-1 revealed a single nucleotide polymorphism (SNP)-rs12979860 in the IL-28B region on chromosome 19 (19q13.13), associated with a more than twofold increased rate of SVR [95]. In a multivariate analysis of HCV-4 patients, baseline viral load, fibrosis and the IL28 (rs8099917 T/T allele) (OR: 0.124, 95% CI: 0.030–0.505) were significantly associated with SVR [95]. The strongest predictor for the final outcome was RVR (OR: 26.00; 95% CI: 7.148–94.545, $p < 0.0001$). If RVR was included into the multivariate model, only the RVR and the fibrosis score remained significant. Thus, deter-

mination of IL28 polymorphism may not be useful to select patients with HCV-4 for abbreviated treatment schedules. However these data need further confirmation.

Duration of treatment

The duration of treatment is based on HCV genotype in current guidelines [96,97]. Forty-eight-week regimens are recommended for patients infected with HCV-1 and 4. This recommendation is based on the results of large randomized, international, phase III trials of peginterferon alfa-2 combined with ribavirin [98–100]. Unfortunately these studies included very few patients with HCV-4.

Measurement of the virological response at 4 and 12 weeks of therapy is a simple and reliable tool that allows the treatment regimen to be tailored to the individual. Patients who become HCV-RNA negative (< 50 IU/ml by qualitative PCR assay) or have a $\geq 2 \log_{10}$ drop in serum HCV RNA level by quantitative PCR assay after 12 weeks of therapy are defined as having an early virological response (EVR). Failure to achieve an EVR can predict which patients are unlikely to have a successful outcome with combination therapy. Those who are HCV-RNA negative after 4 weeks of treatment are defined as having a RVR. In contrast to EVR, RVR predicts which patients are most likely to have a successful outcome with combination therapy.

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The probability of SVR increases with the speed of viral load decline. For example, a higher proportion of patients with HCV-1 who become HCV-RNA negative after 4 weeks of treatment will achieve an SVR than those who become HCV-RNA negative after 12 weeks of treatment [101]. SVR rates were much lower in patients who did not clear HCV RNA during the first 12 weeks of treatment. A completely negative test for HCV RNA at week 12 (complete EVR) is a better predictor of an SVR after 48 weeks of combination therapy than a partial EVR ($\geq 2 \log_{10}$ drop in serum HCV RNA at week 12). Those who achieved an RVR had an SVR rate of 91%, those who achieved a complete EVR had an SVR rate of 75%, and among the 22% of patients with a partial EVR and virus negative by week 24 only 27% achieved an SVR [101].

Response-guided therapy

There are only two randomized controlled trials in patients with HCV-4 reporting viral response at week 4 (Table 2). Patients with HCV-4 who achieve an RVR are potential candidates for abbreviated 24-week treatment regimens; provided that no other predictors of poor response (Fig. 1), [53,84]. Response rates are similar to those treated for 48 weeks [87]. The prospect of shorter treatment for these patients is appealing because the overall tolerability is likely to be better and the costs lower with an abbreviated treatment regimen; conversely, slow responders who do not achieve HCV RNA negativity by week 4 or 12 are potential candidates for prolonging treatment up to 72-weeks. Only one randomized study examined prolonged 72-week regimens in HCV4 patients [102] but the number of patients was too small to draw any conclusions.

Fig. 1 presents a proposed algorithm for treating patients with chronic HCV-4 based on the kinetics of viral response (response-guided therapy). In conclusion, measurement of RVR and complete/partial EVR is a simple and reliable tool that allows clinicians to estimate the likelihood of an SVR and to individualize the duration of treatment. Unfortunately, in contrast to patients with HCV-1 the concept of response-guided therapy has not been validated in patents with HCV-4. In the current absence of any firm data on HCV-4 patients, we suggest that similar response-guided approaches used in HCV-1 patients may be considered. Thus those with an RVR are highly likely to achieve SVR and are candidates for abbreviated, 24-week regimens. Patients with a complete EVR at week 12 have a high probability of achieving an SVR with a 48-week regimen. Patients with a partial (slow) EVR (no RVR and detectable HCV RNA but $>2 \log_{10}$ drop at week 12 and virus negative at week 24) may be considered for treatment prolongation to 72 weeks, if they can tolerate this.

Treatment in special populations

Cirrhosis

Treatment of compensated cirrhosis is a relevant issue since sustained HCV clearance is associated with a better survival, reduced HCC occurrence and absence of decompensation [103]. However, severity of liver fibrosis and HCV genotype are the two major determinants of SVR. The published data on the efficacy of Peg-IFN and ribavirin in cirrhotic patients infected by HCV-4 are limited (Table 3) [39,53,79,84,86,73,104–107]. Overall the cumulative response rate is about 30% of treated patients, but this figure includes also incomplete cirrhosis, or F3, that probably overestimates the real treatment efficacy in cirrhotic patients.

HIV coinfection

It has been reported that the prevalence of HCV-4 among HIV-infected individuals is at least 15% of all anti-HCV positive subjects [108]. Data derived from two trials showed that among 75 HIV-infected patients with HCV-4 treated with Peg-IFN alfa-2a plus ribavirin, only 21 (28.0%) achieved SVR [92]. The major factor of low rate of response was related to premature treatment discontinuation due to severe adverse effects.

Hemodialysis

The prevalence of HCV infection in patients with end stage renal disease is highly variable ranging from 3% to 80% between different countries and centers [109]. HCV-4 infected subjects have a shorter graft survival after kidney transplantation due to increased risk of severe infection and liver disease deterioration. As a consequence, the current recommendation is to give antiviral therapy before transplantation with the aim to eradicate the infection. However the use of Peg-IFN and ribavirin in dialysis patients is hampered by fairly frequent side-effects [110]. Two recent meta-analyses have shown that the overall SVR after Peg-IFN with or without ribavirin was 40%, including 33% for genotype 1 [111]. No data on efficacy of combined therapy in patients infected by genotype 4 are available so far.

Children/adolescents

Combination therapy with Peg-IFN-alpha and ribavirin treatment is effective in children with chronic hepatitis C [112,113] with SVR rates across different genotypes comparable to adults. One study reported the results of Peg-IFN plus ribavirin treatment in 12 adolescents infected by genotype 4 [76]. Nine patients (75%) achieved SVR suggesting that combination therapy is effective in this clinical context.

Acute hepatitis C

Scant data are available about the optimal treatment regimen in acute HCV-4 and demonstrated a high SVR with IFN-based therapies compared with no treatment [37,114,115]. The available clinical trials showed that acute hepatitis patients infected with HCV-4 have higher rates of SVR compared with HCV-1 infections. In one study [116], an SVR was achieved in 60% and 88% of genotype 1 patients and in 93% and 100% of HCV-4 patients after 12 and 24 weeks of treatment, respectively [37].

Thalassemia major

Few data are available on the treatment of HCV-4 in patients with thalassemia. Current literature is lacking sufficient evidence about the use of PEG-IFN as monotherapy or in combination with ribavirin in thalassemic patients. Inati et al. evaluated in a randomized study [117] the safety and efficacy of PEG-IFN- α with or without ribavirin therapy in 20 patients with thalassemia and HCV-4. An SVR was achieved in (30% and 62.5%; $p=0.19$) in the monotherapy and combination groups, respectively. They reported an increase in the transfusion requirements by 34% in the combination group ($p=0.08$). In another study, Kamal et al. [118] reported that the overall SVR rates were (46% and 64%) with PEG-IFN alfa-2b vs. PEG-IFN alfa-2b plus ribavirin combination therapy, respectively. However, the reported adverse events were more frequent with combination therapy than with PEG-IFN alfa-2b alone.

Review

Liver transplantation

End-stage liver disease secondary to HCV infection is the major indication for orthotopic liver transplantation (OLT) worldwide [119]. The percentage of HCV-4 patients among recipients of OLT varies depending on the geographic location from around 29% in Saudi Arabia [120] to more than 90% in Egypt, [121] while it represents a relatively uncommon indication in the western world [122,123].

The natural history of HCV-4 re-infection after liver transplantation is inadequately described in the literature. Re-infection of the graft with HCV is universal after liver transplantation regardless of the genotype, leading to an accelerated course of liver injury in many cases [124]. Most studies conducted worldwide have investigated disease recurrence in HCV genotypes 1, 2, and 3 [119]. However, there are few reports on post-OLT recurrence of HCV-4.

Four studies have been reported from liver transplant centers in Europe and Australia. Gane et al. reported on 14 patients with recurrent HCV-4 post-OLT and found that about 50% of these patients have progressive liver disease [125]. They also found that patients infected with genotypes 1b and 4 had the worst outcomes, while genotype 2 and 3 patients had less severe disease recurrence. Similarly, an analysis of 182 patients transplanted for HCV in Australia and New Zealand (16 of whom had HCV-4) found that among the many factors studied in univariate and multivariate analyses, genotype 4 was associated with an increased risk for re-transplantation and death [123]. By contrast, a study from another Australian center, including patients with HCV-4, showed that genotype 1b, but not 4, was associated with higher recurrence rates after transplantation [126]. In a more detailed study from the UK, 32 of 128 patients who underwent OLT for hepatitis C were infected with HCV-4 [122]. A statistically significant greater fibrosis progression rate was observed in HCV-4 patients compared to non-genotype-4, although their rates of survival were similar. The authors attributed the difference between these two groups to the significantly older donor age in the HCV-4 group and the ethnic background of these patients (predominantly Egyptian). On the other hand, studies from the Middle East show a more favorable outcome of HCV-4 patients. In a study from Saudi Arabia on biopsy proven recurrence HCV post-OLT there were no significant differences between genotype 1 and 4 patients in terms of epidemiological, clinical, and histological factors as well as outcome (patients and graft survival) [127]. Among many epidemiologic, laboratory, virologic factors included in that analysis, the only factor predictive of an advanced histological score was the HCV RNA level at the time of biopsy.

In Egyptian studies of living-related liver transplantation of HCV-4 patients a similar good outcome was observed. HCV clinical recurrence was observed in 31% of patients and was mostly mild, as 91% of patients had fibrosis scores less than F2 [128]. After 36 months of follow-up, 91% of patients were alive with good graft function. Similar to the study from Saudi Arabia, recurrent HCV was associated with pre-transplant and post-transplant viral load and to the presence of antibodies to hepatitis B core antigen.

Published studies on the response rates and outcome of antiviral therapy in patients with HCV-4 post-OLT are lacking. In an abstract from Saudi Arabia, 25 patients infected with HCV-4 were treated with PEG-IFN α -2a at a dose of 180 μ g/week plus ribavirin 800 mg/day, (dose was adjusted as tolerated range 400–

1200 mg) [129]. Fourteen patients (56%) achieved sustained virological response (SVR). The results of this study suggest that the post-transplant treatment outcome in HCV-4 is probably better than genotype 1 and less favorable than genotypes 2 and 3. This response pattern among the different genotypes parallels the response pattern in the immunocompetent population.

More studies are warranted to further understand HCV4 and OLT and to establish effective strategies for limiting the progression of liver disease post-OLT in HCV-4 patients.

Novel treatments

In spite of the improvement of chronic hepatitis C treatment over the last two decades, treatment with PEG-IFN and ribavirin is still associated with frequent and sometimes severe side effects and many patients cannot be treated because of contraindications [130]. A major step forward in the therapy of HCV infection is expected by the approval of new direct inhibitors of HCV replication. Several compounds, mainly inhibitors of the HCV NS3/4A protease and NS5B polymerase, are currently in phase II and III trials and the first HCV protease inhibitors will hopefully be licensed in 2011–2012. [131,132]. The large majority of the new antiviral drugs are currently developed only for HCV-1 infection. The most advanced compounds in clinical development are two protease inhibitors: telaprevir (VX950) and boceprevir (SCH503034). Telaprevir has shown an improvement of SVR rates in treatment naive HCV-1 patients to almost 70% and up to 40% in previously unresponsive patients [133,134]. In a proof of concept study, telaprevir has also shown activity against HCV-4 during 15 days monotherapy or combination with Peg-IFN and RBV compared to Peg-IFN, RBV and placebo [134]. Boceprevir is effective in HCV-1 patients [135], however, preclinical data suggests, that boceprevir might not be effective in HCV-4 with the currently used dosages [136], and data on boceprevir in HCV-4 patients have not been published. R7128 is another nucleoside analog polymerase inhibitor that has demonstrated potent antiviral activity. In a recent interim analysis at week 12; the combination of R7128 (1500 mg twice daily) plus Peg-IFN α -2a and ribavirin in treatment-naïve patients with HCV-1 and 4; R7128 has shown high rates of early viral response with promising safety profiles and low rates of resistance or breakthrough [137].

Further data have been published for two other compounds with different mode of actions, nitazoxanide (NTZ) and Debio 025. NTZ, a synthetic antiprotozoal agent, is licensed in the United States for the treatment of infections with *Cryptosporidium parvum* and *Giardia lamblia*. Antiviral properties of this compound were discovered when patients with acquired immune deficiency syndrome (AIDS) coinfecting with hepatitis B and C were treated for cryptosporidiosis. As a potential mechanism of action, NTZ activates the protein kinase activated by double-stranded RNA (PKR), a key kinase that regulates the cell's innate antiviral response [138]. These observations could explain the clinical antiviral effect of NTZ. In this context, two clinical studies are of interest. A pilot trial explored NTZ monotherapy in Egyptian hepatitis C patients infected with HCV-4. HCV-RNA became undetectable in seven out of 23 patients who all had a rather low viral load before treatment ($\leq 400,000$ IU/ml). Interestingly, four patients achieved SVR after 24 weeks of NTZ treatment [139]. A recent study by Rossignol et al. explored the combination of NTZ and standard PEG-IFN/RBV combination therapy in Egyptian HCV-4 infected patients [93]. The trial was conducted in two

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Egyptian centers and included 96 treatment-naïve patients – all were infected with HCV-4. Patients were randomized in one of three arms, a control arm with Peg-IFN alfa-2a and ribavirin for 48 weeks ($n=40$), and two arms with a 12 week lead-in monotherapy with NTZ followed by a 36 week course of NTZ in combination with pegylated interferon with ($n=28$) or without ribavirin ($n=28$). SVR rates in the control arm were only 50%, which is relatively low as compared to most other previous HCV-4 trials [140]. Importantly, 79% of the patients receiving triple therapy with NTZ and PEG-IFN/RBV achieved a SVR that reached statistical significance although patients in the triple therapy arm were treated for only 36 weeks with PEG-IFN. Of note, patients receiving NTZ and PEG-IFN without RBV also showed a surprisingly high SVR of 61%. Although these data are promising, several issues need to be considered, for example the rather small overall number of patients with only five of the 96 patients having advanced fibrosis or cirrhosis (Ishak S F4-6) [141]. In addition, there were some differences in patient characteristics between the study arms as the body mass index (BMI) was significantly lower in patients who received PEG-IFN, RBV, and NTZ as compared to the control group. This also could have contributed to the better response in the triple therapy arm although the BMI was not an independent factor associated with SVR. Nevertheless, it is quite obvious that further studies are needed to investigate NTZ in genotype 4 patients. The antiviral efficacy of NTZ was confirmed during the 12 week lead-in phase when NTZ was administered alone. NTZ induced a modest but significant HCV-RNA decline of $-0.27 \log_{10}$, which is in line with the previous monotherapy study [93]. However, only two out of 53 patients treated with NTZ monotherapy had a decline of more than $1 \log_{10}$ and just one patient achieved a complete response (HCV-RNA negative) after 12 weeks, which is in contrast to a previous trial where six out of 23 patients (26%) were HCV-RNA-negative after 12 weeks [141]. Thus, it is very unlikely that NTZ monotherapy will play any role in future treatment of chronic hepatitis C.

The cyclophilin inhibitor Debio 025 inhibits HCV replication by inhibiting endogenous cyclophilin and interaction with the NS5B polymerase, but without immunosuppressive activity. For treatment-naïve HCV-1 monoinfected patients a reduction of HCV-RNA of up to $4.75 \log_{10}$ after 29 days of combination therapy with Debio 025, PEG-IFN alfa-2a, and ribavirin was shown [142].

Q2 Two HCV-4 patients were treated underin this study, one of them in a Debio 025 monotherapy arm. With a dose of 1000 mg per day the mean viral load reduction after 29 days was $2.2 \pm 2.4 \log_{10}$ for the 12 patients in that arm (11 patients with genotype 1, one with HCV-4). Importantly, the one genotype-4 patient also had a viral load decline of $>2 \log_{10}$ [142]. Finally, silibinin, for which the mechanism of action is not yet entirely resolved, administered intravenously (20 mg/kg/day) for 7 days, led to a mean decline of the HCV-RNA concentration by $3 \log_{10}$ IU/ml. So far, mainly genotype 1 and single patients with genotype 2, 3, and 4 infections have been investigated and no data on differences of antiviral activities for the different HCV genotypes are available [143]. Recently, Beinhart et al. reported a case that the use of silibinin IV are associated with prevention of graft re-infection in a patient infected with mixed genotype 1a/4. [144].

Q3 The available data on NTZ is are promising, but has need to be confirmed in larger studies. Other new compounds have been

shown to suppress viral load in HCV-4 patients in proof-of-concept studies. Further larger trials on the combination of new compounds with PEG-IFN and RBV are needed to explore the beneficial effect in terms of SVR improvement in patients with HCV-4.

Conclusion

In recent years the study of HCV kinetics under therapy has been found to be extremely useful to guide the appropriate duration of therapy, motivate the patient and improve the cost/effectiveness of treatment. By the application of the RVR, as well as of the complete (cEVR) and partial early responses (pEVR), the duration of therapy can be individualized between 24 and 48 weeks. In this context it has become clear that in HCV genotype 4 – similar to genotype 1, 2, and 3 – a response guided duration of combination therapy is becoming the current standard of care.

The ongoing spread of HCV-4 to European and other countries is expected to facilitate further therapeutic studies including promising drugs like NTZ and direct acting antiviral agents specifically targeted to the proteins of HCV genotype 4.

Conflict of Interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Key Points 1 Major facts about hepatitis C virus genotype (HCV-4)

- HCV-4 is responsible for more than 20% of worldwide HCV infections.
- Although HCV-4 is common in the Middle East and in Africa, recently, it has become increasingly prevalent in some southern European countries.
- The natural history of HCV-4 is likely similar to that of other genotypes.
- Moderate to severe steatosis with no associated sinusoidal fibrosis may be prominent in this genotype, which is mainly associated with metabolic factors and follows the same pattern as those infected with genotype 1.
- A possible association has been suggested between HCV-4 and HCC based on the similarity of distribution of HCC and HCV-4 in Egypt. A significant association seems to exist not only with the most prevalent subtype 4a, but also with subtype 4o.
- Scarce data are available on correlation between HCV-4 and extra hepatic manifestation of HCV, with no clear specific manifestation.

Review

Key Points 2 Treatment of HCV-4

- Combination therapy with pegylated interferon alpha and ribavirin for 48 weeks represents the current standard of care in chronic HCV-4
- Patients with HCV-4 who achieve an RVR are potential candidates for abbreviated 24-week treatment regimens; provided that no other predictors of poor response. However; slow responders who do not achieve HCV RNA negativity by week 4 or 12 are potential candidates for prolonging treatment up to 72-weeks
- Negative predictive factors at baseline include high viral load, presence of cirrhosis and steatosis, insulin resistance, IL-28B polymorphism TT and HIV coinfection.
- Determination of IL28 polymorphism may not be useful to select patients with HCV-4 for abbreviated treatment schedules. However these data need further confirmation before final conclusion can be extracted.
- Telaprevir has also shown activity against HCV-4, however, preclinical data suggests, that boceprevir might not be effective in HCV-4 with the currently used dosages.
- R7 128 has show high rates of early viral response with promising safety profiles and low rates of resistance or breakthrough.
- The available data on nitazoxanide (NTZ) and Debio 025 is promising, but has to be confirmed in larger studies.
- The use of insulin-sensitizing agents such as pioglitazone in conjunction with HCV-4 treatment increases both SVR and RVR rates.

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Review

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医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日 2010. 12. 17	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称		人血清アルブミン		研究報告の公表状況	公表国	
販売名(企業名)		赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン5%静注12.5g/250mL(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)			ヨーロッパ	
研究報告の概要	○欧州連合におけるHIVとAIDS、2009年 欧州連合(EU)と欧州経済領域(EEA)の28カ国から、2009年に新たにHIV感染と診断された25,917症例について報告された。新規感染者の割合はエストニア、ラトビア、イギリス、ベルギーの順で高かった。主要な感染経路は男性間の性的接触(MSM)(35%)であり、ついで異性間性的接触(24%)であった。既知の感染経路で相当な増加があったのはMSMのみであった。静注薬物使用(IDU)による感染は5%であったが、東ヨーロッパではいまだに主な感染源となっている。全体として2009年のHIV症例数は増加したが、AIDS診断の報告はブルガリアとバルト諸国を除く他の国で減少している。HIV感染と診断された時にCD4細胞数が350 cells/ μ L未満の感染後期初診患者が多いことは、適切な時期に治療を受けられなかったことを示唆しており、考慮すべきである。					使用上の注意記載状況・ その他参考事項等
	報告企業の意見 2009年、欧州連合と欧州経済領域の28カ国からHIVと診断された25,917症例が報告された。HIV感染の診断時にCD4細胞数が350 cells/ μ L未満である感染後期初診患者が多いことが分かり、これは適切な時期に治療を受けられなかったことを示しているため、考慮すべきであるとの報告である。 これまで、本製剤によるHIV感染の報告はない。また本製剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれている。さらに最終製品についてHIV-NAT陰性であることを確認していることから本製剤の安全性は確保されていると考える。					今後の対応 日本赤十字社では、化学発光酵素免疫測定法(CLEIA)により抗体検査を実施することに加えて、20プールのNATについてもHIV-2及びHIVグループOの検出が可能な新NATシステムを導入し、陽性血液を排除している。20プールのNAT導入以来、輸血HIV感染は一例も発生していないが、2008年よりさらにNATの感度を上げ対応している。国内外のHIV感染、AIDS発生の動向やHIV感染に関する新たな知見等について今後も情報の収集に努める。

5

RAPID COMMUNICATIONS

HIV and AIDS in the European Union, 2009

G Likatavicius (giedrius.likatavicius@ecdc.europa.eu)¹, M JW Van de Laar¹

1. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden

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In 2009, 28 European Union and European Economic Area (EU/EEA) countries reported 25,917 newly diagnosed cases of human immunodeficiency virus (HIV). Sex among men who have sex with men was the most common transmission mode (35%) followed by heterosexual contact (24%). Overall, the number of HIV cases in 2009 increased while the number of reported acquired immunodeficiency syndrome (AIDS) diagnoses continued to decline. It is of concern that a high proportion of the patients with known CD4 cells count at the time of HIV diagnosis had a CD4 cell count below 350 cells/ μ l suggesting no timely access to treatment and care.

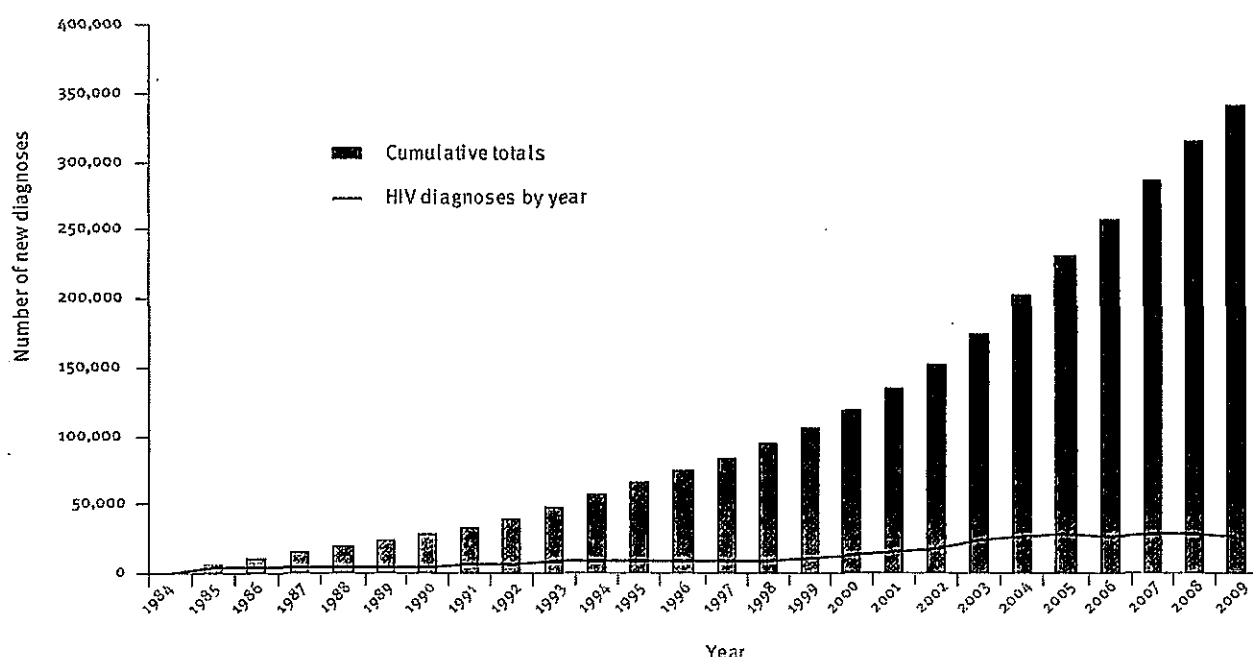
Human immunodeficiency virus (HIV) cases in the European Union and European Economic Area (EU/EEA), 2009

In the EU/EEA, 25,917 people were newly diagnosed with HIV in 2009, reported by 28 countries, a rate of 5.7

per 100,000 population. Data was not reported from Austria or Liechtenstein. The overall rate for men was 8.3 per 100,000 male population and 3.2 for women. The highest rates of new HIV diagnoses were reported by Estonia (30.7), Latvia (12.2), the United Kingdom (UK) (10.7) and Belgium (10.3). The lowest rates (<1.0) were reported by Romania and Slovakia. Twelve per cent of new HIV diagnoses were reported in 15–24 year-old individuals and 28% were female. Sex among men who have sex with men (MSM) is the predominant reported mode of transmission in EU/EEA, accounting for 35% of the HIV diagnoses, followed by heterosexual contact (24%) when cases from countries with generalised HIV epidemics are excluded. Five per cent of HIV cases were reported among intravenous drug users (IDU). Transmission mode was unknown for 20.3% of the cases. The highest proportion of cases classified as heterosexually transmitted and originating from countries with generalised epidemics, was observed

FIGURE 1

Number of diagnosed and reported HIV infections, EU/EEA, 1984–2009



EEA: European Economic Area; EU: European Union.

in Norway (70%), Sweden (69%), Ireland (62%) and Belgium (60%).

Trends in HIV cases in the EU/EEA

Among the 28 EU/EEA countries that have consistently reported HIV data since 2004, the rate of HIV diagnoses per 100,000 population has been relatively stable, ranging from 6.5 in 2004 to 5.7 in 2009. In recent years, more than 25,000 HIV diagnoses were reported each year, resulting in a cumulative total number of nearly 350,000 diagnoses reported since the beginning of the epidemic (Figure 1). Since 2004, the proportion of newly HIV diagnosed women decreased from 36% to 28% in 2009. HIV diagnoses have tripled in Bulgaria, Iceland and Slovakia and doubled in Hungary and Slovenia. HIV diagnoses have decreased by more than 20% in Denmark, Estonia, Italy, Luxembourg and Romania.

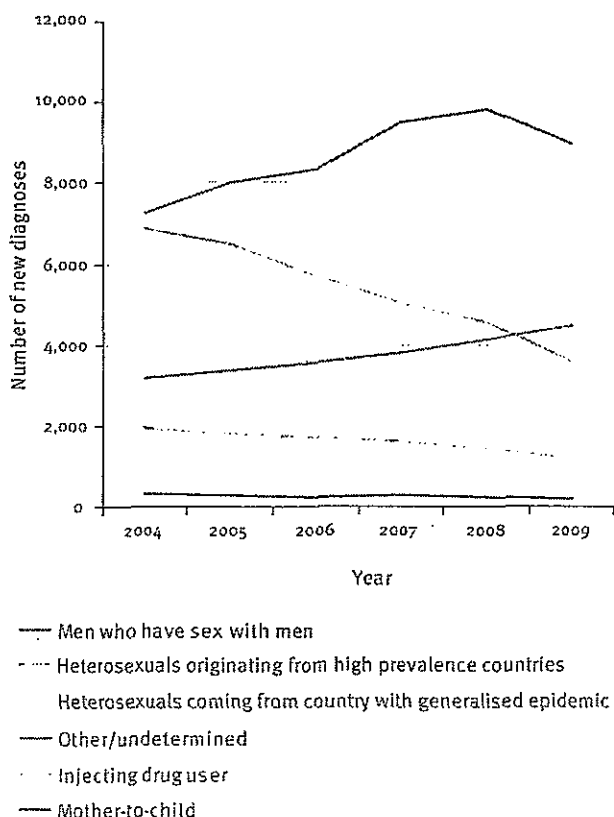
Since 2004, 26 EU/EEA countries have consistently reported data on transmission mode (Estonia and Poland were excluded due to inconsistent reporting). The number of new HIV diagnoses reported as heterosexually acquired decreased by 24% from 13,148

cases to 9,975 cases. The proportion of heterosexually acquired HIV diagnoses from countries with a generalised epidemic, varied from 52% in 2004 to 38% in 2009. In the same period, the number of cases among MSM increased by 24% in, from 7,263 cases to 8,974 cases and declined among IDU by 40% from 1,952 cases to 1,171 cases. The number of cases for which the transmission category was unknown increased by 40% (Figure 2). There are reporting delays for a number of countries which overall limits the ability to interpret the trends in recent years.

In 2009, a total of 4,650 cases of AIDS diagnoses were reported in 27 EU/EEA countries (no data from Austria, Liechtenstein, Sweden), representing a rate of 1.0 cases per 100,000 population. The highest rates were reported by Latvia (4.3), Estonia (2.8), Portugal (2.8), and Spain (2.3). In the EU/EEA, a decline was observed from 9,012 in 2004 to 4,650 in 2009 in all but four countries of the 27 countries reporting AIDS diagnoses consistently. An increase in AIDS diagnoses was reported only in Bulgaria from 22 cases to 30 cases (36%), Estonia 29 cases to 38 cases (31%), Latvia 89 cases to 96 cases (8%) and Lithuania 21 cases to 37 cases (76%).

FIGURE 2

HIV infection by transmission group and origin in EU/EEA countries^a 2004-2009



EEA: European Economic Area; EU: European Union.
Data from Austria, Estonia and Poland not included

Proportion of late presenters

Late presenters are defined as patients with a CD4 cell count below 350 cells/μl at time of HIV diagnosis [1]. Data on CD4 cell counts at the time of diagnosis were available for cases in 18 countries, ranging from 1.2% in Bulgaria to 87% in Spain. For 11 countries, CD4 count information was available for more than 50% of the cases (Table). Half of these were reported as late presenters, also taking into account possible reporting bias for more advanced HIV diagnoses. A slightly higher proportion of female cases (54.4%) were reported as late presenters compared with male cases (49.6%), with high heterogeneity among females across countries ranging from 33% in Cyprus and Luxembourg to 68% in Denmark. Among males, the proportion of late presenters ranged from 20% in Luxembourg to 60% in Denmark.

Among new diagnoses acquired heterosexually the proportion of late presenters ranged from 20% in Luxembourg to 67% in Slovakia, while seven countries reported more than 50% of the newly diagnosed cases as late presenters (Table). For MSM, the proportion of late presenters varied from 25% in Luxembourg to 50% in Slovenia (Table). Among IDU, the proportion of late presenters ranged from 49% in the UK to 60% in Denmark. All countries but Spain and the UK reported more than half of their cases with unknown risk factor as late presenters.

Figure 3 shows that among 10 222 cases with CD4 cell count reported, 51% were "late presenters". The largest proportion of late presenters is among individuals originating from sub-Saharan Africa and southeast/

TABLE

Proportion of CD4 cell counts reported for all HIV diagnosed cases in 2009, CD4 cell counts <200 among all reported, CD4 cell counts <350 among all reported and CD4 cell counts <350 by transmission mode by reporting country, 11 EU Member States*, 2009

Country	Number of cases with CD4 cell count	Completeness (%)	Number of cases CD4 <350	CD4 <350 mm ³ blood in 2009									
				Cases classified as									
				Hetero		IDU		MSM		MTCT		Unknown	
				N	%	N	%	N	%	N	%	N	%
Cyprus	29	76	12	21	42.9			8	37.5				
Czech Republic	117	75	48	34	64.7	4	75.0	78	28.2			1	100.0
Denmark	181	77	91	105	57.6	14	60.0	108	39.0			8	100.0
France	2624	54	1350	1367	56.8	71	53.5	916	40.9	22	40.9	245	60.8
Luxembourg	33	70	8	15	20.0			16	25.0			2	50.0
Netherlands	653	80	306	179	61.5	1	0.0	435	39.3			37	64.9
Romania	114	80	45	72	30.6	1	0.0					41	56.1
Slovakia	41	77	17	6	66.7	1	100.0	32	34.4			2	50.0
Slovenia	42	88	22	6	33.3			28	50.0			8	75.0
Spain	1968	87	987	709	58.8	152	59.2	899	42.3	7	42.9	197	47.7
United Kingdom	4365	66	2268	2205	62.5	89	49.4	1771	39.5	12	75.0	264	46.6

EU: European Union; IDU: injecting drug user; MSM: men having sex with men; MTCT: mother-to-child transmission.

*Member states not included in table: Belgium, Bulgaria, Finland, Germany, Latvia, Lithuania, Portugal.

eastern Asia, followed by cases from the Caribbean and Latin America. The proportion of late presenters is slightly higher in eastern Europe compared with central and western Europe. The areas are defined in the most recent ECDC/WHO HIV/AIDS surveillance report [4].

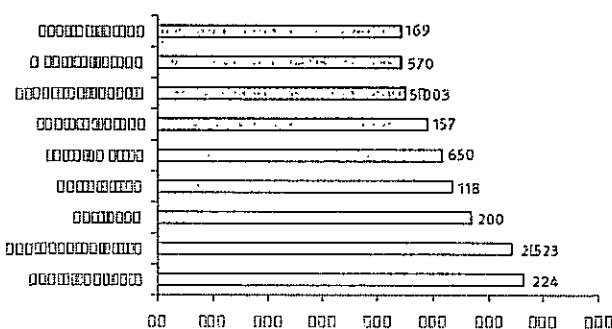
Conclusions

As in previous years, the highest proportion of the total number of new HIV diagnoses in 2009 in EU/EEA countries was reported among MSM, followed by heterosexuals. For the known transmission modes, a substantial increase was reported only in MSM. Heterosexual HIV transmission continues to be frequently reported; the proportion of cases classified as heterosexually acquired in persons originating from countries with generalised epidemics is decreasing, although it is still high in several countries. Although there is an apparent decline in the number of HIV diagnoses among IDU, injecting drug use is still the predominant transmission mode in several Eastern European countries. The transmission mode for a significant proportion of cases was reported as unknown, highlighting the importance of the improvement of surveillance data to better target public health interventions. The number of AIDS cases is decreasing in most of the countries except Bulgaria and Baltic States.

It is important to obtain more rigorous HIV surveillance data to better reflect changing epidemiological conditions. Inclusion of CD4 cell count at diagnosis provides an opportunity to interpret the data in greater depth. However, the reporting of CD4 cell counts needs to be improved as it was reported only for 40% of all new HIV diagnoses in 2009. Half of those were diagnosed as late presenters*, and it appears that late presenters are more often from outside of Europe. Timely HIV diagnosis is beneficial for the patient as it decreases morbidity and mortality and reduce HIV transmission [2-3]. Many factors influence the interpretation of the data such as the stage of the epidemic in the region, migration patterns and reporting bias. However the data suggest that access to testing and treatment needs to be improved among those at risk. Recently, ECDC has launched guidance in HIV testing to support

FIGURE 3

All cases with CD4 cell count reported, proportion of late presenters by region of origin (n=10,222)



Member States in the increasing the uptake of HIV testing in Europe [4-7]

Surveillance of HIV and AIDS in Europe provides the large scale picture of the HIV epidemics within its regions and of its main characteristics and risk groups affected, which is necessary to monitor the epidemic and guide the public health response to control HIV transmission of infections. Ensuring high quality of the data is of utmost importance to follow up and achieve the objectives set up in the EU Commission communication and action plan 'Combating HIV/AIDS in the European Union and Neighbouring countries, 2009-2013'.

Acknowledgements

We would like to thank all participating countries and national institutions of the European network for HIV/AIDS surveillance as well as colleagues at ECDC for their important contributions. In particular we would like to acknowledge M Maly, M Mardarescu, C Semaille, F Cazein and V Delpech for their valuable comments and suggestions.

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医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日 2010. 12. 17	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	人血清アルブミン	研究報告の公表状況	Brennan CA, Yamaguchi J, Devare SG, Foster GA, Stramer SL.. Transfusion. 2010 Dec;50(12):2707-12. doi: 10.1111/j.1537- 2995.2010.02767.x.	公表国 米国	
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン5%静注12.5g/250mL(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)				
研究報告の概要	○HIV-1非B型及び抗レトロウイルス薬剤耐性株に関する米国の供血者における広範な評価:2005~2007年 背景:HIVに感染した米国の供血者66人を対象にしたこれまでの研究(1999~2005年)では、HIV感染のうち4.7%がHIV-1非B型、6.5%が抗レトロウイルス薬剤耐性HIV-1であった。今回、2005年1月~2007年12月に収集した208症例(最近感染した症例11例と感染確定例197例)を対象として調査を行った。 方法:HIV感染供血者の検出は、FDAが認可した検査により行った。プロテアーゼ及び逆転写酵素遺伝子の薬剤耐性プロファイルは、遺伝子タイピングシステム(ViroSeq, Celera Diagnostics)を使用し決定した。サブタイプは、これらの配列を系統発生解析することにより得た。 結果:208検体のうち203検体について、薬剤耐性プロファイルが得られ、そのうち9.9%が薬剤耐性変異を持っていた。10例が1つの薬剤に耐性を示し(9例が非核酸系逆転写酵素阻害剤(NNRTIs)に、1例が核酸系逆転写酵素阻害剤(NRTIs)に耐性)、8例が2つの薬剤に耐性を示し(5例がNNRTIとNRTIに、2例がNRTIとプロテアーゼ阻害剤(PI)に、1例がNNRTIとPIに耐性)、2例が3つの薬剤に耐性を示した。 非B型株はHIV感染供血者の2.5%で同定され、サブタイプA1、D、CRF02_AG、CRF43-02G、およびURF_BFから構成されていた。 結論:本研究と従来の研究から、抗レトロウイルス薬剤耐性HIV-1が1999~2007年のHIV感染供血者の9.1%に存在(感染確定例の9.3%、最近感染した例の6.9%)することが示された。また、米国の供血者において、HIV-1非B型はHIV感染症の3.0%を占めた。				使用上の注意記載状況・ その他参考事項等 赤十字アルブミン20 赤十字アルブミン25 赤十字アルブミン5%静注 12.5g/250mL 赤十字アルブミン20%静注 4g/20mL 赤十字アルブミン20%静注 10g/50mL 赤十字アルブミン25%静注 12.5g/20mL 血液を原料とすることによる 感染症伝播等
報告企業の意見		今後の対応			
2005~2007年の米国のHIV感染供血者において、9.9%が抗レトロウイルス薬剤耐性、2.5%が非B型のHIV-1に感染していることが分かったとの報告である。 これまで、本製剤によるHIV感染の報告はない。また本製剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれている。さらに最終製品についてHIV-NAT陰性であることを確認していることから本製剤の安全性は確保されていると考える。		日本赤十字社では、化学発光酵素免疫測定法(CLEIA)により抗体検査を実施することに加えて、20プールNATについてもHIV-2及びHIVグループOの検出が可能な新NATシステムを導入し、陽性血液を排除している。20プールNAT導入以来、輸血HIV感染は一例も発生していないが、2008年よりさらにNATの感度を上げ対応している。国内外のHIV感染、AIDS発生の動向やHIV感染に関する新たな知見等について今後も情報の収集に努める。			

BLOOD DONORS AND BLOOD COLLECTION

Expanded evaluation of blood donors in the United States for human immunodeficiency virus type 1 non-B subtypes and antiretroviral drug-resistant strains: 2005 through 2007

Catherine A. Brennan, Julie Yamaguchi, Sushil G. Devare, Greg A. Foster, and Susan L. Stramer

BACKGROUND: In a previous study of 66 human immunodeficiency virus (HIV)-infected US blood donors from 1999 to 2005, HIV-1 non-B and antiretroviral drug-resistant strains accounted for 4.7 and 6.5% of HIV infections, respectively. This study was expanded to include an additional 11 recently acquired infections and 197 established infections collected from January 2005 through December 2007.

STUDY DESIGN AND METHODS: HIV-infected donors were detected using FDA-licensed assays. Drug resistance profiles for protease and reverse transcriptase (RT) genes were determined using a genotyping system (ViroSeq, Celeris Diagnostics); genetic subtype was determined by phylogenetic analysis of these sequences.

RESULTS: Drug resistance profiles were obtained for 203 of 208 specimens; 9.9% had mutations that confer drug resistance. Ten showed resistance to a single drug class: nine to nonnucleoside RT inhibitors (NNRTIs) and one to nucleoside RT inhibitors (NRTIs). Eight showed two drug class resistance: five NRTI plus NNRTI, two NRTI plus protease inhibitor (PI), and one NNRTI plus PI. Two showed three drug class resistance. Non-B strains were identified in 2.5% of donors and consisted of subtypes A1 and D, CRF02_AG, CRF43-02G, and URF_BF.

CONCLUSIONS: Data from this and the previous study show that antiretroviral drug-resistant HIV-1 is present in 9.1% of HIV-infected donors from 1999 through 2007; 9.3% of established infections and 6.9% of recent infections. Diverse HIV-1 non-B strains presently account for 3.0% of HIV infections in US donors.

The molecular characterization of human immunodeficiency virus (HIV) from infected blood donors provides data on the types, groups, and subtypes and drug resistance profiles of viral strains circulating in the donor population. This information can be used to monitor changes in the epidemic, direct prevention efforts, and identify risk groups. In addition, the data show the level of genetic diversity in HIV that screening tests must be able to detect.

In a previous study of 66 HIV infections identified in blood donors in the United States from 1999 through 2005,¹ HIV-1 non-subtype B strains accounted for 4.7% (3 of 64) of the HIV infections; RNA levels were insufficient for amplification in two donors. Antiretroviral drug-resistant HIV strains accounted for 11% (2 of 18) of recently acquired infections defined as RNA-positive, antibody-negative (RNA+/Ab-) and 6.5% (4 of 62) of all infected donors; RNA levels were insufficient for genotyping in four donors. Here we report the results following an expansion of the initial study to include an additional 11 RNA+/Ab- infections and 197 established infections (defined as HIV antibody confirmed positive [Ab+]).

ABBREVIATIONS: Ab antibody; ARC American Red Cross; NNRTI(s) nonnucleoside reverse transcriptase inhibitor(s); NRTI(s) nucleoside reverse transcriptase inhibitor(s); PI(s) protease inhibitor(s).

Address reprint requests to: Catherine Brennan, Abbott Diagnostics, Building AP20/Dept 09NG, 100 Abbott Park Road, Abbott Park, IL 60068-6015; e-mail: catherine.brennan@abbott.com.

From Abbott Diagnostics, Abbott Park, Illinois; and the American Red Cross, Gaithersburg, Maryland.

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TRANSFUSION 2010;50:2707-2712.

MATERIALS AND METHODS

The study population consisted of HIV-infected blood donors, irrespective of donation type, who donated at American Red Cross (ARC) centers or ARC external customer centers between January 2005 and December 2007. Eleven recently infected donors identified from December 2005 through June 2007 are characterized in this study. Specimens collected from 197 donors with established infection were selected based on the availability of stored specimens and exclusion from an ongoing NHLBI-sponsored molecular surveillance study.² Donors were screened for anti-HIV Ab and RNA as previously described.^{1,3} Briefly, donors were screened for HIV Ab by an HIV-1/2 enzyme immunoassay (EIA; Abbott Laboratories, Abbott Park, IL) and confirmed by Western blot (Calyptra Biomedical, Rockville, MD) or immunofluorescence assay (Sanochemia, Vienna, Austria). HIV-1 RNA was detected by nucleic acid testing (NAT) in pools of 16 specimens with reactive pools resolved to the individual reactive donation; each reactive individual donation was resolved as HIV-1 RNA reactive and confirmed positive by further RNA and Ab testing of the index donation and by testing follow-up specimens from consenting donors. The drug resistance profile of the protease and reverse transcriptase (RT) genes was determined using an HIV-1 genotyping system (ViroSeq, v2.0, Software v2.7, Celera Diagnostics, Alameda, CA). HIV genetic subtype was determined by phylogenetic analysis of the protease and RT viral sequences.¹ The Fisher's exact two-tailed test was used to determine *p* values. This retrospective study was approved by the ARC Institutional Review Board; donors harboring drug-resistant or non-B subtypes were notified of these results.

RESULTS

Since the implementation of HIV-1 NAT in 1999, through the end of 2009, 49 HIV-1 RNA+/Ab- donors were identified by the ARC and its external customers (Fig. 1) for all donation types representing an overall yield rate of 1 per 1.5 million donations. The number of donations screened each year was consistent at approximately 7.2 million donations, which represented almost one-half of blood donations screened in the United States. The geographical distribution of HIV-infected donors (RNA+/Ab- and Ab+) was disproportionate with 52.1% (5.82 per 100,000 donations) identified in southern states compared to 21.4% (2.48 per 100,000 donations) in the northeast, 16.4% (1.60

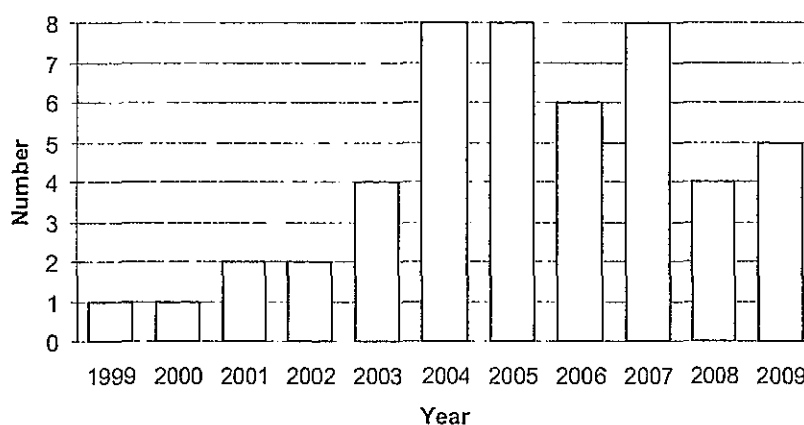


Fig. 1. Number of RNA+/Ab- donors identified by the ARC by year. From 1999 when HIV-1 NAT was implemented through 2009, a total of 49 recently infected donors were identified.

per 100,000 donations) in the midwest, and 10.2% (2.25 per 100,000 donations) in the west. The demographics of RNA+/Ab- donors shows that 61% were repeat donors and 86% were male with a median age of 26 years. Of the 49 HIV-1 RNA+/Ab- specimens, 20 collected before December 2005 were previously characterized¹ and 11 of 12 identified from December 2005 through June 2007 were evaluated in this study. The demographics for the 197 Ab+ donors in this study are similar to previous reports,^{1,4} primarily male, first-time donors with a median age of 30 years. During the same period (1999-2009), the Ab+ donation frequency for the study population was 1 per 34,900.

Antiretroviral drug resistance profiles were obtained for 203 of 208 donor specimens, all 11 RNA+/Ab- and 192 of 197 Ab+ specimens. Five specimens were not amplified in the ViroSeq assay that has a lower detection limit of 2000 RNA copies/mL. No drug-resistant strains were identified in the 11 RNA+/Ab- infections, while 20 of the 192 strains in the Ab+ group had mutations that confer resistance (Table 1). Of donors harboring drug-resistant HIV-1 strains 70% were first-time donors, 90% were male, and the median age was 42 years. Resistance to nonnucleoside RT inhibitors (NNRTIs) was found in 85% (17 of 20) of resistant strains with the most common mutation being K103N (88%; 15 of 17). Mutations that confer resistance to nucleoside RT inhibitors (NRTIs) were present in 40% (8 of 20) of resistant strains; the most common mutation was M184V (75%; six of eight). Mutations that confer resistance to protease inhibitors (PIs) were present in 25% (5 of 20) of resistant strains with no dominant mutation found. Strains with resistance to a single drug class accounted for 50% (10 of 20) of infections, 9 of 10 with resistance to NNRTI and one of 10 with NRTI resistance. Forty percent (8 of 20) of strains had two drug class resistance: NNRTI and NRTI (five of eight), NNRTI and PI (one of eight), and

TABLE 1. Donors with established HIV-1 infection and infected with drug-resistant HIV-1

Coded ID	Collection date ^a	Donor status ^b	Sex	Age (years)	State of residence	NRTI mutations ^c	NNRTI mutations ^c	PI mutations ^c	Drug class resistance ^c	Resistance ^c
ARC-A67	06/17/2005	1	Male	48	Georgia	M41L, T215D, K219R	None	I13V	1	NRTI resistance. ^d
ARC-A69	02/02/2005	2	Male	46	Georgia	M41L, D67N, K70R, M184V, T215F, K219E, K219Q	K103N, Y181C, V108I	V77I	2	Resistance or possible resistance to all NRTIs and resistance to all NNRTIs.
ARC-A73	07/13/2005	1	Female	19	Georgia	none	K103N	V77I	1	Resistance to all NNRTIs.
ARC-A80	08/24/2005	1	Male	46	California	M41L, D67N, L74I, L74V, Y115F, V118I, M184V, L210W, T215Y, K219N	K101E, Y181C, G190S	L10I, I13V, K20R, L33F, M36I, K43T, M46L, I54L, A71V, G73S, V82A, L90M	3	Resistance to all NRTIs, NNRTIs, and PIs.
ARC-A92	07/19/2005	1	Male	36	California	V118I	K103N	K20R, M36I	1	Resistance to all NNRTIs.
ARC-A128	12/22/2005	1	Male	42	Michigan	none	Y188L	V77I	1	Resistance or possible resistance to all NNRTIs.
ARC-A130	10/05/2005	2	Male	42	Michigan	none	K103N	L10I	1	Resistance to all NNRTIs.
ARC-A140	04/14/2005	1	Male	20	Michigan	none	K103N	none	1	Resistance to all NNRTIs.
ARC-A150	08/05/2005	2	Male	22	Oregon	none	K103N	K20R	1	Resistance to all NNRTIs.
ARC-A163	06/18/2005	1	Male	48	Pennsylvania	none	K103N	K45R, V77I	1	Resistance to all NNRTIs.
ARC-A164	10/25/2005	1	Male	42	Pennsylvania	M41L, D67N, V75M, F77L, L210W, T215Y	K101E, G190A	L10F, I13V, M36I, M46I, I54L, I84V	3	Resistance or possible resistance to all NRTIs, NNRTIs, and PIs.
ARC-A199	01/17/2007	1	Male	36	Illinois	None	K103N	V77I	1	Resistance to all NNRTIs.
ARC-A200	10/01/2005	1	Male	31	Illinois	None	K103N	None	1	Resistance to all NNRTIs.
ARC-A202	06/04/2005	1	Female	43	Ohio	A62V, V75I, M184V	K103N	I13V, V77I	2	NRTI resistance ^e and resistance to all NNRTIs.
ARC-A203	03/08/2005	2	Male	47	Ohio	M41L, D67N, T215Y	none	L10I, L33F, I54V, A71V, G73S, V77I, V82A, L90M	2	Resistance ^d or possible resistance ^f to NRTIs and resistance or possible resistance to all PIs.
ARC-A211	12/05/2005	1	Male	17	Texas	A62V, M184V	G190S	M36I, V77I	2	NRTI resistance ^e and NNRTI resistance. ^g
ARC-A222	08/25/2005	1	Male	30	Pennsylvania	None	K103N, Y188L	M46I, A71T, L90M	2	Resistance to all NNRTIs and resistance ^h or possible ⁱ resistance to PIs.
ARC-A230	04/08/2005	2	Male	47	Puerto Rico	D67N, T69N, T215L, K219E	L100I, K103N	None	2	Resistance ^d or possible resistance ^j to NRTIs and resistance to all NNRTIs.
ARC-A240	03/27/2007	2	Male	45	Arkansas	M184V	none	K20R, M36I, V82A, I84V	2	NRTI resistance ^h and resistance or possible resistance to all PIs.
ARC-A242	08/25/2005	1	Male	53	California	M184V	K103N	V77I	2	NRTI resistance ^e and resistance to all NNRTIs.

^a Date read month/day/year.

^b 1 indicates first-time donor; 2 indicates repeat donor.

^c Drug resistance profile determined using ViroSeq. Mutations shown in bold alone either confer resistance or the possibility of resistance. Mutations not in bold must appear with at least one other mutation to confer possibility of resistance. All viruses are subtype B.

^d Zidovudine and stavudine.

^e Lamivudine and emtricitabine.

^f Didanosine, abacavir, and tenofovir.

^g Efavirenz and nevirapine.

^h Nelfinavir.

ⁱ Amprenavir, fosamprenavir, indinavir, saquinavir, ritonavir, and atazanavir.

^j Didanosine.

NRTI and PI (two of eight). Only 10% (2 of 20) of resistant strains had resistance to all three drug classes. In 19 of 20 donors infected with HIV drug-resistant strains, the EIA signal-to-cutoff values were 16 or higher; the single specimen (ARC-A203) with lower reactivity (signal-to-cutoff value, 5.4) showed two drug class-resistance (NRTIs and PIs).

HIV-1 non-subtype B strains were identified only in the established infection group and accounted for 2.5% (5 of 203) of all infections. Phylogenetic analysis of the protease and RT sequences identified the non-B strains as subtypes A1 and D, circulating recombinant forms CRF02_AG and CRF43-02G, and unique recombinant form URF_BF (Table 2). The geographic origin of each donor's HIV infection is unknown; no risk questioning was done as part of the study protocol. Donors infected with non-B strains were primarily first-time donors (four of five) with males (three of five) and females (two of five) about equally represented. In contrast, males accounted for 70% or greater of HIV-infected donors with and without drug-resistant virus. In all five donors infected with non-subtype B strains, the EIA signal-to-cutoff values were higher than 18 (Table 2). Therefore, the current EIA in use at the time of this study demonstrated robust reactivity for both drug resistant and non-subtype B HIV strains.

DISCUSSION

The overall frequency of 9.9% (20 of 203) drug resistance observed in 2005 to 2007 was not significantly higher ($p = 0.61$) than the 6.5% (4 of 62) reported in the earlier study from 1999 to 2005.¹ Combining the data from this and the previous study shows that 9.1% (24 of 265) of HIV-infected blood donors harbor virus with mutations that confer resistance to antiretroviral drugs (Table 3). The prevalence of drug-resistant virus in donors is similar to previous reports including a recent CDC national surveillance study that found a prevalence of 10.4%.⁵ The majority (58%; 14 of 24) of strains showed resistance to a single drug class including 10 to NNRTIs, two to NRTIs, and two to PIs. Thirty-three percent (8 of 24) showed two drug class resistance: five NRTI and NNRTI, two NRTI and PI, and one NNRTI and PI. Only 8% (2 of 24) showed three drug class resistance. Drug resistance was more frequent in established infections (9.3%; 22 of 236) compared to recent infections (6.9%; 2 of 29); in the previous study, but not this study, two recently infected donors harbored resistant strains demonstrating transmission of drug-resistant HIV-1. Drug-resistant HIV-1 was identified in donors from nine different states and Puerto Rico showing broad distribution of these strains throughout the United States (Table 1).¹

Infections with HIV-1 non-B strains occurred at a low frequency (2.5%; 5 of 203). Including the non-B strains

TABLE 2. Donors with established HIV-1 infection and infected with non-B strain

Coded ID	Collection date*	Donor status†	Sex	Age (years)	State of residence	HIV EIA (s/co)‡	HIV-1 subtype (PR-RT)	Drug resistance§
ARC-A59	10/25/2005	2	Female	40	Georgia	19.13	CRF02_AG	None
ARC-A95	08/19/2005	1	Male	21	Arizona	20.95	URF_BF	None
ARC-A127	09/01/2005	1	Male	28	Michigan	18.80	CRF43_02G	None
ARC-A206	07/16/2005	1	Female	33	Texas	18.64	D	None
ARC-A216	04/06/2005	1	Male	55	Maryland	18.64	A1	None

* Date read month/day/year.

† 1 indicates first-time donor; 2 indicates repeat donor.

‡ s/co = signal/cutoff; values > 1.0 are reactive. All reactive specimens were confirmed by HIV-1 Western blot.

§ Drug resistance profile determined using ViroSeq.

CRF = circulating recombinant form; URF = unique recombinant form.

TABLE 3. HIV-1-infected donors 1999 through 2007

Years	Percentage of HIV-infected donors (number/number from which RNA was amplified)			
	Non-B strains	Antiretroviral drug resistant		Total of all infections
		Recently acquired infection (RNA+/Ab-)	Established infection (Ab+)	
1999-2005*†	4.7 (3/64)	11.1 (2/18)	4.5 (2/44)	6.5 (4/62)
2005-2007†	2.5 (5/203)	0 (0/11)	10.4 (20/192)	9.9 (20/203)
Total: 1999-2007	3.0 (8/267)	6.9 (2/29)	9.3 (22/236)	9.1 (24/265)

* Data from Brennan et al.¹

† The differences between the 1999-2005 and 2005-2007 data sets are not significant; all p values are greater than 0.38.

identified in the earlier study (4.7%; 3 of 64),¹ the overall prevalence of non-B strains from 1999 through 2007 was 3.0% (8 of 267) of HIV-infected US blood donors, all of which were detected in Ab+ donors (Table 3). It appears that there has been a slow increase in non-B prevalence since 1986, although there are differences in sampling populations across the studies.^{1,6,7} The prevalence of HIV-1 non-B strains in the CDC surveillance study was similar at 5.1%.⁵ Each non-B strain was identified in a different state with the states distributed across the country (Table 2).¹ In contrast to females accounting for approximately 30% of HIV-infected donors, approximately 50% of donors with non-B infections were female; this is consistent with heterosexual contact being the primary mode of transmission for non-B strains.⁸ Despite a low prevalence, diverse non-B strains were found including two subtypes (A1 and D), three CRFs (01, 02, and 43), and one URF. Subtypes A1 and D and CRF02_AG are found primarily in Africa.⁹ CRF01_AE is endemic to southeast Asia and URF_BF to South America.⁹ CRF43-02G is an uncommon HIV-1 strain found in Saudi Arabia;¹⁰ this is the first report of it in the United States. The high signal-to-cutoff ratios (Table 2) show robust detection of these variants using an FDA-licensed screening assay, as is true for all specimens included in this and the previous study. Based on multiplying the probabilities of HIV RNA+, Ab- donations (1 per 1.5 million) and non-B strains (3% of HIV-infected donations), the risk of a non-B infection during the RNA+, Ab- phase of infection is very low, estimated at 1 in 50 million donations.

There is an ongoing national NHLBI-sponsored study, Retrovirus Epidemiology Donor Study-II (REDS-II), that is conducting molecular surveillance of HIV, hepatitis B virus, and hepatitis C virus for which the ARC is the largest participant.² Preliminary results for 2006 through 2007 from a unique set of 146 HIV-infected donors, which were excluded from this study, found results similar to ours for drug-resistant HIV-1. Approximately 12% (17 of 146) of infected donors harbored a drug-resistant strain with the most prevalent strains being single drug class resistant (15 of 17) and resistance to NNRTIs occurring the most frequently (14 of 17). In contrast to our data, the preliminary results from REDS-II identified only one

non-B strain, subtype C; the 0.7% (1 of 146) frequency of non-B infections is lower than the 3% reported here ($p = 0.17$). A partial explanation for the different results may be methods used for polymerase chain reaction amplification or specimen selection. In the REDS-II study, sequence was obtained from only 63% (146 of 232) of submitted specimens while we were able to obtain protease-RT sequences from 97% (265 of 274) of specimens.

In conclusion, this study, with an expanded number of HIV-infected donors whose virus has been characterized for subtype and drug resistance, provides a more accurate profile of HIV strains that are circulating in the US donor population. The larger data set, from the combined data of this and the earlier study, demonstrate that the prevalence of non-B strains is 3.0% and the prevalence of drug-resistant HIV is 9.1% (Table 3). The frequencies of drug resistance mutations and non-B subtypes are relatively low (<10%) and appear to be stable. The length of time of HIV infection in those donors with established infection is unknown whereas those with recent HIV infection are defined by the duration of the HIV RNA+/Ab- window period. It is likely that those with established infection represent a continuum ranging from relatively early infected individuals to those with long-term HIV infection. Follow-up of these donors was not performed. In any event, over an almost 8-year period of time, dramatic changes in the molecular characteristics of HIV in infected donors were not observed. Finally, the results demonstrate that the HIV screening assays in use in the United States have the ability to detect diverse HIV strains.

CONFLICT OF INTEREST

CAB, JY, and SGD are employees of Abbott Diagnostics. SLS and GAF have no conflict of interest.

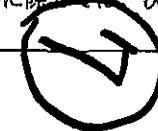
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識別番号・報告回数		報告日		第一報入手日 2010 年 11 月 24 日	新医薬品等の区分	厚生労働省処理欄
一般的名称	①②③④人血清アルブミン ⑤⑥⑦乾燥濃縮人アンチトロンビンⅢ ⑧人ハプトグロビン ⑨乾燥濃縮人血液凝固第Ⅶ因子				公表国 中国	
販売名 (企業名)	①献血アルブミン 25%静注 5g/20mL「ベネシス」 (ベネシス) ②献血アルブミン 25%静注 12.5g/50mL「ベネシス」 (ベネシス) ③献血アルブミン 5%静注 5g/100mL「ベネシス」 (ベネシス) ④献血アルブミン 5%静注 12.5g/250mL「ベネシス」 (ベネシス) ⑤ノイアート静注用 500 単位 (ベネシス) ⑥ノイアート静注用 1500 単位 (ベネシス) ⑦ノイアート (ベネシス) ⑧ハプトグロビン静注 2000 単位「ベネシス」 (ベネシス) ⑨コンコエイトーHT (ベネシス)			研究報告の公表状況	Vector-Borne and Zoonotic Diseases 2010; 10(8765): 765-770	
研究報告の概要	中国の家畜の HEV 感染保有率を評価するために、3579 の血清サンプル(1967 匹のブタ、700 匹のヤギと 912 匹のウシ血清を含む)が 26 の省から集められ、酵素免疫測定法を用いて HEV 抗体および抗原の試験を実施した。 結果、ブタサンプルは 82.2%で抗 HEV 抗体陽性であったが、ウシ血清では 10.4%、ヤギ血清では 28.2%であった。動物中の抗 HEV 抗体の保有率は省毎に違っていた(ブタは 10.9%~100%、ヤギは 0%~48%、ウシは 0%~92.9%であった)。 ブタの約 1.9%、ヤギの約 1.6%、ウシの約 0.8%が HEV 抗原が陽性であった。いくつかのサンプル(全ての HEV 抗原陽性のサンプルを含む)について、逆転写ポリメラーゼ連鎖反応を用いて、HEV 特有の RNA の検査をした。ヤギやウシとは異なり、15 のブタサンプルは、HEV RNA 陽性が判明した。 全てのブタ HEV を配列と系統分析で分離したところ、HEV 遺伝子型 4 に分離された。そして、更に 4 つのサブグループに分けられた。この研究は、HEV 感染が中国の家畜(特にブタ)で広範囲にわたることを証明した。					使用上の注意記載状況・ その他参考事項等
	代表としてノイアート静注用 500 単位の記載を示す。 2. 重要な基本的注意 (1) 本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-I 抗体陰性で、かつ ALT(GPT)値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査(NAT)を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分から人アンチトロンビンⅢを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及びウイルス除去膜による過処理を施しているが、投与に際しては、次の点に十分注意すること。					
報告企業の意見					今後の対応	
万一、ヘパリンの原料であるブタ小腸粘膜に HEV が混入したとしても、HPV1 及び PPV をモデルウイルスとしたウイルスバリデーション試験成績から、ヘパリンの製造工程において十分に不活化・除去されると考えている。					本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。	





Serological Prevalence of Hepatitis E Virus in Domestic Animals and Diversity of Genotype 4 Hepatitis E Virus in China

Yansheng Geng,^{1,2} Chuanbin Wang,³ Chenyan Zhao,² Xiuling Yu,³
 Tim J. Harrison,⁴ Kegong Tian,³ and Youchun Wang^{1,2}

Abstract

Pigs have been confirmed to be reservoirs of some genotypes of hepatitis E virus (HEV), and other nonhuman species are also likely infected with the virus. To assess the prevalence of HEV infection in domestic animals in China, 3579 serum samples, including 1967 swine, 700 goat, and 912 cattle sera, were collected from 26 provinces across the country and tested for HEV antibodies and antigen using enzyme immunoassays. The results showed that 82.2% of the swine samples, but only 10.4% and 28.2% of cattle and goat sera, were anti-HEV positive respectively. The prevalence of anti-HEV antibody in animals varied from province to province, ranging from 10.9% to 100% in pigs, 0% to 48% in goats, and 0% to 92.9% in cattle. About 1.9% of pigs, 1.6% of goats, and 0.8% of cattle tested in the study were positive for HEV antigen. Some samples, including all HEV antigen-positive samples, were tested for HEV-specific RNA using reverse transcription polymerase chain reaction. Fifteen swine samples, but none from the goats or cattle, were found to be HEV RNA positive. Sequence and phylogenetic analyses classified all the swine HEV isolates into HEV genotype 4, which was further divided into four subgroups. This study demonstrated that HEV infection is widespread in domestic animals, particularly pigs, in China. The HEV genotype infecting pigs in China was genotype 4. However, the isolates displayed considerable genetic diversity.

Key Words: Anti-HEV antibody—Cattle—EIAs—Goat—HEV antigen—RT-PCR—Swine.

Introduction

HEPATITIS E IS AN IMPORTANT public health problem in many developing countries and is also endemic in some developed countries. Hepatitis E virus (HEV), the etiological agent of hepatitis E, is a nonenveloped virus approximately 27–34 nm in diameter with a single-stranded, positive-sense RNA genome about 7.2 kb in size. The genome consists of three open reading frames (ORFs) 1, 2, and 3 and short 5' and 3' untranslated regions (Tam et al. 1991). Four major genotypes of HEV, based on nucleotide sequence analyses, are widely accepted (Emerson and Purcell 2003, Okamoto et al. 2007), and subtypes have been proposed within each genotype (Lu et al. 2006). However, only one serotype has been recognized (Lu et al. 2006, Purcell and Emerson 2008). Molecular epidemiological investigations have revealed the

geographical distribution of HEV genotypes: genotype 1 was isolated from Asia and Africa, genotype 2 from Mexico and West Africa, genotype 3 has been found almost worldwide but most human isolates were from industrialized countries, and genotype 4 strains were isolated from sporadic cases in Asia (Lu et al. 2006, Purcell and Emerson 2008).

Natural antibodies against HEV have been detected in numerous animal species, suggesting the spread of HEV among these animals. Successful experimental transmission of human HEV to animals indicates that HEV can infect across species (Huang et al. 2008, Meng 2003, 2009). HEV sequences have been isolated from swine in many countries and, thus far, these isolates belong to genotype 3 or 4. Further, human infections with these two genotypes are found to be caused by HEV genetically closely related to, and in some cases identical to, genotypes 3 and 4 strains found in pigs in the same

¹The Graduate School of Peking Union Medical College, Beijing, China.

²Department of Cellular Biology, National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China.

³Veterinary Diagnostic Lab, China Animal Disease Control Center, Beijing, China.

⁴Division of Medicine, University College London Medical School, London, United Kingdom.

geographical area (Wang et al. 2002, Meng 2003). Accumulating evidence suggests that HEV infections in humans, at least those caused by genotypes 3 and 4, may be acquired zoonotically, and domestic pigs, and perhaps other animals, are reservoirs of the virus (Meng 2009).

Hepatitis E was first recognized in China after a large epidemic in the southern part of the Xinjiang Uighur autonomous region (Zhuang et al. 1991), and small epidemics and sporadic cases have been reported from various regions of the country. Thus, HEV is known to be endemic in China. The Xinjiang epidemic was attributed to HEV genotype 1, but genotype 4 has been isolated more often from sporadic cases in the country than is genotype 1 (Wang et al. 1999, Zhai et al. 2006, Yu et al. 2009, Tai et al. 2009). Reports suggest that HEV infection is endemic in domestic animals in some regions of China and the anti-HEV antibody is particularly highly prevalent in pigs. The aim of this study was to understand more fully the prevalence and genetic variety of HEV present in domestic animals in China, including pigs, goats, and cattle.

Materials and Methods

Serum samples

A total of 3579 serum samples, including 1967 swine, 700 goat, and 912 cattle sera, were collected from 26 provinces in China in the autumn of 2008. Most of the samples were collected from industrialized farms, but some were collected from small groups of animals raised by peasants. All the serum samples were stored in dry ice for transportation and then transferred to and stored in a -70°C freezer until use.

Detection of anti-HEV antibody and antigen

Anti-HEV antibody and antigen were detected by enzyme immunoassay (EIA), using a direct-sandwich EIA kit and a monoclonal antibody-based EIA kit (Wantai Biopharmaceutical, Beijing, China), respectively, according to the manufacturer's instructions. Fifty microliters of each sample were used for antibody detection and 100 μL for antigen detection (Wang et al. 2002, Zhang et al. 2006).

RNA extraction

RNA was extracted from each sample using a spin-column kit and following the procedures of the Kinghawk Biopharmaceutical Company (Beijing, China). The protocols were essentially as described previously (Wang et al. 2002, Li et al. 2008), except that 100 μL serum were used for RNA extraction. The RNA from each sample was recovered in 50 μL of elution buffer.

Nested reverse transcription polymerase chain reaction

The nested reverse transcription polymerase chain reaction (RT-PCR) primers derived from the ORF2 region are capable of detecting divergent HEV strains (genotypes 1 to 4) and have been described previously (Li et al. 2008). The external primer pair was 5'-AAYTATGCWCAGTACCGGGTTG-3' and 5'-CCCTTATCCTGCTGAGCATTCTC-3'; the internal primer pair was 5'-GTATGTYTGCATACATGGCT-3' and 5'-AGCCGACGAAATYAATTCTGTC-3'. Nested RT-PCR

was carried out in 50 μL reaction mixtures containing 7 μL RNA extract, 20 pmol of each of the external primers, 5 U AMV reverse transcriptase (from avian myeloblastosis virus), and 5 U Taq polymerase. The reaction was performed at 50°C for 30 min, followed by 35 cycles of amplification at 94°C for 1 min, 50°C for 45 s, and 72°C for 1 min, with a final extension at 72°C for 10 min. Two microliters of the PCR-amplified product were transferred into a second PCR reaction mixture with a total volume of 50 μL , containing 20 pmol of each of the internal primers and 5 U Taq polymerase. The amplification parameters were the same as for the first PCR. A final amplification product of 348 bp was then analyzed by electrophoresis through 1.0% agarose gel and viewed by ethidium bromide staining.

DNA sequencing and sequence analyses

Amplified PCR products were separated on 1.0% agarose gel. Bands of the predicted size were excised from the gel and purified using a Quick Spin™ column kit (Qiagen, Hilden, Germany). The purified products were sequenced directly on both strands with the specific PCR primers synthesized by Invitrogen (Beijing, China). Sequences were analyzed using ClustalW2 and Mega4 software. The swine HEV sequences were subsequently compared with selected reference sequences from human and swine HEV strains available in the GenBank database. These included the following: for genotype 1, Burma (M73218, human), China Hebei (M94177, human); genotype 2, Mexico (M74506, human); and genotype 3, US1 (AF060668, human), swJHK2-3 (AB194479, Japan, pig), SAAS-JDY5 (FJ527832, Shanghai, pig). The representative strains for each subgenotype of genotype 4 identified by Lu et al. (2006) were as follows: 4a, T21 (AF151963, China Beijing, human), Ch87 (AJ344171, China Beijing, human); 4b, Ch181 (AJ344188, China Beijing, human); 4c, swJ13-1 (AB097811, Japan, pig), swJ14 (AB080575, Japan, pig); 4d, Ch108 (AJ344181, China Beijing, human), swCH72 (AY596308, China Uighur, pig); 4f, HE-JA2 (AJ082558, Japan, human, pig); and 4g, CCC220 (AB108537, China Beijing, human). Chinese swine strains with complete genome sequences in GenBank were swCH189 (FJ610232, Gansu), swGX40 (EU676712, Guangxi), swGX32 (EU366959, China), SH-SW-2SI (EF570133, Shanghai), SW-DQ (DQ279091, Heilongjiang), swCH31 (DQ450072, Jiangsu), and swCH25 (AY594199, Jiangsu).

Statistical analysis

The chi-square test was used to compare HEV prevalence among provinces. To compare HEV antibody and antigen levels expressed as the S/CO (the ratio of Sample Value to Cutoff Value) mean $\pm$ standard error of mean (SEM), statistical analysis was performed using the unpaired Student's *t*-test. *p*-Values <0.05 were considered significant.

Results

Prevalence and levels of anti-HEV antibody in domestic animals

Of the 1967 swine sera collected from 25 provinces in China, 1617 (82.2%) were anti-HEV antibody positive (Table 1). Positive samples were found in all 25 provinces, and their prevalence varied considerably among the provinces, rang-

TABLE 1. DETECTION OF HEPATITIS E VIRUS ANTIBODIES AND ANTIGEN IN PIGS, GOATS, AND CATTLE

Geographic location	Pig			Goat			Cattle		
	No. of samples	Antibody positive (%)	Antigen positive (%)	No. of samples	Antibody positive (%)	Antigen positive (%)	No. of samples	Antibody positive (%)	Antigen positive (%)
Beijing	15	15 (100)	0						
Tianjin	70	60 (85.7)	2 (2.9)	10	2 (20)	0	20	6 (30)	0
Hebei	13	12 (92.3)	1 (7.7)						
Shanxi	40	40 (100)	4 (10.0)	25	12 (48)	0	30	3 (10)	0
Nei Mongle	506	399 (78.9)	9 (1.8)	359	30 (8.4)	2 (0.6)	495	197 (39.8)	6 (1.2)
Shannxi	44	40 (90.9)	2 (4.5)	20	3 (15)	0	35	6 (17.1)	0
Ningxia	44	27 (61.4)	1 (2.3)	13	3 (23.1)	0	30	2 (6.7)	0
Qinghai	15	15 (100)	0	55	7 (12.7)	1 (1.8)	27	2 (7.4)	0
Xinjiang	64	48 (75)	2 (3.1)	79	8 (10.1)	7 (8.9)	40	11 (27.5)	0
Tibet				61	3 (4.9)	0	13	0	0
Shandong	60	57 (95.0)	2 (3.3)			0	37	2 (5.4)	0
Anhui	70	65 (92.9)	2 (2.9)						
Sichuan	46	5 (10.9)	0	22	1 (4.5)	0			
Chongqing	52	9 (17.3)	0						
Hunan	33	23 (69.7)	0				33	0	0
Hubei	35	35 (100)	1 (2.9)						
Shanghai	34	31 (91.2)	0				22	1 (4.5)	1 (4.5)
Jiangsu	37	37 (100)	1 (2.7)	10	0	0	26	0	0
Zhejiang	467	452 (96.8)	5 (1.1)				24	0	0
Jiangxi	57	56 (98.2)	1 (1.8)						
Guizhou	33	24 (72.7)	1 (3.0)	15	4 (26.7)	0	25	1 (4.0)	0
Yunnan	21	21 (100)	0	16	0	0	27	12 (44.4)	0
Guangxi	59	47 (79.7)	2 (3.4)						
Guangdong	49	48 (98)	1 (2.0)				14	13 (92.9)	0
Fujian	54	32 (59.2)	1 (1.9)	15	0	1 (6.7)			
Hainan	49	19 (38.8)	0				14	1 (7.1)	0
Total (26)	1967	1617 (82.2)	38 (1.9)	700	73 (10.4)	11 (1.6)	912	257 (28.2)	7 (0.8)

ing from 10.9% to 100%. The lowest prevalence of 10.9% was for samples from Sichuan, and the neighboring province Chongqing also had a low prevalence (17.3%), both being significantly below the average.

Seven hundred goat serum samples collected from 13 provinces and 912 cattle serum samples collected from 17 provinces also were tested for anti-HEV antibody. The prevalence ranged from 0% to 48% in goats and 0% to 92.9% in cattle. The average prevalence for both species was significantly lower than for swine (Table 1, $p < 0.001$). However, some individual groups showed exceptionally high positivity rates. For example, 13 of 14 (92.9%) cattle sera from Guangdong were anti-HEV positive. The average titer of HEV antibody (expressed as S/CO mean $\pm$ SD) in pigs (10.7 $\pm$ 4.7) was also significantly higher than in goats (4.6 $\pm$ 2.9) and cattle (3.7 $\pm$ 3.4).

HEV antigen and HEV RNA in domestic animals

All serum samples were tested for the presence of HEV antigen. The positivity rate for pigs 1.9% (38/1967) was almost equal to that for goats 1.6% (11/700) but a little higher than for cattle 0.8% (7/912) (Table 1). Cattle sera from most provinces were negative except 6 of 495 samples from Inner Mongolia and 1 of 22 samples from Shanghai. The average titer of HEV antigen (expressed as S/CO mean $\pm$ SD) in pigs (3.94 $\pm$ 2.92) was statistically higher than in goats (1.85 $\pm$ 0.87) and cattle (1.90 $\pm$ 1.74).

On the basis of the report that HEV RNA is present mainly in HEV antigen-positive animal sera (Yu et al. 2009), in this

study all of the HEV antigen-positive and HEV antibody-positive samples with OD values below 1.0 were tested for HEV RNA by RT-PCR. All goat samples from Shanxi and Guizhou, and all cattle samples from Guangdong, Xinjiang, Yunnan, and Tianjin with relatively high anti-HEV prevalence were also tested for HEV RNA. Fifteen swine sera were HEV RNA positive and all of them were also positive for HEV antigen. RNA positivity rate of the antigen-positive samples was 39.5% (15/38), and the overall prevalence of HEV RNA in pigs was 0.8% (15/1976). None of the goat or cattle samples was positive for HEV RNA.

Phylogenetic analysis of the HEV sequences

The PCR products from the 15 positive swine samples were sequenced. The 348 bp sequences of the ORF2 capsid region were compared and aligned with known HEV sequences and subjected to phylogenetic analysis. Sequence analysis showed that the 15 swine isolates had 82–100% nucleotide sequence identity with each other. All the 15 sequences clustered within genotype 4 and were further divided into four distinct subgroups as shown in the phylogenetic tree (Fig. 1). One isolate from Shanxi and one from Tianjin are in the 4a subgroup. Two identical isolates from Xinjiang and two from Shanxi cluster in the 4d subgroup. Three identical isolates from the same farm in Hangzhou and six from Fujian, Hubei, Guizhou, Tianjin, and Anhui cluster closely together and form a novel subgroup. An isolate from Jiangsu, along with other strains reported previously, falls into another subgroup. The

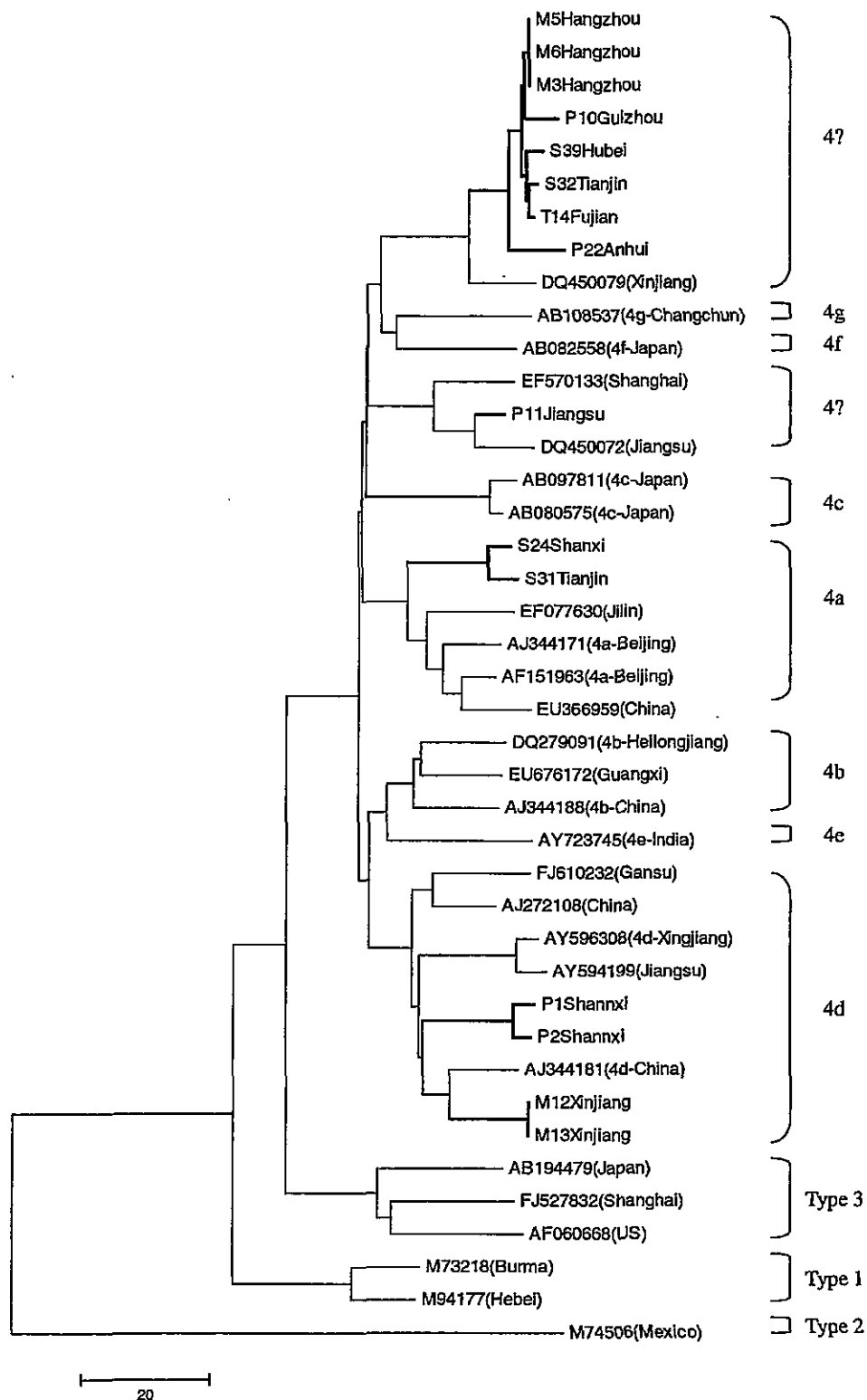


FIG. 1. Phylogenetic tree of hepatitis E virus isolates based on partial ORF2 sequences (348 nt). The tree was constructed using the Neighbor-Joining method by Mega 4.1 software. The scale bar indicating the number of character state changes is proportional to the genetic distance. Hepatitis E virus isolates from this study are indicated by thick lines.

nucleotide differences among the subgroups ranged from 4.7% to 11.6%, the mean difference being 9.6%.

Discussion

Hepatitis E is considered by many to be a zoonotic disease, and domestic pigs have been confirmed to be reservoirs of HEV genotypes 3 and 4 (Meng 2009). Our previous investigations showed anti-HEV antibody prevalence in pigs from the northeast of China (including three provinces: Heilongjiang, Jilin, Liaoning) and from Hunan to be 81.6% and 68.3%, respectively (Li et al. 2008, Yu et al. 2009). A similar result was obtained in the present study. The average positivity rate of anti-HEV antibody in swine serum samples from 25 different provinces was 82.2%. The positivity rate varied greatly from province to province, ranging from 10.9% to 100%. Because samples were collected from only a few farms in each province, this variability may reflect differences among farms rather than among provinces. The age of pigs, breeding environment, and other factors can affect the positivity rate of HEV in a farm (Li et al. 2009a, 2009b). Therefore, these results indicate that HEV is prevalent in pig herds across China and the prevalence is relatively high overall.

Mammalian HEV is divided into four genotypes, and these genotypes have a distinct geographic distribution (Okamoto et al. 2007). A candidate fifth genotype was described recently (Zhao et al. 2009). Genotype 4 is the most common in patients with sporadic hepatitis E and in pigs in China, including Hong Kong (Wang et al. 2002, Lam et al. 2009, Tai et al. 2009). Although each HEV genotype tends to diverge to different subtypes, there is no consensus as to how many subtypes each genotype contains. Further, new variants are constantly being discovered, especially within genotypes 3 and 4. On the basis of the HEV sequences currently deposited in the GenBank database, HEV genotype 4 comprises at least seven subtypes, named 4a–4g (Lu et al. 2006). The subtypes 4a, 4b, 4c, 4d, and 4g include Chinese strains isolated from humans or swine. Candidate new subgroups, which are clearly different from these seven subtypes, were found in Hunan province and in northeast China (Li et al. 2008, Yu et al. 2009). To understand completely the genetic diversity and distribution of swine HEV in China, representative strains from each subtype of HEV genotype 4 described in Lu's report, along with the full-length Chinese swine HEV isolates in GenBank, were used to construct a phylogenetic tree using the isolates of this study. Nine subgroups were found within genotype 4, including subgroups 4a–4g identified previously by Lu et al. (2006) and two new subgroups. The 15 swine isolates from the current study were dispersed among 4a, 4d, and the two new subgroups. Strains identified in the same region tend to cluster together. However, one region may harbor more than one subgroup as described previously (Li et al. 2008, Yu et al. 2009), and each subgroup may contain strains with similar sequences from distinct regions. For instance, in this study, six strains from five different provinces clustered clearly to form a new subgroup, while one Jiangsu isolate clustered with two other previously reported isolates to form another new subgroup. These results indicate that genotype 4 is predominantly prevalent among pigs, and genotype 4 HEV is highly diverse in China. However, the geographic heterogeneity of swine HEV seems becoming blurred. One probable reason is

that pig trading across the country leads to the rapid spread of HEV strains from one region to another.

Genotype 3 HEV also has been implicated as a possible zoonotic agent and may be responsible for sporadic human cases. On the basis of the sequences reported previously, genotype 3 probably originated in European pigs, which were later exported around most of the world (Lu et al. 2006). Genotype 3 HEV strains have been isolated in Taiwan and were considered to be imported from the United States through pig trading (Wu et al. 2002). Recent studies report that this genotype is present exclusively in pig farms scattered in the suburbs of Shanghai, China (Ning et al. 2007, 2008). However, no genotype 3 strain was identified in the present study.

HEV antibody was detected in goat and cattle samples collected from various provinces, the prevalence and titers in both being significantly lower than in pigs in this study. The average HEV prevalence in goats and cattle were 10.4% (73/700) and 28.2% (257/912), respectively. However, anti-HEV positivity rates in some populations of goats and cattle were significantly higher than the average. Twelve of 25 (48%) goat serum samples from Shanxi and 13 of 14 (92.8%) cattle serum samples from Guangdong were positive for anti-HEV antibody. Although no HEV RNA was recovered from the goat and cattle using RT-PCR, some serum samples were HEV antigen positive. These results indicate that cattle and goats may also be infected by HEV and are likely to be potential reservoirs of HEV, as are pigs. Because cattle and goats are popular domestic animals of China and most peasants raise them in the backyard, they have more opportunity to come into contact with people than do pigs. Hence, we cannot neglect their potential roles in the transmission of HEV to humans. A possible reason for our inability to amplify HEV nucleic acid from goats and cattle is that the genomic sequences of HEV in these animals are quite likely divergent from those in pigs and humans, and thus the PCR primers based on swine and human HEV genomes do not bind the goat or cattle HEV RNA efficiently. As noted, none of the antigen-positive goat or cattle samples was positive for HEV RNA. Besides the possibility that the animals were infected with divergent genotypes of HEV, the possibility of false positivity in the antigen tests should be addressed. If this is the case, the antigen test should be improved further.

In conclusion, our study showed that HEV infection is common among pigs in China, genotype 4 HEV being the most common and highly diverse. Goats and cattle also may be infected with HEV, albeit the prevalence being significantly lower than in pigs and no viral RNA detected in them. One possible reason for our inability to detect HEV RNA in cattle and goats is that HEV genotypes from different animal species may have divergent sequences.

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Disclosure Statement

The authors have no potential conflicts of interest.

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Address correspondence to:

Youchun Wang

Department of Cellular Biology

National Institute for the Control of Pharmaceutical

and Biological Products

No 2 Tiantianxili

Beijing 100050

China

E-mail: wychun3@yahoo.com; wangyc@nicpbp.org.cn

Kegong Tian

Veterinary Diagnostic Lab

China Animal Disease Control Center

No 2 Yuanmingyuan West Road

Haidian District

Beijing 100193

China

E-mail: tiankg@263.net

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 2010. 11. 16	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	人血清アルブミン	研究報告の公表状況	Takeda H, Matsubayashi K, Sakata H, Sato S, Kato T, Hino S, Tadokoro K, Ikeda H. Vox Sang. 2010 Nov;99(4):307-13. doi: 10.1111/j.1423-0410.2010.01362.x.	公表国 日本	
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン5%静注12.5g/250mL(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)				
研究報告の概要	○日本の献血適格者におけるE型肝炎ウイルス抗体(HEV IgG)陽性率に関する全国調査 目的: 日本各地域の献血適格者について、HEV IgGの陽性率に関する全国調査を行った。 方法: 日本のほぼ全域を代表する7つの血液センターにおける献血適格者(現行の血液スクリーニング試験: HBV、HCV、HIV-1/2、HTLV-1、梅毒、ヒトパルボウイルスB19 が全て陰性の献血者)からの検体を1,800検体ずつ、計12,600検体採取した。検体は、日本赤十字社内部のHEV IgG ELISAを使用してスクリーニングし、陽性検体は市販のHEV IgG ELISAによって確認検査した。 結果: 12,600検体のうち、431検体(3.4%)がHEV IgG陽性であった。HEV IgGの陽性率は西日本(1.8%)より東日本(5.6%)で高く(P<0.001)、年齢依存的であり、女性(2.9%)より男性(3.9%)で高かった(P=0.002)。 結論: 日本の献血適格者において、HEV感染の国内での広がりが見られた。HEV IgGは、男性、高齢者及び東日本居住者でより高い陽性率を示した。日本におけるHEVの輸血感染に関する潜在的リスクを明らかにするため、更なる研究が必要とされる。				使用上の注意記載状況・ その他参考事項等 赤十字アルブミン20 赤十字アルブミン25 赤十字アルブミン5%静注 12.5g/250mL 赤十字アルブミン20%静注 4g/20mL 赤十字アルブミン20%静注 10g/50mL 赤十字アルブミン25%静注 12.5g/20mL 血液を原料とすることによる 感染症伝播等
報告企業の意見		今後の対応			
日本各地域の献血適格者からの12,600検体について調査したところ、HEV IgGの陽性率は3.4%で、男性、高齢者、東日本居住者で高かったとの報告である。 HEVは脂質膜のないRNAウイルスである。本剤の製造工程にはコーン分画及び液状加熱の2つのウイルス除去・不活化工程が含まれている。最近ある遺伝子型のHEVは耐熱性であるとの成績が発表され、液状加熱の有効性に一部疑念を生じている。しかし、血漿分画製剤で最も長い歴史を持つアルブミンにはHEVの侵淫度が遥かに高い過去から現在に至るまで世界的にHEV感染例がないとの疫学的事実があることから、本剤の安全性は確保されていると考える。		日本赤十字社では、輸血による肝炎ウイルス感染防止のため、血液中のALT高値の献血血液を排除している。また、厚生労働科学研究「経口感染する肝炎ウイルス(A型、E型)の感染防止、遺伝的多様性、および治療に関する研究」班と共同して、献血者におけるHEV感染の疫学調査を行っている。今後もHEV感染の実態に関する情報の収集及び安全対策に努める。			

A nationwide survey for prevalence of hepatitis E virus antibody in qualified blood donors in Japan

H. Takeda,¹ K. Matsubayashi,¹ H. Sakata,¹ S. Sato,¹ T. Kato,¹ S. Hino,² K. Tadokoro² & H. Ikeda¹

¹Japanese Red Cross Hokkaido Blood Center, Sapporo, Japan

²Blood Service Headquarters, Japanese Red Cross Society, Tokyo, Japan

Vox Sanguinis

Background and Objectives In previous studies, we reported the transmission of hepatitis E virus (HEV) by transfusion, and the frequent detection of HEV markers in Japanese blood donors with elevated ALT levels. For the current study, we carried out a nationwide survey of the prevalence of IgG anti-HEV in qualified blood donors throughout Japan.

Materials and Methods The 12 600 samples from qualified blood donors were collected from seven blood centres (1800 per centre) representing nearly all regions of Japan. Samples were from age- and sex-matched blood donors who tested negative for all the current blood screening tests. The samples were screened using the in-house IgG anti-HEV ELISA. Sequentially, the positive samples were tested by the commercial IgG anti-HEV ELISA.

Results Of 12 600 samples, 431 (3.4%) were regarded as positive for IgG anti-HEV. The prevalence of IgG anti-HEV was higher in eastern Japan (5.6%) than in western Japan (1.8%) ($P < 0.001$), and was also age-dependent and higher in men (3.9%) than in women (2.9%) ($P = 0.002$).

Conclusion The spread of the domestic infection of HEV was observed in qualified blood donors in Japan. A higher prevalence of IgG anti-HEV was observed in male donors, older donors and in donors residing in eastern Japan. Further studies are necessary to clarify the potential risk of transfusion-transmission of HEV in Japan.

Key words: blood donors, hepatitis E, HEV antibody, screening.

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Introduction

Hepatitis E virus (HEV) is one of the major causative agents of acute hepatitis in many developing countries, such as in South-East Asia, Africa, Central and South America and in the Middle East [1]. HEV infection in endemic countries is reported to occur mainly via the faecal-oral route of transmission through contaminated water supplies. HEV-associated hepatitis also occurs among industrialized countries including Japan, where hepatitis E had previously been believed to be non-endemic [2–8]. Of 4 HEV genotypes, genotype 3 and genotype 4 are responsible for indigenous

hepatitis E in industrialized countries, while genotype 1 is dominant in the endemic countries. Recent studies have documented that wild boars, wild deer or pigs may act as reservoirs for HEV infection in Japan [9–13], where the major route of infection is likely to be food-borne zoonosis through HEV-infected animal meat. A potential risk of blood-borne transmission exists, because HEV-infected individuals displayed long-term viremia [14–16]. Abe *et al.* [4] found that transmission routes remained obscure in most cases (approximately 60%), whereas about 30%, 8% and 2% were attributed to zoonotic food-borne transmission, imported infection and via blood transfusion, respectively. We previously reported two cases of transfusion-transmitted hepatitis E in Hokkaido, Japan [14,16]. HEV RNA and/or anti-HEV were detected in some Japanese blood donors with elevated ALT levels [14,17–19].

Correspondence: Hiromi Takeda, Japanese Red Cross Hokkaido Blood Center, 2-2 Yamamoto, Nishi-ku, Sapporo 063-0002, Japan
 E-mail: niwa@hokkaido.bc.jrc.or.jp

We reported the nationwide survey for HEV prevalence among Japanese blood donors with elevated ALT levels, where HEV markers (HEV RNA and anti-HEV) were frequently detected in such donor samples collected throughout Japan [19]. However, the real situation of HEV prevalence in blood donors was unknown in the previous report because donor samples with elevated ALT levels were unevenly distributed in men, and the sample size of age groups of 10s, 50s and 60s was small. Consequently, it is important to study HEV prevalence in the typical donor population in Japan. A few studies have been reported on the prevalence of IgG anti-HEV in Japanese healthy individuals in certain areas of Japan [17,20]. The results can be summarized as follows: seroprevalence is (1) highly variable (1.9–14.1%), (2) higher in men and (3) shows age-dependency. In those reports, samples were collected in limited areas and/or the sample sizes were rather small. There has been no report that tried to compare the prevalence of HEV antibodies in age- and sex-matched healthy population at the same period all over Japan so far. Accordingly, further systemic study with a larger number of those individuals from more different parts of Japan will be necessary to draw a definitive conclusion.

To verify the real situation of HEV prevalence in healthy blood donors, we carried out a nationwide survey for the prevalence of IgG anti-HEV in qualified blood donors who were collected separately by age group and sex. IgG anti-HEV are reported to remain long after the disappearance of the virus in the infected individuals and can serve as the marker of the HEV prevalence [14,16]. In this study, we collected 12 600 donor samples from seven Japanese Red Cross (JRC) Blood Centers representing most regions of Japan and tested for the presence of IgG anti-HEV.

Materials and methods

HEV specimens

For the preliminary study, we used serum samples of HEV patients ($n = 55$) that had been obtained for our investigations including trial HEV nucleic acid testing for transfusion in Hokkaido [19] and qualified random blood donor samples ($n = 16$) that were collected at JRC Hokkaido Blood Center in 2005. These samples were tested with the commercial IgG anti-HEV ELISA (Cosmic corporation, Ltd., Tokyo, Japan) and the in-house IgG anti-HEV ELISA. The tests were performed as described later. The samples were kept frozen below -20°C until testing.

Qualified blood donor samples

In JRC Blood Centers, donated blood is screened for infectious diseases (HBV, HCV, HIV-1/2, human

T-lymphotropic virus type 1, syphilis, human parvovirus B19). In addition, ALT level has been used as a surrogate test for hepatitis caused by viruses other than HBV or HCV. A total of 12 600 serum samples were collected from qualified Japanese blood donors who had an ALT level of 60 IU/l or lower and tested negative for all the current blood screening tests in the seven blood centres at Hokkaido, Miyagi, Tokyo, Aichi, Osaka, Okayama and Fukuoka from November 2005 through February 2006. Donors were categorized into six age groups: 16–19 (10s), 20–29 (20s), 30–39 (30s), 40–49 (40s), 50–59 (50s) and 60–69 (60s). The samples at each blood centre were collected separately by age group and by sex, resulting in 150 male and 150 female samples in each age group (a total of 1800 samples).

ELISA for detecting IgG anti-HEV

In the absence of specific confirmatory assay that detects IgG anti-HEV, the samples were screened by the in-house IgG anti-HEV ELISA and the positive samples were tested by the commercial IgG anti-HEV ELISA (Fig. 1). An in-house ELISA was developed according to the methods of Li *et al.* [20], with some modifications [19]. In the in-house assay, virus-like particles (VLPs) consisting of recombinant ORF2 proteins of HEV genotype 1 expressed in cultured recombinant baculovirus-infected insect cells were used as antigens [21]. Wells of microplates (Number 2592, 96-well Stripwell, flat bottom; Corning Life Sciences, Corning, NY) were coated with 50 μl of the recombinant ORF2 protein [3 $\mu\text{g}/\text{ml}$ in phosphate-buffered saline (PBS)], and the plates were incubated at room temperature (RT) for 2 h followed by incubation with 100 μl of blocking buffer containing 40% (v/v) calf serum (Gibco-BRL, Tokyo, Japan) at RT for 1 h. To test for IgG anti-HEV, 50 μl of each sample

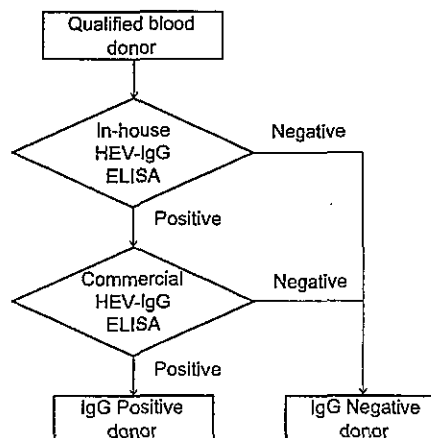


Fig. 1 The algorithm of IgG anti-hepatitis E virus test.

was added to each well at a dilution of 1:100 in saline containing 40% calf serum. The microplates were incubated at RT for 1 h and then washed five times with 0.05% Tween 20 in PBS (PBS-T). Fifty microlitre of horseradish peroxidase-conjugated mouse anti-human IgG (IGB22; Institute of Immunology Co., Ltd., Tokyo, Japan; 1:2000) in PBS-T containing 25% (v/v) foetal calf serum (PAA Laboratories GmbH, Pasching, Austria) was added to each well and incubated at RT for 1 h. The mouse anti-human IgG used in the in-house ELISA was not crossreactive to human IgM at the conditions in the test. The wells were washed five times with PBS-T. Fifty microlitre of tetramethylbenzidine-soluble reagent (Dako Co., Ltd., Carpinteria, CA) as a substrate was added to each well. The plate was incubated at RT for 10 min in the dark, and then 50 µl of 1 N sulphuric acid (Kanto Chemical Co., Inc., Tokyo, Japan) as tetramethylbenzidine stop buffer was added to each well. The optical density (OD) of each sample was read at 450 nm. Test samples with OD values equal to or greater than the cut-off value were considered positive for the presence of IgG anti-HEV in this ELISA. The cut-off value for the in-house ELISA was set tentatively at 7 SDs above the mean value of 81 HEV RNA-negative sera for IgG anti-HEV. The reactive samples were subsequently tested with a commercial IgG anti-HEV ELISA (Cosmic corporation, Ltd., Tokyo, Japan), which was developed according to the methods of Mizuo *et al.* [3] and utilizes VLPs consisting of recombinant ORF2 proteins of HEV genotype 4 expressed in recombinant baculovirus-infected silkworm pupae. The commercial ELISA was performed according to the manufacturer's protocol. Samples were determined to be positive, if they were reactive by both ELISA methods.

Statistical analysis

Statistical analysis was performed by the chi-square test. A difference with a *P*-value of < 0.05 was considered significant.

Results

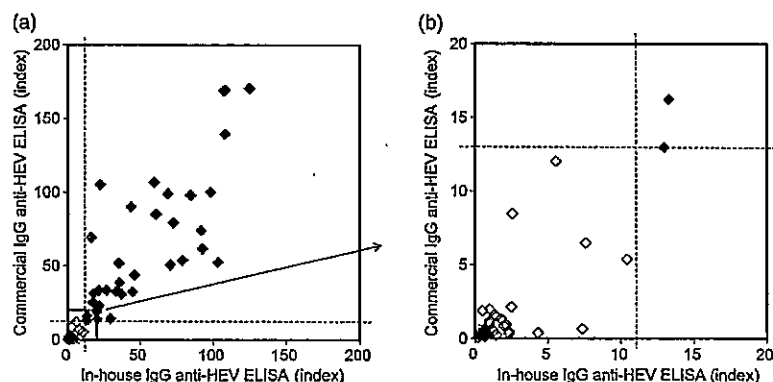
The Comparison of the in-house IgG anti-HEV ELISA and the commercial IgG anti-HEV ELISA among the HEV specimens

In the preliminary study, the 71 HEV specimens were tested with the in-house and the commercial IgG anti-HEV ELISA (Fig. 2). Of the 55 sera from the HEV patients, 34 (62%) were reactive in the commercial assay and all of which were also reactive in the in-house assay. Twenty-one were from the patients with HEV RNA and/or IgM anti-HEV in very early stage of HEV infection. Of the 21 sera, 20 sera were not reactive with both ELISAs and one sera was borderline positive (index was 12.9 and cut-off index was 11) in the in-house assay and borderline negative (index was 12.9 and cut-off index was 13) in the commercial assay. In addition, 16 samples were not reactive for IgG anti-HEV by both ELISAs among 16 qualified random blood donor samples. The result of preliminary study with the HEV specimens indicated that the in-house IgG anti-HEV ELISA and the commercial IgG anti-HEV ELISA was 99% concordant and could discriminate IgG anti-HEV-positive samples from negative samples. Overall, the index of the in-house IgG anti-HEV ELISA appeared to be lower than that of the commercial IgG anti-HEV ELISA.

IgG anti-HEV in qualified Japanese blood donors

Of the 12 600 donor samples, 431 (3.4%) showed the presence of IgG anti-HEV (Figs 3–5). The positivity rate of IgG anti-HEV among each age group: 10s, 20s, 30s, 40s, 50s and 60s was 0.5% (10/2100), 1.0% (21/2100), 2.4% (50/2100), 4.3% (90/2100), 6.0% (126/2100) and 6.4% (134/2100), respectively (Fig. 3). The prevalence of IgG anti-HEV showed clear age-dependence and increased with age in both sexes. The peak of the positivity rate was in 60s age group in men (92/1050 = 8.8%) and in 50s age group

Fig. 2 Correlation of the two ELISAs for IgG anti-hepatitis E virus among the hepatitis E virus specimens ($n = 71$). The closed and open diamonds represent the hepatitis E virus patients samples ($n = 55$) and qualified random blood donor samples ($n = 16$), respectively. Horizontal and vertical broken lines represent cut-off index of the in-house ELISA for IgG anti-hepatitis E virus (cut-off index = 11) and the commercial kit for IgG anti-hepatitis E virus (cut-off index = 13), respectively. The box area in the (A) was magnified as shown in the (B).



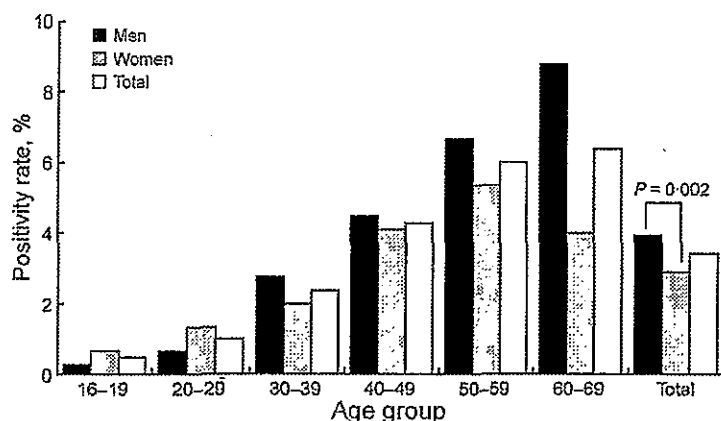


Fig. 3 IgG anti-hepatitis E virus prevalence by sex and age group in qualified blood donors. Each age group consisted of 1050 men (black bars) and 1050 women (grey bars).

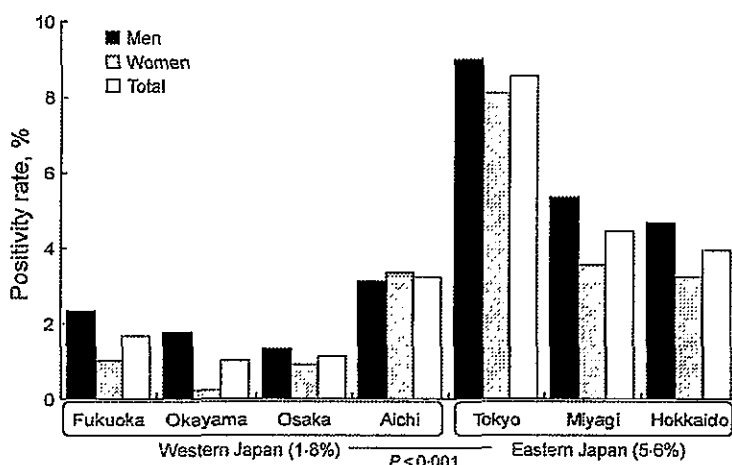


Fig. 4 IgG anti-hepatitis E virus prevalence by blood centre in qualified blood donors. Samples from each blood centre consisted of 900 men (black bars) and 900 women (grey bars).

in women (56/1050 = 5.3%), respectively. Overall, a significant difference in the positivity rate was found between male and female samples (248/6300 = 3.9% vs. 183/6300 = 2.9%; $P = 0.002$). In 60s age group, the positivity rate among men was significantly higher than women (92/1050 = 8.8% vs. 42/1050 = 4.0%; $P < 0.001$).

The positivity rate of IgG anti-HEV in Hokkaido, Miyagi, Tokyo, Aichi, Osaka, Okayama and Fukuoka was 3.9% (71/1800), 4.4% (80/1800), 8.6% (154/1800), 3.2% (58/1800), 1.1% (20/1800), 1.0% (18/1800) and 1.7% (30/1800), respectively (Fig. 4). There was a significant difference in the positivity rate between eastern Japan (Hokkaido, Miyagi and Tokyo) and western Japan (Aichi, Osaka, Okayama and Fukuoka) (305/5400 = 5.6% vs. 126/7200 = 1.8%; $P < 0.001$). Moreover, in most areas except Aichi, the positivity rate among men was higher than among women.

Figure 5 shows the geographic prevalence of IgG anti-HEV classified by sex and age in the seven regions. The age-dependence was observed more or less in each region, and it was most clear in Tokyo. In Hokkaido, the positivity

rates among men in 30s–60s age groups were very similar (> 5%) and the age-dependence was less clear.

Discussion

Here, we have shown a nationwide survey for IgG anti-HEV among qualified Japanese blood donors in seven districts representing most regions of Japan for the first time. The 12 600 donors tested for HEV seroprevalence were qualified donors who were sex- and age-matched, and can be regarded as healthy individuals representing a well-balanced population living in various regions of the country. The HEV seroprevalence in Japanese blood donors have been reported [17,20]. However, in those reports, the samples were collected in some limited areas and/or sample sizes were small, which may not represent nationwide HEV seroprevalence.

In this study, the samples were screened first by the in-house IgG anti-HEV ELISA and the positive samples were tested by the commercial kit because it was not realistic to screen 12 600 samples with the expensive commercial kit.

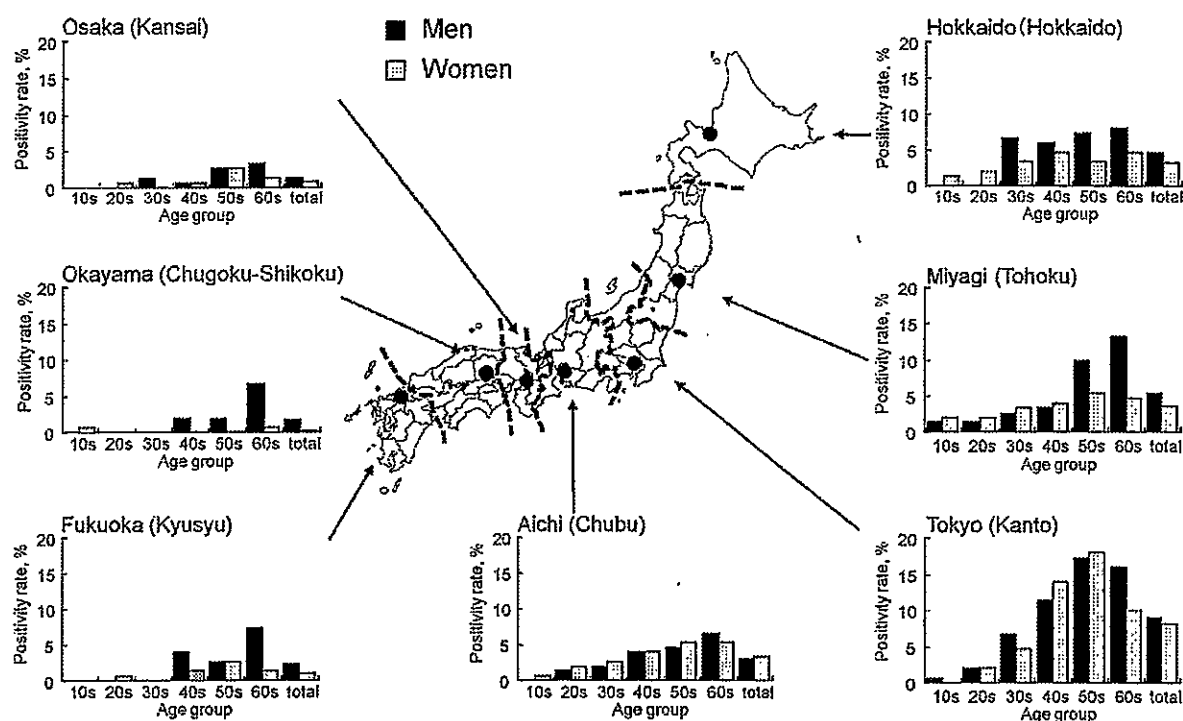


Fig. 5 Geographical prevalence of IgG anti-hepatitis E virus by sex and age group in the seven blood centres. The map of Japan shows the locations of seven geographic regions (every region has been divided in heavy broken line). JRC Blood Centers belong to eastern Japan (three regions: Hokkaido, Tohoku and Kanto) or western Japan (four regions: Chubu, Kansai, Chugoku-Shikoku and Kyusyu) from north to south. The closed circle shows the place of the main JRC Blood Center. Regional names of each JRC Blood Center are written down in parentheses. Samples in each blood centre consisted of 900 men (black bars) and 900 women (grey bars).

In the preliminary study, the HEV specimens were tested with the commercial kit as well as the in-house IgG anti-HEV ELISA. Although the sensitivity of the two assays appeared comparable, we are not certain whether there may be false-negative samples for the in-house assay. The overall sensitivity of the algorithm is dependent on the in-house assay as samples reactive on the in-house assay are tested on the commercial kit. Thus, the study might underestimate the seroprevalence.

IgG anti-HEV appears to be present for a more prolonged period after the disappearance of HEV in blood [14,16]. Of the 12 600 qualified blood donors, 431 (3.4%) samples were positive for IgG anti-HEV. The positivity rate of IgG anti-HEV among male donors was significantly higher than that among female donors (3.9% vs. 2.9%; $P = 0.002$). Fukuda *et al.* [17] reported the prevalence of IgG anti-HEV among blood donors with normal ALT (ALT level of 60 IU/l or lower) at the two JRC Blood Centers, where the prevalence of IgG anti-HEV was higher among male donors than female donors. The prevalence of hepatitis E in Japan was 3.5 times higher among men than women [4], which may be relevant to the higher prevalence in IgG anti-HEV among male donors. The reasons for the higher prevalence

in men are not very clear, although the dietary pattern of the Japanese male may be associated with the higher HEV seroprevalence.

In our results, there was a significant difference in the positivity rate of IgG anti-HEV between eastern Japan (Hokkaido, Miyagi and Tokyo) and western Japan (Aichi, Osaka, Okayama and Fukuoka) (5.6% vs. 1.8%, $P < 0.001$). Li *et al.* [20] reported that the positivity rate of IgG anti-HEV in healthy individuals was 1.9% in the southern (western) prefecture, 3.3% in the northern (eastern) prefecture and 14.1% in the central (eastern) prefecture, respectively, although the sample size was rather small in each age group. Also, Fukuda *et al.* [17] showed that the detection rate of IgG anti-HEV at one blood centre, 5.5% (located near Tokyo) was significantly higher than that at another blood centre, 2.1% (located near Fukuoka). The results were in agreement with our present report. We previously reported that among donors with ALT levels at or > 200 IU/l, the positivity rate of IgG anti-HEV in western Japan and eastern Japan was 2.1% and 5.0%, respectively [19]. Although the donor samples with elevated ALT levels were unbalanced in age and sex, the results corresponded to our present results using the large number of non-biased

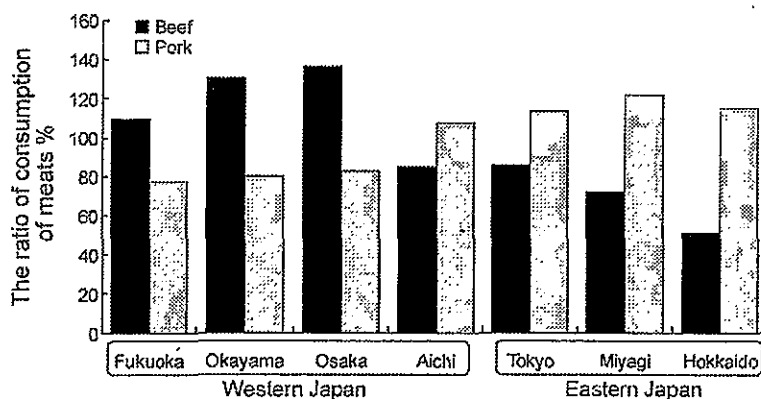


Fig. 6 Regional trend of consumption of beef and pork in Japan. Data was Annual Report of Family Income and Expenditure Survey in 2002 through 2005 Statistic Bureau, Ministry of Internal Affairs and Communications, Japan. The ratio of consumption of meats was the ratio to the national average of consumption of meats in weight.

samples. Depending on the region, the positivity rate varied from 1.0% in Okayama to 8.6% in Tokyo in this study. The difference in the dietary pattern of consumption of meat between eastern Japan and western Japan may be relevant for the higher seroprevalence in eastern Japan; people in eastern Japan are more likely to consume pork than beef (Fig. 6). A number of reports have pointed out that pigs are reservoirs for HEV [13,16,22–25]. IgG anti-HEV were detected in 90% of pigs between 5 and 6 months of age at commercial swine farms in Japan [23]. HEV RNA was detected in packages of pork purchased from local grocery stores in Japan and in the United States [13,24]. In addition, Abe *et al.* [4] described that the zoonotic food-borne route appeared to be the most common cause of HEV infection in Japan, 70% of which have a connection with consumption of pork. Another example is reported in Indonesia where differences in the gastronomic culture have induced a difference in the positivity rate of IgG anti-HEV. Among pregnant women in Bali, anti-HEV was significantly less frequent in Muslims, who were strictly prohibited from eating or touching pigs, than Hindus, who have no such restrictions [25]. Also in industrialized countries including Japan, the widespread distribution of HEV infection among humans and animals has been reported [2,5–8].

Overall, similar to the results found in other studies, the prevalence of IgG anti-HEV increased with age; however, the degree of the age-dependency was varied by region. The clear age-dependent increase of IgG anti-HEV observed in Tokyo may represent a past prevalence of HEV infection. On the other hand, the trend in Hokkaido was less clear, which may indicate that there may be a more recent or an on-going spread of HEV infection. Recently, hepatitis E has been frequently reported in Hokkaido [4].

In conclusion, the spread of the domestic infection of HEV was observed widely in qualified blood donors in Japan and older donors, male donors and donors living in eastern Japan were associated with a higher prevalence of IgG anti-HEV. Further studies are necessary to clarify the

infectious routes and the potential risk of transfusion-transmission of HEV in Japan.

Acknowledgements

We are grateful to Dr T.C. Li and Dr N. Takeda (National Institute of Infectious Diseases) for donation of the recombinant HEV virus-like particles used as antigen for ELISA testing and to JRC Blood Centers for collecting the blood donor samples. We are also grateful to Joann Sustache and John Salituro for their help in preparing the manuscript. This study was supported in part by grants from the Ministry of Health, Labor and Welfare of Japan.

Conflict of interest

The authors declare no conflict of interest.

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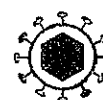
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医薬品
医薬部外品 研究報告 調査報告書
化粧品

識別番号・報告回数		報告日		第一報入手日 2011 年 1 月 24 日	新医薬品等の区分	厚生労働省処理欄
一般的名称	①②③④⑤ポリエチレングリコール処理人免疫グロブリン ⑥⑦⑧人免疫グロブリン			研究報告の公表状況	公表国 イタリア	
販売名 (企業名)	①献血グェノグロブリン IH5% 静注 0.5g/10mL (ベネシス) ②献血グェノグロブリン IH5% 静注 1g/20mL (ベネシス) ③献血グェノグロブリン IH5% 静注 2.5g/50mL (ベネシス) ④献血グェノグロブリン IH5% 静注 5g/100mL (ベネシス) ⑤献血グェノグロブリン-IH ヨシトミ (ベネシス) ⑥グロブリン筋注 450mg/3mL「ベネシス」 (ベネシス) ⑦グロブリン筋注 1500mg/10mL「ベネシス」 (ベネシス) ⑧グロブリン-Wf (ベネシス)					
研究報告の概要	背景：PARV4 は既知のヒトパルボウイルスの何れにも関連しないパルボウイルス属科の新種である。ウイルス血症は PARV4 感染症の特徴を示し、ウイルス DNA の持続は幾つかの組織で実証された。現在まで、PARV4 はいかなる疾病にも関連せず、ヒトでの保有率も明確になっていなかった。 本研究は、PARV4 が持つ組織分布と持続感染する能力をパルボウイルス B19 (B19V) と比較し、評価した。 結果：リアルタイム PCR と nested PCR により、PARV4 と B19V の DNA 検出は、ORF2 と ORF1 を対象に、急性ウイルス感染の疑いのない人の様々な組織で実施された。 PARV4 DNA の低い量が成人の心臓と肝臓で 40%、肺と腎臓(23.5%、18%)、骨髄と皮膚と滑膜検体中(5.5%、4%、5%)で見つかった。 B19V DNA 配列は肝臓よりも高頻度で同じ組織に存在し、血漿中では B19V の頻度は PARV4 よりもかなり低かった。 結論：肝臓と心臓への PARV4 の特徴的な親和性は、ウイルス複製の標的の可能性や、肝疾患や心疾患における PARV4 感染の役割の可能性についての研究に焦点を当てることを示唆している。					使用上の注意記載状況・ その他参考事項等
						代表として献血グェノグロブリン IH5% 静注 0.5g/10mL の記載を示す。 1. 慎重投与（次の患者には慎重に投与すること） (5) 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。〕 (6) 免疫不全患者・免疫抑制状態の患者〔ヒトパルボウイルス B19 の感染を起こす可能性を否定できない。感染した場合には、持続性の貧血を起こすことがある。〕 2. 重要な基本的注意 (1) 略 1) 血漿分画製剤の現在の製造工程では、ヒトパルボウイルス B19 等のウイルスを完全に不活化・除去することが困難であるため、本剤の投与によりその感染の可能性を否定できないので、投与後の経過を十分に観察すること。 6. 妊婦、産婦、授乳婦等への投与 妊婦又は妊娠している可能性のある婦人には、治療上の有益性が危険性を上回ると判断される場合のみ投与すること。〔妊娠中の投与に関する安全性は確立していない。本剤の

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報告企業の意見	今後の対応	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。）
パルボウイルス 4（PARV4）は、パルボウイルス科パルボウイルス亜科のどの属にも分類されないウイルスである。また、PARV4が発見されたのは2005年であり、その病原性は現時点では明らかではない。万一、PARV4が原料血漿に混入したとしても、CPVをモデルウイルスとしたウイルスバリデーション試験成績から、本剤の製造工程において十分に不活化・除去されていると考えられる。	PARV4 及び PARV5 に関する追加情報の入手に努める。	



RESEARCH

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Human parvovirus PARV4 DNA in tissues from adult individuals: a comparison with human parvovirus B19 (B19V)

Fabiana Corcioli¹, Krystyna Zakrzewska¹, Rosa Fanci², Vincenzo De Giorgi³, Massimo Innocenti⁴, Matteo Rotellini⁵, Simonetta Di Lollo⁵, Alberta Azzi^{1*}

Abstract

Background: PARV4 is a new member of the Parvoviridae family not closely related to any of the known human parvoviruses. Viremia seems to be a hallmark of PARV4 infection and viral DNA persistence has been demonstrated in a few tissues. Till now, PARV4 has not been associated with any disease and its prevalence in human population has not been clearly established. This study was aimed to assess the tissue distribution and the ability to persist of PARV4 in comparison to parvovirus B19 (B19V).

Results: PARV4 and B19V DNA detection was carried out in various tissues of individuals without suspect of acute viral infection, by a real time PCR and a nested PCR, targeting the ORF2 and the ORF1 respectively. Low amount of PARV4 DNA was found frequently (>40%) in heart and liver of adults individuals, less frequently in lungs and kidneys (23,5 and 18% respectively) and was rare in bone marrow, skin and synovium samples (5,5%, 4% and 5%, respectively). By comparison, B19V DNA sequences were present in the same tissues with a higher frequency (significantly higher in myocardium, skin and bone marrow) except than in liver where the frequency was the same of PARV4 DNA and in plasma samples where B19V frequency was significantly lower than that of PARV4.

Conclusions: The particular tropism of PARV4 for liver and heart, here emerged, suggests to focus further studies on these tissues as possible target for viral replication and on the possible role of PARV4 infection in liver and heart diseases. Neither bone marrow nor kidney seem to be a common target of viral replication.

Background

PARV4 is a member of the Parvoviridae family discovered in 2005 in plasma from an intravenous drug user, with symptoms consistent with acute HIV infection, but confirmed to be HIV RNA negative [1]. It seemed to be not closely related to any of the known human or animal parvoviruses until 2008 when novel porcine and bovine parvoviruses highly similar to PARV4 were identified so that it was proposed the inclusion of PARV4 in a new genus Hokovirus [2]. Three genotypes of PARV4 have been identified at present [3,4]. Till now, it has not been associated with any disease and its spread in the human population has not been clearly assessed. In fact, the serological assays for anti-PARV4 antibody

detection, only recently developed, till now have been applied on small groups of individuals only [5]. Viremia seems to be a hallmark of PARV4 infection. PARV4 was found in 5% of plasma pools at levels suggesting the existence of donations with high viral load [6], in 16% of clotting factor VIII concentrates [7], in 2% of plasma samples from healthy donors [8] and in 7,95% of serum samples from intravenous drug users in Thailand [9]. Several data indicate that the transmission of the virus predominantly occurs by parenteral route [5,10] whereas the respiratory transmission seems to be unlikely [11]. As regards tissue distribution, PARV4 DNA was demonstrated frequently in bone marrow and lymphoid tissue from HIV infected individuals [12] and also in liver of non HIV-infected adults [13]. One study reports that PARV4 DNA can be detected, with a low frequency, in healthy and pathological skin samples [14].

* Correspondence: alberta.azzi@unifi.it

¹Department of Public Health, University of Firenze, Viale Morgagni 48, Firenze, Italy

Full list of author information is available at the end of the article



It is known that B19V frequently persist in solid tissues in asymptomatic individuals and, less frequently, also in bone marrow [15].

The aim of this study was to assess whether PARV4 is able to persist in different tissues, like B19V, or if it shows a particular tissue tropism.

Results

With the aim to assess the tissue distribution and the ability to persist of PARV4 in comparison to B19V, we searched for DNA sequences of both parvoviruses in various tissues of individuals without suspect of acute viral infection. Table 1 shows the results of PARV4 DNA detection in different tissues, in comparison with B19 DNA detection. The frequency of PARV4 DNA finding was low in skin, synovium and bone marrow (4-5,5%) whereas in myocardium samples PARV4 sequences were shown in 17 out of 35 (49%), a frequency significantly higher than in the other tissues examined but significantly lower than that of B19V. PARV4 DNA was found in 8 out of 39 plasma specimens (20,5%) while B19V DNA was demonstrated in one of these specimens only (2,5%). It is noteworthy that these 39 specimens were from 21 immunodepressed hematological patients and 18 immunocompetent individuals and the frequency of viremia was 33% (7 positives out of 21) in the former group and 5,5% (1 positive out of 18) in the second group (however, this difference is not statistically significant). The viral load in these sera was low: below $3,8 \times 10^3$ copies/ml.

From 17 out the 35 individuals dead for various causes, without known viral myocarditis or dilated cardiomyopathy, lungs, liver, kidney and blood specimens could be examined, in addition to the myocardium, with the aim to verify the distribution of PARV4 and B19V DNA in the different organs of the same individual. In none of these cases viral DNA was detectable in the blood while PARV4 as well as B19V DNA were found frequently in the different organs analyzed (Table 2). PARV4 DNA was present more frequently in the liver

Table 2 Detection of PARV4 and B19V DNA in different tissues from 17 individuals without known viral myocarditis or dilated cardiomyopathy

Patient	myocardium		lung		liver		kidney	
	PARV4	B19V	PARV4	B19V	PARV4	B19V	PARV4	B19V
1	-	+	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-
3	+	+	-	+	-	-	-	+
4	-	+	-	-	-	-	-	-
5	-	+	-	+	-	-	-	+
6	+	+	-	+	-	-	+	-
7	+	+	+	+	+	+	-	+
8	+	+	-	-	+	-	-	-
9	-	-	-	-	+	-	+	-
10	+	+	+	+	+	+	-	+
11	-	+	+	+	+	+	-	+
12	+	+	-	+	+	+	-	+
13	-	+	-	-	-	-	-	-
14	-	+	-	-	-	+	-	-
15	+	-	+	-	+	-	+	-
16	-	+	-	-	-	+	-	+
17	-	+	-	-	-	+	-	+
Tot*	7 (41)	14 (82)	4 (23)	7 (41)	7 (41)	7 (41)	3 (18)	8 (47)

* number of positives (%)

and in the myocardium (41%) than in lungs and kidneys (23,5% and 18% respectively). The frequency of PARV4 DNA in the liver was similar to that of B19V DNA in this tissue and lower in the others. In one individual PARV4 DNA and in 4 individuals B19V DNA were demonstrated in myocardium samples only. In one individual PARV4 DNA and in 4 individuals B19V DNA were present in all the tissues examined. In two cases PARV4 DNA sequences were not demonstrable in the myocardium and were detectable in other tissues, whereas B19V infected individuals had always DNA sequences in the myocardium.

The concentration of PARV4 DNA in all the positive tissues was generally low, below or near the level of quantization by the real time PCR here employed, corresponding to ≤ 380 copies/ 10^6 cells.

As previously reported, the copy number of B19V DNA varied from below the level of quantization by the real time PCR to 7×10^4 copies per 10^6 cells, except for two myocardium samples where about 10^6 copies per 10^6 cells were found (15).

PARV4 sequence analysis, aimed to establish the genotype of PARV4 present in the myocardium samples, could be performed on 10 isolates only, out of 17 positive samples, because of the low level of viral DNA. All were included in the genotype 2 (PARV5) after comparison with consensus sequences of the three genotypes

Table 1 Detection of PARV4 DNA in tissues and plasma in comparison with B19 DNA in the same specimens*

Samples	N°	PARV4 DNA positive (%)	B19V DNA positive (%)
Bone marrow ^a	26	2 (5,5)	9 (25)
Skin ^a	25	1 (4)	18 (72)
Synovium	21	1 (5)	6 (28,5)
Myocardium ^a	35	17 (49)	26 (74)
Plasma ^a	39	8 (20,5)	1 (2,5)

*part of the results of B19 DNA detection have been previously reported (12)

*difference between percentages of PARV4 and B19V DNA statistically significant

(GenBank accession numbers HQ245657-HQ245666). As regards B19V, either genotype 1 or genotype 2 were present, with a slightly higher frequency of the 2.

PARV4 DNA was undetectable in the urine specimens of the bone marrow transplantation patients.

Discussion and Conclusions

In this study low amount of PARV4 DNA was found frequently (>40%) in heart and liver of adults individuals, less frequently in lungs and kidneys (23,5 and 18% respectively) and was rare in bone marrow, skin and synovium samples (5,5%, 4% and 5%, respectively). By comparison, B19V DNA was present in the same tissues with a higher frequency (significantly higher in myocardium, skin and bone marrow) except than in liver where its frequency was the same of PARV4 DNA and in plasma samples where B19V frequency was significantly lower than that of PARV4. Some of these results are fairly in agreement with the literature where the frequency of PARV4 DNA in bone marrow varies from 0% (0/8) in HIV uninfected individuals (like the patients enrolled in this study), to 46% and 65% in HIV infected patients [12,16] and is 7,7% in the skin of HIV-negative individuals [14]. The frequency of PARV4 DNA in the liver reported in literature [13] was lower (15%) in comparison with 41% in this study. It is to note that the study of Schneider [13] and the present study are very different as regards the number and the source of the specimens analyzed as well as the methods used for PARV4 DNA detection; in fact in the former study 87 liver specimens, obtained mainly in occasion of liver transplantation for various causes, were analyzed by four PCRs. The presence of PARV4 DNA in myocardium samples is reported here for the first time and with a high frequency. The viral load was low also in this tissue and 10 isolates out of the 17 positive myocardium samples that could be analyzed by sequencing showed to be genotype 2 (PARV5), a result that could be related with the age of these patients, all born before 1956 [12], in agreement also with B19V genotype distribution, as previously discussed [15].

Low level PARV4 viremia, which seems characteristic of persistent more than of acute infection, was more common in hematological immunodepressed patients (33%), in comparison with 5.5% in immunocompetent individuals. This result, if confirmed on a larger number of patients, could indicate that reactivation or persistence of PARV4 may occur under immunodepression conditions and manifest as viremia more frequently than B19V. In fact, B19V viremia was not reported in this group of hematological immunodepressed patients. Considering that PARV4 DNA was found in bone marrow from 2 out of 36 hematological patients only (5,5%) whereas viremia occurred in 7 out of 21 (33%) patients

of the same group, these results could suggest that it is unlikely that the site of latency/persistence and reactivation of this virus is the bone marrow. In fact, the two bone marrow positive patients were also PARV4 DNA plasma positive, but at least for the other 5 plasma positive samples another source of viremia has to be hypothesized. On this point, the failure to detect urinary PARV4 shedding in the small group of bone marrow transplantation patients enrolled in this study seems indicate that viral reactivation in the kidney do not occur commonly. In addition, the failure to detect PARV4 DNA in BAL [14] seems indicate that lung is not a site of viral active infection, despite the presence of PARV4 sequences in 23% of individuals, as our results show. Further studies are necessary to detect the tissue target of PARV4 infection and the results here reported could suggest to focus in particular on the liver.

If more than 40% of individuals may have viral DNA sequences in liver or myocardium the parenteral route may not be the only way of PARV4 transmission. However, in agreement with the absence of respiratory transmission, it seems likely that PARV4 infection is less frequently transmitted than B19V infection and then it is less frequently detectable in the host tissues. When PARV4 infection occurs, the virus spreads to various organs where it can persist, more or less frequently. If confirmed, the observation that the frequency of PARV4 DNA persistence in the liver is of the same order than that of B19 DNA, while in all the other tissues here considered is lower, could indicate that the liver is a main target of PARV4 infection. Its particular tropism for myocardium and liver, here reported, suggests to further study the possible role of PARV4 infection in heart and liver diseases, considering also that PARV4 infection has been frequently detected in HCV infected patients. It is to note that also B19V is frequently detectable in liver and myocardial tissue both in acute and persistent B19V infection and its importance in liver and heart diseases still remains unclear [17-21].

Methods

Clinical samples

Tissue biopsies were collected in 2006, from 72 Italian adult patients, non HIV-infected, after informed consent: twenty six bone marrow samples from patients (mean age 60; range: 22-82) with B19-unrelated hematological disorders referred to the Department of Hematology, submitted to different chemotherapeutic regimens; twenty one synovial tissue samples from healthy individuals (mean age:70, range:36-82) who underwent surgery for arthrosis or joint trauma, referred to the Department of Orthopedics and Traumatology; twenty five skin biopsies from patients (mean age: 67,

range 33-93) with B19-unrelated dermatological lesions, referred to the Department of Dermatological Sciences. All the patients were immunocompetent except those with hematological disorders. From 39 out of the 72 patients a serum sample was collected at the same time of the biopsy. In addition, from 2006 to 2010, myocardium autoptic samples were obtained from 35 Italian individuals, non HIV-infected (mean:70, range: 53-84) without known viral myocarditis or dilated cardiomyopathy. From 17 out of 35 cadavers autoptic samples from lungs, liver, kidney and blood were also available. In addition, 93 urine specimens from 29 bone marrow transplantation recipients, at different times after infusion were obtained for PARV4 DNA detection.

PARV4 DNA detection and sequencing

After DNA extraction using the High Pure PCR Template Preparation Kit (Roche, Basel, Switzerland) PARV4 DNA detection was performed by two PCR able to amplify sequences of the three genotypes. A real time PCR using the primers PVORF2F and PVORF2R reported by Fryer (8) and Syber Green as intercalating dye, was developed for virus detection and viral load assessment. One μ l (25 pmol) of each primer and 5 μ l of extracted DNA were added to 18 μ l of Quantitec SYBR Green PCR Master Mix (Qiagen, Hilden, Germany). A first step of 10 min at 94°C, to activate the Taq polymerase, was followed by 40 cycles of 94°C for 30 s, 62°C for 30 s, 72°C for 60 s, with a reading step at 79°C for 15 s. The reaction was performed using the Rotor-Gene 3000 real time apparatus (Corbett Research, Sidney, Australia).

Melting curve analysis was performed to control the specificity of the PCR products.

To construct the calibration curve, serial dilutions of the target sequence, 268 bp long, cloned in the p-GEM-T vector system (Promega, Milano, Italy) were used. The range of linearity of the quantitative reaction was $1,96 \times 10^1$ - $1,96 \times 10^6$ copies/ml. A real time PCR for the β -globin gene was performed to calculate the number of cells of the tissues extracted, as already described [15]. About 50.000 cells per reaction were analyzed. In addition, a nested PCR described in literature [8], with primers targeting a conserved region of ORF1 of the three known PARV4 genotypes was used to confirm the results of the previous assay.

The sensitivity of these two assays was similar, both being able to detect 2 copies of its target/ μ l in 100% of cases.

A sample was considered PARV4 DNA positive if positive by both reactions.

Amplicons obtained by the real time PCR (ORF2) and by the nested PCR (ORF 1) were sequenced using the primers PVORF2F and PVORF2R and the inner primers,

respectively. Sequencing was carried out by the dideoxynucleotide chain termination method on an ABI Prism 377 automatic sequencer (Applied Biosystems), using the ABI PRISM Dye Terminator cycle sequencing Ready Reaction kit.

B19 DNA detection and genotyping was performed as previously described [15].

Statistical analysis

The frequencies of PARV4 and B19V DNA positive samples were compared by the chi-square test with Yates correction.

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Author details

¹Department of Public Health, University of Firenze, Viale Morgagni 48, Firenze, Italy. ²Department of Hematology, University of Firenze, Viale Morgagni 85, Firenze, Italy. ³Department of Dermatological Sciences, University of Firenze, Viale Morgagni 85, Firenze, Italy. ⁴Department of Orthopedics, Traumatology, Plastic surgery and Rehabilitation, University of Firenze, Viale Morgagni 85, Firenze, Italy. ⁵Department of Human Pathology, University of Firenze, Viale Morgagni 85, Firenze, Italy.

Authors' contributions

FC participated in planning of the study and carried out the assays for qualitative and quantitative PARV4 and B19V DNA detection and for sequencing. KZ participated in planning of the study and helped in editing the manuscript. RF, VDG, MI, MR and SDL provided clinical samples. AA conceived and planned the study and wrote the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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識別番号・報告回数		報告日		第一報入手日 2011 年 1 月 24 日	新医薬品等の区分	厚生労働省処理欄
一般的名称	①②③④人血清アルブミン ⑤⑥⑦乾燥濃縮人アンチトロンビンⅢ ⑧人ハプトグロビン ⑨乾燥濃縮人血液凝固第Ⅷ因子		研究報告の公表状況	Virus genes 2010; 41(2): 305-308	公表国 中国	
販売名 (企業名)	①献血アルブミン 25%静注 5g/20mL 「ベネシス」 ②献血アルブミン 25%静注 12.5g/50mL 「ベネシス」 ③献血アルブミン 5%静注 5g/100mL 「ベネシス」 ④献血アルブミン 5%静注 12.5g/250mL 「ベネシス」 ⑤ノイアート静注用 500 単位 (ベネシス) ⑥ノイアート静注用 1500 単位 (ベネシス) ⑦ノイアート (ベネシス) ⑧ハプトグロビン静注 2000 単位 「ベネシス」 ⑨コンコエイト-HT (ベネシス)					
研究報告の概要	パルボウイルスは、動物の DNA ウイルスの中で最も小さい。ビリオンは直径 18~26nm で、タンパク質と DNA から構成されている。パルボウイルス科 (パルボウイルス亜科とデンスウイルス亜科の 2 つの亜科に分類される) は、脊椎動物と昆虫に感染し、昆虫、動物およびヒトにおいて広範囲にわたる疾病に関連する。ゲノムは一般的に約 5kb をもつ線形、シングルポリデオキシヌクレオチド本鎖からなる。そして、通常 2 つの大きな転写読断枠 (ORF1 と ORF2) を含む。 ウイルスの分類上、パルボウイルス亜科は Parvovirus、Erythrovirus、Dependovirus、Amdovirus と Bocavirus を含む。近年では、Lau ほかは、もう一つの属 (ブタ hokovirus (PHoV) とウシ hokovirus (BHoV) を含む Hokovirus) を提案した。 この亜科の改良された分類学的理解を容易にするために、十分に特徴づけられなかったメンバーに関する詳しい情報を得ることが必要である。このレポートでは、中国南東部で集められるブタ血清から、いくつかの新しいパルボウイルスを特定した。 2006 年前半から 2007 年に、「高熱」病 (商業的なブタの呼吸器、生殖器症候群や離乳後の Multisystemic 消耗症候群) が中国南東部の浙江省の幾つかの地域で流行した。感染が疑われる 3 つの発生地域のブタから血清サンプルが収集された。血清サンプルからの未知のウイルスゲノム DNA、或いは RNA のランダム検出について、いくらかの改良した方法を使用して実施した。増幅 PCR 製品は、アガロースジェル電気泳動によって分析された。 系統樹は、ORF1 と ORF2 によって暗号化された推定アミノ酸配列からも得られた。両方の代表的な系統樹は類似していた。完全なゲノム配列同一性と進化の距離の分析は、さらに観察された樹形を助長した。 78 の配列は build-in ClustalW 方法に合わせ、そして異なる株間の配列同一性は DNASTAR の MegAlign プログラムを使用して計算した。進化の距離は、ClustalX 整理するシーケンスを使っている MEGA 4.0 ソフトウェアプログラムで計算した。全ゲノム配列のために、40% 以上の同一性は属の中の異なる株の間で観察された、その一方で、異なる属の種の間での配列同一性は 30% 以下だった。属の間の進化の距離値は 0.62~1.97、ところが同じ属の種の間での進化の距離値は 0.00~0.55 だった。 配列同一性の解析と ORF1 と ORF2 によってコード化されたアミノ酸配列から推定される進化の距離は、同等の結果が得られた。上記の全てのデータは、我々が複製した株が「Cnvirus」と名付けた新しいパルボウイルスを意味したという事実を支持した。 「Cnvirus」の分子疫学を調査するために、合計 296 のブタ血清サンプルは、YiWu、YuHang、JinHua、DeQing、LongYou、JiangShan、HangZhou、JianDe、JinYun、WenLing、YuHuan、WenZhou と ZhuJi を含む中国東部の幾つかの都市の養豚場から入手した。全ての血清サ					使用上の注意記載状況・ その他参考事項等 代表としてノイアート静注用 500 単位の記載を示す。 1. 慎重投与 (次の患者には慎重に投与すること) (1) 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。] (2) 免疫不全患者・免疫抑制状態の患者 [ヒトパルボウイルス B19 の感染を起こす可能性を否定できない。感染した場合には、持続性の貧血を起こすことがある。] 2. 重要な基本的注意 (1) 略 1) 血漿分画製剤の現在の製造工程では、ヒトパルボウイルス B19 等のウイルスを完全に不活化・除去することが困難であるため、本剤の投与によりその感染の可能性を否定できないので、投与後の経過を十分に観察すること。 6. 妊婦、産婦、授乳婦等への投与

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ンプルの PCR 検出結果は、下記の通り： 296 のサンプルの間で、PCR は YiWu の 55 の血清サンプルの内の 9 つ (16. 3%)、YuHang の 20 の内の 3 つ (15. 0%)、JinHua の 50 の内の 2 つ (4. 0%)、DeQing の 20 の内の 4 つ (20. 0%)、LongYou の 30 の内の 6 つ (20. 0%)、JiangShan の 30 の内の 1 つ (3. 3%)、HangZhou の 42 の内の 1 つ (2. 4%) で陽性、他の都市のサンプルは陰性であった。我々は、いくつかの PCR 製品の複製をつくって、配列決定した。決定配列の間の同一性は、95. 1－ 99. 6%であった。現在まで、1 つの類似したウイルスがミャンマーからあった。そのようなウイルスが同様に他の国を現在循環しているかどうかは、わかっていない。 3 つの近似した完全長ゲノムが 3 つの異なる発生地域から集められた血清から得られたけれども、我々はこのウイルスと所謂「高熱」病の間に関係があったかどうか、分らない。早熟ブタが居る些細な問題のある農場 (12 箇月の間に 3 回) の調査では、血清中に検出された「Cnvirus」 DNA のピークは、徴候の出現より約 2－3 週間早かった。しかし、有病率のレベルと流行の重大度 (データは示していない) の間には関係がなかった。ブタの呼吸器、生殖症候群や離乳後の Multisystemic 消耗症候群を伴う「高熱」病は、中国南部で 2006 年夏に最初に起こった。流行は、2006 年の夏で初期の秋に大流行した。 結論として、我々は中国南東部で幾つかの新しいパルボウイルスを特定した。広範囲なゲノム配列分析法は、新しく検出されたパルボウイルスがパルボウイルス亜科と異なる集団であることを示した。試験管内で我々の複製された株の成長特性を調査するために、PK15 細胞、293 の細胞に加えて援助プラスミド (David Pintel 教授 (ミズーリ大学) からの寛大な贈り物) と 293 の細胞に加えてアデノウイルス (Hangping Yao 教授 (浙江大学の医科大学) からの寛大な贈り物) 等を含む数種類の細胞は、ウイルスを繁殖させるのに用いられた。パルボウイルス アデノ関連ウイルス type 2 (AAV2) が増殖感染の設立においてヘルパーウイルス (一般的にアデノウイルス、或いはヘルペスウイルス) を利用する一本鎖 DNA ウイルスであると報告した。しかし、成長の明らかな兆候は現在まで観察されていない。	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
万一、ヘパリンの原料であるブタ小腸粘膜にパルボウイルスが混入したとしても、PPV をモデルウイルスとしたウイルスバリデーション試験成績から、ヘパリンの製造工程において十分に不活化・除去されると考える。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。 パルボウイルスに関する情報収集に努める。

Novel parvovirus sublineage in the family of *Parvoviridae*

Fang Wang · Yongwei Wei · Chun Zhu ·
 Xianbo Huang · Yaping Xu · Lian Yu ·
 Xuping Yu

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Abstract *Parvoviridae*, which are classified into two subfamilies *Parvovirinae* and *Densovirinae*, can infect both vertebrate and insects and are related to a wide range of diseases in insects, animals, and humans. In this report, several new parvoviruses were identified in swine sera collected in southeastern China. The sequence analyses showed that the parvoviruses detected in southeastern China formed a distinct sublineage within the subfamily *Parvovirinae*. Based on these results, we propose a novel parvovirus sublineage, Cnvirus, to describe these parvoviruses.

Keywords *Parvoviridae* · Novel sublineage · China

The parvoviruses are the smallest among the animal DNA viruses. The virion has a diameter of 18 to 26 nm and is composed entirely of protein and DNA. *Parvoviridae*, which are classified into two subfamilies *Parvovirinae* and *Densovirinae*, can infect both vertebrate and insects and are related to a wide range of diseases in insects, animals, and humans. The genome is a linear, single-stranded polydeoxynucleotide chain with typically about 5 kilobases (kb) in size and it usually contains two large open reading frames (ORF1 and ORF2) [1]. According to the report of International Committee on Taxonomy of Viruses, the subfamily *Parvovirinae* contains Parvovirus, Erythrovirus,

Dependovirus, Amdovirus, and Bocavirus (<http://www.ncbi.nlm.nih.gov/ICTVdb/Ictv/index.htm>). Recently, Lau et al. proposed another genus, Hokovirus, including porcine hokovirus (PHoV) and bovine hokovirus (BHoV) [2]. To facilitate an improved taxonomic understanding of this subfamily, it is necessary to gain further information about the poorly characterized members. In this report, we identified several new parvoviruses from swine sera collected in southeastern China. Based on the nucleic acid sequence, these new identified parvoviruses formed a distinct cluster within the subfamily *Parvovirinae*.

In early 2006–2007, severe field outbreaks of “High Fever” disease [3, 4] were observed in commercial swine with Porcine Respiratory and Reproductive Syndrome and/or Post-weaning Multisystemic Wasting Syndrome in Zhejiang province, southeast China. Serum samples were harvested from swines with suspected infection from three field outbreak. Random detection of unknown virus genomic DNA or RNA from serum samples was performed using the method as previously described with some modification [5]: Restriction enzyme TaqI (Sangon) was used to replace Csp6.I. And hence Ntaq I Adaptor (which is composed of hybridized oligonucleotides Ntaq24 and Ntaq11, whose sequences will be available upon request) was used to replace NCsp Adaptor and Ntaq24, instead of NBam24, was used as primer to amplify target DNA. The Amplified PCR products were then analyzed by agarose gel electrophoresis. The distinct DNA bands were gel-purified using Gel Extraction Mini Kit (WATSON Biotechnologies). The target DNA fragments were cloned into pMD18-T vector (Takara) and sequenced using universal M13 primers (Invitrogen). To obtain the full-length sequences of the target virus genomes, PCR was used to amplify the intervening DNA sequences and a modified RACE method [6] was performed to amplify its 5' and 3'

Fang Wang and Yongwei Wei both contributed equally to this study.

F. Wang · Y. Wei · C. Zhu · X. Huang · Y. Xu · L. Yu ·
 X. Yu (✉)

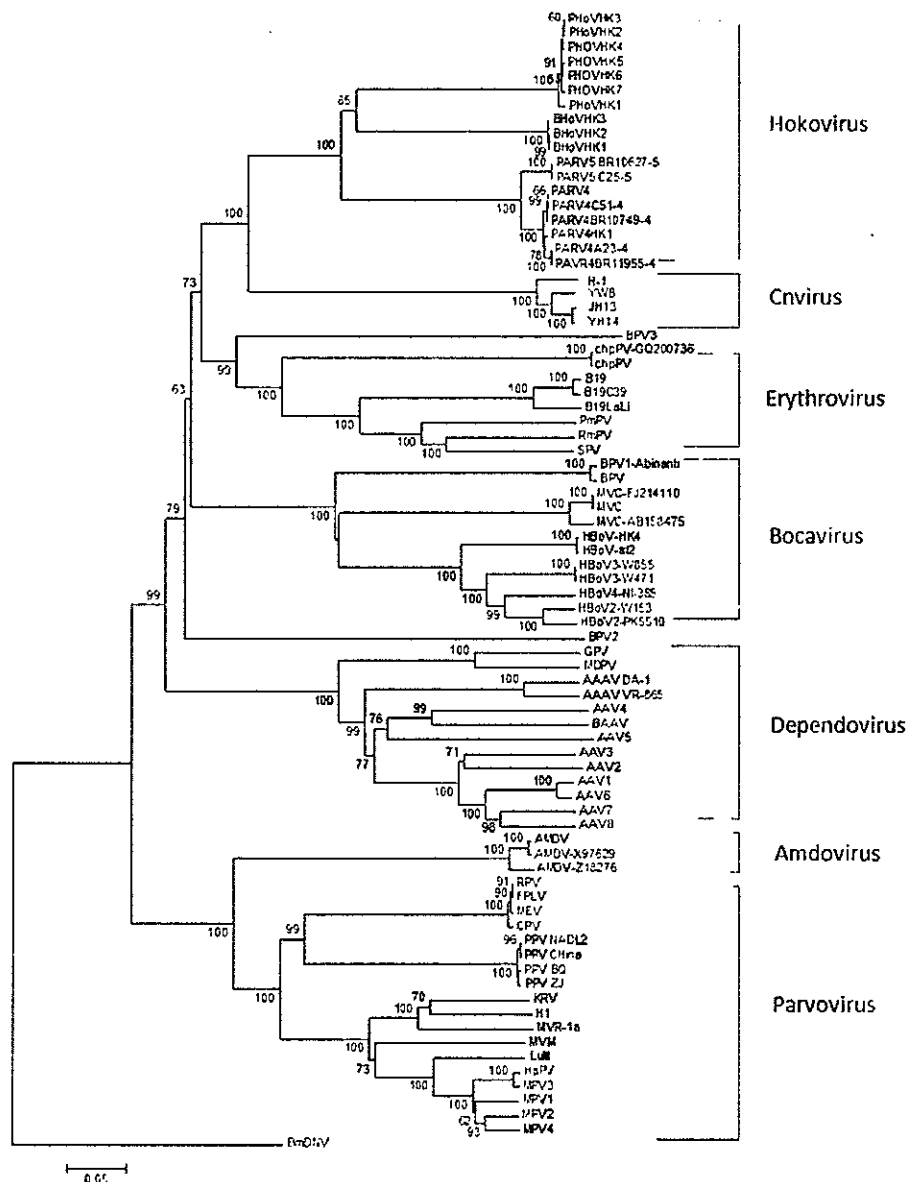
Key Lab of Animal Epidemic Etiology and Immunological
 Prevention of MOA, Department of Veterinary Medicine,
 College of Animal Sciences, Zhejiang University, Hangzhou
 310029, People's Republic of China
 e-mail: xpyu@zju.edu.cn

ends. Finally, the sequences were assembled and edited using Seqman program in DNASTar software. The three strains with nearly full-length sequences were named as YW8, JH13, and YH14. All the sequences were deposited in the GenBank with Accession Nos. GU938299, GU938300, and GU938301, respectively.

Sequence analyses and alignments were performed with the aid of ClustalX 2.0, MEGA 4.0 and DNASTar 7.1 softwares. Seventy-eight (including our new sequences) of *Parvovirinae* with nearly full-length sequences were included in the analysis and one BmDNV sequence of *Densovirinae* was used as outgroup. These sequences were all obtained from GenBank. The sequences (total of 79

sequences) were aligned with ClustalX 2.0 and the phylogenetic tree for parvoviruses was then generated using the neighbor-joining method with 1000 bootstrap replicates with the MEGA 4.0 software program. The phylogenetic relationship of the cloned virus with other members of subfamily *Parvovirinae* was obtained by comparing nucleotide sequences of their entire genomes. The resulting phylogenetic tree is depicted in Fig. 1. The overall branching patterns of the tree constructed for the nucleotide sequence of parvoviruses was split into several distinct branches and each branch represents an individual genus of the subfamily *Parvovirinae*, as follows: *Hokovirus*, *Erythrovirus*, *Bocavirus*, *Dependovirus*, *Amdovirus*, *Parvovirus*, and our cloned

Fig. 1 The phylogenetic tree depicting evolutionary relationship between the members of the subfamily *Parvovirinae*. Phylogenetic analysis was based on the nucleotide sequence, using the neighbor-joining method with 1000 bootstrap replicates. Bootstrap values based on the consensus tree are plotted at the main internal branches to show support values. BmDNV was included in the analysis and used as outgroup. All sequences of other 76 parvoviruses were obtained from GenBank



strains (Fig. 1). The phylogenetic trees were also obtained from the deduced amino acid sequences that were encoded by ORF1 and ORF2. Both the representative phylogenetic trees were similar to Fig. 1 (data not shown). Analysis of full genome sequence identity and evolutionary distance additionally supported the observed tree topology. Seventy-eight sequences were aligned with the build-in ClustalW method and the sequence identity between different strains was calculated using the MegAlign program of DNASTar. The evolutionary distances were calculated with the MEGA 4.0 software program using ClustalX aligned sequences. Both the sequence identity and evolutionary distance within and between genera were then summarized in Tables 1 and 2. For the full genome sequence, over 40% identity was observed between different strains within a genus, while less than 30% sequence identity between species of different genera (Table 1). The evolutionary distance value between genera was 0.62–1.97, whereas it was 0.00–0.55 between species in the same genus (Table 2). Analysis of sequence identity and evolutionary distance obtained from the deduced amino acid sequences that encoded by ORF1 and ORF2 yielded comparable results (data not shown). All data above supported the fact that our cloned strains together with the reported strain H-1 [7] represented a different novel parvovirus which are named as Cnvirus.

To investigate the molecular epidemiology of Cnvirus in southeastern China, a total of 296 serum samples were obtained from pig farms in several cities in eastern China,

including YiWu, YuHang, JinHua, DeQing, LongYou, JiangShan, HangZhou, JianDe, JinYun, WenLing, YuHuan, WenZhou, and ZhuJi. The results of the PCR detection in all the serum samples are summarized below: among the 296 samples, PCR was positive in 9 (16.3%) of the 55 serum samples in YiWu, 3 (15.0%) of the 20 in YuHang, 2 (4.0%) of the 50 in JinHua, 4 (20.0%) of the 20 in DeQing, 6 (20.0%) of the 30 in LongYou, 1 (3.3%) of the 30 in JiangShan, 1 (2.4%) of the 42 in HangZhou, and no positive in other cities' samples. We cloned and sequenced several PCR products. The identities between determined sequences were 95.1–99.6%. To date, there is only one similar virus from Myanmar [7]. It is not known whether such viruses are currently circulating in other countries as well.

Though the three nearly full-length genomes were obtained from sera collected from three different outbreaks, we do not know whether there was any relation between this virus and the so-called “High Fever” disease. In a survey of a farm (3 times in a 12 months period) with minor problem in early growing finishing pigs, the peak of the Cnvirus DNA detected in sera was about 2–3 weeks earlier than the appearance of symptom. However, there was no relation between the levels of prevalence and the severity of the outbreak (data not showed). The “High Fever” disease, with Porcine Respiratory and Reproductive Syndrome and/or Post-weaning Multisystemic Wasting Syndrome, broke out first in the summer of 2006 in southern China. The

Table 1 Sequences identity(%) within and between genera of *Parvovirinae*

	Hokovirus	Cnvirus	Erythrovirus	Bocavirus	Dependovirus	Amdovirus	Parvovirus
Hokovirus	60.6–99.9						
Cnvirus	4.6–10.3	95.1–98.6					
Erythrovirus	2.4–8.1	2.3–4.3	56.6–98.7				
Bocavirus	0.7–5.0	1.4–4.0	2.7–6.6	40.1–99.9			
Dependovirus	3.6–8.5	3.7–9.4	3.1–7.4	1.9–10.1	43.2–95.9		
Amdovirus	1.5–3.9	1.7–2.9	2.3–4.3	0.9–5.4	0.5–3.6	96.3–99.5	
Parvovirus	1.0–5.0	0.7–3.3	1.9–5.5	1.0–6.8	0.7–5.7	9.3–27.2	56.6–99.8

Table 2 Sequence distances within and between genera of *Parvovirinae*

	Hokovirus	Cnvirus	Erythrovirus	Bocavirus	Dependovirus	Amdovirus	Parvovirus
Hokovirus	0.00–0.42						
Cnvirus	0.75–0.82	0.00–0.06					
Erythrovirus	1.00–1.25	1.20–1.25	0.00–0.45				
Bocavirus	1.09–1.30	1.30–1.39	1.28–1.57	0.00–0.55			
Dependovirus	1.27–1.54	1.44–1.67	1.53–1.82	1.50–1.78	0.00–0.55		
Amdovirus	1.53–1.63	1.62–1.70	1.73–1.86	1.63–1.85	1.59–1.60	0.00–0.03	
Parvovirus	1.32–1.59	1.64–1.84	1.57–1.76	1.63–1.86	1.53–1.72	0.62–0.73	0.00–0.51

outbreak was severe in the summer and early fall of 2006. In many farms, the outbreak could be in all pigs including suckling piglets, weaned piglets, growing finishing pigs, sows and boars and the mortality was also high (even up to over 60%). Though there were still a few farms outbreak severely later, the morbidity and mortality in many farms were lowed and usually restricted to piglets (mainly after weaning) or even to growing finishing pigs.

In conclusion, we identified several new parvoviruses in southeastern China. Extensive genome sequence analysis showed that the new detected parvoviruses formed a distinct cluster within the subfamily *Parvovirinae*. To investigate the growth characteristic of our cloned strains in vitro, several kinds of cell, including PK15 cell, 293 cell plus a help plasmid (a generous gift from Prof. David Pintel, University of Missouri) and 293 cell plus adenovirus (a generous gift from Prof. Hangping Yao, Medical School of Zhejiang University) etc. were used to propagate the viruses. It was reported that the parvovirus adeno-associated virus type 2 (AAV2) is a single-stranded DNA virus which utilizes a helper virus (typically adenovirus or herpesvirus) in the establishment of productive infection [8–10]. However, there was no apparent sign of growth yet observed to date.

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医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日 2011. 3. 16	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	解凍人赤血球濃厚液	研究報告の公表状況	Satake M, Hoshi Y, Taira R, Momose S, Hino S, Tadokoro K. Transfusion. 2011 Feb 18. doi: 10.1111/j.1537-2995.2010.03047.x.	公表国	
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)			日本	
研究報告の概要	○輸血用血液製剤による症候性パルボウイルスB19感染 背景: 輸血によるヒトパルボウイルスB19感染(TT-B19V)のリスクは懸念事項となっているが、B19Vを含む成分製剤の輸血に起因する臨床的に重要なTT-B19Vの報告はきわめて少ない。したがって、適切な感度をもつユニバーサルB19Vスクリーニング検査が必要であるかどうかについて議論となっている。 研究デザインと方法: 供血者のB19Vスクリーニング(感度10^{10} IU/mL)が実施され、保管血液検体が入手可能であった1999年から2008年までの期間に、日本赤十字社のヘモビジランスシステムを通じて、TT-B19Vの可能性のある臨床報告を収集した。 結果: 成分輸血に起因するTT-B19V患者8人が同定された。4人の患者が持続性の貧血と赤芽球癆を発症し、1人の患者が汎血球減少症を発症した。これら5人の患者の基礎疾患は、悪性血液疾患または溶血性疾患のいずれかであった。これらの症例について原因となる製剤中のウイルス量は、$10^3 \sim 10^8$ IU/mLの範囲であった。血液疾患に罹患しておらず外科的治療を受けた患者2人は、マイルドな症状を示しただけであった。8人中5人の患者において患者とその供血者のB19VのDNA配列が一致した。8症例のすべてにおいて、原因となる成分製剤は抗B19V IgM陽性であった。 結論: 輸血により重篤なB19V関連疾患を発症するかどうかは、輸血した製剤中のウイルス量ではなく、患者の基礎疾患に依存した。臨床的に重要なTT-B19Vを防ぐためには、赤芽球癆または汎血球減少症などをきたすリスクのある患者を対象とした対策が必要であることが示唆された。				使用上の注意記載状況・ その他参考事項等 解凍赤血球濃厚液「日赤」 照射解凍赤血球濃厚液「日赤」 解凍赤血球-LR「日赤」 照射解凍赤血球-LR「日赤」 血液を介するウイルス、 細菌、原虫等の感染 vCJD等の伝播のリスク
報告企業の意見		今後の対応			
パルボウイルスB19関連疾患を発症するかどうかは、輸血した成分製剤のウイルス量ではなく、患者の基礎疾患に依存することが明らかとなったとの報告である。		日本赤十字社では、以前よりRHA法によるB19抗原検査を導入、ウイルス量の多い血液を排除してきた。また、2008年には検査法をより感度の高いCLEIA法に変更した。今後も引き続き、ヒトパルボウイルスB19に関する新たな知見及び情報の収集に努める。			



ORIGINAL ARTICLE

Symptomatic parvovirus B19 infection caused by blood component transfusion

Masahiro Satake, Yuji Hoshi, Rikizo Taira, Shun-ya Momose, Satoru Hino, and Kenji Tadokoro

BACKGROUND: Although a risk of transfusion-transmitted human parvovirus B19V (TT-B19V) infection has been a concern, there have been very few reports of clinically relevant TT-B19V caused by the transfusion of a B19V-containing blood component. It has therefore been a matter of debate whether a universal B19V screening with an appropriate sensitivity is required.

STUDY DESIGN AND METHODS: Through the Japanese Red Cross hemovigilance system, clinical reports on possible TT-B19V were collected from 1999 to 2008, during which B19V donor screening (sensitivity, 10^{10} IU/mL) was conducted and repository blood samples from donors were available.

RESULTS: Eight patients with TT-B19V caused by component transfusion have been identified. Four patients developed sustained anemia and pure red blood cell (RBC) aplasia and one patient developed pancytopenia. The underlying diseases in these five patients were either hematologic malignancy or hemolytic diseases. The viral loads of the responsible components for these cases ranged from 10^3 to 10^8 IU/mL. Two patients who underwent surgical treatment without any hematologic disorder exhibited only moderate symptoms. The B19V DNA sequence identity between a patient and the linked blood donor was confirmed in five of the eight patients. All of the components responsible for the eight cases were positive for anti-B19V immunoglobulin (Ig)M.

CONCLUSION: Vulnerability to serious B19V-related hematologic disorders depended on the patient's underlying disease state of an enhanced erythropoiesis, not on the viral load of the component transfused. To prevent clinically relevant TT-B19V, a strategy is suggested in which patients at risk of acquiring RBC aplasia or pancytopenia are targeted.

Infection by human parvovirus B19 (B19V) is common in any geographic area and causes erythema infectiosum or fifth disease in childhood. The infection in adults is usually asymptomatic but may cause a transient red blood cell (RBC) aplasia in patients in the state of an enhanced erythropoiesis or may cause persistent anemia in immunocompromised patients.¹⁻³ The prevalence of anti-B19V immunoglobulin (Ig)G increases steadily even after childhood, suggesting that B19V infection occurs frequently during adulthood.³⁻⁶ In acute infection, the viral load in peripheral blood reaches as high as 10^{12} IU/mL.⁷ The frequent primary infection among adults and the high viral load without symptoms imply the presence of a considerable number of blood donors with a high viral load, which raises the possibility of a considerable risk of transfusion-transmitted B19V infection (TT-B19V).

Although TT-B19V cases have often been reported after the transfusion of plasma derivatives,⁸⁻¹⁰ there have been very few reports of clinically relevant B19V infection that is considered as a result of the transfusion of a B19V-containing blood component (i.e., RBCs, fresh-frozen plasma [FFP], or platelet [PLT] concentrates).¹¹⁻¹⁵ It has

ABBREVIATIONS: AML = acute myeloid leukemia; B19V = human parvovirus B19; JRC = Japanese Red Cross; nt = nucleotide(s); RHA = receptor-mediated hemagglutination; TT-B19V = transfusion-transmitted human parvovirus B19V; TTI(s) = transfusion-transmitted infection(s).

From the Japanese Red Cross Central Blood Institute and the Japanese Red Cross Blood Service Headquarters, Tokyo, Japan.

Address correspondence to: Masahiro Satake, Japanese Red Cross Tokyo Metropolitan West Blood Center, Midori-cho 3256, Tachikawa, Tokyo 190-0014, Japan; e-mail ma-satake@tokyo.bc.jrc.or.jp.

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TRANSFUSION **;***-**.

therefore been a matter of debate whether a universal B19V screening with an appropriate sensitivity is indeed required for securing blood component safety. In this regard, several studies have been conducted to establish the frequency of blood products harboring TT-B19V risk and the frequency of clinically relevant TT-B19V cases. In most such studies, a reason to implement a B19V screening method that covers all blood donations has not been verified because no case of clinically relevant TT-B19V was identified during the study period.¹⁶⁻¹⁸

Japanese Red Cross (JRC) blood centers established a hemovigilance system in 1993 and have been collecting voluntary reports on transfusion-transmitted infections (TTIs) and other adverse effects as a result of transfusion. Through the system, JRC has so far obtained five established and three probable cases of TT-B19V. In this article, we describe the details of TT-B19V cases, each of which represented a typical clinical course of B19V infection.

PATIENTS AND METHODS

Hemovigilance system

JRC blood centers are the sole facilities in Japan that deal with blood procurement and processing, testing, and delivery of blood components. The JRC hemovigilance system has been functioning since 1993 and covers the entire country with 1 million patients being transfused every year. Although the reporting of suspected TTI cases to JRC is not mandatory, it is obligatory for every physician to report TTI cases to the Japanese Ministry of Health, Labour and Welfare, which in turn refers this information to JRC. Eventually, JRC is able to obtain all information on suspected TTIs and other serious adverse reactions caused by blood transfusion. The system also includes a complete sample archive from all blood donations since 1999, which enables us to investigate the cause of a TTI using the repository blood samples obtained from the donation associated with the TTI. For TT-B19V cases, a lookback study for the possibility of previous donation with viremia has not been performed.

Receptor-mediated hemagglutination assay

In 1998, JRC implemented a receptor-mediated hemagglutination (RHA) assay as a screening test for B19V and used it for all donated blood until 2007. The theoretical basis of the RHA assay system was described elsewhere^{19,20}; briefly, it is a B19V antigen detection method in which the indicator RBCs agglutinate via B19V particle binding to globosides on the RBC membrane at a critical pH ($\text{pH } 5.6 \pm 0.1$). The sensitivity of RHA is approximately 10^{10} IU/mL, and by this method, 300 to 400 B19V-positive donors with very high titer viremia have been identified every year.²¹ Although the sensitivity of RHA is not sat-

isfactory, it has greatly contributed to the lowering of the viral load in a plasma pool that is manufactured into plasma derivatives.²² In 2008, JRC implemented a chemiluminescence enzyme immunoassay-based screening assay that is also an antigen detection assay with a sensitivity of approximately 10^7 IU/mL.

Polymerase chain reaction analysis of B19V and antibody detection

On receiving a report of a suspected case of TT-B19V, polymerase chain reaction (PCR) analysis was carried out to detect the B19V DNA in patient sera obtained before and after the index blood transfusion as well as in repository tube(s) obtained from the donation(s) from which the blood component(s) suspected of causing TT-B19V had been processed. B19V DNA was extracted using a total nucleic acid isolation kit (Magna Pure LC, Roche Diagnostics, Tokyo, Japan) and amplified and quantified using a B19V quantification kit (LightCycler, Roche Diagnostics, Tokyo, Japan). The forward and reverse primers were located at Nucleotides (nt) 2046 to 2064 and nt 2110 to 2092, respectively. The probe used was mapped at 24 bp of nt 2067 to 2090. The 95% detection limit of the PCR system is 289 IU/mL, as determined by probit analysis. Direct B19V DNA sequencing was performed targeting 1069 bp (nt 1884-2952) in the NS1/VP1 region for Cases 1 to 4.²³ As for Case 5, B19V DNA was sequenced for a total of 1913 bp covering the NS (489 bp [nt 1396-1884] and 225 bp [nt 1962-2187]), NS-VP1 (597 bp [nt 2370-2966]), and VP1 (602 bp [nt 2984-3585]) regions. Sequence identity was assessed for these regions between the patient sample and the blood donor sample. IgM and IgG specific for B19V were detected by enzyme-linked immunosorbent assay (Parvo-IgM and Parvo-IgG, Denka Seiken, Tokyo, Japan).

RESULTS

The annual number of blood donations in Japan is approximately 5 million and the annual number of components released to medical facilities for RBCs, FFP, and PLT concentrate are 3.3 million, 960,000, and 730,000, respectively. Data presented in this article were collected during the period from 1999 through 2008 when repository blood samples from donors were available. During that period, JRC received only 15 reports of suspected TT-B19V from physicians, among which we were able to identify eight cases of TT-B19V. This indicates that the case frequency of documented TT-B19V is eight per 50 million or approximately one in 6 million donations. Details of the five TT-B19V cases that were confirmed by B19V-DNA sequence analysis are described below (Table 1).

Case 1

A 41-year-old man with hairy cell leukemia underwent a treatment regimen including a course of cladribine

TABLE 1. Cases established as having TT-B19V infection

Case	Patient profile	Before transfusion	After transfusion	Symptoms and laboratory findings	Transfused components
1	41, male Hairy cell leukemia After chemotherapy		DNA (+) IgM (+) IgG (+)	RBC aplasia (3 months) Reticulocytopenia (1 month) Viremia level of 1×10^{12} copies/mL	RBCs (irradiated) DNA (+) 1.8×10^5 IU/mL* 2×10^6 IU/bag† IgM (+) IgG (+)
2	57, male AML (M4) After chemotherapy	DNA (-) IgM (-) IgG (-)	DNA (+) IgM (+) IgG (+)	Pure RBC aplasia (approx. 2 months)	PC (irradiated) DNA (+) 9.7×10^8 IU/mL 2×10^{10} IU/bag IgM (+) IgG (-)
3	35, female Placenta previa	DNA (-) IgM (-) IgG (-)	DNA (+) IgM (+) IgG (+)	Fever Systemic eruption (3 weeks)	RBCs (irradiated) DNA(+) 3.0×10^5 IU/mL 3×10^6 IU/bag IgM (+) IgG (+)
4	59, male Rectal cancer	DNA (-) IgM (-) IgG (-)	DNA (+) IgM (+) IgG (+)	Sustained high fever (5 days)	RBCs (irradiated) DNA(+) 5.1×10^5 IU/mL 5×10^4 IU/bag IgM (+) IgG (+)
5	61, male AML After chemotherapy	DNA (-)	DNA (+)	High fever Disseminated erythema Pure RBC aplasia (7 weeks) Reticulocytopenia	PC (irradiated) DNA (+) IgM (+) IgG (+)

* Viral concentration in the donated blood.

† Estimated viral load in the component.

PC = PLT concentrate.

(2-chlorodeoxyadenosine) for 7 days in May 2005. On Day 0, 9 days after the completion of cladribine treatment, he was transfused with 1 unit of RBCs because of anemia. The reticulocyte proportion in his peripheral blood decreased from 3.6% on Day 6 to 2.4 and 0.3% on Days 8 and 10, respectively. B19V PCR analysis was performed on his Day 11 serum, which revealed a viremia level of 1×10^{12} copies/mL. The JRC central laboratory also detected B19V DNA and anti-B19V of both IgM and IgG classes in sera obtained on Day 22. On the basis of these findings, the diagnosis of RBC aplasia due to TT-B19V was made. Reticulocyte counts ranged from 0.1% to 0.2% since then, and the patient remained RBC transfusion dependent requiring occasional granulocyte-colony-stimulating factor (G-CSF) administration. His reticulocyte count started to increase in late June and the complete resolution of anemia was confirmed in late August. The repository sample from the index donation for the RBCs was found to contain 1.8×10^5 IU/mL B19V DNA as well as anti-B19V of both IgM and IgG classes. The patient was administered G-CSF because of existing leukopenia caused by the preceding chemotherapy with cladribine, not by B19V infection (Y. Tsukada, manuscript in preparation).

Case 2

A 57-year-old man received chemotherapy for acute myeloid leukemia (AML, M4). After the completion of chemotherapy in May 2005, he received multiple blood transfusions because of sustained marrow suppression. The index blood transfusion of the PLT concentrate responsible for TT-B19V was carried out on June 14 (Day 0). A delayed recovery of RBC generation was noted despite a complete recovery of white blood cell and PLT generation. Marrow examination was carried out on Day 21 revealing pure RBC aplasia as well as a complete remission of AML. His peripheral blood sample collected on Day 24 was positive for B19V DNA and anti-B19V of both IgM and IgG classes. He became negative for anti-B19V IgM on Day 35 and RBC generation recovery was recognized 1 month later. A pretransfusion sample obtained on Day -21 was negative for both B19V DNA and anti-B19V of both classes. The repository sample from the index donation for the PLT concentrate contained 9.7×10^8 IU/mL B19V DNA and the anti-B19V of the IgM class but not that of the IgG class. Seventeen blood components had been transfused to this patient before the marrow examination, but only the repository sample from the index PLT concentrate was positive for B19V DNA.

Case 3

A 35-year-old woman with placenta previa received a transfusion of 5 units of RBCs in June 2005 because of massive bleeding on delivery. On Day 7 after the index transfusion, she developed a fever of 38°C. Four days later she developed systemic small eruptions, which led her physician to suspect a viral infection. Laboratory testing revealed the presence of anti-B19V IgM in the blood sample collected on Day 12. All the symptoms disappeared 1 month after transfusion without specific medication. The pretransfusion sample collected on Day 0 was found to be negative both for B19V DNA and anti-B19V of both IgM and IgG classes. Posttransfusion samples obtained on both Day 47 and Day 60 contained B19V DNA and anti-B19V of both classes. A retrospective study revealed that a repository sample from 1 of the 5 units of the RBCs contained 3.0×10^5 IU/mL B19V DNA as well as anti-B19V of both classes.

Case 4

A 59-year-old man with rectal cancer received a transfusion of 2 units of RBCs during a surgical operation in April 2006. Although his postoperative course was uneventful for 5 days, he developed a high fever of 38 to 40°C on Day 6 postoperation and the fever remained for 5 days despite medication with antibiotics and antipyretics. The fever thereafter resolved spontaneously. It was reported that a surgical complication was unlikely from the viewpoints of the operative procedure and postoperative course. A posttransfusion sample collected on Day 22 was found to be positive for B19V DNA and anti-B19V of both IgM and IgG classes: these three markers were all negative in a pretransfusion sample obtained 1 day before operation. A repository sample from the donation for 1 of the 2 units of the RBCs transfused contained 5.1×10^3 IU/mL B19V DNA as well as anti-B19V of both IgM and IgG classes.

Case 5

A 61-year-old man undergoing chemotherapy for AML received 24 blood transfusions for 4 months in 2002. He developed a high fever and disseminated erythema 22 and 26 days after the index transfusion (Day 0), respectively. Reticulocytopenia developed and pure RBC aplasia was confirmed by marrow examination. B19V DNA was not detected in his pretransfusion sample collected on Day -49 but was detected in his posttransfusion sample collected on Day 25. He recovered from pure RBC aplasia 7 weeks after the transfusion. The responsible blood component was a PLT concentrate transfused in mid-May, which was found to be positive for anti-B19V of both IgM and IgG classes and B19V DNA. Data on the B19V DNA concentration in the repository sample from the index component are not available.

In the first four cases presented above, complete genome sequence identity was established for nt 1884 to 2952 of B19V between the patient posttransfusion samples and the associated donor repository samples. For Case 5, viral sequence identity was established for a total of 1913 bp (see Patients and Methods). Figure 1 presents a phylogenetic tree of the B19V DNA sequences for the five established cases.

In addition to these five cases with established B19V DNA sequence identity between donors and recipients, JRC blood centers have three reports of probable cases of TT-B19V (Table 2). In all three cases, B19V DNA was detected in posttransfusion samples and linked donor repository samples, but data on B19V DNA sequence analysis were not available. The first case was reported elsewhere in detail¹⁵; briefly, a 52-year-old woman with paroxysmal nocturnal hematuria was regularly receiving RBC transfusions with prednisolone administration. She developed pancytopenia with high fever and general malaise 13 days after the index transfusion (Day 0). She thereafter received a continuous course of transfusions with RBCs and PLT concentrates together with G-CSF. She recovered from pancytopenia approximately 1 month after the index transfusion, although the reticulocyte proportion remained low for a longer period. Anti-B19V IgM was detected in her posttransfusion samples with increasing titer in the following months. Before the onset of pancytopenia, the patient had received transfusion of 1 unit of RBCs on Days -56, -32, 0, and +1. Repository blood samples from the four components were subjected to PCR analysis and only one sample from the index blood component was verified to be positive for B19V DNA. Strikingly, the B19V DNA-positive blood component attributed to this infection was a washed RBC unit processed from a donation containing 6.8×10^3 IU/mL B19V and anti-B19V of both IgM and IgG classes. The blood obtained 4 months later from the same donor still had a viral load of 1.6×10^3 IU/mL but no specific IgM. The second patient was a 28-year-old woman with hemolytic anemia. She sustained a prolonged severe anemia after transfusion of 1 unit of RBCs. Marrow examination verified hypoplasia of a RBC lineage. The proportion of peripheral blood reticulocytes remained as low as 0.1% for 1 month. B19V DNA was not detected in her pretransfusion sample. The blood component responsible for this case was an RBC unit positive for anti-B19V of both IgM and IgG classes. The third patient was a 79-year-old man who received transfusion of 1 unit of RBC during pelvic tumor resection. He did not develop any clinical symptoms after the transfusion but routine postoperative blood analysis revealed a decreased reticulocyte count that lasted for 1 week. Further investigation verified the anti-B19V IgM conversion. The responsible RBC unit contained anti-B19V of both IgM and IgG classes.

In one of the three probable cases presented above, B19V DNA was negative in the pretransfusion sample,

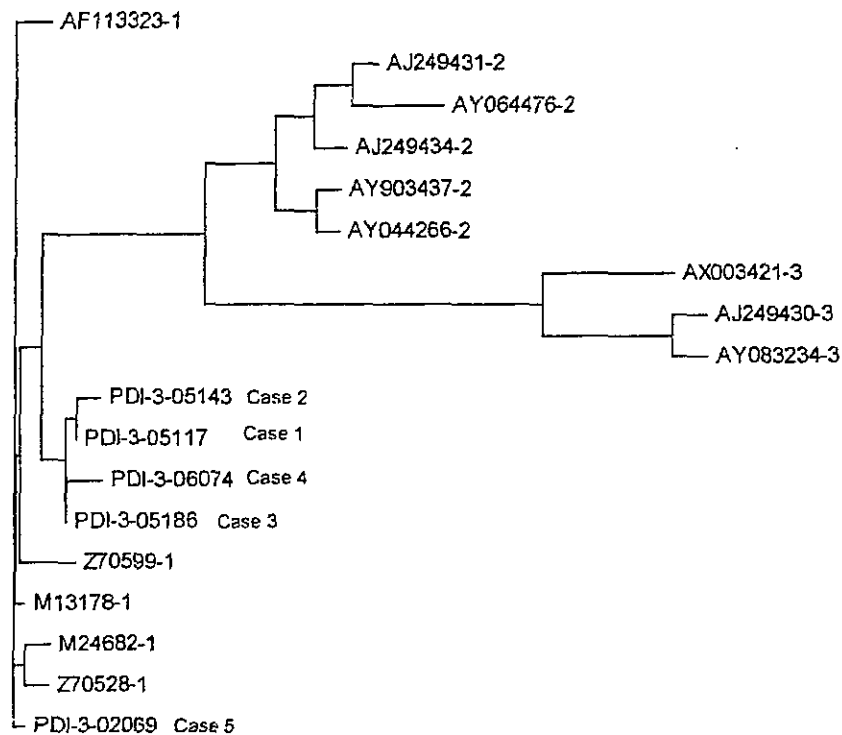


Fig. 1. Phylogenetic tree of B19V DNA for five established cases. PDI-3-05117, -05143, -05186, -06074, and -02069 correspond to Cases 1, 2, 3, 4, and 5, respectively. For these cases, the B19V DNA sequence was identical between those obtained from blood donors and those from transfused patients for the region indicated under Patients and Methods. As references, other sequences published in GenBank from Genotypes 1 to 3 are also shown.

TABLE 2. Probable cases of TT-B19V infection

Case	Patient profile	Before transfusion	After transfusion	Symptoms and laboratory findings	Transfused components
1	52, female Paroxysmal nocturnal hematuria		DNA (+) IgM (+)	High fever General malaise Pancytopenia (1 month)	Washed RBCs (irradiated) DNA (+) 6.8 × 10 ³ IU/mL* IgM (+) IgG (+)
2	28, female Hemolytic anemia	DNA (-)	DNA (+)	Prolonged severe anemia RBC hypoplasia Reticulocytopenia (1 month)	RBCs (irradiated) DNA (+) IgM (+) IgG (+)
3	79, male Pelvic tumor		DNA (+) IgM (+)	No symptoms Reticulocytopenia (1 week)	RBCs (irradiated) DNA(+) IgM (+) IgG (+)

* The washed RBCs were processed from blood containing 6.8 × 10³ IU/mL B19V DNA.

whereas in the other two cases, anti-B19V IgM was positive in the posttransfusion samples, which supports the notion that these three are TT-B19V cases as well.

DISCUSSION

We described in this report five established and three probable TT-B19V cases, seven of which showed overt

clinical illness. The association of the B19V infection with transfusion was proved by serologic data available and viral sequence homology analysis. From a phylogenetic tree of B19V DNA for the five established cases (Fig. 1), it is apparent that the five pairs of B19V DNA all belonging to Genotype 1 are very close but distinct genomes.

There have been only five previously reported cases of TT-B19V caused by the transfusion of blood

components,¹¹⁻¹⁵ one of which was cited in this article as a probable case.¹⁵ Although the TRIP Dutch National Hemovigilance reported one possible case of TT-B19V in 2007,²⁴ there has been no report of TT-B19V in the literature published by hemovigilance systems in other countries including SHOT in the United Kingdom and hemovigilance in France. One asymptomatic case of TT-B19V has recently been reported during a donor-linked prospective study.¹⁸ The possible reasons for the paucity of the reports are as follows: 1) a considerable proportion of transfusion recipients are immune to B19V. 2) B19V infection usually does not cause a serious illness even in nonimmune adults but results in a deleterious outcome only in a small proportion of patients who are in the specific conditions described above. 3) Passive immunization by the transfer of neutralizing anti-B19V often occurs with concurrent transfusion.⁶ 4) The risk factors that increase susceptibility of recipients to severe B19V disease, and the signs and symptoms of TT-B19V infection, are not well recognized among physicians.

In view of almost the same B19V seroprevalence in Japan and western countries,^{2,13,25} it is inconceivable that we encountered these cases because B19V infection is more common in Japan. It is also unlikely that the B19V genotype commonly found in Japan is more virulent and causes more serious illnesses in transfusion recipients because the B19V genome variance is small in a defined genotype and the B19V genotypes found in the established cases are all Genotype 1, the type commonly found in western countries. Moreover, B19V-related serious illnesses as a result of infusion of B19V-contaminated plasma derivatives have often been reported in western countries.² We speculate that we were able to collect TT-B19V cases owing to the efficient hemovigilance system of JRC. It should also be noted that physicians are very cooperative in reporting suspected TTIs to blood centers, which is possibly facilitated because JRC laboratories are capable of investigating the causality of TTIs using complete sample archives from all blood donations.

If the implementation of a universal B19V screening is under consideration or required by a national authority regulating blood program, it is necessary to establish a cutoff level for screening. In this regard, the infectious dose of B19V DNA in blood components that might cause TTIs has been a subject of debate.^{17,26-28} In general, a viral load of approximately 10^5 IU/mL is becoming to be accepted as an infectious dose for TT-B19V. In our clinical observation, the viral concentrations of the components that caused RBC aplasia in susceptible patients were 1.8×10^5 IU/mL in Case 1 and 9.7×10^8 IU/mL in Case 2. The viral concentrations of the components that caused less severe symptoms such as febrile reaction or skin eruption were 3.0×10^5 IU/mL in Case 3 and 5.1×10^3 IU/mL in Case 4. Another patient with probable TT-B19V in our case series developed a sustained pancytopenia after the trans-

fusion of a washed RBC unit processed from a donation containing 6.8×10^3 IU/mL B19V. Although these data may indicate that the components with very low viral loads are infectious, it is yet to be determined whether the lower limit of infectivity is 10^3 or 10^5 IU/mL, because most previous studies have not dealt with the blood components with viral loads in this range and few data are available to determine the lower limit.

While data necessary to determine the infectious dose of B19V in the blood components are still lacking, clinical symptoms due to B19V infection acquired by blood component transfusion appear to be related to the clinical state of the transfusion recipient. Each clinical course of the five established cases described in this report represents typical characteristics of acute B19V infection after a respiratory tract infection that have been described in the literature.^{1,3} When a patient with an enhanced erythropoiesis receives a transfusion with a B19V-containing component, the cell production of a lineage susceptible to B19V, mainly the RBC lineage,^{29,30} is totally impaired by B19V, which directly leads to RBC aplasia in the marrow and anemia in the peripheral blood because the RBC count or hemoglobin level in their peripheral blood was maintained by the enhanced erythropoiesis as presented in Cases 1, 2, and 5. If a patient has been in the state of immunosuppression, it will delay the elimination of B19V and the recovery of the affected cell lineage in the marrow. The underlying diseases for the two of the three probable cases (i.e., paroxysmal nocturnal hematuria and hemolytic anemia) also represent the typical conditions that could lead to serious hematologic disorders after B19V infection. On the other hand, in recipients who are immunologically competent and not in the state of an enhanced erythropoiesis, B19V transmission may not occur or if TT-B19V occurs it may result in outcomes limited to laboratory findings (e.g., seroconversion or DNA conversion) or moderate symptoms not more severe than a sustained fever, generalized skin lesions, or arthropathy, as shown in Cases 3 and 4. These symptoms subsequently resolved without medication possibly owing to a rapid viral neutralization by their intact immune response before anemia developed. It is possible that the patient in Case 4 presented only mild symptoms not because the viral load of the component was low but because he was not in the state of an enhanced erythropoiesis when transfused. It is, thus, more explainable to consider that the clinical state of the patient rather than the viral load of the component determines the clinical course of TT-B19V. In Cases 2 and 5, there is a slight possibility that the clinical course of acute B19V infection was modified by the passive transfer of neutralizing antibodies from other PLT concentrates transfused. None of the patients presented above received FFP transfusion.

It could, however, be argued that we have identified TT-B19V cases associated with a relatively low viral load

and specific IgM because these cases were identified under the screening by RHA, which detects and excludes very high titer viremic donations. It is therefore possible that TT-B19V associated with low-viral-load component transfusion is only a portion of all TT-B19V cases that could be represented by cases transfused with high-viral-load components.

It is of note that all the components for the three probable TT-B19V cases as well as the five established cases were positive for anti-B19V IgM. In the four cases of TT-B19V reported in the literature except for one probable case described in this article, 2 of the 3 units of transfused components tested were also specific IgM positive.^{11,12,14} These findings strongly suggest that the presence of specific IgM in the component with or without specific IgG is a risk factor for TT-B19V. The positivity for specific IgM implies that the donors were in the early recovery phase from an acute primary infection. It is therefore conceivable that the seven blood donors in this study who were also positive for specific IgG had anti-B19V IgG of insufficient titer or an immature specificity that is incapable of full neutralization of B19V, although it has been considered that infectivity is absent or at least modulated once specific IgG is present.

Deep insight is needed to determine whether universal preventive measures should be implemented to eradicate TT-B19V. First, the degree of seriousness of B19V-related illnesses has to be taken into consideration. Sustained fever is uncomfortable and skin lesions are painful to patients but they are essentially benign and self-limited with bearable duration. Although the condition of TT-B19V-related pure RBC aplasia sometimes requires RBC transfusion, patients eventually recover from anemia. Pancytopenia presents a real problem necessitating a course of intensive therapy.³¹ It must be carefully deliberated which of these illnesses should be the target of a new preventive measure that might be implemented. Second, cost-effectiveness must be considered relative to the frequency of TT-B19V. In Japan, more than 5 million people donate blood and approximately 1 million patients receive blood transfusion annually. In such a circumstance, TT-B19V was found to occur at such a low frequency that only seven cases with symptoms were reported during the past 10 years, four of which had an impaired RBC production and one had pancytopenia. These cases were among only 15 reports of suspected TT-B19V, suggesting either that TT-B19V occurrence is very rare or that most clinicians are not aware of TT-B19V. Moreover, most symptoms eventually spontaneously resolve, which might lead to the overlooking or misdiagnosis of the infection. These clinical outcomes of TT-B19V may not support the idea of implementation of a universal donor screening strategy to cope with TT-B19V. However, if more evidence is accumulated showing TT-B19V-related serious illnesses, its implementation may be required in the future.

The universal screening of donated blood for B19V by nucleic acid testing (NAT)-based algorithm is currently carried out in Germany.²⁷ With the detection limit of 10^5 IU/mL, it is surely contributing to the decrease in not only the viral load in pooled source plasma but also the frequency of seroconversion or symptomatic infection after component transfusion. Our finding of the infectivity of blood components with 10^3 IU/mL viral load suggests that this measure may not completely eliminate TT-B19V cases with serious hematologic disorders. The implementation of NAT screening with a much higher sensitivity for B19V is, however, unlikely because it would impair the current blood program because it would result in the discarding of a considerable number of components.

Another strategy may be feasible in which an indication for the transfusion of B19V-safer blood components is defined and components with negligible B19V infectivity are identified, the strategy currently being recommended in the Netherlands.³² Our experience with TT-B19V enabled us to define patients at risk of B19V-related serious illnesses; that is, patients with the indication of TT-B19V-safer components, namely, B19V-seronegative patients with an enhanced erythropoiesis or with hereditary RBC disorders having an increased RBC turnover.³ Seronegative pregnant women are another population at risk because of the high risk of hydrops fetalis.¹ Blood components with no or negligible viral loads will be identified by screening a proportion of the current component inventory using NAT with high sensitivity. To avoid a donation during the NAT window period and early recovery phase after acute infection, components should be selected among donations that show positivity for specific IgG as well as negativity for specific IgM continuously over a long period (e.g., >6 months or 1 year).³²

Pathogen reduction and/or inactivation is a novel strategy for the prevention of TTIs.³³ It is, however, considered to be difficult in general to mitigate the B19V infectivity of blood components because of the rigid viral capsid of B19V that hinders the entry of the photosensitizer and the extremely high viral load found in blood donors in acute-phase B19V infection.

In conclusion, eight cases of TT-B19V caused by transfusion with B19V-contaminated blood components have been identified through the hemovigilance system. Whether a patient developed a serious B19V-related hematologic disorder as a result of component transfusion depended on the patient's underlying disease state such as an enhanced erythropoiesis, not on the viral concentration of the component transfused.

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CONFLICT OF INTEREST

The authors have no conflict of interest for this article.

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医薬品
医薬部外品 研究報告 調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2011 年 4 月 12 日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①②③ポリエチレングリコール処理抗破傷風人免疫グロブリン ④⑤乾燥抗破傷風人免疫グロブリン		研究報告の 公表状況	Vox Sanguinis 2011; (E-PUB AHEAD):1-8	公表国 ドイツ	
販売名 (企業名)	①テタノブリン IH 静注 250 単位 (ベネシス) ②テタノブリン IH 静注 1500 単位 (ベネシス) ③テタノブリン・IH (ベネシス) ④テタノブリン筋注用 250 単位 (ベネシス) ⑤テタノブリン (ベネシス)					
研究報告の概要	13 種類の異なる血漿由来と組換え凝固因子製品の少なくとも 3 つの異なるバッチを、定量 PCR を使用して、パルボウイルス B19、ヒトボカウイルス、ヒトパルボウイルス 4、A 型肝炎ウイルスと E 型肝炎ウイルスの核酸の存在と量を試験した。組換え製品は如何なるウイルスも陽性反応を示さなかったのに対して、5 つの血漿由来製品については 2×10^1 と 1.3×10^3 の間の遺伝子量/mL のパルボウイルス B19 の DNA を検出した。パルボウイルス B19 の遺伝子型 1 に加えて遺伝子型 2 と 3 が、第Ⅷ因子/フォン・ヴィルブランド因子製品の 2 バッチで観察された。二つの製品（一つは第Ⅷ因子濃縮製剤、一つは活性化プロトロンビン複合濃縮製剤）は、パルボウイルス B19 の遺伝子型 1 と 2 の両方が検出された。データは、幾つかの関連するノンエンベロープウイルスからの核酸が、凝固因子濃縮製剤中で検出可能レベルで見つからないことを示した。いくつかのケースで、パルボウイルス B19 の DNA は低レベルで検出された。全てのパルボウイルス遺伝子型についての血漿プールでの検査が、製品の安全性を確保するために提唱される。					使用上の注意記載状況・ その他参考事項等 代表としてテタノブリン IH 静注 250 単位の記載を示す。 1. 慎重投与（次の患者には慎重に投与すること） (1) 略 (2) 略 (3) 溶血性・失血性貧血の患者〔ヒトパルボウイルス B19 の感染を起こす可能性を否定できない。感染した場合には、発熱と急激な貧血を伴う重篤な全身症状を起こすことがある。〕 (4) 免疫不全患者・免疫抑制状態の患者〔ヒトパルボウイルス B19 の感染を起こす可能性を否定できない。感染した場合には、持続性の貧血を起こすことがある。〕 2. 重要な基本的注意 (1) 略 1) 血漿分画製剤の現在の製造工程では、ヒトパルボウイルス B19 等のウイルスを完全に不活化・除去することが困難であるため、本剤の投与によりその感染の可能性を否定できないので、投与後の経過を十分に観察すること。 6. 妊婦、産婦、授乳婦等への投与 妊婦又は妊娠している可能性のある婦人には、治療上の有益性が危険性を上回ると判断される場合にのみ投与すること。〔妊娠中の投与に関する安全性は確立していない。本剤の投与によりヒトパルボウイルス B19 の感染の可能性を否定できない。感染した場合には胎児への障害（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	
ヒトパルボウイルス B19 (human parvovirus B19 : B19) は、脂質エンベロープを持たない極めて小さな（約 20～26nm）DNA ウィルスで、輸血や血漿分画製剤による伝播が報告されている。他のウィルスに比べて、血漿分画製剤の製造工程での不活化・除去が困難であり、本ウィルスの伝播リスクを完全に否定することはできないため、1996 年 11 月より、使用上の注意に B19 についての記載を行い注意喚起を図ってきた。万一、B19 が原料血漿に混入したとしても、CPV をモデルウィルスとしたウィルスバリデーション試験成績及び B19 を用いた不活化・除去試験の結果から、製造工程において十分に不活化・除去されると考えている。				本報告は本剤の安全性に影響を与えないものと考えるので、特段の措置はとらない。		

Prevalence of nucleic acid sequences specific for human parvoviruses, hepatitis A and hepatitis E viruses in coagulation factor concentrates

S. Modrow,¹ J. J. Wenzel,¹ S. Schimanski,^{1*} J. Schwarzbeck,² U. Rothe,² J. Oldenburg,³ W. Jilg¹ & A. M. Eis-Hübing⁴

¹Institute for Medical Microbiology and Hygiene, University of Regensburg, Regensburg, Germany

²Pharmacy of the University Hospital Regensburg, Regensburg, Germany

³Institute of Experimental Haematology and Transfusion Medicine, University of Bonn Medical Center, Bonn, Germany

⁴Institute of Virology, University of Bonn Medical Center, Bonn, Germany



Background and Objectives Due to their high resistance to inactivation procedures, nonenveloped viruses such as parvovirus B19, human bocavirus (HBoV), human parvovirus 4 (PARV4), hepatitis A (HAV) and hepatitis E virus (HEV) pose a particular threat to blood products. Virus transmission to patients treated with blood products presents an additional burden to disease. We determined the frequency and the amount of nucleic acid specific for nonenveloped viruses in recently manufactured preparations of commercial coagulation factor concentrates.

Materials and Methods At least three different batches of each of 13 different plasma-derived and recombinant coagulation factor products were tested for the presence and the amount of nucleic acid for parvovirus B19, HBoV, human parvovirus 4, hepatitis A virus and HEV by using quantitative polymerase chain reaction.

Results Whereas none of the recombinant products tested positive for any of these viruses, parvovirus B19 DNA with amounts ranging between 2×10^1 and 1.3×10^3 genome equivalents/ml was detected in five plasma-derived products. In addition to parvovirus B19 genotype 1, genotypes 2 and 3 were observed in two batches of a factor VIII/von-Willebrand factor product. In two products (one factor VIII concentrate and one activated prothrombin complex concentrate), a combination of both genotypes 1 and 2 of parvovirus B19 was detected.

Conclusion The data show that nucleic acids from several relevant nonenveloped viruses are not found at detectable levels in coagulation factor concentrates. In some cases, parvovirus B19 DNA was detectable at low levels. Testing of the plasma pools for the full range of parvovirus genotypes is advocated for ensuring product safety.

Key words: hepatitis virus, human bocavirus, iatrogenic transmission, parvovirus B19, plasma-derived coagulation factor products, recombinant coagulation factor products.

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Correspondence: Susanne Modrow, Institute for Medical Microbiology and Hygiene, University of Regensburg, Regensburg, Franz-Josef-Strauß Allee 11, 93053 Regensburg, Germany
E-mail: susanne.modrow@klinik.uni-regensburg.de

*Present address: Institute of Laboratory Medicine and Microbiology, Central Hospital Bayreuth, Preuschwitzer Straße 101, 95445 Bayreuth, Germany.

Introduction

Nonenveloped viruses, such as hepatitis A virus (HAV), hepatitis E virus (HEV) and parvovirus B19 (B19V); human bocavirus (HBoV) and human parvovirus 4 (PARV4), are characterized by resistance against inactivation by heat and detergent [1–3]. Consequently, blood and plasma donations from individuals who are acutely or persistently infected with any of these viruses pose a particular problem as

contamination sources in blood-derived products. Despite the use of donor selection criteria, screening of donations for serological markers indicative for virus infection, testing of donations and/or plasma pools by polymerase chain reaction (PCR) and the application of inactivation procedures, the risk of viral transmission cannot be completely eliminated [4]. As most viruses are characterized by high genetic variability, the generation of novel and rare variants or genotypes undetectable by routine assays may also represent additional problems [5–7]. The situation is further complicated by the fact that a balance has to be achieved between maximal viral inactivation and preservation of the functional activity of labile proteins, such as coagulation factors following specific inactivation procedures.

Viral infections that are transmitted by transfusion to patients requiring fractionated plasma products (e. g., haemophiliacs, transplant recipients, cancer patients and pregnant women) can seriously add to the burden of the respective disease. Whereas HAV and HEV predominantly cause hepatitis, B19V infection is associated with a variety of conditions, including febrile illness, erythema infectiosum, transient aplastic crisis, transient arthropathy and pure red cell aplasia [8]. Infection during pregnancy can result in hydrops foetalis and foetal death [9]. In addition, B19V is known to trigger various autoimmune conditions. HBoV infections have been mainly reported in young children with respiratory diseases including bronchiolitis and pneumonia, with viremia detectable during acute infection [10,11]. Currently little knowledge is available concerning the clinical consequences of PARV4 infection. The virus was originally identified in a HBV-infected patient displaying fatigue, vomiting and diarrhoea, sore throat, neck stiffness and joint pains [12]. PARV4 DNA has been predominantly detected in blood and tissue from individuals at risk for parenterally transmitted viruses such as HIV-/HCV-infected intravenous drug users and, to a lower degree, in tissue of individuals without any history of injection-drug use [13–15].

Hepatitis A virus and B19V have in the past been well-documented potential contaminants of plasma pools [16]. Recently identified viruses such as HEV, HBoV, PARV4 and newly identified B19V/genotypes 2 and 3 present new risks to product safety. HAV was undetectable in most plasma products tested with the exception of some brands of solvent-detergent (S/D)-treated factor VIII concentrates [5,17]. There are however reports of transfusion-transmitted HAV infections, involving either single or multiple patients [18,19]. During the last few years, most commercial manufacturers of plasma products have introduced HAV nucleic acid testing of plasma pools. Studies on HEV-specific IgG in blood samples from commercial blood donors and haemophiliacs in hyperendemic areas have shown prevalence rates of approximately 24% [20].

Furthermore, transfusion-transmitted hepatitis E has been reported following transfusion of blood components in both endemic regions [21,22] and also in non- or low-endemic areas [23,24]. Currently, however, there are no regulatory requirements to test the HEV load of plasma pools.

Acute B19V infection is associated with high levels of viremia of up to 10^{13} viral particles per millilitre of blood during the days preceding illness which may be followed by low to medium level viremia for several months. Consequently, B19V was detected in more than 60% of manufacturing plasma pools prior to the general introduction of plasma pool testing for B19V-DNA by PCR [25–27]. Siegl *et al.* reported the presence of B19V DNA in 50–80% of nonvirally inactivated coagulation factor VIII preparations and in 30–80% of S/D-treated coagulation factor IX preparations [28]. More recently, B19V- and PARV4-derived nucleic acid sequences were detected in 40% and 16% of factor VIII concentrates, respectively [29]. To reduce the risk of parvovirus B19 transmission by plasma derivatives, the Food and Drug Administration (FDA) of the United States has limited the B19V-DNA load in manufacturing plasma pools to 10^4 IU/ml or $6-8 \times 10^3$ geq/ml [30]. In Europe, nucleic acid testing and a maximal B19V DNA load of 10^4 IU/ml are obligatory for S/D-treated human plasma [31]. The European Medicines Agency (EMA) has also recommended B19V nucleic acid testing. This is not however mandatory, particularly so when inactivation and removal procedures that result in a significant reduction in the load of nonenveloped viruses are in place [32]. A higher prevalence of viral contamination (B19V, 45% versus 30%; PARV4, 23% versus 2%) was observed in products expiring pre-1990 when compared to those expiring post-1995 [29]. This likely reflects improvements in overall safety of the purification methods introduced over this period. A similar or even more pronounced difference between older and newer clotting factor concentrates was observed in a second study comparing clotting factor concentrates used up to the beginning of the 1980s and from 2000 to 2003 (B19V, 81% versus 43%; PARV4, 33% versus 9%) [33].

Confidence in the viral safety of plasma products remains a basic concern. We have undertaken a study to determine the level of contamination in currently used commercial plasma-derived and recombinant coagulation factor concentrates with both well-known viruses as HAV, HEV and B19V/genotype 1, and with recently discovered agents as HBoV, PARV4 and B19V/genotypes 2 and 3.

Methods

Coagulation factor concentrates

One vial of at least three different batches of coagulation factor concentrates, produced and distributed by eight

different manufacturers (A-H), were purchased between December 2007 and December 2008. All coagulation factor products were randomly selected. The plasma-derived products were as follows: one coagulation factor VII concentrate, three different coagulation factor VIII products, two different coagulation factor IX products, three different factor VIII/von-Willebrand factor (VIII/vWF) products and one activated prothrombin complex concentrate (APCC) (Table 1). A number of recombinant products were also evaluated. These comprised one factor VIIa, one factor VIII and one factor IX product (Table 2). All products are approved for the treatment of haemophilic patients in Germany. Most coagulation factor products were produced in Central Europe or in the United States of America and are used in a number of different countries. One factor VIII product (manufacturer C) was produced and distributed exclusively in Germany.

Viral genome amplification

The coagulation factor concentrates were reconstituted in accordance with the manufacturer's instructions. Nucleic acid was isolated in triplicate from 280 µl of the reconstituted product using the QIAamp Viral RNA kit (Qiagen, Hilden, Germany). Each nucleic acid preparation (120 µl final eluate) was either used immediately or else stored at -20°C. All nucleic acid preparations were quantitatively analysed in duplicates in two independent test runs. B19V DNA was detected using a quantitative real-time TaqMan PCR assay established for amplification of B19V genomes, genotypes 1-3 [34]. The sensitivity of detection of parvovirus B19 genomes was determined using the international standard 99/800 and the methodology described before [34]. The 100% detection limit of the TaqMan PCR is 10 copies per reaction. When positive results were obtained, additional assays were performed using probes specific for B19V genotypes 1, 2 and 3 in separate test runs. HBoV- and PARV4-PCR assays were performed as described previously and the respective sensitivities were adjusted using plasmid-cloned viral genome segments [11,35,36]. For detection of HAV- and JIEV-genomes quantitative RT-PCR was done according to Houde *et al.* [37] and Brost *et al.* [38]. Results were considered positive if viral nucleic acid was detected in either two of three independent nucleic acid preparations and/or in both independent qPCR runs.

Results

Nucleic acid specific for HBoV, PARV4, HAV and JIEV was not detected in any of the plasma-derived coagulation factor concentrates (Table 1). Detectable amounts of B19V DNA (range $\sim 2 \times 10^1$ – 1.3×10^3 geq/ml), however, were found in one of three batches of the factor VII product, in

one of three batches of two different factor VIII products (manufacturers B and C), in three of four batches of one factor VIII/von-Willebrand factor product and in four of eight batches of the APCC. The overall detection rate was 26% (10/39). The highest viral loads were observed in the factor VIII/vWF concentrate. Using separate PCR assays specific for amplification of genotypes 1, 2 and 3, the B19V DNA sequences detected in one clotting factor VIII concentrate (manufacturer C) were identified as simultaneous presence of genotypes 1 and 2 DNA (Table 3). Two of three B19V DNA-positive batches of the factor VIII/vWF product (manufacturer D) contained a combination of all three genotypes, whereas only B19V/genotype 1 was observed in the third positive batch of that product. One B19V DNA-positive batch of the APCC contained a combination of genotypes 1 and 2. In the remaining batches that had been tested positive by the screening PCR, B19V/genotype 1 DNA was identified. None of the lots of the recombinant factor products (manufacturers G and H) contained nucleic acids derived from any of the viruses studied (Table 2).

Discussion

The importance of avoiding transmissible diseases was recognized early in the development of plasma fractionation. For decades, various procedures for virus inactivation and removal have been in use and improved continually. Pooling of plasma donations for the production of coagulation factor concentrates, however, increases the risk of viral transmission, especially for those viruses that are associated with high levels of viremia [39]. The presence of B19V DNA in plasma-derived coagulation factor products occurs not only because of incomplete virus elimination but also to the high-level viremia in acutely infected individuals [3,40]. In the study presented here, B19V DNA was found in 10 of 39 (26%) lots of plasma-derived products and in none of the recombinant products tested. The prevalence was 20% when the product exhibiting the highest B19V detection rate (a factor VIII/vWF product) was excluded and 11% when excluding both the first and second most frequently contaminated product (a factor VIII/vWF product and an APCC). B19V/genotype 1 is currently the most prevalent variant worldwide and consistent with this was detected most frequently in this study. In four lots, however, genotype 2 virus was found. This variant is assumed to have been more prevalent decades ago but is currently circulating at a very low level. In two lots, B19V/genotype 3 was detected. B19V/genotype 3 is prevalent in West- and South-Africa, but has only occasionally been detected also in other countries [41-43].

Our B19V-DNA detection rate is the range of that reported by Geng and co-workers who reported an overall

Table 1 Detection of viral nucleic acids in plasma-derived coagulation factor concentrates

Products	Manufacturer	Type* and origin of plasma pools	Manufacturing and inactivation procedure	IU	Batch	Expiry date (month/year)	B19V	HBoV	PARV4 geq/ml	HAV	HEV
Factor VII	A	Apheresis plasma Central Europe ^b , USA	Ion-exchange chromatography, nanofiltration (35 nm), vapour-heat (60°C/10 h, 80°C/1 h)	600	1	07/2009	5.2×10^1	n.d.	n.d.	n.d.	n.d.
					2	04/2009	n.d.	n.d.	n.d.	n.d.	n.d.
					3	10/2009	n.d.	n.d.	n.d.	n.d.	n.d.
Factor VIII	B	Recovered plasma Central Europe, USA	Anion exchange chromatography, S/D, dry heat (100°C/30 min)	250	1	10/2008	1.0×10^2	n.d.	n.d.	n.d.	n.d.
					2	04/2009	n.d.	n.d.	n.d.	n.d.	n.d.
					3	01/2010	n.d.	n.d.	n.d.	n.d.	n.d.
	C	Central Europe, USA	Anion exchange chromatography, S/D, dry heat (100°C/30 min)	250	1	02/2009	1.0×10^2	n.d.	n.d.	n.d.	n.d.
					2	10/2009	n.d.	n.d.	n.d.	n.d.	n.d.
					3	03/2010	n.d.	n.d.	n.d.	n.d.	n.d.
	D	Unclear Central Europe, USA	Ion exchange chromatography, S/D, terminal dry heat (100°C/30 min) at residual moisture	1000	1	08/2007	n.d.	n.d.	n.d.	n.d.	n.d.
					2	09/2007	n.d.	n.d.	n.d.	n.d.	n.d.
					3	11/2007	n.d.	n.d.	n.d.	n.d.	n.d.
					4	01/2008	n.d.	n.d.	n.d.	n.d.	n.d.
					5	02/2008	n.d.	n.d.	n.d.	n.d.	n.d.
					6	05/2008	n.d.	n.d.	n.d.	n.d.	n.d.
Factor IX	E	Apheresis plasma USA	Ion-exchange and polysaccharide ligand chromatography, S/D, nanofiltration	500	1	05/2009	n.d.	n.d.	n.d.	n.d.	n.d.
					2	09/2009	n.d.	n.d.	n.d.	n.d.	n.d.
					3	01/2010	n.d.	n.d.	n.d.	n.d.	n.d.
Factor VIII/vWF	F	Recovered plasma Central Europe, USA	DEAE-Sephadex, heparin affinity chromatography, pasteurization (60°C/10 h)	600	1	09/2009	n.d.	n.d.	n.d.	n.d.	n.d.
					2	11/2009	n.d.	n.d.	n.d.	n.d.	n.d.
					3	11/2009	n.d.	n.d.	n.d.	n.d.	n.d.
	D	Unclear Central Europe, USA	Ion exchange and size exclusion chromatography, S/D, terminal dry heat (100°C/2 h) at residual moisture	450	1	03/2009	1.1×10^3	n.d.	n.d.	n.d.	n.d.
					2	03/2009	1.3×10^3	n.d.	n.d.	n.d.	n.d.
					3	11/2009	n.d.	n.d.	n.d.	n.d.	n.d.
				900	4	11/2005	3.0×10^2	n.d.	n.d.	n.d.	n.d.
					1	05/2009	n.d.	n.d.	n.d.	n.d.	n.d.
					2	10/2009	n.d.	n.d.	n.d.	n.d.	n.d.
	E	Apheresis plasma USA	Heparin ligand chromatography, S/D, dry heat (80°C/72 h)	250	3	02/2010	n.d.	n.d.	n.d.	n.d.	n.d.
					1	09/2009	n.d.	n.d.	n.d.	n.d.	n.d.
					2	04/2010	n.d.	n.d.	n.d.	n.d.	n.d.
	F	Recovered plasma Central Europe, USA	Multiple precipitations, pasteurization (60°C/10 h)	250	3	04/2010	n.d.	n.d.	n.d.	n.d.	n.d.

Table 1 (Continued)

Products	Manufacturer	Type ^a and origin of plasma pools	Manufacturing and inactivation procedure	IU	Batch	Expiry date (month/year)	B19V	HBoV	PARV4 gcq/ml	HAV	HEV
APCC	A	Apheresis plasma Central Europe, USA	DEAE-sephadex, nanofiltration (35 nm), vapour-heat (60°C/10 h, 80°C/1 h)	500	1	03/2009	4.3×10^1	n.d.	n.d.	n.d.	n.d.
					2	10/2009	2.1×10^1	n.d.	n.d.	n.d.	n.d.
					3	08/2009	n.d.	n.d.	n.d.	n.d.	n.d.
					4	02/2008	3.0×10^1	n.d.	n.d.	n.d.	n.d.
					5	08/2008	3.3×10^1	n.d.	n.d.	n.d.	n.d.
					6	03/2008	n.d.	n.d.	n.d.	n.d.	n.d.
					7	04/2008	n.d.	n.d.	n.d.	n.d.	n.d.
					8	09/2008	n.d.	n.d.	n.d.	n.d.	n.d.

B19V, parvovirus B19; HBoV, human bocavirus; PARV4, human parvovirus 4; HAV, hepatitis A virus; HEV, hepatitis E virus; n.d., nondetectable according to the criteria given in Methods; gcq, genome equivalent; APCC, activated prothrombin complex concentrate; vWF, von-Willebrand factor.

^aInformation concerning the type (apheresis plasma, recovered plasma) and the origin of plasma was obtained from 'Guide for the Assessment of Clotting Factor Concentrates' [57] and from the manufacturers' information sheets, respectively.

^bCentral Europe: Following countries were indicated by the individual manufacturers as source for plasma pools: Germany, Austria, Czech Republic, Poland, Sweden, Belgium, Netherlands. Respective information was not provided by the manufacturer.

prevalence of 28% for B19V DNA in clotting factor VIII concentrates produced during 2001–2004 [44]. They observed a prevalence of 19% when excluding a factor VIII product made from recovered plasma that had not been screened for B19V DNA. In this particular factor VIII product, the B19V DNA detection rate was as high as 90%. Consistent with our findings, all positive lots made from sourced plasma contained $<10^3$ genome equivalents/ml, with 84% containing $<10^2$ genome equivalents/ml. This level of B19V-DNA is consistent with the screening of plasma pools that generally takes place in order to ensure that high-titre donations are excluded in order to meet regulatory guidance that the pool must contain $<10^4$ IU of B19V-DNA per ml [30,31]. We observed viral DNA concentrations of $>10^3$ genome equivalents/mL only in the product presenting the highest B19 detection rate. Thus, our data are in line with those from previous studies showing an ongoing but continuously decreasing B19V contamination in blood-derived coagulation factor concentrates.

Despite the presence of B19V-DNA in blood products, transmission of clinically overt infections is rare in adults [3,45–47]. Since various effective methods for virus inactivation are applied during the manufacturing process, the detection of viral genome equivalents cannot be equated with infectious viral particles. Several additional factors probably contribute to this fact including the generally mild course of B19V infection in immunocompetent individuals, the destruction of infectivity during the manufacturing procedures, the presence of B19V-specific neutralizing IgG in plasma pools as well as the fact that B19V IgG seroprevalence is high in the adult population [48]. Even in haemophiliacs receiving large amounts of plasma-derived products, symptomatic B19V infections are relatively uncommon [49]. Three groups of patients are, however, particularly vulnerable to B19V infections, i.e. seronegative pregnant women in whom acute infection may result in hydrops foetalis and foetal death, patients with low erythropoietic reserves and immunocompromised patients, particularly children [50,58]. In immunodeficient individuals, B19V infection can lead to severe persisting anaemia and, occasionally, pancytopenia [8]. As the immune system of many children has not been stimulated by prior B19V infection, they are particularly affected by complications. In individuals with increased red cell turnover, a potentially life threatening aplastic crisis may occur. Although detection of B19V DNA is not synonymous with infectivity, it would be wise to ensure that blood products given to any of the at-risk patient groups are completely free of B19V.

The absence of HBoV in coagulation factor concentrates is consistent with the findings of others investigating blood donations [29,51]. The absence of detection of HAV is in agreement with the low risk level of transfusion-

Table 2 Analysis of viral nucleic acids in recombinant coagulation factor products

Product	Manufacturer	Batch	Expiry data (month/year)	B19V	HBoV	Parv4	HAV	HEV
Factor VIIa	H	1	02/2010	n.d.	n.d.	n.d.	n.d.	n.d.
		2	04/2010	n.d.	n.d.	n.d.	n.d.	n.d.
		3	05/2010	n.d.	n.d.	n.d.	n.d.	n.d.
Factor VIII	G	1	10/2008	n.d.	n.d.	n.d.	n.d.	n.d.
		2	11/2008	n.d.	n.d.	n.d.	n.d.	n.d.
		3	06/2009	n.d.	n.d.	n.d.	n.d.	n.d.
Factor IX	G	1	01/2010	n.d.	n.d.	n.d.	n.d.	n.d.
		2	03/2010	n.d.	n.d.	n.d.	n.d.	n.d.
		3	06/2010	n.d.	n.d.	n.d.	n.d.	n.d.

B19V, parvovirus B19; HBoV, human boca-virus; PARV4, human parvovirus 4; HAV, hepatitis A virus; HEV, hepatitis E virus; n.d., not detectable.

Table 3 Analysis of the parvovirus B19 genotypes in B19V DNA-positive coagulation factor concentrates^a

Product	Manufacturer	Batch	B19V genotype 1 (geq/ml)	B19V genotype 2 (geq/ml)	B19V genotype 3 (geq/ml)
Factor VIII	C	1	2.1×10^1	3.4×10^1	n.d.
Factor VIII/vWF	D	1	1.9×10^3	1.1×10^3	6.8×10^3
		2	2.1×10^3	7.8×10^2	4.8×10^3
APCC	A	2	1.1×10^1	3.5×10^1	n.d.

APCC, activated prothrombin complex concentrate; n.d., not detectable.

^aConcentrates that contained parvovirus B19 genotypes 2 and/or 3.

transmitted HAV infection which might be due to the lack of a carrier state. Concerning HEV, there are a number of case reports on transfusion-associated infections but to the best of our knowledge there is no systematic study on HEV presence in coagulation factor concentrates. Although we were unable to detect HEV RNA, further and larger studies are needed to exactly assess the risk of HEV transmission by blood products.

Due to its recent discovery, the situation regarding PARV4 is unclear. In two studies, decreasing rates were observed for PARV4 DNA prevalence in coagulation factor concentrates over the past three decades [29,33]. In these studies, PARV4 DNA prevalence was 1.7% in concentrates with expiry dates from 1997 to 2005 and 8.9% in products used in 2000–2003. It may be questioned whether this decline reflects the impact of improved viral inactivation and removal procedures that are now routinely applied during the manufacturing process, or whether it is the result of an altered spread of the virus. The epidemiologic pattern of PARV4 is also unclear. Initially, the infections seemed to be restricted to intravenous drug users and HIV-/HCV-infected individuals indicating parenteral transmission [13,15]. Very recently, however, a PARV4 prevalence of 24% found in a study involving 304 volunteer blood donors from south-eastern France was reported

[52]. In contrast, of 478 blood donors from Paris, France, no confirmed positive donation was found [53] and similarly, no positives were detected when analysing diagnostic minipools representing 69 632 individual donations obtained from voluntary donors in Southern Germany [51]. Whether seasonal or geographic or other reasons account for these striking differences in the prevalence of PARV4 has to be elucidated. Moreover, further studies are needed in order to assess the risk to the safety of the blood products.

The introduction of recombinant coagulation factors has helped avoid viral contamination and transmission. In older products, however, addition of human plasma proteins, e.g. albumin was used to stabilize isolated coagulation factors, and B19V DNA was detected in a small proportion of investigated lots [54]. Today, third-generation recombinant products are available. These are formulated using a plasma/albumin-free method (i.e. free of animal and human protein additives) further reducing the risk of viral contamination and transmission [55]. However, the higher costs of these products must be taken into account when selecting treatment options [56].

In conclusion, our findings reflect the frequent contamination of human plasma pools with parvovirus B19. The data point to the necessity for screening methods that

detect all variants of contaminating virus in donor blood, plasma mini pools and for further improvements in the methods used to remove and/or inactivate the infectivity of nonenveloped viral pathogens from blood products. The lack of HAV, HEV, HBV and PARV4 contamination in the products tested in this study is encouraging.

Acknowledgement

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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 2011 年 1 月 5 日	新医薬品等の区分	厚生労働省処理欄
一般的名称	①②③④⑤ポリエチレングリコール処理人免疫グロブリン ⑥⑦⑧人免疫グロブリン			研究報告の公表状況	公表国 アメリカ	
販売名 (企業名)	①献血グロブリン IH5% 静注 0.5g/10mL (ベネシス) ②献血グロブリン IH5% 静注 1g/20mL (ベネシス) ③献血グロブリン IH5% 静注 2.5g/50mL (ベネシス) ④献血グロブリン IH5% 静注 5g/100mL (ベネシス) ⑤献血グロブリン-IH ヨシトミ (ベネシス) ⑥グロブリン筋注 450mg/3mL「ベネシス」 (ベネシス) ⑦グロブリン筋注 1500mg/10mL「ベネシス」 (ベネシス) ⑧グロブリン-WF (ベネシス)					
研究報告の概要	2010 年 12 月 3 日金曜日、ワシントン発 現時点では、慢性疲労症候群(CFS)、及びマウス白血病ウイルス関連ウイルス(XMRV)感染症に関連付けられている他の疾患の患者の献血延期に関して、特異的な連邦政府の勧告はない。それでも、米国赤十字社は患者とドナーの安全のために、CSF と診断されていることが問診において判明したドナーを無期限に延期する。 XMRV 感染症はいくつかの研究で前立腺癌と慢性疲労症候群と関連付けられているが、現時点ではこれらの疾患との関連は確かめられていない。 現在、XMRV が輸血を介して伝播すると結論づけるにはデータが不十分である。しかし、国立心肺血液研究所(NHLBI)の専門委員会は、ドナーのウイルス頻度を測定するために、輸血感染により、受血者が感染し、病気になるかどうかの調査を実施している。 AABB の組織横断的専門委員会は、利用可能な全てのデータを再評価し、輸血を介して XMRV が伝播する危険性を評価し、必要に応じて緩和戦略を策定し、献血者、受血者および市民に情報を提供するために、更なる行動を推奨している。 米国赤十字社は AABB の推奨を実行し、医学的に CFS との診断歴のあるドナーの献血を AABB の推奨を超えて無期限に延期する。 AABB の専門委員会は、採血機関が一ドナー側で利用できるドナー教材を用いることにより一慢性疲労症候群(CFS) (別名、慢性疲労免疫不全症候群(CFIDS)、または筋痛性脳脊髄炎(ME)) と医者によって診断された潜在ドナーを“献血”、又は“血液成分”から積極的に阻止することを推奨した協会公報 (#10-03) を 2010 年 6 月にリリースした。加えて、献血の日に「あなたは今日、体調は良いですか？」との質問に否定的に応じた場合、CFS 症状のドナーは延期される。 米国赤十字社は AABB の推奨を実行し、CFS 診断の既往歴があるドナーの無期限延期を実施している。					使用上の注意記載状況・ その他参考事項等 代表として献血グロブリン IH5% 静注 0.5g/10mL の記載を示す。 2. 重要な基本的注意 (1) 本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-I 抗体陰性で、かつ ALT(GPT) 値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査(NAT)を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分からポリエチレングリコール 4000 処理、DEAE セファデックス処理等により人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及びウイルス除去膜によるろ過処理及び pH3.9~4.4 の条件下での液状インキュベーション処理を施しているが、投与に際しては、次の点に十分注意すること。

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報告企業の意見

マウス白血病ウイルス (murine leukemia virus : MLV) は、レトロウイルス科ガンマレトロウイルスに属し、ビリオンは球形で直径約100nmの脂質エンベロープを有する比較的大きなRNAウイルスである。万一、MLVが原料血漿に混入したとしても、HIV-1をモデルウイルスとしたウイルスバリデーション試験結果から、本剤の製造工程において十分に不活化・除去され则认为している。

今後の対応

本報告は本剤の安全性に影響を与えないものと考えるので、特段の措置はとらない。

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BENESIS 2011-001

American Red Cross Statement on XMRV and Chronic Fatigue Syndrome

National Headquarters
2025 E Street, N.W.
Washington, DC 20006
www.redcross.org

Contact: Public Affairs Desk
FOR MEDIA ONLY
media@usa.redcross.org
Phone: (202) 303-5551

WASHINGTON, Friday, December 03, 2010 — At present, there are no specific federal recommendations regarding deferral of individuals with Chronic Fatigue Syndrome (CFS) or other diseases that have been associated with Murine Leukemia Virus-related virus (XMRV) infection. Nevertheless, in the interest of patient and donor safety, the American Red Cross will defer indefinitely any donor who reveals during the donor interview that they have been diagnosed with CFS.

XMRV infection has been associated in some studies with prostate cancer and chronic fatigue syndrome, but at the present time these disease associations have yet to be confirmed.

There is currently insufficient data to conclude that XMRV is transmitted through blood transfusion. However, the National Heart, Lung and Blood Institute (NHLBI) Task force is conducting research to determine the frequency of the virus in the donor population, whether it is transfusion-transmitted, and whether recipients become infected and develop the disease.

An AABB Interorganizational Task Force is charged with reviewing all available data, making recommendations for further action to assess the risk of XMRV transmission through blood transfusion, develop mitigation strategies as needed, and to provide information for blood donors, recipients and the public.

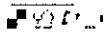
The AABB Taskforce released Association Bulletin #10-03 in June 2010, recommending that blood collecting organizations — through the use of donor education materials available at the donation site — actively discourage potential donors who have ever been diagnosed by a physician with chronic fatigue syndrome (CFS), also known as chronic fatigue and immune dysfunction syndrome (CFIDS) or myalgic encephalomyelitis (ME), from donating blood or blood components. In addition, any donor with symptoms of CFS would be deferred if, on the day of donation, they respond negatively to the question, "Are you feeling well today?"

The Red Cross has implemented the AABB recommendations and has gone further to implement indefinite deferral for donors who reveal a history of a

medical diagnosis of CFS.

About the American Red Cross:

The American Red Cross shelters, feeds and provides emotional support to victims of disasters; supplies nearly half of the nation's blood; teaches lifesaving skills; provides international humanitarian aid; and supports military members and their families. The Red Cross is a charitable organization — not a government agency — and depends on volunteers and the generosity of the American public to perform its mission. For more information, please visit www.redcross.org or join our blog at <http://blog.redcross.org>.

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医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日 2010. 12. 21	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称		人血清アルブミン		古田里佳, 杉山武毅, 宮沢孝幸, 佐藤英次, 倉恒弘彦, 保井一太, 平山文也, 第58回日本ウイルス学 会学術集会; 2010 Nov.7-9; 徳島 県	公表国	
販売名(企業名)		赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン5%静注12.5g/250mL(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)			日本	
研究報告の概要	○Xenotropic murine leukemia virus-related virus(XMRV)抗体特異性の解析 目的:XMRVの輸血感染リスクを評価するには、国内での流行状況の把握が必要である。今回、これまでに同定されたXMRV抗体陽性例が真のXMRV感染によるものかどうかを知るためのひとつのアプローチとして、これら抗体陽性血清の反応特異性を精査した。 材料と方法:抗体スクリーニングには、100倍希釈血清を用いて、XMRVウイルス粒子を抗原としたウエスタンブロッティング(WB)を行った。スクリーニング対象は、一般感染症検査に合格した献血検体から無作為抽出したもの500検体、前立腺がん患者67名、慢性疲労症候群(CFS)患者87名の計654名である。抗体陽性検体の反応特異性はWBにおけるMoloney murine leukemia virus(Mo-MuLV)に対する交差反応性およびNative gelを用いたWBで解析した。 結果:WBスクリーニングで陽性となったのは献血ドナー8名(1.6%)、前立腺がん患者2名(3.0%)、CFS患者2名(2.3%)であり、グループ間の抗体保有率に有意差はなかった。これら陽性検体のGag p30抗体には3パターン of 反応特異性が存在した。XMRV p30のみに結合するもの、Mo-MuLV p30に弱いながらも結合するもの、Mo-MuLV p30にもXMRVと同程度の強さで結合するものである。反応パターンと対象グループとの間に有意な関連性は認められなかった。Envに対する抗体反応は通常のWBでもNative gelを用いたWBでも認められなかった。 考察:XMRV GagとMo-MuLV Gagのアミノ酸レベルでの相同性は約87%である。XMRV抗体陽性者の一部がMo-MuLVと交差反応し、反応強度にもばらつきがあることから、現在までに同定されたXMRV Gag抗体陽性例の必ずしも全てがXMRV既感染を示すものではない可能性が示唆される。					使用上の注意記載状況・ その他参考事項等
	赤十字アルブミン20 赤十字アルブミン25 赤十字アルブミン5%静注 12.5g/250mL 赤十字アルブミン20%静注 4g/20mL 赤十字アルブミン20%静注 10g/50mL 赤十字アルブミン25%静注 12.5g/20mL 血液を原料とすることによる 感染症伝播等					
報告企業の意見			今後の対応			
これまでに同定されたXMRV抗体陽性血清の反応特異性を精査したところ、一部がMo-MuLVと交差反応を示し、反応強度にもばらつきがあることから、現在までに同定された抗体陽性例の必ずしも全てがXMRV既感染を示すものではないという可能性が示唆されたとの報告である。 XMRVの病原性の有無は未だ定かではない。XMRVはマウス白血病ウイルスと類縁な脂質膜を持つ大型RNAウイルスである。この性状から本製剤の製造工程でウイルスが不活化・除去されると期待しうることから、仮にXMRVに病原性が有ったとしても本製剤の安全性は確保されていると考える。			日本赤十字社では、献血の問診時に献血者の健康状態を把握・確認している。平成22年5月18日に開催された平成22年度第1回薬事・食品衛生審議会血液事業部会運営委員会において、献血者におけるCFSについて現時点では緊急的な対応をとる必要はないものの、XMRVとCFSとの関連に関しては引き続き情報収集を行い、新たな知見等が得られれば、同委員会において対応を検討することとされた。今後も引き続き、情報の収集に努める。			

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02-4-16

Xenotropic murine leukemia virus-related virus (XMRV) 抗体特異性の解析

古田 里佳¹⁾、杉山 武毅²⁾、宮沢 孝幸³⁾、佐藤 英次³⁾、
倉恒 弘彦⁴⁾、保井 一太¹⁾、平山 文也¹⁾

1) 大阪府赤十字血液センター 研究部、

2) 兵庫県西脇市立西脇病院 泌尿器科、

3) 京都大学ウイルス研究所 信号伝達学研究分野、

4) 大阪市立大学大学院医学研究科 疲労クリニカルセンター

✉ furuta@osaka.bc.jrc.or.jp

【目的と意義】 XMRV の輸血感染リスクを評価するには、国内での流行状況の把握が必要である。ヒト体内での XMRV 局在やウイルス量に関する情報が現在非常に限られていることから、われわれは抗体スクリーニング検査を行ってきた。今回は、これまでに同定された XMRV 抗体陽性例が真の XMRV 感染によるものかどうかを知るためのひとつのアプローチとして、これら抗体陽性血清の反応特異性を精査した。

【材料と方法】 抗体スクリーニングには、100 倍希釈血清を用いて、XMRV ウイルス粒子を抗原としたウエスタンブロッティング (WB) を行った。スクリーニング対象は、大阪府赤十字血液センターで一般感染症検査を合格した献血検体から無作為抽出したもの 500 検体、2007 年 4 月～2010 年 3 月兵庫県西脇市立西脇病院にて受診した前立腺がん患者 67 名、2010 年 4 月～2010 年 6 月大阪市立大学付属病院疲労クリニカルセンターにて受診した慢性疲労性症候群 (CFS) 患者 87 名の計 654 名である。抗体陽性検体の反応特異性は、WB における Moloney murine leukemia virus (Mo-MuLV) に対する交差反応性および Native Gel を用いた WB で解析した。

【結果】 WB スクリーニングで陽性となったのはドナー 8 名 (1.6%)、前立腺がん患者 2 名 (3.0%)、CFS 患者 2 名 (2.3%) であり、グループ間の抗体保有率に有意差はなかった。これら陽性検体の Gag p30 抗体には 3 パターンの反応特異性が存在した。すなわち XMRV p30 のみに結合するもの、Mo-MuLV p30 に弱いながらも結合するもの、Mo-MuLV p30 にも XMRV と同程度の強さで結合するものである。反応パターンと対象グループとの間に有意な関連性は認められなかった。Env に対する抗体反応は通常の WB でも Native gel を用いた WB でも認められなかった。

【考察】 XMRV Gag と Mo-MuLV Gag のアミノ酸レベルでの相同性は約 87% である。XMRV 抗体陽性者の一部が Mo-MuLV と交差反応し、反応強度にもばらつきがあることから、現在までに同定された XMRV Gag 抗体陽性例の必ずしも全てが XMRV 既感染を示すものではない可能性が示唆される。当日は、現在継続中の患者核酸増幅検査結果と併せて考察する予定である。

医薬品 研究報告 調査報告書

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一般的名称		人血清アルブミン		研究報告の公表状況 佐藤英次, 古田里佳, 倉恒弘彦, 庄嶋貴之, 中富康仁, 保井一太, 宮沢孝幸. 第58回日本ウイルス学 会学術集会; 2010 Nov.7-9; 徳島 県	公表国 日本	
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研究報告の概要	○慢性疲労症候群患者血液におけるxenotropic murine leukemia virus-related virus の核酸調査 目的と意義: 2006年アメリカで, xenotropic murine leukemia virus-related virus (XMRV) が前立腺癌患者から発見され, さらに2009年, 慢性疲労症候群(CFS)患者でXMRVの感染が高率にみられるという報告があった。それ以来, CFSとXMRVの関連性の調査が各国で行われており, 日本でのXMRVの感染状況を調べることは, 緊急かつ重要な課題であると考えられる。 材料と方法: ヒト胎児腎由来株化細胞(HEK 293T)にXMRV持続感染前立腺癌細胞である22Rv1細胞の上清を接種し, この感染細胞から抽出したDNAを鋳型にしてXMRVのgag及びenv遺伝子領域を標的としたプライマー及びXMRV特異的なプライマーを用いてPCRの検出感度を調べた。次にCFS患者87名の血液細胞からゲノムDNAを抽出し, 上記プライマーを用いてPCRを行った。陽性が疑われる検体は再度同じプライマーで試験した。gag及びenvのプライマーではコンタミでマウス白血病ウイルスを検出してしまいう可能性があるため, XMRV特異的なプライマーも用いた。 結果及び考察: 上記プライマーは全て10個の感染細胞を検出できる感度であった。今回調査したCFS患者87名においてPCR解析で陽性は見られなかった。今後, ウイルス分離を含め, より正確で感度が高い検出方法を開発する必要があると考えられる。					使用上の注意記載状況・ その他参考事項等 赤十字アルブミン20 赤十字アルブミン25 赤十字アルブミン5%静注 12.5g/250mL 赤十字アルブミン20%静注 4g/20mL 赤十字アルブミン20%静注 10g/50mL 赤十字アルブミン25%静注 12.5g/20mL 血液を原料とすることによる 感染症伝播等
	報告企業の意見		今後の対応			
国内の慢性疲労症候群患者87名においてXMRVの感染状況を調査した結果, PCR解析で陽性は見られなかったとの報告である。 XMRVの病原性の有無は未だ定かではない。XMRVはマウス白血病ウイルスと類縁の脂質膜を持つ大型RNAウイルスである。この性状から本製剤の製造工程でウイルスが不活化・除去されると期待することから, 仮にXMRVに病原性があったとしても本製剤の安全性は確保されていると考える。		日本赤十字社では, 献血の間診時に献血者の健康状態を把握・確認している。平成22年5月18日に開催された平成22年度第1回薬事・食品衛生審議会血液事業部会運営委員会において, 献血者におけるCFSについて現時点では緊急的な対応をとる必要はないものの, XMRVとCFSとの関連に関しては引き続き情報収集を行い, 新たな知見等が得られれば, 同委員会において対応を検討することとされた。今後も引き続き, 情報の収集に努める。				

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02-4-18

慢性疲労症候群患者血液における xenotropic murine leukemia virus-related virus の核酸調査

佐藤 英次¹⁾、古田 里佳²⁾、倉恒 弘彦³⁾、庄嶋 貴之¹⁾、
中富 康仁³⁾、保井 一太²⁾、宮沢 孝幸¹⁾

1) 京都大学 ウイルス研究所 細胞生物学研究部門 信号伝達学研究分野、

2) 大阪府赤十字血液センター、

3) 大阪市立大学医学部疲労クリニカルセンター

✉ eijisato2@hotmail.com

【目的と意義】2006年アメリカで、マウス白血病ウイルス (murine leukemia virus: MLV) に類似した xenotropic murine leukemia virus-related virus (XMRV) が前立腺癌患者から発見された。さらに昨年、慢性疲労症候群 (chronic fatigue syndrome: CFS) 患者で XMRV の感染が高率にみられるという報告があった (Lombardi et al., 2009 326 (5952): 585-9 Science)。それ以来、CFS と XMRV の関連性の調査が各国で行われている。日本での XMRV の感染状況を調べることは、緊急かつ重要な課題であると考えられる。今回我々は、国内の CFS 患者において XMRV の感染状況を調査した。

【材料と方法】まずヒト胎児腎由来株化細胞 (HEK 293T) に XMRV 持続感染前立腺癌細胞である 22Rv1 細胞の上清を接種し、この感染細胞から抽出した DNA を鋳型にして XMRV の gag 及び env 遺伝子領域を標的としたプライマー (Lombardi et al., 2009 326 (5952): 585-9 Science) 及び XMRV 特異的なプライマー (Hohn et al., 2009 (6): 92 Retrovirology) を用いて PCR の検出感度を調べた。次に2010年4月～2010年6月、大阪市立大学付属病院疲労クリニカルセンターにて受診した CFS 患者87名の血液細胞からゲノム DNA を抽出し、上記 gag 及び env のプライマーを用いて PCR を行った。PCR 産物はアガロースゲルに泳動し、エチジウムブロマイド染色で確認した。陽性が疑われる検体については再度同じプライマーで試験した。また、上記 gag 及び env のプライマーは実験室内でのコンタミで MLV を検出してしまう可能性があるため、XMRV 特異的なプライマーも用いて PCR を行った。

【結果及び考察】上記プライマーは全て10個の感染細胞を検出できる感度であった。今回調査した CFS 患者87名においては、上記 PCR 解析で陽性は見られなかった。今後ウイルス分離を含め、より正確で感度が高い検出方法を開発する必要があると考えられる。なお当日は、現在継続中の患者抗体検査結果と併せて考察する予定である。

医薬品 研究報告 調査報告書

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一般的名称	人血清アルブミン	研究報告の公表状況	Sato E, Furuta RA, Miyazawa T. Retrovirology. 2010 Dec 20;7:110.	公表国 日本	
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン5%静注12.5g/250mL(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)				
研究報告の概要	○市販のRT-PCRキット内の内在性ネズミ白血病ウイルスゲノム(MLV)汚染は、XMRVのための標準プライマーの使用で増幅される 日本で慢性疲労症候群(CFS)患者からの血清でXMRV感染症のウイルスRNAの存在を調査するパイロットスタディの間、XMRVの部分的gag領域を検出するRT-PCRキットを使用すると、陰性コントロール(水)でも陽性のバンドが頻繁に認められた。そのため、キット自体がMLVまたはXMRVで汚染されていることを疑い、2つの独立した研究所で、4つのワンステップRT-PCRキット(Invitrogen、TaKaRa、Promega、QIAGEN)の品質を評価した。 InvitrogenのワンステップRT-PCRキットの使用により、増幅される物質の部分的gag領域のヌクレオチド配列は、7番染色体のpolytropic内在性MLV(PmERV)とほとんど一致し(99.4%)、最近特定されたCFS患者に由来するMLV様のウイルスと非常に類似していた(96.9~97.6%一致)。また部分的env領域のヌクレオチド配列を決定し、それがPmERVとほとんど一致(99.6%)することも発見した。 これらの結果より、InvitrogenのワンステップRT-PCRキットの酵素混合物が、PmERVに由来するRNAで汚染されていることが明らかとなった。 CFSや前立腺がん患者のXMRV感染の調査において、使用する検出キットも慎重に評価しなくてはならない。				使用上の注意記載状況・ その他参考事項等
	赤十字アルブミン20 赤十字アルブミン25 赤十字アルブミン5%静注 12.5g/250mL 赤十字アルブミン20%静注 4g/20mL 赤十字アルブミン20%静注 10g/50mL 赤十字アルブミン25%静注 12.5g/20mL 血液を原料とすることによって由来する感染症伝播等				
報告企業の意見		今後の対応			
XMRVの検出に使用されるRT-PCRキット4種類を評価した結果、Invitrogenのキットが内在性マウス白血病ウイルスに由来するRNAで汚染されていたことが明らかとなったとの報告である。 XMRVの病原性の有無は未だ定かではない。XMRVはマウス白血病ウイルスと類縁の脂質膜を持つ大型RNAウイルスである。この性状から本製剤の製造工程でウイルスが不活化・除去されると期待しうることから、仮にXMRVに病原性があっても本製剤の安全性は確保されていると考える。		日本赤十字社では、献血の間診時に献血者の健康状態を把握・確認している。平成22年5月18日に開催された平成22年度第1回薬事・食品衛生審議会血液事業部会運営委員会において、献血者におけるCFSについて現時点では緊急的な対応をとる必要はないものの、XMRVとCFSとの関連に関しては引き続き情報収集を行い、新たな知見等が得られれば、同委員会において対応を検討することとされた。今後も引き続き、情報の収集に努める。			

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SHORT REPORT

Open Access

An Endogenous Murine Leukemia Viral Genome Contaminant in a Commercial RT-PCR Kit is Amplified Using Standard Primers for XMRV

Eiji Sato¹, Rika A Furuta², Takayuki Miyazawa^{1*}

Abstract

During pilot studies to investigate the presence of viral RNA of xenotropic murine leukemia virus (MLV)-related virus (XMRV) infection in sera from chronic fatigue syndrome (CFS) patients in Japan, a positive band was frequently detected at the expected product size in negative control samples when detecting a partial *gag* region of XMRV using a one-step RT-PCR kit. We suspected that the kit itself might have been contaminated with small traces of endogenous MLV genome or XMRV and attempted to evaluate the quality of the kit in two independent laboratories. We purchased four one-step RT-PCR kits from Invitrogen, TaKaRa, Promega and QiAGEN in Japan. To amplify the partial *gag* gene of XMRV or other MLV-related viruses, primer sets (419F and 1154R, and GAG-I-F and GAG-I-R) which have been widely used in XMRV studies were employed. The nucleotide sequences of the amplicons were determined and compared with deposited sequences of a polytropic endogenous MLV (PmERV), XMRV and endogenous MLV-related viruses derived from CFS patients. We found that the enzyme mixtures of the one-step RT-PCR kit from Invitrogen were contaminated with RNA derived from PmERV. The nucleotide sequence of a partial *gag* region of the contaminant amplified by RT-PCR was nearly identical (99.4% identity) to a PmERV on chromosome 7 and highly similar (96.9 to 97.6%) to recently identified MLV-like viruses derived from CFS patients. We also determined the nucleotide sequence of a partial *env* region of the contaminant and found that it was almost identical (99.6%) to the PmERV. In the investigation of XMRV infection in patients of CFS and prostate cancer, researchers should prudently evaluate the test kits for the presence of endogenous MLV as well as XMRV genomes prior to PCR and RT-PCR tests.

Findings

Xenotropic murine leukemia virus (MLV)-related virus (XMRV), which resembles endogenous MLV, was discovered in prostate cancer patients in 2006 [1,2]. In 2009, a high incidence of XMRV infection was also documented in chronic fatigue syndrome (CFS) patients in the United States [3]. Since then, surveys on XMRV infection of CFS patients have been conducted in several countries [4-9]; however, there is a vigorous debate over conflicting results in CFS patients [10-12]. Moreover, recently, Lo et al. detected MLV-related viruses which are distinct from XMRV but resemble polytropic endogenous MLVs in CFS patients and healthy blood donors [13].

In studies investigating XMRV infection, a PCR approach to detect proviral DNA and/or a RT-PCR approach to detect viral RNA have been commonly employed [1,3-6,8,13-15]. We (the Japanese Red Cross [JRC]) have been studying the prevalence of XMRV infection in Japanese patients with prostate cancer and CFS as well as healthy blood donors. To study the presence of XMRV RNA in plasma from CFS patients, we selected a commercial one-step RT-PCR kit. In the pilot study, we encountered a puzzling result. A positive band was frequently detected at the expected product size in the negative control (water) using primer sets to detect a partial *gag* region of XMRV. We suspected that the test kit itself might have been contaminated with small traces of endogenous MLV genome or XMRV and attempted to evaluate the quality of the kit in two independent laboratories, in JRC and Institute for Virus Research (IVR), Kyoto University (Kyoto, Japan).

* Correspondence: takavet@goo.jp

¹Laboratory of Signal Transduction, Institute for Virus Research, Kyoto University, 53 Shogoin-Kawaracho, Sakyo-ku, Kyoto 606-8507, Japan
 Full list of author information is available at the end of the article



We used the following RT-PCR kits which were purchased in Japan: SuperScript[®] III One-Step RT-PCR System with the Platinum[®] Taq High Fidelity Kit (Cat. no. 12574-030) (Invitrogen, Carlsbad, CA, USA) (abbreviated as Kit I); AccessQuick[™] RT-PCR System (Cat. no. A1701) (Promega, Madison, WI, USA) (abbreviated as Kit P); One Step RT-PCR Kit (Cat. no. PRO24A) (TaKaRa, Ohtsu, Shiga, Japan) (Abbreviated as Kit T); One Step RT-PCR Kit (Cat. no. 210210) (QIAGEN GmbH, Hilden, Germany) (Abbreviated as Kit Q).

To amplify the partial *gag* gene of XMRV or other MLV-related viruses, primers 419F (5'-ATCAGTT AACCTACCCGAGTCGGAC-3') and 1154R (5'-GCC GCCTCTTCTTCATTGTTCTC-3') [3], and GAG-I-F (5'-TCTCGAGATCATGGGACAGA-3') and GAG-I-R (5'-AGAGGGTAAGGGCAGGGTAA-3') [1] were used. To amplify the partial *env* gene of polytropic endogenous MLV, primers p-env1f (5'-AGAAGGTCCAGCGTCTCAA-3'), p-env1r (5'-TTGCCACAGTAGCCCTCTCT-3'), p-env3f (5'-GATGAGACTGGACTCGGGTG-3') and p-env5r (5'-GTGGAGGCCTGGGGAGCATGATC-3') were designed based on the sequence of a polytropic endogenous MLV (PmERV) present in mouse (*Mus musculus*) chromosome (chr) 7 [GenBank: AC167978]. To enhance one-step RT-PCR reactions, 2.5 µl of 1 µg/µl carrier RNA from QIAamp UltraSens[™] Virus Kit (Cat. no. 53704) (QIAGEN) was added to the reaction mixtures of the one-step RT-PCR reactions as indicated in Figure 1. To examine whether the contaminant was RNA, 2 µl of 10 µg/ml RNaseA (Cat. no. 19101) (QIAGEN) were added in the one-step RT-PCR reaction mixture as indicated in Figure 1C. The RT-PCR was conducted in 25 µl (Kit I, Kit T, and Kit Q) or 25.5 µl (Kit P) of reaction mixture according to manufacturers' instructions.

By adding carrier RNA in the samples to enhance the RT-PCR reaction, we consistently detected a positive band using Kit I in negative controls using two primer sets (419F and 1154R, and GAG-I-F and GAG-I-R) which are widely used to amplify XMRV (Figure 1A). These results were confirmed by two independent laboratories (JRC and IVR) under the same experimental conditions. The positive reaction was observed in all four batches (derived from four different lots) of the kit tested. To exclude the possibility that water, the carrier RNA or the primers used were contaminated with an XMRV-like genome, we tested additional one-step RT-PCR kits, termed Kit P and Kit T, from two different manufacturers. Consequently, we could not detect positive bands utilizing these kits (Figure 1B) strongly suggesting that the component(s) of Kit I contained XMRV-like viral genomes. Most of the contaminants appeared to be RNA because the positive bands

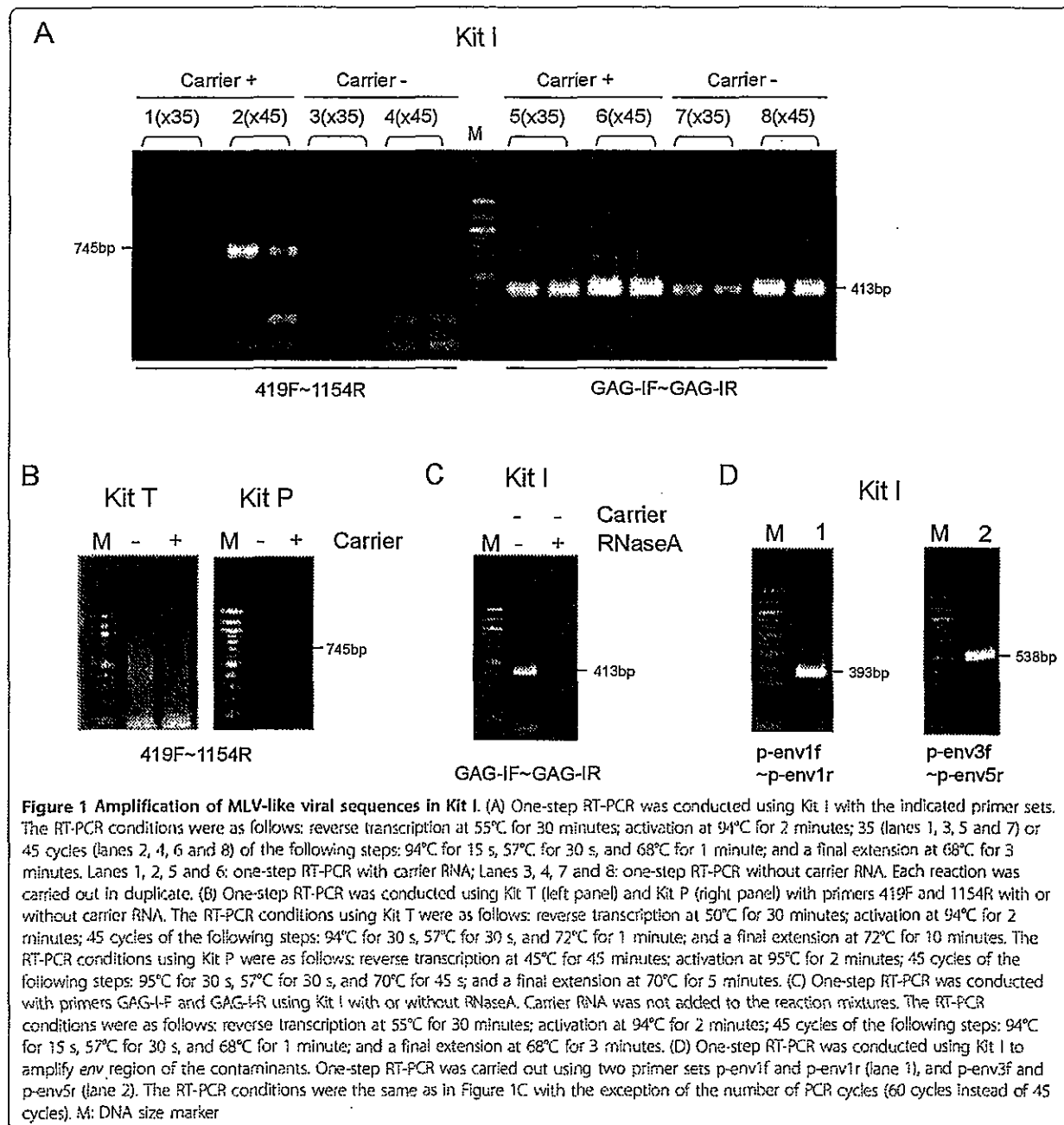
disappeared after adding RNaseA in the reaction mixture from Kit I (Figure 1C).

To further investigate the contaminant in Kit I, nucleic acids purified from the enzymes (a mixture of reverse transcriptase and *Taq* DNA polymerase) and the buffer contained in the kit were tested by adding the individual components to three different one-step RT-PCR kits (Kit T, Kit P, and Kit Q) (Figure 2A-C). As a result, we detected positive bands when the nucleic acids purified from the enzymes of Kit I were added to RT-PCR Kit T, Kit P or Kit Q using two primer sets (419F and 1154R, and GAG-I-F and GAG-I-R). On the contrary, we could not detect the presence of MLV genomes in the buffer of Kit I. These data indicated that the enzyme mixture of Kit I was contaminated with XMRV-like viral RNA.

PCR products amplified using primers 419F and 1154R were cloned into pCR4Blunt[™]-TOPO (Invitrogen) and sequenced for both strands. Three clones (two clones at JRC and one clone at IVR) were sequenced and found to be nearly identical (one nucleotide difference between one another). These sequences have a 9 nucleotide deletion observed in some endogenous polytropic MLVs in place of the XMRV-specific 24 nucleotide deletion in the 5' *gag* leader region and are nearly identical to polytropic endogenous MLVs encoded in multiple chromosomal locations of the C57BL/6J mouse genome. The nucleotide sequences of the representative clone [GenBank: AB597300] were aligned with sequences deposited in GenBank as follows: MLV-like virus from CFS patients types 1, 2 and 3 [GenBank: HM630562, HM630558, and HM630559] [13], XMRV strain VP62 [GenBank: NC_007815] [2] and one representative PmERV on chr 7 [GenBank: AC167978; nt 65,391-64,647] (Figure 3). The contaminant was nearly identical (99.4% identity) to the PmERV chr 7 with only 4 nucleotide differences in the sequenced region. In addition, the contaminant was quite similar (96.9-97.6% identity) to the MLV-like viral sequences (CFS types 1, 2, and 3) derived from CFS patients.

To further characterize the contaminant, we conducted additional RT-PCRs (Figure 1D) amplifying partial *env* regions with two primer sets (p-env1f and p-env1r, and p-env3f and p-env5r) based on the sequence of the PmERV chr 7, and then sequenced the amplicons directly. We determined 674 bp of the N-terminal *env* region [GenBank: AB597301] and found that the contaminant was nearly identical (99.6% identity) to the PmERV chr 7 [GenBank: AC167978; nt 59,992-59,319] (Figure 4).

It should be noted that Kit I contains an anti-DNA polymerase monoclonal antibody to accomplish hot start-PCR and to reduce non-specific amplification.



Mice have enormous copy numbers of endogenous retroviruses in their genomes; and hybridomas, for manufacturing monoclonal antibodies, have been found to produce high amounts of retroviral particles [16]. Therefore, we suspect that the *Taq* DNA polymerase in Kit I was contaminated with the endogenous MLVs. This possibility has been also pointed out by others [17,18]. Because the reverse transcriptase (SuperScriptIII) and the *Taq* DNA polymerase (Platinum Taq) in Kit I can

be purchased separately from the manufacturer, we attempted to detect the MLV genome in the Platinum Taq polymerase using the same protocol as the one performed in Figure 2A-B. As a result, we detected MLV genomes in the Platinum Taq DNA polymerase using the RT-PCR Kit P and Kit T (Figure 2D for Kit P and data not shown for Kit T).

Surveys have been conducted by several research groups on XMRV infection in CFS patients, but the

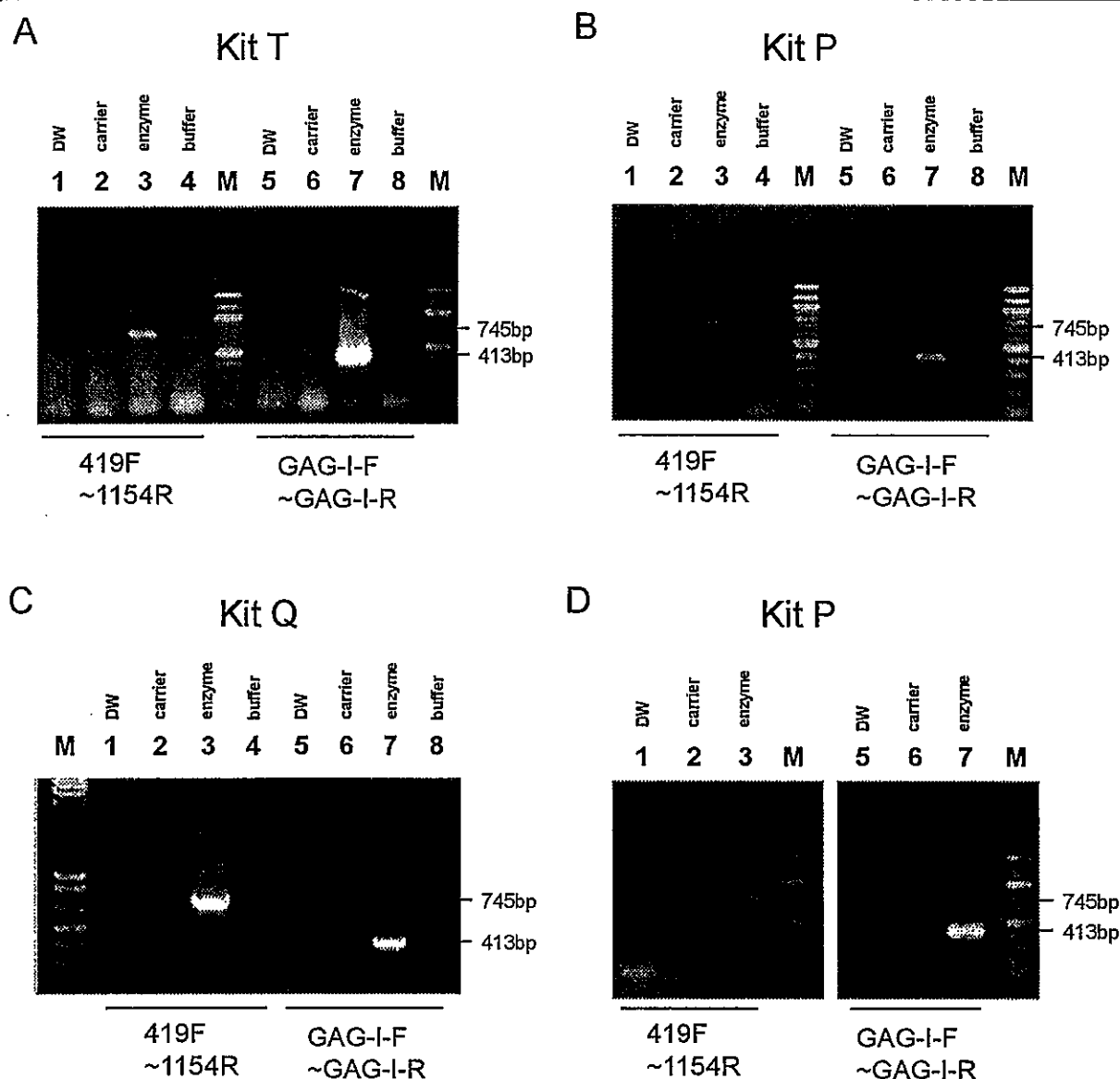
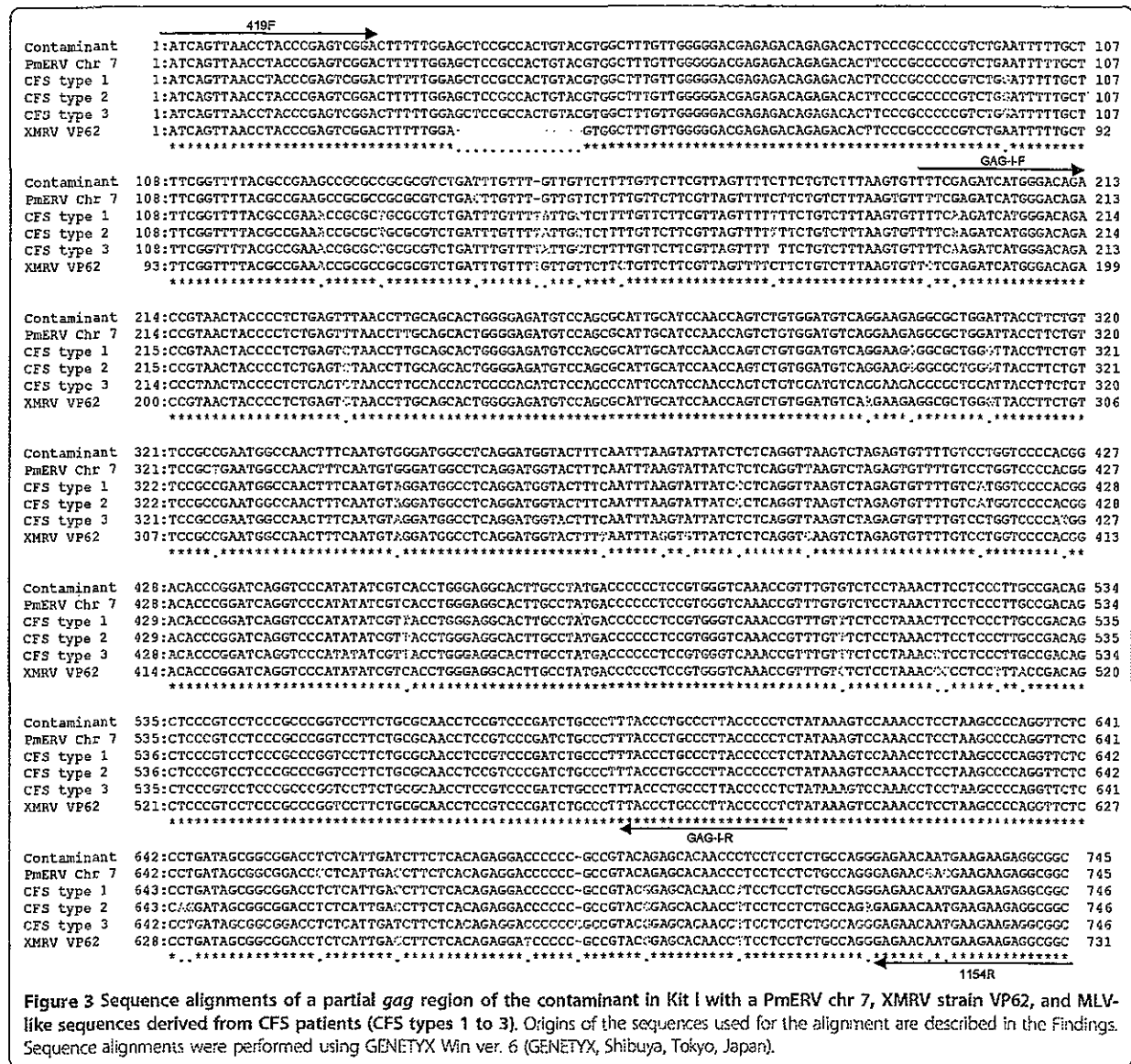


Figure 2 One-step RT-PCR for identification of contaminants in Kit I and Platinum Taq. (A-C) One-step RT-PCR for identification of a contaminated component in Kit I. The experiments were conducted in two independent laboratories, IVR and JRC. In IVR, nucleic acids were extracted from 50 μ l of the enzyme mix of the RT-PCR Kit I using an RNA purification column (QIAamp viral RNA mini kit [Cat. no. 52904] [QIAGEN]) and the presence of polytropic endogenous MLV was examined by using the RT-PCR Kit T (A) and Kit P (B). In JRC, nucleic acids were extracted from 75 μ l of the enzyme mix of RT-PCR Kit I using an RNA/DNA purification column (PureLinkTM Viral RNA/DNA Kit [Cat. no. 12280-050] [Invitrogen]), and the presence of polytropic endogenous MLV was examined using Kit Q (C). Five μ l of test samples were examined with primers indicated below the corresponding lanes. The RT-PCR conditions for Kit T and Kit P were the same as in Figure 1B. The RT-PCR conditions for Kit Q were as follows: reverse transcription at 50°C for 30 minutes; activation at 95°C for 15 minutes; 45 cycles of the following steps: 94°C for 30 s, 57°C for 30 s, and 72°C for 1 minute; and a final extension at 72°C for 10 minutes. Lanes 1 and 5, DW; lanes 2 and 6, column-purified carrier RNA (carrier); lanes 3 and 7, column-purified nucleic acids from enzyme mix (enzyme) of the Kit I; lanes 4 and 8, 1 μ l buffer of the Kit I plus 4 μ l DW (buffer). (D) One-step RT-PCR for the detection of MLV RNA in Platinum Taq. Nucleic acids were extracted from 50 μ l of the Platinum Taq using an RNA purification column (QIAamp viral RNA mini kit [QIAGEN]) and the presence of MLV RNA was examined by using the RT-PCR Kit P. Five μ l of test samples were examined with primers indicated below the corresponding lanes. The RT-PCR condition was the same as in Figure 1B with the exception of the PCR cycles (60 cycles instead of 45 cycles). Abbreviation; DW: distilled water. M: DNA size marker.



results have been inconsistent. Although all research groups carefully performed their experiments to test XMRV infection by PCR and/or RT-PCR, it is still difficult to conclude that the positive results linking XMRV with CFS are not laboratory artifacts. Xenotropic (or polytropic) MLVs are widespread, and there may be many opportunities for samples to get contaminated with such ubiquitous viruses in laboratories when conducting biological or medical research [17]. In this study, we evaluated several one-step RT-PCR kits and a *Taq* DNA polymerase for the contamination of MLV-related genomes and found that the test kit and the *Taq*

DNA polymerase from Invitrogen were contaminated with MLV-related genomes.

The findings in the present study indicate that contaminating nucleic acids in the test kits can potentially produce false-positive PCR results in studies of XMRV and other MLV-related viruses. In particular, our results raise the possibility that the PCR products described by Lo et al. [13] were derived from contaminating MLV RNA and/or DNA. It should be noted, however, that in contrast to our data which shows MLV contamination even in water controls, their report demonstrated that polytropic MLV sequences

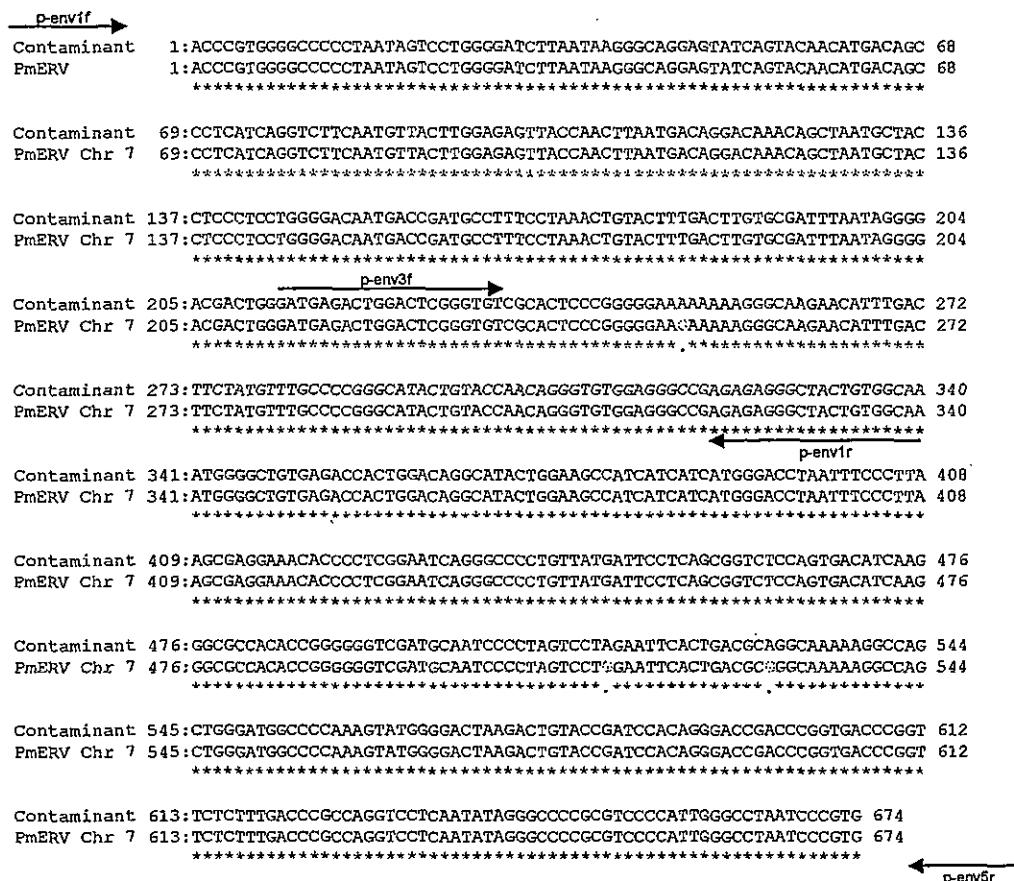


Figure 4 Sequence alignments of a partial *env* region of the contaminant in Kit I with a PmERV chr 7. Origins of the sequences used for the alignment are described in the Findings. Sequence alignments were performed using GENETYX Win ver. 6 (GENETYX, Shibuya, Tokyo, Japan).

were found more frequently in CFS patients than in healthy controls and not at all in water controls. Nonetheless, Lo et al. mentioned in the report that Platinum Taq from Invitrogen gave them the best results among Taq polymerases from several suppliers and was used to test the patient samples [13]. They also used Invitrogen's Superscript II RT and Platinum Taq for RT-PCR, albeit in a two-step cDNA synthesis and PCR amplification procedure [13]. As pointed out by Erlwein et al. in the comments [19] responding to the report by Lo et al. [13], assurance that control samples were assayed simultaneously with the positively identified ones in a blinded, randomized way was missing in their study, unfortunately.

The requirement for quality control to avoid contamination of endogenous retroviral genomes in test kits may differ depending on the intended purpose. However, in XMRV studies, many researchers conduct ultra-sensitive PCR or RT-PCR to detect extremely small amounts of XMRV. Therefore, in the investigation of

XMRV infection, researchers should prudently evaluate test kits for the presence of genomes of endogenous MLV as well as XMRV.

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Author details

¹Laboratory of Signal Transduction, Institute for Virus Research, Kyoto University, 53 Shogoin-Kawaracho, Sakyo-ku, Kyoto 606-8507, Japan.

²Japanese Red Cross Osaka Blood Center, 2-4-43 Morinomiya, Joto-ku, Osaka 536-8505, Japan.

Authors' contributions

RAF, ES, and TM designed the experiments. RAF and ES performed the experiments. TM wrote the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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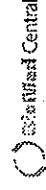
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識別番号・報告回数		報告日	第一報入手日 2011. 1. 18	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	人血清アルブミン	研究報告の公表状況	Smith RA. Retrovirology. 2010 Dec 20;7:112.	公表国 米国	
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン5%静注12.5g/250mL(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)				
研究報告の概要	○MLVをコードしている核酸による臨床検体汚染:XMRVと他のヒトレトロウイルス候補との関連 前立腺がん患者と慢性疲労症候群(CFS)患者でXMRVの感染を評価する研究は、臨床検体のPCRに基づく検査に頼り、大きく異なる結果を与えた。 Retrovirology誌において今回報告された4つの独立した研究は、XMRVのPCR分析において、DNAまたはRNAの汚染を除外するために必要な処置を示した。また、以前報告されたXMRV配列が、一般的に用いられる前立腺がん細胞株(22Rv1)に由来することを示唆している系統発生解析が提示された。 これらの報告をまとめると、XMRVのPCR分析における汚染の3つの潜在的な原因が示された。 i)市販のPCR試薬に、マウス白血病毒ウイルス(MLV)がエンコードされている核酸が存在する。 ii)ヒトの血液や組織において、マウスゲノムDNAが微量存在する。 iii)ヒトの腫瘍細胞株がXMRVまたは他の近縁のガンマレトロウイルスに感染している。 今回の報告は、前立腺がん患者とCFS患者におけるMLV関連ウイルスの存在を裏付けるのに用いられているPCRの結果に重大な疑問を投げかけた。 XMRV流行の将来の評価は、汚染の可能性を除外するために、より厳格なPCR分析と系統発生解析を含まなくてはならない。				使用上の注意記載状況・ その他参考事項等 赤十字アルブミン20 赤十字アルブミン25 赤十字アルブミン5%静注 12.5g/250mL 赤十字アルブミン20%静注 4g/20mL 赤十字アルブミン20%静注 10g/50mL 赤十字アルブミン25%静注 12.5g/20mL 血液を原料とすることに由来する 感染症伝播等
報告企業の意見		今後の対応			
前立腺がん患者とCFS患者におけるMLV関連ウイルスの存在を裏付けるのに用いられているPCRの結果に重大な疑問を投げかける4つの研究が報告されたとのことである。 XMRVの病原性の有無は未だ定かではない。XMRVはマウス白血病毒ウイルスと類縁の脂質膜を持つ大型RNAウイルスである。この性状から本製剤の製造工程でウイルスが不活化・除去されると期待しうることから、仮にXMRVに病原性があっても本製剤の安全性は確保されていると考える。		日本赤十字社では、献血の問診時に献血者の健康状態を把握・確認している。平成22年5月18日に開催された平成22年度第1回薬事・食品衛生審議会血液事業部会運営委員会において、献血者におけるCFSについて現時点では緊急的な対応をとる必要はないものの、XMRVとCFSとの関連に関しては引き続き情報収集を行い、新たな知見等が得られれば、同委員会において対応を検討することとされた。今後も引き続き、情報の収集に努める。			

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VIEWPOINTS

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Contamination of clinical specimens with MLV-encoding nucleic acids: implications for XMRV and other candidate human retroviruses

Robert A Smith

Abstract

Efforts to assess the prevalence of xenotropic murine leukemia virus-related virus (XMRV) in patients with prostate cancer and chronic fatigue syndrome have relied heavily on PCR-based testing of clinical samples and have yielded widely divergent findings. This week in *Retrovirology*, reports from four independent research groups illustrate the extreme care needed to exclude DNA or RNA contamination in PCR analyses of XMRV. In addition, phylogenetic evidence suggesting that previously-published XMRV sequences originated from a commonly-used prostate carcinoma cell line (22Rv1) is presented. These findings raise important questions regarding the provenance of XMRV and its potential connection to human disease.

Introduction

Reports of a newly-discovered gammaretrovirus (xenotropic murine leukemia virus-related virus; XMRV) in patients diagnosed with prostate cancer [1,2] and chronic fatigue syndrome (CFS) [3] have attracted the attention of investigators throughout the retroviral research community. XMRV was initially identified in prostate tumor samples from individuals harboring a specific polymorphism in *RNASEL*, a gene important for interferon-mediated antiviral defense [1]. Studies describing the receptor usage and integration site preference of the virus were soon followed by a second report of XMRV infection in an unrelated cohort of prostate cancer patients [2]. Although an association between XMRV and the aforementioned *RNASEL* polymorphism was not found [2], the idea that defects in innate immunity might be linked to XMRV infection prompted others to look for the virus in patients with CFS [3]. Remarkably, PCR assays identified XMRV DNA in peripheral blood samples from 68 of 101 CFS patients and 8 of 218 healthy controls. These and other findings provided compelling evidence that XMRV is the first known example of an exogenous human gammaretrovirus.

In contrast, subsequent efforts to assess the prevalence of XMRV in patients with CFS and prostate cancer have

reached widely disparate conclusions [[4]; see also reference [5] for review]. The underlying factors responsible for this discord are unclear; but from the beginning, researchers have repeatedly voiced concerns that at least some accounts of PCR-positive results are attributable to the inadvertent contamination of human specimens or reagents with mouse DNA. These concerns were revisited following a recent report by Lo *et al.* that described the existence of sequences closely related to polytropic and modified-polytropic murine leukemia viruses (MLVs)—but not XMRV—in blood samples from CFS patients [6]. Such skepticism is justified by previous examples of alleged human retroviruses that later turned out to be laboratory artifacts [7].

Evidence for contamination of human samples

With this history in mind, four independent studies published this week in *Retrovirology* reinforce the need to take extreme precautions in excluding mouse DNA contamination. Robinson *et al.* [8] performed a PCR analysis of 437 prostate tissue specimens from patients in the United Kingdom (UK), Thailand and Korea using primers that targeted the 5'-leader region of XMRV *gag*. Initial PCR results showed that 14 of 292 samples from the UK contained XMRV or MLV-related sequences. However, 78 of the UK samples, including all 14 XMRV/MLV-positive specimens, contained amplifiable levels of LTR sequences from interstitial A-type

Correspondence: smithra@u.washington.edu
 Department of Pathology, University of Washington, Seattle WA, USA



particles (IAPs), a class of endogenous retroelements found in the mouse genome. Similarly, Oakes and colleagues [9] identified 2 of 112 blood samples from CFS patients and 17 of 36 samples from healthy controls that were PCR-positive for XMRV *gag*-leader DNA, but later found that all of the XMRV-positive specimens contained amplifiable levels of mouse mitochondrial or IAP sequences. These data strongly suggest that the XMRV sequences recovered by Robinson [8] and Oakes [9] originated from mouse DNA that contaminated the study samples prior to PCR.

Evidence for contamination of PCR reagents

A third report by Sato *et al.* [10] describes the detection of MLV-encoding nucleic acids in PCR reagents obtained from a commercial supplier (Invitrogen). Analyses of individual components from the PCR kit (SuperScript[®] III One-Step RT-PCR System with Platinum[®] Taq High Fidelity) suggest that the mixture of reverse transcriptase and Taq DNA polymerase supplied by the manufacturer was contaminated with MLV RNA. This contamination likely originated from a monoclonal antibody preparation used in the polymerase mixture to facilitate hot-start PCR.

Further details of the contamination found by Robinson [8], Oakes [9] and Sato [10] were obtained by DNA sequence analysis of the PCR-amplified products. All three studies identified sequences that were closely related to endogenous MLV. In particular, Oakes and coworkers obtained a broad array of polytropic, modified-polytropic and xenotropic MLV-like sequences [9], a result strikingly similar to the findings of Lo *et al.* in their analysis of CFS patient samples [6]. Both Oakes and Robinson also identified subsets of amplicons that encoded a 24-nt *gag*-leader deletion previously thought to be specific for XMRV (see below). Collectively, these data show the ease with which contamination can lead to false-positive MLV/XMRV signals.

Phylogenetic support for contamination in previous studies of XMRV

Finally, Hué and colleagues [11] present multiple lines of evidence suggesting that contamination has occurred repeatedly in previous studies of XMRV. The authors begin by showing that the 24-nt *gag*-leader deletion is not unique to XMRV; PCR primers targeting the deletion readily amplified endogenous MLV sequences from 12 different inbred and wild-derived mouse strains commonly used in laboratory experiments, as well as MLV sequences present in 5 of 411 human tumor cell lines. The latter result is consistent with previous reports of xenotropic MLV contamination in human cell cultures [11] and references therein].

Next, Hué *et al.* PCR-amplified, cloned and sequenced XMRV *gag*, *pol* and *env* segments from 22Rv1 prostate carcinoma cells, an immortalized line known to harbor multiple integrated copies of the virus. Remarkably, the 22Rv1 sequences displayed average pairwise genetic distances that equaled or exceeded those of previously-published XMRV sequences from prostate cancer [1] and CFS patients [3], despite the fact that these patients were from epidemiologically unlinked cohorts. In addition, phylogenetic analyses of the 22Rv1 and patient-derived XMRV sequences strongly suggest that the patient sequences obtained to date [1,3] originated from one or more XMRV proviruses present in the 22Rv1 cell line [11].

Conclusions

The reports discussed above [8-11] collectively identify three potential sources of contamination in PCR-based studies of XMRV: (i) MLV-encoding nucleic acids present in commercial PCR reagents, (ii) trace amounts of mouse genomic DNA in human blood and tissue samples, and (iii) DNA or RNA from human tumor cell lines infected with XMRV or other closely-related gammaretroviruses. PCR testing for IAP sequences [8,9] should prove useful in further studies of XMRV, as well as other candidate human retroviruses, in which the confounding effects of mouse DNA contamination must be minimized. However, the findings of Hué *et al.* clearly show that contamination cannot be assessed by PCR testing for mouse DNA alone, since several human cell lines harbor xenotropic MLVs that are closely related to XMRV [11]. Additional findings from the Hué study suggest that previously-published XMRV sequences [1,3] were derived from copies of the virus present in 22Rv1 cells, which likely acquired XMRV during xenografting of the tumor cells in athymic mice [11] and references therein]. Collectively, these results cast serious doubts on the PCR evidence used to support claims of MLV-related viruses in prostate cancer and CFS patients. Future assessments of the prevalence of XMRV should include more rigorous PCR and phylogenetic tests to exclude the possibility of contamination.

Abbreviations

XMRV: xenotropic murine leukemia virus-related virus; MLV: murine leukemia virus; PCR: polymerase chain reaction; CFS: chronic fatigue syndrome; IAP: interstitial A-type particle

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Competing interests

The author declares that he has no competing interests.

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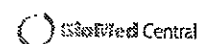
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研究報告 調査報告書

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一般的名称	①②③④⑤ポリエチレングリコール処理人免疫グロブリン ⑥⑦⑧人免疫グロブリン			研究報告の公表状況	公表国 中国	
販売名 (企業名)	①献血グロブリン IH5% 静注 0.5g/10mL (ベネシス) ②献血グロブリン IH5% 静注 1g/20mL (ベネシス) ③献血グロブリン IH5% 静注 2.5g/50mL (ベネシス) ④献血グロブリン IH5% 静注 5g/100mL (ベネシス) ⑤献血グロブリン-IH ヨシトミ (ベネシス) ⑥グロブリン筋注 450mg/3mL「ベネシス」 (ベネシス) ⑦グロブリン筋注 1500mg/10mL「ベネシス」 (ベネシス) ⑧グロブリン-WF (ベネシス)					
研究報告の概要	背景：最近の論争として、異種指向性マウス白血病関連ウイルス (XMRV) が慢性疲労症候群 (CFS) の病因に寄与するかどうかの問題がある。中国での調査をするために、65 人の CFS 患者と 85 人の献血者コントロールを登録した。複合的なリアルタイム PCR、または逆転写酵素 PCR (RT-PCR) が被験者の XMRV 感染状態を分析するために開発された。分析評価は、それぞれ、XMRV 標準と競争的内部コントロール (CICs) としてプラスミド DNAs と装甲 RNA を造ることによって標準化した。 結果：複合的なリアルタイム PCR と RT-PCR 分析法の特異性の感度は 100% で、それぞれ 20 コピー/反応、10IU/mL だった。操作中の精度変異係数 (CV) は 1.76%~2.80% および 1.70%~2.59%、一方操作間の CV は 1.07%~2.56% および 1.06%~2.74% の範囲だった。XMRV は、65 人の CFS 患者と 65 人の健康人において検出されなかった。 結論：本研究では、中国の CFS 患者の末梢血単核細胞と血漿で XMRV は検出されなかった (XMRV 感染と CFS の発症の間に関連はなかった)。					使用上の注意記載状況・ その他参考事項等 代表として献血グロブリン IH5% 静注 0.5g/10ml の記載を示す。 2. 重要な基本的注意 (1) 本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-I 抗体陰性で、かつ ALT (GPT) 値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査 (NAT) を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分からポリエチレングリコール 4000 処理、DEAE セファデックス処理等により人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及びウイルス除去膜によるろ過処理及び pH3.9~4.4 の条件下での液状インキュベーション処理を施しているが、投与に際しては、次の点に十分注意すること。
	報告企業の意見				今後の対応	
マウス白血病ウイルス (murine leukemia virus: MLV) は、レトロウイルス科ガンマレトロウイルスに属し、ビリオンは球形で直径約 100nm の脂質エンベロープを有する比較的大きな RNA ウイルスである。万一、MLV が原料血漿に混入したとしても、HIV-1 をモデルウイルスとしたウイルスバリデーション試験結果から、本剤の製造工程において十分に不活化・除去されと考えている。				本報告は本剤の安全性に影響を与えないものと考えるので、特段の措置はとらない。		



RESEARCH

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Failure to detect Xenotropic murine leukaemia virus-related virus in Chinese patients with chronic fatigue syndrome

Ping Hong^{1,2}, Jinming Li^{1,2*}, Yongzhe Li^{3*}

Abstract

Background: Recent controversy has surrounded the question of whether xenotropic murine leukaemia virus-related virus (XMRV) contributes to the pathogenesis of chronic fatigue syndrome (CFS). To investigate the question in a Chinese population, 65 CFS patients and 85 blood donor controls were enrolled and multiplex real-time PCR or reverse transcriptase PCR (RT-PCR) was developed to analyze the XMRV infection status of the study participants. The assay was standardized by constructing plasmid DNAs and armored RNAs as XMRV standards and competitive internal controls (CICs), respectively.

Results: The sensitivities of the multiplex real-time PCR and RT-PCR assays were 20 copies/reaction and 10 IU/ml, respectively, with 100% specificity. The within-run precision coefficient of variation (CV) ranged from 1.76% to 2.80% and 1.70% to 2.59%, while the between-run CV ranged from 1.07% to 2.56% and 1.06% to 2.74%. XMRV was not detected in the 65 CFS patients and 65 normal individuals out of 85 controls.

Conclusions: This study failed to show XMRV in peripheral blood mononuclear cells (PBMCs) and plasma of Chinese patients with CFS. The absence of XMRV nucleic acids does not support an association between XMRV infection and the development of CFS in Chinese.

Background

Chronic fatigue syndrome (CFS) is a multisystem disease which is characterized by severe and debilitating fatigue, abnormal sleep behaviour, impaired memory and concentration, and musculoskeletal pain [1]. The constellation of symptoms is non-specific and can be caused by a variety of diseases. Studies have identified various features relevant to the pathogenesis of CFS, such as viral infection, abnormal immune function, exposure to toxins, chemicals and pesticides, stress, hypotension, abnormal lymphocyte levels, and neuroendocrine dysfunction. However, the precise underlying mechanisms of the disease and the means by which they interact in patients with CFS remain to be clarified [2].

Recent works have emphasized the frequent association of CFS with gammaretrovirus (XMRV) infection,

although the role of XMRV as a causative agent of CFS remains controversial. In a recent US study, Lombardi et al [3] found that about 67% (68/101) of patients with CFS carried detectable levels of XMRV DNA in their peripheral blood mononuclear cells (PBMCs). Moreover, replicating virus was isolated from stimulated PBMCs in vitro. If confirmed, these findings would have a serious impact on understanding the pathogenesis of this complex and debilitating disease. However, another 3 recent reports showed that XMRV was absent in PBMCs from European CFS patients [4-6]. The results were similar in a recent report from the US [7], leading to an intense scientific debate over the relationship between the virus and CFS [8]. It is not yet clear whether the distribution of this virus is primarily dependent on geographic restrictions or, more likely, the diagnostic techniques used, such as PCR and real-time PCR. In terms of methodology, one of the risks associated with testing samples by PCR is the frequent occurrence of false negatives as a result of PCR inhibition [9]. The inclusion of an internal control (IC) serves as a monitor for false negatives

* Correspondence: ljm63hn@yahoo.com.cn; yongzheli@pumc@yahoo.com.cn

¹Graduate School, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, People's Republic of China

³Peking Union Medical College Hospital, Beijing, People's Republic of China
 Full list of author information is available at the end of the article

caused by DNA degradation or inhibitory factors that may be present in clinical samples [10]. In previous studies of XMRV detection [4-7], non-competitive ICs, such as GAPDH, were used to monitor for false-negative reactions. In the non-competitive IC strategy, separate primer pairs are used to detect the IC and the target nucleic acid. Nevertheless, the non-competitive ICs may introduce different amplification efficiencies due to their natural inter-individual variation, and may produce false-negative results. This matter is of considerable importance in the extensive controversy surrounding XMRV detection in CFS patients.

A review of the diverse results from previous studies reveals several questions about worldwide distribution and whether the retrovirus is linked to CFS, at all. Until now, no information has been published regarding XMRV infection in Chinese CFS sufferers. We developed sensitive multiplex real-time PCR and reverse transcriptase PCR (RT-PCR) assays, using a competitive internal control (CIC) strategy to ensure PCR integrity and eliminate false-negative results, to detect XMRV proviral DNA and viral RNA, respectively, in the PBMCs and plasma of Chinese CFS patients. The assays were standardized using constructed XMRV DNA or armored RNA standards, and their performances were evaluated.

Materials and methods

Study subjects and samples

Sixty-five CFS patients and 85 blood donors, including 65 healthy controls and 20 controls with hepatitis B, hepatitis C, human immunodeficiency virus type 1 infection, or human T-cell leukaemia virus infection (confirmed at the blood bank) were enrolled. Patients and controls were closely matched for age, sex, and place of residence. Both groups were aged between 20 and 55 years, and the ratios of women to men were 35:30 (CFS) and 44:41 (blood donors). Samples were collected from 2007 to 2009. CFS patients were recruited from clinics in Peking Union Medical College Hospital. All patients underwent full medical and neurological evaluations and were tested to exclude alternative explanations for their symptoms. Additionally, they fulfilled the 1994 international research criteria for diagnosis of CFS [1], which requires the presence of fatigue with 4 or more additional symptoms and was established to help distinguish CFS from other illnesses that cause fatigue. Blood donors were enrolled from the Beijing blood centre. All subjects provided informed consent prior to their participation in the study.

Whole blood was obtained by venipuncture from 85 blood donors and 65 CFS patients. PBMCs and plasma were isolated immediately by Ficoll-Hypaque-1077 (Sigma) from whole blood and stored at -80°C within 2 hours of sampling.

DNA and RNA preparation

DNA from 100 µl PBMCs (about 5.0×10^2 to 2×10^3 cells) or RNA from 140 µl plasma was isolated according to the manufacturer's instructions (QIAamp DNA Blood Mini Kit, QIAamp Viral RNA Mini kit, QIAGEN GmbH, Germany); extracted DNA was eluted in 100 µl DNase-free water, while RNA was eluted in 60 µl RNase-free water. Both were immediately stored at -80°C.

Primers and probes

Primers and probes for the XMRV real-time detection assay were designed to amplify regions of the XMRV *gag* gene (nt 462-523). Primer and probe sequences were optimized using Primer Express (Applied Biosystems) and were synthesized as previously described [11], to detect both XMRV proviral DNA and XMRV viral RNA. In order to calibrate the constructed armored RNAs to an international (IU) value, primers were designed to amplify regions of the HCV 5' UTR. Probes for the detection of XMRV and CIC were 5'-labelled with 6-carboxyfluorescein (FAM) or 6-carboxyhexachlorofluorescein (HEX), and all were 3'-labelled with Black Hole Quencher Dye (BHQ). The sequences and characteristics of the primers and probes are listed in Table 1.

Preparation of the XMRV DNA standard and the CIC: recombinant plasmids pACYC-MS2-2V and pACYC-MS2-IC-2V

An exogenous chimeric sequence 1584 bp in length was inserted into pACYC-MS2 [12] (previously constructed by our laboratory) with three C-variant *pac* sites inserted at the beginning, middle, and end. This sequence was obtained by overlapping extension PCR [11,12] amplification of XMRV (nt 33 to 1149, 1117 bp amplified from plasmid VP62 [3], kindly provided by Lombardi; [Genbank: EF185282]) and HCV (nt 25 to 445, 420 bp amplified from pNCCL-HCV [13], constructed by our laboratory; [Genbank: AF139594]). The primers used in this method are shown in Table 1.

CIC sequences were identical to the 1584-bp exogenous chimeric sequence, except for the probe-binding sites which were replaced by internal probe sequences using overlapping extension PCR [14]. The sequence of these 22 artificial random nucleotides shared a similar nucleotide composition as the wild type XMRV probe (Fig. 1).

The resulting recombinant plasmids pACYC-MS2-2V and pACYC-MS2-IC-2V were validated by sequencing. The concentrations of DNA standard and CIC were assessed by UV-spectrophotometry and DNA copy numbers were calculated.

Table 1 Primer and probe sequences

Primer or probe	Sequence (5'-3')
Gag-1S	5'-TTGCCCGGCCACATGAGGATCACCCTGTCGTGTTCCCAATAAAGCCTTTTGTCTGTTG-3'
Gag-1A	5'-ATTCAGACGGGGCGGGAATGTCGGCTTTGAGGGGGCCTGAGTGTCTCTGTCTCTCGTC-3'
Gag-2S	5'-GACGAGAGACAGAGACACTCAGGCCCCCTCAAAGCCGACATTCGCCGCCCTGTCTGAAT-3'
Gag-2A	5'-GAGTGATCTATGGTGGAGACATGGGTGATCCTCATGTGCCGCTCTTCTTCATTG-3'
HCV- S	5'-CAATGAAGAAGAGGCGGCACATGAGGATCACCCTGTCCTCCACCATAGATCACTC-3'
HCV-A	5'-CCTTAATTAACATGGGTGATCCTCATGTGGTGGTGTACGTTTGGTT-3'
Gag-3S	5'-GGACTTTTGGAGTGGCTTTGTT-3'
Gag-3A	5'-GCGTAAACCGAAAGCAAAAT-3'
Gag p	FAM5'-ACAGAGACACTTCCGCCCCCG-3'BHQ
IC p	HEX5'-CAGGCCCCCTCAAAGCCGACAT-3'BHQ
β-actin A	5'-CCTGGCACCCAGCACAAT-3'
β-actin S	5'-GCTGATCCACATCTGCTGGAA-3'
β-actin p	FAM5'-ATCAAGATCATTGCTCCTCTGAGCGC-3'TAKARA

FseI and *PacI* restriction enzyme sites are underscored; a C-variant *pac* site is indicated by boldface type. The broken line indicates the internal control sequences inserted in the CIC. FAM: 6-carboxyfluorescein; HEX: 6-carboxyhexachlorofluorescein; BHQ: Black hole quencher dye.

Preparation of the viral RNA standard and its CIC (armored RNAs)

Both pACYC-MS2-2V and pACYC-MS2-IC-2V were transformed into *E. coli* strain BL21 (DE3). The armored RNAs were expressed, purified, and verified [12,13], and their stabilities were also verified [12,13].

The purified armored RNAs were calibrated against the World Health Organization (WHO) Second International Standard for HCV RNA (National Institute for Biological Standards and Controls [NIBSC], code 96/798, UK), using an HCV RNA PCR fluorescence quantitative diagnostic kit (Shanghai, Kehua) [13]. The samples were tested in triplicate and the quantification values were averaged. The concentration of the CIC was evaluated by the same method.

Multiplex real-time PCR and RT-PCR for XMRV detection

Standard DNA and armored RNA were quantified, diluted to obtain 10^5 to 10^6 copies/μl and IU/ml, respectively, and used to determine the linearity, sensitivity, specificity, and reproducibility of the multiplex real-time assays. They also served as external positive controls (EPCs) in the multiplex real-time PCR and RT-PCR assays.

To determine the optimal CIC concentration for the real-time assay, a chequer-board assay was performed in which XMRV standards (10^5 to 10^2 DNA copies/μl or 10^5 to 10^2 RNA IU/ml) were spiked with 3 different concentrations (10^5 to 10^3 copies/μl or IU/ml, respectively) of the CIC, and the template mixture was assayed. Thereafter, it was coamplified or coextracted and coamplified with the samples in the same reaction tube. The final optimized PCR mixture (25 μl) contained 12.5 μl QuantiTect Probe PCR or RT-PCR Master Mix (QIAGEN, QuantiTect Multiplex PCR or RT-PCR kit), 0.4 μM XMRV-specific primers, 0.4 μM XMRV-specific

probes, and 0.2 μM IC-specific probe, 8.3 μl sample (2.0 μl of XMRV DNA, 1.0 μl CIC DNA (1000 copies/μl added during the amplification step), and 5.3 μl DEPC-treated water) or 0.25 μl QuantiTect RT Mix, 10 μl RNA (1000 IU/ml armored RNA CIC added to each sample prior to extraction). PCR was performed with an ABI 7500 sequence detection system as follows: an initial denaturation step at 95°C for 15 min, 45 cycles at 94°C for 15 s and 60°C for 1 min. In addition, the RT-PCR included an initial reverse transcription step of 50°C for 30 min.

The linearity and sensitivity of the XMRV assay were determined by using a dilution series of the DNA or armored RNA standard (10 copies to 10^6 copies/μl or IU/ml, respectively) in PBMCs DNA or plasma from a healthy donor. To mimic the conditions of the multiplex real-time PCR or RT-PCR procedures, we also included a steady concentration of CIC (1000 copies/μl or IU/ml, respectively). Experiments were performed in triplicate at each concentration.

Forty-five controls, which included 20 subjects with hepatitis B, hepatitis C, human immunodeficiency virus type 1 infection, or human T-cell leukaemia virus infection and 25 out of 65 healthy controls, were used to determine the specificity of the real-time assay.

The within-run precision of the quantitative real-time assay was assessed by evaluating 10 replicates of 3 dilutions of the DNA plasmid or armored RNA standard (10^5 , 10^4 , and 10^2 copies/μl or IU/ml, respectively), while the between-run precision was assessed by testing the same samples 10 times in 10 separate experiments. The coefficients of variance (CV) of the threshold cycles (Ct) were calculated.

Samples from 65 CFS cases and 65 healthy controls were tested using the standard curve method by multiplex

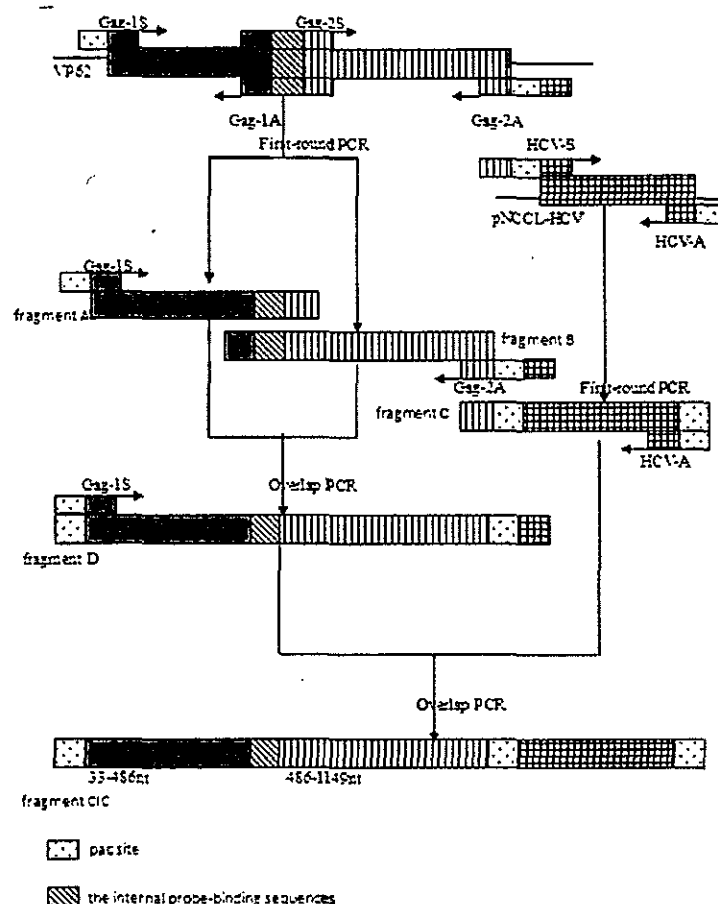


Figure 1 Construction of CIC by overlapping PCR. During the first-round of PCR, 3 fragments (A, B and C: VP62 33-486 nt, VP62 486-1149, and the HCV 5'UTR) were amplified from plasmid VP62 (kindly provided by Lombardi) and pNCCL-HCV (constructed by our laboratory) using primers Gag-1S and Gag-1A, Gag-2S and Gag-2A, and HCV-S and HCV-A. In the first overlapping PCR, fragment D was amplified from fragments A and B using primers Gag-1S and Gag-2A; the internal probe-binding sequences were introduced into fragment D. Fragment CIC was obtained by a second overlapping PCR using primers Gag-1S and HCV-A to amplify from fragments D and C.

real-time PCR or RT-PCR with CICs (10^3 copies/ μ l or IU/ml, respectively, present in each sample). The standard curves were generated from serial dilutions of the standards (10^6 to 10^2 copies/ μ l or IU/ml, respectively). In addition, pACYC-MS2-2V or armored RNA standard was used as an EPC. To control for the integrity of the DNA or RNA, the cellular β -actin gene was amplified in all clinical specimens and was tested under the same conditions, but with 0.4 μ M β -actin specific primers and 0.4 μ M β -actin specific probe (5' FAM, 3' TAKARA-labelled) (see Table 1).

Results

Construction of XMRV DNA standard and CIC plasmid

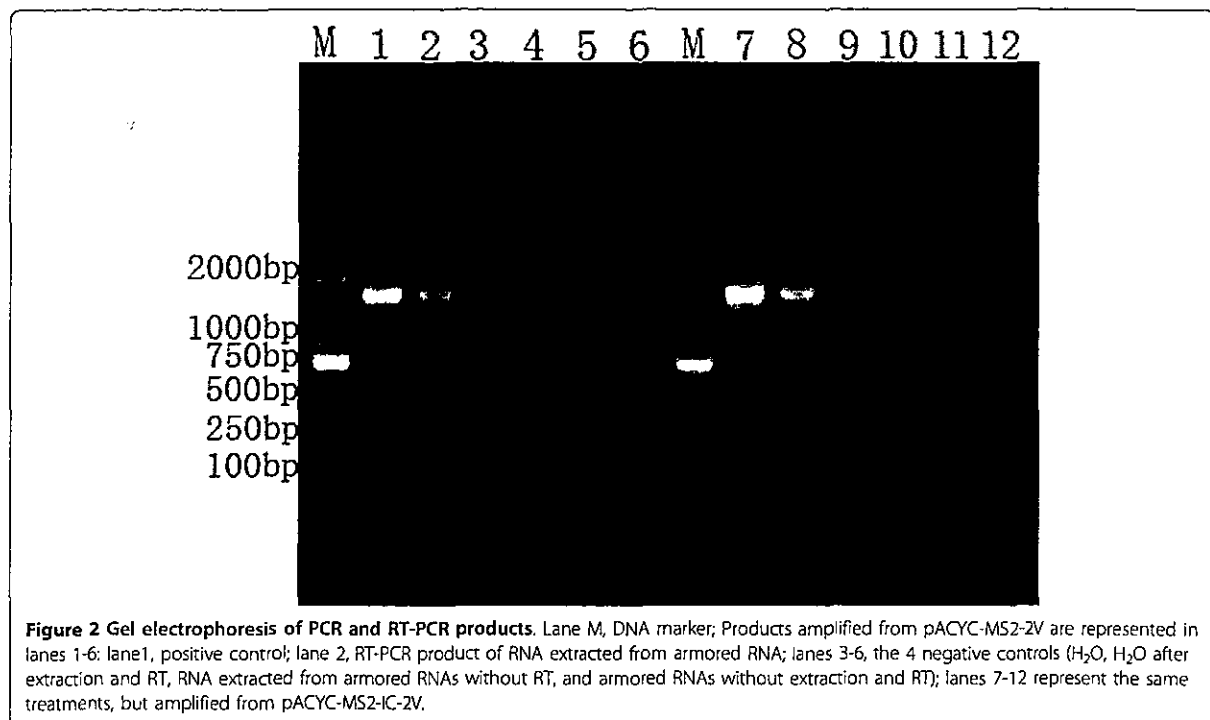
The PCR amplification products from the DNA standard or CIC plasmid (using primer Gag-1S and HCV-A) were full length (1584 bp and 1606 bp, respectively).

Sequencing demonstrated that the exogenous chimeric sequences were successfully inserted into the pACYC-MS2 vector. The PCR products were analyzed by electrophoresis on an agarose gel (1%) (Fig. 2).

Preparation of the viral RNA standard and its internal control (armored RNAs)

The purified armored RNAs were electrophoresed and a single band of approximately 1.0 kb could be seen by agarose gel analysis (Fig. 3). The RT-PCR amplification products of the RNA extracted from armored RNAs were analyzed by agarose gel electrophoresis (Fig. 2). PCR products were then verified by sequencing.

Armored RNAs in EDTA-preserved human plasma incubated at 4°C, 37°C, and room temperature were stable for over 3 months (data not shown).



To evaluate the performance characteristics of armored RNA as a calibrator for XMRV RNA assays, we used the HCV international standard to calibrate serially diluted armored RNA. The concentrations of the chimeric armored RNA for the 5 dilutions (10^6 , 10^5 , 10^4 , 10^3 , and 10^2) were 5.63×10^6 IU/ml, 6.01×10^5 IU/ml, 5.47×10^4 IU/ml, 5.36×10^3 IU/ml, and 5.75×10^2 IU/ml. The concentrations of the CIC were evaluated in the same way, at 1.12×10^6 IU/ml, 9.78×10^4 IU/ml, 1.03×10^4 IU/ml, 1.15×10^3 IU/ml, and 1.07×10^2 IU/ml.

Multiplex real-time PCR and RT-PCR for XMRV detection

A dilution series of the DNA or RNA XMRV standard was spiked with 3 different concentrations (10^5 to 10^3 copies/ μ l or IU/ml) of the CIC and used as a mixed template. We determined that 1000 copies/ μ l of DNA plasmid or 1000 IU/ml armored RNA was the optimal CIC concentration for the multiplex real-time assay (Table 2).

Linear regression analysis of the Ct values against the $\log_{10}$ XMRV DNA or armored RNA standard concentration yielded $r^2 = 0.999$. The lowest detectable concentration of XMRV DNA or armored RNA standard was 20 copies/reaction, calculated as 10 copies/ μ l $\times$ 2.0 μ l XMRV DNA standard per reaction, or 10 IU/mL, respectively (Fig 4).

The specificity of the multiplex real-time assay was 100% in testing of the 45 controls.

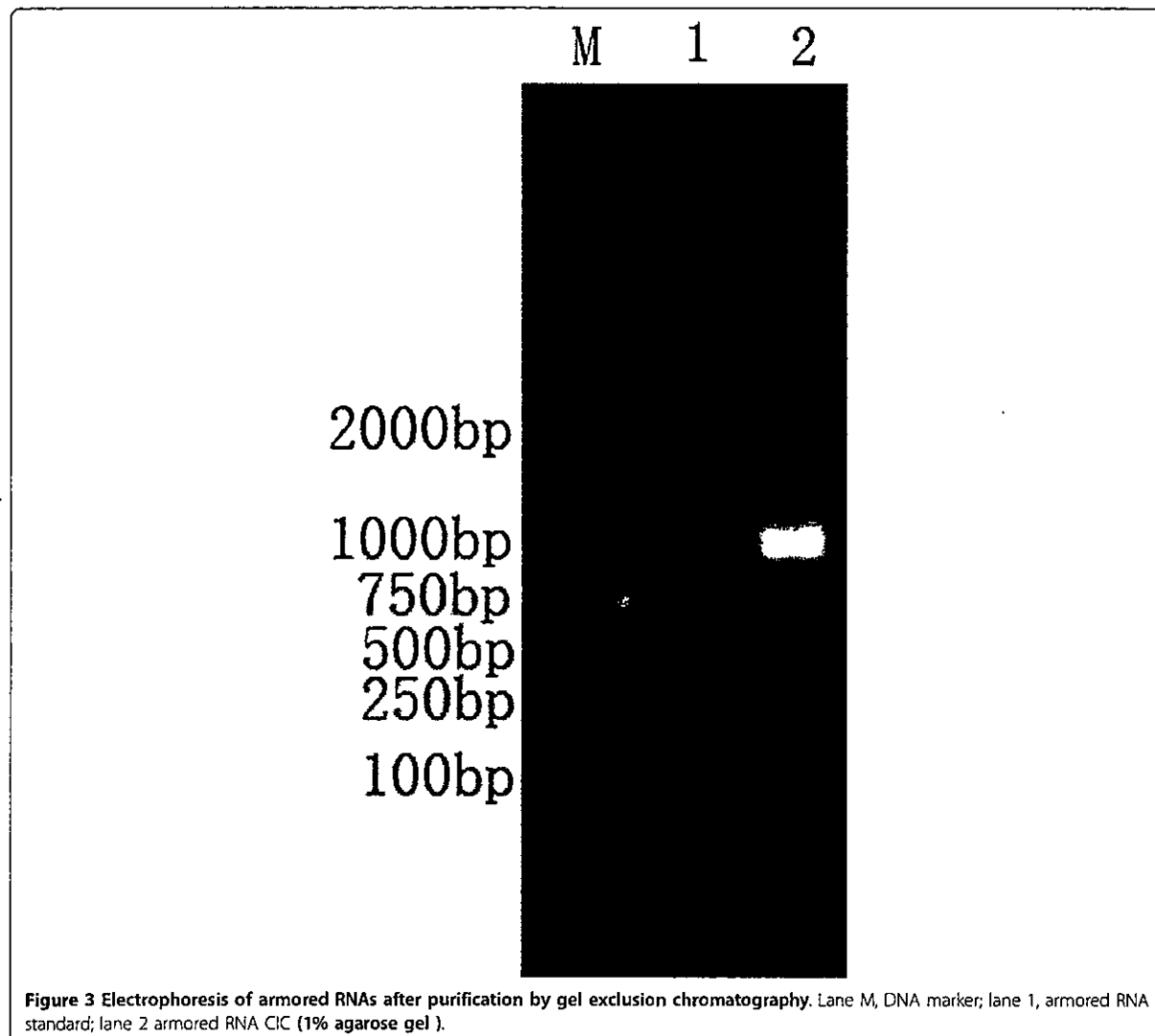
Reproducibility was established based on the Ct values obtained within each run (within-run) and between runs. The within-run precision CV ranged from 1.76% to 2.80% or 1.70% to 2.59%, while the between-run CV ranged from 1.07% to 2.56% or 1.06% to 2.74% (Table 3).

The amounts of XMRV DNA derived from PBMC and RNA derived from plasma were determined by using the standard curve method. No signals for the XMRV-specific probe were detected, while all 65 CFS cases and 65 healthy controls generated positive CIC (1000 copies/ μ l or IU/ml, respectively) signals with Ct values between 32 and 36 (Figure 5). The β -actin gene was detected in all clinical specimens.

Discussion

Sensitive multiplex real-time PCR and RT-PCR assays with CICs were established to detect XMRV proviral DNA in PBMCs or viral RNA in plasma, respectively, from Chinese patients with CFS. The virus was not detected in any of our study subjects; these results do not support an association between XMRV and CFS in Chinese.

Our findings appeared to be inconsistent with a previous report of XMRV DNA isolation from PBMCs of CFS patients in the US [3]. Technical differences can be ruled out as a reason for the failure to detect XMRV. The sensitivity of the multiplex real-time PCR (20 copies/reaction) was likely to be as sensitive, if not more



so, as the end point PCR method previously used [3], thus suggesting that multiplex real-time PCR can be used for the detection of XMRV proviral DNA. The end-point PCR method used in the previous study requires multiple manipulations of the sample after the amplification step, thus increasing the risk of carryover contamination. The possibility that proviral DNA degradation during the extraction process may have led to our negative results seems unlikely. The β -actin gene was positive for all clinical specimens, confirming the integrity of the DNA. In addition, samples used in this research were representative of typical patients with CFS, which met the 1994 Centers for Disease Control and Prevention case definition of CFS (called the Fukuda criteria). Although the patients studied by Lombardi et al [3] were reported to fulfil the same criteria, a

clear description of their patient and control cohorts was lacking.

Several PCR-based methodologies have been developed for the detection of XMRV DNA, including end point and real-time PCR methodologies [4-7]. Beta-globin gene, GAPDH, and β -actin were used as non-competitive ICs to validate DNA integrity in 4 recent studies of XMRV [4-7]. However, DNA concentrations may vary widely between clinical samples. Van Kuppeveld et al [6] amplified a known amount of phocine distemper virus (PDV) that had been added to clinical samples to monitor RNA quality and to detect amplification inhibition. Although attractive, the use of live viruses as internal controls may raise concerns regarding safety and consistency between preparations. Additionally, the performance of non-competitive ICs is imperfect due to

Table 2 Optimization of CIC concentration

(1) Multiplex real-time PCR									
pACYC-MS2-IC-2V plasmid CIC concentration (copies/μl)	pACYC-MS2-2V plasmid standard 1 × 10 ⁵ copies/μl		pACYC-MS2-2V plasmid standard 1 × 10 ⁴ copies/μl		pACYC-MS2-2V plasmid standard 1 × 10 ³ copies/μl		pACYC-MS2-2V plasmid standard 1 × 10 ² copies/μl		XMRV 0 copies/μl
	CIC (HEX)Ct	XMRV (FAM)Ct	CIC (HEX)Ct	XMRV (FAM)Ct	CIC (HEX)Ct	XMRV (FAM)Ct	CIC (HEX)Ct	XMRV (FAM)Ct	CIC (HEX)Ct
100000	39.22	28.79	32.18	31.58	28.92	34.81	28.50	No Ct	28.34
10000	37.85	28.12	31.26	31.06	32.36	34.56	31.45	44.26	31.55
1000	39.52	24.73	37.20	27.76	31.50	31.18	35.79	34.28	34.11
0	No Ct	24.69	No Ct	28.47	No Ct	30.73	No Ct	33.81	No Ct
(2) Multiplex real-time RT-PCR									
Armored RNA concentration (IU/ml)	Armored RNA standard 1 × 10 ⁵ IU/ml		Armored RNA standard 1 × 10 ⁴ IU/ml		Armored RNA standard 1 × 10 ³ IU/ml		Armored RNA standard 1 × 10 ² IU/ml		XMRV 0 IU/ml
	CIC (HEX)Ct	XMRV (FAM)Ct	CIC (HEX)Ct	XMRV (FAM)Ct	CIC (HEX)Ct	XMRV (FAM)Ct	CIC (HEX)Ct	XMRV (FAM)Ct	CIC (HEX)Ct
100000	39.50	25.24	30.89	28.28	27.12	No Ct	26.35	No Ct	26.07
10000	37.96	25.00	30.70	28.22	30.37	31.17	27.79	37.06	29.49
1000	No Ct	25.25	30.65	28.03	33.18	31.14	33.87	34.40	33.27
0	No Ct	25.18	No Ct	27.82	No Ct	30.78	No Ct	37.11	No Ct

Ct values indicate the standard concentrations. A sample with Ct > 45 cycles was considered to be negative. Concentrations of XMRV DNA/RNA and pACYC-MS2-2V plasmid/armored RNA standard were indicated by FAM and HEX signals, respectively.

differences in the amplification efficiencies of different target nucleic acids [15]. Here, we used CIC as a substitute. The CIC was a constructed plasmid which mimicked the template with the same length and primer binding sites, and similar GC content. In order to avoid suppression of target amplification and possible competition between the target and CIC, we optimized the concentration of CIC added to the samples. IC concentrations of more than 1000 copies/μl altered the Ct of almost all standards which yielded an underestimation

of the concentration. CIC at 1000 copies/μl was determined to be optimal for our real-time assay. Our results indicated that no inhibitory effects were at play in the multiplex real-time assay we used to screen our study population.

The pathogenesis and outcome of XMRV infection may be associated or even causally linked with plasma viral RNA loads, as well as proviral loads. In addition to XMRV proviral DNA detection, we developed a sensitive multiplex real-time RT-PCR assay to detect XMRV

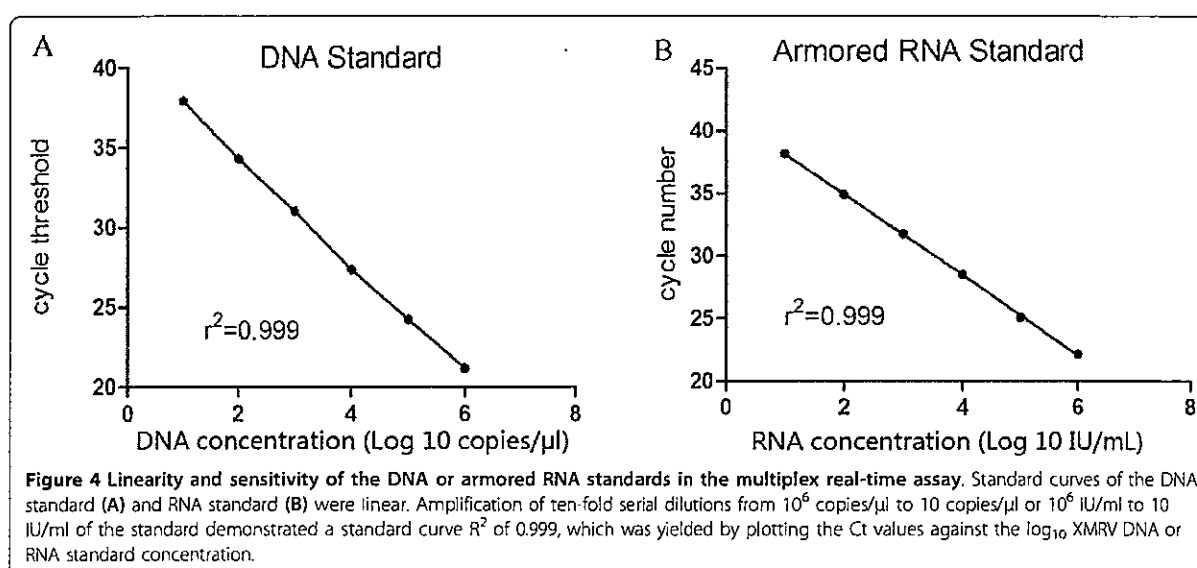


Table 3 Reproducibility of the real-time PCR/RT-PCR assay

Reproducibility	XMRV DNA/RNA (copies/μl/IU/ml)	Number of determinations	Mean Ct (DNA/RNA)	SD (DNA/RNA)	CV(%) (DNA/RNA)
within-run	10 ⁵	10	24.47/25.13	0.50/0.52	2.03/2.06
	10 ⁴	10	27.25/27.99	0.48/0.48	1.76/1.70
	10 ²	10	33.81/34.62	0.94/0.90	2.80/2.59
between-run	10 ⁵	10	24.97/26.17	0.28/0.32	1.14/1.23
	10 ⁴	10	27.98/29.14	0.30/0.31	1.07/1.06
	10 ²	10	34.43/35.94	0.88/0.99	2.56/2.74

viral RNA in plasma. We constructed armored RNAs to serve as the XMRV viral RNA standard and CIC to evaluate the analytical linearity, sensitivity, specificity, and reproducibility of the detection assay. Both were stable in normal human EDTA-preserved plasma at 4°C, 37°C, and room temperature for over 3 months. Armored RNA is a kind of non-infectious recombinant virus-like particle (VLP) containing target exogenous RNA. In comparison to naked RNA, as armored RNA is a more suitable candidate for a positive control or standard in the quantification of RNA viruses, because it is RNase-resistant, stable, non-infectious, and easily extracted by conventional methods [16-19]. Moreover, armored RNA can serve as a control for all stages of the RT-PCR assay, from extraction through amplification. The inclusion of the HCV 5'UTR made it easy to assign an IU value to the XMRV RNA and the CIC within the armored RNA, avoiding the necessity for the complex procedures involved in value assignment of calibrators or standards when international standards (IS) are not available [12]. These characteristics of armored RNA ensure the validity of our data. Conflicting results have made the associations between XMRV and CFS unclear; it is therefore important to produce a 'universal' XMRV standard so that the results of different assays may be compared. By using an armored RNA standard, different research groups and clinical laboratories may directly

compare their quantitative data. Nevertheless, we did not detect XMRV viral RNA with our armored RNA-standardised method in plasma samples from a Chinese study population.

These findings may not be generalisable beyond the study population because XMRV infection rates may vary geographically. Similarly, although XMRV was initially discovered in tumour tissues of a subset of patients with prostate cancer [20], other studies have shown a variable incidence of the virus in prostate tumours. One US study found XMRV in up to 23% of prostate cancer tumours [21]; however, a recent German study found a 0% incidence of XMRV [22]. Additional research is needed to determine what, if any, role XMRV plays in CFS in Chinese patients.

Conclusions

This study failed to show the presence of XMRV in PBMCs and plasma of Chinese patients with CFS using the multiplex real-time PCR assay; these results do not support an association between XMRV and CFS in people of Chinese ancestry.

Acknowledgements

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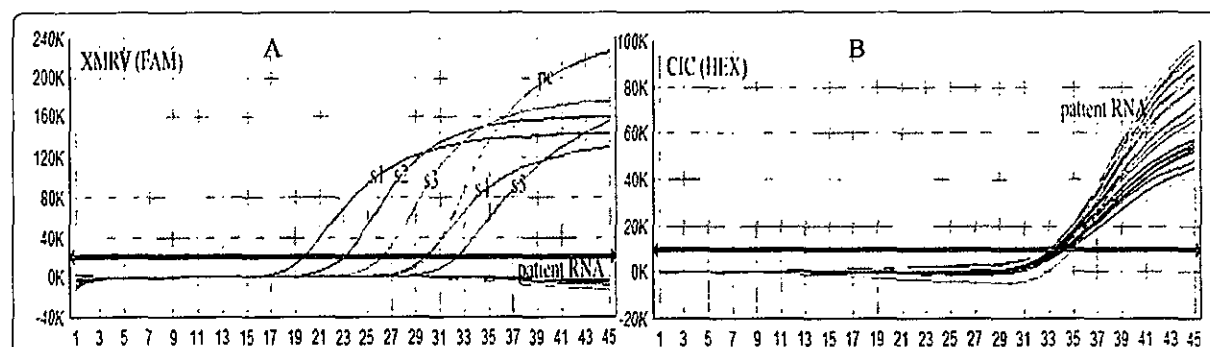


Figure 5 Multiplex real-time assay for detection of patient XMRV proviral DNA or viral RNA Examples of viral RNA screen of 25 CFS patient plasma samples including CICs in each reaction. (A) Amplification plot for a plasma sample obtained from a CFS patient: No XMRV-specific signals were detected. S1-S5 were armored RNA standards (from 10⁷ to 10³ IU/ml), pc was an armored RNA EPC. (B) Amplification plot of the CICs (1000 IU/ml), which were added to patient samples.

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Author details

¹Graduate School, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, People's Republic of China. ²National Center for Clinical Laboratories, Beijing Hospital, Beijing, People's Republic of China. ³Peking Union Medical College Hospital, Beijing, People's Republic of China.

Authors' contributions

PH planned the experimental design and drafted the manuscript. JL generated the concept for the study, participated in its design and coordination, and helped to revise the manuscript. YZL collected specimens and data from the study population. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日 2010. 11. 15	新医薬品等の区分 該当なし	総合機構処理欄
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販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン5%静注12.5g/250mL(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)			イタリア	
研究報告の概要	○エジプトから帰国した旅行者におけるAlkharma出血熱、2010年 Alkharmaウイルス(ALKV)はフラビウイルス属のダニ媒介性出血熱グループに属し、現在、Kyasanur森林病ウイルスの変種であると考えられている。この新興病原体は発熱、頭痛、関節痛、嘔吐、血小板減少などを引き起こし、重症例では出血症状や脳炎となる可能性があり、これまでの報告例によると致死率は25%程度である。ALKVはヒトに経皮的(感染脊椎動物の血液への接触、感染ダニの刺咬)に感染するか、ウイルスで汚染された未殺菌のミルクを飲むことで経口感染すると推定されている。蚊が媒介するとの仮説もある。ここでは、2010年にエジプトからイタリアに帰国した2名の旅行者でのAlkharma出血熱を報告する。 1例目の患者は1週間の南エジプト旅行の途中、ラクダ市場を訪問し、節足動物に足を刺咬された。その48時間後に高熱、悪寒、戦慄、摂食障害、倦怠感、嘔気、嘔吐、霧視が出現し、その後5日間継続した。PCR検査、シーケンス解析によりALKV感染が認められた。2例目の患者も1例目と同じ地域に1か月後に訪問し、同じラクダ市場を訪れて罹患したが、比較的軽症であった。ALKVは今までサウジアラビアだけで検出されていたが、今回エジプトからの帰国者で報告されたことで、このウイルスの地理分布は以前より広範囲である可能性が疑われる。ALKVの地理的分布に関して、また地元住民や旅行者の危険性を評価するため、さらなる調査が必要である。				使用上の注意記載状況・ その他参考事項等
報告企業の意見		今後の対応		血液を原料とすることに由来する感染症伝播等	
エジプト南部からイタリアに帰国した2名の旅行者で、Alkharmaウイルス感染による出血熱が認められたとの報告である。 Alkharmaウイルスは脂質膜を持つ中型のRNAウイルスである。 これまで、本製剤によるAlkharmaウイルス感染の報告はない。 本製剤の製造工程には平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれていることから、本製剤の安全性は確保されていると考える。		日本赤十字社では、輸血感染症対策として受付時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。			

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Alkhurma Hemorrhagic Fever in Travelers Returning from Egypt, 2010

Fabrizio Carletti, Concetta Castilletti, Antonino Di Caro, Maria R. Capobianchi, Carla Nisii, Fredy Suter, Marco Rizzi, Alessandra Tebaldi, Antonio Goglio, Cristiana Passerini Tosi, and Giuseppe Ippolito

Two travelers returning to Italy from southern Egypt were hospitalized with a fever of unknown origin. Test results showed infection with Alkhurma virus. The geographic distribution of this virus could be broader than previously thought.

Alkhurma virus (ALKV) is a recently described member of the tick-borne hemorrhagic fever group of the genus *Flavivirus*. It was initially isolated in the late 1990s (1,2) and is today considered a variant of the Kyasanur Forest disease virus, sharing 89% nt sequence homology (3,4). This emerging pathogen causes signs and symptoms such as fever, headache, joint pain, muscle pain, vomiting, and thrombocytopenia; severe cases may have hemorrhagic manifestations (epistaxis, ecchymoses, petechiae, hematemesis) and encephalitis, which can result in death (reported case-fatality rate as high as 25%) (5–8). Camels and sheep are thought to be the natural hosts of ALKV, but whether other mammals are also involved in its life cycle remains unknown. ALKV RNA was recently detected in an *Ornithodoros savignyi* tick collected near Jeddah, Saudi Arabia (9); on the Arabian Peninsula, these ticks have been associated with camels and their resting places and can be found where cases of ALKV infection in humans have been reported. These ticks seek multiple hosts, are nocturnal and cryptic, and commonly attack humans and other animals resting under trees (10). The hypothesis that mosquitoes could also be vectors has been suggested by 2 studies (6,7); despite the absence of data to substantiate it, this possibility cannot be excluded.

Author affiliations: "Lazzaro Spallanzani" National Institute for Infectious Diseases, Rome, Italy (F. Carletti, C. Castilletti, A. Di Caro, M.R. Capobianchi, C. Nisii, G. Ippolito); and "Ospedale Riuniti di Bergamo," Bergamo, Italy (F. Suter, M. Rizzi, A. Tebaldi, A. Goglio, C.P. Tosi)

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Evidence suggests that ALKV infects humans either transcutaneously (by contamination of a skin wound with the blood of an infected vertebrate or through the bite of an infected tick) or orally through consumption of unpasteurized contaminated milk. Transmission to humans has been associated with butchering of sheep and camels. No human-to-human transmission has been reported. ALKV is classified in different countries as a BioSafety Level 3 or 4 agent.

ALKV has been detected only in Saudi Arabia, but the closely related Kyasanur Forest disease virus has spread as far as India and the People's Republic of China (4). We describe 2 cases of Alkhurma hemorrhagic fever in 2 travelers who returned to Italy from Egypt in 2010.

The Cases

The first patient, a 64-year-old man from Italy, spent 1 week (April 25–May 1, 2010) in a touristic village in southern Egypt, near the Sudan border. While visiting a camel and dromedary market in Shalatin on April 29, he was bitten on the foot by an unidentified arthropod (although not formally identified, was described as tick shaped). Soon after, a small, papular lesion developed. During his return flight to Italy, ≈48 hours after the bite, the patient experienced high fever, shaking chills, anorexia, malaise, nausea and vomiting, and blurred vision. During the next 5 days, these signs and symptoms worsened, and the man was admitted to the "Ospedali Riuniti di Bergamo" in northern Italy. His medical history was unremarkable, but he frequently traveled abroad and had been vaccinated against yellow fever in 1998.

Laboratory test results showed leukopenia (2,250 cells/mm³), thrombocytopenia (67,000 platelets/mm³), and increased liver enzymes (aspartate transaminase 469 U/L, reference 3–46 U/L; alanine transaminase 406 U/L, reference 3–46 U/L). The patient was given acetaminophen, and fever and general malaise progressively decreased over the next 5 days. He was discharged 11 days later, on May 17, in good general condition despite persistence of asthenia.

Acute-phase and convalescent-phase serum samples (collected on May 10 and 27, respectively) were sent to the virology laboratory of the "Lazzaro Spallanzani" National Institute for Infectious Diseases in Rome to be tested for dengue and West Nile viruses. Immunoglobulins (Ig) G and M for both viruses were detected by immunofluorescence of both samples; for each virus, IgG titer was ≥640 and IgM titer was ≥20. No evidence of rising antibody titers was found in the convalescent-phase specimen, raising suspicion of cross-reactivity to a previous *Flavivirus* infection or yellow fever vaccination. A genus-specific reverse transcription-PCR selective for the nonstructural protein (NS) 5 gene of flaviviruses (11) was positive for the acute-phase and negative for the convalescent-phase

samples. Sequence analysis of the amplicon (GenBank accession no. HM629507) showed high similarity with ALKV sequences in GenBank (BLAST [www.ncbi.nlm.nih.gov/blast/Blast.cgi] submission showed 97% identity with AF331718). This unexpected result called for further investigations to confirm the diagnosis of an ALKV infection. Thus, an ALKV-specific nested reverse transcription-PCR selective for a wider region of a different gene (E) was designed by using the following primers: outer forward 5'-TGGAACCCACACGGGTGACT-3'; outer reverse 5'-ATGCCACTGTCTGGTTGGCG-3'; inner forward 5'-CCCACAGCAATCGAAAAACGGCATC-3'; inner reverse 5'-GCCACATCACAGGTGACATGACC-3'.

All residual biological samples collected during the patient's hospital stay were sent to the virology laboratory of the Spallanzani Hospital (Italy's national reference labora-

tory for viral hemorrhagic fever viruses, BioSafety Level 4) in compliance with biosafety procedures. The new ALKV PCR result was positive, and the sequence of the amplicon (GenBank accession no. HM629508) showed high homology with ALKV (99% identity with AF331718). The phylogenetic trees based on partial sequences of NS5 (Figure 1) and E (Figure 2) genes confirmed the diagnosis of ALKV infection.

After submitting this article, we detected ALKV infection in a second patient. This patient had traveled to the same area ≈ 1 month later, visited the same camel market, and was affected by a milder disease. NS5 (HQ218942) and E (HQ218941) gene sequences obtained from this patient have been included in the phylogenetic tree, showing that they cluster together with those from the first patient (Figures 1, 2).

Conclusions

The 2 patients had traveled to an area of the world where ALKV had not been previously reported. Although viremia was demonstrated 10 days after symptom onset, and we can reliably suppose that it started when fever and chills appeared, the probability of a susceptible vector in Europe is small, and the infection seems not to be transmissible from human to human.

Laboratory diagnosis of this infection is not easy to obtain and requires a specialized laboratory because of antibody cross-reactivity with other members of the family *Flaviviridae* and because of the absence of commercially available serologic tests and reference biologic materials for their development. However, surveillance of travelers returning from areas where highly dangerous infectious diseases are endemic should be improved and should include ALKV. The finding that the distribution of this virus is wider than previously thought and that it includes the African continent is in line with the hypothesis that tick-borne flaviviruses originated in Africa (12). The low genetic distance between the Egypt and Saudi Arabia sequences supports the hypothesis of a recent divergence from Kyasanur Forest disease virus, i.e., the closest flavivirus (5), and a slow microevolution of ALKV, as for other tick-borne flaviviruses (13). The higher genetic divergence in the NS5 gene than in the E gene of ALKV strains confirms previous observations for viruses isolated from human samples after inoculation of suckling mice (5) and deserves more detailed evolutionary analysis.

The detection of 2 independent infection events for travelers who visited the same area in a restricted period strongly supports the hypothesis of sustained local ALKV circulation. Further veterinary and entomologic investigations are needed to expand understanding of the geographic distribution of ALKV and to assess the danger for local populations and visitors. It would be advisable to inform

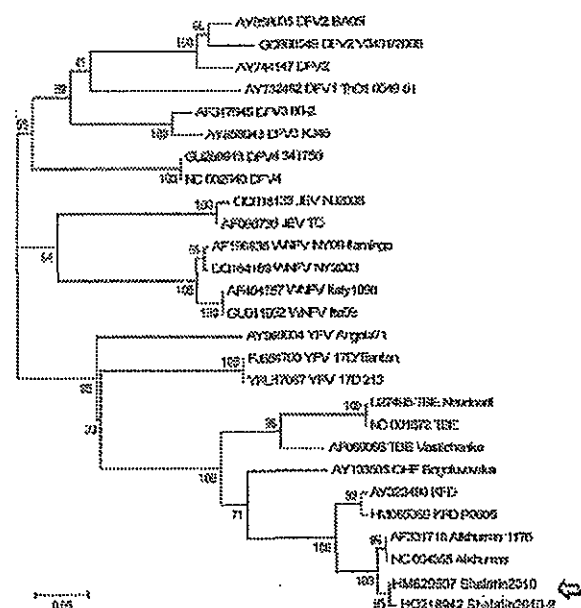


Figure 1. Phylogenetic tree based on sequences of the amplicon produced by the flavivirus nonstructural protein (NS) 5 gene reverse transcription-PCR (amplicon size, 208 bp; position in reference AF331718, nt 9077–9275), performed on the acute-phase serum samples of 2 travelers returning to Italy from Egypt (open arrow) showing relationship with other flaviviruses. Sequences are identified by name and GenBank accession number. Multiple alignment of other flavivirus sequences available in GenBank was generated by use of the ClustalW 1.7 software (www.clustal.org) included in the Bioedit package (www.mbio.ncsu.edu/BioEdit/BioEdit.html). The phylogenetic tree was constructed by nucleotide alignment, the Kimura 2-parameter algorithm, and the neighbor-joining method implemented in MEGA 4.1 software (www.megasoftware.net). The robustness of branching patterns was tested by 1,000 bootstrap pseudo-replications. Scale bar indicates nucleotide substitutions per site. DFV, dengue fever virus; JEV, Japanese encephalitis virus; WNV, West Nile fever virus; TBEV, tick-borne encephalitis virus; OHFV, Omsk hemorrhagic fever virus; KFDV, Kyasanur Forest disease virus.

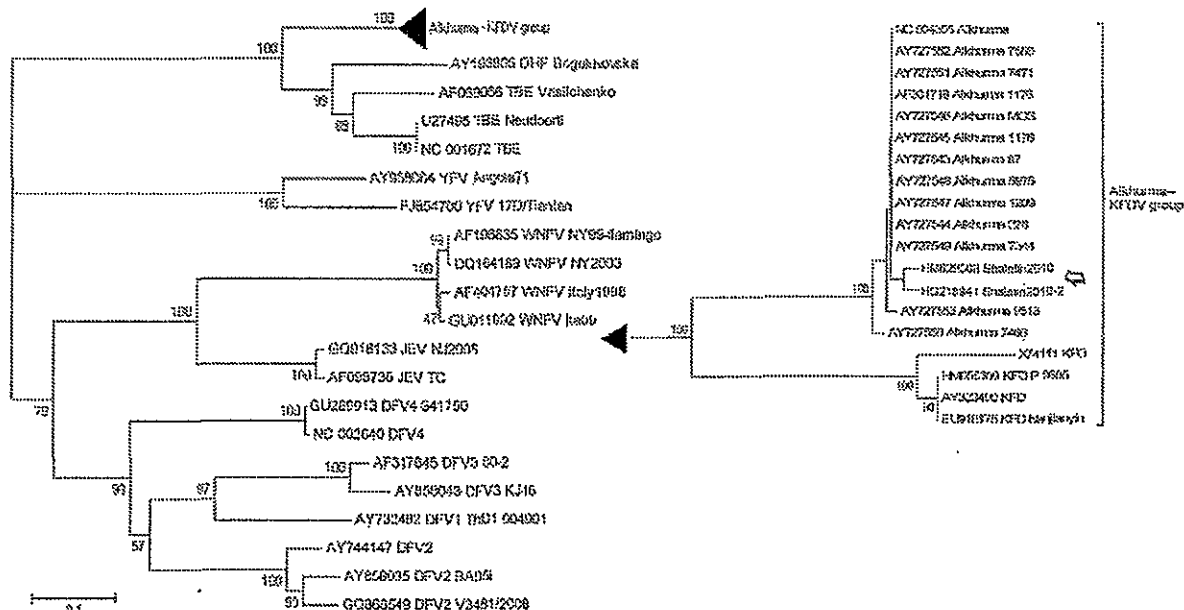


Figure 2. Phylogenetic tree based on the sequences of Alkhurma hemorrhagic fever virus E gene amplicon (amplicon size, 516 bp; position in reference AF331718, nt 1398–1913), obtained from acute-phase serum samples from a patient who had traveled to Egypt (open arrow) with respect to other flaviviruses. Sequences are identified by name and GenBank accession number. The phylogenetic tree was constructed by nucleotide alignment, the Kimura 2-parameter algorithm, and the neighbor-joining method implemented in MEGA 4.1 software (www.megasoftware.net). The robustness of branching patterns was tested by 1,000 bootstrap pseudo-replications. Scale bar indicates nucleotide substitutions per site. DFV, dengue fever virus; JEV, Japanese encephalitis virus; WNV, West Nile fever virus; TBEV, tick-borne encephalitis virus; OHFV, Omsk hemorrhagic fever virus; KFDV, Kyasanur Forest disease virus. The relevant part of the tree is enlarged at right.

travelers about the danger of coming into contact with infected animals in areas where the virus has been reported. Avoidance of or minimization of exposure to infected ticks should be recommended as the most effective prevention measure.

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Dr Carletti is a scientist at the Virology Laboratory of the “Lazzaro Spallanzani” National Institute for Infectious Diseases in Rome. His research interests include emerging and reemerging infections, especially virologic aspects of host–pathogen interactions and development of tools and protocols for diagnosis of emerging viral diseases, including viral hemorrhagic fevers.

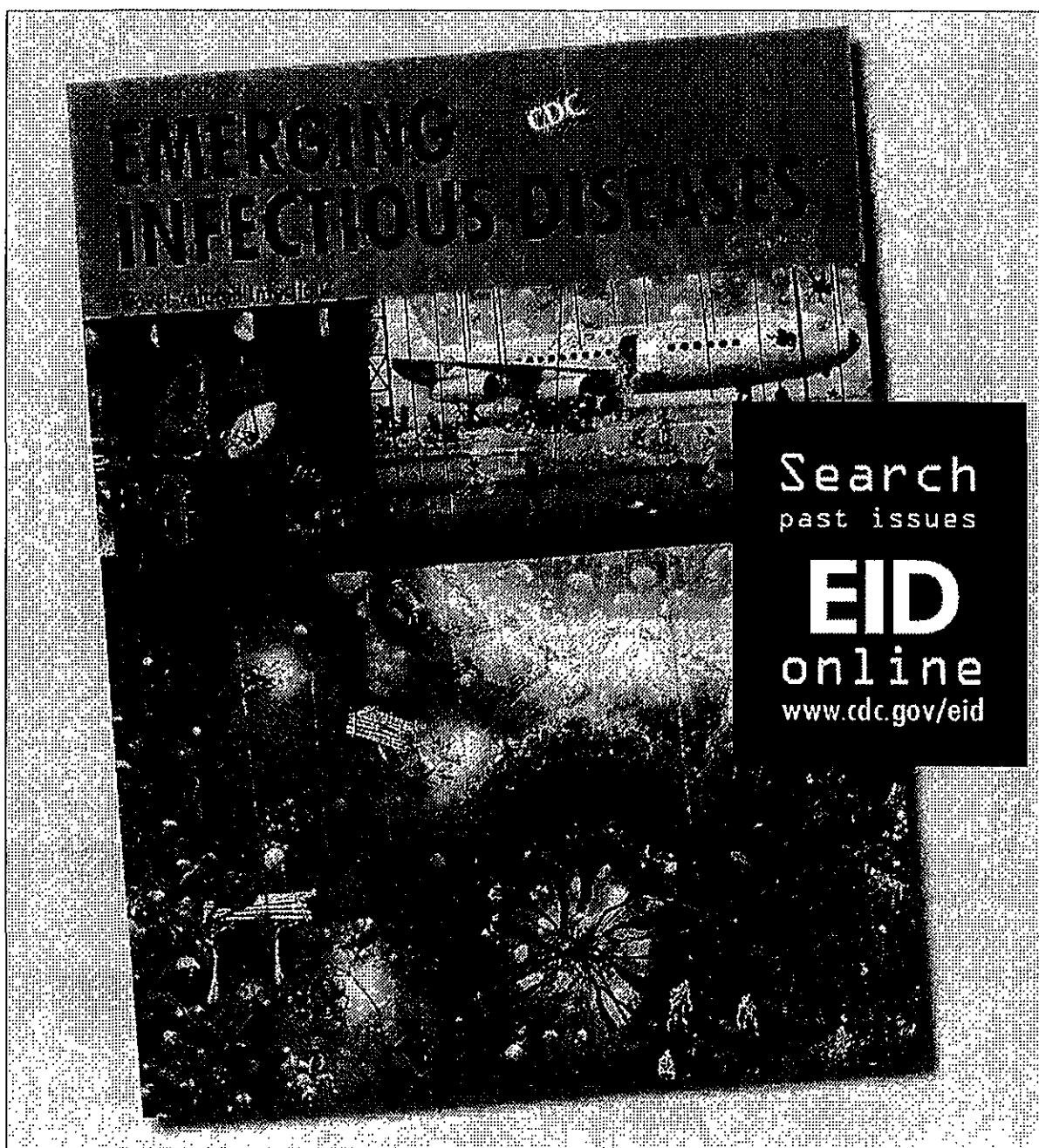
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11. Scaramozzino N, Crance JM, Jouan A, DeBriel DA, Stoll F, Garin D. Comparison of flavivirus universal primer pairs and development of a rapid, highly sensitive heminested reverse transcription-PCR assay for detection of flaviviruses targeted to a conserved region of the NS5 gene sequences. *J Clin Microbiol*. 2001;39:1922-7. DOI: 10.1128/JCM.39.5.1922-1927.2001
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Address for correspondence: Giuseppe Ippolito, National Institute for Infectious Diseases "L. Spallanzani," 292 Via Portuense, 00149 Rome, Italy; email: giuseppe.ippolito@inmi.it



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 2011. 3. 16	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	解凍人赤血球濃厚液	研究報告の公表状況	Quinlivan ML, Ayres KL, Kelly PJ, Parker SP, Scott FT, Johnson RW, Maple C, Breuer J. J Clin Virol. 2011 Feb;50(2):130-5.	公表国 英国	
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	○帯状疱疹を呈する患者における水痘帯状疱疹ウイルス(VZV)によるウイルス血症の持続 背景: 帯状疱疹は、VZVが感覚ニューロンでの潜伏状態から再活性化されるときに生じる。帯状疱疹に伴う最も一般的な合併症は、帯状疱疹後神経痛(PHN)という慢性痛である。 目的: 帯状疱疹が消退した後のVZVウイルス血症の動態とウイルス量、及びPHN発症との関連性について調査すること。 研究方法: 血液サンプルは活動性の帯状疱疹を呈する患者63人を対象とした前向き調査のベースライン時である1カ月、3カ月、6カ月時に採取された。全血のVZV DNAの定量化は、リアルタイムPCRアッセイを用いて実施した。 結果: 急性期には全ての患者の血液においてVZV DNAが検出できた。6カ月の患者血液でも、レベルは有意に低下したものの、91%でVZV DNAが検出できた($p < 0.0001$)。前駆症状の既往歴($p = 0.005$)やベースライン時に強い痛み($p = 0.038$)のあった患者は、抗ウイルス薬を服用している患者($p = 0.046$)や免疫不全の患者($p = 0.043$)と同様に、PHNから回復するまでの時間が長かった。ウイルスDNA量は一貫してPHNのリスク因子を有する患者で多かった。また、ウイルスDNA量が多いほど、回復までの時間が長かった(全体で$p = 0.058$、免疫正常で$p = 0.038$)。 結論: これらの結果は、VZVの増殖が急性の帯状疱疹を持続させること及び高ウイルスDNA量がPHNのリスク因子となることを示唆する。				使用上の注意記載状況・ その他参考事項等
		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			
活動性の帯状疱疹を呈する患者を対象とした前向き調査により、水痘帯状疱疹ウイルスの増殖は急性の帯状疱疹を持続させることや、高ウイルスDNA量が帯状疱疹後神経痛のリスク因子となることが示唆されたとのことである。		日本赤十字社では受付時に3週間以内の帯状疱疹発症の有無を確認し、該当者を献血不適としている。また、皮疹が治癒した後でも合併症が残っている場合は献血不適としている。今後も引き続き情報の収集に努める。			

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Persistence of varicella-zoster virus viraemia in patients with herpes zoster

M.L. Quinlivan^{a,*}, K.L. Ayres^b, P.J. Kelly^c, S.P. Parker^a, F.T. Scott^a, R.W. Johnson^d, C. Maple^e, J. Breuer^a

^a Department of Infection, The Windeyer Institute, University College London, 46 Cleveland Street, London WC1T 4JF, England, UK

^b Department of Mathematics and Statistics, School of Mathematical and Physical Sciences, University of Reading, Whiteknights, Reading RG6 6AX, England, UK

^c School of Public Health, Edward Ford Building, A27 The University of Sydney, NSW 2006, Australia

^d University of Bristol Department of Ophthalmology, Bristol Eye Hospital, Bristol BS1 2LX, UK

* Virus Reference Department, Health Protection Agency Centre for Infections, Colindale, London NW9 5HT, UK

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ABSTRACT

Background: Herpes zoster is caused by the reactivation of varicella-zoster virus from sensory neurons. The commonest complication following zoster is chronic pain termed post herpetic neuralgia.

Objectives: To investigate the dynamics of VZV viraemia and viral load following the resolution of zoster and its relationship to PHN development.

Study design: Blood samples were collected at baseline, 1 month, 3 months and 6 month from a prospective study of 63 patients with active zoster. Quantification of VZV DNA in whole blood was performed using a real-time PCR assay.

Results: During acute zoster, all patients had detectable VZV DNA in their blood. VZV DNA remained detectable in the blood of 91% of patients at 6 months although levels declined significantly ($p < 0.0001$). A history of prodromal symptoms ($p = 0.005$) and severity of pain at baseline ($p = 0.038$) as well as taking antivirals ($p = 0.046$) and being immunocompromised ($p = 0.043$) were associated, with longer time to recovery from PHN. Viral DNA loads were consistently higher in patients with risk factors for PHN and higher viral DNA loads over time were associated with longer time to recovery ($p = 0.058$ overall and 0.038 in immunocompetent).

Conclusions: Based on these observations we hypothesise that VZV replication persists following acute shingles and that higher viral DNA loads contribute to the risk factors for PHN.

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1. Background

Varicella-zoster virus (VZV) is the cause of varicella (chickenpox) following which the virus remains latent in sensory root ganglia, reactivating usually in later life to cause a unilateral dermatomal rash known as herpes zoster (HZ, shingles). Up to 25% of individuals will develop shingles, and greater age is the commonest risk factor, although the incidence also increases with suppression of cell mediated immunity.^{1,2} The most frequent and debilitating complication of shingles is neuropathic pain persisting beyond the duration of rash. Pain which lasts more than 3 months after rash onset is known as post-herpetic neuralgia (PHN). The risk of developing PHN, its severity and duration all increase with age.^{3,4} Other risk factors include the presence of prodromal pain, the extent of rash, the severity of acute pain and in some studies, location of rash in the ophthalmic division of the trigeminal nerve and female

gender.⁵⁻⁸ However, the predictive value of these risk factors is poor.^{6,9}

VZV DNA can be detected in the blood during acute HZ, although the prevalence varies from 16% to 100% depending on the method used and the patient population.¹⁰⁻¹⁴ Gilden et al.¹⁵ have detected VZV DNA and gene expression in patients with PHN but not in controls and hypothesise that VZV might persist in ganglia at a greater level in patients with PHN, with spill over into blood mononuclear cells. Others have failed to detect VZV DNA or RNA in peripheral blood mononuclear cells (PBMCs) from 16 patients with PHN of three months to seven years duration.¹⁶

We have previously found an association between the presence of VZV viral DNA in PBMCs during the acute phase of HZ and development of PHN on univariate, but not multivariate, analysis.^{13,17} More recently, others have shown that viral DNA load in saliva measured during acute HZ was significantly correlated with higher pain scores.¹⁸

2. Objectives

To investigate further the relationship between VZV viraemia and the natural history of HZ, including the development of PHN,

Abbreviations: VZV, varicella-zoster virus; HZ, herpes zoster; PHN, post-herpetic neuralgia; PBMCs, peripheral blood mononuclear cells; ZBPI, Zoster Brief Pain Inventory.

¹ Corresponding author. Tel.: +44 020 7679 9559; fax: +44 020 76799652.

E-mail address: mark.quinlivan@hartsandthelondon.nhs.uk (M.L. Quinlivan).

by measuring the frequency of viraemia and magnitude of VZV DNA loads in the blood of 63 patients with acute zoster who were followed for 6 months following resolution of the rash.

3. Study design

3.1. Patient recruitment

Blood samples were obtained at baseline and 1 month, 3 months and 6 month follow-up visits from a prospective study of 63 patients with active zoster presenting to family doctors in London between 2001 and 2003. Herpes Zoster was confirmed both clinically and by PCR of VZV DNA from vesicle fluid. Patients were consented for inclusion in the study and were asked to complete a detailed questionnaire providing information on age, gender, ethnicity, whether they were born in the UK, immune status (including any underlying illness and whether they were receiving any treatment), antiviral therapy, rash age (time between their rash appearing and their visit to the GP), and experience of prodromal symptoms. Patients were also asked to rate the severity of their acute pain from 1 to 10 on average over the last 24 h using the Zoster Brief Pain Inventory (ZBPI).¹⁹ Allodynic symptoms, i.e. inappropriate pain in response to light touch, heat and cold was also recorded as this is typical of zoster associated neuropathic pain.²⁰ At each follow-up visit, patients were asked to rate the severity of any persisting pain or, in the absence of pain, whether they had any abnormal sensations (e.g. allodynia).

3.2. Preparation of PBMCs, DNA extractions and real-time PCR

Separation of PBMCs was performed using a Ficoll-Hypaque gradient as previously described.¹⁷ DNA was extracted from PBMCs or whole blood using a QIAamp DNA blood mini kit (Qiagen Ltd., United Kingdom), according to the manufacturer's instructions. The eluted DNA was stored at -20°C . Detection and quantification of VZV DNA was performed using a real-time PCR assay with a sensitivity of 10 copies/ μl as previously described.¹⁷

4. Measurement of antibodies

Antibody titres were measured using the in-house time resolved fluorescence immunoassay (TRFIA) which has been validated for detection and quantification of VZV antibody.^{21,22}

4.1. Univariate and multivariate analysis

Fisher's exact test was used to compare VZV DNA loads in whole blood and PBMCs. Before analysis of viral DNA loads, a $\log_{10}$ transformation was applied to reduce skewness, and a *t*-test or an Analysis of Variance was performed for each factor, depending on whether it was binary or not: recorded viral DNA loads of exactly zero were assigned a value of 1.0 before log transformation. For visits where more than one factor was found to be significant, a General Linear Model was obtained via stepwise selection to identify factors that remained independently predictive of mean log viral DNA load in a multivariate model. To investigate fac-

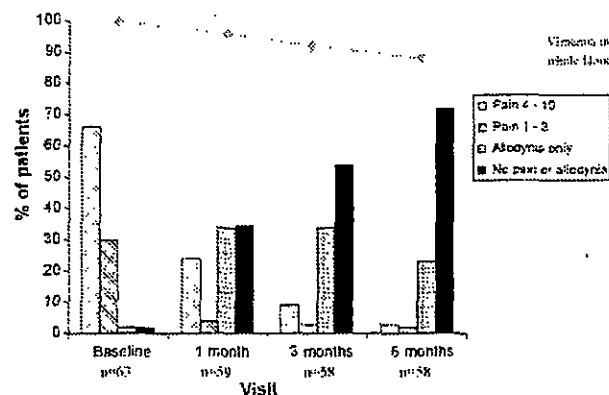


Fig. 1. Frequency and severity of neuropathic symptoms and VZV viraemia.

tors that influenced viral DNA loads over time, a multivariate linear mixed model was obtained. To account for the repeated measurements, visit number and a subject random effect (assumed to be normally distributed) were included in the model, and estimated variances for regression coefficients were empirically adjusted using the sandwich estimator.²³ Residuals were checked and were normally distributed. The effect of baseline variables on time to recovery from pain or abnormal sensations was investigated with Kaplan-Meier curves and a Cox proportional hazards model. To investigate changing viral DNA load over the course of the study (baseline and maximum of three follow-up measurements), a univariate Cox regression model was fitted with log viral DNA load treated as a time-dependent variable. For this analysis, as well as all patients, we separately analysed only immunocompetent patients ($n=56$) as the natural history of both pain and viral DNA load dynamics are likely to be different from those of immunocompromised patients.²⁴ The association between antibody titres and viral DNA loads was assessed with Pearson's correlation coefficient with associated *p*-values for a test of zero correlation. Depending on the analysis, Minitab version 15, SAS 9.2 or SPSS version 16.0 were used. For all multivariate models, variables entered the model using a stepwise approach and remained in the model if the multivariate *p*-value was less than 10%.

5. Results

5.1. Demographic characteristics

Table 1 shows the demographic characteristics of the patients from whom samples were obtained. When stratified by age (≤ 50 vs. >50 years), the majority of patients in each age group were immunocompetent, UK-born Caucasians who had acquired chicken pox in the UK.

5.2. Frequency and severity of neuropathic symptoms

The frequency and severity of neuropathic symptoms is shown in Fig. 1. At baseline 62 (98%) patients had neuropathic symptoms. Sixty six percent (42/63) had pain which was considered clinically

Table 1
Patient demographics.

Age (years)	Gender (% male)	Ethnicity (% UK Caucasian) ^a	Place of birth (% UK) ^b	Country of chicken pox acquisition (% UK) ^c	Immune status (% competent)
≤ 50	57	61	61	57	89
>50	51	71	66	67	89

^a Compared to non-UK Caucasian ($n=21$) Turkish-Caucasian 9, Asian 3, Afro-Caribbean 4, Other 5.

^b Compared to outside of UK ($n=23$) Africa 2, Turkey 5, New Zealand 2, Latvia 1, Bangladesh 2, South America 2, Cyprus 4, Romania 1, EIRE 2, Caribbean 2.

^c Compared to outside of UK ($n=24$) Africa 2, Turkey 5, New Zealand 1, Latvia 1, Bangladesh 2, South America 2, Cyprus 4, Romania 1, EIRE 2, Caribbean 2, Lybia 1, Israel 1.

Table 2
Detection of VZV in peripheral blood mononuclear cells vs. whole blood.

Patient	VZV copies per g of total DNA	
	Whole blood	PBMCs
A	638	NEG
B	2444	7186
C	2766	NEG
D	1626	NEG
E	2108	1988
F	1558	NEG
G	NEG	NEG
H	8111	7133
I	433	NEG
J	426	1430
Mean	2011	1174

significant (ZBPI score 4–10) while in 30% the pain scores were lower (ZBPI score 1–3).¹⁹ Two patients were not in pain, one of whom had allodynia and one had no neuropathic symptoms. Over the 6 month follow-up period, the percentage of patients with pain decreased (Fig. 1). By 6 months 3% (2/58) still had clinically significant pain. As pain decreased, the incidence of allodynia increased to 34% (20/59) at 1 month remaining stable thereafter (Fig. 1)

5.3. Detection of VZV in peripheral blood mononuclear cells vs. whole blood

In a previous study we measured VZV DNA load in PBMCs.¹⁷ One report comparing viral DNA load in PBMCs and whole blood found the latter to be more sensitive.¹⁰ We therefore compared VZV DNA copy number in 200 µl of PBMCs, separated by Ficoll and 200 µl of whole blood taken at baseline from 10 randomly selected patients with acute zoster (Table 2). VZV DNA was detected more frequently in whole blood (90%) than in matched PBMC samples (40%) (Table 2) and VZV DNA load per µg of DNA was significantly higher in whole blood compared with PBMCs ($p=0.029$). We therefore used whole blood viral DNA load in this study.

5.4. Prevalence and levels of VZV viraemia during acute zoster and follow-up visits

The percentage of patients who were positive for VZV DNA in whole blood at baseline and up to 6 months following acute zoster is shown in Fig. 1. Overall viral DNA was detectable in nearly 91% of patients up to 6 months following rash, although the viral DNA load decreased significantly over time ($p<0.0001$) (Fig. 2).

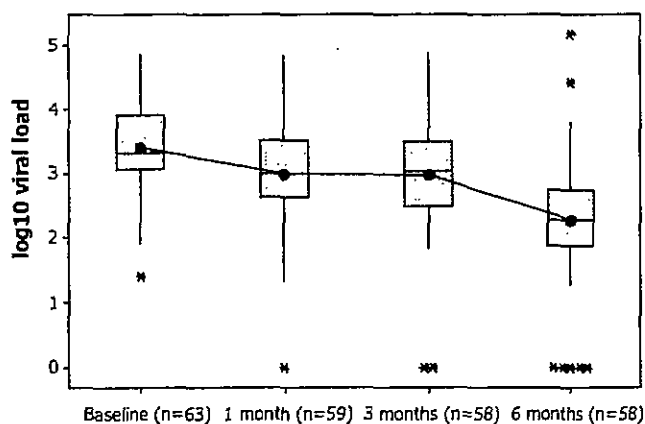


Fig. 2. Decrease in viral DNA load over time. Means are shown as solid circles and lines are drawn between them, medians are shown as horizontal lines, the box shows the interquartile range. Asterisks show outliers.

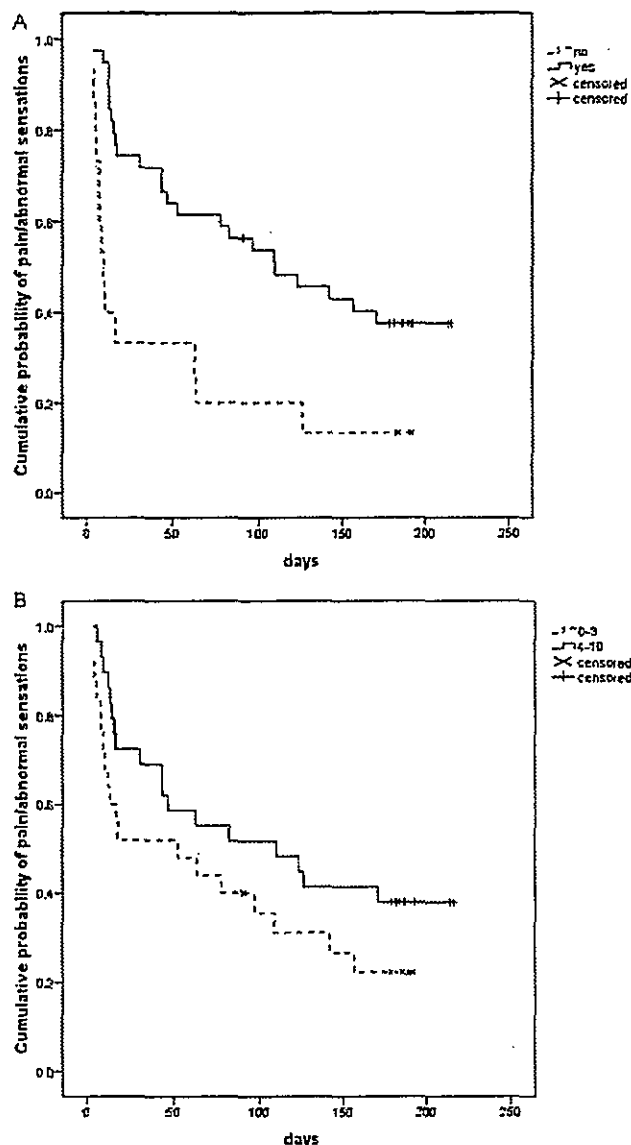


Fig. 3. Kaplan-Meier survival curves for immunocompetent patients regarding (A) prodromal symptoms and (B) average ZBPI score. Censored observations are for those who still reported pain or abnormal sensations at the last point they were contacted.

5.5. Risk factors for longer time to recovery

To explore the effect of baseline viral DNA load and other factors on the time to recovery from pain or abnormal sensations we used Cox proportional hazards regression (Table 3) and Kaplan-Meier curves (Fig. 3). Only greater severity of pain at baseline ($p=0.053$) and the presence of prodromal symptoms (0.004) were associated with longer time to recovery by univariate analysis. These remained significantly associated with longer time to recovery on multivariate analysis (Table 3). In addition, on multivariate analysis, taking antivirals, and being immunocompromised, were also associated with longer time to recovery from pain ($p=0.046$ and 0.043 respectively) (Table 3). Although higher baseline viral DNA load was not associated with longer time to recovery on univariate ($p=0.152$, Table 3) or multivariate analysis, when a univariate Cox regression model was fitted with log viral DNA load treated as a time-dependent variable, there was evidence that higher log viral DNA loads over time were weakly associated with a reduced chance

Table 3

Cox proportional hazards regression analysis of baseline patient demographic and pain characteristics and time to recovery.

Variable (all at baseline, except where stated)	All patients (n = 58)		Immunocompetent patients only (n = 50)	
	Hazard ratio (95% CI)	p-Value	Hazard ratio (95% CI)	p-Value
Age (years)	0.988 (0.973,1.003)	0.108	0.989 (0.974,1.005)	0.185
Gender (female vs. male)	0.915 (0.502,1.667)	0.771	0.946 (0.496,1.804)	0.866
Immunocompetent (yes vs. no)				
UVP	1.294 (0.543,3.086)	0.561	–	–
MVP	2.758 (1.034,7.355)	0.043		
Rash age at enrollment (days)	0.937 (0.840,1.046)	0.247	0.923 (0.815,1.045)	0.206
Prodromal symptoms (no vs. yes)				
UVP	2.850 (1.493,5.439)	0.001	2.762 (1.395,5.469)	0.004
MVP	3.171 (1.571,6.398)	0.001	2.796 (1.369,5.711)	0.005
On antivirals (no vs. yes)				
UVP	1.460 (0.785,2.712)	0.232	1.578 (0.822,3.028)	0.171
MVP	2.077 (1.052,4.101)	0.035	1.994 (1.012,3.927)	0.046
Disseminated rash (no vs. yes)	1.687 (0.600,4.737)	0.321	1.988 (0.608,6.504)	0.256
Trigeminal involvement (no vs. yes)	1.266 (0.562,2.851)	0.569	1.376 (0.534,3.541)	0.509
Severity of acute pain, average ZBPI score over last 24 h				
UVP	0.857 (0.740,0.991)	0.038	0.851 (0.723,1.002)	0.053
MVP	0.846 (0.723,0.991)	0.038	0.846 (0.708,1.011)	0.067
Allodynia (no vs. yes)	1.467 (0.651,3.305)	0.355	1.886 (0.826,4.307)	0.132
Viral load (log ₁₀), baseline	0.679 (0.400,1.153)	0.152	0.743 (0.413,1.339)	0.324
Viral load (log ₁₀), time-dependent	0.650 (0.417,1.015)	0.058	0.588 (0.355,0.972)	0.038

UVP: univariate probability; MVP: multivariate probability.

of recovery ($p = 0.058$) for all patients (Table 3), but only in univariate analysis. The strength of the association increased when only immunocompetent patients were analysed ($p = 0.038$). The association was no longer significant however in a multivariate analysis.

5.6. Factors affecting viral load

To identify factors that might affect viral DNA load, we separately analysed mean log₁₀ viral DNA loads measured at each time point (baseline, 1, 3 and 6 months) following acute infection, in relation to demographic and zoster-associated factors listed in the methods. While viral DNA loads tended to be higher in those with risk factors for PHN (older age, female gender, prodromal symptoms, trigeminal nerve involvement, higher pain score and persisting pain), the differences were rarely statistically significant (Table 4). For other factors such as disseminated rash and being immunocompromised, viral loads were lower at most time points (Table 4). Analysis of viral DNA loads over the four time points in relation to the above parameters using a linear mixed model showed that only increasing time ($p < 0.0001$) and taking antivirals during acute HZ ($p = 0.02$), were associated with reducing DNA loads in multivariate analysis, while greater age was weakly associated with a lower reduction in viral DNA load over time ($p = 0.06$).

5.7. Antibody titres and viral load

Baseline viral DNA loads were strongly correlated ($p = 0.001$) with antibody titres at 4 weeks and slightly less so at 12 weeks ($p = 0.021$). There were no other significant correlations between viral DNA loads and antibody.

6. Discussion

Although our study was small, we were able to show that greater severity of pain ($p = 0.038$) and the presence of prodromal symptoms at baseline ($p = 0.005$) were associated with longer time to recovery (Table 3). These risk factors for PHN have been identified in previous studies suggesting that even though this study was not powered to demonstrate these associations, our population is comparable. Our data also showed an association between taking antivirals and longer time to pain resolution ($p = 0.035$ in multivariate analysis), which probably reflects current UK prac-

tice to direct treatment to those with most risk factors for PHN. In addition, our paper highlights two new findings. First we show that VZV DNA in whole blood, which in our hands is more sensitive than PBMCs, is detectable in the majority of patients for at least 6 months following acute herpes zoster. The use of whole blood is supported by De Jong et al.¹⁰ who calculated that almost 40% of VZV in whole blood was present in the serum or plasma fraction suggesting only 60% is present in PBMCs. While the most likely explanation is that whole blood includes VZV DNA from lysed cells, we cannot exclude the possibility that cell-free virus is also circulating, something that has not previously been investigated. Persistence of VZV DNA contrasts with experience from cases of varicella, where VZV DNA was no longer detectable by an equally sensitive assay at 12–14 days following acute varicella infection in 25 immunocompetent patients.^{11,12} The results fit with recent observations that prolonged shedding of VZV DNA occurs in saliva following HZ¹⁸ and are consistent with ongoing viral replication in affected ganglia, with shedding into the blood or persistence of VZV DNA following HZ in PBMCs.

Second the data suggest a relationship between persistent detection of VZV DNA, and longer time to recovery from neuropathic symptoms. For patients with normal immune function, higher viral DNA loads over time were associated, in univariate analysis, with longer time to recovery from pain as measured by ZBPI pain scores ($p = 0.038$) (Table 3). A multivariate mixed model showed that falling viral DNA load over time was independently associated with time since rash ($p < 0.0001$) and having taken antivirals ($p = 0.02$), whereas older subjects were less likely to show falls ($p = 0.06$). Whether ongoing viral replication is responsible, either by directly causing tissue damage or indirectly through an immunopathologic response is not known. The evidence for a class 1 HLA association with PHN^{25,26} strongly supports the importance of host cell mediated immune (CMI) responses in the pathogenesis of PHN. Higher CMI during acute zoster has been associated with greater PHN,²⁷ further suggesting that acute viral replication increases immune-mediated cytopathology and promotes the development of PHN. At the same time, higher acute VZV antibody titres have also been observed for subjects who develop PHN following HZ.^{27,28} We found an association between baseline viral DNA loads (which were generally the highest) and antibody titres at four weeks (again, generally the highest) $p = 0.001$. Since antibodies peak at around 4 weeks following rash,²⁸ this suggests that

Table 4

Mean log₁₀ viral loads at each visit for factors measured at baseline and for the presence of pain or abnormal sensations assessed at 1 month, 3 months and 6 months visits. *p*-Values obtained from a two-sample *t*-test with equal variances or Analysis of Variance are also shown.

Baseline variable	Category	Mean log ₁₀ viral loads (sample size) at visit, with <i>p</i> -value			
		Baseline	1 month	3 months	6 months
Age (years)	≤50	3.373 (28)	2.795 (26)	2.880 (26)	2.340 (26)
	>50	3.446 (35)	3.164 (33)	3.04 (32)	2.223 (32)
		<i>p</i> = 0.672	<i>p</i> = 0.112	<i>p</i> = 0.499	<i>p</i> = 0.671
Gender	Female	3.342 (29)	3.059 (27)	2.98 (27)	2.276 (27)
	Male	3.474 (34)	2.952 (32)	2.96 (31)	2.270 (31)
		<i>p</i> = 0.441	<i>p</i> = 0.648	<i>p</i> = 0.920	<i>p</i> = 0.984
Immunocompetent	No	3.084 (7)	3.074 (7)	2.04 (7)	1.920 (6)
	Yes	3.454 (56)	2.991 (52)	3.099 (51)	2.314 (52)
		<i>p</i> = 0.171	<i>p</i> = 0.818	<i>p</i> = 0.003*	<i>p</i> = 0.364
Rash age (days)	0–2	3.660 (7)	3.180 (6)	2.775 (6)	2.591 (5)
	3–4	3.436 (21)	2.993 (21)	2.916 (21)	2.105 (20)
	5–6	3.338 (22)	3.055 (20)	3.163 (19)	2.455 (20)
	>6	3.370 (13)	2.836 (12)	2.861 (12)	2.129 (13)
		<i>p</i> = 0.741	<i>p</i> = 0.870	<i>p</i> = 0.724	<i>p</i> = 0.575
Any prodromal symptoms	Yes	3.422 (46)	3.103 (43)	2.989 (42)	2.270 (42)
	No	3.390 (17)	2.726 (16)	2.923 (16)	2.292 (16)
		<i>p</i> = 0.872	<i>p</i> = 0.147	<i>p</i> = 0.806	<i>p</i> = 0.928
On antivirals	Yes	3.328 (43)	2.943 (40)	2.910 (39)	2.120 (38)
	No	3.597 (20)	3.123 (19)	3.087 (19)	2.563 (20)
		<i>p</i> = 0.140	<i>p</i> = 0.470	<i>p</i> = 0.504	<i>p</i> = 0.106
Disseminated rash	Yes	3.177 (7)	2.846 (7)	3.010 (7)	1.480 (7)
	No	3.397 (53)	2.995 (49)	2.908 (48)	2.395 (48)
		<i>p</i> = 0.399	<i>p</i> = 0.659	<i>p</i> = 0.781	<i>p</i> = 0.013*
Trigeminal involvement	Yes	3.439 (11)	3.170 (10)	3.387 (10)	2.130 (10)
	No	3.408 (52)	2.967 (49)	2.884 (48)	2.304 (48)
		<i>p</i> = 0.889	<i>p</i> = 0.513	<i>p</i> = 0.114	<i>p</i> = 0.609
Average ZBPI pain score in last 24 h	0–3	3.280 (29)	2.951 (26)	2.935 (25)	2.336 (25)
	4–10	3.527 (34)	3.040 (33)	3.000 (33)	2.230 (33)
		<i>p</i> = 0.149	<i>p</i> = 0.705	<i>p</i> = 0.797	<i>p</i> = 0.678
Allodynia	Yes	3.355 (53)	2.982 (49)	2.950 (48)	2.280 (49)
	No	3.720 (10)	3.093 (10)	3.070 (10)	2.260 (9)
		<i>p</i> = 0.117	<i>p</i> = 0.723	<i>p</i> = 0.704	<i>p</i> = 0.966
Pain or abnormal sensations at 1 month	Yes	3.533 (39)	3.119 (38)	2.990 (38)	2.120 (36)
	No	3.280 (22)	2.790 (21)	2.935 (20)	2.520 (22)
		<i>p</i> = 0.135	<i>p</i> = 0.168	<i>p</i> = 0.831	<i>p</i> = 0.132
Pain or abnormal sensations at 3 months	Yes	3.537 (28)	3.282 (27)	3.080 (27)	2.253 (26)
	No	3.360 (33)	2.764 (32)	2.876 (31)	2.290 (32)
		<i>p</i> = 0.280	<i>p</i> = 0.024*	<i>p</i> = 0.403	<i>p</i> = 0.888
Pain or abnormal sensations at 6 months	Yes	3.575 (16)	3.214 (16)	3.219 (16)	2.290 (16)
	No	3.362 (43)	2.896 (42)	2.947 (41)	2.266 (42)
		<i>p</i> = 0.251	<i>p</i> = 0.223	<i>p</i> = 0.269	<i>p</i> = 0.929

p-value; mvp, multiple regression *p*-value <0.05.

* Significant at 5% level.

antibody titres reflect levels of viral replication.²⁷ However, a recent report has also suggested that the pathogenesis of PHN is related to cross reactive binding by IE62 antibodies to the Brain Derived Neurotropic Factor²⁹; thus levels of antibody to IE62 in this population may be important.

A more extensive survey than ours would address the unexpected finding that viral loads were lower at most time points in patients who had disseminated rashes or who were immunosuppressed. Severity of rash at presentation was also, unlike in previous studies, not associated with more severe pain (Table 3). One possible explanation for this is that, previous studies analysed data from clinical trials where patient recruitment and initiation of treatment occurred ≤72 h after the onset of rash. Patients in our study presented up to 9 days following rash onset and at different times in relation to starting antivirals. In summary, our data show persistence of VZV DNA in whole blood for up to 6

months following acute HZ in over 90% of individuals and a trend towards an association of higher levels with persistent neuropathic symptoms or PHN. The possibility that ongoing VZV replication in ganglia is contributing to PHN pathogenesis, is supported by recent reports that neuronal expression of viral antigens occurs up to 3 months beyond HZ rash-healing but not in controls.³⁰ Alternatively, persistence of VZV DNA in blood cells could reflect severity of acute infection resulting in high acute viral DNA loads with persistence in long lived cells. In this small study we did not find a relationship between baseline viral loads, or peak antibody titres and PHN.¹⁷ To resolve these and other findings and the underlying biology, larger studies are now needed.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日 2011. 1. 17	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	新鮮凍結人血漿	研究報告の公表状況	ABC Newsletter- #46. December 17; 2010	公表国	
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社)			米国	
研究報告の概要	○ <i>Trypanosoma cruzi</i> (<i>T. cruzi</i>) に関する血液製剤諮問委員会の最新情報 米国食品医薬品局(FDA)の血液製剤諮問委員会(BPAC)において、米国における <i>T. cruzi</i> 感染率に関する最新情報が報告された。 FDAが2010年12月に公布した「輸血用の全血及び血液成分における <i>T. cruzi</i> 感染の輸血リスクを減少するための血清学的検査の使用に関するガイダンス」についての説明に続き、2009年4月にBPACが推奨した「米国の供血者における <i>T. cruzi</i> 感染の発生率を更に明らかにするための研究」の結果について、米国赤十字社から説明があった。この研究は、米国赤十字社の高有病率地域の3カ所及びUnited Blood Systemsの高有病率地域の1カ所で実施中である。 この研究では、2007年1月末から、22カ月間は全ての供血に関して <i>T. cruzi</i> に対する抗体を検査していたが、それ以降は、高有病率地域では全ての供血を検査対象とすることを継続し、それ以外の地域は1回でも陰性結果を受けた供血者には、追加の検査と新たな感染リスクに関する質問を求められることなく供血資格が与えられる特定検査体制に移行した。 43カ月目まで継続した結果は、合計460万人年の観察について、346万人の供血者(複数回の供血)の中で陽転化した供血者は1例も同定されなかった。95%信頼区間の上限信頼限界は、0.08例/10万人年又は1例/100万人年未満であった。この結果は、この研究のために設定された許容リスク基準である2.4例/100万人年より低い。よって、<i>T. cruzi</i> は、輸血に関連する他の感染疾患リスクと同程度であるとみなすことができる。 米国赤十字社は、上述の特定検査体制を取り入れることにより、受血者の安全性を損なうことなく、必要な検査の数が有意に減少したと結論を下した。				使用上の注意記載状況・ その他参考事項等
	報告企業の意見 FDAの血液製剤諮問委員会において <i>T. cruzi</i> 感染率についての最新情報が報告され、その中で、米国赤十字社等の行った供血者における <i>T. cruzi</i> 検査についての研究の結果、特定検査体制を取り入れることにより、受血者の安全性を損なうことなく、必要な検査の数が有意に減少したと結論を下したとの報告である。				新鮮凍結血漿「日赤」 新鮮凍結血漿-LR「日赤」 新鮮凍結血漿-LR「日赤」成分採血 血液を介するウイルス、細菌、原虫等の感染 vCJD等の伝播のリスク
報告企業の意見		今後の対応			
FDAの血液製剤諮問委員会において <i>T. cruzi</i> 感染率についての最新情報が報告され、その中で、米国赤十字社等の行った供血者における <i>T. cruzi</i> 検査についての研究の結果、特定検査体制を取り入れることにより、受血者の安全性を損なうことなく、必要な検査の数が有意に減少したと結論を下したとの報告である。		日本赤十字社は、シャーガス病の既往がある場合には献血不適としている。日本在住の中南米出身献血者については、厚生労働科学研究「献血血の安全性確保と安定供給のための新興感染症等に対する検査スクリーニング法等の開発と献血制限に関する研究」班と共同して検討している。今後も引き続き情報の収集に努める。			

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BPAC: Defer CFS Patients (continued from page 6)

issued in June, that blood collectors distribute CFS education materials to “discourage” potential donors diagnosed with CFS from giving blood. The Red Cross is now indefinitely deferring donors who reveal a history of CFS (see *ABC Newsletter*, 12/10/10).

A working group of blood researchers and virologists sponsored by the National Heart, Lung, and Blood Institute and the Department of Health and Human Services is attempting to determine if the disorder is tied to xenotropic murine leukemia virus-related virus (XMRV) infection and poses a threat to the blood supply.

Three researchers described recent studies on the epidemiology of MLV-related retroviruses. Judy Mikovits, PhD, of the Whittemore Peterson Institute, gave a presentation on a study in the UK that sought to detect MLV-related viruses in blood cells and plasma from a cohort of patients with myalgic encephalomyelitis post-viral fatigue, which is another name for CFS. The study found gene sequences from an MLV-related virus in 48 percent of patients.

Dr. Mikovits and other researchers from the National Cancer Institute and the Cleveland Clinic in October 2009 reported that they had detected the DNA of XMRV in 68 of 101 (67 percent) of CFS patients,

(continued on page 8)

Speakers Update BPAC on T. Cruzi

The Food and Drug Administration’s Blood Products Advisory Committee (BPAC), on Wednesday morning, heard two updates about the incidence of infection by *Trypanosoma cruzi* in the US.

First, Robert Duncan, PhD, from the FDA’s Division of Emerging and Transfusion Transmitted Diseases, made a presentation on the recently issued FDA “Guidance for the Use of Serological Tests to Reduce the Risk of Transmission of *Trypanosoma cruzi* Infection in Whole Blood and Blood Components Intended for Transfusion.” Susan Stramer, PhD, of the American Red Cross (ARC), then reviewed results of the “Study to Further Define the Incidence of T. cruzi Infection in the U.S. Blood Donor Population,” which had been recommended by BPAC in April 2009 as it voted in favor of a selective testing strategy.

Under the recommended approach, one negative test qualifies a donor for all future donations without further testing or the need of asking questions regarding risk of a newly acquired infection. The study discussed by Dr. Stramer is being carried out in three of ARC’s high-prevalence regions and one of United Blood Systems’ high-prevalence regions.

The study tests all donors for antibodies to T. cruzi on all donations since the end of January 2007. The 43-month results did not identify a single seroconverting donor among the 3.46 million donors who gave two or more donations for a total of 4.6 million person years of observation. The upper 95 percent confidence limit was 0.08 cases per 100,000 person years, or less than one case per million person years. That result is lower than the criterion of an acceptable risk of 2.4 cases per million person years established for the study, so that T. cruzi can be considered comparable with other infectious disease risks associated with transfusion. Dr. Stramer concluded that the selective testing strategy has significantly reduced the amount of testing required without compromising recipient safety.

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 2010. 12. 22	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	新鮮凍結人血漿	研究報告の公表状況	佐々木年則, 星野啓太, 比嘉由紀子, 伊澤晴彦, 小林陸生, 沢辺京子, 他. 病原体微生物検出月報 (IASR). Vol.31 No.12. Available from: http://idsc.nih.go.jp/iasr/31/370/dj3704.html	公表国 日本	
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社)				
研究報告の概要	○わが国の都市部における路上生活者より採取されたコロモジラミからの <i>Bartonella quintana</i> 検出 背景:世界的に、大都市に住む路上生活者に寄生するコロモジラミから壱壊熱の病原体である <i>Bartonella quintana</i> (<i>B. quintana</i>) の遺伝子が検出され、菌株も分離されている。 方法:路上生活者から採取したコロモジラミからゲノムDNAを抽出し、<i>B. quintana</i> の同定を行った。また、インフォームドコンセントを行い了承を得て路上生活者から採血し、<i>B. quintana</i> DNA の検出、および抗体検査を行った。 結果: (1) 東京の検出事例 東京において、1999年5月～2005年6月までに路上生活者62名から採取したコロモジラミのうち、6名由来のコロモジラミから <i>B. quintana</i> の遺伝子を検出した(9.7%)。同様に、東京において、2001年5月～2004年11月に路上生活者429名の血液から <i>B. quintana</i> の遺伝子を調べ、11名から <i>B. quintana</i> の遺伝子を検出した(2.6%)。 <i>B. quintana</i> に対する抗体価(IgG)は、一般人(200名)に比べ路上生活者(151名)の方が有意に高かった($p<0.001$)。また、路上生活歴の長い人ほど、有意に <i>B. quintana</i> 抗体価(IgG)が高かった($p<0.01$)。 (2) 大阪の検出事例 大阪において、2009年5月～2010年1月までに、ある医療機関を受診した路上生活者(42歳以上)10名から採取したコロモジラミのうち、6名由来のコロモジラミから <i>B. quintana</i> の遺伝子を検出した(60%)。 考察:世界各国で採取したコロモジラミの <i>B. quintana</i> 遺伝子検出率は1.4%～90%と、その地域の状況によって大きく異なる。一方、東京において <i>B. quintana</i> の遺伝子の検出率が約10%で、壱壊熱が特殊な集団において蔓延している可能性が懸念される。大阪においては東京と比べて、異常に高い <i>B. quintana</i> 遺伝子の検出率が確認され、2地域における路上生活者の生活習慣の違いなど種々の要因を含めて、今後より詳細な調査が必要である。				使用上の注意記載状況・ その他参考事項等
	報告企業の意見	東京と大阪の路上生活者から採取したコロモジラミから、壱壊熱の病原体である <i>Bartonella quintana</i> の遺伝子が検出されたとの報告である。			
今後の対応		日本赤十字社では輸血による細菌感染予防対策として、すべての輸血用血液製剤を対象に、保存前白血球除去及び初流血除去を導入している。さらに、輸血情報リーフレット等により、細菌感染やウイルス感染について医療機関へ情報提供し注意喚起しているほか、細菌感染が疑われる場合の対応を周知している。細菌やウイルスの検出や不活化する方策について検討している。			

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わが国の都市部における路上生活者より採取されたコロモジラミからの *Bartonella quintana* 検出

(Vol. 31 p. 354-355: 2010年12月号)

背景

世界的に、大都市に住む路上生活者に寄生するコロモジラミから塹壕熱の病原体である *Bartonella quintana* (*B. quintana*) の遺伝子が検出され、菌株も分離されている。塹壕熱は、第一次世界大戦や第二次世界大戦の時に、塹壕内で敵と対峙していた兵士に蔓延した。その後大きな流行はみられなかったが、1990年代になって、開発途上国の刑務所や、衣服を取り替えることが困難な施設、先進国の路上生活者等で散発的に流行がみられるようになった。わが国で2003年に厚生労働省が行った路上生活者の調査では、全国で25,296人が路上生活者として報告されたが、2007年の同調査では一時的な景気回復によって18,564人となっている¹⁾。中高年男性が95%を占め、平均年齢は57.5歳である。実数で減少しているように見えるが、2007年のネットカフェ難民に関する調査では、ネットカフェで生活していると考えられる人が推定で5,400人ほど確認されており、今後の経済の動向によって増加する可能性があり、コロモジラミの寄生に関し注視する必要がある。

方法

コロモジラミからゲノムDNAを抽出し、*Bartonella* 属特異的プライマーにてPCRを行った。その後、ダイレクトシーケンスにより塩基配列を決定し、*B. quintana* と同定した。また、インフォームドコンセントを行い、承諾を得て採血し、血液培養ボトルで35℃のCO₂インキュベーターで7日間培養し、その後、一部を血液寒天培地で3週間培養した。培養ボトルから抽出したDNAを用いて、*Bartonella* 属特異的プライマーにてPCRを行った。その結果、ダイレクトシーケンスにより塩基配列を決定し、*B. quintana* と同定した。*B. quintana* に対する抗体検査は、間接蛍光抗体法にて行った。

結果

(1) 東京の検出事例

東京において、1999年5月～2005年6月までに路上生活者62名から採取したコロモジラミのうち、6名から *B. quintana* の遺伝子を検出した(9.7%) (表2, 3)。同様に、東京において、2001年5月～2004年11月に路上生活者429名の血液から *B. quintana* の遺伝子を調べ、11名から *B. quintana* の遺伝子を検出した(2.6%)⁴⁾。*B. quintana* に対する抗体価(IgG)は、一般人(200名)に比べ路上生活者(151名)の方が有意に高かった($p < 0.001$)⁴⁾。また、路上生活歴の長い人ほど、有意に *B. quintana* に対する抗体価(IgG)が高かった($p < 0.01$)。しかし、現在までに *B. quintana* の分離には成功していない。一方、シラミ媒介性病原体として *Borrelia recurrentis* が知られている。*Borrelia* 属に対するプライマーを用いてPCRを行ったが、1999年5月～2003年10月の47名由来コロモジラミにおいて、すべて陰性であった。

(2) 大阪の検出事例

大阪において、2009年5月～2010年1月までに、ある医療機関を受診した路上生活者(42歳以上)10名から採取したコロモジラミのうち、6名由来のコロモジラミから *B. quintana* の遺伝子を検出した(60%) (表3)。なお、42歳男性の血餅から *B. quintana* の遺伝子を検出した。しかし、*B. quintana* の分離には成功していない。

考察

表1に示すように、世界各国で採取したコロモジラミの *B. quintana* 遺伝子検出率は1.4%～90%と、その地域の状況によって大きく異なる。一方、東京において *B. quintana* の遺伝子の検出率が約10%で、塹壕熱が特殊な集団において蔓延している可能性が懸念される。大阪においては東京と比べて、異常に高い *B. quintana* 遺伝子の検出率が確認され、2地域における路上生活者の生活習慣の違いなど種々の要因を含めて、今後より詳細な調査が必要である。

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国立感染症研究所昆虫医科学部

佐々木年則 星野啓太 比嘉由紀子 伊澤晴彦 小林睦生 沢辺京子

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 2010. 12. 21	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	新鮮凍結人血漿	研究報告の公表状況	Pitassi LU, Barjas-Castro M, Drummond MR, Gilioll R, Lania B, Scorpio DG, Castro V, Diniz PP, Velho PN. AABB Annual Meeting and CTTXPO; 2010 Oct.9-12; Baltimore; USA.	公表国 ブラジル	
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社)				
研究報告の概要	○供血者における <i>Bartonella henselae</i> 菌血症 背景: <i>Bartonella</i> 種は新興人獣共通病原体と考えられており、スナバエ、コロモジラミ、ノミなどの種々の媒介動物によって伝播する。供血者の、バルトネラ種に関する血清陽性率は世界中で異なっており、50%以上に達する場合もある。本研究では、ブラジル・カンピーナスの大規模な供血者集団において、<i>Bartonella</i> 菌の感染及び暴露の調査を行った。 方法: 供血者 (n=500) に対し、ペット(イヌ、ネコ、トリなど)や野生動物、昆虫との接触や旅行歴、病歴などについて問診を行った。また、血液検体の培養を行い、細菌増殖を確認した。 結果: 現在までに <i>Bartonella henselae</i> による感染が2人の供血者において確認された。2人ともイヌに接触しており、ダニに咬まれていた。そのうち1人は、ネコおよびコロモジラミとも接触していた。 結論: 供血者による <i>Bartonella</i> 菌伝播の潜在的リスクは過小評価されていた。しかし <i>Bartonella</i> 菌に感染した供血者から採取した血液は、重度免疫不全患者において致命的な疾患を引き起こす可能性がある。受血者における <i>Bartonella</i> 菌感染を排除するために、診断検査の改善が必要である。				使用上の注意記載状況・ その他参考事項等
	新鮮凍結血漿「日赤」 新鮮凍結血漿-LR「日赤」 新鮮凍結血漿-LR「日赤」成分採血				血液を介するウイルス、 細菌、原虫等の感染 vCJD等の伝播のリスク
報告企業の意見		今後の対応			
大規模な供血者集団 (n=500) において <i>Bartonella</i> 菌感染の調査を行ったところ、2人の供血者に <i>Bartonella henselae</i> 感染が確認されたとの報告である。		今後も情報の収集に努める。			

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SP370

A Novel Application of a Commercial Multiplex Real-Time PCR Assay for High Sensitive Screening and Detection of Bacterial Contamination in Platelet ConcentratesM Spreafico¹ (ma.spreafico@ospedale.lecco.it), B Foglieni¹, L Raffaele¹, I Guarnori¹, B Alessandra¹, G R Brigante², F Luzzaro², D Prati¹.¹Transfusion Medicine, Alessandro Manzoni Hospital, Lecco, Italy;²Microbiology and Virology, Alessandro Manzoni Hospital, Lecco, Italy

Background: Since the impressive reduction of transfusion-transmitted viral infections, bacterial infections by blood transfusion represent the most important infection risk. Platelet concentrates (PC) are the current focus of attention, as the ambient storage temperature conditions make them most likely to facilitate bacterial growth. In Italy, blood banks usually perform a random bacterial contamination test by gold-standard culture-based assays on 2% of PCs production. In January 2010, a regional Italian project for the systematic microbiological screening of all PCs units by conventional culture and other culture-independent tests was started in 4 Transfusion Centres, in order to determine the prevalence of bacterial contamination in donor platelet concentrates in Lombardy. Aim of the study was to evaluate the performance in PCs matrix of the LightCycler SeptiFast Test (Roche Diagnostics), a commercial high sensitive multiplex real-time PCR assay designed to detect 90% of the sepsis-associated microorganisms, in comparison with the reference culture BacT/Alert system. **Methods:** PCs from buffy coats were spiked, in double, with different concentrations (1, 10, 100 colony-forming units (CFU)/mL), of three micro-organisms (*E. coli*, *St. aureus*, *Candida krusei*) detected by the SeptiFast assay and potentially associated with PC contamination. Spiked PCs were maintained at room temperature in agitation. After 5 days, the mandatory European platelet concentrate shelf life, all PCs were tested: two 5 mL aliquots by the culture assay (BacT/Alert), and 1.5 mL by the SeptiFast assay. A second 1.5 mL aliquot and a 1:10 dilution of each spiked PC were stored at -30°C for the evaluation of the molecular assay on the frozen matrix. **Results:** Preliminary results showed that SeptiFast is suitable to be applied to the PC matrix. At day 5 after the inoculum, 1 CFU/mL of *E. coli* and *St. aureus* and 10 CFU/mL of *Candida krusei* were analytically detected in about 6 hours, starting from both fresh and frozen samples. The same results were obtained on 1:10 diluted samples, indicating that the sensitivity for *E. coli* and *St. aureus* might be even higher. Results were confirmed by the reference culture method. **Conclusion:** Our preliminary studies showed a good performance of the multiplex real-time PCR-based assay in the detection of bacterial contamination in fresh and frozen PCs. The assay showed a high sensitivity and a rapid execution, demonstrating to be clinically valuable add-on to conventional culture methods for rapid aetiological detection. Other studies are required to determine the performance of the assay with respect to other microorganisms potentially involved in platelets bacterial contamination and to establish the optimal sampling time.

Disclosure of Commercial Conflict of Interest

B. Alessandra: Nothing to disclose; G. R. Brigante: Nothing to disclose; B. Foglieni: Nothing to disclose; I. Guarnori: Nothing to disclose; F. Luzzaro: Nothing to disclose; D. Prati: Nothing to disclose; L. Raffaele: Nothing to disclose; M. Spreafico: Nothing to disclose

Disclosure of Grants Conflict of Interest

B. Alessandra: Nothing to disclose; G. R. Brigante: Nothing to disclose; B. Foglieni: Nothing to disclose; I. Guarnori: Nothing to disclose; F. Luzzaro: Nothing to disclose; D. Prati: Nothing to disclose; L. Raffaele: Nothing to disclose; M. Spreafico: Nothing to disclose

SP371

Molecular Relatedness of Coagulase Negative Staphylococci Found in Platelet Concentrates

I Rood^{1,2} (igh.rood@sanquin.nl), A Pettersson², S Ramirez-Arcos³, P M Savelkoul², D de Korte^{1,4}. ¹Blood Cell Research, Sanquin Research, Amsterdam, Netherlands; ²Medical Microbiology and Infection Control, VUMC, Amsterdam, Netherlands; ³R&D, Canadian Blood Services, Ottawa, ON, Canada; ⁴R&D, Sanquin Blood Bank North West, Amsterdam, Netherlands

Background: Currently, bacterial contamination is the major infectious hazard of platelet transfusion. In the Netherlands, screening of platelet concentrates (PC) for the presence of bacteria is done with the BacT/Alert culturing system (BioMerieux). It is assumed that contamination of PCs occurs from the introduction of low concentrations of skin bacteria at the time of phlebotomy and less common from asymptomatic donor bacteraemia or from contaminant during processing. This is reflected in the types of bacteria that are most commonly implicated in these events. In the Nether-

lands, the most frequently identified bacterial contaminants of PCs are *Propionibacterium acnes* and coagulase negative staphylococci (CNS). Adverse transfusion reactions with *P. acnes* are not known and therefore contamination of PCs with *P. acnes* is considered as clinically not relevant. On the other hand, several adverse transfusion reactions due to PCs contaminated with CNS have been reported. Aim of this study was to gain more insight which CNS strains are involved in bacterial contamination of PCs. For this purpose, CNS strains that were found in PCs during screening were collected and determined to species level with three different methods. **Methods:** Throughout 2007 and 2008, 66 CNS strains that were found with the BacT/Alert culture system in Nijmegen and Amsterdam were collected. Sixteen confirmed positive CNS cultures from the Canadian blood service and 2 CNS strains found during re-culturing of outdated PCs were included in the study as well. The strains were typed with amplified fragment length polymorphism (AFLP) analysis, and determined to species level by sequencing of 500 bp of the 16S rRNA gene and by MALDI TOF mass spectrometry (MS) analysis (Shimadzu). **Results:** A total of 84 CNS strains found in BacT/Alert cultures from PCs were determined/typed with three different methods. All but one CNS strains could be determined to species level by 16S rRNA sequencing. Nine different species were found most of which (n = 38) were *S. epidermidis*. Other frequently found species were *S. capitis* (n = 13), *S. warneri* (n = 11) and *S. hominis* (n = 7). The MALDI TOF MS data did not show many discrepancies with the sequencing data. However, the MALDI TOF MS was not able to determine *S. caprae* and *S. saccharolyticus* to species level and some *S. capitis* strains were determined as *S. capitis/caprae*. Typing by AFLP revealed that all Staphylococcal strains that belonged to the same species as determined by sequencing clustered together. **Conclusion:** 1. This study gives an overview of CNS species found in BacT/Alert cultures from PCs. 2. Nine different staphylococcal species were found by 16S rRNA sequencing and AFLP. Not all species could be determined by MALDI TOF MS analysis. 3. The found species are able to cause serious disease in immunocompromised patients. Therefore, the source of contamination should be further investigated.

Disclosure of Commercial Conflict of Interest

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D. de Korte: BCSI, Consulting or Board of Director Fees; A. Pettersson: Nothing to disclose; S. Ramirez-Arcos: Nothing to disclose; I. Rood: Nothing to disclose; P. M. Savelkoul: Nothing to disclose

SP372

Bartonella Henselae Bacteremia in Blood Donors

L U Pitassi¹ (plassi@yahoo.com), M Barjas-Castro², M R Drummond¹, R Gilioli³, B Lania¹, D G Scorpio⁴, V Castro⁵, P P Diniz⁵, P N Velho¹.

¹Department of Dermatology, UNICAMP University of Campinas, Campinas, Brazil; ²Hemocentro, UNICAMP University of Campinas, Campinas, Brazil; ³Laboratory of Animal Quality Control-Multidisciplinary Center of Biological Investigation—(CEMIB), UNICAMP University of Campinas, Campinas, Brazil; ⁴Molecular and Comparative Pathobiology, Johns Hopkins University, Baltimore, MD, United States; ⁵Department of Small Animal Internal, Medicine Western University of Health Sciences, Pomona, CA, United States

Background: Species of *Bartonella* are considered emerging zoonotic pathogens, transmitted by a variety of vectors including sand flies, body lice and fleas. In the last years, the wide spectrum of clinical manifestations associated with these bacterial infections has rapidly increased and the potential risk of these agents transmitted from the blood donors to recipients should be considered. Seroprevalence rates among blood donors for these bacteria vary widely around the world, and can reach over 50%. This data reinforce the possibility of the risk of *Bartonella* spp. exposure among donors. Previous studies published by our group have demonstrated the ability of *Bartonella* sp to survive in stored red blood cell units (Magalhães et al, 2008; 2009; 2010; Pitassi et al, 2007). The objective of this study was to investigate the occurrence of *Bartonella* spp. infection and/or exposure in a large blood donor population of Campinas Brazil. **Methods:** Blood donors (n = 500) were submitted to an interview carried out face-to-face by a professional of our group under a qualified physician's supervision. The interview included questions regarding contact with pets (dogs, cats, birds...), wild animals, insect expositions, trips and detailed medical history. Blood samples (2 mL) were inoculated into liquid *Bartonella* alpha-Proteobacteria growth medium (8 mL) as an enrichment culture step, and incubated at 35°C in 5% CO₂ for 14 days. Subsequently, 1 mL from liquid culture was sub-inoculated onto blood-agar plates and incubated for 45 days, and checked

weekly for evidence of bacterial growth. **Results:** Liquid culture with 14 days of growth have been tested for *Bartonella henselae* DNA by double round amplification PCR assay using the cell division *FtsZ* protein, as target gene region. To date, infection by *Bartonella henselae* was confirmed in two blood donors. The profile of both donors showed close contact with dogs and tick bites. One of them had cats also and body lice contact. **Conclusion:** The potential risk of transmission of these agents transmitted by blood donors has been underestimated. Nonetheless, blood collected from donors infected with *Bartonella* sp may result in serious illness that can be fatal, especially in heavily immunocompromised patients. Efforts should be made towards improving diagnostic tests with sufficient sensitivity to rule out the presence of *Bartonella* spp. infection in transfusion receptors.

Disclosure of Commercial Conflict of Interest

M. Barjas-Castro: Nothing to disclose; V. Castro: Nothing to disclose; P. P. Diniz: Nothing to disclose; M. R. Drummond: Nothing to disclose; R. Gilioli: Nothing to disclose; B. Lania: No Answer; L. U. Pitassi: Nothing to disclose; D. G. Scorpio: Nothing to disclose; P. N. Velho: Nothing to disclose

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M. Barjas-Castro: Nothing to disclose; V. Castro: Nothing to disclose; P. P. Diniz: Nothing to disclose; M. R. Drummond: Nothing to disclose; R. Gilioli: Nothing to disclose; B. Lania: No Answer; L. U. Pitassi: Nothing to disclose; D. G. Scorpio: Nothing to disclose; P. N. Velho: Nothing to disclose

SP373

Evaluating the Utility of an Increased Platelet Inoculum in BACT/Alert Aerobic Culture Bottles

L. Su^{1,2} (lsu@bloodsystems.org), M. Bravo², S. K. Vanderpool², S. E. Souza², H. Kamel², P. A. Tomasulo². ¹United Blood Services, Scottsdale, AZ, United States; ²Blood Systems, Scottsdale, AZ, United States; ³Blood Systems Laboratories, Tempe, AZ, United States

Background: In the setting of bacterial detection of apheresis platelets, the manufacturer recommended platelet inoculation volume for BACT/Alert culture bottles ranges from 4-10 ml. Since the inception of bacterial detection testing at United Blood Services, BACT/Alert culture bottles have been inoculated with 4 ml of platelet sample. In November of 2008, the decision was made to increase the volume of platelet sample per culture bottle to 8 ml. The purpose of this study was to compare the rate of capture of true positive contaminations between aerobic (BPA) culture bottles inoculated with either 4 ml or 8 ml of sample and to assess if a larger sample volume would result in decreased time to detection. **Methods:** Detection of true positives (defined as growth of the same organism in both the culture bottle and platelet component) and average time to detection were determined for two time periods. The first time period, lasting from September 1, 2003 to November 23, 2008, represented platelet collections tested with 4 ml in the aerobic culture bottle only (7 day platelets tested with both aerobic and anaerobic culture bottles were excluded). The second time period, lasting from November 23, 2008 to February 28, 2010, represented platelet collections tested with 8 ml in the aerobic culture bottle only. **Results:** 95,146 platelet collections were tested with 8 ml in the aerobic bottle and 191,521 platelet collections were tested with 4 ml in the aerobic bottle. 16 true positives in the 8 ml group and 30 true positives in the 4 ml group were identified. Analysis of true positive culture rates by volume sampled show a modest increase in the rate of detection of true positives with the 8 ml sample relative to the 4 ml sample (1.68 versus 1.56 per 105 collections, OR, 1.07; 95% CI, 0.58-1.97). Comparison of mean time to detection in true positives shows a statistically significant decrease in average time to detection using 8 ml compared with 4 ml (12.63 $\pm$ 3.7 hrs to 15.97 $\pm$ 6.3 hrs, $p = 0.030$). **Conclusion:** Doubling the sample volume to 8 ml increased the rate of detection for true positives by 7% and shortened the mean time of detection for true positives by 21% when compared to a sample volume of 4 ml. The observation of a decrease in mean time to detection for true positives using a larger sample volume suggests that platelets that are currently released after 18 hours of incubation by current UBS policy could be reduced to a shorter incubation period without significantly increasing patient risk.

Disclosure of Commercial Conflict of Interest

M. Bravo: Nothing to disclose; H. Kamel: Nothing to disclose; S. E. Souza: Nothing to disclose; L. Su: Nothing to disclose; P. A. Tomasulo: Nothing to disclose; S. K. Vanderpool: Nothing to disclose

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SP374

Logistical Considerations in Using Platelet PGD Assay for Point-of-Release Testing of Apheresis Platelets

G. Giannarelli¹ (Gina.Giannarelli@ucsfmedctr.org), D. Johnson¹, T. Chapman¹, A. Nambiar¹. ¹Transfusion Service, University of California San Francisco Medical Center, San Francisco, CA, United States

Background: The Verax Platelet PGD assay is a rapid qualitative immunoassay designed to detect aerobic and anaerobic gram-positive and gram-negative bacteria in platelets. It may be used as an adjunct quality control measure following testing with an FDA approved bacterial detection test for leukoreduced apheresis platelets (LAP). We participated in a multicenter, post market surveillance study of this assay to detect contaminated LAP that may be falsely negative by culture performed 24 hours post-collection. Our center transfuses over 9000 LAP annually. All LAP undergo standard bacterial detection testing using the BacT aerobic culture method (8 ml sample volume). **Methods:** PGD testing was performed only on day 5 LAP, eliminating the need to test again during the remainder of their shelf-life. PGD testing was not required for issuing LAP. The number of LAP tested each day was largely dictated by work flow considerations; almost all tests were performed on weekdays, by night and day shift staff. A 500 μ l sample was obtained in a sterile manner from each LAP bag. Manufacturer's recommended protocols were followed. LAP showing 'Reactive' results were removed from inventory. All co-component LAP were quarantined and PGD tested. The blood supplier was asked to quarantine co-components. The retest algorithm included repeat PGD testing of a freshly drawn sample from the LAP bag, culture of another sample under aerobic and anaerobic conditions, and identification of any bacterial colonies found. **Results:** Testing was performed on 137/210 days. 1502/4640 LAP received on these days were tested. Of the 1502 tested, 1211 required sterile welding of additional tubing to obtain samples from the LAP bag for testing. 14 LAP (from 9 apheresis collections) tested positive on initial PGD testing. Only 8 tested repeat positive. However, all 8 results were determined to be false positive as bacterial contamination was not detected on gram stain and cultures performed on additional samples obtained from these LAP bags. Also, culture bottles from the BacT test performed 24 hours after collection remained negative at the end of 5 days of incubation. Minor modifications to assay conditions to minimize drying of test device helped decrease the rate of false positive reactions during the latter part of the study. **Conclusion:** The PGD assay did not detect bacterial contamination in the 1502 LAP tested. Sampling issues and workload considerations posed the biggest challenge to performance of this assay in our fast-paced transfusion service. Although this point-of-release assay has the potential to enhance patient safety by further decreasing the risk of transfusion of bacterially contaminated platelets, full scale adaptation to complex transfusion services requires risk-benefit assessment and allocation of additional resources.

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SP375

Solid Phase Syphilis Test Utilizing Kode Technology

S. E. Chesla¹ (schesla@immucor.com), S. Henry², R. A. Eatz¹, L. Sinor¹. ¹R&D, ImmucorGamma, Norcross, GA, United States; ²KODE Biotech Limited, Auckland, New Zealand

Background: Syphilis, caused by the spirochete *Treponema pallidum*, can be acquired after sexual contact with an infected individual and can be transmitted through transfusion of blood and blood components. Blood establishments instituted testing of blood donations for syphilis in 1938. Such testing has been required by regulation since 1958 and is also used as a surrogate marker for high risk behavior. Although rigorous screening has helped reduce the incidence rate for transfusion transmitted diseases to an almost non-existent level, continual improvement in sensitivity, specificity, and cost effectiveness is highly valued. Syphilis tests that detect antibody to reagin (nontreponemal) agents are cost effective but suffer in specificity. More specific testing for antibodies against treponemal targets is usually more costly. It is now possible to synthesize individual peptide sequences and attach them to cells and these synthesized peptides are relatively inexpensive to obtain in highly purified form. We report here a method to attach synthesized peptide from the protein content of *Treponema*

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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	○アフエレーシス血小板細菌汚染を防止するための皮膚消毒において、シングルステップ2%クロルヘキシジン・スワブはツーステップポビドンヨード法よりも有効である 背景: 皮膚に生息する共生細菌は、アフエレーシス血小板(PLT)輸血後の敗血症性副作用の主な原因となる。血小板採取時の細菌汚染を防止するための皮膚消毒について、2つの方法の効果を評価した。 研究デザインと方法: 症例群として3つの血液センターでワンステップ2%クロルヘキシジン/70%イソプロピルアルコール(2% CHX/IPA)皮膚消毒法を評価し、対照群として32の血液センターでツーステップのポビドンヨード(P-I)法を用いた。細菌培養の結果と供血者における有害事象について、2009年4月1日～12月31日までの期間、比較した。 結果: 2009年の初回の細菌培養陽性率は、対照群よりも症例群の方が有意に低かった(143/321/100万、オッズ比[OR] 0.44、95%信頼区間[CI] 0.23-0.86)。症例群は、培養陽性率(100/214/100万、OR 0.47、95%CI 0.21-1.03)と培養偽陽性(汚染)率(43/96/100万、OR 0.45、95%CI 0.14-1.49)の双方で低い傾向にあった。全センターが皮膚用ポビドンヨード製剤を使用していた2007年と2008年の同期においては、相違はみられなかった。症例群の73,247例のアフエレーシス採血のうち89例(0.12%)にアレルギー反応が報告された。これは、対照群と比較して16倍(OR 15.9、95%CI 9.9-25.6)であった。 結論: アフエレーシス血小板の細菌汚染の防止において、2%CHX/IPAシングルステップ皮膚スワブは、ツーステップP-I 皮膚消毒法より効果があり、敗血症性輸血副作用のリスクを低下させる可能性がある。皮膚刺激とアレルギー反応は、症例群の供血者でより起こりやすかった。しかし、その反応は軽度で自然に治癒した。				使用上の注意記載状況・ その他参考事項等
	解凍赤血球濃厚液「日赤」 照射解凍赤血球濃厚液「日赤」 解凍赤血球-LR「日赤」 照射解凍赤血球-LR「日赤」				
報告企業の意見		今後の対応			
アフエレーシス血小板の細菌汚染の防止において、2%クロルヘキシジン/70%イソプロピルアルコールのシングルステップ皮膚スワブは、ツーステップポビドンヨード法より効果があり、敗血症性輸血副作用のリスクを低下させる可能性があることが分かったとの報告である。		日本赤十字社では、採血時に穿刺部位のアルコール清拭及び10%ポビドンヨードエタノール液による消毒を行っている。これまでの研究報告により10%ポビドンヨードより2%クロルヘキシジンによる消毒の方が効果が高いことが示唆されているが、日本国内において2%クロルヘキシジンは手指以外の皮膚への使用が認められていない。また、クロルヘキシジンエタノールの使用によるアナフィラキシーショックが国内で報告されている。これらのことから、早急にクロルヘキシジンを使用することは難しいと考えるが、今後も情報の収集に努める。			

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BLOOD DONORS AND BLOOD COLLECTION

Skin disinfection with a single-step 2% chlorhexidine swab is more effective than a two-step povidone-iodine method in preventing bacterial contamination of apheresis platelets

Richard J. Benjamin, Beth Dy, Ryan Warren, Michael Lischka, and Anne F. Eder

BACKGROUND: Skin commensal bacteria account for most septic reactions after apheresis platelet (PLT) transfusion. Consequently, we evaluated the effectiveness of two skin disinfection methods in preventing bacterial contamination of PLT collections.

STUDY DESIGN AND METHODS: Three regional blood centers evaluated a one-step 2% chlorhexidine/70% isopropyl alcohol (2% CHX/IPA) skin disinfection method (trial group), while 32 blood centers (control group) continued to use a two-step povidone-iodine (P-I) method. Aerobic quality control bacterial culture results and adverse donor events were compared between April 1, 2009, and December 31, 2009.

RESULTS: The rate of initial positive bacterial cultures was significantly lower in the trial regions compared to control regions in 2009 (143 vs. 321 per million; odds ratio [OR] 0.44, 95% confidence interval [CI] 0.23-0.86). Trial regions also tended to have lower rates of both true-positive cultures (100 vs. 214 per million; OR 0.47, 95% CI 0.21-1.03) and false-positive (contamination) cultures (43 vs. 96 per million; OR 0.45, 95% CI 0.14-1.49). No differences were seen in the corresponding periods in 2007 and 2008 when all centers used P-I skin preparation. Allergic reactions were reported after 89 of 73,247 apheresis procedures (0.12%) in the trial regions representing a 16-fold increase (OR 15.9, 95% CI 9.9-25.6) compared to control regions.

CONCLUSION: The 2% CHX/IPA single-step skin swabs are more effective in preventing bacterial contamination of apheresis PLT components than a two-step P-I skin disinfection method and may reduce the risk of septic transfusion reactions. Skin irritation and allergic reactions were more likely among donors in trial regions, but reactions were generally mild and self-limiting.

Cleansing and decontamination procedures can significantly reduce the bacterial load on human skin, which in turn prevents surgical and line infections, blood culture contamination, and the contamination of blood components collected by whole blood or apheresis technologies.¹⁻⁶ Early studies evaluated the efficacy of skin decontamination methods using a direct culture plating technique from skin swabs and supported the use of three disinfectants for blood donation in North America: the iodophor povidone-iodine (P-I), chlorhexidine (CHX), and tincture of iodine (TI), often in association with 70% isopropyl alcohol (IPA).^{1,2} A two-step process incorporating a 70% IPA cleansing step and a 2% TI decontamination step was shown to produce the most complete skin decontamination when compared to CHX and P-I two-step procedures and was adopted by many national blood services.^{7,8} The TI process, however, garnered considerable dissatisfaction among donors and collection staff owing to its propensity to stain skin and clothing and cause allergic reactions. These factors led to the use of a two-step P-I disinfection process in the American Red Cross (ARC).

Recently, an enhanced 2% CHX/IPA one-step procedure has been developed that is highly bactericidal and shows equivalent degrees of skin decontamination

ABBREVIATIONS: ARC = American Red Cross; ARC HP = American Red Cross Hemovigilance Program; CHX = chlorhexidine; IPA = isopropyl alcohol; P-I = povidone-iodine; TI = tincture of iodine.

From Biomedical Services, Medical Office, Jerome H. Holland Laboratory, Rockville, Maryland; and the National Headquarters, American Red Cross, Washington, DC.

Address reprint requests to: Richard J. Benjamin, MD, PhD, Biomedical Services, Medical Office, 15601 Crabbs Branch Way, Rockville, MD 20855; e-mail: BenjaminR@usa.redcross.org.

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as the two-step 70% IPA/70% TI process as demonstrated by direct plating cultures, while promising better donor acceptability due to its clear colorless form.⁸⁻¹¹ Skin irritation and allergic reactions were associated with the use of 2% CHX/IPA; however, these reactions were generally mild, self-limiting, and manageable in the donor room setting. CHX gluconate is a water-soluble, cationic biguanide that binds to the negatively charged bacterial cell wall, altering the bacterial cell osmotic equilibrium. At low concentrations, CHX affects membrane integrity, but at high concentrations, cytoplasmic contents precipitate, resulting in cell death. CHX (0.05% solution) has broad activity against Gram-positive and Gram-negative bacteria; facultative anaerobes; and aerobes, yeasts, and some lipid-enveloped viruses, including human immunodeficiency virus, but is not sporicidal.¹²

The need for enhanced skin decontamination is highlighted by the residual risk of septic reactions after transfusion of apheresis platelet (PLT) components, based on reports to the American Red Cross Hemovigilance Program (ARC HP). Despite recent improvements in limiting and detecting bacteria in apheresis PLT collections with universal inlet-line sample diversion during collection and optimized sampling for aerobic quality control (QC) cultures, skin commensal bacteria are still the leading cause of contamination in septic transfusion reactions.^{13,14} We therefore performed an operational trial comparing our standard two-step P-I process and the one-step 2% CHX/IPA procedure in regional blood centers utilizing a common set of procedures and protocols. We evaluated bacterial contamination of apheresis PLT donations as assessed by QC culture and reported donation-related adverse reactions.

MATERIALS AND METHODS

Collection of apheresis PLTs

Apheresis PLTs were collected utilizing standard protocols from volunteer donors at 35 regional blood centers of the ARC with one of two apheresis devices: Amicus (Fenwal, Inc., Lake Zurich, IL) or Trima (CaridianBCT, Lakewood, CO) as previously described.¹⁴ All collections utilized inlet-line diversion of the initial 30 to 40 mL of blood.

Skin preparation

Before phlebotomy, the donor's antecubital fossa was prepared using standard procedures. In the control group of 32 regional ARC blood centers, a two-step process used a 30-second swab with 7.5% P-I (0.75% available iodine) followed by 30-second spiral swab with 10% P-I (1% available iodine; Duo-Swab P-Ie

swabsticks, Professional Disposables International, Inc., Orangeburg, NY) and 30-second drying period. For donors who had a history of allergic reactions to P-I, a single-step 2% CHX gluconate/70% IPA impregnated brush was used (Chloraprep one-step Frepp applicator, Cardinal Health, Leawood, KS), with a 30-second application period followed by a 30-second drying period.

In the trial group of three large, geographically dispersed blood centers, each with many fixed and mobile apheresis collection sites, skin preparation was performed using a single-step 2% CHX/70% IPA impregnated swab (Chloraprep swabstick, Cardinal Health) with a 30-second application period followed by a 30-second drying period. Donors who experienced skin irritation or an allergic reaction to 2% CHX/70% IPA were switched back to the P-I technique for subsequent donations, as described above. Staff training and full implementation of the trial protocol were completed in March 2009.

Detection of bacterial contamination

An automated microbial detection system (BacT/ALERT 3D, bioMérieux, Durham, NC) was used for aerobic cultures according to the manufacturer's instructions, as previously described,¹⁴ using a sample volume of 8 mL. Investigation of an initial positive bottle culture result was performed as previously described.¹³ A true-positive result is defined as an initial-positive culture with a subsequent positive confirmatory culture result for the PLT component or cocomponent. A false-positive result is defined as a positive initial signal but a negative result on subsequent confirmatory culture, which was further characterized as either possible sampling contamination if the initial culture bottle was shown to grow bacteria or instrument error (machine failure) if the initial culture bottle was sterile. If PLT components were not available for confirmatory culture, because they were transfused or destroyed during the manufacturing process, or if the case information reported to the ARC HP was incomplete, the results were classified as "indeterminate." Indeterminate results include cases where the initial culture bottle may or may not have been shown to harbor viable bacteria. Suspected transfusion reactions were investigated by the 35 ARC regional blood centers and reported to the ARC Hemovigilance Program and were classified as probable or definite septic reactions as previously described.^{14,15}

Statistical analysis

Sample size was determined based on 2008 QC bacterial cultures results for all initial positive culture results that were demonstrated to be bacterially contaminated

(approx. 1:2000 [0.05%], true-positive plus false-positive [contamination]). Approximately 70,000 test cultures and 282,000 control cultures would detect a 50% reduction in positive cultures with 80% power and 95% confidence.¹⁶ Because skin preparation was the only known variable that differed between the trial and control centers, univariate analysis was appropriate. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to compare bacterial contamination rates and risk of allergic reactions. The ARC Institutional Review Board determined that the study was exempt under 45CFR46, 21CFR50.

RESULTS

Residual risk of septic reactions to apheresis PLT transfusion

Between January 1, 2007, and December 31, 2009, the ARC HP received 25 reports of probable or definite septic transfusion reactions involving split products from 22 collections (Table 1). Five reports were received in 2007, 11 reports in 2008, and nine reports in 2009 (involving split products from five, eight, and nine collections, respectively) revealing no significant change over time in the number of collections involved in septic reactions. A trial region reported three of the cases (one in 2007; two in 2008); the remainder were from the control regions. All collection procedures performed during this period utilized inlet-line sample diversion and an 8-mL sample in aerobic QC culture; these measures were previously shown to reduce contamination with skin commensal organisms and improve detection of bacteria. Eleven of the 25 cases from 2007 and 2008 were included in previous reports.^{13,17} Four cases were reported as fatalities including three with *Staphylococcus aureus* and one with coagulase-negative *Staphylococcus*. Thus, skin commensal bacteria that contaminate the apheresis donation are still the main cause of morbidity and

mortality from septic transfusion reactions, despite improvements in detecting and limiting bacteria in apheresis PLT collections.

Comparison of QC bacterial culture results in test and control regional blood centers

The operational trial compared a one-step 2% CHX/IPA swab to the standard two-step P-I process. After converting to the use of 2% CHX/IPA swabs as the primary skin preparation method, the three trial centers monitored 69,863 apheresis PLT donations for bacterial contamination using standard QC bacterial aerobic cultures between April 1 and December 31, 2009. The bacterial culture results in the trial regions were compared with results from 261,165 collections performed in the remaining 32 regional blood centers that continued to use the standard two-step P-I process during the same time period (control group). The overall rate of all initial positive results was significantly lower in the trial regions compared to the control group (358 vs. 559 per million cultures; OR 0.64, 95% CI 0.42-0.98). These data included false-positive (machine failure) signals and some indeterminate results that were also associated with a sterile initial culture bottle, signifying an erroneous alarm. These results reflect the functioning of the device, are not influenced by skin preparation techniques, and are excluded from further analysis.

The primary outcome measure for power calculations was the identification of a positive bacterial culture from the initially inoculated bottle in the trial and control regions during the study period. These results include true (confirmed) positives as well as unconfirmed initial results (false positives attributed to sampling contamination and some indeterminates). This analysis is therefore similar to that presented by other authors^{4,18-20} and is consistent with our previous observation that both true- and false-positive (contaminant) culture-positive results are similarly reduced by measures that limit skin contamination.^{13,21} The trial regions were 56% less likely than the

control regions to have positive initial culture results during the study period (143 vs. 321 positive cultures per million donations; OR 0.44, 95% CI 0.23-0.86; Table 2; Fig. 1). Both true-positive (100 vs. 214 per million donations; OR 0.47, 95% CI 0.21-1.03; Fig. 1) and false-positive (contaminant; 43 vs. 96 per million donations; OR 0.45, 95% CI 0.14-1.49) cultures tended to be lower in the test regions compared to the control regions during the study period.

Skin commensal bacteria accounted for four of seven (57%) true-positive culture results in the trial group and 38 of 56 (68%) in the control

TABLE 1. Septic transfusion reactions reported to the ARC HP between January 1, 2007, and December 31, 2009

Clinical case classification	Bacterial isolate	Number
Definite	Coagulase-negative <i>Staphylococcus</i>	13*
	<i>S. aureus</i>	3*
Probable	Coagulase-negative <i>Staphylococcus</i>	5
	<i>S. aureus</i>	1
	<i>Streptococcus</i> (α -hemolytic) spp.	1
	<i>Klebsiella</i> spp.	1
	<i>Enterobacter cloacae</i>	1
	Total	25

* Four cases were reported fatalities: three with *S. aureus*; one with coagulase-negative *Staphylococcus*; 11 septic reactions in 2007 and 2008 were included in a previous report;^{13,17} definite and probable cases meet clinical case definition for posttransfusion sepsis; definite cases are further confirmed by culture results of patient and residual component or cocomponents.

TABLE 2. Bacterial culture results in the trial and control regions

Site	Interpretation	Baseline (n)		Study period (n) 2009	Temporal trends (secondary analysis) OR (95% CI)	
		2007	2008		2009 vs. 2007	2009 vs. 2008
Trial regions (3)	Collections	68,991	68,797	69,863		
	True positive (confirmed)	14	10	7	NS	NS
	False positive (contaminant)	13	7	3	0.23 (0.06-0.80)	NS
	False positive (machine failure)	19	29	15	NS	0.51 (0.27-0.95)
	Indeterminate	1	1	0	ND	ND
	Total positive	47	47	25	0.53 (0.32-0.85)	0.52 (0.32-0.85)
	Initial culture positive*	28	18	10	0.35 (0.17-0.73)	NS
Control regions (32)	Collections	263,531	267,505	261,165		
	True positive (confirmed)	44	48	56	NS	NS
	False positive (contaminant)	50	45	25	0.50 (0.31-0.82)	0.57 (0.35-0.93)
	False positive (machine failure)	95	92	54	0.57 (0.41-0.80)	0.60 (0.43-0.84)
	Indeterminate	14	16	11	NS	NS
	Total positive	203	201	146	0.73 (0.59-0.90)	0.74 (0.60-0.92)
	Initial culture positive*	106	102	84	NS	NS
Trial vs. control regions (OR [95%CI])						
		Baseline		Study period (primary outcome measure) 2009		
	Interpretation	2007	2008			
	True positive (confirmed)	NS	NS	NS (0.47 [0.21-1.03])		
	False positive (contaminant)	NS	NS	NS (0.45 [0.14-1.49])		
	False positive (machine failure)	NS	NS	NS		
	Total positives	NS	NS	0.64 (0.42-0.98)		
	Initial culture positive*	NS	NS	0.44 (0.23-0.86)		

* Initial culture positive includes true positive, false positive (contaminant), and indeterminate cultures in which bacteria were cultured from the initial bottle.
ND = not determined; NS = not significant.

regions; enteric organisms constitute the remainder. Enhanced skin preparation techniques are unlikely to affect the rates of contamination by enteric organisms that are thought to result from asymptomatic donor bacteremia. These data support the premise that 2% CHX/IPA decreases the absolute rate of contamination with skin bacteria; however, the number of skin contaminants was small and the study was not powered to demonstrate such a difference.

In a secondary post hoc analysis, the numbers of culture-positive collections in trial and control regions during the period April through December were compared in 2007 and 2008 when all centers utilized P-1 skin preparation. This analysis confirms that there was no significant difference in the rates of initial-positive, true-positive, or false-positive cultures between trial and control regions in the 2 prior years (Table 2). In the trial regions, the number of initial bacterial-positive cultures was lower in 2009 compared to 2007 but not compared to 2008 (Table 2). The control regions showed significantly

lower rates of total-positive, false-positive (contaminant), and false-positive (machine failure) cultures in 2009 when compared to 2007 and 2008 (Table 2); however, there was no significant difference in initial bacterial culture-positive or true (confirmed)-positive rates.

Adverse reactions to 2% CHX/70% IPA skin preparation

Adverse reactions to 2% CHX/IPA were assessed as the number of major and minor allergic reactions recorded by collection staff on the blood donation record at the time of donation and the number of events, including those requiring outside medical care reported after the donors had left the donor center.²² The numbers of reactions were not significantly different between trial and control regions in the years 2007 and 2008. Although the absolute number of allergic reactions was relatively low in the trial regions during the study period, and the majority of reactions were minor localized skin irritation, a notable

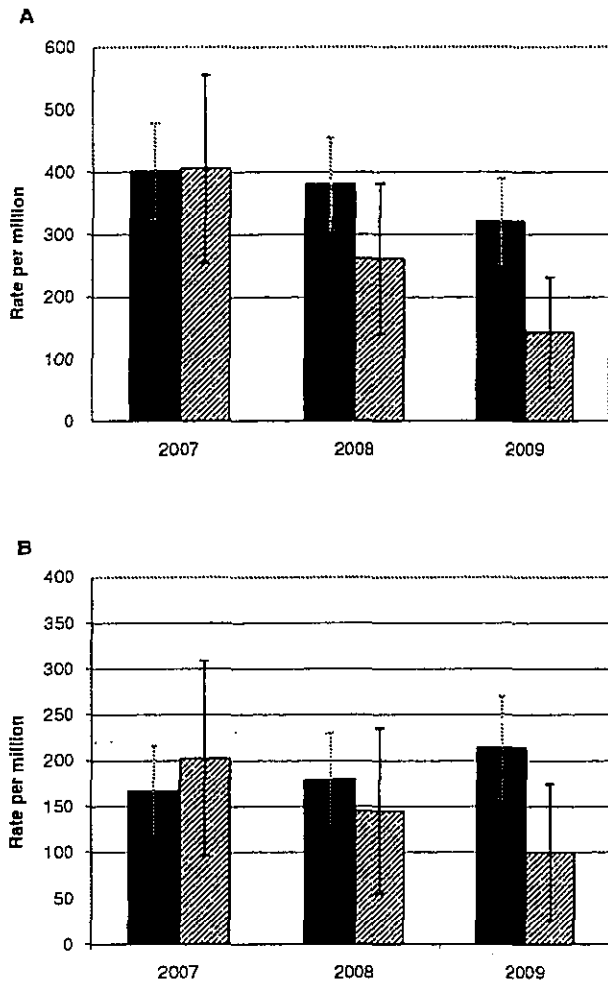


Fig. 1. (A) Rate per million of positive QC bacterial cultures in which bacteria were isolated from the initial aerobic culture bottle shows a significant 56% lower rate in 2009 in trial regions compared to control regions; (B) rate of true (confirmed)-positive cultures in which bacterial contamination was confirmed by reculture shows a strong declining trend in 2009 in trial regions compared to control regions. (■) Thirty-two control regions; (▨) 3 trial regions.

increase of reports compared to the control regions was apparent, with a fivefold increase at the collection site (0.3 vs. 1.4 per 10,000 registrations; OR 5.4, 95% CI 2.0-14.1) and a 16-fold increase in postdonation reports (0.8 vs. 12.2 per 10,000 registrations; OR 15.9, 95% CI 9.9-25.6; Table 3). The increase in allergic reactions after apheresis procedures in the trial regions likely reflects the increased documentation of skin irritation and localized allergic reactions to CHX. Seven donors in the trial regions sought further medical care after donation for local skin irritation and treatment of pruritic rashes at the venipuncture site during the trial period; two donors in the control regions sought additional medical care for systemic allergic reac-

tions unrelated to the skin preparation. Even among the cases of outside medical care, the allergic reactions were relatively mild and self-limiting. Donors who experienced skin irritation or suspected allergy to the CHX scrub had the option during the trial to request the use of the standard P-I skin preparation when they returned to donate. Reversion to the P-I skin preparation was documented in 519 donations during the trial or approximately 0.95% of donations.

DISCUSSION

Bacterial contamination of apheresis PLTs components remains a major risk of transfusion despite the introduction of inlet-line sample diversion and QC bacterial culture of all products in the ARC. We previously reported that these measures substantially reduce the number of positive bacterial culture tests and the incidence of septic reactions; however, the ARC HP continues to receive reports of septic transfusion reactions and fatalities, at a rate of 1:108,000 and approximately 1:500,000 distributed components, respectively.^{13,14} All of these septic reactions implicated bacteria in apheresis PLT components that escaped detection and had acceptable (negative) QC culture results through to expiration. The major limitation of current QC testing is the requirement to sample the component and inoculate the culture bottle 24 to 36 hours after collection, which will fail to detect bacteria present initially at very low concentrations and with long lag phases of growth. This limitation of current detection methods is further illustrated by studies that have cultured outdated components and estimate an even higher risk of contamination at approximately 1:1500 transfusions.^{19,23} The large discrepancy between measured contamination rates of stored apheresis PLT components and reported septic reactions likely reflects the absence of a discernable reaction in many patients, the failure to recognize or report septic reactions, or both.

We continue to observe that the residual risk of reported sepsis principally involves organisms known to be skin commensals or common contaminants, especially *Staphylococcus* species. We therefore investigated the utility of a potentially improved skin disinfection process in an operational trial with the primary outcome measure being the number of bacterially contaminated apheresis PLT collections detected by QC bacterial culture. The data from the three regional blood centers utilizing the 2% CHX/IPA one-step skin preparation swabs demonstrate that the overall rate of initial bacterial-positive cultures was 56% lower compared with 32 blood centers using a conventional two-step P-I skin preparation technique. Similarly, the rate of true (confirmed)-positive cultures showed a similar strong decreasing trend in the trial regions compared to the control regions, although the number of observations was small and did not reach

TABLE 3. Allergic reactions after apheresis donation procedures in the trial and control regions

		Baseline		Study period	
		2007	2008	2009	Rate per 10,000
Trial regions	Registrations*	72,290	72,120	73,247	
	On-site reaction	5	2	10	1.4
	Postdonation reaction†	1	4	89	12.2
	Outside medical care	1	0	7	1.0
Control regions	Registrations*	278,290	281,247	274,307	
	On-site reaction	8	7	7	0.3
	Postdonation reaction†	21	23	21	0.8
	Outside medical care	3	2	2	0.1
Trial vs. control regions, OR (95% CI)					
		Baseline		Study period (primary outcome measure)	
		2007	2008	2009	
On-site reaction		NS	NS	5.35 (2.0-14.1)	
Postdonation reaction†		NS	NS	15.9 (9.9-25.6)	
Outside medical care		NS	NS	13.1 (2.7-63.1)	

* Includes all donation procedures (productive and nonproductive units).
† Postdonation reactions include major (systemic) allergic reactions that occurred at the collection site that require follow-up and major (systemic) and minor (localized) allergic reactions that were reported to the ARC after an apheresis donation procedure.
NS = not significant.

significance during the study period. A secondary analysis of a before-and-after comparison in the trial regions also demonstrated a decreasing trend in the rate of initial-positive and true (confirmed)-positive bacterial cultures in the 2009 study period compared to the same time period in the 2 prior years (2007 and 2008; Fig. 1). These data suggest that 2% CHX/IPA is a superior skin disinfectant to P-I and that this translates into decreased component contamination, as detected by QC culture. The 2% CHX/IPA skin preparation is also predicted to decrease the total number of collections discarded because of initial-positive cultures and to reduce time spent on performing the skin preparation process, because the one-step process is quicker to perform than the two-step P-I process.¹⁰ Finally, 2% CHX/IPA skin preparation may reduce the overall rate of septic transfusion reactions reported to the ARC HP, because most breakthrough cases implicate skin commensal bacteria.

We used the overall rate of initial bacterially contaminated cultures as the primary endpoint in our study, because initial sample size calculations showed that more than 880,000 cultures would be necessary to demonstrate a 50% reduction with 95% confidence if the true-positive (confirmed) culture rate was used instead. Such an experiment was not feasible with available resources or desirable considering the required duration of study. The decision to rely on initial bacterially contaminated culture results (i.e., both confirmed and unconfirmed bacterial-positive cultures) for this trial is consistent with the manner in which culture data have been previously reported by other authors^{4,18-20} and is consistent with data from our two prior reports that demonstrate similarly decreased rates of both true-positive and unconfirmed

false-positive (contamination) culture results with in-line sample diversion.^{19,21} Diversion strategies reduce component contamination by skin commensal organisms disturbed during the phlebotomy puncture^{7,24} and are thought to act synergistically with improved skin preparation techniques to reduce blood component contamination. The observation that diversion strategies reduced the rates of false-positive (contamination) cultures, as well as true-positive results, suggests that the false-positive result may not represent inadvertent contamination of the sample or culture bottle as assumed, but may instead reflect a failure of a truly contaminated component to confirm as positive, due either to low-level contamination and inadequate sampling or to overgrowth and death of the contaminating organisms in the primary culture bottle.²⁵ Our results in this study showing a comparable decline in true- and false-positive (contamination) cultures with 2% CHX/IPA skin decontamination further supports this hypothesis.

Interestingly, we noted an overall decrease in the number of total positive culture results in 2009 in the control group compared to the prior years. The decline was seen in the false-positive (contamination) and (machine failure) groups and not in the true-positive subset (Table 2). The decline in false positives attributed to machine failure likely resulted from a corrective action that was taken to perform more frequent calibration of the QC bacterial incubators as they near the end of their expected operational life. A possible explanation for the decreasing trends with time for the false-positive (contamination) results in the control regions is not immediately apparent.

We documented a relatively low rate but still substantial increase in localized allergic reactions reported to the

ARC HP in the trial centers during the study compared to the control regions. In addition, about 1% of donors requested P-I disinfection instead of 2% CHX/IPA when they returned to the trial regions to donate apheresis PLTs, presumably because of localized skin irritation or allergic reactions. Our results are similar to those reported by Wong and colleagues⁹ and Ramirez-Arcos and Goldman,⁸ who noted that reports of these reactions decreased over time, as donors were instructed to wash the area to remove residual CHX after donation or as donors reverted to P-I skin disinfection. Donors in the Red Cross system that experience reactions to 2% CHX/IPA have the option of using conventional P-I skin disinfection, unlike the practice in the United Kingdom, where donors experiencing allergic reactions to 2% CHX/IPA would be indefinitely deferred.¹⁰

As noted by Ramirez-Arcos and Goldman,⁸ the efficacy of skin disinfection depends on multiple factors, including the chemical disinfectant utilized, its concentration and duration of exposure, the use of a one-step or two-step process, the training of the staff performing the process, and the method of application (swab, scrub, ampoule).⁸ The effectiveness of these interventions has been assessed principally using a direct plating technique of skin swabs, where the residual number of bacteria remaining on the skin is directly assessed.^{1,2} Early work by Goldman and colleagues² documented that a two-step P-I process was less effective than a 70% IPA/TI process, more effective than the use of "green soap"/70% IPA, and equivalent to a 0.5% CHX/70% IPA process. McDonald and coworkers⁷ showed that a 70% IPA/TI process is better at reducing bacterial contamination, as measured by QC BacT/ALERT cultures and by plating cultures, than a one-step 0.5% CHX/70% IPA process and also the two-step P-I process. These data led the Canadian and British blood services to adopt a two-step 70% IPA/TI standard technique. More recently, many hospitals and blood centers have evaluated a single-step CHX swab technique that incorporates higher concentrations of CHX (1%-2%) in 70% IPA. For example, Adams and colleagues⁶ demonstrated higher *in vitro* bacterial kill rates with 2% CHX/70% IPA than with aqueous 2% or 0.5% CHX, 0.5% CHX in 70% IPA, or 10% (wt/vol) P-I. Wong and coworkers⁹ showed that 1% CHX/70% IPA is effective in skin disinfection by direct plating culture, but 3% of donors reported allergic reactions. Barenfanger and colleagues¹¹ reported that the rates of bacterial contamination of blood cultures were not different when using TI versus a one-step 2% CHX swab technique; McDonald and coworkers¹⁰ showed that a two-step 70% IPA/2% TI technique gave equivalent results to a one-step 2% CHX technique by direct plating cultures. Recently Ramirez-Arcos and Goldman⁸ reported that one-step 2% CHX/70% IPA swabs are equivalent to a two-step 2% CHX/70% IPA swab technique and also to a two-step 70% IPA/TI tech-

nique in plating cultures. Finally, no difference was seen in component QC bacterial culture rates comparing 2% CHX/IPA skin preparation and historical data using a two-step 70% IPA/TI protocol.⁸

Taken together, these data suggest that the one-step 2% CHX/IPA skin preparation technique is comparable with the best techniques described in the literature and substantially more effective than the current two-step P-I technique. Based on these results, the ARC will introduce 2% CHX/IPA as the primary method for skin disinfection for apheresis PLT collection. Although we predict that this will improve safety, we accept that risk cannot be eliminated with the current approach because some skin commensal organisms will continue to evade our best attempts at neutralization, diversion, or detection. Clinicians should continue to monitor patients who receive blood components and to promptly report suspected septic transfusion reactions to blood centers, so that blood centers can take immediate actions to prevent transfusion of other cocomponents from the same donation and assess the residual risk to evaluate further process improvements in limiting and detecting bacteria in apheresis PLT collections.

CONFLICT OF INTEREST

The authors have no conflicts regarding this submission.

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医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日 2011. 3. 16	新医薬品等の区分 該当なし	総合機構処理欄
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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	○臓器移植による <i>Cryptococcus neoformans</i> の伝播 背景: クリプトコッカス髄膜炎の1人のドナーから臓器移植を受けた3人のレシピエントに、<i>Cryptococcus neoformans</i> が伝播した症例を報告する。 方法: 診察記録を調査し、クリプトコッカスのドナー伝播の可能性を決定するため、分離株の遺伝子タイピングを実施した。 結果: 3人のレシピエントがクリプトコッカス症と診断された。これらのレシピエントは、死亡時に未解明の神経病態であったドナーから臓器移植を受けていた。このドナーは、後の剖検においてクリプトコッカス髄膜炎であったと診断された。肝臓を移植されたレシピエントと片方の腎臓を移植されたレシピエントはクリプトコッカス敗血症と肺炎を発症した。もう片方の腎臓を移植されたレシピエントはクリプトコッカス敗血症と髄膜炎を発症した。腎臓を移植された2人の患者は、長期間の抗真菌治療により回復した。多遺伝子配列タイピングによりレシピエント分離株4つを検査し、それらが同じ対立遺伝子を有する事を認めた。 結論: 今回の調査により、臓器移植による <i>Cryptococcus neoformans</i> の伝播が証明された。未解明の神経病態を呈するドナーにおいて、クリプトコッカス症に関する評価は強く考慮されるべきである。				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 未解明の神経病態であった1人のドナーからの臓器移植で、3人のレシピエントに <i>Cryptococcus neoformans</i> の伝播が認められたとの報告である。 日本赤十字社では発熱などの体調不良者を献血不適としている。今後も引き続き情報の収集に努める。			

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MAJOR ARTICLE

Transmission of *Cryptococcus neoformans* by Organ Transplantation

John W. Baddley,^{1,2} Denise C. Schain,³ Asmita A. Gupte,³ Sundus A. Lodhi,³ Liise K. Kayler,³ João P. Frade,⁴ Shawn R. Lockhart,⁴ Tom Chiller,⁴ J. Steve Bynon Jr,¹ and William A. Bower⁴

¹Department of Medicine, University of Alabama at Birmingham, Birmingham, Alabama; ²Birmingham Veterans Affairs Medical Center, Birmingham, Alabama; ³Department of Medicine, University of Florida School of Medicine, Gainesville, Florida; and ⁴Centers for Disease Control and Prevention, Atlanta, Georgia

Background. This article describes transmission of *Cryptococcus neoformans* by solid organ transplantation.

Methods. We reviewed medical records and performed molecular genotyping of isolates to determine potential for donor transmission of *Cryptococcus*.

Results. Cryptococcosis was diagnosed in 3 recipients of organs from a common donor with an undifferentiated neurologic condition at the time of death. Cryptococcal meningoencephalitis was later diagnosed in the donor at autopsy. The liver and 1 kidney recipient developed cryptococchemia and pneumonia and the other kidney recipient developed cryptococchemia and meningitis; 2 patients recovered with prolonged antifungal therapy. We tested 4 recipient isolates with multilocus sequence typing and found they had identical alleles.

Conclusions. Our investigation documents the transmission of *Cryptococcus neoformans* by organ transplantation. Evaluation for cryptococcosis in donors with unexplained neurologic symptoms should be strongly considered.

Cryptococcosis is a systemic mycosis caused by the encapsulated yeasts *Cryptococcus neoformans* and *Cryptococcus gattii*, organisms found in soil and often associated with pigeon droppings or trees. Infection typically involves the lungs or central nervous system, and less frequently the blood, skin, skeletal system, or prostate. Because the incidence of cryptococcosis is greatly increased in patients with deficits in cellular immunity, cryptococcosis is considered an opportunistic fungal infection.

Cryptococcosis is the third most common fungal infection among solid-organ transplant (SOT) recipients [1–3]. Recent surveillance data suggest the 12-month cumulative incidence is low, <.25% [2, 3]. The disease

usually occurs >1 year posttransplant and is generally considered to represent reactivation of latent infection [4, 5]. Although acquisition of the organism in most cases occurs via inhalation, there is risk of transmission through donor organs or other tissues. Previous studies have described 2 cases of *Cryptococcus* transmission through solid organs and 2 cases of transmission via corneal tissue [6–9]. We report the investigation of *C. neoformans* infection that developed in 3 recipients of cadaveric organ transplants from 1 donor with unrecognized cryptococcal meningoencephalitis. We used multilocus sequence typing (MLST), a rapid, reproducible, and discriminatory methodology for genotyping isolates of *C. neoformans*, to investigate genetic relatedness among the isolates [10, 11].

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Correspondence: John W. Baddley, MD, University of Alabama at Birmingham, Department of Medicine, Division of Infectious Diseases, 1900 University Boulevard, 229 Tinsley Harrison Tower, Birmingham, AL 35294-0006 (jbaddley@uab.edu).

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CASE REPORTS

Donor

The organ donor was a 51-year-old woman who presented to the emergency department after 2 weeks of seizures, urinary incontinence, headaches, and slurred speech. She had a 10-year history of sarcoidosis for which she received daily corticosteroid therapy. On

presentation to the emergency department, she was hypotensive and in respiratory distress, which progressed and necessitated mechanical ventilation. Admission chest radiography showed no infiltrates. Computed tomography (CT) of the brain revealed only marked hydrocephalus. Results of routine blood and urine cultures drawn on admission were negative, as was the HIV test result. Lumbar puncture and cerebrospinal fluid (CSF) studies were not performed. The patient developed cardiac arrest 2 days after admission, was unresponsive to resuscitation, and died. The family granted permission for organ donation. The liver and kidneys were used for transplantation, and the lungs were retained for research purposes. Other organs were discarded.

At organ procurement, the lungs, liver, and kidneys looked grossly normal. Brain biopsy showed inflammation, but routine staining revealed neither malignancy nor organisms. Autopsy results, available 30 days after the organs were transplanted, showed meningoencephalitis, and stains revealed fungal organisms consistent with *C. neoformans*. Cryptococcal antigen titer performed on stored serum was 1:64. Lung examination at autopsy revealed non-caseating granulomas, fibrosis, and calcifications consistent with sarcoidosis. No organisms were demonstrated with periodic acid-Schiff, Grocott's methenamine silver, or mucicarmine staining.

Recipient 1

A 72-year-old white woman with cirrhosis and hepatocellular carcinoma received the liver from the donor on 30 November 2009. Prior to the transplant, she had no fever, chills, or respiratory complaints. Corticosteroids were given as induction immunosuppression, with maintenance consisting of tacrolimus, mycophenolate, and prednisone. Her course after the transplantation was complicated by lower gastrointestinal bleeding and immune thrombocytopenic purpura (ITP) refractory to medications. The patient underwent splenectomy and liver biopsy 2 weeks after transplantation. Unexpectedly, frozen sections from spleen and liver showed organisms consistent with *C. neoformans* (Figure 1), and both spleen and liver tissue grew *C. neoformans*. Blood cultures were drawn and grew *C. neoformans*. Serum cryptococcal antigen titer was 1:1024.

The patient was placed on a lipid formulation of amphotericin B (AMB) and was transitioned to fluconazole after 8 weeks of AMB therapy. Flucytosine was not administered due to severe thrombocytopenia and anemia. A lumbar puncture was not performed due to severe thrombocytopenia. The patient had a complicated respiratory course and developed diffuse cryptococcal pneumonia, diagnosed by lung tissue histopathology. The patient died from aspiration pneumonia 6 months post-transplant. Serum cryptococcal antigen test was negative 1 week before death, and the patient had remained on fluconazole therapy.

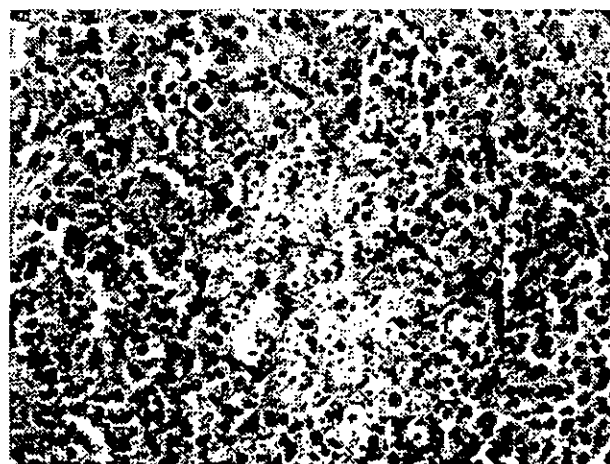


Figure 1. Tissue showing *Cryptococcus* organisms in biopsy specimen from Recipient 1. The spleen shows areas of dark yeast nuclei surrounded by nonstaining capsule (hematoxylin-eosin stain; low-power magnification).

Recipient 2

A 58-year-old white man with end-stage renal disease secondary to IgA nephropathy and a previous renal transplant in 2004 received a cadaveric renal transplant on 1 December 2009. Antithymocyte globulin and steroids were given as induction immunosuppression, with maintenance consisting of tacrolimus, mycophenolate, and prednisone. On postoperative day 16, the patient was readmitted for evaluation of malaise with fever, and blood cultures yielded *C. neoformans*. Serum cryptococcal antigen titer was 1:256, and CSF antigen test result was negative. The result of CSF analysis was normal. Chest CT revealed new focal airspace consolidation with effusions, and sputum cultures yielded oral flora. The patient was treated with a lipid formulation of AMB and flucytosine for 14 days, during which he clinically improved and blood cultures sterilized. Therapy was changed to oral fluconazole, and the patient was discharged home. The patient was readmitted for fever and dry cough 1 month later, and chest CT showed diffuse micronodules throughout the lungs. Bronchoalveolar lavage cytology (Figure 2) and transbronchial biopsy showed cryptococcal organisms. The patient received a second 2-week induction course of lipid AMB and flucytosine. He was discharged home on oral fluconazole and is doing well 6 months posttransplant.

Recipient 3

A 46-year-old white man with a history of end-stage renal disease secondary to Alport syndrome who received a cadaveric renal transplant on 1 December 2009 was admitted with complaints of fever, neck stiffness, and photophobia, which began 24 days posttransplant. Immunosuppression included induction with basiliximab and steroids, then maintenance on tacrolimus, mycophenolate, and prednisone. Lumbar puncture revealed an

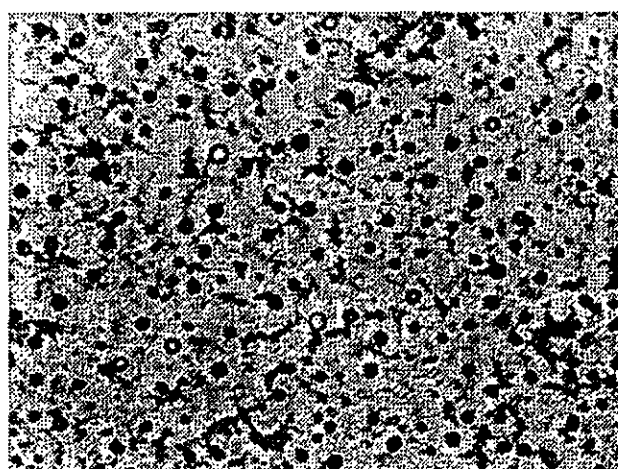


Figure 2 Cytology from bronchoalveolar lavage of Recipient 2 showing darkly stained *Cryptococcus* capsules (mucicarmine stain; magnification $\times 20$).

opening pressure of 36 mm H₂O, 264 white blood cells (86% polymorphonuclear leukocytes), elevated protein level, and normal glucose level. CSF cryptococcal antigen was 1:256. Cultures of blood and CSF yielded *C. neoformans*, and diagnosis of cryptococcosis was made on day 38 posttransplant. The patient was treated with a lipid formulation of AMB and flu-cytosine for 17 days, after which oral fluconazole was started. Serial lumbar punctures were performed to reduce elevated opening CSF pressures. The patient's CSF culture became sterile on treatment day 10. During this treatment period, the patient's course was further complicated by diverticulitis with intra-abdominal abscess requiring temporary diverting colostomy and by the development of *Clostridium difficile* colitis. The patient was clinically improved and on oral fluconazole as an outpatient 6 months posttransplant.

METHODS

The institutions of origin sent 4 available isolates from the 3 organ recipients to the Centers for Disease Control and Prevention. We grew isolates on yeast extract, peptone, and

dextrose medium (YPD) with 0.5M sodium chloride, and we purified DNA using the Utraclean DNA isolation kit from MoBio Laboratories as instructed by the manufacturer. We performed multilocus sequence typing (MLST) according to the consensus *C. neoformans* typing scheme described by Meyer et al [10] with the loci *URA5*, *IGS1*, *GPD1*, *LAC1*, *PLB1*, *SOD1*, and *CAP59*, comprising a combined total of 3892 nucleotides. We assigned allele numbers using the archived online *C. neoformans* database onMLST.net (<http://cneoformans.mlst.net/>).

RESULTS

For each of the 7 loci tested, the 4 patient isolates had identical alleles (Table 1), indicating a probable clonal origin for this set of isolates. The relative frequency of this sequence type in a US population cannot be determined because there is currently no MLST data set of US isolates. Unpublished data from the CDC suggest that this type is an uncommon strain in the United States (S. R. Lockhart, personal communication). Query of this sequence type using www.MLST.net (<http://cneoformans.mlst.net>) reveals 3 additional isolates with this sequence type [11].

Other than each receiving an organ from the same donor, Recipient 2 and recipient 3 had no known contact, direct or indirect, and lived in different states. Transplantation for Recipients 2 and 3 occurred in a different geographical region from that for Recipient 1.

DISCUSSION

In this report, we confirm with reasonable certainty that *C. neoformans* was transmitted from the donor to geographically separated patients by transplantation of liver and kidneys. This report emphasizes the importance and difficulty of recognizing fungal infection pretransplant, specifically in a donor with an undifferentiated neurologic condition.

Cryptococcus causes up to 8% of invasive fungal infections in SOT recipients and is third in frequency behind *Aspergillus* and *Candida* species [1–3]. Disease most likely represents reactivation of latent infection [12], but epidemiologic investigations also suggest acquisition of primary infection following

Table 1. Multilocus Sequence Typing Analysis of Patient Isolates

Locus	Alleles						
	<i>CAP59</i>	<i>GPD1</i>	<i>IGS1</i>	<i>LAC1</i>	<i>PLB1</i>	<i>SOD1</i>	<i>URA5</i>
Allele length	501	489	725	471	533	536	637
Patient 1 blood	8	10	15	8	12	3	11
Patient 2 blood	8	10	15	8	12	3	11
Patient 3 blood	8	10	15	8	12	3	11
Patient 3 cerebrospinal fluid	8	10	15	8	12	3	11

transplant [13–15]. Cryptococcosis in SOT recipients usually presents late (>1 y) after transplant [1–3, 16]. In a recent surveillance study of cryptococcosis among SOT patients, the median onset time was 575 days posttransplant, with 75% of cases occurring <3 years posttransplant [3]. Among our recipients, disease was early in onset (<4 wk after transplant), prompting suspicion of donor transmission.

The donor presented with clinical signs and symptoms of cryptococcal meningitis that included headache, seizures, neurologic deficits, and findings of hydrocephalus on brain imaging, and was at risk because of underlying sarcoidosis and corticosteroid therapy; however, cryptococcal meningitis was not diagnosed at the time of death or organ procurement.

Only the donor liver and kidneys were used for transplantation. The liver was transplanted at a single institution (Recipient 1); and both kidneys were transplanted at a separate institution in a different geographical area (Recipients 2 and 3). None of the recipients had contact with each other, nor did they receive antifungal prophylaxis. All recipients developed disseminated disease, and at the 6-month follow-up, 2 were alive and improved. The third died of unrelated causes and had a negative serum cryptococcal antigen test result 1 week before death. Molecular typing confirmed the relatedness of all recipient isolates, showing an identical sequence type for all 4 of the isolates across 3892 nucleotides. Without the availability of a donor isolate, it cannot be completely ruled out that each transplant recipient independently acquired an isolate identical to that of the other recipients; however, this scenario is highly unlikely.

Transmission of cryptococcosis via donor solid organs is extremely unusual, with only 2 cases previously reported [7, 9]. However, transmission of fungi by organ transplantation or through organ preservation fluids has been described for several fungi, including *Candida*, *Histoplasma*, *Aspergillus*, *Zygomycetes* and *Coccidioides* [17–20]. The first case of transmission of cryptococcosis, described by Qoi and colleagues in 1971, was reported in a 29-year-old female who received a kidney from a 20-year-old donor with elevated intracranial pressure and suspected brain tumor. On the fifth postoperative day, examination of the nontransplanted kidney showed the presence of 2 cryptococcal granulomas. The patient developed cryptococcuria on the 18th day, and positive cultures for *Cryptococcus* were obtained over subsequent weeks [9]. In 1996, Kanj and colleagues described a case in which the recipient of a bilateral lung transplant developed cryptococcal left-lower-lobe pneumonia 2 days after transplantation [7]. Endotracheal sputum cultures from postoperative day 2 were positive for *C. neoformans* and *Penicillium* species, although pre-implantation donor lung cultures were positive only for *Rhodotorula* species. Transmission was not proven but was suspected based on early development of cryptococcosis in the posttransplant period.

Donor evaluation to identify transmissible infections must be rapid and specific but is often limited by laboratory technology at specific donor institutions and by time constraints during which the potential donated organs can be used and by available. Donors with altered mental status or other neurologic conditions are particularly challenging because the cause of abnormal neurologic findings may be an infection that can then be transmitted unknowingly to the recipient. Previous reports have documented transmission of rabies virus, West Nile virus, and lymphocytic choriomeningitis virus from donors with altered neurologic presentations [21–23]. In addition, other cases not yet published of *Balamuthia mandrillaris* and *Coccidioides immitis* indicate transmission of donor-derived infections in the clinical setting of unexplained meningoencephalitis (D. C. Schain, personal communication). In donors whose presentations included low-grade fever, headache, and unexplained abnormal mental status, subsequent findings of head trauma–related diagnoses or cerebrovascular accident have been assumed to be the primary cause of the abnormal neurologic presentation. In retrospect, documented epidural hematoma, hemiplegia, cerebrovascular accident, and subarachnoid hemorrhage occurred most likely secondarily to underlying central nervous system infection [21–23]. This underscores the importance of considering infectious etiologies for donors with altered mental status, signs of infection, or for whom there is an incomplete medical history, even though the apparent cause may be trauma or other cerebrovascular disease. In addition, the importance of donor autopsy should be emphasized, particularly in patients with possible meningoencephalitis. Testing for meningoencephalitis should include stains to detect fungal organisms. Where possible, autopsy testing should be expedited, and results should be communicated to the organ procurement organization and transplant centers.

At present, uniform guidelines for donor screening for fungal infections are not available [24]. For endemic mycoses such as coccidioidomycosis and histoplasmosis, both of which have been transmitted by organ transplantation [17, 18], donor pretransplant screening with serologic testing or cultures is not recommended routinely; however, carefully taken donor history including locations of residence may help in determining risk of these geographically restricted fungi. Because *C. neoformans* is ubiquitous, location of residence does not aid detection, and it is unclear whether a history of soil or pigeon-feces exposure would be of benefit. Although detection of cryptococcosis using latex antigen has proven to be a valuable diagnostic tool, especially among HIV-infected patients [25, 26], the use of this test as a screening tool in the donor population has not been studied and is not recommended routinely. Based on this report of probable transmission, evaluation for cryptococcosis in donors with unexplained neurologic symptoms should be strongly considered.

Acknowledgments

The findings and conclusions of this article are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

Potential conflicts of interest. J.W.B. is an advisory board member at Merck and Co., a consultant for Pfizer, and a Pfizer research grant recipient. All other authors: no conflicts.

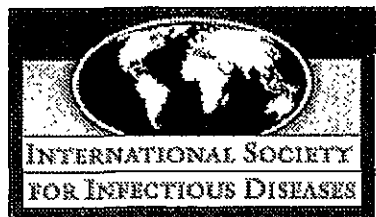
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医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日 2011. 1. 14	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	人血清アルブミン	研究報告の公表状況	ProMED 20110110.0119, 10-JAN-2011	公表国 英国・台湾・イタリア	
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン5%静注12.5g/250mL(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)				
研究報告の概要	○プリオン疾患最新情報2011年 ・英国:国立CJDサーベイランスユニット 月次vCJD・CJD統計、2011年1月10日現在 英国のCJDサーベイランスユニットから公表されたvCJDを始めとするプリオン病の患者数に関する最新情報である。 2010年1年間で146件の疑い例の照会があり、確定例または疑い例の死亡者数は、弧発性CJD:69名、vCJD:3名、医原性CJD:2名、家族性CJD:5名、GSS:1名であった。 vCJD確定例または疑い例の死亡者総数は2010年までで170名である。 死亡患者数のピークは2000年の28名であり、以降、2001年:20名、2002年:17名、2003年:18名、2004年:9名、2005年:5名、2006年:5名、2007年:5名、2008年:1名、2009年:3名、2010年:3名となっている。全体的な英国のvCJD発生は低下しているとの見解と一致している。 ・台湾:vCJD症例の疑い 2010年5月、36歳男性がvCJD疑いで死亡した。この患者は1989年から1997年まで英国に居住していた。 ・イタリア:2例目のvCJD症例 2011年1月、44歳女性がvCJDにより死亡した。イタリアでは2002年に次ぐ2番目の死亡例である。				使用上の注意記載状況・ その他参考事項等 赤十字アルブミン20 赤十字アルブミン25 赤十字アルブミン5%静注 12.5g/250mL 赤十字アルブミン20%静注 4g/20mL 赤十字アルブミン20%静注 10g/50mL 赤十字アルブミン25%静注 12.5g/20mL 血液を原料とすることによる 感染症伝播等
報告企業の意見		今後の対応			
英国CJDサーベイランスユニットの統計によると、2011年1月10日現在、vCJD確定例または疑い例の死亡者総数は170名であり、2010年の死亡者数は3名で、全体的な死亡者数は低下する傾向が続いているとの報告である。 プリオン病の原因とされる異常プリオンがコーン分画工程で効果的に除去されるとの成績と併せて、これまでの疫学研究では如何なるプリオン病も、アルブミンを介して伝播するという証拠は無い。また本製剤の使用は一時的かつ限定的であることから伝播のリスクは非常に低いものと考ええる。		これまでアルブミンを介してvCJDが伝播したとの証拠はないが、輸血あるいは第Ⅷ因子製剤によりvCJDに感染する可能性が示唆されていることから、今後も引き続き情報の収集に努める。なお、日本赤十字社は、CJD、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)、CJDの既往歴(本人、血縁者)、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。			

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Archive Number 20110110.0119

Published Date 10-JAN-2011

Subject PRO/AH/EDR> Prion disease update 2011 (01)

PRION DISEASE UPDATE 2011 (01)

A ProMED-mail post

<<http://www.promedmail.org>>

ProMED-mail is a program of the
International Society for Infectious Diseases

<<http://www.isid.org>>

[With the continuing decline of the number of cases in the human population of variant Creutzfeldt-Jakob disease -- abbreviated previously as vCJD or CJD (new var.) in ProMED-mail -- it has been decided to broaden the scope of the occasional ProMED-mail updates to include other prion-related diseases. In addition to vCJD, data on other forms of CJD: sporadic, iatrogenic, familial, and GSS (Gerstmann-Straussler-Scheinker disease) are included also since they may have some relevance to the incidence and etiology of vCJD. - Mod.CP]

In this update:

[1] UK: National CJD Surveillance Unit - monthly statistics as of Mon 10 Jan 2011 - no new vCJD cases

[2] France: Institut de Veille Sanitaire - monthly statistics as of Wed 4 Jan 2011 - no new vCJD cases

[3] USA: National Prion Disease Pathology Surveillance Center - cumulative case numbers for 2010 up to 1 Nov 2010 - no vCJD cases

[4] Taiwan: suspected vCJD case, ex UK, DOH statement

[5] Taiwan: suspected vCJD case, 'ex UK'

[6] Italy (Livorno): 2nd vCJD case

[1]

UK: National CJD Surveillance Unit - monthly statistics as of Mon 10 Jan 2011 - no new vCJD cases

Date: Mon 10 Jan 2011

Source: UK National CJD Surveillance Unit, monthly statistics [edited]

<<http://www.cjd.ed.ac.uk/figures.htm>>

The number of deaths due to definite or probable vCJD during 2010 was 170. A total of 4 definite/probable patients are still alive so the total number of definite or probable vCJD recorded during 2010 was 174.

Although 3 new deaths due to vCJD were recorded in 2009 and 3 deaths in 2010, the overall picture is still consistent with the view that the vCJD outbreak in the UK is in decline, albeit now with a pronounced tail. The 1st cases were observed in 1995, and the peak number of deaths was 28 in the year 2000, followed by 20 in 2001, 17 in 2002, 18 in 2003, 9 in 2004, 5 in 2005, 5 in 2006, 5 in 2007, one in 2008, 3 in 2009, and 3 in 2010.

Totals for all types of CJD cases in the UK in the year 2010

During 2010, there were 146 referrals, 69 fatal cases of sporadic CJD, 3 fatal cases of vCJD, 2 cases of iatrogenic CJD, 5 cases of familial CJD, and one case of GSS.

Since records began in 1990 there have been 2729 referrals, 1199 fatal cases of sporadic CJD, 170 cases of vCJD, 64 cases of

iatrogenic CJD, 82 cases of familial CJD and 43 of GSS.

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[2] France: Institut de Veille Sanitaire - monthly statistics as of 4 Jan 2011 - no new vCJD cases
Date: Tue 4 Jan 2011
Source: IVS - Maladie de Creutzfeldt-Jakob et maladies apparentees [in French, trans. & summ. Mod.CP, edited]
<http://www.invs.sante.fr/display/?doc=publications/mcj/donnees_mcj.html>

During the 12 months of 2010, there were 1610 referrals, 98 confirmed cases of sporadic CJD, 6 cases of familial CJD, and no cases of iatrogenic CJD or vCJD.

A total of 25 cases of confirmed or probable vCJD have been recorded in France since records began in 1992. There was 1 case in 1996, 1 in 2000, 1 in 2001, 3 in 2002, 2 in 2004, 6 in 2005, 6 in 2006, 3 in 2007, 2 in 2009, and none in 2010.

The 25 confirmed cases comprise 13 females and 12 males. All 25 are now deceased. Their median age is 37 (between 19 and 58). 7 were resident in the Ile-de-France and 18 in the provinces. All the identified cases have been Met-Met homozygotes. No risk factor has been identified. One of the 25 had made frequent visits to the United Kingdom, during about 10 years from 1987.

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[3] USA: National Prion Disease Pathology Surveillance Center - cumulative case numbers for 2010 up to 1 Nov 2010 - no vCJD cases
Date: Mon 1 Nov 2010
Source: US National Prion Disease Pathology Surveillance Center [edited]
<<http://www.cjdsurveillance.com/pdf/case-table.pdf>>

Cumulative data 1 Jan 2010 to 31 October 2010

During the 10-month period 1 Jan 2010 to 31 Oct 2010, there were 333 referrals, 213 of whom were classified as prion disease cases, comprising 158 cases of sporadic CJD, 33 of familial CJD, and none of iatrogenic CJD or vCJD.

Overall of 3831 referrals examined since screening began in 1996 or thereabouts, diagnosis is still pending in 18 cases, and another 18 cases have been considered inconclusive. [This is a measure of the difficulty in achieving unequivocal diagnoses. - Mod.CP]

During the same period a total of 2265 prion disease cases have been screened, a figure which includes 23 (22 during 2010) cases with type determination pending, but in which a diagnosis of vCJD has been excluded. 3 cases of vCJD recorded in the USA during the same period have been attributed to infection in the United Kingdom in 2 cases, and in Saudi Arabia in the other.

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[4] Taiwan: suspected vCJD case, ex UK, DOH statement
Date: Thu 9 Dec 2010
Source: Taiwan Today [edited]
<<http://www.taiwantoday.tw/ct.asp?xItem=135270&ctNode=445>>

A 36-year-old Taiwanese male is very likely to have died from Creutzfeldt-Jakob disease, the Cabinet-level Department of Health (DOH) said on [8 Dec 2010].

The patient, who died this May [2010], resided in the United Kingdom between 1989 and 1997, when bovine spongiform encephalopathy (BSE) in cattle was at its peak in the UK. It is unlikely that the patient contracted his disease in Taiwan, the DOH said.

Scientists believe that many years after eating cattle infected with BSE, patients can develop symptoms of CJD. In the case of the 36-year-old patient, he began to show CJD symptoms, including memory loss and hypersomnia, in the 2nd half of 2008. Doctors reported to health authorities in March 2009 that they suspected he had CJD.

After the patient died this year [2010], his family refused to give doctors permission to perform [an autopsy], the only way the doctors could determine the cause of death with certainty. His body has since been cremated. "Though tests were not conducted, the man is an extremely likely case of CJD, based on his symptoms, travel history, and the results of magnetic resonance imaging and electroencephalograms," the Centers for Disease Control under the DOH said.

The DOH further specified that the patient probably died from variant CJD [vCJD]. "The World Health Organization has listed the man as a vCJD case as his brain waves and cerebrospinal fluid showed symptoms of CJD and vCJD simultaneously," said Chen Shun-seng, convener of DOH's CJD advisory committee and a neurologist at Kaohsiung Chang Gung Memorial Hospital.

According to the World Health Organization, CJD is a fatal human degenerative condition characterized by progressive brain dysfunction and is categorized into 4 forms: sporadic, familial, iatrogenic, and variant. "It is believed to be caused by self-replicating host-encoded protein or prion protein," the WHO said.

According to the UK Department of Health, vCJD differs from CJD in that the former is more likely to strike younger people. The average age of death for vCJD victims is around 30 years old, it said.

As of October 2010, there have been 243 possible or extremely possible cases of CJD reported in Taiwan, resulting in 102 deaths. Of the deaths, 5 have been confirmed as resulting from CJD, the DOH said.

[byline: Grace Kuo]

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[This press report provides background to the case described in the preceding update (PromED-mail Prion disease update 2010 (11) 20101206.4364, part [4], which was an abstract reproduced from the journal Psychiatry and Clinical Neurosciences
<<http://onlinelibrary.wiley.com/doi/10.1111/j.1440-1819.2010.02151.x/abstract>>. The press report above states that the World Health Organization has listed the man as a vCJD case, but I have been unable to verify this statement. - Mod.CP]

[The HealthMap/ProMED-mail interactive map of Taiwan is available at
<<http://healthmap.org/r/00tD>>. - Sr.Tech.Ed.MJ]

[5] Taiwan: suspected vCJD case, ex UK

Date: Sat 18 Dec 2010

Source: Focus Taiwan, Central News Agency (CNA) report [edited]

<http://focustaiwan.tw/ShowNews/WebNews_Detail.aspx?ID=201012180020&Type=aLIV>

The 1st probable human case of mad cow disease in Taiwan was listed posthumously Saturday [18 Dec 2010], following the death in May [2010] of a man who had symptoms of the fatal brain-wasting illness.

[The clinical investigation of this case was reported in the preceding update: Prion disease update 2010 (11) 20101206.4364, part [4] -- Yang CW, Fuh JL, Wang SJ, et al: Probable variant Creutzfeldt-Jakob disease in Asia: A case report from Taiwan and review of two prior cases. Psychiatry Clin Neurosci, 64(6): 652-8. doi: 10.1111/j.1440-1819.2010.02151.x. published online 25 Nov 2010; abstract available at <http://onlinelibrary.wiley.com/doi/10.1111/j.1440-1819.2010.02151.x/abstract>]

The listing was made after officials and experts on a Department of Health (DOH) panel on Creutzfeldt-Jakob Disease (CJD) held a meeting to determine whether the man had died from the disease as result of eating infected beef.

"We believe it is very likely that the man who died in May this year [2010] contracted the disease during his stay in the United Kingdom," the DOH said in a press statement. "We cannot rule out the possibility that he ate infected beef during that time."

Scientists believe that many years after eating cattle infected with bovine spongiform encephalopathy (BSE), commonly known as mad cow disease, humans can develop symptoms of new variant CJD (vCJD), also known as human mad cow disease.

The 36-year-old man had lived in the UK between 1989 and 1997, when mad cow disease was at its peak in that country. He began to show vCJD symptoms, including memory loss and hypersomnia, in 2008. Doctors reported the case to the DOH's Centers for Disease Control in March 2009.

As his family refused to give doctors permission to perform an autopsy, the DOH said, it could not list the patient as a confirmed case of vCJD. His body has since been cremated.

Though the cause of death could not be determined by means of an autopsy, it was an extremely likely case of vCJD, based on the man's symptoms, travel history, and the results of magnetic resonance imaging and electroencephalograms, the DOH said.

According to the DOH's CJD advisory panel, it was unlikely that the patient contracted the disease in Taiwan.

"His death could be counted as an imported case of vCJD and unlikely to pose any threat to Taiwan's disease control network," the DOH statement said.

Citing its tracking records, the DOH said the man had donated blood to 2 patients 10 years ago. "Neither of them has contracted the disease," the statement said, adding that precautionary measures had been taken to prevent any spread of the disease during the time the man was hospitalized in Taiwan.

According to the World Health Organization, CJD is a fatal human degenerative condition characterized by progressive brain dysfunction and is categorized into four forms -- sporadic, familial, iatrogenic, and variant.

The UK Department of Health has said vCJD differs from CJD in that the former is more likely to strike younger people.

Since 1997 when the DOH set up a CJD reporting and monitoring system, 436 suspected CJD or vCJD have been reported in Taiwan, DOH figures show. Of that number, 246 were listed as possible or extremely possible cases of sporadic or familial forms of CJD, 5 were confirmed as traditional forms of CJD and one was listed as probable vCJD, according to the DOH tallies.

The others were ruled out as possible cases of CJD.

[byline: Chen ching-fang, Sofia Wu]

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[In view of the fact that the patient's body has been cremated, the investigation cannot proceed further and the case must remain suspected rather than confirmed vCJD. If the patient's disease was vCJD, it is probable that it was contracted during the victim's 8 years residence in the UK. - Mod.CP]

[6] Italy (Livorno): 2nd vCJD case

Date: Thu 6 Jan 2011

Source: Adnkronos International (AKI) [edited]

<<http://www.adnkronos.com/IGN/Aki/English/Security/Italy-Mad-cow-disease-claims>

The human variant of the brain-wasting 'mad cow' disease claimed a 2nd victim in Italy after a 44-year-old woman died in hospital in the northwestern port city of Livorno, the local health authority reported on Thursday [6 Jan 2011]. The health authority said the 44-year-old Livorno woman died on Wednesday 5 Jan 2011 and [had been] admitted to a city hospice in July [2010] in the final stages of her battle against variant Creutzfeldt-Jakob disease (vCJD), commonly known as 'mad cow' disease. There is no known cure for vCJD.

Italy's 1st case, a Sicilian woman, died in 2002, a year after she contracted the disease.

Variant Creutzfeldt-Jakob disease is the human form of the fatal brain-wasting illness first identified in cattle -- BSE or bovine spongiform encephalopathy. A total of 275 people have been infected with the disease over the past decade, 170 in Britain, the worst-affected country, 56 in Israel, and 25 in France. [As of 31 Dec 2010 the current totals of vCJD cases according to the European and Allied Countries CJD Study Group (<<http://www.cjd.ed.ac.uk/vcjdworld.htm>>) are UK -171, France - 25, Eire - 4, Italy - 4, USA - 3*, Canada - 1, Saudi Arabia - 1*, Japan - 1*, Netherlands - 3, Portugal -2, Spain - 5, where * indicates non-indigenous. - Mod.CP]

Nearly 200 000 cattle have died from BSE over the same period. The human form of BSE is believed to be caused by eating meat infected with the bovine disease. EU restrictions on the sale of T-bone beef steaks and beef offal were lifted in 2006 when officials declared the 'mad cow' outbreak was over.

A massive cull in Britain prevented the spread of the disease but isolated cases are still reported. Almost 190 000 cattle have died from BSE over the past 10 years, the vast majority in the UK.

Italy's health ministry and various local health authorities have always denied vCJD patients can infect their family members and carers with the disease.

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[The HealthMap/ProMED-mail interactive map of Italy is available at <<http://healthmap.org/r/01V5>>. - Sr.Tech.Ed.MJ]

[see also:

2010

Prion disease update 2010	(11)	20101206.4364
Prion disease update 2010	(10)	20101105.4008
Prion disease update 2010	(09)	20101006.3622
Prion disease update 2010	(08)	20100911.3285
Prion disease update 2010	(07)	20100809.2720
Prion disease update 2010	(06)	20100706.2248
Prion disease update 2010	(05)	20100507.1488
Prion disease update 2010	(04)	20100405.1091
Prion disease update 2010	(03)	20100304.0709
Prion disease update 2010	(02)	20100205.0386
Prion disease update 2010		20100107.0076]

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医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
一般的名称	—	研究報告の 公表状況	Nat Med. 2011 Feb;17(2):175-8. Epub 2011 Jan 30.	公表国	
販売名(企業名)	—			日本	
研究報告の概要	伝達性海綿状脳症又はプリオン病は、正常な細胞性プリオン (PrP^C) が誤った構造の異常プリオン (PrP^{Sc}) へと自己触媒的に複製されることにより、中枢神経に PrP^{Sc} が集積する特徴がある。いくつかの例外を除き、PrP^{Sc} はプリオン病において最も特徴的で信頼性の高いマーカーである。生前の CJD の確定診断には、生検による PrP^{Sc} の検出が必要となるが、生検は侵襲的であり、また、医療関係者にとってもリスクを有するため奨励できない。 異常プリオン (PrP^{Sc}) の増幅技術の発展は、高感度の検出法につながる可能性がある。近年、amyloid seeding アッセイ法や QUIC 法のような PrP^{Sc} 増幅技術が開発され、脳脊髄液 (CSF) を含む様々な組織から直接的かつ高感度に PrP^{Sc} を検出することが可能となった。 QUIC 法は超音波処理の必要がないため amyloid seeding アッセイ法より簡便である。QUIC 法によりハムスターやヒツジのプリオン病モデルで正常及びスクレイピーに感染した CSF を正確に判別することが可能であるが、CJD 感染検体の CSF から高感度に PrP^{Sc} を検出することはできなかった。そのため、著者らは、QUIC 法を改良し、新たな PrP^{Sc} 増幅アッセイ法、(real-time quaking-induced conversion: RT-QUIC) を開発し、クロイツフェルト・ヤコブ病 (CJD) の脳ホモジネート内の 1fg (10⁻¹⁵) 以上の PrP^{Sc} を 48 時間以内に検出することを可能とした。さらに、日本の 18 検体の試験及びオーストラリアの 30 検体の CSF を用いた盲検試験を行った結果、感度は 80% 以上、特異性は 100% であった。これらの結果から CSF を用いた RT-QUIC 法による超高感度の PrP^{Sc} 検出は、CJD を疑われる症例の生前の評価において、早期の迅速かつ特異的な CJD の新たな検出法になり得ることが示唆された。				使用上の注意記載状況・ その他参考事項等 重要な基本的注意 現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病 (vCJD) 等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的な vCJD 等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。
報告企業の意見	異常プリオンの検出法に関する報告である。現時点では評価検討中とのことであり、方法が確立した場合にはスクリーニングに応用できるかどうかを検討する。				今後の対応 今後とも vCJD に関する安全性情報等に留意していく。

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Ultrasensitive human prion detection in cerebrospinal fluid by real-time quaking-induced conversion

Ryuichiro Atarashi^{1,2}, Katsuya Satoh¹, Kazunori Sano^{1,3}, Takayuki Fuse¹, Naohiro Yamaguchi¹, Daisuke Ishibashi¹, Takehiro Matsubara¹, Takehiro Nakagaki¹, Hitoki Yamanaka⁴, Susumu Shirabe⁵, Masahito Yamada⁶, Hidehiro Mizusawa⁷, Tetsuyuki Kitamoto⁸, Genevieve Klug⁹, Amelia McGlade⁹, Steven J Collins⁹ & Noriyuki Nishida^{1,3}

The development of technologies for the *in vitro* amplification of abnormal conformations of prion protein (PrP^{Sc}) has generated the potential for sensitive detection of prions. Here we developed a new PrP^{Sc} amplification assay, called real-time quaking-induced conversion (RT-QUIC), which allows the detection of ≥ 1 fg of PrP^{Sc} in diluted Creutzfeldt-Jakob disease (CJD) brain homogenate. Moreover, we assessed the technique first in a series of Japanese subjects and then in a blind study of 30 cerebrospinal fluid specimens from Australia, which achieved greater than 80% sensitivity and 100% specificity. These findings indicate the promising enhanced diagnostic capacity of RT-QUIC in the antemortem evaluation of suspected CJD.

Transmissible spongiform encephalopathies, or prion diseases, are characteristically associated with the accumulation of PrP^{Sc} in the central nervous system through autocatalytic conversion of normal cellular PrP (PrP^C) into replicate misfolded isomers^{1,2}. Despite a few other reported markers^{3,4}, PrP^{Sc} remains the best characterized and most reliable marker of prion disease.

Definitive antemortem confirmation of CJD requires the detection of PrP^{Sc} in biopsy specimens, the practice of which is discouraged because it is invasive and poses risks to health care personnel. Recently, however, *in vitro* PrP^{Sc} amplification techniques, including protein misfolding cyclic amplification⁵⁻⁷, the amyloid seeding assay⁸ and QUIC, have been reported to enable the direct and highly sensitive detection of PrP^{Sc} in various tissues, including cerebrospinal fluid (CSF). QUIC assays involve the use of soluble recombinant PrP

(rPrP-sen) as a substrate, which is seeded with PrP^{Sc} and then subjected to intermittent automated shaking. This technique can be performed more easily than the protein misfolding cyclic amplification, which requires repeated sonication. Previous studies have shown that QUIC assays correctly discriminate between normal and scrapie-infected CSF samples in both hamster and sheep prion disease models^{9,10}. However, ultrasensitive PrP^{Sc} detection in CSF from subjects with CJD has not yet been accomplished. Accordingly, we further refined the QUIC assay to improve its sensitivity and practicability and then applied the technique in a blind pilot study to detect PrP^{Sc} in CJD CSF specimens.

Given that a correlation between protease-resistant rPrP aggregate (rPrP-res) abundance and thioflavin T (ThT) fluorescence had been shown previously⁷, we sought to determine the relative kinetics of rPrP-res formation by monitoring levels of ThT fluorescence in the QUIC assay. This was intended to minimize the time needed to detect rPrP-res. We first tested whether PrP^{Sc}-dependent rPrP-res (rPrP-res^{Sc}) formation could be induced in a microplate reader with intermittent shaking. We used human rPrP-sen (rHuPrP-sen) and a 10⁻⁷ dilution of CJD (molecular subtype MM1) brain homogenate as the substrate and seed, respectively. We conducted QUIC reactions at various concentrations (0, 0.25, 0.5 and 1.0 M) of guanidine-HCl (GdnHCl), because it has been shown that GdnHCl greatly enhances conversion of PrP-sen to PrP-res in cell-free conversion reactions¹¹. Unexpectedly, we observed positive PrP^{Sc}-dependent ThT fluorescence within 24 h, both in the presence and in the absence of GdnHCl (Fig. 1a). In contrast, the negative control reactions without seed and in the absence of GdnHCl resulted in no increase in ThT fluorescence over 24 h; however, *de novo* formation of rPrP-res (rPrP-res^{spont}) was rapidly induced in the presence of GdnHCl when shaking was added (Fig. 1a,b). These results indicate that shaking accelerates PrP^{Sc}-dependent rPrP-res^{Sc} formation even without GdnHCl (Supplementary Fig. 1), albeit with a lower peak of fluorescence.

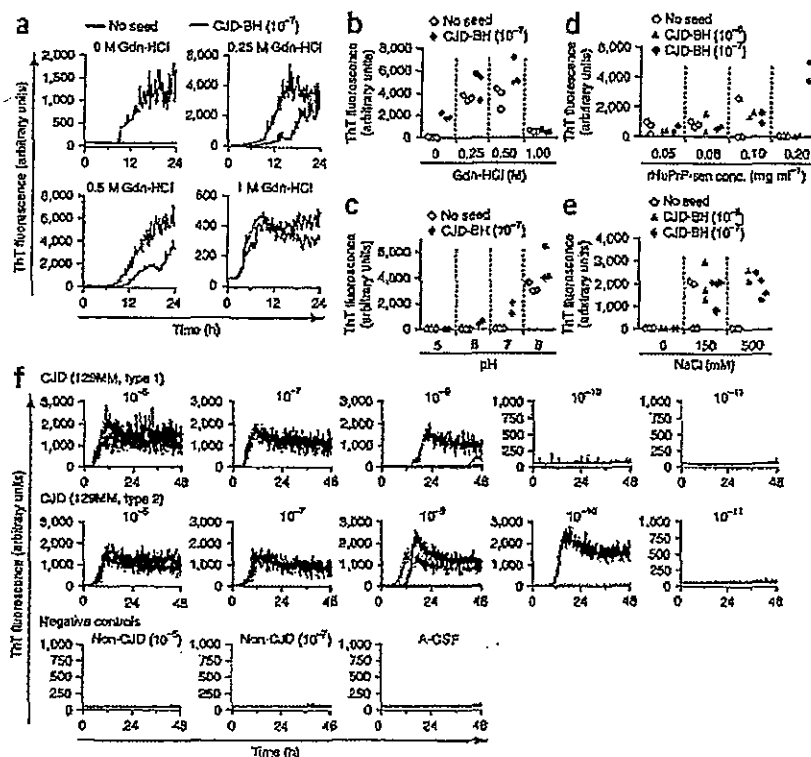
Shaking is thought to cause partial unfolding of a portion of rPrP-sen by increasing the air-water interface¹². Moreover, shaking enhances the interaction between rPrP-sen and PrP^{Sc} and promotes the fragmentation of rPrP-res polymers¹³. It is generally accepted that the energetic barrier of seed-dependent fibril formation and elongation is lower than that of spontaneous fibril formation, which first requires nucleation as the rate-limiting step¹⁴. The partial unfolding of rPrP-sen by shaking seems to be more heterogeneous than that facilitated by a denaturant such as GdnHCl, perhaps because the air-water interfaces created by shaking are unequally distributed

¹Department of Molecular Microbiology and Immunology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan. ²Nagasaki University Research Centre for Genomic Instability and Carcinogenesis, Nagasaki, Japan. ³Global Centers of Excellence Program, Nagasaki University, Nagasaki, Japan. ⁴Division of Comparative Medicine, Center for Frontier Life Sciences, Nagasaki University, Nagasaki, Japan. ⁵Organization of Rural Medicine and Residency Education, Nagasaki University Hospital, Nagasaki, Japan. ⁶Department of Neurology and Neurobiology of Aging, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan. ⁷Department of Neurology and Neurological Science, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan. ⁸Division of Creutzfeldt-Jakob Disease Science and Technology, Department of Prion Research, Tohoku University Graduate School of Medicine, Sendai, Japan. ⁹Department of Pathology, Australian National Creutzfeldt-Jakob Disease Registry, University of Melbourne, Melbourne, Australia. Correspondence should be addressed to R.A. (atarashi@nagasaki-u.ac.jp).

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Figure 1 QUIC reactions induce PrP^{Sc}-dependent rHuPrP-res formation under GdnHCl-free conditions. (a,b) The effect of the indicated concentration of Gdn-HCl on the kinetics of rHuPrP fibril formation with or without 10⁻⁷ dilution of CJD brain homogenate (CJD-BH) (type 1, 129MM). The reaction buffer contained 150 mM NaCl, 50 mM PIPES, pH 7.0, 1 mM EDTA and 10 μM ThT. The concentration of rHuPrP-sen was 0.1 mg ml⁻¹. The graphs in a depict one representative of triplicates. The maximal fluorescence intensity of each single reaction for 24 h is plotted in b. (c–e) The effect of pH (c), the concentration of rHuPrP-sen (d) and the concentration of NaCl (e) were tested with the indicated dilutions of CJD-BH (type 1, 129MM) as seeds. Buffers used in c were pH 5 50 mM sodium acetate buffer, pH 6 50 mM MES, pH 7 50 mM PIPES and pH 8 50 mM HEPES. The concentration of NaCl in c and d was 150 mM, the pH of the buffer in d and e was 7.0 (50 mM PIPES) and the rHuPrP-sen concentration in c and e was 0.1 mg ml⁻¹. Each symbol represents the maximal fluorescence intensity from an individual reaction for 48 h. (f) Detection limit of RT-QUIC with the indicated dilutions of CJD-BH (129MM, type 1) and CJD-BH (129MM, type 2) as seeds. We used the indicated dilutions of non-CJD-BH (dissecting aneurysm) or artificial CSF (A-CSF) as negative controls. We performed the RT-QUIC reactions as described in the Supplementary Methods. The colored curves represent the kinetics of ThT fluorescence from an individual reaction seeded with the same dilution of BH. The graphs are representative of two independent experiments, each performed in triplicate.



In solution. We found that the addition of GdnHCl to QUIC reactions leads to an increase in the nucleation rate, and increased spontaneous fibril formation. The early appearance of rPrP-res^{spont} decreases the specificity of QUIC, because ThT fluorescence cannot distinguish between rPrP-res^{Sc} and rPrP-res^{spont}. Therefore, we chose not to use GdnHCl in subsequent analyses.

To further optimize the conditions, we examined the effects of pH, as well as of the concentrations of rHuPrP-sen and salt on QUIC reactions in GdnHCl-free conditions with shaking (Fig. 1c–e and Supplementary Fig. 2). After assessment, we successfully established a method for the real-time monitoring of the kinetics of rPrP fibril formation seeded with CJD brain homogenate (see Supplementary Methods), without the generation of rPrP-res^{spont} and designated the assay RT-QUIC.

To determine the minimum amount of PrP^{Sc} detectable by RT-QUIC, we diluted CJD brain homogenate (MM1 and MM2) serially with artificial CSF (A-CSF) and used these dilutions to seed the reactions. We observed increased PrP^{Sc}-dependent ThT fluorescence within 48 h in more than half the replicates of CJD brain homogenate, with dilutions ranging from 10⁻⁵ to 10⁻⁹ (Fig. 1f and Supplementary Table 1). With 10⁻¹⁰ brain homogenate dilutions, we observed a marginally lower rate of positive reactions, and 10⁻¹¹ dilutions of the CJD-brain homogenates produced no ThT fluorescence response (Fig. 1f and Supplementary Table 1). The negative controls seeded with 10⁻⁵ and 10⁻⁷ dilutions of non-CJD brain homogenate or A-CSF alone (no seed) did not produce an increase in the fluorescence (Fig. 1f and Supplementary Table 1). The 10⁻⁹ dilutions of MM1 and MM2 CJD brain homogenate contained approximately 0.8 and 1.9 fg of

PrP^{Sc}, respectively, according to our estimation (data not shown). Consequently, the results indicate that this assay consistently enables us to detect more than or equal to about 1 fg of PrP^{Sc} in the diluted CJD brain homogenates within 48 h. Moreover, the fact that there was no rPrP-res^{spont} formation under the conditions used implies a lower and acceptable risk of false-positive reactions. Whether the RT-QUIC has the same sensitivity to CJD brain homogenate with 129MV or 129VV as 129MM remains to be determined.

CSF is routinely used in the evaluation of central nervous system disorders and presumably contains more PrP^{Sc} and fewer impurities than blood. This prompted us to compare the RT-QUIC seeding activity in CSF samples from subjects with CJD and subjects without CJD but with other neurodegenerative diseases. For the pilot study, we initially tested CJD CSF samples from 18 definite cases of CJD in Japan (Table 1) and 35 non-CJD controls from subjects with other neurodegenerative diseases (Supplementary Table 2). We saw a minimal ThT fluorescence increase in the controls, with no false positives in the assay. In contrast, increased PrP^{Sc}-dependent fluorescence was seen in at least one of four replicates in 15/18 (83.3%) of the CJD CSF samples (Table 1 and Supplementary Table 3).

To further confirm the reliability of RT-QUIC, we conducted a blind trial using 30 CSF samples from the Australian National CJD Registry and 155 CSF samples containing 25 probable cases of CJD and 130 cases of other neurological diseases obtained in Japan. In the Australian samples, we were able to detect PrP^{Sc} in 14/16 (87.5%) definite CJD CSF samples, as opposed to 0/14 of the non-CJD controls (Table 1, Supplementary Fig. 3 and Supplementary Table 3). It should be noted that 3/4 129VV and 2/2 129MV cases were positive by RT-QUIC.

Table 1 Clinical data and RT-QUIC reactions seeded with CSF samples

CSF from 18 definite CJD samples in Japan						
Sample	Age (years)/sex	CJD type	Molecular subtype ^a	14-3-3 (γ -isoform) ^b	RT-QUIC ^c	
C1	68/M	sCJD	MM 1	+	+(3/4)	
C2	66/F	sCJD	MM 1	+	+(2/4)	
C3	71/F	sCJD	MM 1	+	+(1/4)	
C4	67/F	sCJD	MM 2	+	+(2/4)	
C5	70/M	sCJD	MM 2	+	+(2/4)	
C6	66/M	sCJD	MM 2	+	+(2/4)	
C7	60/F	sCJD	MM 2	-	-(0/4)	
C8	73/F	sCJD	MM 2	+	+(4/4)	
C9	74/M	sCJD	MM 2	+	-(0/4)	
C10	79/F	sCJD	MM 2	-	+(3/4)	
C11	65/F	sCJD	MM 2	+	+(4/4)	
C12	69/M	sCJD	MM 2	+	-(0/4)	
C13	69/F	sCJD	MM 2	+	+(3/4)	
C14	54/F	sCJD	MM 2	+	+(3/4)	
C15	76/F	sCJD	MM 2	-	+(2/4)	
C16	68/M	sCJD	MM 2	-	+(4/4)	
C17	58/F	iCJD	MM 1 ^d	+	+(3/4)	
C18	79/F	iCJD	MM 1	-	+(3/4)	
Blind trial of 30 CSF samples in Australia						
Sample	Age (years)/sex	Diagnosis ^d	Codon 129	Profile of PrP ^{Sc} ^e	14-3-3 (all isoforms) ^f	RT-QUIC ^g
A1	53/F	PN/MC			-	-(0/4)
A2	59/F	PN/ MC			-	-(0/4)
A3	85/M	AD			-	-(0/4)
A4	60/F	ICD			+	-(0/4)
A5	83/M	AD			+	-(0/4)
A6	73/M	sCJD	VV	2	+	+(2/4)
A7	67/F	sCJD	MM	1	+	+(4/4)
A8	82/F	sCJD	MM	1	+	+(2/4)
A9	67/M	sCJD	MV	1	-	+(2/4)
A10	50/M	sCJD	MM	1	+	+(3/4)
A11	66/M	PN/MC			-	-(0/4)
A12	61/M	PN/MC			-	-(0/4)
A13	84/F	sCJD	MM	1	+	+(3/4)
A14	76/M	sCJD	MM	1	+	+(2/4)
A15	69/M	sCJD	MV	1	+	+(4/4)
A16	67/M	AD			-	-(0/4)
A17	75/F	PN/MC			+	-(0/4)
A18	93/M	DLB/PD			-	-(0/4)
A19	67/F	sCJD	ND	2	+	+(2/4)
A20	53/M	DLB/PD			-	-(0/4)
A21	71/F	sCJD	VV	2	+	-(0/4)
A22	62/F	sCJD	MM	2	-	-(0/4)
A23	90/M	sCJD	VV	ND	+	+(1/4)
A24	61/F	DLB/PD			-	-(0/4)
A25	74/M	sCJD	MM	ND	$\pm^h$	+(2/4)
A26	74/F	AD			-	-(0/4)
A27	68/F	sCJD	ND	ND	+	+(2/4)
A28	69/F	sCJD	VV	2	+	+(1/4)
A29	82/M	DLB/PD			+	-(0/4)
A30	70/F	sCJD	ND	2	+	+(4/4)

^aCJD can be divided into six molecular subtypes on the basis of whether methionine (M) or valine (V) is present at codon 129 of the gene encoding prion protein combined with the profile of PrP^{Sc} (type 1 or type 2) as determined by western blotting¹⁷. ^bAmounts of the γ -isoform of 14-3-3 protein in CSF were determined by western blotting with a polyclonal antibody specific for the γ -isoform of the 14-3-3 protein¹⁸. +, positive reaction; -, negative reaction. ^cRT-QUIC was performed as described in the Supplementary Methods. The number of positive reactions over the number of replicates is shown in parentheses. Samples with at least one positive reaction were defined as +, representing a positive result in the RT-QUIC. ^dThe final diagnosis was made by the Australian National CJD Registry. ^eAmounts of 14-3-3 protein (all isoforms) in CSF were determined by western blotting. ^fAdditional atypical bands were observed. ^gKinetics graphs are provided in Supplementary Figure 3. Subjects or their families agreed with the aims and role of our research and gave appropriate informed consent. The investigation protocol was approved by the Ethics Committee of Nagasaki University Hospital (ID: 10042823), and the study was registered with the University Hospital Medical Information Network (ID: UMIN000003301). sCJD, sporadic Creutzfeldt-Jakob disease; iCJD, Iatrogenic Creutzfeldt-Jakob disease; PN/MC, paraneoplastic/malignant cancer; ICD, inflammatory central nervous system disorder; DLB/PD, dementia with Lewy bodies/Parkinson's disease; AD, Alzheimer's disease; ND, not determined.

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These results indicate that RT-QUIC has the ability to discriminate CJD CSF samples that include 129MM, 129MV and 129VV cases from non-CJD CSF samples. In addition, none of the 130 Japanese cases of other neurological diseases were positive, further confirming the reliability of this assay (Supplementary Table 4). Collectively, the RT-QUIC assays showed more than 80% sensitivity and 100% specificity. The sensitivity was equivalent to and the specificity was much higher than that achieved by 14-3-3 (refs. 14,15), a nonspecific marker of rapid neuronal damage (Supplementary Table 3).

Although we have never experienced a false-positive reaction among the hundreds of non-CJD neurodegenerative disease samples we have so far tested, it remains possible that certain conditions may evoke a positive reaction, and further studies will be required to eliminate this possibility. Furthermore, scrupulous attention to the conditions of the assay is essential to avoid false positives in the clinical setting. Nevertheless, we believe that the ultrasensitive detection of PrP^{Sc} in CSF by RT-QUIC represents a valuable new means for the early, rapid and specific diagnosis of CJD.

Note: Supplementary information is available on the Nature Medicine website.

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AUTHOR CONTRIBUTIONS

R.A. designed the project, performed experiments and wrote the manuscript. K. Satoh, K. Sano, T.E., N.Y., D.J., T.M., T.N. and H.Y. performed experiments. K. Satoh, S.S., M.Y., H.M., T.K., G.K., A.M. and S.J.C. contributed to the collection of human specimens and provided information about subjects. N.N. supervised the project. K. Satoh, K. Sano, A.M., S.J.C. and N.N. helped with the editing of the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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医薬部外品 研究報告 調査報告書
化粧品

識別番号・報告回数		報告日		第一報入手日 2011 年 4 月 4 日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①②③ポリエチレングリコール処理抗破傷風人免疫グロブリン ④⑤乾燥抗破傷風人免疫グロブリン			研究報告の 公表状況	公表国 イギリス	
販売名 (企業名)	①テタノブリン IH 静注 250 単位 (ベネシス) ②テタノブリン IH 静注 1500 単位 (ベネシス) ③テタノブリン-IH (ベネシス) ④テタノブリン筋注用 250 単位 (ベネシス) ⑤テタノブリン (ベネシス)					
研究報告の概要	背景： ヒトプリオン病の医原性伝播は内科的、或いは外科的処置を介して起こりうるもので、そのような処置としては、死体の脳下垂体から抽出したゴナドトロピン (=性腺刺激ホルモン) などのホルモン注射剤が含まれる。米国とカナダでは、毎年 30 万人以上の女性が不妊治療のために尿由来のゴナドトロピンを処方されている。閉経期の尿ドナーは症候性神経疾患の有無でスクリーニングされているが、クロイツフェルト・ヤコブ病 (CJD) が潜伏していた場合、非侵襲性の検査で排除することは不可能である。変異型 CJD (vCJD) は何十年間も末梢で潜伏する可能性のある疾患で、あるドナーがその vCJD のキャリアー状態である危険性は、英国では人口 100 万人当たり 100 人のオーダーであると推定されている。 腎疾患を有する、または有しない実験動物の尿中の感染性プリオンの存在を示した幾つかの研究では、プリオンが無症候性の尿ドナーの体内に存在していることが示唆されている。幾つかのヒト排卵誘発剤はドネーションされた尿由来品であり、最近、プリオンたん白質がヒト閉経期ゴナドトロピン (hMG) 製剤中に検出された。					使用上の注意記載状況・ その他参考事項等
	方法/主な知見： 古典的なプロテオミクスのアプローチを使って、尿性ヒト絨毛性ゴナドトロピン (u-hCG) 中に 33 種類、そして高純度尿性ヒト閉経期ゴナドトロピン (hMG-HP) 製剤中に 34 種類のゴナドトロピン以外のたん白質がそれぞれで特定された。プリオンたん白質が初めて u-hCG 製剤中に主要な夾雑物として特定された。その後、プリオンたん白質を標的とした高度なプロテオミクスのアプローチを用いて、ゴナドトロピン製剤の調査を行った。このアプローチで、hCG、hMG、および hMG-HP を含有する尿由来の注射用排卵誘発剤中にヒトプリオンたん白質のペプチドを検出したが、遺伝子組換え製剤中には検出しなかった。					代表としてテタノブリン IH 静注 250 単位の記載を示す。 2. 重要な基本的注意 (1) 略 1) 略 2) 現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病 (vCJD) 等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的な vCJD 等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。
	結論/意義： hCG、hMG、および hMG-HP を含有する尿由来の注射用排卵誘発剤中にプロテアーゼ感受性のプリオンたん白質が存在することは、プリオンがこれらの製剤中に同時精製されていることを示唆している。ヒトプリオン疾患の伝播経路として筋肉内注射は比較的効率の高い経路で、プリオンに暴露された若い女性は最小限の接種 (プリオンの取込み) に伴う潜伏期間を生存し続けるものと考えうる。尿由来排卵誘発剤の危険性は、特に遺伝子組換え製剤が利用可能であることを考慮すると、今ではそれらの利点を上回ることが出来る。					

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報告企業の意見	今後の対応	
血漿分画製剤は理論的な vCJD 伝播リスクを完全には排除できないため、投与の際には患者への説明が必要である旨を 2003 年 5 月から添付文書に記載している。2009 年 2 月 17 日、英国健康保護庁 (HPA) は vCJD に感染した供血者の血漿が含まれる原料から製造された第Ⅷ因子製剤の投与経験のある血友病患者一名から、vCJD 異常プリオン蛋白が検出されたと発表した。弊社の原料血漿採取国である日本及び米国では、欧州滞在歴のある献 (供) 血希望者を一定の基準で除外し、また国内での BSE の発生数も少数であるため、原料血漿中に異常型プリオン蛋白が混入するリスクは 1999 年以前の英国に比べて極めて低いと考える。また、製造工程においてプリオンが低減される可能性を検討するための実験を継続して進めているところである。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。	

Detection of Prion Protein in Urine-Derived Injectable Fertility Products by a Targeted Proteomic Approach

Alain Van Dorsselaer^{1*}, Christine Carapito¹, François Delalande¹, Christine Schaeffer-Reiss¹, Daniele Thierse¹, Hélène Diemer¹, Douglas S. McNair², Daniel Krewski³, Neil R. Cashman^{4*}

1 Laboratoire de Spectrométrie de Masse Bio-Organique, Université de Strasbourg, IPHC, CNRS, UMR7178, Strasbourg, France, **2** Cerner Corporation, Kansas City, Missouri, United States of America, **3** McLaughlin Centre for Population Health Risk Assessment, University of Ottawa, Ontario, Canada, **4** Brain Research Centre, Department of Medicine (Neurology), University of British Columbia, Vancouver, British Columbia, Canada

Abstract

Background: Iatrogenic transmission of human prion disease can occur through medical or surgical procedures, including injection of hormones such as gonadotropins extracted from cadaver pituitaries. Annually, more than 300,000 women in the United States and Canada are prescribed urine-derived gonadotropins for infertility. Although menopausal urine donors are screened for symptomatic neurological disease, incubation of Creutzfeldt-Jakob disease (CJD) is impossible to exclude by non-invasive testing. Risk of carrier status of variant CJD (vCJD), a disease associated with decades-long peripheral incubation, is estimated to be on the order of 100 per million population in the United Kingdom. Studies showing infectious prions in the urine of experimental animals with and without renal disease suggest that prions could be present in asymptomatic urine donors. Several human fertility products are derived from donated urine; recently prion protein has been detected in preparations of human menopausal gonadotropin (hMG).

Methodology/Principal Findings: Using a classical proteomic approach, 33 and 34 non-gonadotropin proteins were identified in urinary human chorionic gonadotropin (u-hCG) and highly-purified urinary human menopausal gonadotropin (hMG-HP) products, respectively. Prion protein was identified as a major contaminant in u-hCG preparations for the first time. An advanced prion protein targeted proteomic approach was subsequently used to conduct a survey of gonadotropin products; this approach detected human prion protein peptides in urine-derived injectable fertility products containing hCG, hMG and hMG-HP, but not in recombinant products.

Conclusions/Significance: The presence of protease-sensitive prion protein in urinary-derived injectable fertility products containing hCG, hMG, and hMG-HP suggests that prions may co-purify in these products. Intramuscular injection is a relatively efficient route of transmission of human prion disease, and young women exposed to prions can be expected to survive an incubation period associated with a minimal inoculum. The risks of urine-derived fertility products could now outweigh their benefits, particularly considering the availability of recombinant products.

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Competing Interests: Dr. McNair is Vice-President of Cerner Corporation, which had no role in the initiation or conduct of this study. This does not alter the authors' adherence to all the PLoS ONE policies on sharing data and materials.

* E-mail: neil.cashman@vch.ca (NRC); vandors@unistra.fr (AVD)

Introduction

Transmissible spongiform encephalopathies (TSEs), or prion diseases, such as scrapie, bovine spongiform encephalopathy (BSE) and Creutzfeldt-Jakob disease (CJD), are sub-acute neurodegenerative disorders in which the infectious agent is an aggregated, misfolded host prion protein that propagates through 'template directed misfolding'. The disease-associated protein isoform, generically designated PrP^{TSE}, is derived from a normal, non-infectious, host-derived cellular isoform termed PrP^C. The most common human TSE, CJD, occurs in forms which are sporadic (sCJD), iatrogenic (iCJD) or genetic (e.g. familial CJD: fCJD). The emergence of a new variant CJD (vCJD) in the UK

and France, almost certainly the result of human consumption of BSE-contaminated bovine tissues, has affected 216 individuals in 11 countries worldwide as of March 2010 [1]. Although the primary zoonotic epidemic of vCJD is probably in decline, a survey of lymphoreticular tissues in the UK estimated that 237 people per million population (95% confidence interval: 49–692 per million) were 'silent carriers' of vCJD [2]. These results suggest that many countries may have asymptomatic citizens 'incubating' the disease for decades. Thus, it is possible that secondary outbreaks may occur through iatrogenic transmission via organ transplantation, contaminated surgical instruments, and human-derived therapeutic products; indeed, four cases of blood-borne transmission of the vCJD prion agent have already

been documented in the UK. Iatrogenic transmission of classical CJD has also been demonstrated through dural and corneal transplantation and neurosurgical instruments. Notably, in the 1990s 194 individuals were infected through intramuscular injections of pituitary growth hormone derived from human cadavers [3]. The latter episode not only demonstrated the high infectivity of parenterally injected CJD prions (recipient disease rates exceeded 10% in some French batches), but also raised the possibility that chromatography systems used to purify therapeutic hormones and peptides may enrich human prions. Four cases of iatrogenic transmission of CJD through fertility treatment involving human pituitary-derived gonadotropin have also been reported in Australia [4].

It is now clear that infectious prions are excreted into the urine in renal disease states. Studies from the Aguzzi laboratory in 2005 convincingly demonstrated the presence of fully infectious prion particles in the urine of scrapie mice with co-existing renal inflammation [5]. Although PrP^{TSE} levels were below the limits of detection by immunoblotting in this study, intracerebral inoculation of concentrated urine transmitted scrapie in host mice. More recent work has demonstrated that renal pathology is not required for urinary prion excretion in experimental animals. In 2006, the Gabizon group [6] first demonstrated prionuria in the absence of renal inflammation by inducing prion disease in naïve hamsters through inoculation of scrapie urine. In 2007, Gregori and colleagues [7] and Murayama and colleagues [8] demonstrated detectable levels of PrP^{TSE} in the urine of scrapie hamsters for the first time. Urinary PrP^{TSE} was only detectable at the symptomatic stage of the disease. However, these findings do not imply that urine is 'safe' if PrP^{TSE} is undetectable; Aguzzi and colleagues failed to detect PrP^{TSE} in urine subsequently proven to be infectious [5]. Furthermore, the studies of both Aguzzi [5] and Gabizon [6] found infectivity in the urine during the presymptomatic incubation period before

the appearance of clinical disease. Gonzalez-Romero and colleagues recently confirmed the presence of templating-competent PrP^{TSE} in the urine of experimentally infected animals using protein misfolding cyclic amplification [9]. Using a rigorous quantitative method, Gregori and colleagues [7] have recently reported that the titer of prions in urine of scrapie-infected hamsters was several units of infectivity per mL, similar to that observed in hamster blood.

Collectively, these studies suggest that the urine of prion-infected individuals may be infectious before the appearance of clinical signs – even before PrP^{TSE} is detectable in the urine. These and other studies [10,11] also indicate that, while renal disease may facilitate prionuria, such pathology is not a prerequisite for urinary prion transmission. Recently, Kuwabara and colleagues [12] have identified prion protein in urine-derived gonadotropins. Although this class of products is not administered to patients for extended periods, the number of injections and the amount of total protein administered could expose patients to a potential risk of prion transmission. Using advanced proteomics methods, we now report the detection of prion protein in commercially available urine-derived gonadotropins, but not in recombinant fertility products.

Results

Identification of non-gonadotropin proteins in human chorionic gonadotropin (hCG) preparations using electrophoresis and mass spectrometry (MS)

After 2D gel electrophoresis of hCG products (Figure 1), protein spots found to be present in the molecular weight range of approximately 14–70 kDa in u-hCG and recombinant-hCG (r-hCG) were analyzed by electrospray ionization MS (ESI-MS/MS), and identified using internationally available databases.

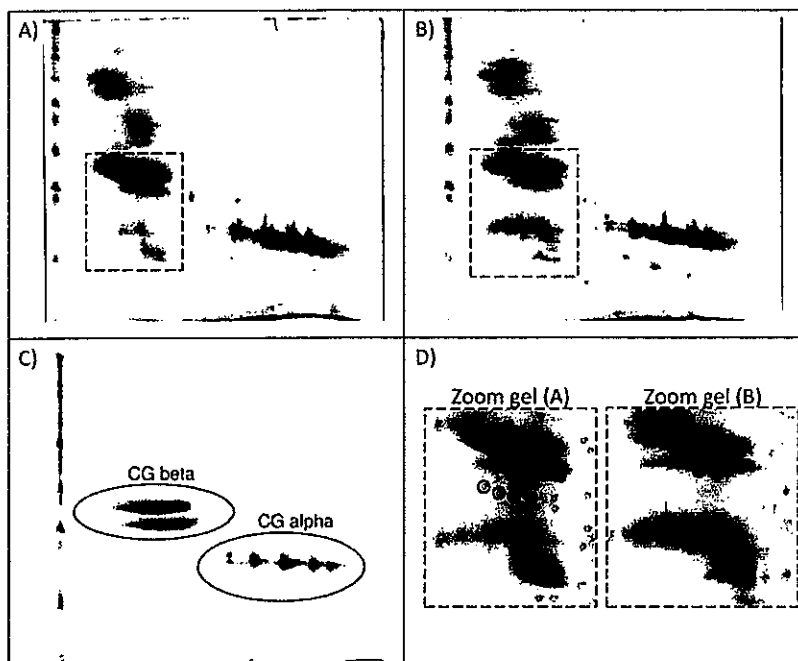


Figure 1. 2D gel electrophoresis separation of urinary and recombinant hCG products (6,500 IU loaded). (A) u-hCG manufacturer A. (B) u-hCG Manufacturer B. (C) r-hCG Manufacturer D. (D) Zoom views on the regions of gels (A) and (B) with spots containing PrP peptides encircled. doi:10.1371/journal.pone.0017815.g001

Several non-gonadotropin proteins were identified in the u-hCG derived preparations (Table 1) but not in the r-hCG preparation. For u-hCG-manufacturer A, prion protein was identified as one of the major non-gonadotropin proteins present. Within urinary impurities, major prion protein (Swissprot accession number P04156) was identified in several 2D gels as a series of spots in the mass range 21–25 kDa for u-hCG-manufacturer A and at about 29 kDa for u-hCG-manufacturer B. The isoelectric point (pI) ranged from four to five for both samples. The fact that major prion protein was identified in several spots is probably due to heterogeneous glycosylation and degraded prion protein forms [13]. In contrast, no prion protein was identified in the r-hCG preparation.

Considering the importance of these findings, liquid chromatography tandem MS (LC-MS/MS) was conducted to confirm the

presence of prion protein in both u-hCG products. The tryptic peptides identified by LC-MS/MS in u-hCG preparations are reported in Figure 2. A total of five peptides were identified from the mature human prion protein sequence covering amino acids 121–228 which is in agreement with recently published characterization of the truncated prion protein forms in human urine [14]. MS/MS spectra of all the peptides identified are provided in Figure S1.

Separation of gonadotropin and non-gonadotropin proteins in human menopausal gonadotropin (hMG) using electrophoresis

Two samples of hMG and one of a combination of recombinant human follicle-stimulating hormone and recombinant human luteinizing hormone (r-hFSH/r-hLH) were analyzed by 2D gel

Table 1. Non-gonadotropin protein profile identified in u-hCG products.

N	Identified human proteins	Accession number	u-hCG manufacturer A	u-hCG manufacturer B
1	Major prion protein	P04156	X	X
2	Apolipoprotein D	P05090	X	X
3	Beta-2-glycoprotein 1	P02749	X	X
4	Complement decay-accelerating factor	P08174	X	X
5	Alpha-1-microglobulin (protein AMBP)	P02760	X	X
6	CD59 glycoprotein	P13987	X	X
7	Uromodulin	P07911	X	X
8	Growth/differentiation factor 15	Q99988	X	X
9	Endosialin	Q9HCU0	X	X
10	Protein delta homolog 1	P80370	X	X
11	Fibrillin-1	P35555	X	X
12	Matrix metalloproteinase-9	P14780	X	X
13	Plasminogen	P00747	X	
14	Complement factor B	P00751	X	
15	Tumor necrosis factor receptor superfamily member 1B	P20333	X	
16	Hepatocyte growth factor activator	Q04756	X	
17	EGF-containing fibulin-like extracellular matrix protein 1	Q12805	X	
18	Tumor necrosis factor receptor superfamily member 1A	P19438	X	
19	Connective tissue growth factor	Q18739	X	
20	Basement membrane-specific heparan sulfate proteoglycan core protein	P98160	X	
21	Plasma serine protease inhibitor (protein C inhibitor)	P05154		X
22	Insulin-like growth factor-binding protein 7	Q16270		X
23	Zinc-alpha-2-glycoprotein	P25311		X
24	Folate receptor alpha	P15328		X
25	Kininogen-1	P01042		X
26	Protein S100-A7 (Psoriasin)	P31151		X
27	Pro-epidermal growth factor	P01133		X
28	Fibulin-2	P98095		X
29	Collagen alpha-1(III) chain	P02461		X
30	Apolipoprotein(a)	P08519		X
31	Probable carboxypeptidase PM20D1	Q6GT58		X
32	Complement factor H-related protein 1	Q03591		X
33	Cystatin-M	Q15828		X

u-hCG = urinary human chorionic gonadotropin.
doi:10.1371/journal.pone.0017815.t001

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      10      20      30      40      50      60
MANLGCNMLV LFVATWSDLG LCKKRPKPGG WNTGGSRYPG QGSPGGNRYF PQGGGGWGQP
      70      80      90      100     110     120
HGGGHWQPHG GGWQPHGGG WGQPHGGGNG QGGGTHSQWN KPSKPKTNMK HMAGAAAAGA
      130     140     150     160     170     180
VVGGLGGYML GSAMSRPIIH FGSDYEDRYR RENMHRYPNQ VYYRPMDEYS NQNNFVHDC
      190     200     210     220     230     240
NITIKQHTVT TTTKGENFTE TDVKMMERVV EQMCITQYER ESQAYYQGRS SMVLFSPPV
      250
ILLISFLIFL IVG

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▽ Potential human PrP fragments

*Tryptic peptides identified in human chorionic gonadotropin (hCG) preparations (PrP₁₂₁₋₁₃₆, PrP₁₃₇₋₁₄₈, PrP₁₅₇₋₁₆₄, PrP₂₀₉₋₂₂₀, and PrP₂₂₁₋₂₂₈) are underlined.

Figure 2. Human prion protein (PrP) sequence (mature form in bold). *Tryptic peptides identified in human chorionic gonadotropin (hCG) preparations (PrP₁₂₁₋₁₃₆, PrP₁₃₇₋₁₄₈, PrP₁₅₇₋₁₆₄, PrP₂₀₉₋₂₂₀, and PrP₂₂₁₋₂₂₈) are underlined.
doi:10.1371/journal.pone.0017815.g002

electrophoresis (Figure 3). According to the high amount of impurities known to be present in hMG (manufacturer A), a lower amount (600 IU FSH) of this sample was loaded. Since no information was available on the purity of hMG (manufacturer C), it was handled as hMG-HP preparation by loading a total of 1,125 IU FSH. Comparative analysis indicated that both hMGs tested were less pure than hMG-HP and contained high levels of total proteins, mainly urinary impurities.

Identification of non-gonadotropin proteins in hMG-HP preparations using electrophoresis and MS

After 2D gel electrophoresis of two batches of hMG-HP (Figures 4A and 4B), protein spots were analyzed by LC-MS/MS, and identified using internationally available databases.

In total, 34 non-gonadotropin proteins were identified in the protein profile of the hMG-HP preparations (Table 2). No impurities were identified in the r-hFSH/r-hLH preparation

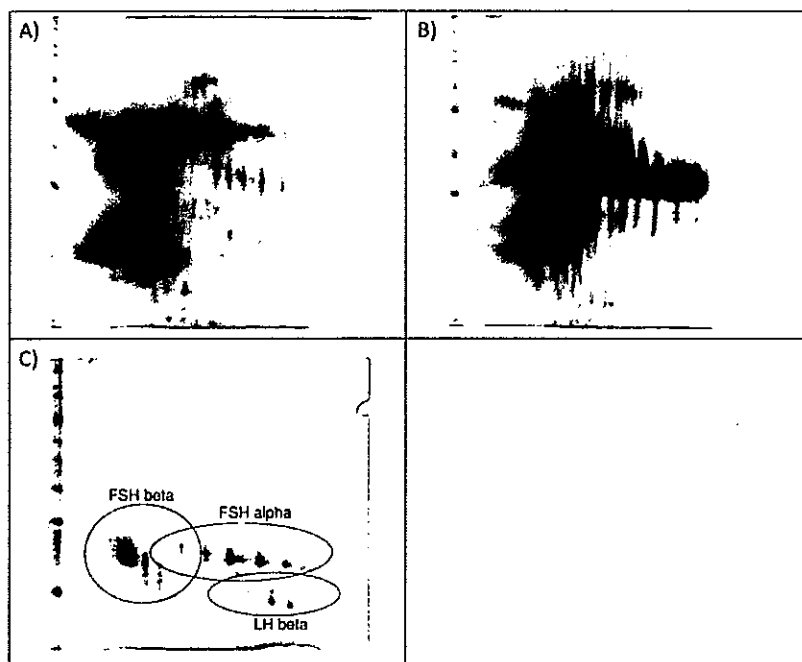


Figure 3. 2D gel electrophoresis separation of urinary hMG and recombinant hFSH/hLH products. (A) hMG Manufacturer A (600 IU loaded). (B) hMG Manufacturer C (1,125 IU loaded). (C) r-hFSH/r-hLH Manufacturer D (1,125 IU loaded).
doi:10.1371/journal.pone.0017815.g003

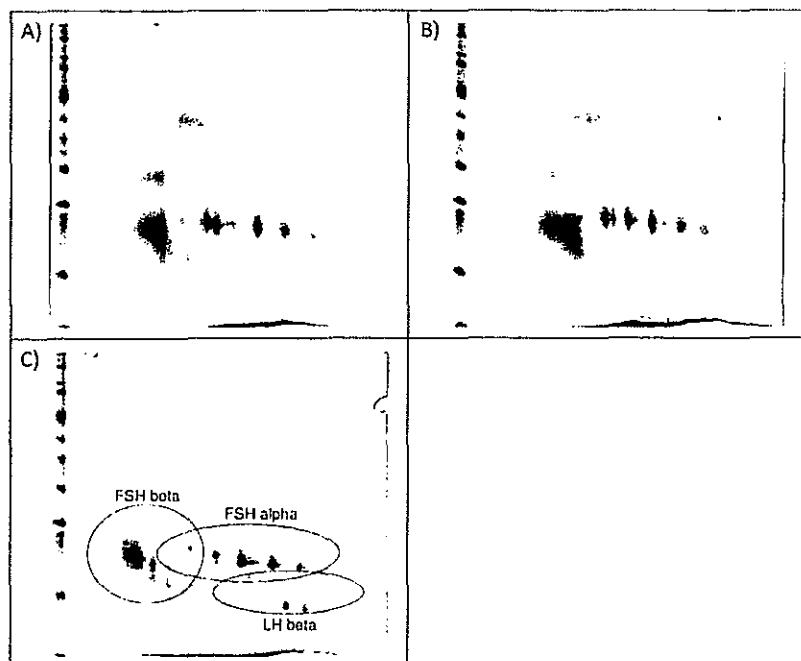


Figure 4. 2D gel electrophoresis separation of highly purified urinary hMG and recombinant hFSH/hLH products (1,125 IU loaded). (A) hMG-HP Manufacturer A-Product 1. (B) hMG-HP Manufacturer A-Product 2. (C) r-hFSH/r-hLH Manufacturer D.
doi:10.1371/journal.pone.0017815.g004

(Figure 4C). Considering previous proteomics studies [15], the new impurities identified in hMG-HP products by manufacturer A increased the number of non-gonadotropin proteins identified by the scientific community to 39.

Targeted prion protein quantification in gonadotropin preparations using liquid chromatography selected reaction monitoring (LC-SRM)

LC-SRM analysis was performed directly on all hCG samples to allow specific detection of tryptic peptides from the prion protein, confirming unambiguously the 2D gel data from urine-derived hCG samples. The same technique was used to test the other products included in this study (u-hCG, hMG and hMG-HP), and was able to detect and quantify the human prion protein (Table 3).

It should be noted that primary experiments were performed using only one vial of each product. Among the urinary preparations, hMG-HP showed the presence of prion protein but the level was not quantifiable (data not shown). The experiment was then repeated with 6 pooled vials and prion presence was confirmed. No trace of prion protein was detected when the same process (using a larger number of vials) was carried out for the recombinant preparation r-hLH (Table 3).

The tryptic prion peptides PrP (209–220) and PrP (221–228) were used for SRM detection and peptide PrP (209–220) for quantification. As the human PrP peptide sequence (221–228) is identical to the related bovine PrP peptide, it was used to simultaneously evaluate the presence of human and bovine prion sequence. These tryptic prion peptides are located in the human prion carboxy-terminal sequence, and thus allow the detection of several potential truncated prion protein forms (see Figure 2) [13,16]. LC-SRM is therefore suitable to evaluate the potential presence of bovine prion peptides in recombinant products, if bovine prion protein is present at levels higher than the limit of

detection (LOD). The LOD was experimentally established and found to be product-dependent. According to the results reported in Table 3, no human or bovine prion peptides were detected in r-hCG, r-hLH, and r-hFSH/r-hLH products from manufacturer D.

Discussion

Using classical proteomic analyses, prion protein was detected for the first time in two u-hCG preparations, and was among 33 different non-gonadotropin proteins identified as contaminants of these pharmaceutical products. In contrast, r-hCG preparations were negative for prion proteins.

In one of the two u-hCG products tested, human prion protein was among the ten major contaminants. The fact that prion protein sequences were identified in several spots on our 2D electrophoresis gels is likely due to the presence of heterogeneous glycosylation and degraded prion protein forms. It is also worth noting that in the u-hCG preparation of manufacturer A, plasminogen was identified among the urinary impurities; plasminogen has been identified as a binding protein for disease-associated prion protein [17].

Both hMG and hMG-HP were tested using 2D gel electrophoresis. The results confirmed that the two hMGs tested were less pure than hMG-HP and contained a large number of total proteins (mainly represented by urinary impurities). Non-gonadotropin proteins present in hMG-HP products were identified by MS, resulting in 34 co-purified contaminants. Only gonadotropin proteins were seen in all the recombinant preparations analyzed by 2D gel electrophoresis. Using this 2D-gel/LC-MS/MS proteomic workflow, prion proteins were identified only in u-hCG and not in hMG-HP preparations. In parallel, a targeted proteomic approach (LC-SRM) was developed to detect human prion proteins which are sensitive to proteases. The method was optimized to provide quantitative data in each container of

Table 2. Non-gonadotropin protein profile identified in hMG-HP products.

N	Identified impurities	Accession number	hMG-HP manufacturer A Product 1	hMG-HP manufacturer A Product 2
1	Plasma serine protease inhibitor (protein C inhibitor)	P05154	XX	XX
2	Apolipoprotein D	P05090	XX	XX
3	Insulin-like growth factor-binding protein 7	Q16270	XX	XX
4	Leukocyte elastase inhibitor	P30740	XX	XX
5	Alpha-2-antiplasmin	P08697	XX	X
6	Zinc-alpha-2-glycoprotein	P25311	X	XX
7	Serum albumin	P02768	X	X
8	Folate receptor alpha	P15328	X	X
9	Urokinase plasminogen activator surface receptor	Q03405	X	X
10	Afamin	P43652	X	X
11	Kininogen-1	P01042	X	X
12	Reversion-inducing cysteine-rich protein with Kazal motifs	O95980	X	X
13	Alpha-1-antitrypsin	P01009	X	X
14	Plasminogen	P00747	X	X
15	Beta-2-glycoprotein 1	P02749	X	X
16	Thrombospondin type-1 domain-containing protein 4	Q6ZMP0	X	X
17	Protein S100-A7 (Psoriasin)	P31151	X	X
18	Alpha-1-acid glycoprotein 1	P02763	X	X
19	Complement decay-accelerating factor	P08174	X	X
20	Sialate O-acetyltransferase	Q9HAT2	X	
21	Alpha-1-microglobulin (protein AMBP)	P02760	X	
22	Ig kappa chain C region	P01834	X	
23	Ig gamma chain C region	P01857	X	
24	Peptidoglycan recognition protein	O75594	X	
25	Golgi phosphoprotein 2	Q8NBJ4		X
26	Delta-like protein 1	O00548		X
27	Death-associated protein kinase 1	P53355		X
28	CD27 antigen	P26842		X
29	Serotransferrin	P02787		X
30	TNF receptor superfamily member 21	O75509		X
31	CD59 glycoprotein	P13987		X
32	Folate receptor gamma	P41439		X
33	Platelet endothelial aggregation receptor 1	Q5VY43		X
34	TNF receptor superfamily member 4	P43489		X

hMG-HP = highly purified urinary human menopausal gonadotropin.
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product. This is the most sensitive MS-based quantification technique currently available with a limit of detection in the low femtomolar range. This approach for prion protein detection, identification and quantification was used on all gonadotropin pharmaceutical preparations included in our study, including both urinary (hCG, hMG, hMG-HP) and recombinant products.

All urine-derived preparations tested, produced by different manufacturers, showed the presence of human prion proteins in varying amounts. These findings demonstrate that the purification processes for different urine-derived preparations are unable to remove prion proteins from the source material and that the process controls employed do not permit the identification of this contaminant.

Do the prion protein peptides detected in this study originate from infectious prions? Preparation of tryptic peptides is preceded

by solubilization in 8M urea, which is adequate to disaggregate and denature the disease-associated isoform of the prion protein rendering it susceptible to trypsin digestion. It is also clear that native and diseased isoforms of the prion protein share affinity for chromatography substrates utilized to purify peptide hormones [3]. Finally, infectious prions can range down in size to oligomers of a few dozen prion protein molecules [18], which would be undetectable by existing biochemical methodologies including MS methods employed in this study.

Although no cases of human prion disease due to the use of urinary gonadotropins have been recognized to date, the epidemiological signal for transmission may be difficult to detect. Each year, more than 300,000 young women in the US and Canada are prescribed urine-derived gonadotropins for infertility. Although the Food and Drug Administration and Health Canada

Table 3. Results of prion protein peptides analysis in gonadotropin preparations by LC-SRM.

Commercial product	Manufacturer	Concentration	Prion detection (peptide 209-220, peptide 221-228)	Prion quantification (peptide 209-220)		
				Prion content (pmoles/vial)	CV (%)	Limit of detection (pmoles/vial)
u-hCG	A	5,000 IU	Yes	116.21	5	2.88
	B – product 1	5,000 IU	Yes	30.38	7	2.00
	B – product 2	5,000 IU	Yes	15.78	6	0.62
hMG	A	75 IU	Yes	6.78	6	1.35
	C	75 IU	Yes	5.38	12	0.96
hMG-HP	A – product 1*	75 IU	Yes	0.21	10	0.01
	A – product 2*	75 IU	Yes	0.10	13	0.01
r-hCG	D	250 µg	No	<LOD	-	0.47
r-hFSH/r-hLH	D	150 IU/75 IU	No	<LOD	-	0.09
r-hLH	D*	75 IU	No	<LOD	-	0.04

For all products a single vial was used for measurements. For products with *, six vials were pooled in order to minimize protein material losses. LC-SRM = liquid chromatography selected reaction monitoring; CV = coefficient of variation; u-hCG = urinary human chorionic gonadotropin; hMG = urinary human menopausal gonadotropin; hMG-HP = highly purified hMG; r-hCG = recombinant hCG; r-hFSH = recombinant human follicle-stimulating hormone; r-hLH = recombinant human luteinizing hormone.
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once considered these products to be in the lowest category of risk for prion disease transmission, the discovery of full infectivity in the urine of nephritic scrapie-infected mice in 2005 led to new requirements for product labeling and a review of donor procedures and manufacturing processes. Additional recent findings suggest that urinary prion excretion can occur without renal pathology [6,7]. These results warrant a reassessment as to whether the risks of urine-derived fertility products could now outweigh their benefits, particularly considering the availability of recombinant products that do not require human urine as a substrate.

Although urinary gonadotropins have been previously characterized as safe [19,20], this opinion may be overly optimistic in view of the present findings, supported by results from other recently published studies. Notably, blood products were once also considered 'safe', based on the lack of detectable prions in vCJD using an inadequately sensitive mouse bioassay [21]. In line with recent published studies, the 2010 updated World Health Organization tables on 'Tissue infectivity distribution in transmissible spongiform encephalopathy' moved urine from the category of 'Tissues with no detectable infectivity' to the category of 'Lower-infectivity tissue' (the latter category includes blood) [22].

Current urine collection systems pool the urine of thousands of donors and, unlike the blood collection system, do not allow for donor tracing. There is also no mechanism of ensuring that the designated donor is actually the one who provides the urine, as donation is normally done at home. However, even if donor management and tracing were flawless, the fact that prionuria may exist well before the onset of clinically overt prion disease, without being detectable by current methods, remains a cause for concern. Furthermore, the now indisputable detection of prions in urine of experimental animals, the lack of a species barrier for human-to-human transmission, the relative efficiency of the intramuscular injection route for prion transmission, and the young age of fertility drug recipients all support application of the 'precautionary principle' for urinary derived pharmaceuticals. As risk management paradigms shift towards more proactive approaches intended to 'anticipate and prevent' emerging risks [23–26], a

careful examination of the risk of transmission of human prion disease through the use of urine-derived hormones and peptides would appear to be warranted.

Materials and Methods

Source of material

Commercial products of urinary human chorionic gonadotropin (u-hCG) from Ferring (Switzerland) and Organon (Denmark), human menopausal gonadotropin derived from urine (hMG) from Ferring (Switzerland) and Livzon (China); and hMG highly purified (hMG-HP) from Ferring (Switzerland) were used (Table 4). In addition, recombinant human chorionic gonadotropin (r-hCG), serum-free recombinant human follitropin alfa in association with lutropin alfa (r-hFSH + r-hLH) and human

Table 4. Characteristics of commercial products analyzed.

Commercial product	Manufacturer	Strength	Country producer
u-hCG	A	5000 IU	Switzerland
	B – product 1	5000 IU	Denmark
	B – product 2	5000 IU	Denmark
hMG	A	75 IU	Switzerland
	C	75 IU	China
hMG-HP	A – product 1	75 IU	Switzerland
	A – product 2	75 IU	Switzerland
r-hCG	D	250 µg	Switzerland
r-hFSH/r-hLH	D	150IU/75IU	Switzerland
r-hLH	D	75 IU	Switzerland

u-hCG = urinary human chorionic gonadotropin; hMG = urinary human menopausal gonadotropin; hMG-HP = highly purified hMG; r-hCG = recombinant hCG; r-hFSH = recombinant human follicle-stimulating hormone; r-hLH = recombinant human luteinizing hormone.

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lutropin alfa alone (r-hLH) were all from Merck Serono (Switzerland) (Table 4).

Identification of non-gonadotropin proteins using electrophoresis and MS

a) Sample preparation. A total amount of 6,500 IU of hCG samples, 600 IU of hMG sample A and 1,125 IU of hMG sample C, 1,125 IU of hMG-HP samples and 1,125 IU r-hFSH/r-hLH sample were pretreated by ultrafiltration to remove non-protein product excipients such as lactose or mannitol which are incompatible with the first dimension of 2D SDS-PAGE (VIVASPIN-2 filter, Sartorius VS0291; cut off: 3,000 Da, centrifugation at 4,000 g, temperature was constantly maintained at 4°C). The volume of solution was reduced to about 0.6 mL of pure water.

b) SDS-PAGE. Samples were loaded onto a 2D SDS-PAGE gel. Proteins were separated on a first dimension according to their pI at 86,000 VH, and then on a second dimension according to their molecular weight. Following 2D separation, the gels were stained by silver or Coomassie blue staining. Detected proteins were removed from the gel using a gel cutter (PROTEINEERsp, Bruker Daltonics, Bremen, Germany), washed, reduced, alkylated and dehydrated automatically with a MassPrep robot (Waters, Milford, MA). The gel pieces were digested with porcine trypsin (Promega, Madison, WI, USA). The resulting tryptic peptides were extracted with 60% acetonitrile in 0.5% formic acid and analyzed by nanoflow liquid chromatography tandem mass spectrometry (nanoLC-MS/MS).

c) Protein identification by NanoLC-MS/MS analysis. NanoLC-MS/MS analysis was performed using an Agilent 1100 series nanoLC-Chip system (Agilent Technologies, Palo Alto, USA) coupled to an HCT Plus ion trap (Bruker Daltonics, Bremen, Germany). The chip was composed of a Zorbax 300SB-C18 analytical column (43 mm × 75 µm, with a 5 µm particle size) and a Zorbax 300SB-C18 enrichment column (40 nL, 5 µm). Elution of the peptides was performed at a flow rate of 300 nL·minute⁻¹ with a 8–40% linear gradient (solvent B, 98%ACN/0.1%FA) over 7 minutes.

The voltage applied to the capillary cap was optimized to −1,750 V and the mass range was 250–2,500 *m/z*. For tandem MS experiments, the system was operated in data-dependent acquisition mode with automatic switching between MS and MS/MS. The three most abundant precursor ions were selected to be further isolated and fragmented. The MS/MS scanning was performed in the ultrascan resolution mode at a scan rate of 26,000 *m/z* per second. A total of six scans were averaged to obtain an MS/MS spectrum.

Mass data collected during nanoLC-MS/MS analysis were processed, converted into *.mgf files and were interpreted using a local Mascot server (Matrix Science, London, UK). Searches were performed against the SwissProt database (57.4 version, 470,369 entries) using a target/decoy approach and without molecular weight or isoelectric point restrictions. The tolerance on mass measurements was set to 0.20 Da for both MS and MS/MS modes. One tryptic missed cleavage per peptide was allowed and some variable modifications were taken into account such as carbamidomethylation for cysteine and oxidation for methionine. Validation criteria were applied in order to retain no decoy hit.

Targeted prion protein quantification in gonadotropin preparations using LC-SRM

a) Sample preparation. After removal of the non-protein product excipients as described for the 2D gel electrophoresis, the total protein content was determined by Bradford assay using the

Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions for the Microplate Protocol, with a working range of 1–25 µg. Bovine serum albumin was used as standard. Samples were evaporated to dryness and rediluted with 200 µL of 8 M urea, 0.1 M NH₄HCO₃. Reduction was performed with addition of 3.5 µL of 700 mM DTT, 37°C, 30 minutes (final concentration 12 mM) and alkylation with addition of 12 µL of 700 mM IAA, 25°C, dark, 1 hour (final concentration 40 mM). In order to perform the tryptic digestion, this solution was diluted with 1,500 µL of 0.1 M fresh NH₄HCO₃. Digestion was carried out by adding 1 µg of bovine trypsin (Promega) per 100 µg of proteins and maintained at 37°C overnight. Desalting and further concentration was carried out by adsorption using C18 Sep-Pak cartridges (Sep-Pak Vac 1cc 50 mg, tC18, Waters) and peptides were eluted with 600 µL of 50%ACN/0.1%FA. After evaporation to dryness, the peptide fraction was re-dissolved in water to a final concentration of 1 µg/µL.

b) LC-SRM quantification of human PrP. The approach developed was mainly based on the AQUA strategy previously described by Gerber et al. for the absolute quantitative analysis of proteins [27].

Stable isotope labeled internal standard peptides (Sigma Aldrich, St. Louis, MO) were introduced in protein samples just before proteolytic digestion. Both the isotope labeled AQUA peptide and the unlabeled native peptide (produced during proteolytic cleavage of the target protein) were then measured by LC-SRM. Similar to small molecule isotope dilution strategies, the isotope-labeled standard and the analyte are identical except for their masses. In an analogous fashion to classic isotope dilution approaches, the mass difference allows the mass spectrometer to differentiate between the two nearly identical species. Since the amount of internal standard is known, and the ratio between amounts of internal standard and analyte can be determined from the mass spectra, the amount of the analyte peptide can be calculated.

The quantification was performed directly on vials or pooled vials. Peptides already detected and identified in 2D gel spots by the classical LC-MS/MS proteomic approach were targeted by LC-SRM allowing a specific detection of the four peptide sequences of the human PrP: 137–148, 157–164, 209–220, and 221–228. A fifth peptide, corresponding to peptide 209–220 with the methionine oxidized, was also targeted. Three different amounts of standard peptides were spiked in (250, 750, and 2,250 femtomoles) in order to make sure that the final quantification was performed in the linear range. All analyses were performed in triplicate to evaluate the coefficient of variance. Results were given in picomoles per vial.

Because of the low amounts of standard peptides spiked in the samples, special care had to be taken to prepare these standard solutions. To minimize expected losses of peptides by irreversible adsorption on the walls of the tubes at very low concentrations, dilution of the stock solution was made in low-binding 500 µL tubes. The dilutions from these stock solutions were made extemporaneously, briefly mixed and spiked in samples within less than 2 min after dilution. We have verified that at the final diluted concentrations, there is no signal intensity loss when 1 µL is analyzed in less than 3 min. The following dilution process has been used: 1 nanomole of lyophilized AQUA peptides was dissolved in 20 µL of 10%FA, sonicated for 3 min and then 180 µL of 0.1%FA was added and vortexed to obtain a stock solution at 5 pmol/µL.

c) Micro LC separation. Separation was made on an Agilent 1100 HPLC system equipped with a C18 column (Zorbax 300 SB C18 Agilent, 150×0.3 mm, 3.5 µm) using a water/acetonitrile gradient at 4 µL·minute⁻¹ (2–22% acetonitrile

Table 5. Optimized transitions for prion protein native peptides and isotope labeled standard peptides.

Peptides	Precursor ion m/z (charge state)	Quantifier (A) m/z (charge state, fragment type)	Qualifier 1 (B) m/z (charge state, fragment type)	Qualifier 2 (C) m/z (charge state, fragment type)	B/A	C/A
Native 209-220	778,4 (2+)	696,4 (1+, y5)	1228,6 (1+, y9)	1100,5 (1+, y8)	0,35+/-25%	1+/-25%
AQUA 209-220	783,4 (2+)	706,4 (1+, y5)	1238,6 (1+, y9)	1110,6 (1+, y8)	0,35+/-25%	1+/-25%
Native 221-228	522,7 (2+)	629,3 (1+, y4)	466,3 (1+, y3)	700,3 (1+, y5)	1+/-30%	1+/-30%
AQUA 221-228	527,8 (2+)	639,4 (1+, y4)	476,3 (1+, y3)	710,4 (1+, y5)	1+/-30%	1+/-30%

SRM was used to follow peptide fragments, the most intense fragment was used as a quantifier (A) and the two secondary fragments were used as qualifiers (B and C).
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in 17 minutes) and a preconcentration system (Zorbax SB C18 Agilent, 17×1 mm, 3.5 μm, reverse mode).

d) SRM method setup. A triple quadrupole mass spectrometer (QQQ G 6410, Agilent Technologies) was used. The best transitions per peptide were optimized using the Peptide Optimizer software tool. One fully tryptic peptide (209-220) was used for quantification. Peptide (221-228) gave in all samples in which PrP was detected a lower amount than peptide (209-220) indicating that the PrP must undergo C-terminal cleavages. Hence, the quantification using the more internal peptide (209-220) was more relevant. The other two peptides (137-148 and 157-164) were excluded for quantification as they correspond to proline cleavages and displayed incomplete and irreproducible trypsin cleavages. The list of the optimized transitions is given in Table 5 for the native peptides and the internal isotope labeled standard peptides. Dynamic SRM method setup was used with a dwell time of 200 ms per transition. The most intense fragment was used as a quantifier

and the two other as qualifiers; the tolerances for the fragments' ratios are listed in Table 5.

Supporting Information

Figure S1 MS/MS spectra of peptides identified in the 2-D gel electrophoresis spots as PrP tryptic peptides. (PDF)

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Author Contributions

Conceived and designed the experiments: AVD CC NRC. Performed the experiments: CC FD DT HD CS-R. Analyzed the data: AVD CC FD DT HD CS-R. Contributed reagents/materials/analysis tools: AVD CC FD DT HD CS-R. Wrote the paper: NRC AVD DSM DK.

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医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日 2011. 3. 16	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称		解凍人赤血球濃厚液		Edgeworth JA, Farmer M, Sicilia A, Tavares P, Beck J, Campbell T, Lowe J, Mead S, Rudge P, Collinge J, Jackson GS. Lancet. 2011 Feb 5;377(9764):487-93.	公表国	
販売名(企業名)		解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)			英国	
研究報告の概要	○異型クロイツフェルトヤコブ病におけるプリオン感染の検出:血液検査 背景:プリオン感染は、長期間の潜伏期を伴う。異型クロイツフェルトヤコブ病(vCJD)プリオン感染を呈する無症候性の患者の数は知られておらず、輸血、血液製剤、臓器や組織移植片、汚染医療機器を通して他へ感染するリスクとなっている。vCJDプリオン感染を検出するための血液ベースアッセイについて、その感度と特異性を確定することを目的とし研究を行った。 方法:疾患に関連するプリオン蛋白を捉えて濃縮するための固体結合マトリックスを開発し、この方法を表面結合物質の直接免疫検出法と合わせた。定量的アッセイの感度は、ヒト全血中に10^{-6}の正常脳ホモジネートを加えてベースラインとし、ヒト全血中に、10^{-7}から10^{-10}の一連の希釈系列のvCJDプリオン感染脳ホモジネートを加えて感度を評価した。内因性vCJDを検出するためのアッセイの感度と特異性を確定するために、vCJD患者21人、孤発性CJD患者27人、その他の神経性疾患患者42人、正常対照群100人からの全血190検体を盲検化したパネルを分析した。検体は、アッセイや分析とは独立した個人によって盲検化しナンバリングされた。各検体は、それぞれのアッセイにつき2回検査し、2回とも反応した検体のみを陽性とした。 所見: 10^{-10}倍に希釈した外因性vCJDプリオン感染脳と、10^{-6}倍に希釈した正常脳を識別することが出来た。アッセイの感度は、過去に報告されていたものよりも桁違いに高かった。盲検化したパネルのうち15検体が陽性であった。15検体はすべてvCJD患者からのものであり、vCJDのアッセイ感度が71.4%(95%信頼区間47.8-88.7)、特異性が100%(95%信頼区間97.8-100)であることが示された。 解釈: これらの研究によって症候性の患者におけるvCJD診断のための血液検査の基本を確立し、無症候性のvCJDプリオン感染のための大規模なスクリーニング検査の開発を見込むことができた。					使用上の注意記載状況・ その他参考事項等
	報告企業の意見 vCJDプリオンを検出するための血液検査が開発された。そのアッセイ感度は71.4%、特異性は100%であることが示され、症候性患者の診断のための血液検査や、無症候性vCJD感染のスクリーニング検査の開発を見込むことができたとの報告である。					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、受付時に過去の海外渡航歴(旅行及び居住)を確認し、欧州等38カ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、1980～96年に1カ月以上の英国滞在歴のある人の献血を制限している。今後もプリオン除去フィルター等の技術を含め、CJD等プリオン病に関する新たな知見及び情報の収集に努める。

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Detection of prion infection in variant Creutzfeldt-Jakob disease: a blood-based assay

Julie Ann Edgeworth, Michael Farmer, Anita Sicilia, Paul Tavares, Jonathan Berk, Tracy Campbell, Jessica Lowe, Simon Mead, Peter Rudge, John Collinge, Graham S Jackson

Summary

Background Variant Creutzfeldt-Jakob disease (vCJD) is a fatal neurodegenerative disorder originating from exposure to bovine-spongiform-encephalopathy-like prions. Prion infections are associated with long and clinically silent incubations. The number of asymptomatic individuals with vCJD prion infection is unknown, posing risk to others via blood transfusion, blood products, organ or tissue grafts, and contaminated medical instruments. We aimed to establish the sensitivity and specificity of a blood-based assay for detection of vCJD prion infection.

Methods We developed a solid-state binding matrix to capture and concentrate disease-associated prion proteins and coupled this method to direct immunodetection of surface-bound material. Quantitative assay sensitivity was assessed with a serial dilution series of 10^{-7} to 10^{-10} of vCJD prion-infected brain homogenate into whole human blood, with a baseline control of normal human brain homogenate in whole blood (10^{-6}). To establish the sensitivity and specificity of the assay for detection of endogenous vCJD, we analysed a masked panel of 190 whole blood samples from 21 patients with vCJD, 27 with sporadic CJD, 42 with other neurological diseases, and 100 normal controls. Samples were masked and numbered by individuals independent of the assay and analysis. Each sample was tested twice in independent assay runs; only samples that were reactive in both runs were scored as positive overall.

Findings We were able to distinguish a 10^{-10} dilution of exogenous vCJD prion-infected brain from a 10^{-6} dilution of normal brain (mean chemiluminescent signal, 1.3×10^5 [SD 1.1×10^4] for vCJD vs 9.9×10^4 [4.5×10^3] for normal brain; $p < 0.0001$)—an assay sensitivity that was orders of magnitude higher than any previously reported. 15 samples in the masked panel were scored as positive. All 15 samples were from patients with vCJD, showing an assay sensitivity for vCJD of 71.4% (95% CI 47.8–88.7) and a specificity of 100% (95% CIs between 97.8% and 100%).

Interpretation These initial studies provide a prototype blood test for diagnosis of vCJD in symptomatic individuals, which could allow development of large-scale screening tests for asymptomatic vCJD prion infection.

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Introduction

Prion disease encompasses various closely related and uniformly fatal neurodegenerative disorders affecting the CNS in human beings and animals. These disorders include Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker disease, fatal familial insomnia and kuru in man, bovine spongiform encephalopathy (BSE) in cattle, chronic wasting disease of deer and elk, and scrapie in sheep.^{1,2} The emergence of variant CJD (vCJD) and the confirmation that it originates from exposure to BSE³ has raised a plethora of public health concerns affecting endoscopy, surgery, dentistry, organ transplantation, and blood transfusion.

Exposure of the UK population to BSE has been widespread, with more than 181 000 cases confirmed in cattle and estimates of total infections of 1–3 million.⁴ Although the number of clinical cases of vCJD has been fairly small, the number of infected individuals remains unclear.⁵ A retrospective study⁶ of archived surgical lymphoreticular specimens estimated a prevalence of infection in the UK population of 237 per million (95% CI 49–692 per million), which is far higher than the number of clinical cases of vCJD thus far. On the

basis of this study, a prevalence estimate of one in 4000 was recommended by the Spongiform Encephalopathy Advisory Committee for management of public health risks.^{7,8} Further investigations have not refined these estimates,^{9,10} and a major caveat to these studies is the unknown sensitivity of the methods used to detect subclinical or preclinical cases of vCJD infection. The long incubation periods seen in natural human prion infections, which can exceed 50 years,¹¹ and the existence of subclinical carrier states of prion infection in animal models¹² offer a potential explanation for the discrepancy between estimates of infection prevalence and clinical cases.

Concern regarding secondary transmission of vCJD has had extensive effects on public health policy in the UK and elsewhere. Since the prevalence of preclinical or subclinical vCJD prion infection is poorly defined, the extent of future transfusion-transmission of vCJD cannot be accurately risk-assessed. Secondary infection from asymptomatic donors has already been confirmed in four recipients of blood transfusion.^{13–15} Although the number of transfusion recipients positively identified as having received packed red cells contaminated with vCJD is



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MRC Prion Unit, Department of Neurodegenerative Disease, UCL Institute of Neurology, London, UK (J A Edgeworth PhD, M Farmer BSc, A Sicilia MSc, P Tavares MSc, J Beck BSc, T Campbell BSc, J Lowe BSc, S Mead PhD, Prof J Collinge FRS, G S Jackson PhD); and National Prion Clinic, National Hospital for Neurology and Neurosurgery, London, UK (S Mead, P Rudge FRCP, Prof J Collinge)

Correspondence to: Prof John Collinge or Dr Graham S Jackson, MRC Prion Unit, Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK (j.collinge@prion.ucl.ac.uk or g.s.jackson@prion.ucl.ac.uk)

small, a much larger cohort of around 7000 recipients of contaminated plasma products have already been identified and notified of their at-risk status.¹⁴ Concern for this cohort has been heightened by post-mortem evidence of infection with vCJD prions in the spleen of an individual from this at-risk group with haemophilia.¹⁵

The infectious agents or prions responsible for transmission of disease are composed principally, if not entirely, of a misfolded form of host cellular prion protein (PrP^C). This unique pathogenesis has posed major challenges to the development of diagnostic tests, which for infectious agents generally involves detection of a host humoral immune response or agent-specific nucleic acid, neither of which apply in vCJD. PrP^C is expressed ubiquitously, although at highest concentrations in the CNS and cells of the immune system. When recruited during prion propagation, PrP^C is remodelled to aggregated, detergent insoluble isoforms designated PrP^{Sc}, which are chemically identical but conformationally distinct from PrP^C. Detection of PrP^{Sc} by the depletion of PrP^C with proteinase K (PK) in CNS and lymphoreticular tissues generally correlates with the presence of prion infectivity^{16,17} and remains wholly specific for prion disease. However, in recent years, researchers have found that additional PK-sensitive but disease-related abnormal isoforms of PrP might have an important role in prion disease pathogenesis,^{18,19} and only 10% of abnormal PrP may be deposited in the form of PrP^{Sc}.²⁰

Although the quantities of PrP^{Sc} deposited in neural tissues are sufficient during the symptomatic phase of illness for detection by conventional immunoassays such as western blotting and ELISA, concentrations in peripheral tissues are substantially lower.²¹ Quantification of infectious titre in rodent models has suggested that the levels of infectivity in blood are very low, with buffy coat fractions containing between 2 and 10 intracerebral LD₅₀ units per mL during the asymptomatic phases of disease, rising to 100 LD₅₀ units per mL during the clinical stage.^{22,23}

To successfully identify infection in blood, an assay has to be able to detect abnormal PrP in a range that is several orders of magnitude lower than the sensitivity of conventionally used immunoassays. Furthermore, the ratio of background PrP^C (which is chemically identical to PrP^{Sc}) is higher in blood than in any other tissue, with the high lipid and protein content of blood potentially contributing to non-specific background signals.²⁴ Conventionally, immunoassays for PrP^{Sc} have depended on protease pretreatment of tissues to degrade PrP^C and other proteins, thereby reducing cross-reactivity.

The finding that prions can bind avidly to some surfaces, including metals,²⁵ has been used to develop quantitative assays for infectivity²⁶ that approach the high sensitivity needed to detect the low concentrations of prions and abnormal PrP associated with blood. We have adopted a similar approach for the capture and enrichment of abnormal PrP from whole blood using an

optimised solid-state capture matrix derived from investigation of an extensive range of potential binding surfaces coupled with direct immunodetection of the surface-bound material. This approach avoids use of any proteolytic processing, ensuring that all abnormal PrP isoforms are available for detection. We aimed to establish the sensitivity and specificity of this test for detection of vCJD prion infection in blood.

Methods

Setting

These studies were approved by the local research ethics committee of the UCL Institute of Neurology and the National Hospital for Neurology and Neurosurgery (NHNN; London, UK). Blood samples were obtained with informed consent from patients who were enrolled in the MRC PRION-1 trial²⁷ or the National Prion Disease Monitoring Cohort study,¹⁰ or who were referred to the National Prion Clinic or Dementia Research Centre at the NHNN. Samples were collected in EDTA blood tubes and stored frozen at -70°C. Diagnosis of variant or sporadic CJD was made according to established criteria.^{21,22} Normal control blood samples were obtained as EDTA samples from the National Blood Transfusion Service (NBTS) of England and Wales.

Laboratory procedures

We assayed blood spiked with vCJD, blood spiked with normal brain homogenate, and whole human blood samples from patients. In the assay of spiked samples, an exogenous spike of 5 µL of 10% (w/v) vCJD-infected brain homogenate or normal human brain homogenate was diluted into 9995 µL of whole human blood. From this mixture, a serial dilution series of 10⁻⁷ to 10⁻¹⁰ of vCJD-infected brain homogenate into whole human blood was prepared, with a baseline control of a high concentration of normal human brain in whole blood (10⁻⁶). Each sample was further diluted two-fold into capture buffer (200 mmol/L Tris, 4% [w/v] bovine serum albumin, 4% [w/v] CHAPS, 2x concentration of complete protease inhibitors [Roche, Mannheim, Germany], 80 units benzamide [grade II, Merck, Darmstadt, Germany]) for assay. To aliquots of capture matrix (45 µm stainless steel particles [Goodfellow, Cambridge, UK]), 800 µL of the spiked blood-capture buffer mix was added and incubated overnight. In the assay of blood samples from patients, a whole human blood-capture buffer mix was added to aliquots of capture matrix and incubated overnight at 18°C with agitation.

After incubation with either spiked or whole blood, capture matrix was isolated on a magnet and the supernatant discarded. Capture matrix was washed repeatedly with 1 mL phosphate-buffered saline (PBS) plus 0.05% (v/v) Tween-20 (PBST). After the final wash, all liquid was removed and the capture matrix was heat-treated at 110°C for 5 min. To each tube, an aliquot of 50 µL of biotinylated primary antibody 1CSM18 (D-Gen, London,

UK) prepared at 1 µg/mL in PBS plus 1% v/v Tween-20 was added and incubated at 37°C for 1 h. Samples were washed repeatedly with 1 mL PBST, isolating capture matrix each time. Each sample was then incubated with high sensitivity NeutrAvidin-HRP (Pierce, Rockford, USA) prepared at a 1:100 000 dilution in PBS plus 1% (v/v) Tween-20 at 37°C for 45 min.

Finally, samples were washed repeatedly with 1 mL PBST, isolating capture matrix each time. To each sample, 60 µL of SuperSignal ELISA Femto chemiluminescent substrate (Pierce) was added and 20 µL of capture matrix was transferred into three replica wells of a black flat bottom ELISA plate (Greiner, Frickenhausen, Germany). Immediately before plates were read, a further 80 µL of SuperSignal ELISA Femto chemiluminescent substrate was added per well. A dilution series of 1:100 000 to 1:10 million of the high sensitivity NeutrAvidin-HRP (Pierce) was prepared to allow for correction in absolute chemiluminescent readings across multiple plates. Plates were scanned with an M1000 plate reader (Tecan, Männedorf, Switzerland).

Analyses

We analysed a panel of 190 whole blood samples, consisting of samples from 21 patients with vCJD, 100 normal controls (provided by NBST), 16 patients with probable sporadic CJD, 11 patients with definite sporadic CJD, and 42 patients from other neurodegenerative diseases (25 Alzheimer's disease, four frontotemporal dementia, six familial Alzheimer's disease, and seven neurological referrals to the National Prion Clinic subsequently confirmed as not prion disease). Samples were masked and numbered from 1 to 190 by individuals independent of sample collection, testing, or analysis. Each of the masked panel samples were tested twice in independent assay runs as described previously. Samples were processed and analysed in groups of 19 masked panel samples per 96-well plate with a set of eight quality control samples containing six normal control blood samples and two blood samples from patients infected with vCJD.

Masked panel samples were scored as reactive if the ratio of the mean chemiluminescence signal from three replicate wells exceeded a cutoff threshold determined for each plate. The threshold was set at the mean plus three SDs from the mean of the six normal blood samples on each plate. Thus, samples with a ratio of greater than 1 were regarded as reactive. To reduce potential false positive reactions, each sample was tested in two independent assay runs. Only samples that were reactive in both assays were scored as positive. On completion of testing of all samples in duplicate, the results were declared to an independent party and the samples decoded.

Instat (GraphPad Software, La Jolla, USA) was used for all analyses. Unpaired *t* tests, two-tailed, with Welch correction, were used for all comparisons.

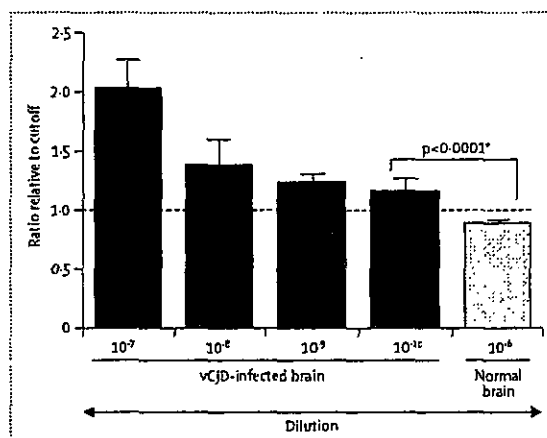


Figure 1: Assay sensitivity to exogenous vCJD

A serial dilution of vCJD prion-infected brain homogenate in whole human blood compared with a baseline control of normal human brain in whole blood; data shown are the mean of six replicates and are expressed as a ratio relative to the cutoff value (three SDs greater than the mean chemiluminescent signal for a normal brain sample; dashed line). Error bars show SDs. vCJD=variant Creutzfeldt-Jakob disease. *Two tailed, unpaired *t* test with Welch correction.

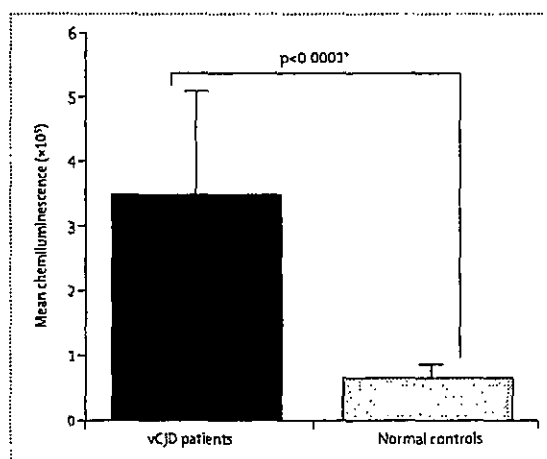


Figure 2: Discrimination of endogenous vCJD blood samples from normal control samples

Data shown are the mean chemiluminescent signal (arbitrary units) of 14 blood samples from individual patients with confirmed vCJD compared with the mean signal from 100 normal controls. Error bars show SDs for the mean signals. vCJD=variant Creutzfeldt-Jakob disease. *Two tailed, unpaired *t* test with Welch correction.

Role of the funding source

The sponsors provided financial support for the research but were not involved in the project design, interpretation of data or drafting of this manuscript. GSJ had full access to all the data in this study and had final responsibility for the decision to submit for publication.

Results

To establish the sensitivity of the assay compared with other methods, we analysed serial dilutions of vCJD brain homogenate diluted into whole blood. Exogenous

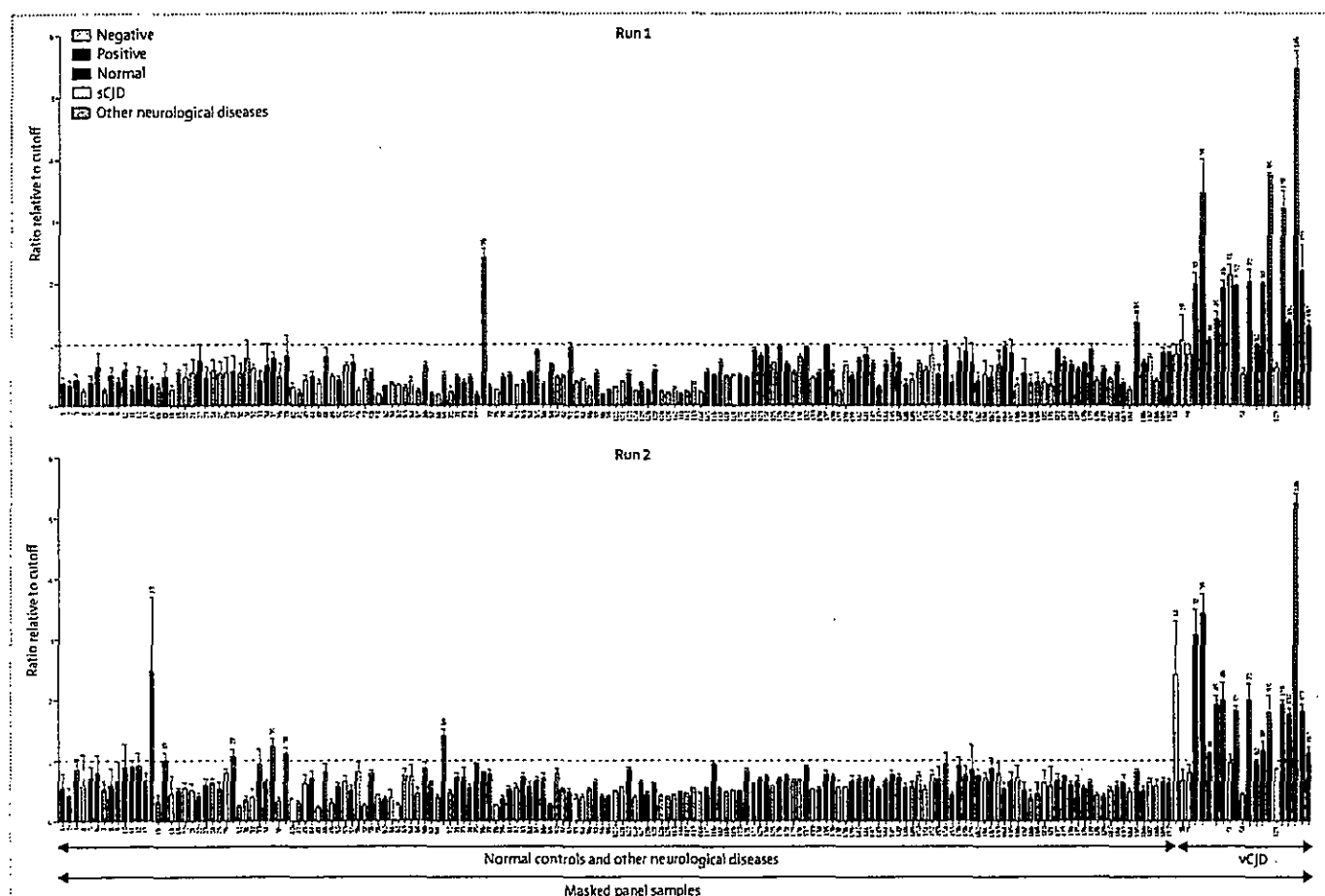


Figure 3: Two independent assay runs for a masked panel of 190 blood samples

The masked panel consisted of blood samples from 21 patients infected with vCJD, 100 healthy normal controls (dark blue bars), 27 patients with sCJD (green bars), and 42 controls with other neurological diseases (turquoise bars). Data are shown as the chemiluminescent signal ratio relative to a cutoff established as the mean of the signal for normal controls plus three SDs. Samples shown in grey are from patients infected with vCJD that had a ratio of less than 1 in one or both assays and were therefore scored as negative. Red bars show all samples that had a ratio greater than 1 relative to cutoff in both assays and were scored as positive. vCJD=variant Creutzfeldt-Jakob disease. sCJD=sporadic Creutzfeldt-Jakob disease.

spikes of vCJD-infected brain homogenate could clearly be distinguished from normal brain homogenate, even at a 10^{10} -fold dilution (figure 1), a sensitivity more than four orders of magnitude higher than previously achieved for immunoassay of vCJD tissue.²⁶ A chemiluminescent signal of mean 1.3×10^5 (SD 1.1×10^4) was obtained with 10^{-10} dilution of vCJD-infected brain versus 9.9×10^4 (SD 4.5×10^3) for a 10^{-6} dilution of normal control brain, corresponding to a ratio relative to the cutoff threshold of 1.2 (SD 0.1). The difference was highly significant ($p < 0.0001$). The highest dilution of vCJD brain could still be distinguished from the normal control when a threshold of mean normal signals plus three SDs was applied.

Initial studies of exogenous spikes of vCJD-infected brain homogenate in whole human blood showed that our assay was capable of discriminating between infected and non-infected samples with a sensitivity theoretically

sufficient to detect infection in blood samples from patients with vCJD, on the basis of estimates of titre obtained from rodent models.^{24,25} However, the biochemical nature of infectivity and abnormal PrP associated with blood is unknown. To ensure that this level of discrimination could be achieved with endogenous blood samples, we tested a subset of blood samples from 14 patients with confirmed vCJD from whom larger volumes of blood were available, and compared these with normal control blood samples (figure 2). The samples were analysed as groups and had mean chemiluminescent signals that were significantly different (normal, mean 65 693 [SD 20 538] vs vCJD, mean 348 842 [160 721]; $p < 0.0001$).

We then tested the masked panel of 190 samples from 21 patients with confirmed vCJD (including the 14 tested above), 69 patients with other neurological disease, and 100 normal healthy controls. 19 samples from the panel

were reactive in assay run 1, and 22 in run 2 (figure 3). A subset of 15 samples gave signals greater than the cutoff threshold in both assays and were hence scored as positive. Subsequently, all 15 samples were decoded as true positives obtained from patients with vCJD, showing an assay sensitivity of 15/21 or 71.4% (exact 95% CI 47.8–88.7).

Samples from six patients with vCJD did not score positive during testing of the masked panel. Of those samples, three had been reactive in either assay run 1 or 2 (figure 3), but did not react in both assays. The three remaining samples were scored as negative in both of the test runs and were indistinguishable from normal controls. Of the controls, eight samples gave single reactions (two in assay run 1, six in run 2). These eight reactions all came from normal controls obtained from the NBTS, with no reactions seen from sporadic CJD samples or samples from other neurological diseases. No samples other than those from patients with vCJD gave repeat reactive results and thus all of the normal and other neurological disease controls were correctly identified as true negatives (169/169), suggesting 100% specificity after two independent assays (exact 95% CIs between 97.8% and 100%).

Discussion

Evidence shows that there is a risk of iatrogenic (secondary) vCJD prion infection from transfusion of blood and blood products^{11–13} and, by inference, from many forms of surgical and dental interventions. Taken together with the existence of subclinical carrier states of prion disease¹⁴ and the long incubation periods for clinical disease,¹⁵ the implications for current and future public health could be substantial. Current risk reduction strategies in the UK, of uncertain efficacy or necessity, are very costly to the National Health Service. These strategies include leucodepletion of all transfused packed red cells, sourcing of plasma from the USA, and importing of blood for transfusion to young children. Studies modelling the use of leucocyte depletion have shown reductions in blood infectivity of between 58% and 72%.^{16,17} The use of prion blood filters is also being considered by the UK Department of Health.¹⁸

Our research efforts have focused on sensitive methods for detection of vCJD prion infection in whole blood, since this sample is not only easily obtained for clinical purposes, but is also the target for protection of the donated blood supply. Application of a capture matrix for enrichment of abnormal PrP and optimised immunodetection of bound material has allowed detection of vCJD brain homogenate diluted 10¹⁰-fold in exogenous spiking experiments (figure 1). This concentration of abnormal PrP is about 100 000 times lower than is encountered during tonsil biopsy and high sensitivity western blotting¹⁹ currently used at the National Prion Clinic for diagnosis of vCJD. It is also 10 000-fold beyond the most sensitive reported

immunoassays for vCJD,²⁰ and is more sensitive than conventional rodent bioassays that typically do not report dilutions of prion-infected brain tissue greater than 10⁴-fold for rodent-adapted scrapie strains, and which are far less sensitive for assay of human vCJD prions because of transmission barrier effects.²¹ Although this finding might at first seem contradictory, rodent bioassays report infectious titre and do not detect PrP molecules directly. The number of PrP monomers that constitute an infectious particle is not defined and indeed is likely to be heterogeneous.²² However, a single infectious unit contains many PrP monomers, as an aggregate all of which are potentially available to our assay, allowing sensitivity beyond that previously achieved.

As a rare disease, blood samples from patients with vCJD are inevitably scarce, and validation of assay results with the high numbers of samples conventionally expected is impossible. We have applied our assay to samples of whole blood that were readily available and for which sufficient volumes were present to prevent significant depletion of this scarce resource. 21 samples were tested, of which 15 were positively identified in our masked panel, yielding a sensitivity of 71%. Although this finding is a very significant step forward in prion diagnostics, it should be interpreted within the context of associated specificity. Indeed, for consideration as a screening assay of blood and tissue donations, specificity is expected to exceed a minimum of 99.85% to avoid large numbers of false positive tests.²³

Our panel contained 100 normal blood samples from the NBTS and 69 neurological disease controls. We included samples obtained from patients with Alzheimer's disease because, in addition to being an important differential diagnosis in suspected CJD, abnormal PrP deposition can accompany amyloid β accumulation in these diseases.²⁴ In addition to Alzheimer's disease, other disorders that form part of the differential diagnosis for prion disease were also included in the panel, including frontotemporal dementia, young onset familial Alzheimer's disease, and cases referred to the National Prion Clinic as suspect cases, but for whom prion disease was later excluded as a diagnosis. Encouragingly, none of this small series of neurological disease controls provided any reactions in either of the two independent assay runs. The single reactive samples were all normal controls obtained from the NBTS. The most likely explanations for occasional reactions in the assay are cross-contamination or incomplete removal of primary antibody during washing. For these reasons, samples were assayed twice and had to be reactive in both assays to be deemed positive. Although no false-positive results were recorded by our assay criteria (0/169), the exact 95% CIs for specificity are 97.8% to 100%. This level of specificity would be acceptable in neurological diagnostic use, but screening of large (>10 000) numbers of negative controls from countries with low BSE prion exposure is necessary to

accurately determine specificity before considering the assay's wider application as a screen of asymptomatic individuals.

Our testing has to date been restricted to samples from patients with clinical vCJD. Serial blood samples are being obtained under the auspices of the National Prion Disease Monitoring Cohort³⁰ from several vCJD prion-exposed at-risk individuals who might become symptomatic in the future and on whom longitudinal clinical and neuroradiological studies are being performed. Such samples could in due course provide data for the stage at which prionemia is detectable by our or other future tests. Notably, evidence from rodent models of prion infection suggest involvement of blood at early preclinical stages.⁴ Extended longitudinal study of individuals testing positive will be needed to establish the proportion of patients with vCJD prionemia that go on to develop clinical vCJD and how many are chronic carriers.⁴¹

Despite these uncertainties, our findings demonstrate the ability to detect prion infection in blood and show that a donor blood screening test is technically feasible. The use of this test in differential diagnosis of suspected vCJD will be further investigated in large case series.

Contributors

JAE and GS designed the experiments with contributions and direction from JC, MF, AS, and PT assisted in development of the methods and analysed all samples with JAE, JB, TC, JL, SM, PR, and JC investigated the patients and obtained, processed, and documented clinical samples. JAE, JC, and GS drafted the report.

Conflicts of interest

JC is a director and JC and GS are shareholders and consultants for D-Gen (London, UK), an academic spinout company working in the field of prion disease diagnosis, decontamination, and therapeutics. D-Gen markets the antibody used in this study. All other authors declare that they have no conflicts of interest.

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医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
一般的名称	—	研究報告の 公表状況		公表国	
販売名(企業名)	—				
研究報告の概要	本情報は2004年5月に発効された「血漿由来製剤の製品概要および添付文書の感染性物質に関する警告の記載」についてのガイドライン(CPMP/BPWG/BWP/561/03)の改訂案で、vCJD関連の更新と添加物としてのアルブミンについても触れている。 血漿分画製剤における注意として記載すべき事項として、次のことをあげている。 ・ 供血者のスクリーニングを実施していること ・ 製造工程中に不活化/除去工程があること ・ 既知又は未知のウイルス、他の感染因子の伝播の可能性は否定できないこと 特に脂質膜を持たないウイルス(HAV、パルボウイルスB19)の潜在的危険性 ・ 投与した際には氏名と製造番号を記録することを推奨すること vCJDについては、2003年に言及すべきかどうか検討されたが、vCJDは複雑で最新の知識も不完全であることから、追記した場合には感染の危険性が増加したとの印象を与えると結論づけられ、2010年にも同様な結論となったことから、感染因子の伝達の可能性を除外することはできないとの一般的な警告を続けるとしている。 添加物としてのアルブミンについては、確立した方法(European Pharmacopoeia specifications)で製造された製剤による感染報告がないことから、特定の警告は必要ないとしている。 西ナイルウイルスについても血漿分画製剤での感染例がないことから、特定の警告は必要ないとしている。				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血液については、HBs抗原、抗HCV抗体、抗HIV抗体(製剤毎の実施項目)・・・抗体陰性で・・・プールした試験血漿については、HIV、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。その後の製造工程(製品毎の不活化/除去工程)・・・は、HIVをはじめとする各種ウイルスに対し、不活化・除去作用を有することが確認されているが、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。 2) 現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病(vCJD)等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。 取扱上の注意 記録の保存：本剤は特定生物由来製品に該当することから、本剤を投与した場合は、医薬品の名称(販売名)、製造番号、投与日、投与を受けた患者の氏名、住所等を記録し、少なくとも20年間保存すること。
	報告企業の意見	今後の対応			
本邦で販売されている血漿分画製剤の添付文書にはスクリーニング項目、不活化/除去工程や、vCJD、パルボウイルスB19を含め感染リスクを完全には否定できない旨を記載している。	今後ともウイルスに関する安全性情報等に留意していく。				

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EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

1 12th January 2011
2 EMA/CHMP/BWP/360642/2010

3 Guideline on the warning on transmissible agents in
4 summary of product characteristics (SmPCs) and package
5 leaflets for plasma-derived medicinal products
6 Draft

Draft Agreed by Biologics Working Party	Dec 2010
Draft Agreed by Blood Products Working party	Dec 2010
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End of consultation (deadline for comments)	30 th April 2011
Agreed by Biologics Working Party	<Month YYYY>
Adoption by Committee for medicinal products for human use	<DD Month YYYY>
Date for coming into effect	<DD Month YYYY> ¹

7
8 This guideline replaces 'Guideline on the warning on transmissible agents in summary of product
9 characteristics (SmPCs) and package leaflets for plasma-derived medicinal products'
10 (CPMP/BPWG/BWP/561/03).

11
12 Comments should be provided using this [template](#). The completed comments form should be sent to
alberto.ganan@ema.europa.eu

Keywords	<i>Warning statements, plasma derived medicinal products, immunoglobulins, albumin, SmPC, package leaflet.</i>
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¹ First day of the 7th month.



14	Guideline on the warning on transmissible agents in	
15	summary of product characteristics (SmPCs) and package	
16	leaflets for plasma-derived medicinal products	
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Executive summary

This guideline provides standard texts for warning statements on transmissible agents to be included in summary of product characteristics (SmPCs) and package leaflets for plasma-derived medicinal products. The original guideline (CPMP/BPWG/BWP/561/03) was adopted by CHMP in October 2003 and came into operation in May 2004. This revision affects only the introduction, where an update related to vCJD and an addition concerning albumin as excipient are now included.

1. Introduction (background)

When medicinal products prepared from human blood or plasma are administered, infectious diseases due to the transmission of infective agents cannot be totally excluded. The measures taken to prevent infection resulting from the use of these products include selection of donors, screening of individual donations and plasma pools for specific markers of infection and the inclusion of effective steps for the inactivation / removal of a wide range of viruses in manufacturing processes.

All these measures are critically evaluated by the relevant Competent Authority(ies) for medicines for the granting and maintenance of the Marketing Authorisation of each plasma-derived medicinal product.

In 1994, CPMP recommended a standard text for the Summary of Product Characteristics (SmPC) and the user Package Leaflet to inform doctors and patients about the risk of transmission of infective agents associated with the administration of any human blood or plasma-derived medicinal products².

This warning text on transmissible agents has been reviewed and updated by the Blood Products Working Group (BPWG) and Biotechnology Working Party (BWP) in the core SmPCs for specific plasma-derived medicinal products approved since June 2000. The text can be modified if certain warnings are not valid for a specific product.

Additionally, since potential safety problems may be batch-related, a strong recommendation to health professionals is included that, every time that a plasma-derived medicinal product is administered to a patient, the name and batch number of the product are recorded in order to maintain a link between the patient and the batch of the product. Patients are also made aware of this recommendation through the warning statement in the user Package Leaflet.

This document updates the previous recommendations and states the warning to be included in the SmPC and Package Leaflet of any plasma-derived medicinal product.

This warning is part of Section 4.4, "Special warnings and special precautions for use" of the SmPC. As indicated by the title of this section, it is intended for clinically important warnings and precautions for use. Therefore, the recommended text should not be extended by other information that is not a warning or precaution (e.g. description of viral inactivation / removal steps or tests for specific viruses)³. The same considerations apply to the user Package Leaflet.

There are two changes in the approach to the warning statement from the previous recommendations. Firstly, reference to specific mandatory measures is removed, as it is not a warning or precaution, the information is available elsewhere⁴, and the important message of the resulting text is clearer. Secondly, the warning gives information on the overall effectiveness of measures for the safety of the product, rather than highlighting whether inactivation / removal procedures can be considered

² CPMP "Background document on medicinal products derived from human blood or plasma", 16 March 1994

³ It is not the purpose of the SmPC or Package Leaflet to give technical details of manufacturing processes. Manufacturing details are not listed in Article 11 or Article 59 with Article 62 of Directive 2001/83/EC, where the information to be included in SmPC and Package Leaflets respectively is specified.

⁴ Mandatory measures are published in the European Pharmacopoeia monograph for Human Plasma for Fractionation.

effective. Focussing on inactivation / removal procedures may be misleading, particularly in the case of B19 (i.e. capable of inactivating / removing several logs of infectivity) but the capacity of the step may be exceeded if there is a high viral load in the plasma pool.

This warning statement should indicate the remaining potential risk of transmitting infective agents by plasma-derived medicinal products. Guidance on assessing the risk of virus transmission is in preparation to support the use of the warning statements in this Note for Guidance.

The warning statements make specific reference to the viruses that have been transmitted in the past by plasma-derived medicinal products. The measures taken to prevent the transmission of enveloped viruses such as HIV, HBV and HCV are considered effective for all marketed products. Non-enveloped viruses, such as HAV and parvovirus B19, are more difficult to inactivate / remove and the effectiveness of measures for non-enveloped viruses differs among marketed products. Therefore, the information given in the SmPC and user Package Leaflet should highlight the remaining potential risk of transmission of the non-enveloped viruses, HAV and parvovirus B19, taking into account the characteristics of the safety measures taken and the results of the viral inactivation / removal studies performed by the Marketing Authorisation Holder.

No specific statement can be made about remaining potential risks with non-enveloped viruses in general. It is an objective, for all plasma-derived medicinal products, to incorporate effective steps for inactivation / removal of a wide range of viruses of diverse physico-chemical characteristics. This would provide some assurance of effectiveness for viruses that are at present unknown or emerging.

West Nile virus (WNV) has recently emerged in North America and has been transmitted by blood components. However, no plasma-derived medicinal product has been implicated in WNV transmission. A CPMP Position Statement on WNV and plasma-derived medicinal products was published in July 2003, which concludes that the steps currently in place are adequate to assure safety of plasma-derived medicinal products with respect to WNV. Considering these factors, no specific reference to WNV is included in the warning statements.

Consideration has been given to whether to include a specific reference to vCJD in the warning statements. Variant CJD is a complex subject, where current knowledge is incomplete. In 2003 it was concluded that inclusion of a specific reference at that time would give the impression that there was increased concern about potential transmissibility by plasma-derived medicinal products when this was not the case. Therefore, it was concluded that it is better to continue with the practice of providing specific information through CPMP Position Statements. This position has been reconfirmed in 2010 in conjunction with the update of the CHMP Position Statement on CJD.

The warning statement will continue to include a general warning that the possibility of transmitting infective agents cannot be totally excluded.

There are no reports of virus infections with albumin manufactured to European Pharmacopoeia specifications by established processes. When albumin is used as excipient in medicinal products, there is no need to include any specific warning statement related to albumin. This is based on the good safety records of human albumin. In these products, human albumin should be declared in the List of excipients.

The following documents can be consulted for further information on plasma-derived medicinal products and transmissible agents:

- Note of Guidance on plasma-derived medicinal products (CPMP/BWP/269/95 rev 3.)
<http://www.ema.europa.eu/pdfs/human/bwp/026995en.pdf>
- Report of EMEA Workshop on viral safety of plasma-derived medicinal products with particular focus on non-enveloped viruses (CPMP/BWP/BPWG/4080/00, 21 March 2001) and Addendum:

- Conclusions and recommendations of the Biotechnology Working Party (BWP) and Ad-hoc Working Group on Blood Products (BPWG) (CPMP/BWP/BPWG/93/01, 28 March 2001)
<http://www.ema.europa.eu/pdfs/human/bwp/408000en.pdf>
- CPMP Position Statement on West Nile Virus and plasma-derived medicinal products (EMA/CPMP/BWP/3752/03)
<http://www.ema.europa.eu/pdfs/human/bwp/375203en.pdf>
 - CPMP Position Statement on Creutzfeldt-Jakob disease and plasma-derived and urine-derived medicinal products (EMA/CPMP/BWP/2879/02)
<http://www.ema.europa.eu/pdfs/human/press/pos/287902en.pdf>
 - Investigation of Manufacturing Processes for Plasma-Derived Medicinal Products with regard to vCJD risk (EMA/CPMP/BWP/55136/03)
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003741.pdf
 - European Public Assessment Reports for plasma-derived medicinal products authorised under the Centralised Procedure
<http://www.ema.europa.eu / Home / Find medicine / Human medicines>

2. Warning on transmissible agents in SmPCs for plasma-derived medicinal products

In the following recommendations for the warning in section 4.4 "Special warnings and special precautions for use", the choice of text indicated between < > depends on whether the measures taken are considered effective for the specified virus.

2.1. Plasma-derived medicinal products (except immunoglobulins and albumin)

"Standard measures to prevent infections resulting from the use of medicinal products prepared from human blood or plasma include selection of donors, screening of individual donations and plasma pools for specific markers of infection and the inclusion of effective manufacturing steps for the inactivation / removal of viruses. Despite this, when medicinal products prepared from human blood or plasma are administered, the possibility of transmitting infective agents cannot be totally excluded. This also applies to unknown or emerging viruses and other pathogens.

The measures taken are considered effective for enveloped viruses such as HIV, HBV and HCV

<, and for the non-enveloped virus<es> <HAV><and parvovirus B19>.>

<The measures taken may be serious for pregnant women (fetal infection) and for individuals with immunodeficiency or increased erythropoiesis (e.g. haemolytic anaemia).>⁵

It is strongly recommended that every time that {name product} is administered to a patient, the name and batch number of the product are recorded in order to maintain a link between the patient and the batch of the product.

Examples:

⁵ Note: the statement about parvovirus B19 risk groups does not need to be included for products where the measures are considered effective for B19.

162 *Measures effective for HAV and parvovirus B19*

163 "The measures taken are considered effective for enveloped viruses such as HIV, HBV and HCV, and
164 for the non-enveloped viruses HAV and parvovirus B19."

165 *Measures effective for HAV but not parvovirus B19*

166 "The measures taken are considered effective for enveloped viruses such as HIV, HBV and HCV, and
167 for the non-enveloped virus HAV. The measures taken may be of limited value against non-enveloped
168 viruses such as parvovirus B19. Parvovirus B19 infection may be serious for pregnant women (fetal
169 infection) and for individuals with immunodeficiency or increased erythropoiesis (e.g. haemolytic
170 anaemia)."

171 *Measures not effective for HAV or parvovirus B19*

172 "The measures taken are considered effective for enveloped viruses such as HIV, HBV and HCV. The
173 measures taken may be of limited value against non-enveloped viruses such as HAV and parvovirus
174 B19. Parvovirus B19 infection may be serious for pregnant women (fetal infection) and for individuals
175 with immunodeficiency or increased erythropoiesis (e.g. haemolytic anaemia)."

176 ***2.2. Additional text for plasma-derived medicinal products***
177 ***regularly/repeatedly administered except immunoglobulins***

178 For coagulation factor products, antithrombin products, and other plasma-derived medicinal products
179 regularly/repeatedly administered except immunoglobulins, include the following additional text before
180 the final sentence on recording name and batch number of the product:

181 "Appropriate vaccination (hepatitis A and B) should be considered for patients in regular/repeated
182 receipt of human plasma-derived {product class e.g. factor VIII products, antithrombin products}."

183 ***2.3. Immunoglobulins***

184 "Standard measures to prevent infections resulting from the use of medicinal products prepared from
185 human blood or plasma include selection of donors, screening of individual donations and plasma pools
186 for specific markers of infection and the inclusion of effective manufacturing steps for the inactivation /
187 removal of viruses. Despite this, when medicinal products prepared from human blood or plasma are
188 administered, the possibility of transmitting infective agents cannot be totally excluded. This also
189 applies to unknown or emerging viruses and other pathogens.

190 The measures taken are considered effective for enveloped viruses such as HIV, HBV and HCV

191 <, and for the non-enveloped virus<es> <HAV><and parvovirus B19>.>

192 <The measures taken may be of limited value against non-enveloped viruses such as <HAV> <and>
193 <parvovirus B19>.

194 There is reassuring clinical experience regarding the lack of hepatitis A or parvovirus B19 transmission
195 with immunoglobulins and it is also assumed that the antibody content makes an important
196 contribution to the viral safety.

197 It is strongly recommended that every time that {name of the product} is administered to a patient,
198 the name and batch number of the product are recorded in order to maintain a link between the
199 patient and the batch of the product."

2.4. Albumin

"Standard measures to prevent infections resulting from the use of medicinal products prepared from human blood or plasma include selection of donors, screening of individual donations and plasma pools for specific markers of infection and the inclusion of effective manufacturing steps for the inactivation/removal of viruses. Despite this, when medicinal products prepared from human blood or plasma are administered, the possibility of transmitting infective agents cannot be totally excluded. This also applies to unknown or emerging viruses and other pathogens.

There are no reports of virus transmissions with albumin manufactured to European Pharmacopoeia specifications by established processes.

It is strongly recommended that every time that {name of the product} is administered to a patient, the name and batch number of the product are recorded in order to maintain a link between the patient and the batch of the product."

3. Text for section 4.8 Undesirable effects in SmPCs for plasma-derived medicinal products

"For safety with respect to transmissible agents, see 4.4."

4. Warning on transmissible agents in the package leaflets for plasma-derived medicinal products

A warning statement compatible with the text in the SPC is included in Section 2 **Before you take** {name of the product}.

In the following recommendations, the choice of text indicated between < > depends on whether the measures taken are considered effective for the specified virus.

4.1. Plasma-derived medicinal products (except immunoglobulins and albumin)

<Rapporteur to include text>

"When medicines are made from human blood or plasma, certain measures are put in place to prevent infections being passed on to patients. These include careful selection of blood and plasma donors to make sure those at risk of carrying infections are excluded, and the testing of each donation and pools of plasma for signs of virus/infections. Manufacturers of these products also include steps in the processing of the blood or plasma that can inactivate or remove viruses. Despite these measures, when medicines prepared from human blood or plasma are administered, the possibility of passing on infection cannot be totally excluded. This also applies to any unknown or emerging viruses or other types of infections.

The measures taken are considered effective for enveloped viruses such as human immunodeficiency virus (HIV), hepatitis B virus and hepatitis C virus

<, and for the non-enveloped <hepatitis A >< and parvovirus B19> virus<es>.>

<The measures taken may be of limited value against non-enveloped viruses <such as> <hepatitis A virus> <and > <parvovirus B19>.

237 <Parvovirus B19 infection may be serious for pregnant women (fetal infection) and for individuals
238 whose immune system is depressed or who have some types of anaemia (e.g. sickle cell disease or
239 haemolytic anaemia).>⁶

240 It is strongly recommended that every time you receive a dose of {name of product} the name and
241 batch number of the product are recorded in order to maintain a record of the batches used."

242 Examples:

243 *Measures effective for HAV and parvovirus B19:*

244 "The measures taken are considered effective for enveloped viruses such as human immunodeficiency
245 virus (HIV), hepatitis B virus and hepatitis C virus, and for the non-enveloped hepatitis A and
246 parvovirus B19 viruses."

247 *Measures effective for HAV but not parvovirus B19:*

248 "The measures taken are considered effective for enveloped viruses such as human immunodeficiency
249 virus (HIV), hepatitis B virus and hepatitis C virus, and for the non-enveloped hepatitis A virus. The
250 measures taken may be of limited value against non-enveloped viruses such as parvovirus B19.
251 Parvovirus B19 infection may be serious for pregnant women (fetal infection) and for individuals whose
252 immune system is depressed or who have some types of anaemia (e.g. sickle cell disease or
253 haemolytic anaemia)."

254 *Measures not effective for HAV or parvovirus B19:*

255 "The measures taken are considered effective for enveloped viruses such as human immunodeficiency
256 virus (HIV), hepatitis B virus and hepatitis C virus. The measures taken may be of limited value against
257 non-enveloped viruses such as hepatitis A virus and parvovirus B19. Parvovirus B19 infection may be
258 serious for pregnant women (fetal infection) and for individuals whose immune system is depressed or
259 who have some types of anaemia (e.g. sickle cell disease or haemolytic anaemia)."

260 **4.2. Additional text for plasma-derived medicinal products**
261 **regularly/repeatedly administered except immunoglobulins**

262 For coagulation factor products, antithrombin products, and other plasma-derived medicinal products
263 regularly/repeatedly administered except immunoglobulins, include the following additional text before
264 the final sentence on recording name and batch number of the product:

265 "Your doctor may recommend that you consider vaccination against hepatitis A and B if you
266 regularly/repeatedly receive human plasma-derived {product class e.g. Factor VIII products,
267 antithrombin products}."

268 **4.3. Immunoglobulins**

269 "When medicines are made from human blood or plasma, certain measures are put in place to prevent
270 infections being passed on to patients. These include careful selection of blood and plasma donors to
271 make sure those at risk of carrying infections are excluded, and the testing of each donation and pools
272 of plasma for signs of virus/infections. Manufacturers of these products also include steps in the
273 processing of the blood or plasma that can inactivate or remove viruses. Despite these measures,
274 when medicines prepared from human blood or plasma are administered, the possibility of passing on

⁶ Note: The statement about parvovirus B19 risk groups does not need to be included for products where the measures are considered effective for B19.

infection cannot be totally excluded. This also applies to any unknown or emerging viruses or other types of infections.

The measures taken are considered effective for enveloped viruses such as human immunodeficiency virus (HIV), hepatitis B virus and hepatitis C virus

<, and for the non-enveloped <hepatitis A >< and parvovirus B19> virus<es>.>

<The measures taken may be of limited value against non-enveloped viruses <such as> <hepatitis A virus> <and > <parvovirus B19>.

Immunoglobulins have not been associated with hepatitis A or parvovirus B19 infections possibly because the antibodies against these infections, which are contained in the product, are protective.

It is strongly recommended that every time you receive a dose of {name of product} the name and batch number of the product are recorded in order to maintain a record of the batches used."

4.4. Albumin

"When medicines are made from human blood or plasma, certain measures are put in place to prevent infections being passed on to patients. These include careful selection of blood and plasma donors to make sure those at risk of carrying infections are excluded, and the testing of each donation and pools of plasma for signs of virus/infections. Manufacturers of these products also include steps in the processing of the blood or plasma that can inactivate or remove viruses. Despite these measures, when medicines prepared from human blood or plasma are administered, the possibility of passing on infection cannot be totally excluded. This also applies to any unknown or emerging viruses or other types of infections.

There are no reports of virus infections with albumin manufactured to European Pharmacopoeia specifications by established processes.

It is strongly recommended that every time you receive a dose of {name of product} the name and batch number of the product are recorded in order to maintain a record of the batches used."

5. Implementation of this note for guidance

The warning statements may be used before the date for coming into operation of this Note for guidance.

5.1. Authorised products

In the case of albumin, no supporting data on the risk assessment for virus transmission are needed to support variation applications to update the product information to include the revised warning statement.

For all other plasma-derived medicinal products, the risk assessment and data to support claims that measures taken are considered effective for HAV and/or parvovirus B19 should be provided in support of a variation application to update the product information. Guidance on assessing the risk of virus transmission is in preparation to support the use of the warning statements. If no claims are made that measures taken are considered effective for HAV and/or parvovirus B19, no supporting data on the risk assessment for virus transmission are needed.

312 **5.2. Application for Marketing Authorisation**

313 See the guidance on assessing the risk of virus transmission for the risk assessments that should be
314 provided.

315

B 個別症例報告概要

- 総括一覧表
- 報告リスト

個別症例報告のまとめ方について

個別症例報告が添付されているもののうち、個別症例報告の重複を除いたものを一覧表の後に添付した（国内症例については、資料3において集積報告を行っているため、添付していない）。

感染症定期報告の報告状況(2011/3/1～2011/6/30)

血対ID	受理日	番号	報告者名	一般名	生物由来成分名	原材料名	原産国	含有区分	文献	症例	適正措置報告
100302	2011/3/18	101028	バクスター	乾燥人血液凝固因子抗体迂回活性化複合体	乾燥人血液凝固因子抗体迂回活性化複合体	人血漿	米国	有効成分	なし	なし	なし
100303	2011/3/18	101029	バクスター	乾燥濃縮人血液凝固第Ⅷ因子	人血清アルブミン	人血漿	米国	添加物	なし	あり	なし
100304	2011/3/18	101030	バクスター	乾燥濃縮人血液凝固第Ⅷ因子	乾燥人血液凝固第Ⅷ因子	人血漿	米国	有効成分	なし	あり	なし
100317	2011/3/29	101093	CSLベーリング	人血清アルブミン 人血液凝固第Ⅲ因子 フィブリノゲン加第Ⅲ因子	人血清アルブミン	ヒト血液	米国、ドイツ、オーストリア	有効成分 添加物	あり	あり	なし
100329	2011/4/20	110078	日本赤十字社	人血小板濃厚液	人血小板濃厚液	人血液	日本	有効成分	あり	あり	なし
100342	2011/4/22	110107	CSLベーリング	抗破傷風人免疫グロブリン	破傷風抗毒素	ヒト血液	米国、ドイツ、オーストリア	有効成分	あり	あり	なし
100376	2011/6/29	110255	バクスター	乾燥イオン交換樹脂処理人免疫グロブリン	人血清アルブミン	人血漿	米国	添加物	なし	あり	なし
100377	2011/6/29	110256	バクスター	乾燥イオン交換樹脂処理人免疫グロブリン	人免疫グロブリンG	人血漿	米国	有効成分	なし	あり	なし

番号		感染症の種類		発現国	性別	年齢 (歳)	発現時期 (年/月/日)	転帰	出典	区分	識別番号	報告日	備考	
器官別大分類	基本語	MedDRA (Ver.)												
第16回	16-1	臨床検査	HIV検査陽性	ブラジル	男性	不明	1992	不明	症例報告	外国製品	10000021	2010/10/06	13.1	
第16回	16-1	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	10000021	2010/10/06	13.1	
第16回	16-2	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	2009	不明	症例報告	外国製品	10000023	2010/10/18	13.1	
第16回	16-3	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	2008	不明	症例報告	外国製品	10000024	2010/10/18	13.1	
第16回	16-4	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	2008	不明	症例報告	外国製品	10000025	2010/10/18	13.1	
第15回	13-2	感染症および寄生虫症	C型肝炎	アメリカ	男性	不明	1999	不明	症例報告	外国製品	09000005	2010/5/27	13.1	第15回症例番号13-2は第13回症例報告番号13-2において報告した
第15回	15-1	臨床検査	HIV検査陽性	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	03000030	2010/3/30	13.1	
第15回	15-2	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	10000010	2010/6/23	13.1	
第15回	15-2	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	10000010	2010/6/23	13.1	
第14回	13-1	感染症および寄生虫症	HIV感染	フランス	男性	49	1983	不明	症例報告	外国製品	08000041	2010/1/19	12.1	第14回症例番号13-1は前回報告における第13回症例番号13-1において報告したものの追加報告
第14回	13-1	感染症および寄生虫症	A型肝炎	フランス	男性	49	1996/5/7	不明	症例報告	外国製品	08000041	2010/1/19	12.1	第14回症例番号13-1は前回報告における第13回症例番号13-1において報告したものの追加報告
第14回	13-1	感染症および寄生虫症	B型肝炎	フランス	男性	49	2003/2/24	不明	症例報告	外国製品	08000041	2010/1/19	12.1	第14回症例番号13-1は前回報告における第13回症例番号13-1において報告したものの追加報告
第14回	13-1	感染症および寄生虫症	C型肝炎	フランス	男性	49	2003/2/24	不明	症例報告	外国製品	08000041	2010/1/19	12.1	第14回症例番号13-1は前回報告における第13回症例番号13-1において報告したものの追加報告
第13回	13-1	感染症および寄生虫症	HIV感染	フランス	男性	49	不明	不明	症例報告	外国製品	08000041	2009/3/18	12.1	
第13回	13-1	感染症および寄生虫症	C型肝炎	フランス	男性	49	1996	不明	症例報告	外国製品	08000041	2009/3/18	12.1	
第13回	13-2	感染症および寄生虫症	C型肝炎	アメリカ	男性	不明	1999	不明	症例報告	外国製品	09000005	2009/5/18	12.1	
第12回	12-1	感染症および寄生虫症	C型肝炎	アメリカ	男性	不明	不明	死亡	症例報告	外国製品	08000023	2008/10/27	11.0	
第12回	12-2	感染症および寄生虫症	急性HIV感染	アメリカ	男性	不明	不明	死亡	症例報告	外国製品	08000023	2008/10/27	11.0	
第11回	5-231	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000274	2008/4/21	11.0	第11回症例番号5-231は第5回症例番号5-231において報告したものの追加報告
第11回	5-231	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000274	2008/4/21	11.0	第11回症例番号5-231は第5回症例番号5-231において報告したものの追加報告
第10回	10-1	臨床検査	C型肝炎ウイルス	ブラジル	男性	小児	2004/5/25	不明	症例報告	外国製品	07000015	2007/10/29	10.1	
第10回	10-1	臨床検査	C型肝炎ウイルス	ブラジル	男性	小児	2004/5/25	不明	症例報告	外国製品	07000015	2007/12/28	10.1	追加報告
第10回	10-2	感染症および寄生虫症	急性HIV感染	アメリカ	男性	34	不明	不明	症例報告	外国製品	07000017	2007/12/6	10.1	
第10回	10-2	臨床検査	C型肝炎ウイルス	アメリカ	男性	34	不明	不明	症例報告	外国製品	07000017	2007/12/6	10.1	
第10回	10-3	感染症および寄生虫症	C型肝炎	ベルギー	男性	不明	1991	未回復	症例報告	外国製品	07000028	2008/2/25	10.1	
第9回	0*	0	0	0	0	0	0	0	0	0	0	0	0	* 当該調査期間に対象となる感染症報告はなかった
第8回	7-012	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	11	2006/5/2	不明	症例報告	外国製品	06000019	2006/9/1	9.0	第8回症例番号7-012は第7回症例番号7-012において報告したものの追加報告
第8回	7-012	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	11	2006/5/2	不明	症例報告	外国製品	06000019	2006/9/25	9.0	第8回症例番号7-012は第7回症例番号7-012において報告したものの追加報告

番号		感染症の種類		発現国	性別	年齢 (歳)	発現時期 (年/月/日)	転帰	出典	区分	識別番号	報告日	備考	
		器官別大分類	基本語									MedDRA (Ver.)		
第8回	7-012	臨床検査	ウイルス負荷増加	アルゼンチン	男性	11	2006/5/2	不明	症例報告	外国製品	06000019	2006/9/25	9.0	第8回症例番号7-012は第7回症例番号7-012において報告したものの追加報告
第7回	7-022	感染症および寄生虫症	A型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000648	2006/3/3	8.1	
第7回	7-007	感染症および寄生虫症	A型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	06000013	2006/5/15	9.0	
第7回	7-023	臨床検査	A型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000649	2006/3/3	8.1	
第7回	7-021	感染症および寄生虫症	B型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000647	2006/3/3	8.1	
第7回	7-001	感染症および寄生虫症	B型肝炎	イギリス	男性	24	不明	不明	症例報告	外国製品	06000007	2006/5/1	9.0	
第7回	7-002	感染症および寄生虫症	B型肝炎	イギリス	男性	9	不明	不明	症例報告	外国製品	06000009	2006/5/10	9.0	
第7回	7-008	感染症および寄生虫症	B型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	06000011	2006/5/10	9.0	
第7回	7-007	感染症および寄生虫症	B型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	06000013	2006/5/15	9.0	
第7回	7-006	感染症および寄生虫症	B型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	06000018	2006/5/22	9.0	
第7回	7-023	臨床検査	B型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000649	2006/3/3	8.1	
第7回	7-024	臨床検査	B型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000650	2006/3/3	8.1	
第7回	7-011	感染症および寄生虫症	C型肝炎	台湾	男性	不明	不明	不明	症例報告	外国製品	05000635	2006/3/2	8.1	
第7回	7-009	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000637	2006/3/3	8.1	
第7回	7-013	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000638	2006/3/3	8.1	
第7回	7-014	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000639	2006/3/3	8.1	
第7回	7-015	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000640	2006/3/3	8.1	
第7回	7-016	感染症および寄生虫症	C型肝炎	アルゼンチン	女性	不明	不明	不明	症例報告	外国製品	05000641	2006/3/3	8.1	
第7回	7-017	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000642	2006/3/3	8.1	
第7回	7-018	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000643	2006/3/3	8.1	
第7回	7-019	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000644	2006/3/3	8.1	
第7回	7-020	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000645	2006/3/3	8.1	
第7回	7-004	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000646	2006/3/3	8.1	
第7回	7-022	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000648	2006/3/3	8.1	
第7回	7-059	感染症および寄生虫症	C型肝炎	イギリス	男性	55	不明	不明	症例報告	外国製品	06000006	2006/5/1	9.0	
第7回	7-001	感染症および寄生虫症	C型肝炎	イギリス	男性	24	不明	不明	症例報告	外国製品	06000007	2006/5/1	9.0	
第7回	7-060	感染症および寄生虫症	C型肝炎	アメリカ	男性	不明	不明	不明	症例報告	外国製品	06000008	2006/5/1	9.0	
第7回	7-002	感染症および寄生虫症	C型肝炎	イギリス	男性	9	不明	不明	症例報告	外国製品	06000009	2006/5/10	9.0	
第7回	7-003	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	06000010	2006/5/10	9.0	
第7回	7-008	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	06000011	2006/5/10	9.0	
第7回	7-007	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	06000013	2006/5/15	9.0	
第7回	7-061	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	06000014	2006/5/15	9.0	
第7回	7-010	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	死亡	症例報告	外国製品	06000015	2006/5/15	9.0	
第7回	7-005	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	06000016	2006/5/15	9.0	
第7回	7-062	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	06000017	2006/5/15	9.0	
第7回	7-006	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	06000018	2006/5/22	9.0	
第7回	7-012	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	11	2006/5/2	不明	症例報告	外国製品	06000019	2006/6/16	9.0	
第7回	5-130	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000065	2006/3/30	9.0	第7回症例番号5-130は第5回症例番号5-130と重複症例のため報告放棄
第7回	5-139	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000104	2006/3/2	8.1	第7回症例番号5-139は第5回症例番号5-139において報告したものの追加報告
第7回	7-023	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000649	2006/3/3	8.1	
第7回	7-024	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000650	2006/3/3	8.1	
第7回	7-025	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000651	2006/3/3	8.1	
第7回	7-026	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000652	2006/3/3	8.1	
第7回	7-027	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000653	2006/3/3	8.1	

感染症の種類														備考	
番号	器官別大分類	基本語	発現国	性別	年齢 (歳)	発現時期 (年/月/日)	転帰	出典	区分	識別番号	報告日	MedDRA (Ver.)			
第7回	7-028	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000654	2006/3/3	8.1		
第7回	7-029	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000655	2006/3/3	8.1		
第7回	7-030	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000656	2006/3/3	8.1		
第7回	7-031	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000657	2006/3/3	8.1		
第7回	7-032	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000658	2006/3/3	8.1		
第7回	7-033	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000659	2006/3/3	8.1		
第7回	7-034	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000660	2006/3/3	8.1		
第7回	7-035	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000661	2006/3/13	8.1		
第7回	7-036	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000662	2006/3/13	8.1		
第7回	7-037	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000663	2006/3/13	8.1		
第7回	7-038	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000664	2006/3/13	8.1		
第7回	7-039	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000665	2006/3/13	8.1		
第7回	7-040	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000666	2006/3/13	8.1		
第7回	7-041	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000667	2006/3/13	8.1		
第7回	7-042	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000668	2006/3/13	8.1		
第7回	7-043	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000669	2006/3/13	8.1		
第7回	7-044	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000670	2006/3/13	8.1		
第7回	7-045	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000671	2006/3/13	8.1		
第7回	7-046	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000672	2006/3/13	8.1		
第7回	7-047	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000673	2006/3/13	8.1		
第7回	7-048	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000674	2006/3/13	8.1		
第7回	7-049	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000675	2006/3/13	8.1		
第7回	7-050	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000676	2006/3/13	8.1		
第7回	7-051	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000677	2006/3/13	8.1		
第7回	7-052	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000678	2006/3/13	8.1		
第7回	7-053	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000679	2006/3/13	8.1		
第7回	7-054	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000680	2006/3/13	8.1		
第7回	7-055	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000681	2006/3/13	8.1		
第7回	7-056	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000682	2006/3/13	8.1		
第7回	7-057	臨床検査	C型肝炎ウイルス	チリ	男性	不明	不明	不明	症例報告	外国製品	05000683	2006/3/13	8.1		
第7回	7-058	臨床検査	C型肝炎ウイルス	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000684	2006/3/13	8.1		
第7回	5-139	感染症および寄生虫症	HIV感染	香港	男性	不明	1985	死亡	症例報告	外国製品	05000104	2006/3/2	8.1	第7回症例番号5-139は第5回症例番号5-139において報告したものの追加報告	
第7回	7-001	感染症および寄生虫症	HIV感染	イギリス	男性	24	1985	不明	症例報告	外国製品	06000007	2006/5/1	9.0		
第7回	7-002	感染症および寄生虫症	HIV感染	イギリス	男性	9	1985	不明	症例報告	外国製品	06000009	2006/5/10	9.0		
第7回	7-003	感染症および寄生虫症	HIV感染	イギリス	男性	不明	1985/10/4	不明	症例報告	外国製品	06000010	2006/5/10	9.0		
第7回	7-004	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1986	不明	症例報告	外国製品	05000646	2006/3/3	8.1		
第7回	7-005	感染症および寄生虫症	HIV感染	イギリス	男性	不明	1986	不明	症例報告	外国製品	06000016	2006/5/15	9.0		
第7回	7-006	感染症および寄生虫症	HIV感染	イギリス	男性	不明	1986	不明	症例報告	外国製品	06000018	2006/5/22	9.0		
第7回	7-007	感染症および寄生虫症	HIV感染	イギリス	男性	不明	1986/3	不明	症例報告	外国製品	06000013	2006/5/15	9.0		
第7回	7-008	感染症および寄生虫症	HIV感染	イギリス	男性	不明	1986/4/9	不明	症例報告	外国製品	06000011	2006/5/10	9.0		
第7回	7-009	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1988	不明	症例報告	外国製品	05000637	2006/3/3	8.1		
第7回	7-010	感染症および寄生虫症	HIV感染	イギリス	男性	不明	1988/5	死亡	症例報告	外国製品	06000015	2006/5/15	9.0		
第7回	7-011	感染症および寄生虫症	HIV感染	台湾	男性	不明	1997/4/17	不明	症例報告	外国製品	05000635	2006/3/2	8.1		
第7回	5-130	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000065	2006/3/30	9.0	第7回症例番号5-130は第5回症例番号5-130と重複症例のため報告破棄	
第7回	7-013	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000638	2006/3/3	8.1		
第7回	7-020	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000645	2006/3/3	8.1		

感染症発生症例一覧

番号		感染症の種類		発現国	性別	年齢 (歳)	発現時期 (年/月/日)	転帰	出典	区分	識別番号	報告日	備考	
第何回	番号	器官別大分類	基本語										MedDRA (Ver.)	
第7回	7-021	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000647	2006/3/3	8.1	
第7回	7-044	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000670	2006/3/13	8.1	
第7回	7-045	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000671	2006/3/13	8.1	
第7回	7-046	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000672	2006/3/13	8.1	
第7回	7-047	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000673	2006/3/13	8.1	
第7回	7-048	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000674	2006/3/13	8.1	
第7回	7-049	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000675	2006/3/13	8.1	
第7回	7-057	感染症および寄生虫症	HIV感染	チリ	男性	不明	不明	死亡	症例報告	外国製品	05000683	2006/3/13	8.1	
第7回	7-058	感染症および寄生虫症	HIV感染	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000684	2006/3/13	8.1	
第6回	6-126	感染症および寄生虫症	A型肝炎	アルゼンチン	男性	不明	不明	回復	症例報告	外国製品	05000534	2006/2/8	8.1	
第6回	6-148	感染症および寄生虫症	A型肝炎	アルゼンチン	男性	不明	不明	回復	症例報告	外国製品	05000559	2006/2/13	8.1	
第6回	6-153	感染症および寄生虫症	A型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000565	2006/2/13	8.1	
第6回	6-159	感染症および寄生虫症	A型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000587	2006/2/16	8.1	
第6回	6-161	感染症および寄生虫症	A型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000589	2006/2/16	8.1	
第6回	6-032	感染症および寄生虫症	B型肝炎	アルゼンチン	男性	14	1994	不明	症例報告	外国製品	05000458	2005/10/28	8.1	
第6回	4-06	感染症および寄生虫症	B型肝炎	イギリス	男性	11	不明	不明	症例報告	外国製品	04000081	2005/9/16	8.1	
第6回	5-136	感染症および寄生虫症	B型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000100	2005/10/27	8.0	
第6回	5-101	感染症および寄生虫症	B型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000404	2005/10/21	8.1	
第6回	6-059	感染症および寄生虫症	B型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000453	2005/10/25	8.1	
第6回	6-087	感染症および寄生虫症	B型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000490	2005/12/20	8.1	
第6回	6-146	感染症および寄生虫症	B型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000558	2006/2/13	8.1	
第6回	6-013	感染症および寄生虫症	B型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000567	2006/2/13	8.1	
第6回	6-002	感染症および寄生虫症	B型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000569	2006/2/13	8.1	
第6回	6-003	感染症および寄生虫症	B型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000585	2006/2/16	8.1	
第6回	6-163	感染症および寄生虫症	B型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000591	2006/2/16	8.1	
第6回	6-166	感染症および寄生虫症	B型肝炎	ペルー	男性	不明	不明	不明	症例報告	外国製品	05000598	2006/2/16	8.1	
第6回	6-176	感染症および寄生虫症	B型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000613	2006/2/22	8.1	
第6回	6-007	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	1985	不明	症例報告	外国製品	05000571	2006/2/13	8.1	
第6回	6-015	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	1986	不明	症例報告	外国製品	05000619	2006/2/22	8.1	
第6回	6-025	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	1990	不明	症例報告	外国製品	05000536	2006/2/8	8.1	
第6回	6-026	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	1990	不明	症例報告	外国製品	05000537	2006/2/8	8.1	
第6回	6-032	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	14	1994	不明	症例報告	外国製品	05000458	2005/10/28	8.1	
第6回	6-035	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	32	1995/5/24	不明	症例報告	外国製品	05000607	2006/2/22	8.1	
第6回	6-037	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	2001	不明	症例報告	外国製品	05000562	2006/2/13	8.1	
第6回	6-038	感染症および寄生虫症	C型肝炎	アルゼンチン	女性	不明	2003	不明	症例報告	外国製品	05000628	2006/2/24	8.1	
第6回	4-06	感染症および寄生虫症	C型肝炎	イギリス	男性	11	不明	不明	症例報告	外国製品	04000081	2005/9/16	8.1	
第6回	5-101	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000404	2005/10/21	8.1	
第6回	6-045	感染症および寄生虫症	C型肝炎	ベネズエラ	男性	不明	不明	死亡	症例報告	外国製品	05000439	2005/9/9	8.0	
第6回	6-049	感染症および寄生虫症	C型肝炎	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000443	2005/9/14	8.0	
第6回	6-050	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	41	不明	不明	症例報告	外国製品	05000444	2005/9/14	8.0	
第6回	6-051	感染症および寄生虫症	C型肝炎	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000445	2005/9/14	8.0	

番号		感染症の種類		発現国	性別	年齢 (歳)	発現時期 (年/月/日)	転帰	出典	区分	識別番号	報告日	備考	
第6回	番号	器官別大分類	基本語										MedDRA (Ver.)	
第6回	6-052	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000446	2005/9/14	8.0	
第6回	6-054	感染症および寄生虫症	C型肝炎	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000448	2005/9/16	8.0	
第6回	6-055	感染症および寄生虫症	C型肝炎	アメリカ	男性	不明	不明	不明	症例報告	当該製品	05000449	2005/9/22	8.1	
第6回	6-056	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000450	2005/10/4	8.1	
第6回	6-057	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000451	2005/10/19	8.1	
第6回	6-058	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000452	2005/10/25	8.1	
第6回	6-059	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000453	2005/10/25	8.1	
第6回	6-060	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000454	2005/10/25	8.1	
第6回	6-061	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	05000455	2005/10/27	8.1	
第6回	6-062	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	24	不明	不明	症例報告	外国製品	05000457	2005/10/27	8.1	
第6回	6-063	感染症および寄生虫症	C型肝炎	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000459	2005/10/28	8.1	
第6回	6-064	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000460	2005/10/28	8.1	
第6回	6-066	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000464	2005/11/2	8.1	
第6回	6-069	感染症および寄生虫症	C型肝炎	コスタリカ	男性	不明	不明	不明	症例報告	外国製品	05000467	2005/11/2	8.1	
第6回	6-070	感染症および寄生虫症	C型肝炎	ドミニカ共和国	男性	不明	不明	不明	症例報告	外国製品	05000468	2005/11/2	8.1	
第6回	6-071	感染症および寄生虫症	C型肝炎	ペルー	不明	不明	不明	不明	症例報告	外国製品	05000469	2005/11/2	8.1	
第6回	6-072	感染症および寄生虫症	C型肝炎	ペルー	男性	不明	不明	不明	症例報告	外国製品	05000470	2005/11/2	8.1	
第6回	6-075	感染症および寄生虫症	C型肝炎	チリ	男性	不明	不明	不明	症例報告	外国製品	05000478	2005/12/2	8.1	
第6回	6-076	感染症および寄生虫症	C型肝炎	チリ	男性	不明	不明	不明	症例報告	外国製品	05000479	2005/12/2	8.1	
第6回	6-077	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	死亡	症例報告	外国製品	05000480	2005/12/2	8.1	
第6回	6-079	感染症および寄生虫症	C型肝炎	チリ	男性	不明	不明	不明	症例報告	外国製品	05000482	2005/12/2	8.1	
第6回	6-080	感染症および寄生虫症	C型肝炎	チリ	男性	不明	不明	死亡	症例報告	外国製品	05000483	2005/12/2	8.1	
第6回	6-086	感染症および寄生虫症	C型肝炎	チリ	男性	不明	不明	死亡	症例報告	外国製品	05000489	2005/12/2	8.1	
第6回	6-087	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000490	2005/12/20	8.1	
第6回	6-088	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000496	2006/2/6	8.1	
第6回	6-125	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000533	2006/2/8	8.1	
第6回	6-126	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	未回復	症例報告	外国製品	05000534	2006/2/8	8.1	
第6回	6-128	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000538	2006/2/8	8.1	
第6回	6-129	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000539	2006/2/8	8.1	
第6回	6-131	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000543	2006/2/10	8.1	
第6回	6-132	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000544	2006/2/10	8.1	
第6回	6-133	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000545	2006/2/10	8.1	
第6回	6-134	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000546	2006/2/10	8.1	
第6回	6-135	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000547	2006/2/10	8.1	
第6回	6-136	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000548	2006/2/10	8.1	
第6回	6-144	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000556	2006/2/13	8.1	
第6回	6-145	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000557	2006/2/13	8.1	
第6回	6-146	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000558	2006/2/13	8.1	
第6回	6-147	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000573	2006/2/14	8.1	
第6回	6-148	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	未回復	症例報告	外国製品	05000559	2006/2/13	8.1	
第6回	6-149	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000560	2006/2/13	8.1	
第6回	6-150	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000561	2006/2/13	8.1	
第6回	6-152	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000564	2006/2/13	8.1	
第6回	6-153	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000565	2006/2/13	8.1	
第6回	6-154	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000566	2006/2/13	8.1	
第6回	6-1013	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000567	2006/2/13	8.1	
第6回	6-155	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000568	2006/2/13	8.1	
第6回	6-157	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000579	2006/2/16	8.1	

番号		感染症の種類		発現国	性別	年齢 (歳)	発現時期 (年/月/日)	転帰	出典	区分	識別番号	報告日	備考	
第6回	番号	器官別大分類	基本語										MedDRA (Ver.)	
第6回	6-158	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000580	2006/2/16	8.1	
第6回	6-001	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000582	2006/2/16	8.1	
第6回	6-159	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000587	2006/2/16	8.1	
第6回	6-160	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000588	2006/2/16	8.1	
第6回	6-161	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000589	2006/2/16	8.1	
第6回	6-162	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000590	2006/2/16	8.1	
第6回	6-163	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000591	2006/2/16	8.1	
第6回	6-014	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000592	2006/2/16	8.1	
第6回	6-018	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000593	2006/2/16	8.1	
第6回	6-019	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000594	2006/2/16	8.1	
第6回	6-004	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000595	2006/2/16	8.1	
第6回	6-164	感染症および寄生虫症	C型肝炎	パラグアイ	男性	不明	不明	不明	症例報告	外国製品	05000596	2006/2/16	8.1	
第6回	6-165	感染症および寄生虫症	C型肝炎	パラグアイ	男性	不明	不明	不明	症例報告	外国製品	05000597	2006/2/16	8.1	
第6回	6-166	感染症および寄生虫症	C型肝炎	ペルー	男性	不明	不明	不明	症例報告	外国製品	05000598	2006/2/16	8.1	
第6回	6-169	感染症および寄生虫症	C型肝炎	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000601	2006/2/21	8.1	
第6回	6-173	感染症および寄生虫症	C型肝炎	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000605	2006/2/21	8.1	
第6回	6-174	感染症および寄生虫症	C型肝炎	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000606	2006/2/21	8.1	
第6回	6-022	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000609	2006/2/22	8.1	
第6回	6-175	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000612	2006/2/22	8.1	
第6回	6-024	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000624	2006/2/24	8.1	
第6回	6-183	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000625	2006/2/24	8.1	
第6回	6-184	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000626	2006/2/24	8.1	
第6回	6-185	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000627	2006/2/24	8.1	
第6回	6-012	感染症および寄生虫症	C型肝炎	台湾	男性	不明	不明	不明	症例報告	外国製品	05000629	2006/2/24	8.1	
第6回	6-010	感染症および寄生虫症	C型肝炎	台湾	男性	不明	不明	不明	症例報告	外国製品	05000630	2006/2/24	8.1	
第6回	6-011	感染症および寄生虫症	C型肝炎	台湾	男性	不明	不明	不明	症例報告	外国製品	05000633	2006/2/24	8.1	
第6回	6-001	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1983	不明	症例報告	外国製品	05000582	2006/2/16	8.1	第6回症例番号5-136は前回報告 における第5回症例番号5-136にお いて報告したものの追加報告 第6回症例番号5-101は前回報告 における第5回症例番号5-101にお いて報告したものの追加報告
第6回	6-002	感染症および寄生虫症	HIV感染	アルゼンチン	不明	不明	1984	不明	症例報告	外国製品	05000569	2006/2/13	8.1	
第6回	6-003	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1984	不明	症例報告	外国製品	05000585	2006/2/16	8.1	
第6回	6-004	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1984	不明	症例報告	外国製品	05000595	2006/2/16	8.1	
第6回	6-005	感染症および寄生虫症	HIV感染	台湾	男性	不明	1984/10/18	不明	症例報告	外国製品	05000620	2006/2/22	8.1	
第6回	6-006	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1985	不明	症例報告	外国製品	05000570	2006/2/13	8.1	
第6回	6-008	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1985	不明	症例報告	外国製品	05000608	2006/2/22	8.1	
第6回	6-009	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1985	不明	症例報告	外国製品	05000610	2006/2/22	8.1	
第6回	6-010	感染症および寄生虫症	HIV感染	台湾	男性	不明	1985/1/3	不明	症例報告	外国製品	05000630	2006/2/24	8.1	
第6回	6-011	感染症および寄生虫症	HIV感染	台湾	男性	不明	1985/11/13	不明	症例報告	外国製品	05000633	2006/2/24	8.1	
第6回	6-012	感染症および寄生虫症	HIV感染	台湾	男性	不明	1985/5/1	不明	症例報告	外国製品	05000629	2006/2/24	8.1	
第6回	6-013	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1986	不明	症例報告	外国製品	05000567	2006/2/13	8.1	
第6回	6-014	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1986	不明	症例報告	外国製品	05000592	2006/2/16	8.1	
第6回	6-015	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	1986	不明	症例報告	外国製品	05000619	2006/2/22	8.1	
第6回	5-136	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1986	不明	症例報告	外国製品	05000100	2005/10/27	8.0	
第6回	5-101	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	1986/7/16	不明	症例報告	外国製品	05000404	2005/10/21	8.1	
第6回	6-016	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1987	不明	症例報告	外国製品	05000541	2006/2/9	8.1	
第6回	6-017	感染症および寄生虫症	HIV感染	アルゼンチン	女性	不明	1987	不明	症例報告	外国製品	05000581	2006/2/16	8.1	
第6回	6-018	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1987	不明	症例報告	外国製品	05000593	2006/2/16	8.1	
第6回	6-019	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1987	不明	症例報告	外国製品	05000594	2006/2/16	8.1	

番号		感染症の種類		発現国	性別	年齢 (歳)	発現時期 (年/月/日)	転帰	出典	区分	識別番号	報告日	備考	
第6回	番号	器官別大分類	基本語										MedDRA (Ver.)	
第6回	6-036	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	2000	不明	症例報告	外国製品	05000542	2006/2/9	8.1	
第6回	6-020	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	1988	不明	症例報告	外国製品	05000576	2006/2/16	8.1	
第6回	6-021	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1988	不明	症例報告	外国製品	05000584	2006/2/16	8.1	
第6回	6-022	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1989	不明	症例報告	外国製品	05000609	2006/2/22	8.1	
第6回	6-023	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1989	不明	症例報告	外国製品	05000611	2006/2/22	8.1	
第6回	6-024	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1989	不明	症例報告	外国製品	05000624	2006/2/24	8.1	
第6回	6-028	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1990	不明	症例報告	外国製品	05000623	2006/2/24	8.1	
第6回	6-029	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	1990/1/3	不明	症例報告	外国製品	05000578	2006/2/16	8.1	
第6回	6-030	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1992	不明	症例報告	外国製品	05000583	2006/2/16	8.1	
第6回	6-034	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1994	不明	症例報告	外国製品	05000586	2006/2/16	8.1	
第6回	5-271	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000403	2005/10/27	8.0	第6回症例番号5-271は第6回症例番号5-101と重複症例のため報告放棄
第6回	6-040	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000434	2005/9/1	8.0	
第6回	6-045	感染症および寄生虫症	HIV感染	ベネズエラ	男性	不明	不明	死亡	症例報告	外国製品	05000439	2005/9/9	8.0	
第6回	6-046	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000440	2005/9/9	8.0	
第6回	6-048	感染症および寄生虫症	HIV感染	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000442	2005/9/9	8.0	
第6回	6-053	感染症および寄生虫症	HIV感染	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000447	2005/9/16	8.0	
第6回	6-065	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000463	2005/11/2	8.1	
第6回	6-066	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000464	2005/11/2	8.1	
第6回	6-067	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000465	2005/11/2	8.1	
第6回	6-068	感染症および寄生虫症	HIV感染	ペルー	男性	不明	不明	不明	症例報告	外国製品	05000466	2005/11/2	8.1	
第6回	6-071	感染症および寄生虫症	HIV感染	ペルー	不明	不明	不明	不明	症例報告	外国製品	05000469	2005/11/2	8.1	
第6回	6-072	感染症および寄生虫症	HIV感染	ペルー	男性	不明	不明	不明	症例報告	外国製品	05000470	2005/11/2	8.1	
第6回	6-076	感染症および寄生虫症	HIV感染	チリ	男性	不明	不明	不明	症例報告	外国製品	05000479	2005/12/2	8.1	
第6回	6-077	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	死亡	症例報告	外国製品	05000480	2005/12/2	8.1	
第6回	6-078	感染症および寄生虫症	HIV感染	パナマ	男性	不明	不明	死亡	症例報告	外国製品	05000481	2005/12/2	8.1	
第6回	6-080	感染症および寄生虫症	HIV感染	チリ	男性	不明	不明	死亡	症例報告	外国製品	05000483	2005/12/2	8.1	
第6回	6-081	感染症および寄生虫症	HIV感染	チリ	男性	不明	不明	死亡	症例報告	外国製品	05000484	2005/12/2	8.1	
第6回	6-082	感染症および寄生虫症	HIV感染	パナマ	男性	不明	不明	死亡	症例報告	外国製品	05000485	2005/12/2	8.1	
第6回	6-083	感染症および寄生虫症	HIV感染	パナマ	男性	不明	不明	死亡	症例報告	外国製品	05000486	2005/12/2	8.1	
第6回	6-084	感染症および寄生虫症	HIV感染	パナマ	男性	不明	不明	死亡	症例報告	外国製品	05000487	2005/12/2	8.1	
第6回	6-085	感染症および寄生虫症	HIV感染	パナマ	男性	不明	不明	死亡	症例報告	外国製品	05000488	2005/12/2	8.1	
第6回	6-086	感染症および寄生虫症	HIV感染	チリ	男性	不明	不明	死亡	症例報告	外国製品	05000489	2005/12/2	8.1	
第6回	6-090	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000498	2006/2/6	8.1	
第6回	6-101	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000509	2006/2/8	8.1	
第6回	6-105	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000513	2006/2/8	8.1	
第6回	6-107	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000515	2006/2/8	8.1	
第6回	6-108	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000516	2006/2/8	8.1	
第6回	6-111	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000519	2006/2/8	8.1	
第6回	6-112	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000520	2006/2/8	8.1	
第6回	6-117	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000525	2006/2/8	8.1	
第6回	6-118	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000526	2006/2/8	8.1	
第6回	6-144	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000556	2006/2/13	8.1	
第6回	6-162	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000590	2006/2/16	8.1	
第6回	6-176	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000613	2006/2/22	8.1	
第6回	4-06	臨床検査	HIV検査陽性	イギリス	男性	11	1981/11/23	不明	症例報告	外国製品	04000081	2005/9/16	8.1	第6回症例番号4-06は前回報告における第4回症例番号4-06において報告したものの追加報告
第6回	6-020	肝胆道系障害	肝炎	ブラジル	男性	不明	1988	不明	症例報告	外国製品	05000576	2006/2/16	8.1	

番号		感染症の種類		発現国	性別	年齢 (歳)	発現時期 (年/月/日)	転帰	出典	区分	識別番号	報告日	備考	
第6回	番号	器官別大分類	基本語										MedDRA (Ver.)	
第6回	6-027	肝胆道系障害	肝炎	ブラジル	男性	不明	1990	不明	症例報告	外国製品	05000575	2006/2/16	8.1	第6回症例番号5-286は第6回症例番号6-033と重複症例のため報告放棄
第6回	6-031	肝胆道系障害	肝炎	ブラジル	男性	不明	1993	不明	症例報告	外国製品	05000618	2006/2/22	8.1	
第6回	5-286	肝胆道系障害	肝炎	ブラジル	男性	13	1994	不明	症例報告	外国製品	05000273	2006/2/15	8.0	
第6回	6-033	肝胆道系障害	肝炎	ブラジル	男性	13	1994	不明	症例報告	外国製品	05000572	2006/2/13	8.1	
第6回	6-036	肝胆道系障害	肝炎	ブラジル	男性	不明	2000	不明	症例報告	外国製品	05000542	2006/2/9	8.1	
第6回	6-151	肝胆道系障害	肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000563	2006/2/13	8.1	
第6回	6-156	肝胆道系障害	肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000577	2006/2/16	8.1	
第6回	6-089	社会環境	伝染病暴露	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000497	2006/2/6	8.1	
第6回	6-093	社会環境	伝染病暴露	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000501	2006/2/6	8.1	
第6回	6-096	社会環境	伝染病暴露	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000503	2006/2/6	8.1	
第6回	6-097	社会環境	伝染病暴露	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000504	2006/2/6	8.1	第6回症例番号5-136は前回報告における第5回症例番号5-136において報告したものの追加報告 第6回症例番号5-271は第6回症例番号5-101と重複症例のため報告放棄
第6回	6-091	臨床検査	A型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000499	2006/2/6	8.1	
第6回	6-137	臨床検査	A型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000549	2006/2/10	8.1	
第6回	6-091	臨床検査	B型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000499	2006/2/6	8.1	
第6回	6-137	臨床検査	B型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000549	2006/2/10	8.1	
第6回	6-142	臨床検査	B型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000554	2006/2/10	8.1	
第6回	6-187	臨床検査	B型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000632	2006/2/24	8.1	
第6回	5-136	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000100	2005/10/27	8.0	
第6回	5-271	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000403	2005/10/27	8.0	
第6回	6-039	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000433	2005/9/1	8.0	
第6回	6-040	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000434	2005/9/1	8.0	
第6回	6-041	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000435	2005/9/1	8.0	
第6回	6-042	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000436	2005/9/1	8.0	
第6回	6-043	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000437	2005/9/1	8.0	
第6回	6-044	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000438	2005/9/9	8.0	
第6回	6-046	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000440	2005/9/9	8.0	
第6回	6-047	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000441	2005/9/9	8.0	
第6回	6-065	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000463	2005/11/2	8.1	
第6回	6-067	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000465	2005/11/2	8.1	
第6回	6-068	臨床検査	C型肝炎ウイルス	ペルー	男性	不明	不明	不明	症例報告	外国製品	05000466	2005/11/2	8.1	
第6回	6-073	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000473	2005/11/21	8.1	
第6回	6-074	臨床検査	C型肝炎ウイルス	パナマ	男性	不明	不明	不明	症例報告	外国製品	05000476	2005/11/30	8.1	
第6回	6-090	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000498	2006/2/6	8.1	
第6回	6-091	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000499	2006/2/6	8.1	
第6回	6-092	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000500	2006/2/6	8.1	
第6回	6-094	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000502	2006/2/6	8.1	
第6回	6-095	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000506	2006/2/6	8.1	
第6回	6-098	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000505	2006/2/6	8.1	
第6回	6-099	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000507	2006/2/8	8.1	
第6回	6-100	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000508	2006/2/8	8.1	
第6回	6-101	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000509	2006/2/8	8.1	
第6回	6-102	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000510	2006/2/8	8.1	
第6回	6-103	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000511	2006/2/8	8.1	
第6回	6-104	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000512	2006/2/8	8.1	

感染症の種類														備考
番号	器官別大分類	基本語	発現国	性別	年齢 (歳)	発現時期 (年/月/日)	転帰	出典	区分	識別番号	報告日	MedDRA (Ver.)		
第6回	6-105	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000513	2006/2/8	8.1	
第6回	6-106	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000514	2006/2/8	8.1	
第6回	6-109	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000517	2006/2/8	8.1	
第6回	6-110	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000518	2006/2/8	8.1	
第6回	6-113	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000521	2006/2/8	8.1	
第6回	6-114	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000522	2006/2/8	8.1	
第6回	6-115	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000523	2006/2/8	8.1	
第6回	6-116	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000524	2006/2/8	8.1	
第6回	6-118	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000526	2006/2/8	8.1	
第6回	6-119	臨床検査	C型肝炎ウイルス	コスタリカ	男性	不明	不明	不明	症例報告	外国製品	05000527	2006/2/8	8.1	
第6回	6-120	臨床検査	C型肝炎ウイルス	コスタリカ	男性	不明	不明	不明	症例報告	外国製品	05000528	2006/2/8	8.1	
第6回	6-121	臨床検査	C型肝炎ウイルス	コスタリカ	男性	不明	不明	不明	症例報告	外国製品	05000529	2006/2/8	8.1	
第6回	6-122	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000530	2006/2/8	8.1	
第6回	6-123	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000531	2006/2/8	8.1	
第6回	6-124	臨床検査	C型肝炎ウイルス	アメリカ	女性	不明	不明	不明	症例報告	外国製品	05000532	2006/2/8	8.1	
第6回	6-127	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000535	2006/2/8	8.1	
第6回	6-130	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000540	2006/2/9	8.1	
第6回	6-137	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000549	2006/2/10	8.1	
第6回	6-138	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000550	2006/2/10	8.1	
第6回	6-139	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000551	2006/2/10	8.1	
第6回	6-140	臨床検査	C型肝炎ウイルス	アメリカ	女性	不明	不明	不明	症例報告	外国製品	05000552	2006/2/10	8.1	
第6回	6-141	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000553	2006/2/10	8.1	
第6回	6-142	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000554	2006/2/10	8.1	
第6回	6-143	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000555	2006/2/10	8.1	
第6回	6-029	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000578	2006/2/16	8.1	
第6回	6-167	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000599	2006/2/16	8.1	
第6回	6-168	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000600	2006/2/21	8.1	
第6回	6-170	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000602	2006/2/21	8.1	
第6回	6-171	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000603	2006/2/21	8.1	
第6回	6-172	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000604	2006/2/21	8.1	
第6回	6-177	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000614	2006/2/22	8.1	
第6回	6-178	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000615	2006/2/22	8.1	
第6回	6-179	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000616	2006/2/22	8.1	
第6回	6-180	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000617	2006/2/22	8.1	
第6回	6-181	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000621	2006/2/22	8.1	
第6回	6-182	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000622	2006/2/22	8.1	
第6回	6-186	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000631	2006/2/24	8.1	
第6回	6-187	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000632	2006/2/24	8.1	
第5回	5-001	感染症および寄生虫症	A型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	04000118	2005/3/18	8.0	
第5回	5-002	感染症および寄生虫症	A型肝炎	ベネズエラ	男性	不明	不明	死亡	症例報告	外国製品	05000244	2005/7/15	8.0	
第5回	5-003	感染症および寄生虫症	A型肝炎	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000245	2005/7/15	8.0	
第5回	5-004	感染症および寄生虫症	B型肝炎	ベネズエラ	男性	不明	1994	不明	症例報告	外国製品	05000225	2005/7/11	8.0	
第5回	5-001	感染症および寄生虫症	B型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	04000118	2005/3/18	8.0	
第5回	5-002	感染症および寄生虫症	B型肝炎	ベネズエラ	男性	不明	不明	死亡	症例報告	外国製品	05000244	2005/7/15	8.0	
第5回	5-003	感染症および寄生虫症	B型肝炎	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000245	2005/7/15	8.0	
第5回	5-005	感染症および寄生虫症	B型肝炎	アメリカ	男性	53	不明	不明	症例報告	当該製品	04000114	2005/3/15	7.1	
第5回	5-006	感染症および寄生虫症	B型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	04000119	2005/3/18	8.0	
第5回	5-007	感染症および寄生虫症	B型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	05000118	2005/6/9	8.0	
第5回	5-008	感染症および寄生虫症	B型肝炎	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000147	2005/6/20	8.0	
第5回	5-004	感染症および寄生虫症	C型肝炎	ベネズエラ	男性	不明	1994	不明	症例報告	外国製品	05000225	2005/7/11	8.0	

感染症の種類													備考
番号	器官別大分類	基本語	発現国	性別	年齢(歳)	発現時期(年/月/日)	転帰	出典	区分	識別番号	報告日	MedDRA(Ver.)	
第5回	5-009	感染症および寄生虫症	C型肝炎	イタリア	男性	不明	1992	不明	症例報告	外国製品	04000127	2005/3/31	8.0
第5回	5-001	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	04000118	2005/3/18	8.0
第5回	5-002	感染症および寄生虫症	C型肝炎	ベネズエラ	男性	不明	不明	死亡	症例報告	外国製品	05000244	2005/7/15	8.0
第5回	5-003	感染症および寄生虫症	C型肝炎	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000245	2005/7/15	8.0
第5回	5-005	感染症および寄生虫症	C型肝炎	アメリカ	男性	53	不明	不明	症例報告	当該製品	04000114	2005/3/15	7.1
第5回	5-006	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	04000119	2005/3/18	8.0
第5回	5-007	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	05000118	2005/6/9	8.0
第5回	5-008	感染症および寄生虫症	C型肝炎	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000147	2005/6/20	8.0
第5回	5-010	感染症および寄生虫症	C型肝炎	アメリカ	男性	52	不明	不明	症例報告	外国製品	04000103	2005/3/3	7.1
第5回	5-011	感染症および寄生虫症	C型肝炎	アメリカ	男性	21	不明	不明	症例報告	外国製品	04000106	2005/3/3	7.1
第5回	5-012	感染症および寄生虫症	C型肝炎	アメリカ	男性	49	不明	不明	症例報告	外国製品	04000111	2005/3/10	7.1
第5回	5-013	感染症および寄生虫症	C型肝炎	アメリカ	男性	24	不明	不明	症例報告	当該製品	04000112	2005/3/15	7.1
第5回	5-014	感染症および寄生虫症	C型肝炎	アメリカ	男性	35	不明	不明	症例報告	当該製品	04000113	2005/3/15	7.1
第5回	5-015	感染症および寄生虫症	C型肝炎	アメリカ	男性	26	不明	不明	症例報告	当該製品	04000115	2005/3/15	7.1
第5回	5-016	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	04000117	2005/3/17	8.0
第5回	5-017	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000005	2005/4/25	8.0
第5回	5-018	感染症および寄生虫症	C型肝炎	スペイン	男性	不明	不明	不明	症例報告	外国製品	05000007	2005/4/25	8.0
第5回	5-019	感染症および寄生虫症	C型肝炎	スペイン	男性	48	不明	不明	症例報告	外国製品	05000091	2005/6/1	8.0
第5回	5-020	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000069	2005/6/1	8.0
第5回	5-020	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000069	2005/6/15	8.0
第5回	5-021	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000070	2005/6/1	8.0
第5回	5-021	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000070	2005/6/15	8.0
第5回	5-022	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000071	2005/6/1	8.0
第5回	5-022	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000071	2005/6/15	8.0
第5回	5-023	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000072	2005/6/1	8.0
第5回	5-023	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000072	2005/6/15	8.0
第5回	5-024	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000073	2005/6/1	8.0
第5回	5-024	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000073	2005/6/15	8.0
第5回	5-025	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000074	2005/6/1	8.0
第5回	5-025	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000074	2005/6/15	8.0
第5回	5-026	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000075	2005/6/1	8.0
第5回	5-027	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000076	2005/6/1	8.0
第5回	5-028	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000092	2005/6/1	8.0
第5回	5-029	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000093	2005/6/1	8.0
第5回	5-030	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000094	2005/6/1	8.0
第5回	5-031	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000095	2005/6/1	8.0
第5回	5-032	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000096	2005/6/1	8.0
第5回	5-033	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000097	2005/6/1	8.0
第5回	5-034	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000077	2005/6/1	8.0
第5回	5-035	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000078	2005/6/1	8.0
第5回	5-035	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000078	2005/6/15	8.0
第5回	5-036	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000079	2005/6/1	8.0
第5回	5-037	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000080	2005/6/1	8.0
第5回	5-038	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000081	2005/6/1	8.0
第5回	5-039	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000082	2005/6/1	8.0
第5回	5-040	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000083	2005/6/1	8.0
第5回	5-041	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000084	2005/6/1	8.0
第5回	5-042	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000088	2005/6/1	8.0
第5回	5-043	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000089	2005/6/1	8.0
第5回	5-043	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000089	2005/6/15	8.0

感染症発生症例一覧

番号		感染症の種類		発現国	性別	年齢 (歳)	発現時期 (年/月/日)	転帰	出典	区分	識別番号	報告日	備考	
第5回	番号	器官別大分類	基本語										MedDRA (Ver.)	
第5回	5-044	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000090	2005/6/1	8.0	
第5回	5-044	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000090	2005/6/15	8.0	
第5回	5-045	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	05000109	2005/6/9	8.0	
第5回	5-046	感染症および寄生虫症	C型肝炎	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000114	2005/6/9	8.0	
第5回	5-047	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000135	2005/6/20	8.0	
第5回	5-048	感染症および寄生虫症	C型肝炎	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000136	2005/6/20	8.0	
第5回	5-049	感染症および寄生虫症	C型肝炎	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000137	2005/6/20	8.0	
第5回	5-050	感染症および寄生虫症	C型肝炎	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000144	2005/6/20	8.0	
第5回	5-051	感染症および寄生虫症	C型肝炎	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000145	2005/6/20	8.0	
第5回	5-052	感染症および寄生虫症	C型肝炎	コロンビア	男性	不明	不明	不明	症例報告	外国製品	05000150	2005/6/27	8.0	
第5回	5-053	感染症および寄生虫症	C型肝炎	ドミニカ共和国	男性	不明	不明	不明	症例報告	外国製品	05000155	2005/6/27	8.0	
第5回	5-054	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000183	2005/7/4	8.0	
第5回	5-055	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000195	2005/7/8	8.0	
第5回	5-056	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000196	2005/7/8	8.0	
第5回	5-057	感染症および寄生虫症	C型肝炎	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000215	2005/7/11	8.0	
第5回	5-058	感染症および寄生虫症	C型肝炎	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000218	2005/7/11	8.0	
第5回	5-059	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000221	2005/7/11	8.0	
第5回	5-060	感染症および寄生虫症	C型肝炎	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000223	2005/7/11	8.0	
第5回	5-061	感染症および寄生虫症	C型肝炎	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000226	2005/7/11	8.0	
第5回	5-062	感染症および寄生虫症	C型肝炎	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000227	2005/7/11	8.0	
第5回	5-063	感染症および寄生虫症	C型肝炎	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000228	2005/7/11	8.0	
第5回	5-064	感染症および寄生虫症	C型肝炎	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000231	2005/7/12	8.0	
第5回	5-065	感染症および寄生虫症	C型肝炎	ドミニカ共和国	男性	不明	不明	不明	症例報告	外国製品	05000234	2005/7/12	8.0	
第5回	5-066	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000238	2005/7/15	8.0	
第5回	5-067	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000239	2005/7/15	8.0	
第5回	5-068	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000243	2005/7/15	8.0	
第5回	5-069	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000246	2005/7/15	8.0	
第5回	5-070	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000253	2005/7/19	8.0	
第5回	5-070	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000253	2005/8/2	8.0	
第5回	5-071	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000262	2005/7/22	8.0	
第5回	5-072	感染症および寄生虫症	C型肝炎	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000263	2005/7/22	8.0	
第5回	5-073	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000281	2005/7/26	8.0	
第5回	5-074	感染症および寄生虫症	C型肝炎	アルゼンチン	女性	不明	不明	不明	症例報告	外国製品	05000310	2005/7/27	8.0	
第5回	5-075	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000311	2005/7/27	8.0	
第5回	5-076	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000312	2005/7/27	8.0	
第5回	5-077	感染症および寄生虫症	C型肝炎	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000324	2005/7/27	8.0	
第5回	5-078	感染症および寄生虫症	C型肝炎	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000325	2005/7/27	8.0	
第5回	5-079	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000327	2005/7/29	8.0	
第5回	5-080	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000328	2005/7/29	8.0	
第5回	5-081	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000347	2005/8/2	8.0	
第5回	5-082	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000348	2005/8/2	8.0	
第5回	5-083	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000349	2005/8/2	8.0	
第5回	5-084	感染症および寄生虫症	C型肝炎	コスタリカ	男性	不明	不明	不明	症例報告	外国製品	05000352	2005/8/2	8.0	
第5回	5-085	感染症および寄生虫症	C型肝炎	パナマ	男性	不明	不明	不明	症例報告	外国製品	05000361	2005/8/2	8.0	
第5回	5-086	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000363	2005/8/2	8.0	
第5回	5-087	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000364	2005/8/2	8.0	
第5回	5-088	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000365	2005/8/2	8.0	
第5回	5-089	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000366	2005/8/2	8.0	
第5回	5-090	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000367	2005/8/3	8.0	
第5回	5-091	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000369	2005/8/3	8.0	

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番号		感染症の種類		発現国	性別	年齢 (歳)	発現時期 (年/月/日)	転帰	出典	区分	識別番号	報告日	備考	
第5回	番号	器官別大分類	基本語										MedDRA (Ver.)	
第5回	5-092	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000371	2005/8/3	8.0	
第5回	5-093	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000372	2005/8/3	8.0	
第5回	5-094	感染症および寄生虫症	C型肝炎	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000375	2005/8/3	8.0	
第5回	5-095	感染症および寄生虫症	C型肝炎	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000376	2005/8/3	8.0	
第5回	5-096	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000379	2005/8/3	8.0	
第5回	5-097	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000380	2005/8/3	8.0	
第5回	5-098	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000386	2005/8/3	8.0	
第5回	5-099	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000392	2005/8/8	8.0	
第5回	5-100	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000393	2005/8/8	8.0	
第5回	5-101	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000404	2005/8/15	8.0	
第5回	5-102	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	05000416	2005/8/26	8.0	
第5回	5-103	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000419	2005/8/26	8.0	
第5回	5-009	感染症および寄生虫症	HIV感染	イタリア	男性	不明	1985/3	不明	症例報告	外国製品	04000127	2005/3/31	8.0	
第5回	5-072	感染症および寄生虫症	HIV感染	ベネズエラ	男性	不明	1986	不明	症例報告	外国製品	05000263	2005/7/22	8.0	
第5回	5-080	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1990	不明	症例報告	外国製品	05000328	2005/7/29	8.0	
第5回	5-002	感染症および寄生虫症	HIV感染	ベネズエラ	男性	不明	不明	死亡	症例報告	外国製品	05000244	2005/7/15	8.0	
第5回	5-003	感染症および寄生虫症	HIV感染	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000245	2005/7/15	8.0	
第5回	5-017	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000005	2005/4/25	8.0	
第5回	5-018	感染症および寄生虫症	HIV感染	スペイン	男性	不明	不明	不明	症例報告	外国製品	05000007	2005/4/25	8.0	
第5回	5-020	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000069	2005/6/1	8.0	
第5回	5-020	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000069	2005/6/15	8.0	
第5回	5-021	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000070	2005/6/1	8.0	
第5回	5-021	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000070	2005/6/15	8.0	
第5回	5-022	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000071	2005/6/1	8.0	
第5回	5-022	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000071	2005/6/15	8.0	
第5回	5-023	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000072	2005/6/1	8.0	
第5回	5-023	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000072	2005/6/15	8.0	
第5回	5-024	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000073	2005/6/1	8.0	
第5回	5-024	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000073	2005/6/15	8.0	
第5回	5-025	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000074	2005/6/15	8.0	
第5回	5-025	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000074	2005/6/1	8.0	
第5回	5-026	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000075	2005/6/1	8.0	
第5回	5-027	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000076	2005/6/1	8.0	
第5回	5-028	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000092	2005/6/1	8.0	
第5回	5-029	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000093	2005/6/1	8.0	
第5回	5-030	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000094	2005/6/1	8.0	
第5回	5-031	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000095	2005/6/1	8.0	
第5回	5-032	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000096	2005/6/1	8.0	
第5回	5-033	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000097	2005/6/1	8.0	
第5回	5-034	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000077	2005/6/1	8.0	
第5回	5-035	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000078	2005/6/1	8.0	
第5回	5-035	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000078	2005/6/15	8.0	
第5回	5-036	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000079	2005/6/1	8.0	
第5回	5-037	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000080	2005/6/1	8.0	
第5回	5-038	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000081	2005/6/1	8.0	
第5回	5-039	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000082	2005/6/1	8.0	
第5回	5-040	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000083	2005/6/1	8.0	
第5回	5-041	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000084	2005/6/1	8.0	
第5回	5-042	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000088	2005/6/1	8.0	
第5回	5-053	感染症および寄生虫症	HIV感染	ドミニカ共和国	男性	不明	不明	不明	症例報告	外国製品	05000155	2005/6/27	8.0	

番号		感染症の種類		発現国	性別	年齢 (歳)	発現時期 (年/月/日)	転帰	出典	区分	識別番号	報告日	備考	
第5回	番号	器官別大分類	基本語										MedDRA (Ver.)	
第5回	5-055	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000195	2005/7/8	8.0	
第5回	5-056	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000196	2005/7/8	8.0	
第5回	5-067	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000239	2005/7/15	8.0	
第5回	5-068	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000243	2005/7/15	8.0	
第5回	5-069	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000246	2005/7/15	8.0	
第5回	5-071	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000262	2005/7/22	8.0	
第5回	5-073	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000281	2005/7/26	8.0	
第5回	5-079	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000327	2005/7/29	8.0	
第5回	5-082	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000348	2005/8/2	8.0	
第5回	5-083	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000349	2005/8/2	8.0	
第5回	5-085	感染症および寄生虫症	HIV感染	パナマ	男性	不明	不明	不明	症例報告	外国製品	05000361	2005/8/2	8.0	
第5回	5-089	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000366	2005/8/2	8.0	
第5回	5-096	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000379	2005/8/3	8.0	
第5回	5-097	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000380	2005/8/3	8.0	
第5回	5-100	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000393	2005/8/8	8.0	
第5回	5-101	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000404	2005/8/15	8.0	
第5回	5-103	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000419	2005/8/26	8.0	
第5回	5-104	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000004	2005/4/25	8.0	
第5回	5-105	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000006	2005/4/25	8.0	
第5回	5-106	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000008	2005/4/28	8.0	
第5回	5-107	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000012	2005/5/12	8.0	
第5回	5-108	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	死亡	症例報告	外国製品	05000013	2005/5/12	8.0	
第5回	5-109	感染症および寄生虫症	HIV感染	香港	男性	不明	不明	不明	症例報告	外国製品	05000015	2005/5/23	8.0	
第5回	5-110	感染症および寄生虫症	HIV感染	香港	男性	不明	不明	不明	症例報告	外国製品	05000016	2005/5/23	8.0	
第5回	5-111	感染症および寄生虫症	HIV感染	香港	男性	不明	不明	不明	症例報告	外国製品	05000017	2005/5/23	8.0	
第5回	5-112	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000018	2005/5/23	8.0	
第5回	5-113	感染症および寄生虫症	HIV感染	香港	男性	不明	不明	不明	症例報告	外国製品	05000019	2005/5/23	8.0	
第5回	5-114	感染症および寄生虫症	HIV感染	香港	男性	不明	不明	不明	症例報告	外国製品	05000020	2005/5/23	8.0	
第5回	5-115	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000021	2005/5/23	8.0	
第5回	5-116	感染症および寄生虫症	HIV感染	香港	男性	不明	不明	不明	症例報告	外国製品	05000022	2005/5/23	8.0	
第5回	5-117	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000023	2005/5/23	8.0	
第5回	5-118	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000025	2005/5/26	8.0	
第5回	5-119	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000026	2005/5/26	8.0	
第5回	5-120	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	死亡	症例報告	外国製品	05000027	2005/5/26	8.0	
第5回	5-121	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000053	2005/5/30	8.0	
第5回	5-122	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000054	2005/5/30	8.0	
第5回	5-123	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000056	2005/5/30	8.0	
第5回	5-124	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000057	2005/5/30	8.0	
第5回	5-125	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000058	2005/5/30	8.0	
第5回	5-126	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000061	2005/5/30	8.0	
第5回	5-127	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000062	2005/5/30	8.0	
第5回	5-128	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000063	2005/5/30	8.0	
第5回	5-129	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000064	2005/5/30	8.0	
第5回	5-130	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000065	2005/5/30	8.0	
第5回	5-131	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000066	2005/5/30	8.0	
第5回	5-132	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000067	2005/5/30	8.0	
第5回	5-133	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000068	2005/5/30	8.0	
第5回	5-134	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000098	2005/6/1	8.0	
第5回	5-135	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	死亡	症例報告	外国製品	05000099	2005/6/1	8.0	
第5回	5-136	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000100	2005/6/1	8.0	

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番号		感染症の種類		発現国	性別	年齢 (歳)	発現時期 (年/月/日)	転帰	出典	区分	識別番号	報告日	備考	
器官別大分類	基本語	MedDRA (Ver.)												
第5回	5-176	感染症および寄生虫症	HIV感染	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000126	2005/6/15	8.0	
第5回	5-177	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000129	2005/6/15	8.0	
第5回	5-178	感染症および寄生虫症	HIV感染	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000133	2005/6/20	8.0	
第5回	5-179	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000134	2005/6/20	8.0	
第5回	5-180	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000148	2005/6/20	8.0	
第5回	5-181	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000156	2005/6/27	8.0	
第5回	5-182	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000159	2005/6/27	8.0	
第5回	5-183	感染症および寄生虫症	HIV感染	チリ	男性	不明	不明	不明	症例報告	外国製品	05000161	2005/6/27	8.0	
第5回	5-184	感染症および寄生虫症	HIV感染	チリ	男性	不明	不明	不明	症例報告	外国製品	05000162	2005/6/27	8.0	
第5回	5-185	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000164	2005/6/27	8.0	
第5回	5-186	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000165	2005/6/27	8.0	
第5回	5-187	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000167	2005/6/27	8.0	
第5回	5-188	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000171	2005/6/30	8.0	
第5回	5-189	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000173	2005/6/30	8.0	
第5回	5-190	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000175	2005/6/30	8.0	
第5回	5-191	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000176	2005/6/30	8.0	
第5回	5-192	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000181	2005/6/30	8.0	
第5回	5-193	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000182	2005/6/30	8.0	
第5回	5-193	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000182	2005/8/8	8.0	
第5回	5-194	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000185	2005/7/4	8.0	
第5回	5-195	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000186	2005/7/4	8.0	
第5回	5-196	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000187	2005/7/4	8.0	
第5回	5-197	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000189	2005/7/4	8.0	
第5回	5-198	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000190	2005/7/4	8.0	
第5回	5-199	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000191	2005/7/4	8.0	
第5回	5-200	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000194	2005/7/4	8.0	
第5回	5-201	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000202	2005/7/8	8.0	
第5回	5-202	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000203	2005/7/8	8.0	
第5回	5-203	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000204	2005/7/8	8.0	
第5回	5-204	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000206	2005/7/8	8.0	
第5回	5-205	感染症および寄生虫症	HIV感染	コロンビア	男性	不明	不明	不明	症例報告	外国製品	05000209	2005/7/8	8.0	
第5回	5-206	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000212	2005/7/8	8.0	
第5回	5-207	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000213	2005/7/11	8.0	
第5回	5-208	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000214	2005/7/11	8.0	
第5回	5-209	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000219	2005/7/11	8.0	
第5回	5-210	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000230	2005/7/11	8.0	
第5回	5-211	感染症および寄生虫症	HIV感染	コスタリカ	男性	不明	不明	不明	症例報告	外国製品	05000232	2005/7/12	8.0	
第5回	5-212	感染症および寄生虫症	HIV感染	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000233	2005/7/12	8.0	
第5回	5-213	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000235	2005/7/12	8.0	
第5回	5-214	感染症および寄生虫症	HIV感染	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000237	2005/7/12	8.0	
第5回	5-215	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000240	2005/7/15	8.0	
第5回	5-216	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000241	2005/7/15	8.0	
第5回	5-217	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000242	2005/7/15	8.0	
第5回	5-218	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000248	2005/7/15	8.0	
第5回	5-219	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000249	2005/7/15	8.0	
第5回	5-220	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000250	2005/7/15	8.0	
第5回	5-221	感染症および寄生虫症	HIV感染	ベネズエラ	男性	不明	不明	死亡	症例報告	外国製品	05000251	2005/7/15	8.0	
第5回	5-222	感染症および寄生虫症	HIV感染	アルゼンチン	女性	不明	不明	死亡	症例報告	外国製品	05000252	2005/7/19	8.0	
第5回	5-223	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000254	2005/7/19	8.0	
第5回	5-224	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000256	2005/7/19	8.0	

感染症の種類			発現国	性別	年齢 (歳)	発現時期 (年/月/日)	転帰	出典	区分	識別番号	報告日	備考 MedDRA (Ver.)	
番号	器官別大分類	基本語											
第5回	5-225	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000257	2005/7/22	8.0
第5回	5-226	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000258	2005/7/22	8.0
第5回	5-227	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000259	2005/7/22	8.0
第5回	5-228	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000261	2005/7/22	8.0
第5回	5-229	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000264	2005/7/22	8.0
第5回	5-230	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000267	2005/7/22	8.0
第5回	5-231	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000274	2005/7/26	8.0
第5回	5-232	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000276	2005/7/26	8.0
第5回	5-233	感染症および寄生虫症	HIV感染	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000279	2005/7/26	8.0
第5回	5-234	感染症および寄生虫症	HIV感染	コスタリカ	男性	不明	不明	不明	症例報告	外国製品	05000297	2005/7/26	8.0
第5回	5-235	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000299	2005/7/26	8.0
第5回	5-236	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000302	2005/7/26	8.0
第5回	5-237	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000282	2005/7/26	8.0
第5回	5-238	感染症および寄生虫症	HIV感染	ブラジル	女性	不明	不明	死亡	症例報告	外国製品	05000283	2005/7/26	8.0
第5回	5-239	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000284	2005/7/26	8.0
第5回	5-240	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000285	2005/7/26	8.0
第5回	5-241	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000286	2005/7/26	8.0
第5回	5-242	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000287	2005/7/26	8.0
第5回	5-243	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000288	2005/7/26	8.0
第5回	5-244	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000292	2005/7/26	8.0
第5回	5-245	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000293	2005/7/26	8.0
第5回	5-246	感染症および寄生虫症	HIV感染	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000295	2005/7/26	8.0
第5回	5-247	感染症および寄生虫症	HIV感染	ブラジル	女性	不明	不明	死亡	症例報告	外国製品	05000296	2005/7/26	8.0
第5回	5-248	感染症および寄生虫症	HIV感染	ブラジル	女性	不明	不明	死亡	症例報告	外国製品	05000305	2005/7/26	8.0
第5回	5-249	感染症および寄生虫症	HIV感染	アルゼンチン	女性	不明	不明	不明	症例報告	外国製品	05000308	2005/7/26	8.0
第5回	5-250	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000314	2005/7/27	8.0
第5回	5-251	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000315	2005/7/27	8.0
第5回	5-252	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000316	2005/7/27	8.0
第5回	5-253	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000317	2005/7/27	8.0
第5回	5-254	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000320	2005/7/27	8.0
第5回	5-255	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000321	2005/7/27	8.0
第5回	5-256	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000334	2005/7/29	8.0
第5回	5-257	感染症および寄生虫症	HIV感染	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000335	2005/7/29	8.0
第5回	5-258	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000342	2005/8/2	8.0
第5回	5-259	感染症および寄生虫症	HIV感染	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000350	2005/8/2	8.0
第5回	5-260	感染症および寄生虫症	HIV感染	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000353	2005/8/2	8.0
第5回	5-261	感染症および寄生虫症	HIV感染	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000356	2005/8/2	8.0
第5回	5-262	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000358	2005/8/2	8.0
第5回	5-263	感染症および寄生虫症	HIV感染	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000360	2005/8/2	8.0
第5回	5-264	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000368	2005/8/3	8.0
第5回	5-265	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000383	2005/8/3	8.0
第5回	5-266	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000387	2005/8/3	8.0
第5回	5-267	感染症および寄生虫症	HIV感染	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000390	2005/8/8	8.0
第5回	5-268	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000396	2005/8/10	8.0
第5回	5-269	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000397	2005/8/10	8.0
第5回	5-270	感染症および寄生虫症	HIV感染	ベネズエラ	男性	不明	不明	死亡	症例報告	外国製品	05000401	2005/8/10	8.0
第5回	5-271	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000403	2005/8/11	8.0
第5回	5-272	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000407	2005/8/23	8.0
第5回	5-273	感染症および寄生虫症	HIV感染	ニュージーランド	男性	不明	不明	不明	症例報告	外国製品	05000408	2005/8/23	8.0
第5回	5-274	感染症および寄生虫症	HIV感染	ニュージーランド	男性	不明	不明	不明	症例報告	外国製品	05000410	2005/8/24	8.0

番号		感染症の種類		発現国	性別	年齢 (歳)	発現時期 (年/月/日)	転帰	出典	区分	識別番号	報告日	備考	
第5回	5-	器官別大分類	基本語										MedDRA (Ver.)	
		275	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000415	2005/8/24	8.0
		276	感染症および寄生虫症	HIV感染	パナマ	男性	不明	不明	死亡	症例報告	外国製品	05000422	2005/8/30	8.0
		277	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000423	2005/8/30	8.0
		278	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000424	2005/8/30	8.0
		279	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000425	2005/8/30	8.0
		280	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000427	2005/8/30	8.0
		281	感染症および寄生虫症	HIV感染	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000428	2005/8/30	8.0
		282	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000429	2005/8/30	8.0
		283	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000430	2005/8/30	8.0
		284	感染症および寄生虫症	HIV感染	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000431	2005/8/30	8.0
		285	感染症および寄生虫症	後天性免疫不全症候群	イギリス	男性	不明	2002	不明	症例報告	外国製品	04000128	2005/3/31	8.0
		115	感染症および寄生虫症	後天性免疫不全症候群	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000021	2005/5/23	8.0
		153	感染症および寄生虫症	後天性免疫不全症候群	香港	男性	4	不明	不明	症例報告	外国製品	05000113	2005/6/9	8.0
		286	肝胆道系障害	肝炎	ブラジル	男性	13	不明	不明	症例報告	外国製品	05000273	2005/7/22	8.0
		287	臨床検査	B型肝炎ウイルス	イギリス	男性	不明	不明	不明	症例報告	外国製品	04000107	2005/3/3	7.1
		288	臨床検査	B型肝炎ウイルス	イギリス	男性	25	不明	不明	症例報告	外国製品	05000003	2005/4/18	8.0
		285	臨床検査	C型肝炎ウイルス	イギリス	男性	不明	1990	不明	症例報告	外国製品	04000128	2005/3/31	8.0
		107	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000012	2005/7/29	8.0
		108	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000013	2005/5/12	8.0
		109	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000015	2005/5/23	8.0
		110	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000016	2005/5/23	8.0
		111	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000017	2005/5/23	8.0
		112	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000018	2005/5/23	8.0
		113	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000019	2005/5/23	8.0
		114	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000020	2005/5/23	8.0
		115	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000021	2005/5/23	8.0
		116	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000022	2005/5/23	8.0
		117	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000023	2005/5/23	8.0
		118	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000025	2005/5/26	8.0
		119	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000026	2005/5/26	8.0
		120	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000027	2005/5/26	8.0
		121	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000053	2005/5/30	8.0
		122	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000054	2005/5/30	8.0
		123	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000056	2005/5/30	8.0
		124	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000057	2005/5/30	8.0
		125	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000058	2005/5/30	8.0
		126	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000061	2005/5/30	8.0
		127	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000062	2005/5/30	8.0
		128	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000063	2005/5/30	8.0
		129	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000064	2005/5/30	8.0
		130	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000065	2005/5/30	8.0
		131	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000066	2005/5/30	8.0
		132	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000067	2005/5/30	8.0
		133	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000068	2005/5/30	8.0
		134	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000098	2005/6/1	8.0
		135	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000099	2005/6/1	8.0
		136	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000100	2005/6/1	8.0
		138	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000103	2005/6/2	8.0
		139	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000104	2005/6/2	8.0
		140	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000105	2005/6/2	8.0

感染症の種類													
番号	器官別大分類	基本語	発現国	性別	年齢 (歳)	発現時期 (年/月/日)	転帰	出典	区分	識別番号	報告日	備考 MedDRA (Ver.)	
第5回	5-144	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000030	2005/5/30	8.0
第5回	5-144	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000030	2005/6/15	8.0
第5回	5-145	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000031	2005/5/30	8.0
第5回	5-145	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000031	2005/6/15	8.0
第5回	5-146	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000032	2005/5/30	8.0
第5回	5-146	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000032	2005/6/15	8.0
第5回	5-147	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000033	2005/5/30	8.0
第5回	5-148	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000034	2005/5/30	8.0
第5回	5-149	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000035	2005/5/30	8.0
第5回	5-151	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000111	2005/6/9	8.0
第5回	5-152	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000112	2005/6/9	8.0
第5回	5-153	臨床検査	C型肝炎ウイルス	香港	男性	4	不明	不明	症例報告	外国製品	05000113	2005/6/9	8.0
第5回	5-154	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000037	2005/5/30	8.0
第5回	5-156	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000039	2005/5/30	8.0
第5回	5-157	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000040	2005/5/30	8.0
第5回	5-158	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000116	2005/6/9	8.0
第5回	5-159	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000041	2005/5/30	8.0
第5回	5-161	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000043	2005/5/30	8.0
第5回	5-162	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000044	2005/5/30	8.0
第5回	5-162	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000044	2005/6/27	8.0
第5回	5-163	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000045	2005/5/30	8.0
第5回	5-163	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000045	2005/6/15	8.0
第5回	5-164	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000046	2005/5/30	8.0
第5回	5-165	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000047	2005/5/30	8.0
第5回	5-166	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000048	2005/5/30	8.0
第5回	5-167	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000049	2005/5/30	8.0
第5回	5-167	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000049	2005/6/15	8.0
第5回	5-168	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000050	2005/5/30	8.0
第5回	5-168	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000050	2005/6/15	8.0
第5回	5-169	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000051	2005/5/30	8.0
第5回	5-169	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000051	2005/6/15	8.0
第5回	5-170	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000052	2005/5/30	8.0
第5回	5-170	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000052	2005/6/15	8.0
第5回	5-173	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000121	2005/6/13	8.0
第5回	5-174	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000122	2005/6/13	8.0
第5回	5-177	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000129	2005/6/15	8.0
第5回	5-180	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000148	2005/6/20	8.0
第5回	5-181	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000156	2005/6/27	8.0
第5回	5-182	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000159	2005/6/27	8.0
第5回	5-185	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000164	2005/6/27	8.0
第5回	5-186	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000165	2005/6/27	8.0
第5回	5-187	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000167	2005/6/27	8.0
第5回	5-187	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000167	2005/8/18	8.0
第5回	5-188	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000171	2005/6/30	8.0
第5回	5-189	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000173	2005/6/30	8.0
第5回	5-190	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000175	2005/6/30	8.0
第5回	5-191	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000176	2005/6/30	8.0
第5回	5-192	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000181	2005/6/30	8.0
第5回	5-194	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000185	2005/7/4	8.0
第5回	5-195	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000186	2005/7/4	8.0

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第5回	番号	器官別大分類	基本語										MedDRA (Ver.)	
第5回	5-258	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000342	2005/8/2	8.0	
第5回	5-260	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000353	2005/8/2	8.0	
第5回	5-262	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000358	2005/8/2	8.0	
第5回	5-265	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000383	2005/8/3	8.0	
第5回	5-266	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000387	2005/8/3	8.0	
第5回	5-268	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000396	2005/8/10	8.0	
第5回	5-269	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000397	2005/8/10	8.0	
第5回	5-270	臨床検査	C型肝炎ウイルス	ベネズエラ	男性	不明	不明	死亡	症例報告	外国製品	05000401	2005/8/10	8.0	
第5回	5-271	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000403	2005/8/11	8.0	
第5回	5-272	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000407	2005/8/23	8.0	
第5回	5-273	臨床検査	C型肝炎ウイルス	ニュージーランド	男性	不明	不明	不明	症例報告	外国製品	05000408	2005/8/23	8.0	
第5回	5-274	臨床検査	C型肝炎ウイルス	ニュージーランド	男性	不明	不明	不明	症例報告	外国製品	05000410	2005/8/24	8.0	
第5回	5-275	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000415	2005/8/24	8.0	
第5回	5-277	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000423	2005/8/30	8.0	
第5回	5-278	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000424	2005/8/30	8.0	
第5回	5-279	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000425	2005/8/30	8.0	
第5回	5-280	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000427	2005/8/30	8.0	
第5回	5-282	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000429	2005/8/30	8.0	
第5回	5-283	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000430	2005/8/30	8.0	
第5回	5-284	臨床検査	C型肝炎ウイルス	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000431	2005/8/30	8.0	
第5回	5-287	臨床検査	C型肝炎ウイルス	イギリス	男性	不明	不明	不明	症例報告	外国製品	04000107	2005/3/3	7.1	
第5回	5-288	臨床検査	C型肝炎ウイルス	イギリス	男性	25	不明	不明	症例報告	外国製品	05000003	2005/4/18	8.0	
第5回	5-289	臨床検査	C型肝炎ウイルス	イギリス	男性	57	不明	不明	症例報告	外国製品	04000102	2005/3/3	7.1	
第5回	5-290	臨床検査	C型肝炎ウイルス	イギリス	男性	58	不明	不明	症例報告	外国製品	04000104	2005/3/3	7.1	
第5回	5-291	臨床検査	C型肝炎ウイルス	イギリス	男性	不明	不明	不明	症例報告	外国製品	04000120	2005/3/18	8.0	
第5回	5-292	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000024	2005/5/26	8.0	
第5回	5-293	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000055	2005/5/30	8.0	
第5回	5-294	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000059	2005/5/30	8.0	
第5回	5-295	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000060	2005/5/30	8.0	
第5回	5-296	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000101	2005/6/1	8.0	
第5回	5-297	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000108	2005/6/7	8.0	
第5回	5-298	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000110	2005/6/9	8.0	
第5回	5-299	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000115	2005/6/9	8.0	
第5回	5-300	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000117	2005/6/9	8.0	
第5回	5-301	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000123	2005/6/13	8.0	
第5回	5-302	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000127	2005/6/15	8.0	
第5回	5-303	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000128	2005/6/15	8.0	
第5回	5-304	臨床検査	C型肝炎ウイルス	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000130	2005/6/15	8.0	
第5回	5-305	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000131	2005/6/15	8.0	
第5回	5-306	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000132	2005/6/15	8.0	
第5回	5-307	臨床検査	C型肝炎ウイルス	コスタリカ	男性	不明	不明	不明	症例報告	外国製品	05000138	2005/6/20	8.0	
第5回	5-308	臨床検査	C型肝炎ウイルス	コスタリカ	男性	不明	不明	不明	症例報告	外国製品	05000139	2005/6/20	8.0	
第5回	5-309	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000140	2005/6/20	8.0	
第5回	5-310	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000141	2005/6/20	8.0	
第5回	5-311	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000142	2005/6/20	8.0	
第5回	5-312	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000143	2005/6/20	8.0	
第5回	5-313	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000146	2005/6/20	8.0	
第5回	5-314	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000149	2005/6/27	8.0	
第5回	5-315	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000151	2005/6/27	8.0	
第5回	5-316	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000152	2005/6/27	8.0	

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第5回	番号	器官別大分類	基本語										MedDRA (Ver.)	
第5回	5-317	臨床検査	C型肝炎ウイルス	パナマ		不明	不明	不明	症例報告	外国製品	05000153	2005/6/27	8.0	
第5回	5-318	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000154	2005/6/27	8.0	
第5回	5-319	臨床検査	C型肝炎ウイルス	カナダ	男性	不明	不明	不明	症例報告	外国製品	05000157	2005/6/27	8.0	
第5回	5-320	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000158	2005/6/27	8.0	
第5回	5-321	臨床検査	C型肝炎ウイルス	コスタリカ	男性	不明	不明	不明	症例報告	外国製品	05000160	2005/6/27	8.0	
第5回	5-322	臨床検査	C型肝炎ウイルス	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000163	2005/6/27	8.0	
第5回	5-323	臨床検査	C型肝炎ウイルス	ドミニカ共和国	男性	不明	不明	不明	症例報告	外国製品	05000166	2005/6/27	8.0	
第5回	5-324	臨床検査	C型肝炎ウイルス		男性	不明	不明	不明	症例報告	外国製品	05000170	2005/6/30	8.0	
第5回	5-325	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000172	2005/6/30	8.0	
第5回	5-326	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000174	2005/6/30	8.0	
第5回	5-327	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000177	2005/6/30	8.0	
第5回	5-328	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000178	2005/6/30	8.0	
第5回	5-329	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000179	2005/6/30	8.0	
第5回	5-330	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000180	2005/6/30	8.0	
第5回	5-331	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000184	2005/7/4	8.0	
第5回	5-332	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000188	2005/7/4	8.0	
第5回	5-333	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000192	2005/7/4	8.0	
第5回	5-334	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000193	2005/7/4	8.0	
第5回	5-335	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000197	2005/7/8	8.0	
第5回	5-336	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000198	2005/7/8	8.0	
第5回	5-337	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000199	2005/7/8	8.0	
第5回	5-338	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000200	2005/7/8	8.0	
第5回	5-339	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000201	2005/7/8	8.0	
第5回	5-340	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000205	2005/7/8	8.0	
第5回	5-341	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000207	2005/7/8	8.0	
第5回	5-342	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000208	2005/7/8	8.0	
第5回	5-343	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000210	2005/7/8	8.0	
第5回	5-344	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000211	2005/7/8	8.0	
第5回	5-345	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000216	2005/7/11	8.0	
第5回	5-346	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000217	2005/7/11	8.0	
第5回	5-347	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000220	2005/7/11	8.0	
第5回	5-348	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000222	2005/7/11	8.0	
第5回	5-349	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000224	2005/7/11	8.0	
第5回	5-350	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000229	2005/7/11	8.0	
第5回	5-351	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000236	2005/7/12	8.0	
第5回	5-352	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000247	2005/7/15	8.0	
第5回	5-353	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000255	2005/7/19	8.0	
第5回	5-354	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000260	2005/7/22	8.0	
第5回	5-355	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000265	2005/7/22	8.0	
第5回	5-356	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000266	2005/7/22	8.0	
第5回	5-357	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000268	2005/7/22	8.0	
第5回	5-358	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000269	2005/7/22	8.0	
第5回	5-359	臨床検査	C型肝炎ウイルス	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000270	2005/7/22	8.0	
第5回	5-360	臨床検査	C型肝炎ウイルス	コスタリカ	男性	不明	不明	不明	症例報告	外国製品	05000271	2005/7/22	8.0	
第5回	5-361	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000275	2005/7/26	8.0	
第5回	5-362	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000277	2005/7/26	8.0	
第5回	5-363	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000278	2005/7/26	8.0	
第5回	5-364	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000298	2005/7/26	8.0	
第5回	5-365	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000300	2005/7/26	8.0	
第5回	5-366	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000301	2005/7/26	8.0	

番号		感染症の種類		発現国	性別	年齢 (歳)	発現時期 (年/月/日)	転帰	出典	区分	識別番号	報告日	備考 MedDRA (Ver.)
器官別大分類		基本語											
第5回	5-367	臨床検査	C型肝炎ウイルス	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000303	2005/7/26	8.0
第5回	5-368	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000304	2005/7/26	8.0
第5回	5-369	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000280	2005/7/26	8.0
第5回	5-370	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000289	2005/7/26	8.0
第5回	5-371	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000290	2005/7/26	8.0
第5回	5-372	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000291	2005/7/26	8.0
第5回	5-373	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000294	2005/7/26	8.0
第5回	5-374	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000306	2005/7/26	8.0
第5回	5-375	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000307	2005/7/26	8.0
第5回	5-376	臨床検査	C型肝炎ウイルス	パナマ	男性	不明	不明	不明	症例報告	外国製品	05000309	2005/7/27	8.0
第5回	5-377	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000313	2005/7/27	8.0
第5回	5-378	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000318	2005/7/27	8.0
第5回	5-379	臨床検査	C型肝炎ウイルス	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000322	2005/7/27	8.0
第5回	5-380	臨床検査	C型肝炎ウイルス	アルゼンチン	女性	不明	不明	不明	症例報告	外国製品	05000323	2005/7/27	8.0
第5回	5-381	臨床検査	C型肝炎ウイルス	アメリカ	男性	41	不明	不明	症例報告	外国製品	05000326	2005/7/27	8.0
第5回	5-382	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000329	2005/7/29	8.0
第5回	5-383	臨床検査	C型肝炎ウイルス	パナマ	男性	不明	不明	不明	症例報告	外国製品	05000330	2005/7/29	8.0
第5回	5-384	臨床検査	C型肝炎ウイルス	パナマ	男性	不明	不明	不明	症例報告	外国製品	05000331	2005/7/29	8.0
第5回	5-385	臨床検査	C型肝炎ウイルス	アルゼンチン	女性	不明	不明	不明	症例報告	外国製品	05000332	2005/7/29	8.0
第5回	5-386	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000333	2005/7/29	8.0
第5回	5-387	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000336	2005/7/29	8.0
第5回	5-388	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000337	2005/7/29	8.0
第5回	5-389	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000338	2005/7/29	8.0
第5回	5-390	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000339	2005/7/29	8.0
第5回	5-391	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000343	2005/8/2	8.0
第5回	5-392	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000344	2005/8/2	8.0
第5回	5-393	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000345	2005/8/2	8.0
第5回	5-394	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000346	2005/8/2	8.0
第5回	5-395	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000351	2005/8/2	8.0
第5回	5-396	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000354	2005/8/2	8.0
第5回	5-397	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000355	2005/8/2	8.0
第5回	5-398	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000357	2005/8/2	8.0
第5回	5-399	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000359	2005/8/2	8.0
第5回	5-400	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000362	2005/8/2	8.0
第5回	5-401	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000370	2005/8/3	8.0
第5回	5-402	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000373	2005/8/3	8.0
第5回	5-403	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000374	2005/8/3	8.0
第5回	5-404	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000377	2005/8/3	8.0
第5回	5-405	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000378	2005/8/3	8.0
第5回	5-406	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000381	2005/8/3	8.0
第5回	5-407	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000382	2005/8/3	8.0
第5回	5-408	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000384	2005/8/3	8.0
第5回	5-409	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000385	2005/8/3	8.0
第5回	5-410	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000388	2005/8/8	8.0
第5回	5-411	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000389	2005/8/8	8.0
第5回	5-412	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000391	2005/8/8	8.0
第5回	5-413	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000395	2005/8/10	8.0
第5回	5-414	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000398	2005/8/10	8.0
第5回	5-415	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000399	2005/8/10	8.0
第5回	5-416	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000400	2005/8/10	8.0

番号	感染症の種類		発現国	性別	年齢 (歳)	発現時期 (年/月/日)	転帰	出典	区分	識別番号	報告日	備考	
	器官別大分類	基本語										MedDRA (Ver.)	
第5回	5-417	臨床検査	C型肝炎ウイルス	パナマ	男性	不明	不明	不明	症例報告	外国製品	05000405	2005/8/18	8.0
第5回	5-418	臨床検査	C型肝炎ウイルス	ニュージーランド	男性	不明	不明	不明	症例報告	外国製品	05000409	2005/8/24	8.0
第5回	5-419	臨床検査	C型肝炎ウイルス	オーストラリア	男性	不明	不明	不明	症例報告	外国製品	05000411	2005/8/24	8.0
第5回	5-420	臨床検査	C型肝炎ウイルス	パナマ	男性	不明	不明	不明	症例報告	外国製品	05000412	2005/8/24	8.0
第5回	5-421	臨床検査	C型肝炎ウイルス	パナマ	男性	不明	不明	不明	症例報告	外国製品	05000413	2005/8/24	8.0
第5回	5-422	臨床検査	C型肝炎ウイルス	パナマ	男性	不明	不明	不明	症例報告	外国製品	05000414	2005/8/24	8.0
第5回	5-423	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000418	2005/8/26	8.0
第5回	5-424	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000420	2005/8/26	8.0
第5回	5-425	臨床検査	C型肝炎ウイルス	ニュージーランド	男性	不明	不明	不明	症例報告	外国製品	05000421	2005/8/30	8.0
第5回	5-426	臨床検査	C型肝炎ウイルス	パナマ	男性	不明	不明	不明	症例報告	外国製品	05000426	2005/8/30	8.0
第5回	5-427	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000432	2005/8/30	8.0
第5回	5-001	臨床検査	HIV検査陽性	イギリス	男性	不明	1986/3	不明	症例報告	外国製品	04000118	2005/3/18	8.0
第5回	5-288	臨床検査	HIV検査陽性	イギリス	男性	25	1985/10/25	不明	症例報告	外国製品	05000003	2005/4/18	8.0
第5回	5-006	臨床検査	HIV検査陽性	イギリス	男性	不明	1985	不明	症例報告	外国製品	04000119	2005/3/18	8.0
第5回	5-287	臨床検査	HIV検査陽性	イギリス	男性	不明	1984/2/16	不明	症例報告	外国製品	04000107	2005/3/3	7.1
第5回	5-285	臨床検査	HIV検査陽性	イギリス	男性	不明	1983/12	不明	症例報告	外国製品	04000128	2005/3/31	8.0
第4回	4-01	感染症および寄生虫症	A型肝炎	イギリス	男性	29	不明	不明	症例報告	外国製品	04000077	2004/10/8	7.1
第4回	4-01	感染症および寄生虫症	B型肝炎	イギリス	男性	29	不明	不明	症例報告	外国製品	04000077	2004/10/8	7.1
第4回	4-02	感染症および寄生虫症	B型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	04000078	2004/10/8	7.1
第4回	4-03	感染症および寄生虫症	B型肝炎	イギリス	男性	11	不明	不明	症例報告	外国製品	04000081	2004/10/8	7.1
第4回	4-04	感染症および寄生虫症	C型肝炎	イギリス	男性	15	1990/7	不明	症例報告	外国製品	04000076	2004/10/8	7.1
第4回	4-01	感染症および寄生虫症	C型肝炎	イギリス	男性	29	不明	不明	症例報告	外国製品	04000077	2004/10/8	7.1
第4回	4-02	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	04000078	2004/10/8	7.1
第4回	4-03	感染症および寄生虫症	C型肝炎	イギリス	男性	11	不明	不明	症例報告	外国製品	04000081	2004/10/8	7.1
第4回	4-05	感染症および寄生虫症	C型肝炎	イギリス	男性	31	不明	不明	症例報告	外国製品	04000080	2004/10/8	7.1
第4回	4-06	感染症および寄生虫症	C型肝炎	イギリス	男性	34	不明	不明	症例報告	外国製品	04000079	2004/10/8	7.1
第4回	4-07	感染症および寄生虫症	C型肝炎	アメリカ	男性	25	不明	不明	症例報告	当該製品	04000100	2005/2/24	7.1
第4回	4-08	感染症および寄生虫症	C型肝炎	アメリカ	男性	41	不明	不明	症例報告	当該製品	04000099	2005/2/24	7.1
第4回	4-09	感染症および寄生虫症	C型肝炎	アメリカ	男性	27	不明	不明	症例報告	外国製品	04000098	2005/2/24	7.1
第4回	4-10	感染症および寄生虫症	C型肝炎	アメリカ	女性	22	不明	不明	症例報告	外国製品	04000095	2005/2/21	7.1
第4回	4-11	感染症および寄生虫症	C型肝炎	アメリカ	男性	17	不明	不明	症例報告	外国製品	04000094	2005/2/21	7.1
第4回	4-12	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	04000075	2004/10/8	7.1
第4回	4-13	感染症および寄生虫症	HIV感染	イギリス	男性	32	1988/8	不明	症例報告	外国製品	04000074	2004/10/8	7.1
第4回	4-06	感染症および寄生虫症	HIV感染	イギリス	男性	34	1985/1/9	不明	症例報告	外国製品	04000079	2004/10/8	7.1
第4回	4-01	感染症および寄生虫症	HIV感染	イギリス	男性	29	1985	不明	症例報告	外国製品	04000077	2004/10/8	7.1
第4回	4-04	感染症および寄生虫症	HIV感染	イギリス	男性	15	1984/12/14	不明	症例報告	外国製品	04000076	2004/10/8	7.1
第4回	4-05	臨床検査	HIV検査陽性	イギリス	男性	31	1987	不明	症例報告	外国製品	04000080	2004/10/8	7.1
第4回	4-03	臨床検査	HIV検査陽性	イギリス	男性	11	1981/11/23	不明	症例報告	外国製品	04000081	2004/10/8	7.1
第3回	3-1	感染症および寄生虫症	C型肝炎	トルコ	男性	44	2001/11/22	不明	症例報告	当該製品	04000001	2004/4/1	7.0

100302	2011/3/18	101028	バクスター	乾燥人血液凝固因子抗体迂回活性化複合体	乾燥人血液凝固因子抗体迂回活性化複合体	人血漿	米国	有効成分	なし	なし	なし
100303	2011/3/18	101029	バクスター	乾燥濃縮人血液凝固第Ⅷ因子	人血清アルブミン	人血漿	米国	添加物	なし	あり	なし
100304	2011/3/18	101030	バクスター	乾燥濃縮人血液凝固第Ⅷ因子	乾燥人血液凝固第Ⅷ因子	人血漿	米国	有効成分	なし	あり	なし

感染症発生症例一覧

	番 号	感染症の種類		発生国	性別	年齢	発現時期	転帰	出典	区分	備考
		器官別大分類	基本語								
第16回	1	臨床検査	B型肝炎ウイルス検査陽性	アメリカ	男	73	2010/8/20	不明	症例報告	外国製品	識別番号3-10000018 報告日:2010年9月29日
	1	臨床検査	A型肝炎抗体	アメリカ	男	73	2010/8/20	不明	症例報告	外国製品	識別番号3-10000018 報告日:2010年9月29日
	2	臨床検査	B型肝炎ウイルス検査陽性	アメリカ	男	73	2010/8/20	不明	症例報告	外国製品	識別番号3-10000019 報告日:2010年9月29日
	2	臨床検査	A型肝炎抗体	アメリカ	男	73	2010/8/20	不明	症例報告	外国製品	識別番号3-10000019 報告日:2010年9月29日
	3	臨床検査	C型肝炎ウイルス検査陽性	カナダ	男	81	2010/1/25	不明	症例報告	外国製品	識別番号3-10000027 報告日:2010年11月26日
	4	感染症および寄生虫症	A型肝炎	ドイツ	女	66	不明	不明	症例報告	外国製品	識別番号3-10000013 報告日:2010年8月6日
	4	臨床検査	A型肝炎抗体陽性	ドイツ	女	66	2009年6月	不明	症例報告	外国製品	識別番号3-10000013 報告日:2010年8月6日
第15回	1	感染症および寄生虫症	C型肝炎	ドイツ	男	28	2010年4月	不明	症例報告	外国製品	識別番号3-10000006 報告日:2010年5月20日
	1	臨床検査	A型肝炎抗体陽性	ドイツ	男	28	2010年4月	不明	症例報告	外国製品	識別番号3-10000006 報告日:2010年5月20日
	2	感染症および寄生虫症	A型肝炎	ドイツ	女	71	2009/12/14	不明	症例報告	外国製品	識別番号3-09000024 報告日:2010年8月20日*
	2	感染症および寄生虫症	B型肝炎	ドイツ	女	71	2009/12/14	不明	症例報告	外国製品	識別番号3-09000024 報告日:2010年8月20日*
	2	感染症および寄生虫症	C型肝炎	ドイツ	女	71	2009/12/14	不明	症例報告	外国製品	識別番号3-09000024 報告日:2010年8月20日*
第14回	1	感染症および寄生虫症	A型肝炎	ドイツ	女	66	不明	不明	症例報告	外国製品	識別番号3-09000010 報告日:2010年8月6日*
	1	臨床検査	A型肝炎抗体陽性	ドイツ	女	66	2009年6月	不明	症例報告	外国製品	識別番号3-09000010 報告日:2010年8月6日*
第13回	1	感染症および寄生虫症	C型肝炎	ドイツ	男	66	2009/5/1	不明	症例報告	外国製品	識別番号3-09000009 報告日:2009年7月22日
	2	感染症および寄生虫症	C型肝炎	ドイツ	女	77	2009/1/5	不明	症例報告	外国製品	識別番号3-08000040 報告日:2009年2月17日
第12回	報告なし										
第11回	1	感染症および寄生虫症	B型肝炎	ドイツ	男	24	2008/1/10	不明	症例報告	外国製品	識別番号3-07000031 報告日:2008年3月25日
	2	臨床検査	C型肝炎抗体陽性	日本	女	37	2007/9/11	不明	症例報告	当該製品	識別番号1-07000251 報告日:2008年4月30日

	番 号	感染症の種類		発生国	性別	年齢	発現時期	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回	1	感染症および寄生虫症	B型肝炎	日本	女	33	2007/8/7	回復	症例報告	当該製品	識別番号1-07000093 報告日:2007年10月11日
第9回	1	感染症および寄生虫症	C型肝炎	ドイツ	男	50	2007/6/18	不明	症例報告	外国製品	識別番号3-07000010 報告日:2007年8月6日
	1	臨床検査	C型肝炎抗体陽性	ドイツ	男	50	2007/6/18	不明	症例報告	外国製品	識別番号3-07000010 報告日:2007年8月6日
	1	臨床検査	C型肝炎RNA陽性	ドイツ	男	50	2007/6/18	不明	症例報告	外国製品	識別番号3-07000010 報告日:2007年8月6日
	2	感染症および寄生虫症	C型肝炎	ドイツ	女	61	2007年1月	不明	症例報告	外国製品	識別番号3-06000032 報告日:2007年3月30日
	2	臨床検査	C型肝炎陽性	ドイツ	女	61	2007年1月	不明	症例報告	外国製品	識別番号3-06000032 報告日:2007年3月30日
第8回	報告なし										
第7回	報告なし										
第6回	1	感染症および寄生虫症	B型肝炎	ドイツ	女	77	2005/9/28	未回復	症例報告	外国製品	識別番号:3-05000493 報告日:2005年12月27日
第5回	1	感染症および寄生虫症	C型肝炎	日本	男	68	2004/7/6	未回復	症例報告	当該製品	識別番号:1-04000325 報告日:2005年3月18日
第4回	1	臨床検査	C型肝炎陽性	フランス	男	68	2004/08	不明	症例報告	外国製品	識別番号:3-04000088 報告日:2004年11月22日
第3回	1	臨床検査	C型肝炎陽性	カナダ	男	81	1996	不明	症例報告	外国製品	識別番号:3-04000048 報告日:2004年07月30日
第2回	1	感染症および寄生虫症	C型肝炎	日本	男	78	2003/10/20	未回復	症例報告	当該製品	識別番号:1-03000030 報告日:2003/12/26
	2	肝胆道系障害	肝機能異常NOS	日本	男	62	2003/04/07	軽快	症例報告	当該製品	識別番号:A03-22 報告日:2003/08/14
第1回	報告なし										

*:今回調査期間に追加報告を行った。

100317	2011/3/29	101093	CSLベーリン グ	人血清アルブミ ン 人血液凝固第X III因子 フィブリノゲン加 第XIII因子	人血清アルブ ミン	ヒト血液	米国、ドイツ、 オーストリア	有効 成分 添加 物	あり	あり	なし
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感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期	転帰	出典	区分	備考
		器官別大分類	基本語								
第16回	1	呼吸器および衛生虫	敗血症	日本	男	62	2011/1/26	①回復	症例報告	当該製品	2011/3/1提出、識別番号1-10000099 完了報告
	2	呼吸器および衛生虫	B型肝炎	日本	男	70	2010/12/6	⑥不明	症例報告	当該製品	2011/1/11提出、識別番号1-10000085 未完了報告
	3	呼吸器および衛生虫	E型肝炎	日本	男	30	2010/12/2	①回復	症例報告	当該製品	2011/3/3提出、識別番号1-10000087 完了報告
	4	呼吸器および衛生虫	気道感染	ブラジル	男	23	2010/12/2	⑤死亡	症例報告	当該製品	2011/1/25提出、識別番号3-10000030 完了報告
	5	呼吸器および衛生虫	敗血症性ショック	ブラジル	男	23	2010/12/2	⑤死亡	症例報告	当該製品	2011/1/25提出、識別番号3-10000030 完了報告
	6	呼吸器および衛生虫	細菌感染	ブラジル	男	46	2010/12/2	①回復	症例報告	当該製品	2011/1/25提出、識別番号3-10000031 完了報告
	7	呼吸器および衛生虫	敗血症性ショック	ブラジル	女	1	2010/12/2	⑤死亡	症例報告	当該製品	2011/1/25提出、識別番号3-10000032 完了報告
	8	呼吸器および衛生虫	敗血症	日本	女	68	2010/11/29	①回復	症例報告	当該製品	2011/1/27提出、識別番号1-10000079 完了報告
	9	呼吸器および衛生虫	C型肝炎	日本	女	43	2010/11/24	③未回復	症例報告	当該製品	2010/12/15提出、識別番号1-10000078 未完了報告
	10	呼吸器および衛生虫	細菌感染	日本	男	68	2010/10/28	①回復	症例報告	当該製品	2010/12/27提出、識別番号1-10000067 完了報告
	11	呼吸器および衛生虫	C型肝炎	日本	男	74	2010/10/22	③未回復	症例報告	当該製品	2010/11/30提出、識別番号1-10000070 未完了報告
	12	呼吸器および衛生虫	C型肝炎	日本	女	73	2010/10/19	③未回復	症例報告	当該製品	2010/11/11提出、識別番号1-10000065 未完了報告
	13	呼吸器および衛生虫	細菌感染	日本	男	54	2010/10/18	①回復	症例報告	当該製品	2010/12/21提出、識別番号1-10000061 完了報告
	14	呼吸器および衛生虫	C型肝炎	日本	男	64	2010/10/15	⑥不明	症例報告	当該製品	2010/12/24提出、識別番号1-10000072 完了報告
	15	呼吸器および衛生虫	細菌感染	日本	男	82	2010/9/28	②軽快	症例報告	当該製品	2010/11/17提出、識別番号1-10000056 完了報告
	16	呼吸器および衛生虫	B型肝炎	日本	男	50	2010/9/27	③未回復	症例報告	当該製品	2010/10/14提出、識別番号1-10000055 未完了報告
	17	呼吸器および衛生虫	A型肝炎	日本	男	1	2010/9/15	①回復	症例報告	当該製品	2010/12/27提出、識別番号1-10000066 完了報告
	18	呼吸器および衛生虫	敗血症	日本	女	9	2010/9/6	①回復	症例報告	当該製品	2010/10/25提出、識別番号1-10000046 完了報告
	19	呼吸器および衛生虫	B型肝炎	日本	男	18	2010/9/6	③未回復	症例報告	当該製品	2010/12/7提出、識別番号1-10000069 完了報告
	20	呼吸器および衛生虫	敗血症	日本	男	67	2010/9/3	①回復	症例報告	当該製品	2010/12/28提出、識別番号1-10000043 完了報告
	21	呼吸器および衛生虫	細菌感染	日本	女	63	2010/9/2	②軽快	症例報告	当該製品	2010/10/29提出、識別番号1-10000045 完了報告
	22	呼吸器および衛生虫	B型肝炎	日本	女	60	2010/8/30	③未回復	症例報告	当該製品	2010/10/25提出、識別番号1-10000053 未完了報告
	23	呼吸器および衛生虫	敗血症性ショック	日本	男	75	2010/8/1	①回復	症例報告	当該製品	2010/9/9提出、識別番号1-10000028 完了報告
	27	呼吸器および衛生虫	B型肝炎	日本	女	61	2009/7/7	③未回復	症例報告	当該製品	2011/2/1提出、識別番号1-10000093 未完了報告
第14回	22	呼吸器および衛生虫	B型肝炎	日本	女	61	2009/5/8	①回復	症例報告	当該製品	2011/3/3提出、識別番号1-09000063 完了報告 完了報告を行ったことから、最新情報に更新した。
第13回	3	呼吸器および衛生虫	B型肝炎	日本	男	50	2009/7/22	⑥不明	症例報告	当該製品	2011/2/28提出、識別番号1-09000055 完了報告 完了報告を行ったことから、最新情報に更新した。
	15	呼吸器および衛生虫	B型肝炎	日本	女	75	2009/2/5	⑥不明	症例報告	当該製品	2011/1/27提出、識別番号1-08000082 完了報告 完了報告を行ったことから、最新情報に更新した。
第12回	4	呼吸器および衛生虫	C型肝炎	日本	男	54	2009/1/13	⑥不明	症例報告	当該製品	2011/1/18提出、識別番号1-080000847 完了報告 完了報告を行ったことから、最新情報に更新した。
	10	呼吸器および衛生虫	B型肝炎	日本	男	76	2008/10/17	⑥不明	症例報告	当該製品	2010/12/24提出、識別番号1-080000829 完了報告 完了報告を行ったことから、最新情報に更新した。
	11	呼吸器および衛生虫	C型肝炎	日本	男	56	2008/10/16	⑥不明	症例報告	当該製品	2011/1/12提出、識別番号1-080000830 完了報告 完了報告を行ったことから、最新情報に更新した。
	30	呼吸器および衛生虫	B型肝炎	日本	女	58	2008/8/21	②軽快	症例報告	当該製品	2011/1/27提出、識別番号1-080000655 完了報告 完了報告を行ったことから、最新情報に更新した。
	31	呼吸器および衛生虫	B型肝炎	日本	女	67	2008/8/18	⑥不明	症例報告	当該製品	2011/2/7提出、識別番号1-08000713 完了報告 完了報告を行ったことから、最新情報に更新した。
	43	呼吸器および衛生虫	C型肝炎	日本	男	72	2008/6/6	⑥不明	症例報告	当該製品	2011/2/3提出、識別番号1-080000721 完了報告 完了報告を行ったことから、最新情報に更新した。
	46	呼吸器および衛生虫	B型肝炎	日本	男	55	2008/5/13	②軽快	症例報告	当該製品	2010/11/5提出、識別番号1-080000656 完了報告 完了報告を行ったことから、最新情報に更新した。
第11回	10	呼吸器および衛生虫	B型肝炎	日本	女	71	2008/6/25	①回復	症例報告	当該製品	2010/9/29提出、識別番号1-08000430 完了報告 完了報告を行ったことから、最新情報に更新した。
	20	呼吸器および衛生虫	B型肝炎	日本	男	56	2008/6/4	⑥不明	症例報告	当該製品	2010/10/25提出、識別番号1-080000541 完了報告 完了報告を行ったことから、最新情報に更新した。
	37	呼吸器および衛生虫	C型肝炎	日本	女	39	2008/2/19	⑥不明	症例報告	当該製品	2011/2/7提出、識別番号1-070000279 完了報告 完了報告を行ったことから、最新情報に更新した。

100329	2011/4/20	110078	日本赤十字社	人血小板濃厚液	人血小板濃厚液	人血液	日本	有効成分	あり	あり	なし
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別紙様式第4

感染症発生症例一覧

	番 号	感染症の種類		発生国	性別	年齢	発現時期	転帰	出典	区分	備考
		器官別大分類	基本語								
第16回	1	感染症および寄生虫症	HIV感染	ドイツ	男	31	2009年4月	不明	症例報告	外国製品	識別番号3-10000026 報告日:2010年10月27日
第15回	報告なし										
第14回	1	感染症および寄生虫症	HIV感染	イタリア	男	28	2004年11月	不明	症例報告	外国製品	識別番号3-09000026 報告日:2010年2月26日 報告対象外報告:2010年3月29日
第13回	報告なし										
第12回	報告なし										
第11回	報告なし										
第10回	報告なし										
第9回	報告なし										
第8回	報告なし										
第7回	報告なし										
第6回	報告なし										
第5回	1	感染症および寄生虫症	C型肝炎	ドイツ	不明	不明	不明	不明	症例報告	外国製品	識別番号3-04000125 報告日:2005年5月27日
第4回	報告なし										
第3回	報告なし										
第2回	報告なし										
第1回	報告なし										

100342	2011/4/22	110107	CSLベーリン グ	抗破傷風人免疫 グロブリン	破傷風抗毒素	ヒト血液	米国、ドイツ、 オーストリア	有効 成分	あり	あり	なし
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感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期 (年/月/日)	転帰	出典	区分	備考
		器官別大分類	基本語								
第16回	16-1	感染症および寄生虫症	C型肝炎	米国	女性	不明	2008/08/08	不明	症例報告	外国製品	識別番号：11035513(完了報告) 報告日：2011年5月30日 MedDRA: Version(14.0)
第15回	15-1	感染症および寄生虫症	B型肝炎	米国	男性	58歳	2010/07/20	未回復	症例報告	外国製品	識別番号：10000017(完了報告、追加報告) 報告日：2010年9月6日、2010年9月22日 MedDRA: Version(13.0)
	15-2	肝胆道系障害	肝炎	米国	男性	14歳	不明	不明	症例報告	外国製品	識別番号：10000016(完了報告) 報告日：2010年8月13日 MedDRA: Version(13.0)
	15-2	臨床検査	抗HBc IgG抗体陽性	米国	男性	14歳	2010/05/29	不明	症例報告	外国製品	識別番号：10000016(完了報告) 報告日：2010年8月13日 MedDRA: Version(13.0)
	15-3	臨床検査	抗HBc IgG抗体陽性	スウェーデン	男性	不明	2009/11/16	回復	症例報告	外国製品	識別番号：10000011(完了報告) 報告日：2010年7月9日 MedDRA: Version(13.0)
	15-3	臨床検査	抗HBs抗体陽性	スウェーデン	男性	不明	2009/11/16	回復	症例報告	外国製品	識別番号：10000011(完了報告) 報告日：2010年7月9日 MedDRA: Version(13.0)
	15-4	肝胆道系障害	肝炎	米国	女性	不明	不明	不明	症例報告	外国製品	識別番号：10000007(完了報告) 報告日：2010年6月8日 MedDRA: Version(13.0)
	15-4	臨床検査	A型肝炎抗体陽性	米国	女性	不明	2006	不明	症例報告	外国製品	識別番号：10000007(完了報告) 報告日：2010年6月8日 MedDRA: Version(13.0)
	15-4	臨床検査	B型肝炎抗体陽性	米国	女性	不明	2006	不明	症例報告	外国製品	識別番号：10000007(完了報告) 報告日：2010年6月8日 MedDRA: Version(13.0)

別紙様式第4

	番号	感染症の種類		発現国	性別	年齢	発現時期 (年/月/日)	転帰	出典	区分	備考
		器官別大分類	基本語								
	14-3	感染症および 寄生虫症	B型肝炎	米国	女性	不明	不明	不明	症例 報告	外国 製品	識別番号： 10000002 (追加報告) 報告日：2010年6月7日 第14回症例番号14-3において報告したものの 追加報告 MedDRA: Version (13.0)
第 14 回	14-1	臨床検査	抗HBc IgG抗体 陽性	米国	男性	3歳	2010/01/28	不明	症例 報告	外国 製品	識別番号： 09000025 (完了報告) 報告日：2010年2月26日 MedDRA: Version(12.1)
	14-2	肝胆道系障害	急性肝炎	米国	女性	75歳	2010/03/01	軽快	症例 報告	外国 製品	識別番号： 09000029 (完了報告) 報告日：2010年3月24日 MedDRA: Version(13.0)
	14-3	感染症および 寄生虫症	B型肝炎	米国	女性	不明	不明	不明	症例 報告	外国 製品	識別番号： 10000002 (完了報告) 報告日：2010年4月27日 MedDRA: Version(13.0)
	14-4	臨床検査	A型肝炎陽性	カナダ	男性	19歳	不明	不明	症例 報告	外国 製品	識別番号： 10000008 (完了報告) 報告日：2010年6月14日 MedDRA: Version(13.0)
第 13 回	13-1	臨床検査	C型肝炎陽性	米国	男性	65歳	2009/09	未回復	症例 報告	外国 製品	識別番号： 09000017 (完了報告) 報告日：2009年11月5日 MedDRA: Version(12.1)
	13-2	臨床検査	B型肝炎抗体陽性	米国	女性	32歳	2009/07/12	未回復	症例 報告	外国 製品	識別番号： 09000013 (完了報告) 報告日：2009年9月24日 MedDRA: Version(12.1)
	13-3	感染症および 寄生虫症	B型肝炎	米国	女性	40歳	2009/05	回復	症例 報告	外国 製品	識別番号： 09000012 (完了報告) 報告日：2009年8月19日 MedDRA: Version(12.1)
	13-4	臨床検査	B型肝炎抗体陽性	米国	女性	37歳	2009/04/23	未回復	症例 報告	外国 製品	識別番号： 09000014 (完了報告) 報告日：2009年10月8日 MedDRA: Version(12.1)

	番号	感染症の種類		発現国	性別	年齢	発現時期 (年/月/日)	転帰	出典	区分	備考
		器官別大分類	基本語								
	13-5	臨床検査	B型肝炎抗体陽性	米国	不明	新生児	2009/04/23	未回復	症例報告	外国製品	識別番号：09000015（完了報告） 報告日：2009年10月8日 MedDRA: Version(12.1)
第12回	12-1	感染症および寄生虫症	肝炎ウイルスキャリアー	米国	不明	不明	1993	不明	症例報告	当該製品	識別番号：08000002（完了報告） 報告日：2008年12月22日 MedDRA: Version(11.1)
	12-2	感染症および寄生虫症	C型肝炎	米国	女性	48	2008/12/09	未回復	症例報告	外国製品	識別番号：08000034（完了報告） 報告日：2009年1月19日 MedDRA: Version(11.1)
	12-3	感染症および寄生虫症	C型肝炎	米国	女性	不明	不明	不明	症例報告	外国製品	識別番号：09000004（完了報告） 報告日：2009年5月18日 MedDRA: Version(12.0)
第11回	11-1	臨床検査	B型肝炎抗体陽性	米国	男性	17	2008/05	不明	症例報告	当該製品	識別番号：08000007（完了報告） 報告日：2008年6月5日 MedDRA: Version(11.0)
	11-2	感染症および寄生虫症	C型肝炎	米国	女性	不明	2008	不明	症例報告	当該製品	識別番号：08000018（追加報告） 報告日：2008年11月12日 第11回症例番号11-2において10月17日に報告したものの追加報告 MedDRA: Version(11.1)
	11-2	感染症および寄生虫症	C型肝炎	米国	女性	不明	2008	不明	症例報告	当該製品	識別番号：08000018（完了報告） 報告日：2008年10月17日 MedDRA: Version(11.0)
	11-3	感染症および寄生虫症	B型肝炎	スペイン	女性	不明	2008/6/3	未回復	症例報告	外国製品	識別番号：08000026（完了報告） 報告日：2008年10月31日 MedDRA: Version(11.1)

別紙様式第4

	番号	感染症の種類		発現国	性別	年齢	発現時期 (年/月/日)	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回		0*	0	0	0	0	0	0	0	0	* 当該調査期間に対象となる感染症報告はなかった
第9回		0	0	0	0	0	0	0	0	0	
第8回		0	0	0	0	0	0	0	0	0	
第7回	7-1	臨床検査	H I V抗体陽性	米国	不明	小児	不明	不明	症例報告	外国製品	識別番号： 06000022 (完了報告) 報告日：2006年8月24日 MedDRA: Version(9.0)
第6回	5-1	感染症および寄生虫症	C型肝炎	米国	男性	51歳	2005年9月	未回復	症例報告	当該製品	識別番号： 05000456 (追加報告) 報告日：2006年2月15日 第6回症例番号 5-1 は前回報告における第5回症例番号 5-1 において報告したものの追加報告 MedDRA: Version(8.1)

	番号	感染症の種類		発現国	性別	年齢	発現時期 (年/月/日)	転帰	出典	区分	備考
		器官別大分類	基本語								
第 5 回	5-1	感染症および 寄生虫症	C型肝炎	米国	男性	51 歳	2005 年 9 月	未回復	症例 報告	当該 製品	識別番号： 05000456(追加報告) 報告日：2005 年 11 月 11 日 MedDRA: Version(8.1)
	5-1	感染症および 寄生虫症	C型肝炎	米国	男性	51 歳	2005 年 9 月	未回復	症例 報告	当該 製品	識別番号： 05000456(完了報告) 報告日：2005 年 10 月 27 日 MedDRA: Version(8.1)
	1-3	感染症および 寄生虫症	C型肝炎	米国	男性	26 歳	2002/11/19	不明	症例 報告	当該 製品	識別番号： 03000006(追加報告) 報告日：2005 年 7 月 4 日 第 2 回症例番号 1-3 において報告したものの追加 報告 MedDRA: Version(8.0)
	1-3	感染症および 寄生虫症	B型肝炎	米国	男性	26 歳	2002/10/4	不明	症例 報告	当該 製品	識別番号： 03000006(追加報告) 報告日：2005 年 7 月 4 日 第 2 回症例番号 1-3 において報告したものの追加 報告 MedDRA: Version(8.0)
	4-1	臨床検査	HTLV-1 血清学的検査 陽性	フランス	男性	6 歳	2005 年	不明	症例 報告	当該 製品	識別番号： 05000001(追加報告) 報告日：2005 年 6 月 27 日 第 4 回症例番号 4-1 において報告したものの追加 報告 MedDRA: Version(8.0)
	4-1	臨床検査	HTLV-2 血清学的検査 陽性	フランス	男性	6 歳	2005 年	不明	症例 報告	当該 製品	識別番号： 05000001(追加報告) 報告日：2005 年 6 月 27 日 第 4 回症例番号 4-1 において報告したものの追加 報告 MedDRA: Version(8.0)

別紙様式第4

	番号	感染症の種類		発現国	性別	年齢	発現時期 (年/月/日)	転帰	出典	区分	備考
		器官別大分類	基本語								
第4回	4-1	臨床検査	HTLV-1 血清学的検査 陽性	フランス	男性	6歳	2005年	不明	症例 報告	当該 製品	識別番号：05000001(追加報告) 報告日：2005年4月25日 MedDRA: Version(8.0)
	4-1	臨床検査	HTLV-1 血清学的検査 陽性	フランス	男性	6歳	2005年	不明	症例 報告	当該 製品	識別番号：05000001(完了報告) 報告日：2005年4月7日 MedDRA: Version(8.0)
	4-1	臨床検査	HTLV-2 血清学的検査 陽性	フランス	男性	6歳	2005年	不明	症例 報告	当該 製品	識別番号：05000001(追加報告) 報告日：2005年4月25日 MedDRA: Version(8.0)
	4-1	臨床検査	HTLV-2 血清学的検査 陽性	フランス	男性	6歳	2005年	不明	症例 報告	当該 製品	識別番号：05000001(完了報告) 報告日：2005年4月7日 MedDRA: Version(8.0)
	4-2	感染症および 寄生虫症	C型肝炎	フランス	男性	不明	不明	不明	症例 報告	外国 製品	識別番号：04000129 報告日：2005年3月31日 MedDRA: Version(8.0)

	番号	感染症の種類		発現国	性別	年齢	発現時期 (年/月/日)	転帰	出典	区分	備考
		器官別大分類	基本語								
第 3 回	3-1	感染症および 寄生虫症	C型肝炎	米国	女性	37 歳	2004/5/21	不明	症例 報告	当該 製品	識別番号：04000023 報告日：2004 年 6 月 30 日 MedDRA: Version(7.0)
	3-2	臨床検査	B型肝炎抗体陽性	米国	女性	63 歳	2004/7/27	不明	症例 報告	当該 製品	識別番号：04000059 報告日：2004 年 9 月 7 日 MedDRA: Version(7.0)
	3-2	臨床検査	A型肝炎抗体陽性	米国	女性	63 歳	2004/8/16	不明	症例 報告	当該 製品	識別番号：04000059 報告日：2004 年 9 月 7 日 MedDRA: Version(7.0)
	3-3	臨床検査	B型肝炎抗体陽性	米国	女性	50 歳代	2004/9 月	不明	症例 報告	当該 製品	識別番号：04000082 報告日：2004 年 10 月 20 日 MedDRA: Version(7.1)
	3-3	臨床検査	A型肝炎抗体陽性	米国	女性	50 歳代	2004/9 月	不明	症例 報告	当該 製品	識別番号：04000082 報告日：2004 年 10 月 20 日 MedDRA: Version(7.1)

別紙様式第 4

	番号	感染症の種類		発現国	性別	年齢	発現時期 (年/月/日)	転帰	出典	区分	備考
		器官別大分類	基本語								
第 2 回	1-3	感染症および 寄生虫症	C型肝炎	米国	男性	26 歳	2003/8/30	軽快	症例 報告	当該 製品	識別番号：03000006 報告日：2004 年 1 月 7 日 第 1 回症例番号 1-3 において報告したもの (FAX 報告) の完了報告 MedDRA: Version(6.1)
	2-2	感染症および 寄生虫症	C型肝炎	ドイツ	女性	6 歳	1994/6/21	未回復	症例 報告	外国 製品	識別番号：04000013 報告日：2004 年 5 月 27 日 MedDRA: Version(7.0)
第 1 回	1-1	臨床検査	C型肝炎ウイルス	米国	男性	不明	不明	未回復	症例 報告	外国 製品	識別番号：D03-31 報告日：2003 年 8 月 6 日 MedDRA: Version(6.1)
	1-2	臨床検査	C型肝炎ウイルス	米国	男性	不明	不明	未回復	症例 報告	外国 製品	識別番号：A03-32 報告日：2003 年 8 月 6 日 MedDRA: Version(6.1)
	1-3	感染症および 寄生虫症	C型肝炎	米国	男	26 歳	2003/8/30	軽快	症例 報告	当該 製品	識別番号：03000006 報告日：2003 年 11 月 28 日 FAX 報告：2003 年 11 月 19 日) MedDRA: Version(6.1)

100376	2011/6/29	110255	バクスター	乾燥イオン交換 樹脂処理人免疫 グロブリン	人血清アルブ ミン	人血漿	米国	添加 物	なし	あり	なし
100377	2011/6/29	110256	バクスター	乾燥イオン交換 樹脂処理人免疫 グロブリン	人免疫グロブ リンG	人血漿	米国	有効 成分	なし	あり	なし