資料2

感染症定期報告に関する今後の対応について

平成１６年度第５回
運営委員会確認事項
（平成１６年９月１７日）

１ 基本的な方針

運営委員会に報告する資料においては、

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

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

２ 具体的な方法

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

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

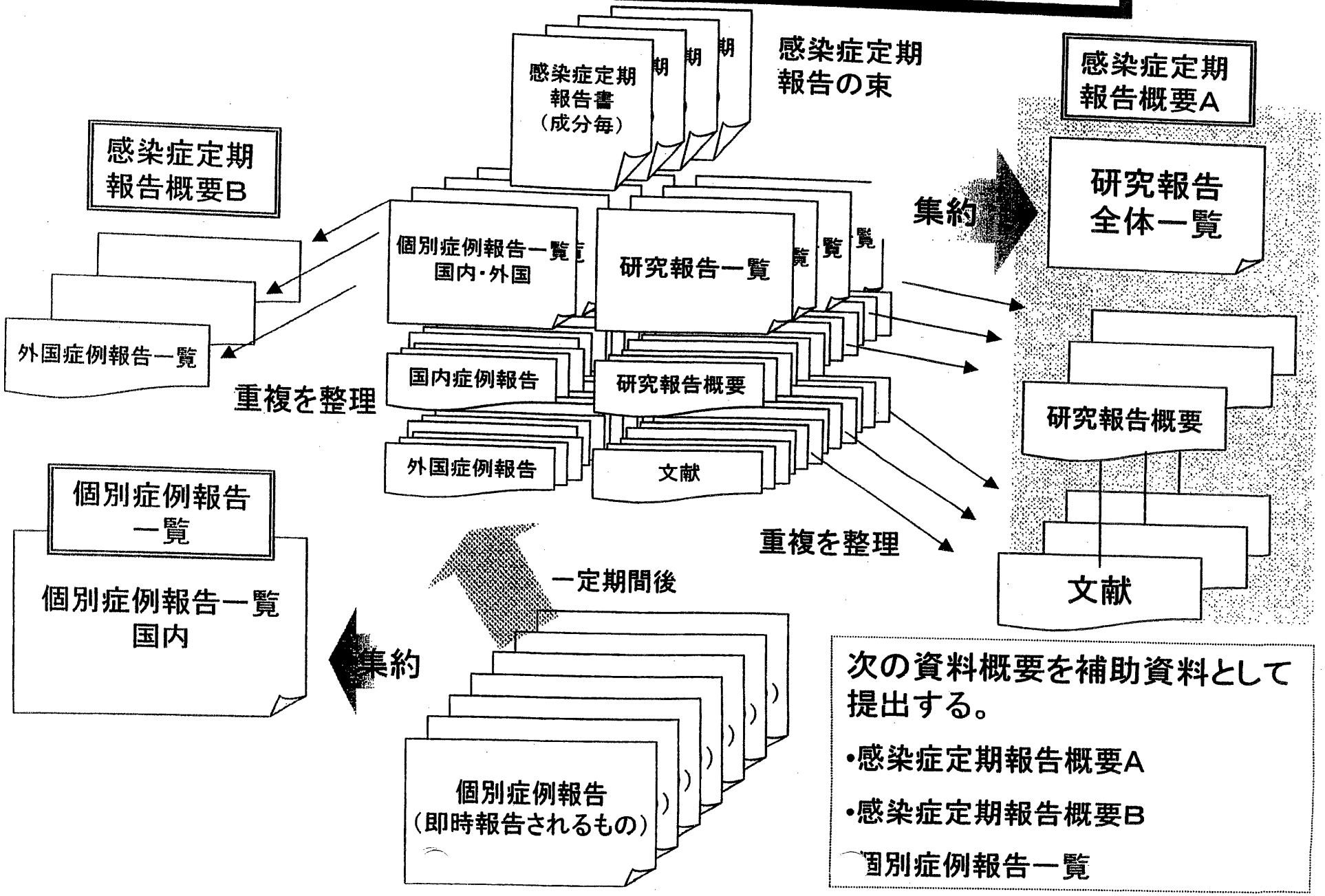
② 感染症発生症例報告のうち、発現国が「外国」の血漿分画製剤の使用による症例は、同一製品毎に報告期間を代表する感染症発生症例一覧（別紙様式第4）をまとめた**「資料概要B」**を事務局が作成し、送付する。

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

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

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

感染症定期報告・感染症個別症例報告の取り扱い



次の資料概要を補助資料として提出する。

- 感染症定期報告概要A
- 感染症定期報告概要B
- 個別症例報告一覧

感染症定期報告概要

（平成２０年７月１５日）

平成１９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２０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（2008/3/1～2008/5/31）

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
80033	2008/03/18	71045	A型肝炎	第55回日本ウイルス学会学術集会 2P213	遺伝子型の異なる複数のHAV細胞馴化株における加熱や加圧による不活化効果を検討した。25%アルブミン存在下60℃10時間加熱処理または室温下300～420MPaの1分間加圧3サイクルに対し、HAV細胞馴化株間で不活化効果に差が見られた。Validation試験に使用する株として、加熱や加圧で不活化されにくく細胞で良く増殖するKRM238が適切と考えられた。血液製剤の製造工程に新規不活化法を導入する場合にはValidation試験に使用する株を適切に選定する必要がある。	
80100	2008/05/30	80207	BSE	OIE／World animal health situation 2008年3月31日	1989年から2008年3月までに、英国以外の世界各国から国際獣疫事務局(OIE)に報告された畜牛におけるBSE症例数である。2006年は、スペイン68頭、アイルランド41頭、ポルトガル33頭、ドイツ16頭、日本およびポーランド10頭、フランス8頭、イタリア7頭、スイスおよびカナダ5頭、チェコ3頭、オーストリア、ベルギーおよびオランダ2頭、スロベニア、スウェーデンおよび米国1頭である。2008年には、これまでにカナダ1頭、アイルランド6頭が報告されている。	1
80100	2008/05/30	80207	BSE	OIE／World animal health situation 2008年4月17日	2008年3月までに、英国から国際獣疫事務局(OIE)に報告されたBSE数である。1987年以前は英国全体で446頭であったが、1992年には37280頭となった。その後、減少し、2007年には67頭となった。2008年は3月31までに10頭報告されている。	2
80054	2008/04/11	80053	B型肝炎	Transfusion 2008; 48: 286-294	最小感染量を求めるために、遺伝型Aまたは遺伝型CのHBVを含む急性期前の接種株をチンパンジーに接種したところ、最小50%チンパンジー感染量(CID50)は各々約10コピーと推定された。最低感染量を接種したチンパンジーにおけるHBV DNA ウィンドウ期は遺伝子型Aでは55-76日、遺伝子型Cでは35-50日、HBs Ag ウィンドウ期は遺伝子型Aでは69-97日、遺伝子型Cでは50-64日であった。またHBV DNAダブリングタイムは遺伝型Cの方が遺伝型Aに比べ有意に短かった。	3
80077	2008/04/25	80124	B型肝炎	Vox Sanguinis 2008 Epub ahead of print	1999年に核酸増幅検査（NAT）によるHBV DNA検出のための最初のWHO国際標準品（サンプル1）が樹立された。同じ血漿から調整され、長期保存された別のDNA検体(サンプル2)およびサンプル1の力価および安定性を多施設で評価した。両サンプルの力価に有意差はなく、凍結乾燥により保存されたHBV DNAが極めて安定であることが確認された。これを受け、WHOは2006年10月にサンプル2を第2の国際標準品として樹立した。	4
80045	2008/03/25	71075	B型肝炎	第31回日本血液事業学会総会 2007年10月 3-5日	平成19年3月、輸血によるHBV感染が疑われるとの報告が千葉県赤十字血液センターにあった。因果関係の確認のために実施した当該輸血用血液製剤に係る保管検体個別NATは陰性であり、献血者追跡調査を行った。1名の献血者が平成19年1月にB型肝炎を発症したとの情報が得られ、調べたところ、献血者のHBV-DNAは患者のそれと塩基配列が一致した。20プールNAT陰性、HBV保管検体個別NAT陰性であったが、献血者追跡調査により輸血用血液製剤からのHBV感染が示唆された症例であった。	
80045	2008/03/25	71075	B型肝炎	第31回日本血液事業学会総会 2007年10月 3-5日 一般演題51	2004年8月よりNATスクリーニングのプールサイズを50から20に縮小した。大阪府赤十字血液センターで検出されたHBV-NAT陽性事例81人を基にプールサイズ縮小の効果等について解析を行った。プールサイズ縮小後に100コピー未満/mLのHBV-NAT陽性者の比率が高くなっていることから、縮小による効果があると思われた。追跡調査、遡及調査及び医師の面談等による総合的な解析によりHBV低濃度キャリアが疑われる献血者がプールサイズ縮小後に多く検出されていることが推察された。	
80059	2008/04/22	80083	B型肝炎	第37回 日本肝臓学会西部会 2007年12月7-8日、肝臓 2007; 48(Suppl 3): A522	輸血によりHBs抗体エスケープ変異株に感染し、肝炎を発症した40歳代女性の症例報告である。献血者、受血者の塩基配列の解析を行って感染が証明された。核酸増幅検査を含む献血者のスクリーニングを行っているにもかかわらず、本邦では年間10-20例のHBV感染が報告されている。その原因の一つがHBs抗体エスケープミュータントであるが、本症例のように献血者、受血者ともに塩基配列の解析を行い感染が証明された例はきわめて稀である。	5
80045	2008/03/25	71075	C型肝炎	American Society for the Study of Liver Diseases 2007年11月2-6日	慢性HCV感染患者1930名(感染群)とHCV陰性患者1941名（対照群）とを比較し、リスク因子を検討した。静注薬物使用、1992年以前の輸血および2つ以上の入れ墨は感染群の方が対照群より有意に高かった。入れ墨はHCV感染リスク要因のない患者群においてもHCV感染と強く相関していた。	6

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80066	2008/04/23	80093	C型肝炎	Clin Vaccine Immunol published online doi:10.1128	抗HCV抗体陰性で、肝組織中のHCV RNA検出により潜在性HCV感染と診断された110例の患者由来の血清中のGOR抗体反応性を調べた。抗GOR IgG陽性患者は22例(20%)で、慢性C型肝炎患者での陽性率(70/110、63.6%)に比べ有意に低かった。HCVに無関係の肝疾患患者120例では抗GOR IgGは全く検出されなかった。市販の検査でHCV特異抗体を検出できず、血清中HCV RNAが検出できない患者で抗GOR IgG検査を行う事は、肝生検なしで潜在性HCV感染を同定する手助けとなりうる。	
80045	2008/03/25	71075	C型肝炎	J Med Virol 2008; 80: 261-267	2003年4～10月にイタリアの血液透析施設で患者4名にHCV抗体セロコンバージョンが認められた。この4名と以前からHCV抗体陽性であった10名のHCV RNAおよびHCV遺伝子型を検査し、系統遺伝学的解析をした結果、新規感染患者4名のHCVは遺伝子型2cで、2c型慢性感染患者1名から分離されたウイルスと近縁であった。感染制御手段の不備と装置による伝播が疑われた。	7
80045	2008/03/25	71075	E型肝炎	Arch Virol 2007; 152: 1623-1635	日本においてHEVの不顕性感染が増加しているかを調べるため、1991-2006年の献血者のうちHEV感染の可能性のあるALT 61IU/L以上の4019名から得られた血清検体中の抗HEV IgG、抗HEV IgMおよびHEV RNAを調べたところ、2004-2006年の献血者のHEV陽性率は1998年のそれと同等であった。またALT 201IU/L以上の献血者についても1991-1995年、1996-1999年および2004-2006年でHEV陽性率の差は見られなかった。	
80045	2008/03/25	71075	E型肝炎	J Med Virol 2008; 80: 283-288	英国サウスハンプシャーの単一施設において2005年6月から13ヶ月間にE型肝炎13例が発生した。これらの患者はルーチンのE型肝炎血清検査を導入開始後に特定された。同一期間中A型肝炎は2例、B型肝炎は4例であったことから、原因不明の急性肝疾患を発症し、関連する渡航歴のない患者全員にルーチンのE型肝炎検査を実施することが重要と考えられる。	8
80054	2008/04/11	80053	E型肝炎	N Engl J Med 2008; 358: 811-817	2004年1月1日～2006年12月31日に腎移植(241名)または肝移植(86名)を受けた患者の移植時の抗HEV IgG保有率は、各々14.5%または10.4%であった。この内、肝移植を受けた3名、腎移植を受けた9名、腎臓と膵臓の移植を受けた2名の計14名で急性HEV感染を同定したが、全員血清HEV RNA陽性であり、内8名が慢性肝炎となった。移植から診断までの時間は短く、慢性肝炎に進展した患者ではリンパ球数並びにCD2、CD3およびCD4 T細胞数が有意に低かった。	9
80033	2008/03/18	71045	E型肝炎	第55回日本ウイルス学会学術集会　2P207	HEVに感染したブタ糞便より精製した4種のHEVは、ウイルス除去膜PLANOVA15Nおよび20Nで全て検出限界以下にまで除去された。液状加熱実験では、PBS組成では加熱開始後短時間で全て検出限界以下となったが、アルブミン存在下では4株とも加熱開始後5時間目でも検出された。HEVは熱に弱いと考えれられていたが、条件によって不活化効果が異なることから、血液製剤や加工食品において慎重に不活化効果を検討しなければならない。	
80100	2008/05/30	80207	HIV	AIDS 2007; 21: 2351-2353	フランスの新規HIV診断例におけるHIV-2およびHIV-1グループO型の感染率を調べた。2003年1月から2006年6月に10184例のHIV新規診断症例が報告されたが、HIV-2およびHIV-1グループO型感染の割合は、各々、1.8%および0.1%であった。これらの症例のほとんどは、異性との接触により感染した流行地域出身の患者であった。HIV-2感染のうち3例は男性と性的関係を持つ非アフリカ系男性であった。	10
80045	2008/03/25	71075	HIV	Clin Infect Dis 2007; 45: e68-71	ボツワナで急性HIV-1感染スクリーニング中に特定された抗体陰性のHIV-1サブタイプC感染の初の症例を報告する。HIV-1抗体検査の結果は、迅速検査、通常の酵素免疫測定法及びウエスタンブロットで全て陰性であった。遺伝子組換えがないHIV-1サブタイプC感染は、ウイルスのgag、pol及びenv遺伝子のジェノタイピングによって確定された。臨床的に安定した状態からAIDS関連死までの期間は約3ヵ月だった。サブタイプCが優勢なアフリカ南部における血清学検査陰性HIV-1感染の調査の重要性が示された。	
80045	2008/03/25	71075	HIV	Eurosurveillance 2007; 12(5): E070524.5 2007年5月24日	AIDS最新号において、LikataviciusらはEuroHIV surveillance network によるヨーロッパの供血血液のHIV陽性率についての14年間のモニタリングデータを提示した。この分析は、1990-2004年のWHO欧州地域のデータが網羅されている。2000-2004年の10万供血中の平均HIV陽性率は西欧1.7、中欧3.4、東欧36.7であった。1990年以降の変化では、西欧で低下、中欧で横ばい、東欧では急激な上昇が認められた。	

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80045	2008/03/25	71075	HIV感染、C型肝炎、B型肝炎	第31回日本血液事業学会総会 2007年10月 3-5日 シンポジウム4-2	日本赤十字社血液事業本部が関わる安全対策の取り組みと感染症リスクについて報告する。平成16年から18年までの3年間に全国の医療機関から日赤血液センターに報告された輸血関連感染症（疑い症例を含む）の報告数は749例であった。日赤の安全対策の実施によりHBV、HCV及びHIVの感染リスクは減少し、安全性は高くなった。しかし、HCV及びHIVも含め遡及調査の実施により確認された感染症例も少なくない。感染拡大を防止するための安全対策を引き続き講じていく必要がある。	
80045	2008/03/25	71075	HTLV	American Society of Hematology 2007年12月8-11日	1999年1月～2006年12月に長崎で献血を行った初回献血者の年齢別、出生年別および期間別HTLV-1血清陽性率の傾向分析を行った。血清陽性率は年齢が高くなるにつれ有意に増加した。また1987～1990年に生まれた献血者では1985～1986年に生まれた献血者と比較して有意に低かった。ウイルスキャリアの母親の授乳を避ける事を指導した県の対応が陽性率の低下に貢献していることが示された。	11
80100	2008/05/30	80207	インフルエンザ	AABB Weekly Report 2008年2月29日	インフルエンザパンデミックと血液供給に関するAABBの作業部会は、パンデミック時に供血間隔の例外的な取り扱いを認めるよう2月14日にFDAに対し要望書を送付した。パンデミック時には適格な供血者数が制限されることが予想されるため、全血および赤血球採取の間隔を短くすることが最も有効であるとしている。	12
80059	2008/04/22	80083	インフルエンザ	Emerg Infect Dis 2007; 13: 1865-1870	カナダの共同農場で生活していた7ヶ月齢の乳児から、A/Canada/1158/2006と名づけられたブタインフルエンザAウイルス(H3N2)が単離された。この農場のメンバー90名の内54名で同ウイルスに対する血清学的検査を行ったところ、54名中9名が陽性であった。また、ブタ10頭のうち1頭で血清陽性が明らかになった。ブタインフルエンザウイルス株は効率的にヒトからヒトへ伝染する形に適応または交雑することから、インフルエンザ流行への備えの一環として養豚者の定期的サーベイランスを検討すべきである。	13
80069	2008/04/24	80108	ウイルス感染	AIDS Res Hum Retroviruses 2007; 23: 1330-1337	Simian Foamy Virus (SFV)感染した男性7名を長期間追跡調査した。男性は非ヒト霊長類と接触する職業であった。男性の全ての末梢血単核球(PBMC)からプロウイルスDNAが検出され、口腔や尿生殖検体から検出されることもあった。長期間（中央値20年）の性的曝露にかかわらず妻たちは陰性であった。特異的な臨床症状は報告されなかった。限定的な追跡調査であるためSFV関連疾患やヒト-ヒト感染を特定できなかった。	14
80045	2008/03/25	71075	ウイルス感染	CDC Press Release 2007年8月22日	米国疾病対策予防センター（CDC）と協力施設の科学者がよく見られるアフリカフルーツコウモリの一種でマールブルグウイルス感染を特定することに初めて成功した。マールブルグウイルスは、ヒトや霊長類に重篤で死に至ることも多い出血熱を引き起こす。コウモリがマールブルグウイルスを保有することが疑われていたが、証拠はなかった。この研究結果はPlos ONEに掲載された。この研究は、マールブルグウイルスの伝播についてより理解し、ヒトにおける感染拡大を予防・減少させる助力になると思われる。	
80045	2008/03/25	71075	ウイルス感染	CDC/MMWR 2007; 56(45): 1181-1184	米国4州における2006-2007年のアデノウイルス血清型14(Ad14)に関連した急性呼吸器疾患に関する報告である。Ad14は稀にしか報告されないが、全ての年齢層の患者に重症で致死的な呼吸器疾患を起こす可能性がある。2006年5月にニューヨーク州で生後12日目の乳児がAd14感染により死亡し、07年3-6月にオレゴン州、ワシントン州およびテキサス州で計140名の感染患者が確認された。これらの患者から新規のAd14変異種が分離された。	15
80045	2008/03/25	71075	ウイルス感染	J Clin Microbiol 2007; 45: 3008-3014	ヨーロッパでの出血熱は主にPuumalaウイルス（PUUV）またはDobravaウイルス感染による。ドイツ南東部Lower Bavariaでハンタウイルス感染患者31名について、酵素免疫測定法、免疫蛍光法、免疫ブロット法による診断を行った。標準的検査による抗体のPUUV特異的タイピングができない症例が2、3あった。3名の患者の急性期血清から得たPUUV RNAをRT-PCR を用いて増幅したところ、同地域で捕獲したハタネズミ から得たウイルス配列と非常に近縁であることが明らかとなった。	
80048	2008/03/25	71078	ウイルス感染	J Med Virol 2008; 80: 365-371	定期的に輸血を受けるサラセミア患者で、Torque Teno virus (TTV)の有無を調べたところ、2-20歳の患者の約10%（118名中12名）がTTV陰性であった。フェリチン、ASTおよびALT値はTTV陽性群より陰性群の方が低かった。TTV-HCV共感染群ではフェリチンおよびALT値がTTV単独感染群より高かった。輸血による高頻度かつ継続的なTTV感染はサラセミア患者における肝機能障害と相関することが示唆された。	16

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
80045	2008/03/25	71075	ウイルス感染	ProMED-mail20070930.3228	オーストラリアQueensland州で蚊が異常発生し、ロスリバーウイルスが拡大している。通常は北部の熱帯地域で優勢であるが、Brisbane南部における過去4週間の感染者数は、昨年（2006年）同時期のほぼ450%である。Queensland保健局の発表によると、過去4週間に報告された感染者数は93例であった。	
80059	2008/04/22	80083	ウイルス感染	ProMED-mail20080218.0645	2008年1月21日、Braziliaで32歳の男性が黄熱のため死亡した。これは、ブラジルにおける15人目の黄熱死亡患者である。Mato Grossoでも1名の感染と死亡が確認された。パラグアイ保健当局は首都Asunciónの病院で集中治療を受けていた39歳の女性が2008年2月16日に死亡したと発表した。同国ではこれまでに、少なくとも6名が黄熱によって死亡した。多くの市民がワクチン投与を求めて病院に殺到している。	17
80045	2008/03/25	71075	ウイルス感染	Transfusion 2007; 47: 1972-1983	供血者血漿検体中のサイトメガロウイルス(CMV) DNA陽性率を検討した。過去にCMV血清陰性で初めて抗CMV IgG陽性を示した供血者82名の血漿検体44%が反復的にCMV DNA陽性であった。1年以上血清反応陽性または血清反応陰性供血者はいずれもCMV DNA陰性であった。白血球除去の実施にもかかわらず、新規血清反応陽性供血者のウイルス血症は輸血伝搬性CMVの残存リスクの重要な原因と考えられる。	18
80045	2008/03/25	71075	ウイルス性脳炎	Neurology 2007; 69: 156-165	同種造血幹細胞移植（HSCT）後に急性大脳辺縁系脳炎を発症した患者9名の臨床、EEG、MRI、ならびに臨床検査特性を調べた。患者は、順行性健忘、不適切な抗利尿ホルモン分泌症候群、軽度CSF多球症、一時的なEEG異常を特徴とした。MRIでは、T2、FLAIR、DWI画像にて、鉤、扁桃体、内側嗅領、海馬領域内に高信号域を認めた。PCRを用いた初回腰椎穿刺CSFの検査では9名中6名がHHV6陽性であり、同脳炎はHHV6と関連がある可能性が示唆された。	
80069	2008/04/24	80108	ウエストナイルウイルス	J Med Virol 2008; 80: 557-563	中央ヨーロッパにおけるウエストナイルウイルス(WNV)の潜在的脅威を調べた。ドイツ人供血者14437名由来の検体中0.03%が抗WNV陽性であった。ドイツ人9976名由来の検体をWNV NAT法を用いてWNV-RNAの有無を調べた結果、全て陰性であった。米国由来血漿プールではWNV-RNAがしばしば検出されたが、ヨーロッパやアジア由来のプールからは検出されなかった。また、血漿製剤製造過程のウイルス不活化によりWNVに関する安全性は保証されることが明らかとなった。	19
80045	2008/03/25	71075	ウエストナイルウイルス	The New York Times 2007年7月26日	米国におけるウエストナイルウイルス症例数は1年前の約4倍であり、大流行がおこる可能性があると政府研究者が報告している。昨年は米国で4,269症例が報告され、この中には1,495例の脳症が含まれ、177人が死亡した。今年はこれまで122症例が報告され、カリフォルニア州と南北ダコタ州で最も多いが、昨年の同時期は33例のみであった。今年は既に脳症が42例および死亡が3例ある。	
80045	2008/03/25	71075	ウエストナイルウイルス	第144回日本獣医学会学術集会 2007年9月2-4日	近い将来、日本にも侵入する可能性があるため、日本産蚊の室内継代株を用いてウエストナイルウイルス増殖・媒介能を調べた。アカイエカ、ヒトスジシマカ、オオクロヤブカでウイルス注入実験を、アカイエカ、ヒトスジシマカで吸血実験をしたところ、全種類の蚊においてウイルスの増殖が観察された。媒介試験では、アカイエカ注入、吸血両群、ヒトスジシマカ2系統の注入群、1系統の吸血群では供試したすべてのマウスが12日以内に死亡し、死亡したマウスからはWNV が検出された。	
80030	2008/03/12	70993	エボラ出血	CDC 2008年1月8日	CDCとウガンダ保健省は、2007年8月から始まったウガンダ西部に位置するBundibugyo地区におけるエボラ出血熱のアウトブレイクを報告した。2008年1月3日までに148人が罹患し、37人が死亡した。患者検体の遺伝子解析により、既知の4つのエボラウイルス株と異なる、新たなウイルス株である可能性が示唆された。確定には更なる研究が必要である。	
80045	2008/03/25	71075	エボラ出血	ProMED-mail20071130.3869	保健当局は、ウガンダ西部において16名が死亡し、他に50人が罹患したエボラウイルスは、新規の株であると2007年11月30日に発表した。最初の症例はコンゴ民主共和国と国境を接するBundibugyo地区において11月10日に報告された。この株では出血はあまり見られず、患者は高熱の後、死亡する。	20

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80045	2008/03/25	71075	クロイツフェルト・ヤコブ病	2007年プリオン研究会 Poster-20	日本の人口動態統計では、CJDによる死亡は過去20年以上に渡り増加傾向を示し、2005年は人口100万対1.23人であった。CJDサーベイランス委員会による調査では過去8年間に918例がプリオン病と判定された。病型別では、孤発性CJD 716例、遺伝性プリオン病 128例、感染性(獲得性)CJD 72例(変異型CJD 1例、硬膜移植後CJD 71例)、および分類不能 2例であった。	
80045	2008/03/25	71075	チクングニヤウイルス感染	Eurosurveillance 2007; 12(9): E070906.1	チクングニヤ熱は2005年以来、大規模な流行がインド洋諸島とインドから報告されているが、これまでヨーロッパ地域内での蚊による感染伝播は発生していなかった。2007年8月にイタリアのエミリア・ロマーニャ州ラヴェンナ県衛生当局は異常に多数の発熱患者発生を検知し、臨床・疫学調査を行った。血清学的検査およびPCR法でチクングニヤ熱と確定された。更にヒトスジシマカからもPCR法によりチクングニヤウイルスが確認された。2007年9月4日までに合計197名の患者が報告されている。	
80045	2008/03/25	71075	チクングニヤウイルス感染	Lancet 2007; 370: 1840-1846	イタリア北東部の隣接する2つの村で原因不明の発熱性疾患患者が多数報告され、ヒトおよび蚊由来の検体を分析した結果、チクングンヤウイルス(CHIKV)が原因であることが明らかとなった。2007年7月4日から9月27日の間に205例のCHIKV感染症例を同定した。村の親戚 を訪問した時に発症したインド出身男性が初発症例と推定された。系統遺伝学的分析により、イタリアのCHIKV株はインド洋諸島での初期のアウトブレイクで分離された株と高い相同性を示した。	21
80045	2008/03/25	71075	チクングニヤウイルス感染	Pediatr Infect Dis J 2007; 26: 811-815	チクングニヤウイルス感染が大流行したレユニオン島の5つの新生児医療部門で同ウイルスの母子感染を調べるため、後ろ向き記述的研究を実施した。母親は出産時に徴候があったか又は新生児が出生初日に発病したかをスクリーニングし、新生児38名を登録した。無症候の2名を除き、全母親が周産期 (分娩4日前～1日後)に症状があった。全新生児が発熱(79%)、疼痛(100%)などの症状を示し、脳脊髄液のPCR法は24名中22名で陽性であった。高い罹患率の周産期母子伝播の可能性が初めて示された。	
80030	2008/03/12	70993	チクングニヤウイルス感染	PLoS Pathogens 2007; 3: 1895-1906	2005～2006年にレユニオン諸島でアウトブレイクしたチクングニヤウイルス(CHIKV)感染は、エンベロープ蛋白遺伝子の変異株(E1-A226V) が関係していた。この変異の、ネッタイシマカおよびヒトスジシマカにおけるCHIKV適合性に対する影響を調べた。その結果、CHIKVのヒトスジシマカに対する感染性が有意に増加し、哺乳マウスへの伝播がより効率的になることが明らかとなった。通常のベクターであるネッタイシマカがいない同地域でCHIVKが大流行したのはこの変異が原因と考えられる。	
80046	2008/03/25	71076	ツツガ虫病	朝鮮日報 2007年8月21日	韓国では最近ツツガムシ病の患者が急増している。2007年8月20日、疾病管理本部の発表によると、2002年に1,919人だったツツガムシ病の患者数が、04年は4,698人、06年には6,420人に増加したことが分かった。1993年末に法定伝染病に指定されて以来、患者数は実に25倍以上増加した。ツツガムシ病は、主に9月以降、ツツガムシ菌に感染したツツガムシ(ダニの一種)の幼虫に刺されることにより感染する。10日間程度の潜伏期を経ると、突然高熱が発生し、目の充血、頭痛、筋肉痛、発疹などの症状が現れる。	
80045	2008/03/25	71075	デング熱	ProMED-mail20071001.3237	2007年9月30日、中国保健当局はFujian省Putian市で39例のデング熱症例が確定されたと発表した。ベトナムでは2007年9月24日時点で約68000人が感染し、内60名が死亡した。パキスタンでは2007年9月26日、Karachiで新たに22例のデング熱症例が報告された。ラテンアメリカとカリブ海諸国ではデング熱の最悪のアウトブレイクが起こっており、2007年になってから何十万もの人々が関節痛を訴え、約200人が死亡した。	
80045	2008/03/25	71075	デング熱	YAHOO!ニュース 2007年10月14日	台湾南部でデング熱が流行している。台南市当局によると2007年10月13日までに市内で511人の感染者が確認された。隣接する高雄市でも2つの区で集団感染が発生しており、感染の広がりは過去最大規模である。行政と軍が協力して大規模な蚊の撲滅作戦を展開する方針である。	
80046	2008/03/25	71076	トリパノソーマ症	ABC Newsletter 2007年9月14日	AABBはCDCからAABBシャーガス病バイオビジランスネットワーク強化をするための資金を受けている。2007年9月13日時点で、反復反応性供血710件でT. Cruziに対する抗体の追加RIPA試験を行った結果、196例がRIPA陽性、486例が無反応で、残りの検体については結果保留となっている。13の検査施設がシャーガスネットワークにデータを報告し、18の検査施設が報告のため同ネットワークにアクセスしている。	

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80059	2008/04/22	80083	トリパノソーマ症	Clin Infect Dis 2008; 46: e44-47	血液製剤の輸血によりシャーガス病に感染し、死亡したスペイン人患者の寄生虫学的、血清学的疾患経過、ならびに供血者の調査の報告である。患者は白血病の既往があり、176名以上の供血者由来の輸血を受けていた。臍帯血移植のための免疫抑制状態で、寄生虫が血液脳関門を通過して神経系に感染したことが確認された。特定された供血者は無症候であった。複数回輸血患者は、免疫抑制剤治療実施前に、抗Trypanosoma cruzi抗体のスクリーニングを受けるべきである。	22
80046	2008/03/25	71076	トリパノソーマ症	CMAJ 2007;177: 242	カナダ血液サービスは、2008年後半の血液製剤製造プロセス見直しの際に北緯49度以北では稀にしか見られないシャーガス病のスクリーニングを開始する。2種類のシャーガス病検査法がカナダ保健省の認可を待っている。供血血液の検査実施は、血小板製剤の製造を「バフィーコート」法に切替えてからとなる。メキシコや中南米では800万人～1,100万人がシャーガス病の保因者であり、毎年45,000人以上死亡している。カナダでは、これまでに輸血による感染が2例マニトバ州で発生した。	
80046	2008/03/25	71076	トリパノソーマ症	第48回　日本熱帯医学会大会　12C-02	日本におけるラテンアメリカ人の慢性シャーガス病キャリアーからの献血についての対策を検討した。カーミC液（CPD液）を用いてT.Cruzi感染マウス血液を4℃にて1-21日間保存処理を行ったところ、マウスへの感染性は無処理のものと差異は無かったが、病原性はかなり減弱することが示された。しかし、T.Cruzi虫体はほとんどの白血球除去フィルターを通過した。現在の保存血液提供システムはシャーガス病の輸血感染防止には不十分であり、対策の改善が必要である。	
80059	2008/04/22	80083	トリパノソーマ症	第48回　日本熱帯医学会大会　2007年10月12-13日　12C-02	日本におけるラテンアメリカ人の慢性シャーガス病キャリアーからの献血についての対策を検討した。カーミC液（CPD液）を用いてT.Cruzi感染マウス血液を4℃にて1-21日間保存処理を行ったところ、マウスへの感染性は無処理のものと差異は無かったが、病原性はかなり減弱することが示された。しかし、T.Cruzi虫体はほとんどの白血球除去フィルターを通過した。現在の保存血液提供システムはシャーガス病の輸血感染防止には不十分であり、対策の改善が必要である。	
80048	2008/03/25	71078	パルボウィルス	J Gen Virol 2007; 88: 2162-2167	ヒト血漿プール中に新規のパルボウイルスPARV4とその変異株であるPARV5が存在することが最近示された。4株のPARV4と2株のPARV5のDNA配列を分析したところ、PARV5はPARV4と同様に2つのオープンリーディングフレームを持ち、PARV4とPARV5は92%近くのヌクレオチド相同性を示した。両者は密接な関係のあるジェノタイプであり、ジェノタイプ1と2(PRV5と呼ばれていたもの）から成るPARV4という1つのウイルス名を使用することを提案する。	
80045	2008/03/25	71075	パルボウィルス	Transfusion 2007; 47: 1756-1764	米国の血液センター7施設において2000-2003年の期間に採取した5020名の供血者由来の保存血漿検体を高感度PCRスクリーニング法を用いてパルボウイルスB19 DNAについて検査した。B19 DNA陽性率は0.88%であった。DNA陽性検体の全てがIgG陽性で、23%がIgM陽性であった。IgM血清陽性率はDNA値と相関した。	
80041	2008/03/24	71071	パルボウィルス	Transfusion 2007; 47: 1765-1774	B19ウイルスの不活性化機構を調べた。熱または低PHによるB19Vの不活性化はカプシド分解によるものではなく、感染性ビリオンがDNA枯渇カプシドへ変換することによって起こった。DNA枯渇カプシドは感染性はないが、標的細胞に接着することは可能であった。Parvoviridaeの他のウイルスとの比較試験の結果、被殻状態でのB19V DNAの著しい不安定性が明らかとなった。B19Vが不活化処理に抵抗性が低いのはこのためと考えられる。	
80077	2008/04/25	80124	パルボウィルス	Transfusion 2007; 47: 1775-1782	ドイツ及びオーストリアで2003-2006年の計280万の供血検体をパルボウイルスB19(B19V)についてミニプールNATによりスクリーニングした。その結果、10万IU/ml以上は10万供血当たり12.7、10万 IU/ml未満は10万供血当たり261.5であった。10万 IU/ml未満のB19Vを含有する検体では全てに中和抗体（VP2)が検出された。10万 IU/ml未満のB19 DNA陽性血液製剤は高濃度の中和抗体を含有するため安全であると思われる。	23
80062	2008/04/22	80086	パルボウィルス	Transfusion 2008; 48: 178-186	B19V IgG力価に関係したB19V中和の役割を検討するため、製造血漿プール1000以上について酵素免疫測定法による検査を実施した。血漿プールは平均33±9IU/mL（最小値11IU/mL）のB19V IgG力価を含有し、これらの11IU/mLのB19V IgGは、B19V遺伝子型1の感染性を4.6 log、遺伝子型2の感染性を3.9 log以上を中和した。このため、このようなプール由来の10%静注用免疫グロブリン製剤（IVIG）は、さらに高いB19V中和活性を含有することが明らかとなった。	24

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80077	2008/04/25	80124	パルボウィルス	Vox Sanguinis 2007; 93: 208-215	2005年3月から2007年3月の間にオランダで約260万の血漿成分検体を対象として2種類のPCRアッセイを用いたパルボウイルスB19(B19V)のスクリーニング試験を実施した。その結果、232検体がB19V DNA値100万IU/ml以上であった。ヨーロッパ人ドナーにおいてはB19V遺伝子2型及び3型の保有率は極めて低いと考えられた。	25
80077	2008/04/25	80124	パルボウィルス	Vox Sanguinis 2007; 93: 216-222	ヒト血漿中のパルボウイルスB19(B19V)抗原を検出するEIAを開発した。本アッセイを用いて無症候性ドナーから採取したウイルス血症性の献血検体を検査したところ、低pHの状態ではB19V検出が大幅に増加した。また、B19抗原の検出はB19 IgMまたはIgG抗体存在下で影響を受けなかった。B19V IgMアッセイと併用することにより、急性B19感染の91%を検出した。B19V IgM検出法とB19V抗原EIAの併用はPCRに替わるB19V感染の有効な検出法となると思われる。	26
80033	2008/03/18	71045	パルボウィルス	Vox Sanguinis 2007; 93: 341-347	過去30～35年間に製造された第Ⅷ因子製剤中にヒトパルボウイルスが存在するかを調べた。175ロットのうち28ロットがPARV4シークエンスを含み、その内2ロットにジェノタイプ1型及び2型の両方が存在した。最大ウイルス量は10^5copies/mL以上であった。PARV4陽性の第Ⅷ因子製剤の大部分は1970年代及び1980年代に製造されていた。B19Vは175ロット中70ロットで陽性であった。	
80077	2008/04/25	80124	パルボウィルス	Vox Sanguinis 2008; 94: 74-80	パルボウイルスB19(B19V)の新規の遺伝子型が発見されていることから、種々の遺伝子型のB19V検出及び定量結果を統一する方法を見いだす目的で国際ワーキンググループ会議が2007年3月に開催された。その会議の要旨である。会議では、B19V株の分類、種々の遺伝子型の有病率、分布、臨床的意義などが検討された。また、特性が十分に明らかになっている標準物質を用いたアッセイの標準化について合意が得られた。	27
80054	2008/04/11	80053	ヒトポリオーマウイルス感染	Science 2008; 319: 1096-1100	メルケル細胞癌(MCC)検体をdigital transcriptome subtraction法を用いて検査し、新種のポリオーマウイルスを同定し、メルケル細胞ポリオーマウイルス(MCVまたはMCPyV)と命名した。このウイルスはMCC腫瘍10検体中8例(80%)で検出されたが、対照組織検体では59例中5例(8%)、対照皮膚組織検体では25例中4例(16%)でしか検出されなかった。MCVがMCCの病原因子である可能性が示唆された。	28
80046	2008/03/25	71076	ブルセラ症	J Travel Med 2007; 14: 343-345	64歳の日本人男性が6週間続く発熱で1998年6月2日に都内の病院に入院した。入院時の血液培養からグラム陰性桿菌が検出され、Brucella melitensis2型と同定された。患者は同年3月にイラクに滞在し、ヒツジのチーズを摂取したことが明らかとなった。患者の妻(60歳)が同年5月31日から発症し、Brucella melitensisが血液と関節液の培養で検出された。イラクの帰国者からその妻へ、ブルセラ症が性感染した可能性がある。	29
80059	2008/04/22	80083	ペスト	Emerg Infect Dis 2007; 13: 1459-1462	2003年6月から7月にアルジェリアOran地区においてペストの集団感染が発生した。同国では、この疾患は50年以上報告されていなかった。腺ペスト症例18名が特定され、Yersinia pestisが6名から分離された。初発患者を除き、全員が回復した。標的予防的化学療法、衛生、ベクターコントロールが、感染制御上重要な役割を果たした。疫学的、分子生物学的な知見から、当該期間中、現地の保菌動物の存在が強く示唆されたが、その起源については特定できなかった。	30
80046	2008/03/25	71076	リケッチア症	Jpn J Infect Dis 2007; 60: 241-243	血清学的、微生物学的に確定された日本紅斑熱の初めての死亡症例を報告する。淡路島在住の77歳男性で、2005年9月2日に食欲低下を呈し、翌日、下腿に皮疹が出現、4日目に38.7℃の高熱、歩行障害、構音障害が出現、肝機能障害が急速に進行し、DIC、消化管出血により8日目に死亡した。右肩にダニ刺し口があった。血液よりDNAを抽出し、PCRを実施したところ、塩基配列はR. japonicaと100%一致した。日本紅斑熱は増加傾向にあり、注意が必要である。	
80030	2008/03/12	70993	ﾘﾝﾊﾟ性脈絡髄膜炎	N Engl J Med 2008; 358 10.1056/NEJMoa073785	オーストラリアで一人のドナーから臓器移植を受けた3例が移植後4-6週後に死亡した。他のいかなる方法でも原因不明であったが、2例のレシピエントの移植肝および腎から得られたRNAを偏りのない迅速シークエンシングで解析することにより、ﾘﾝﾊﾟ性脈絡髄膜炎に関係する新規のアレナウイルスが原因であることが明らかとなった。レシピエントの腎、肝、血液および脳脊髄液からこのウイルスが検出され、また免疫組織学的および血清学的に確認された。この方法は病原体発見の強力な手段である。	
80054	2008/04/11	80053	ﾘﾝﾊﾟ性脈絡髄膜炎	N Engl J Med 2008; 358: 991-998	オーストラリアで一人のドナーから臓器移植を受けた3例が移植後4-6週後に死亡した。他のいかなる方法でも原因不明であったが、2例のレシピエントの移植肝および腎から得られたRNAを偏りのない迅速シークエンシングで解析することにより、ﾘﾝﾊﾟ性脈絡髄膜炎に関係する新規のアレナウイルスが原因であることが明らかとなった。レシピエントの腎、肝、血液および脳脊髄液からこのウイルスが検出され、また免疫組織学的および血清学的に確認された。この方法は病原体発見の強力な手段である。	

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80045	2008/03/25	71075	異型クロイツフェルト・ヤコブ病	2007年プリオン研究会 Poster-38	BSE感染ウシ由来の脳乳剤を用いてPrPresのin vitro感染系の確立を試みた。感染させたヒト由来グリオーマ細胞株から抗プリオン抗体に反応する約30KのPK耐性のバンドが検出された。このバンドは非感染細胞には存在しなかった。また、9ヶ月継代した感染細胞の培養上清に伝達性があることが明らかとなった。さらに20nmのウイルス除去膜によって培養上清の伝達性が減少することが認められた。	
80045	2008/03/25	71075	異型クロイツフェルト・ヤコブ病	Arch Neurol 2007; 64: 1780-1784	運動失調や記憶障害などを呈し、発症後14ヶ月で死亡した患者(39歳女性)の剖検を行ったところ、白質の広汎な変性と皮質および白質におけるPrP沈着を示す非定型孤発性CJDであった。小脳組織由来のPrPScを分子分析した結果、vCJDでみられるPrPSc 4型と似た新規のPrPScであることが示された。典型的vCJDとはEDTA存在下でのプロテアーゼ開裂部位が異なった。この患者のPRNPコドン129はホモバリンであった。	31
80080	2008/04/25	80127	異型クロイツフェルト・ヤコブ病	Biochem Biophys Res Commun 2007; 364: 796-800	正常な脳ホモジネートを慢性消耗性疾患エルクの異常プリオンとともにインキュベートするin vitroアッセイを用いて、プリオンの転換について調べた。標準の条件下(pH 7.4)ではPrPCからPrPScへの転換は同種(トナカイ、ムースなど)でのみ効率的であったが、、酸性条件下(pH3.5)では異種(ヒト、ウシ、ハムスターおよびマウス)においても転換が著しく促進された。基質の部分変性によって構造上の変化が起こり、遠隔種	32
80033	2008/03/18	71045	異型クロイツフェルト・ヤコブ病	Biologicals 2007; doi:10.1016/j.biologicals.2007.04.005	異なるポアサイズのウイルス除去膜を使用し、異なる処理を行ったスクレーピープリオン蛋白(PrPSc)の除去能力を評価した。超音波処理により粒子径分布を至適化するように調製した263K MFをスパイク物質として使用したときは、75nmのろ液中にPrPScが検出された。15nmのろ過のみが全ての条件でウエスタンブロット法の検出限界以下までPrPScが除去されることが示された。しかし、 1条件下の15nmろ液のバイオアッセイの結果では、感染性PrPScが確認された。	
80033	2008/03/18	71045	異型クロイツフェルト・ヤコブ病	J Biol Chem 2007; 282: 35878-35886	トランスジェニックマウス(101LL)を用いた感染性実験の結果、TSE疾患の臨床症状と脳の空胞化という徴候を示すがPrPScのレベルが低いかもしくはイムノブロット法では検出されない動物の脳組織内に、高力価のTSE感染性が存在しうることが明らかとなった。この結果はPrPScのレベルと感染価との間の相関性に疑問を投げかけるものであり、プロテアーゼK抵抗性のPrPをほとんどもしくは全く含まない組織が感染性となりうること、および高力価のTSE感染性を有しうることを示すものである。	33
80100	2008/05/30	80207	異型クロイツフェルト・ヤコブ病	J Virol 2008; 82: 3697-3701	非典型的BSE株の1つであるBASE(またはBSE-L)の感染性およびヒトでの表現型を調べた。BASEウシ由来の脳ホモジネートを、ヒトプリオン蛋白を発現するトランスジェニック(Tg)マウスに接種したところ、60%が20-22ヶ月後に感染し、古典的BSEに関する報告より高い感染率であった。BASE感染ヒト化Tgマウス脳における病因性プリオンのアイソフォームは、元のウシBASEまたは孤発性ヒトプリオン病のものとは異なっていた。またBASEプリオンはリンパ向性であった。	34
80059	2008/04/22	80083	異型クロイツフェルト・ヤコブ病	Microbiol Immunol 2007; 51: 1221-1231	感染動物モデルにおいても、血中のPrPresは白血球を除きめったに検出されない。新規の酸性SDS沈殿法と高感度化学発光法とを組み合わせることにより、プロテイナーゼK耐性3F4反応性タンパクが、スクレイピー感染ハムスターの血漿中からは検出されるが、疑似感染ハムスターでは検出されないことが示された。血漿中においてPrPresは他の血漿タンパクと糖鎖を通じて凝集しており、スクレイピー感染ハムスター血漿において検出可能となったことが示唆された。	35
80062	2008/04/22	80086	異型クロイツフェルト・ヤコブ病	Prion 2007 P04.102 2007年9月26-28日	1987年6月から1998年9月にかけて出荷された計175バッチの血漿製剤中に、後にvCJDと診断された11名からの供血が含まれていたが、これらの製品に関係したvCJD症例は今までのところ全く報告されていない。これは赤血球輸血によると思われるvCJD感染が3例あることと対照的である。血漿分画製剤の製造工程によるプリオン除去効果を調べたところ、2.7～11.5log以上の除去能があることが明らかとなった。	
80045	2008/03/25	71075	異型クロイツフェルト・ヤコブ病	Prion 2007; 2007年9月26-28日 Edinburgh P04.51	73歳の受血者で生前に特定されたvCJDの非典型的症状の報告である。患者は1997年12月に輸血を受けたが、供血後にvCJDを発症した供血者由来の赤血球製剤であった。輸血から6年後、受血者は疲労及び集中困難を訴えたが、神経学的検査及び脳MRIは正常であった。この6ヵ月後に神経学的症状が発現し、進行したが、血清学的検査は正常であった。MRIでは視床背側核全体の顕著な信号変化が示された。vCJDの長期潜伏期間と無症候状態は、重大な公衆衛生問題を提示する。	

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80045	2008/03/25	71075	異型クロイツフェルト・ヤコブ病	ProMED-mail20080107.0087	英国National CJD Surveillance Unitに報告された2008年1月7日現在のCJD数は、vCJD診断確定死亡症例（確定例）114名、vCJD可能性死亡症例（神経病理学的確定診断がない）48名、vCJD可能性死亡症例（神経病理学的確定診断待ち）1名で、vCJD診断確定または可能性例の死亡総数163名であった。生存中のvCJD可能性症例数は3名であった。英国におけるvCJD流行は減少しつつあるという見解に一致する。	36
80095	2008/05/26	80188	異型クロイツフェルト・ヤコブ病	Transfusion 2008; 48: 609-619	ヒツジのリコンビナントPrP（rPrP）のヒツジにおける血液クリアランスならびにスクレイピー関連フィブリル(SAF)静注後のPrPresへの曝露について調べた。rPrPのARR変異型は、VRQ変異型よりもより早く除去された。また、PrPcのARR変異型のクリアランスがVRQ変異型のクリアランスよりも大きいことが示唆された。rPrPの血漿クリアランスは、両腎臓摘出後は52%減少し、rPrP除去に腎臓が重要であることが示された。PrPresはSAF静注後は緩やかに除去された。	37
80080	2008/04/25	80127	異型クロイツフェルト・ヤコブ病	Vet Res 2008; 39: 33	1990年代にウシ海綿状脳症が英国で流行したことを受けて、ヒト及び動物において伝染性海綿状脳症を検出するために開発された様々な技法についての総説である。vCJDが輸血により感染しうることが明らかなことから、vCJDの診断に関する血液検査の開発が最優先事項である。	38
80080	2008/04/25	80127	異型クロイツフェルト・ヤコブ病	Vet Res 2008; 39: 34	伝染性海綿状脳症（TSE）の伝播性を調べるための実験的アプローチ法を要約し、実験における所見と自然発生するTSE（主にウシ海綿状脳症及びスクレイピー）およびコントロール方法との関連性を考察している。	39
80095	2008/05/26	80188	感染	56th Annual Meeting of the American-Society-of-Tropical-Medicine-and-Hygiene 1044	ヒト顆粒球アナプラズマ症(HGA)の発生率は、1999年以来2倍となった。原因病原体のAnaplasma phagocytophilumによる血液の安全リスクを調査するため、間接免疫蛍光法を用いてコネチカット州及びマサチューセッツ州の血液ドナーのA. phagocytophilumに対するヒトIgG抗体を測定した。その結果、2001年から2006年に採取された15,828ドナー血清中432例（2.7%）が抗体陽性であった。比較的高い陽性率が持続していることから、A. phagocytophilumの血液安全性に及ぼす影響を調べる必要がある。	40
80046	2008/03/25	71076	感染	ABC Newsletter 2008年1月11日	血液安全・安定供給諮問委員会は、米国保健社会福祉省事務局に対し、安全で効果的な輸血用血液製剤の病原体低減技術（不活化）の早急な開発を優先して進め、開発され次第実施するよう勧告した。病原体低減の効果と安全性を示すエビデンスの蓄積は、今後蔓延する可能性のある感染症に対し広く適応できるセーフガードとして、この技術の導入を保証するという決議を採択した。	41
80077	2008/04/25	80124	感染	Transfusion 2007; 47: 2180-2184	カナダ血液サービスとHema-Quebecが主催した血漿分画製剤における病原体不活化（PI）に関するコンセンサス会議で得られた結論の考察と主な見解が報告されている。現在ヨーロッパで広く用いられているPIがカナダや米国で実現されようとしている。PIを推進することによって、現在の技術や供血者スクリーニング法では防ぐことができない輸血伝播感染症を減らすことができる。	42
80046	2008/03/25	71076	感染	Transfusion 2007; 47: 2338-2347	2007年3月29-30日、カナダのトロントで行われた病原体不活化(PI)技術に関するコンセンサス会議の報告である。近年の検査技術の発達により、現状の輸血感染症リスクは非常に低く、PIを直ちに導入する事は推奨しない。しかし新興感染症のリスクは未知数であり、PIは予防手段として重要である。広範囲の病原体を不活化できる安全な方法が確立されれば実施すべきである。	43
80045	2008/03/25	71075	感染	Vox Sanguinis 2007; 93(Suppl.2): 31	日本赤十字社(JRC)が全国的ヘモビジランス体制を導入してから14年が経過した。報告された輸血副作用症例数は年間約2000例で、過去3年間はほぼ一定である。非溶血性輸血副作用は報告症例の約80%を占め、輸血関連急性肺障害などが含まれる。輸血感染症の報告数は年々減少している。JRCのヘモビジランスは病院の自発報告に基づいており、病院と血液センターとの協力が不可欠である。	44
80048	2008/03/25	71078	寄生虫感染	Int J Med Microbiol 2007; 297: 197-204	ドイツにおけるヒトバベシア症の初めての症例を報告する。患者は結節性リンパ球性ホジキンリンパ腫が再発し、脾臓摘出されたドイツ人の63歳男性で、リツキシマブ投与後、貧血とヘモグロビン尿による暗色尿のため入院した。末梢血塗抹標本で梨状の寄生虫赤血球封入体が確認されバベシア症と推定され、Babesia特異的18S rDNA PCRによって確認された。シークエンス分析によりEU1と99.7%の相同性があり、EU3と名づけられた。寄生虫が消えるまでにはatovaquoneによる長期治療を要した。	
80046	2008/03/25	71076	細菌感染	ABC Newsletter 2007年9月21日	FDAは輸血前の血小板中の細菌汚染を検出するための初めての迅速検査を販売承認した。Verax Biomedical Inc 製造のPlatelet Pan Genera Detection Test Systemは病院の輸血部で使用するための使い捨て検査機器である。	

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
80100	2008/05/30	80207	細菌感染	Vox Sanguinis 2008; 94: 193-201	ルックバック調査でPropionibacterium acnes汚染が推定される血小板濃縮製剤(PC)の保存から輸血までを追跡したところ、輸血後の有害事象は見られなかった。In vitro試験でプロピオン酸菌属の臨床分離菌をPCに接種し、好気的に22℃で10日間保存という条件下での生育を調べたところ、細菌の生育は緩慢か生育を認めなかった。プロピオン酸菌属はPC保存条件下では増殖しないため、検出されないか、輸血後に検出されると考えられた。	45
80030	2008/03/12	70993	鳥インフルエンザ	China View, www.chinaview.cn 2008-01-10	2007年12月に江蘇省南京で発生した52歳男性の鳥インフルエンザ感染患者は、患者であった息子との濃厚な接触により感染したものであり、ウイルスの変異は認められていない。しかし、息子と父親はいずれも死亡した家禽との接触がないため、息子の感染源は明らかになっていない。息子は11月24日に発症し、12月2日に死亡し、父親は12月3日に発症したが回復した。ヒト用トリインフルエンザワクチンは臨床試験Phase IIの段階にある。	
80045	2008/03/25	71075	鳥インフルエンザ	WHO/CSR 2007年12月9日	中国におけるトリインフルエンザの状況(update5):2007年12月9日、中国衛生省は同ウイルスの新規ヒト感染症例を報告した。Jiangsu省の52才の男性で、12月2日に同ウイルス感染で死亡した24才男性の父親で、現在入院中である。中国での確定例は27例で、うち17例が死亡している。	46
80045	2008/03/25	71075	日本脳炎	Epidemiol Infect 2007; 135: 974-977	2004年11月から2005年2月にかけて、日本の西部に位置する広島県の野生イノシシから血清25検体を採取した。日本脳炎ウイルス(JEV)に対する抗体検査を、IgMキャプチャー及びIgG酵素免疫測定法(ELISA)、並びにプラーク減少中和試験により行った。17検体(68%)がJEV中和抗体陽性だった。中和抗体陽性検体は全てIgG-ELISA陽性だった。1検体はIgMも陽性だった。約70%の野生イノシシが抗JEV抗体陽性であることが示され、この地域のJEV感染サイクルに関与している可能性が提示された。	

別紙様式第2-1

No. 23

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2008. 3. 5	該当なし	

一般的名称	解凍人赤血球濃厚液	研究報告の公表状況	OIE - World Organisation for Animal Health. Available from: URL: http://oie.int/eng/info/en_esbmonde.htm	公表国
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)			OIE

研究報告の概要

○世界(英国を除く)の畜牛におけるウシ海綿状脳症(BSE)症例の報告数
1989年から2008年(3月現在)までに、世界各国から国際獣疫事務局(OIE)に報告されたウシ海綿状脳症の報告数である。2007年にBSE症例が報告されたのはオーストリア(1頭)、カナダ(3頭)、チェコ共和国(2頭)、ドイツ(4頭)、アイルランド(25頭)、イタリア(2頭)、日本(3頭)、ポーランド(7頭)、スロベニア(1頭)、スペイン(26頭)である。2008年には、これまでにカナダ(1頭)とアイルランド(6頭)から報告されている。

報告企業の意見	今後の対応
1989年から2008年(3月現在)までに、世界各国から国際獣疫事務局(OIE)に報告されたウシ海綿状脳症の報告数である。英国を除くヨーロッパでは、1999年から2003年頃をピークに流行は収束しつつある。	日本赤十字社は、vCJDの血液を介する感染防止の目的から、献血時に過去の海外渡航歴(旅行及び居住)を確認し、欧州36ヶ国に一定期間滞在したドナーを無期限に献血延期としている。また、英国滞在歴を有するvCJD患者が国内で発生したことから、平成17年6月1日より1980～96年に1日以上の英国滞在歴のある方からの献血を制限している。今後もCJD等プリオン病に関する新たな知見及び情報の収集に努める。

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

defined JRC2008T-018

* Number of cases in the United Kingdom * Number of reported cases worldwide (excluding the United Kingdom)

* Cases in imported animals only * Annual incidence rate

Number of reported cases of bovine spongiform encephalopathy (BSE) in farmed cattle worldwide*(excluding the United Kingdom)

Country/Year	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
Austria	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	2	1	
Belgium	0	0	0	0	0	0	0	0	1	6	3	9	46	38	15	11	2	2	0	
Canada	0	0	0	0	1(b)	0	0	0	0	0	0	0	0	0	2(a)	1	1	5	3	1(c)
Czech Republic	0	0	0	0	0	0	0	0	0	0	0	0	2	2	4	7	8	3	2	
Denmark	0	0	0	1(b)	0	0	0	0	0	0	0	1	6	3	2	1	1	0		
Finland	0	0	0	0	0	0	0	0	0	0	0	0	1(a)	0	0	0	0	0	0	
France	0	0	5	0	1	4	3	12	6	18	31(a)	161 (d)	274 (e)	239 (f)	137 (g)	54(h)	31	8		
Germany	0	0	0	1(b)	0	3(b)	0	0	2(b)	0	0	7	125	106	54	65	32	16	4	
Greece	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0(k)	
Ireland	15(a)	14(a)	17(a)	18(a)	16	19(a)	16(a)	73	80	83	91	149 (d)	246 (e)	333 (f)	183 (g)	126 (h)	69(i)	41(j)	25(k)	6(c)
Israel	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	
Italy	0	0	0	0	0	2(b)	0	0	0	0	0	0	48	38(a)	29	7	8	7	2(k)	
Japan	0	0	0	0	0	0	0	0	0	0	0	0	3(e)	2	4(g)	5	7	10	3(k)	
Liechtenstein	0	0	0	0	0	0	0	0	0	2(a)	0	0	0	0	0	0	0	0	0	
Luxembourg	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0(k)	
Netherlands	0	0	0	0	0	0	0	0	2	2	2	2	20	24	19	6	3	2		
Poland	0	0	0	0	0	0	0	0	0	0	0	0	0	4(f)	5	11	19	10	7(k)	
Portugal	0	1(b)	1(b)	1(b)	3(b)	12	15	31	30	127	159	149 (a)	110	86	133	92(a)	46	33		
Slovakia	0	0	0	0	0	0	0	0	0	0	0	0	5	6	2	7	3	0		
Slovenia	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	2(a)	1	1	1(k)	
Spain	0	0	0	0	0	0	0	0	0	0	0	2	82	127	167	137	98	68	26(k)	
Sweden	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1		
Switzerland	0	2	8	15	29	64	68	45	38	14	50	33(d)	42	24	21(g)	3	3(i)	5	0	
United Kingdom	see particular table																			
United States of America	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0(k)	

* Cases are shown by year of confirmation.
... Not available

(a) Canada: 1 case diagnosed in Canada in May 2003 + 1 case diagnosed in the United States of America in December 2003 and confirmed as having been imported from Canada.
Finland: date of confirmation of the case: 7 December 2001.
France: includes 1 imported case (confirmed on 13 August 1999).
Ireland: includes imported cases: 5 in 1989, 1 in 1990, 2 in 1991 and 1992, 1 in 1994 and 1995.
Italy: includes 2 imported cases.
Liechtenstein : date of the last confirmation of a case: 30 September 1998.
Portugal: includes 1 imported case.
Slovenia: includes 1 imported case.

(b) Imported case(s).

(c) Canada – Data as of 26 February 2008.
Ireland – Data as of 31 March 2008. Cases detected by the passive surveillance programme = 1. Cases detected by the active surveillance programme = 5.

(d) France year 2000 – Clinical cases = 101. Cases detected within the framework of the research programme launched on 8 June 2000 = 60.
Ireland year 2000 – Clinical cases = 138. Cases identified by active surveillance of at risk cattle populations = 7. Cases identified by examination of depopulated BSE positive herds, birth cohorts and progeny animals = 4.
Switzerland year 2000 – Clinical cases = 17. Cases detected within the framework of the investigation programme = 16.

(e) France year 2001 – Clinical cases = 91. Cases detected at rendering (bovines at risk) = 100 (out of 139,500 bovines tested). Cases detected as result of routine screening at the abattoir = 83 (out of 2,373,000 bovines tested).
Ireland year 2001 – Clinical cases = 123. Cases identified by systematic active surveillance of all adult bovines = 119. Cases identified by examination of depopulated BSE positive herds, birth cohorts and progeny animals = 4.
Japan year 2001 – Clinical cases = 1. Cases detected as result of screening at the abattoir = 2.

(f) France year 2002 – Clinical cases = 41. Cases detected at rendering (bovines at risk) = 124 (out of 274,143 bovines tested). Cases detected as result of systematic screening at the abattoir = 74 (out of 2,915,103 bovines tested). The active BSE surveillance programmes implemented in France in 2002 led to routine examination of cattle aged over 24 months, which were slaughtered for consumption purposes, were euthanised or died due to other reasons.
Ireland year 2002 – Clinical cases = 108. Cases detected by the active surveillance programme = 221. Cases identified by examination of depopulated BSE positive herds, birth cohorts and progeny animals = 4.
Poland year 2002 – Clinical cases = 1. Cases detected as result of routine screening at the abattoir (cattle over 30 months) = 3.

(g) France year 2003 – Clinical cases = 13. Cases detected at rendering (bovines at risk) = 87. Cases detected as result of systematic screening at the abattoir = 37.
Japan year 2003 – The 9th case was a bullock aged 21 months.
Ireland year 2003 – Clinical cases = 41. Cases detected by the active surveillance programme = 140.
Switzerland year 2003 – Clinical cases: 8. Cases detected within the framework of the official surveillance programme: 11. Cases detected through voluntary testing following routine slaughter: 2.

(h) France year 2004 – Clinical cases = 8. Cases detected at rendering (bovines at risk) = 29. Cases detected as result of systematic screening at the abattoir = 17.
Ireland year 2004 – Clinical cases = 31. Cases detected by the active surveillance programme = 94. Cases identified by examination of depopulated BSE positive herds, birth cohorts and progeny animals = 1.

(i)
Ireland year 2005 – Cases detected by the passive surveillance programme = 13. Cases detected by the active surveillance programme = 56.
Switzerland year 2005 – Cases detected by the passive surveillance programme = 1. Cases detected

within the framework of the official surveillance programme: 1. Cases detected through voluntary testing following routine slaughter = 1.

(j) Ireland year 2006 – Cases detected by the passive surveillance programme = 5. Cases detected by the active surveillance programme = 36.

(k) Greece – Data as of 30 June 2007.

Ireland year 2007 – Cases detected by the passive surveillance programme = 5. Cases detected by the active surveillance programme = 20.

Italy – Data as of 30 June 2007.

Japan – Data as of 21 December 2007.

Luxembourg – Data as of 30 June 2007.

Poland – Data as of 20 December 2007.

Slovenia– Data as of 30 June 2007.

Spain– Data as of 30 June 2007.

United States of America – Data as of 30 June 2007.

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Last update : 31-Mar-2008 (fr)

Tel: +33 (0)1 44 15 18 88 – Fax: +33 (0)1 42 67 09 87 – Email: oie@oie.int

別紙様式第2-1

No. 24

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2008. 3. 5	該当なし	

一般的名称	解凍人赤血球濃厚液	研究報告の公表状況	OIE - World Organisation for Animal Health. Available from: URL: http://oie.int/eng/info/en_esbru.htm	公表国
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)			OIE

研究報告の概要

○英国の畜牛におけるウシ海綿状脳症(BSE)症例の報告数
1987年以前から2008年(3月現在)までに、英国から国際獣疫事務局(OIE)に報告されたウシ海綿状脳症の報告数である。2007年にはグレートブリテン島で53頭、北アイルランドで14頭の計67頭が報告された。2008年には、これまでにグレートブリテン島で10頭の症例が報告されている。

報告企業の意見	今後の対応
1987年以前から2008年(3月現在)までに、英国から国際獣疫事務局(OIE)に報告されたウシ海綿状脳症の報告数である。英国では、1992年の37,280例をピークに流行は収束しつつある。	日本赤十字社は、vCJDの血液を介する感染防止の目的から、献血時に過去の海外渡航歴(旅行及び居住)を確認し、欧州36ヶ国に一定期間滞在したドナーを無期限に献血延期としている。また、英国滞在歴を有するvCJD患者が国内で発生したことから、平成17年6月1日より1980～96年に1日以上の英国滞在歴のある方からの献血を制限している。今後もCJD等プリオン病に関する新たな知見及び情報の収集に努める。

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

(N)

idefined JRC2008T-019

■ Number of cases in the United Kingdom ■ Number of reported cases worldwide (excluding the United Kingdom)
■ Cases in imported animals only ■ Annual incidence rate

Number of cases of bovine spongiform encephalopathy (BSE) reported in the United Kingdom [1]

	Alderney	Great Britain	Guernsey [3]	Isle of Man [2]	Jersey	Northern Ireland	Total United Kingdom
1987 and before[4]	0	442	4	0	0	0	446
1988[4]	0	2 469	34	6	1	4	2 514
1989	0	7 137	52	6	4	29	7 228
1990	0	14 181	83	22	8	113	14 407
1991	0	25 032	75	67	15	170	25 359
1992	0	36 682	92	109	23	374	37 280
1993	0	34 370	115	111	35	459	35 090
1994	2	23 945	69	55	22	345	24 438
1995	0	14 302	44	33	10	173	14 562
1996	0	8 016	36	11	12	74	8 149
1997	0	4 312	44	9	5	23	4 393
1998	0	3 179	25	5	8	18	3 235
1999	0	2 274	11	3	6	7	2 301
2000	0	1 355	13	0	0	75	1 443
2001	0	1,113	2	0	0	87	1,202
2002	0	1,044	1	0	1	98	1,144
2003	0	549	0	0	0	62	611
2004	0	309	0	0	0	34	343
2005	0	203	0	0	0	22	225
2006	0	104	0	0	0	10	114
2007	0	53	0	0	0	14	67
2008[5]	0	10	0	0	0	0	10

(1) Cases are shown by year of restriction.

(2) In the isle of Man BSE is confirmed on the basis of a laboratory examination of tissues for the first case on a farm and thereafter by clinical signs only. However, all cases in animals born after the introduction of the feed ban have been subjected to histopathological/scrapie-associated fibrils analysis. To date, a total of 277 animals have been confirmed on clinical grounds only.

(3) In Guernsey BSE is generally confirmed on the basis of clinical signs only. To date, a total of 600 animals have been confirmed without laboratory examination.

(4) Cases prior to BSE being made notifiable are shown by year of report, apart from cases in Great Britain which are shown by year of clinical onset of disease.

(5) Data as of 31 March 2008.

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Last update :17-Avr-2008 (fr)

Tel: +33 (0)1 44 15 18 88 – Fax: +33 (0)1 42 67 09 87 – Email: oie@oie.int

別紙様式第2-1
番号7

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 2008年2月18日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①乾燥抗HBs人免疫グロブリン ②ポリエチレングリコール処理抗HBs人免疫グロブリン		研究報告の公表状況	Transfusion 2008; 48(2): 286-294	公表国 日本	
販売名 (企業名)	①ヘブスブリン（ベネシス） ②静注用ヘブスブリンーIH（ベネシス）					

研究報告の概要

【背景】
HBV血液スクリーニングの最適な戦略を計画するには、最小感染価とHBVの初期動態を測定し、HBs抗原に加えてHBV DNAについてのウインドウ期間を明確にすることが必要である。
【研究デザイン及び方法】
最小感染価を求めるために、遺伝型A、または遺伝型CのHBVを含む急性期前の接種株をそれぞれチンパンジー1対に接種するとともに、遺伝型Aと遺伝型CのHBVの最小感染価を接種した2対のチンパンジーについてHBVマーカーを追跡調査した。
【結果】
遺伝型Aおよび遺伝型Cの50%チンパンジー感染価（CID50）は、約10コピーであると推定された。最小感染価を接種された2頭のチンパンジーにおいて、HBV DNAのウインドウ期は、遺伝型Aおよび遺伝型Cでそれぞれ55-76日及び35-50日であった。HBsAgのウインドウ期は、遺伝型A及び遺伝型Cでそれぞれ69-97日及び50-64日であった。HBV DNAのダブリングタイムは、遺伝型A及び遺伝型Cでそれぞれ3.4日及び1.9日であった。この2つの遺伝型の間でのHBV DNAの複製速度を比較すると、遺伝型Cのダブリングタイムは遺伝型Aよりも著しく短かった。
【結論】
CID50は約10コピーで2つの遺伝型で類似していたが、ダブリングタイムおよび最小感染価に感染したチンパンジーにおけるHBV NATウインドウ期間（<100コピー/mL）は、遺伝型Aよりも遺伝型Cが短いようであった。

報告企業の意見	今後の対応
チンパンジーにおけるHBVの遺伝型Aと遺伝型Cの最小感染価、初期動態(ダブリングタイム、ウインドウ期)に関する報告である。 万一、原料血漿にHBVが混入したとしても、BVD及びBHVをモデルウイルスとしたウイルスバリデーション試験成績から、本剤の製造工程において十分に不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

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

(3)

Minimum infectious dose of hepatitis B virus in chimpanzees and difference in the dynamics of viremia between genotype A and genotype C

Yutaka Komiya, Keiko Katayama, Hisao Yugi, Masaaki Mizui, Harumichi Matsukura, Tetsushi Tomoguri, Yuzo Miyakawa, Ayako Tabuchi, Junko Tanaka, and Hiroshi Yoshizawa

BACKGROUND: In planning optimal hepatitis B virus (HBV) blood screening strategies, the minimum infectious dose and early dynamics of HBV need to be determined for defining the window period for HBV DNA as well as for hepatitis B surface antigen (HBsAg).

STUDY DESIGN AND METHODS: Pairs of chimpanzees were inoculated with preacute-phase inocula containing HBV of genotype A or genotype C to determine the minimum infectious dose, and two pairs of chimps infected with the lowest infectious dose of genotypes A and C were followed for HBV markers.

RESULTS: The minimum 50 percent chimpanzee infectious dose (CID_{50}) was estimated to be approximately 10 copies for genotype A and for genotype C. In the two chimps inoculated with the lowest infectious dose, the HBV DNA window was 55 to 76 days for genotype A and 35 to 50 days for genotype C, respectively. The HBsAg window was 69 to 97 days for genotype A and 50 to 64 days for genotype C, respectively. The doubling times of HBV DNA were 3.4 days (95% confidence interval [CI], 2.6-4.9 days) for genotype A and 1.9 days (95% CI, 1.6-2.3 days) for genotype C. When comparing the replication velocity of HBV DNA between the two genotypes, the doubling time of genotype C was significantly shorter than that of HBV genotype A ($p < 0.01$).

CONCLUSION: Although the CID_{50} of approximately 10 copies was similar for the two HBV genotypes, the doubling time and pre-HBV nucleic acid amplification technology (<100 copies/mL) window period in chimps infected with the lowest infectious dose seemed to be shorter for genotype C than for genotype A.

Posttransfusion infection with hepatitis B virus (HBV) has decreased dramatically since screening for hepatitis B surface antigen (HBsAg) was introduced in the early 1970s. The number of reported posttransfusion hepatitis B cases has been further reduced after screening for antibody to HBV core (anti-HBc) was implemented in the late 1980s in the United States and Japan.[1,2] Japan introduced HBV DNA screening by nucleic acid amplification technology (NAT) in minipools (MPs) in 1999. Since introduction of MP-NAT, more than 500 seronegative donations with detectable HBV DNA have been interdicted, although there are still units of blood in an early or late phase of HBV infection

ABBREVIATIONS: CID_{50} = 50 percent chimpanzee infectious dose; CLIA = chemiluminescent immunoassay; JRC = Japanese Red Cross; MP(s) = minipool(s).

From the Department of Epidemiology, Infectious Disease Control and Prevention, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima; Primate Park, Sanwa Kagaku Kenkyusho, Ltd, Kumamoto; the Department of NAT, Central Blood Institute, Japanese Red Cross Society, Tokyo; the Department of Laboratory Medicine, Japanese Red Cross Hiroshima Blood Center, Hiroshima; the Department of Research and Development of Reagents, Japanese Red Cross Osaka Blood Center, Osaka; and the Miyakawa Memorial Research Foundation, Tokyo, Japan.

Address reprint requests to: Hiroshi Yoshizawa, MD, Department of Epidemiology, Infectious Disease Control and Prevention, Graduate School of Biomedical Sciences, Hiroshima University, Kasumi 1-2-3, Minami-Ku, Hiroshima 734-8551, Japan; e-mail: eidcp@hiroshima-u.ac.jp.

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with low viral load that can escape detection by NAT.[3] Interestingly, the lookback program of the Japanese Red Cross (JRC) demonstrated that low viral load donations in the window phase were more than 10-fold more often implicated in HBV transmission reports than were occult carriers with anti-HBc titers below the exclusion limit of the anti-HBc hemagglutination inhibition screening assay.[3] Bearing in mind the relatively high infectivity of HBV in the window phase, exact knowledge on early dynamics of HBV replication is important for residual risk estimations.[4-7] It will determine the threshold of NAT in identifying blood donors during the preacute phase of HBV infection, which is important for planning and executing evidence-based hepatitis B blood screening strategies.[7,8] In this context, the relative infectivity of HBV in the early window phase is an important factor for measuring the effect of NAT screening systems on the residual risk of HBV transmission by blood transfusion.[7-9]

Chimpanzees are the only experimental animals susceptible to human hepatitis viruses and have been very useful in transmission studies.[10] As early as the mid-1970s, it was demonstrated that blood units from HBV carriers, especially those positive for the presence of hepatitis B e antigen (HBeAg), have a tremendously high infectious potential and can transmit infection to chimps by intravenous inoculation with 1 mL of plasma diluted to $1:10^8$.[11] Now that NAT enables detection of HBV DNA in blood donors even in individual-donation format, it can be estimated by mathematical modeling what the residual risk would be depending on the minimum copy number required for infection.[9] More conservative risk modeling assumes that a single copy of HBV, if it successfully reaches a hepatocyte in susceptible hosts, may be enough to establish infection.[5] To pursue strategies for preventing HBV infections by blood transfusions, additional information on the infectivity of HBV is crucially required. It is imperative to define not only the minimum copy number of HBV DNA or number of virions required for transmission of HBV, but also the early dynamics of HBV replication in the circulation of infected hosts. This can be established more accurately in chimpanzee experiments. In this report on experimental transmission of hepatitis B in chimps, the minimum infectious dose was determined separately for HBV of genotypes A and C, and the copy number of HBV DNA for establishing infection was defined for each of them. Moreover, the doubling time and logarithmic time of HBV DNA were determined by following the viral dynamics in the preacute phase of chimpanzees who had received the minimum infectious dose of HBV.

MATERIALS AND METHODS

Chimpanzees

Six chimps entered this study. Their age, sex, and weight, as well as the HBV inocula that they received are listed in Table 1 along with the infection outcome. Every chimp was kept in an individual cage and received humane care in accordance with all relevant requirements for the use of primates in an approved institution. None of the six chimps had serologic or molecular biologic evidence of past or present HBV infection prior to the inoculation. They were also not infected with hepatitis C virus (HCV) and human immunodeficiency virus type-1. Chimps were inoculated intravenously while they were under anesthesia by intramuscular injection with ketamine hydrochloride. After the inoculation, serum samples were obtained once a week or more frequently as required, until 16 weeks or longer. They were tested for HBV DNA, HBsAg, anti-HBc, anti-HBs, and alanine aminotransferase as well as aspartate aminotransferase.

Inocula containing HBV

The chimpanzees received four kinds of inocula (Table 1). Inocula I and III were fresh-frozen plasma (FFP) units from blood donors acutely infected with HBV genotypes A and C, respectively. Inocula II and IV were plasma samples from chimps infected with inoculum I of genotype A and inoculum III of genotype C, respectively (see Figs. 1A and 1B). Inocula II and IV were: 1) recovered in the preacute phase of HBV infection before immune responses of the host had developed; 2) positive for the presence of HBV DNA in the highest titer before anti-HBc developed; and 3) taken with utmost care to maintain the infectious activity and avoid attenuation during serial processing from blood collection until storage. Immediately after blood drawing

TABLE 1. Six chimpanzees and HBV inocula and HBV infection outcomes

Chimpanzee	Age, sex, weight	HBV DNA copies	Outcome
Inoculum I: FFP from a human donor in the preacute phase of HBV infection of genotype A			
1 Chimp 246	13 years, male, 60.7 kg	1 mL (6.9×10^4 copies/mL)	Infected
Inoculum II: Preacute-phase plasma of Chimp 246 containing HBV (2.6×10^6 copies/mL)			
2 Chimp 272	9 years, male, 58.7 kg	1 mL ($1:10^6$ dilution)	Not infected
3 Chimp 279	8 years, male, 51.4 kg	1 mL ($1:10^6$ dilution)	Not infected
3 Chimp 279	Reinoculation	1 mL ($1:10^5$ dilution)	Infected
4 Chimp 280	8 years, male, 39.4 kg	1 mL ($1:10^5$ dilution)	Infected
Inoculum III: FFP from a human donor in the preacute phase of HBV infection of genotype C			
2 Chimp 272	Reinoculation	5 mL (5.3×10^5 copies/mL)	Infected
Inoculum IV: Preacute-phase plasma of Chimp-272 containing HBV (3.0×10^6 copies/mL)			
5 Chimp 269	11 years, male, 62.5 kg	1 mL ($1:10^6$ dilution)	Not infected
6 Chimp 285	7 years, male, 41.1 kg	1 mL ($1:10^6$ dilution)	Not infected
5 Chimp 269	Reinoculation	1 mL ($1:10^5$ dilution)	Infected
6 Chimp 285	Reinoculation	1 mL ($1:10^5$ dilution)	Infected

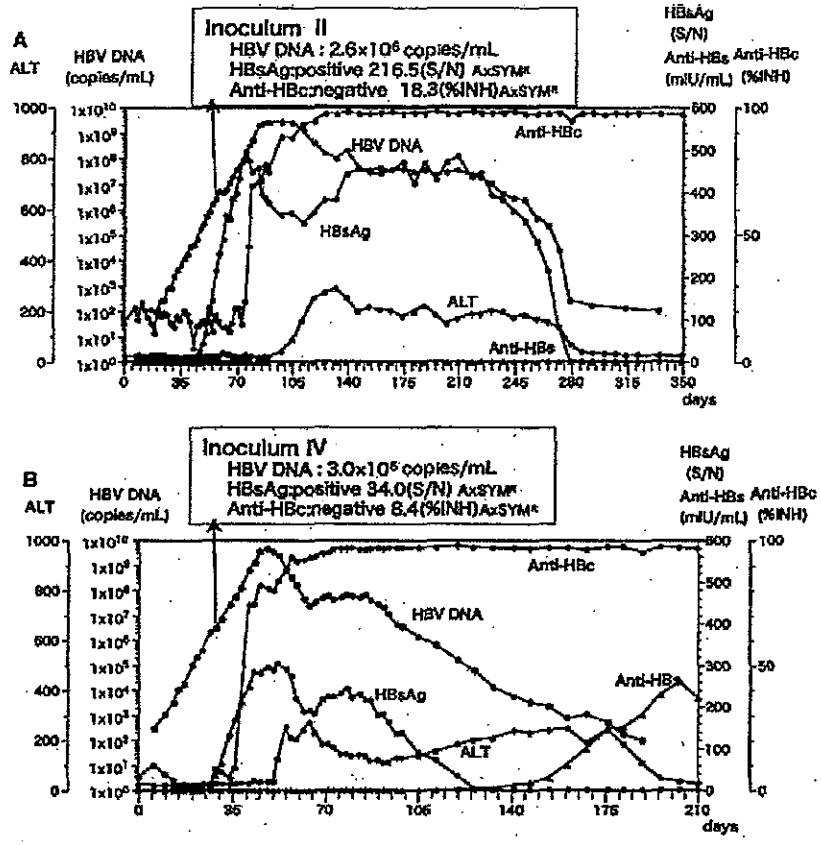

Fig. 1. Time course of HBV serum markers in chimps used as a source of inoculation of HBV genotype A and genotype C. (A) Chimp 246 was inoculated with human plasma of HBV genotype A. Inoculum II for chimp infectivity studies was harvested in the ramp-up phase of viremia before anti-HBc seroconversion at the time HBV DNA had reached a concentration of 2.6×10^6 copies per mL and the HBsAg response had increased to a signal-to-noise (S/N) ratio of 216.5 (cutoff S/N = 2.0). (B) Chimp 272 was inoculated with human plasma of HBV genotype C. Inoculum IV was harvested in the ramp-up phase of viremia before anti-HBc seroconversion at the time HBV DNA had reached a concentration of 3.0×10^6 copies per mL and HBsAg had increased to an S/N ratio of 34.0.

from chimps, plasma samples were separated. They were divided into 15 tubes in 1-mL aliquots, snap-frozen in liquid nitrogen, and kept in a deep freezer at –80°C until used for transmission experiments. For each experiment, the plasma in one tube was thawed gently by immersing it in a water bath at 37°C, and the required amounts were used.

Laboratory tests

HBsAg, anti-HBc, and antibody to HBsAg (anti-HBs) were determined by chemiluminescent immunoassay (CLIA) with commercially available kits (AxSYM, Abbott Japan, KK, Tokyo, Japan), with the index of 2.0 (signal-to-noise [S/N]), 50 percent inhibition, and 5.0 mIU per mL as cutoff values, respectively. Qualitative assay for HBV DNA was performed by polymerase chain reaction (PCR) with primers deduced from the S region of HBV DNA.[12] HBV DNA was quantitated by the PCR (TaqMan, Roche Diagnostics KK, Tokyo, Japan) with a sensitivity of 100 copies per mL. Quantitative assays for HBV DNA were performed simultaneously for an accurate comparison of data.

Calculation for doubling time and logarithmic time of HBV DNA

To calculate the doubling time and the logarithmic time (time for reaching 10 times the amount) of HBV DNA at ramp-up after the infection, HBV DNA copy numbers were evaluated statistically by log linear regression analysis. The comparison of regression slope (the doubling time and the logarithmic time) between HBV genotypes was evaluated by growth curve analysis (Vonesh-Carter-Ohtaki method).[13,14]

RESULTS

Inocula and copy numbers of HBV genotype A or genotype C recovered from chimpanzees in the preacute phase of infection

Chimp 246 was injected intravenously with 1 mL of FFP from a blood donor in the preacute phase of HBV infection (Table 1); the donor had been screened by NAT at a JRC Blood Center. His plasma sample contained 6.9×10^4 copies per mL of HBV DNA genotype A and was positive for the presence of HBsAg but negative for the presence of anti-HBc (inoculum I). Plasma was harvested from Chimp 246, in the preacute phase of HBV infection 57 days after inoculation (inoculum II). It contained 2.6×10^6 copies per mL HBV DNA and was positive for the presence of HBsAg but still negative for the presence of anti-HBc and anti-HBs (Fig. 1A).

Likewise, Chimp 272 was injected intravenously with 5 mL of FFP from a blood donor in the preacute phase of HBV infection who had been screened by NAT at JRC (Table 1). It contained 5.3×10^5 copies per mL of HBV DNA genotype C and was positive for the presence of HBsAg but negative for the presence of anti-HBc and anti-HBs (inoculum III). Thus, Chimp 272 was inoculated with

2.7×10^6 copies of HBV genotype C. The preacute plasma sample was collected from Chimp 272 29 days after challenge (inoculum IV). It contained 3.0×10^6 copies per mL HBV DNA and was positive for the presence of HBsAg but still negative for the presence of anti-HBc and anti-HBs (Fig. 1B).

Estimates of HBV DNA copy numbers in serial 1-in-10-fold dilutions and inocula below the HBV NAT detection limit

Serial 1-in-10 dilutions of inoculum II of genotype A were prepared in preinoculation serum sample from each chimp (e.g., Chimp 272, Chimp 279, and Chimp 280, respectively). Dilutions were delivered to three tubes each in 1-mL aliquots and snap-frozen in liquid nitrogen. Concentration of HBV DNA was determined in one of the three tubes in each dilution so as to guarantee copy numbers of HBV DNA in the other two vials that were inoculated into chimps. These samples had been stored in a deep freezer at –80°C until inoculation.

Table 2 shows the measured HBV DNA concentrations in 1-in-10 dilutions of inoculum II (genotype A). The quantitative HBV DNA results starting from 2.6×10^6 copies per mL in the undiluted sample varied between 2.0×10^5 to 2.3×10^5, 2.0×10^4 to 2.4×10^4, 1.6×10^3 to 2.0×10^3, and 1.7×10^2 to 2.8×10^2 copies per mL, respectively, in the 1:10, $1:10^2$, $1:10^3$, and $1:10^4$ dilutions. These quantitative results are an indication of the accuracy of the dilution and assay procedure. On the premise that dilutions beyond $1:10^4$ had been performed properly, further dilutions to $1:10^5$ and $1:10^6$ would have contained 16 to 28 and 1.6 to 2.8 HBV DNA copies per mL (ranges estimated by variations of HBV DNA measurements in lower dilutions), respectively, although they were below the detection limit of the PCR method used.

Likewise, serial 1-in-10 dilutions of inoculum IV (genotype C) were prepared in the plasma sample from Chimp 269 and Chimp 285. HBV DNA in 3.0×10^6, 3.5×10^5 to 3.8×10^5, 3.6×10^4 to 3.9×10^4, 3.6×10^3 to 4.6×10^3, and 4.3×10^2 to 4.6×10^2 copies per mL were detected in the original serum samples at 1:10, $1:10^2$, $1:10^3$, and $1:10^4$ dilutions thereof, respectively (Table 3). Thus, further experiments were performed on the assumption that serial dilutions of $1:10^5$ and $1:10^6$ of inoculum IV would have contained 35 to 46 and 3.5 to 4.6 HBV DNA copies per mL, respectively.

Determination of the minimum copy number required for transmission of HBV genotype A or genotype C to chimpanzees

When Chimp 272 and Chimp 279 were inoculated intravenously with 1.0 mL of inoculum II diluted $1:10^6$ (equivalent to 1.6 to 2.8 copies of HBV DNA in an in vitro assay), HBV infection did not develop in either of them during monitoring for 119 days (17 weeks) and thereafter. Chimp 279 was then rechallenged with 1.0 mL of inoculum II diluted $1:10^5$ (equivalent to 16-28 copies). He then became infected and developed HBV DNA in his serum 55 days (8 weeks) after the inoculation. Chimp 280 was also inoculated intravenously with 1.0 mL of inoculum II diluted $1:10^5$ (equivalent to 16 to 28 copies of HBV DNA). He developed HBV DNA in the circulation 76 days (11 weeks) after infection. In view of the incubation period of 55 to 76 days (8-11 weeks) for $1:10^5$ dilution of inoculum II, HBV infection would probably not have occurred in chimps who received $1:10^6$ dilution if they had been followed longer than 119 days (17 weeks).

Chimp 269 and Chimp 285 were inoculated with 1.0 mL of inoculum IV diluted $1:10^6$ (equivalent to 3.5-4.6 copies of HBV DNA in an in vitro assay). During follow-up

TABLE 2. Quantification of HBV DNA in serial 1-in-10 dilutions of the standard serum for HBV genotype A (inoculum II)*

		Serial dilutions in preinoculation serum samples of each chimpanzee					
Chimpanzee	Undiluted	1:10	$1:10^2$	$1:10^3$	$1:10^4$	$1:10^5$	$1:10^6$
272	2.6×10^6	2.3×10^5	2.0×10^4	2.0×10^3	1.7×10^2	Not done	<100
279	2.6×10^6	2.0×10^5	2.4×10^4	2.0×10^3	2.4×10^2	<100	<100
280	2.6×10^6	2.3×10^5	2.3×10^4	1.6×10^3	2.8×10^2	<100	Not done

* Data are reported as copies per mL.

TABLE 3. Quantification of HBV DNA in serial 1-in-10 dilutions of the standard serum for HBV genotype C (inoculum IV)*

		Serial dilutions in preinoculation serum of each chimpanzee					
Chimpanzees	Undiluted	1:10	$1:10^2$	$1:10^3$	$1:10^4$	$1:10^5$	$1:10^6$
Chimp 269	3.0×10^6	3.8×10^5	3.9×10^4	3.6×10^3	4.6×10^2	<100	<100
Chimp 285	3.0×10^6	3.5×10^5	3.6×10^4	4.6×10^3	4.3×10^2	<100	<100

* Data are reported as copies per mL.

for 112 days (16 weeks), however, no HBV infection occurred in either of them. Subsequently, they were rechallenged with 1.0 mL of inoculum IV diluted $1:10^5$ (equivalent to 35-46 copies of HBV DNA) 17 weeks after the initial inoculation. They developed HBV DNA in the circulation 35 and 50 days thereafter, respectively, indicating that both of them were infected. Therefore, the 50 percent chimp infectious dose (CID_{50}) for both genotype A and genotype C lies between the lowest infectious dose of approximately 30 copies and the subinfectious dose of approximately 3 copies or at approximately 10 HBV DNA copies.

HBV infection resolved in all six chimps and they never became carriers. Within a few weeks after the peak HBV DNA titer was reached, serum levels of transaminase increased slightly, within 3 times the upper limit of normal.

Replication velocity of HBV DNA in the preacute phase of infection

Doubling time and logarithmic time of HBV genotype A

Figure 2A illustrates the appearance of HBV genotype A in the circulation, when HBV DNA reached more than 10^2 copies per mL, as well as its early dynamics in Chimp 246, Chimp 279, and Chimp 280 during the preacute phase of exponential replication. HBV DNA emerged in the circulation earlier in Chimp 246 than the other two chimps, but

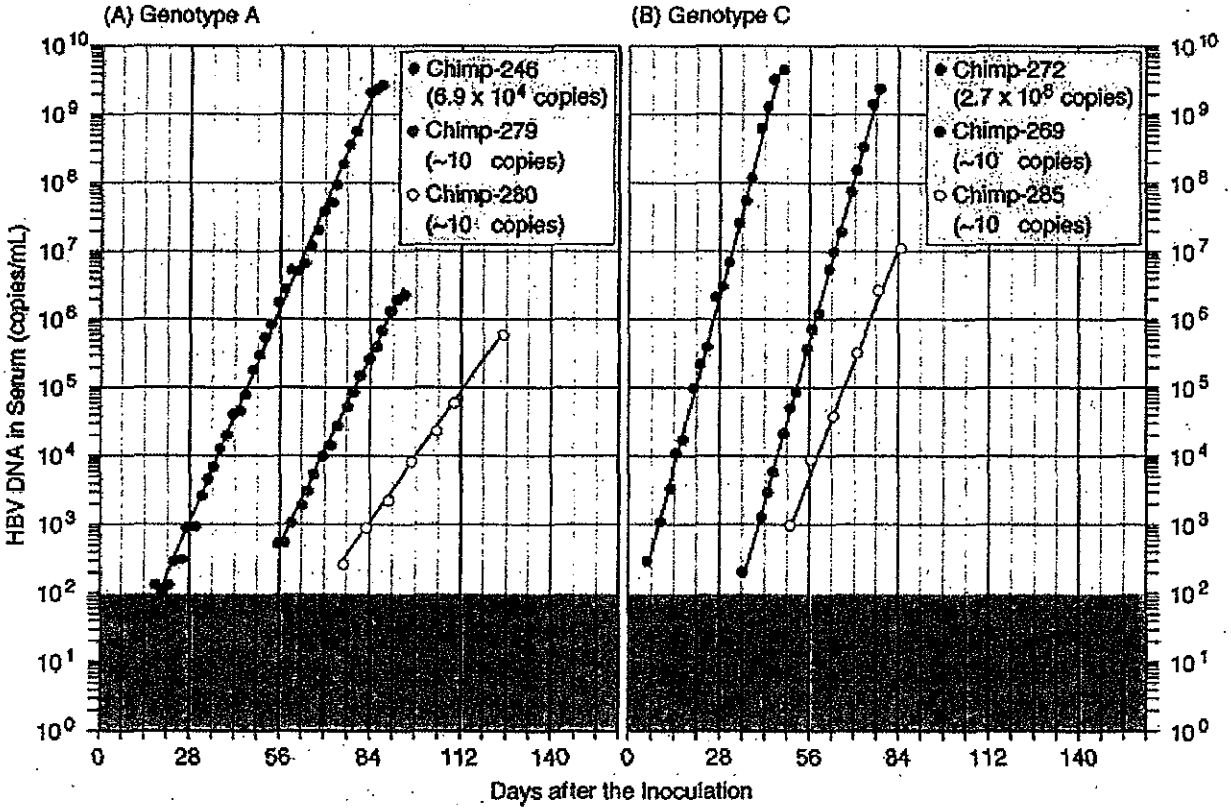

Fig. 2. Log-linear increase of HBV DNA in the circulation of chimpanzees during the early exponential replication phase. (A) Dynamics in the early ramp-up phase of viral DNA for three chimps inoculated with HBV genotype A: one chimp (Chimp 246) received 1 mL of human plasma containing 6.9×10^4 copies and the other two chimps (Chimps 279 and 280) received 1 mL of a 100,000 dilution of chimp plasma taken in the HBsAg ramp-up phase just before appearance of anti-HBc, which dilution contains a measured amount of 16 to 28 copies. (B) Graph summarizes the viral load dynamics for three chimpanzees inoculated with HBV genotype C: one chimp (Chimp 272) received 5 mL of human plasma with 2.7×10^5 copies of HBV DNA and the two other chimps (Chimps 269 and 285) received a measured amount of 35 to 46 copies (1:100,000 dilution) of preacute-phase chimpanzee plasma. Shaded areas are below the detection limit of NAT (<100 copies/mL). Only the phase of exponential replication is shown, and HBV DNA decreased after it reached peak values of 5.7×10^5 to 2.8×10^9 copies per mL in three chimps inoculated with HBV genotype A and 1.1×10^7 to 4.6×10^9 copies per mL in three chimps inoculated with HBV genotype C.

this animal had received more than a 1000-fold larger amount of copies of HBV than the other two chimps. Despite the 1000-fold higher infectious dose, the log-linear increase of HBV DNA in Chimp 246 was the same as in Chimp 279, who had received the minimum infectious dose. In Chimp 246, HBV DNA replicated exponentially from 21 to 97 days (3-13 weeks) until it peaked and then declined. Even though the same minimum infectious dose of HBV was inoculated, Chimp 279 developed detectable HBV DNA about 21 days (3 weeks) earlier than Chimp 280, in whom HBV replicated slightly slower. Despite differences in HBV doses and individual variation, the replication velocity was constant for HBV genotype A in the preacute phase of infection, before innate immune responses of the host developed, while the virus replicated at an exponential rate. The doubling time and the logarithmic time, in the early exponential viral replication phase, were calculated to be 2.7 to 4.4 and 9.0 to 14.7 days, respectively (see Table 4).

TABLE 4. Estimated doubling times and logarithmic times for HBV genotypes A and C with log-linear and growth-curve analysis

Genotype	Doubling time (days)	Logarithmic time (days)	$y = a \times \exp(b \times x)$ a	b	R^2
Genotype A					
Chimp 246	2.71	9.01	0.8491	0.2556	0.997
Chimp 279	3.05	10.14	0.0015	0.2271	0.998
Chimp 280	4.43	14.73	0.0022	0.1563	0.999
Genotype C					
Chimp 272	1.68	5.58	0.2074	0.413	0.998
Chimp 269	1.79	5.96	0.0002	0.3863	0.999
Chimp 285	2.5	8.31	0.0009	0.2771	0.997

TABLE 5. Comparing the replication velocity of HBV DNA between the two genotypes estimated by a growth curve

Genotype	Doubling time (95% CI), days	Logarithmic time (95% CI), days	$y = a \times \exp(b \times x)$ a*	b (95% CI)	p Value
A	3.44 (2.64-4.89)	11.42 (8.80-16.26)	2.299	0.2017 (0.14-0.26)	<0.01
C	1.9 (1.63-2.27)	6.3 (5.41-7.54)	2.299	0.3654 (0.31-0.43)	

* To evaluate the difference of "b" (that is, slope) between the two genotypes, the growth curve model is assuming that "a" is identical.[13]

Doubling time and logarithmic time of HBV genotype C

The replication velocity in the preacute phase of infection in chimpanzees inoculated with genotype C inocula was faster than in the chimps infected with HBV of genotype A (Fig. 2B). Again, slight variation in log-linear increase of HBV DNA was found, and HBV DNA appeared in serum earlier in Chimp 272 who was inoculated with a 100,000-fold higher infectious dose than was administered to Chimps 269 and 285. As seen in the chimps inoculated with HBV genotype A, HBV genotype C increased in a log-linear fashion in the absence of host immune responses. Doubling times of HBV DNA in the circulation of Chimp 272, Chimp 269, and Chimp 285 were calculated to be 1.7 to 2.5 days and logarithmic times were 5.6 to 8.3 days as determined with the regression formula shown in Table 4.

When comparing the replication velocity of HBV DNA between the two genotypes estimated by a growth curve, the difference was significant ($p < 0.01$, Table 5). That is, the doubling time of replications of HBV DNA with genotype A was estimated to be 3.44 days (95% confidence interval [CI], 2.64-4.89 days) and the logarithmic time was estimated to be 11.42 days (95% CI, 8.80-16.26 days). By contrast, those with HBV genotype C were estimated to be 1.90 days (95% CI, 1.63-2.27 days) and 6.30 days (95% CI, 5.41-7.54 days), respectively.

TABLE 6. Window periods before HBV DNA and HBsAg developed in the circulation of chimpanzees inoculated with the minimum infectious dose of genotype A or genotype C

HBV inoculated	Chimp infected	Markers of HBV infection HBV DNA (days)	HBsAg (days)
Genotype A	279	55	69
	280	76	97
Genotype C	269	35	50
	285	50	64

Window periods of HBV DNA and HBsAg in chimpanzees inoculated with the minimum infectious dose of HBV

After inoculation, the time before HBV DNA becomes detectable in the circulation by the single-sample NAT (with a sensitivity of 10^2 copies/mL) and the time before HBsAg was detected by CLIA after inoculation are listed in Table 6. The HBV DNA (<100 copies/mL) NAT window was 55 and 76 days, respectively, in Chimp 279 and Chimp 280 inoculated with the lowest infectious dose of HBV genotype A (approx. 30 copies). These NAT window periods were longer than the 35 and 50 days, respectively, found in Chimp 269 and Chimp 285 inoculated with the lowest infectious amounts of HBV genotype C (approx. 30 copies). Likewise, the HBsAg window was longer in Chimp 279 and Chimp 280 infected with genotype A than in Chimp 269 and Chimp 285 infected with genotype C (69 and 97 days, respectively, vs. 50 and 64 days, respectively).

DISCUSSION

Animal models sensitive to human hepatitis viruses offer robust advantages in obtaining basic data of viral infectivity.[10] By experimental infection of chimps with HCV, we have been able to determine the minimum infectious dose of HCV required for establishing infection.[15,16] The doubling time of HCV was determined to be 6.3 to 8.6 hours in two chimps inoculated with the minimum infectious dose of approximately 10 copies of HCV RNA. During the first 5 days after inoculation, HCV RNA did not increase above the NAT detection limit of 10^2 copies per mL in the circulation.[16] It would not be possible to detect HCV infection during the initial few days after exposure, even if 1-mL samples were used for individual NAT.

In this study, we have determined the minimum infectious dose for two standardized inocula containing defined copy numbers of HBV DNA. They were plasma passages of HBV in chimps harvested during the preacute phase of infection and had been processed with the utmost care for maintaining infectious activity. The minimum infectious dose of HBV or the dose where 50 percent of the chimps would be infected lies between 1-in-1 million and 1-in-100,000 dilution of the original inocula and is estimated to be of the order of 10 copies, as was the case for HCV.[15] On the basis of HBV DNA concentrations measured in serial dilutions of inocula (Tables 2 and 3), the minimum infectious dose can be determined to be 16 to 28 copies for HBV genotype A and 35 to 46 copies for HBV genotype C.

There are two definitions of the minimum infectious dose of HBV. Theoretically, it is a single copy of HBV. Not all HBV virions entering the circulation of recipients, however, will succeed in reaching hepatocytes, because some of them are phagocytized by circulating macrophages and Kupffer cells in the sinusoids of the liver. In a mathematical window-phase risk model, Weusten and colleagues[9] have proposed a minimum infectious dose approximately 10 copies of HBV, on the basis of the CID_{50}.[17-19] Recently the inocula derived from chronic HBV carriers used in older chimpanzee studies[17,18] were requantified by Hsia and coworkers[20] with real-time TaqMan PCR. The estimated HBV copy number per CID_{50} (geq) was 169 for genotype A *adw*, 78 for genotype D *ayw*, and 3 for genotype C *adr*, calculated by mathematical division, respectively. These viral load data, performed on cryopreserved aliquots from an inocula derived from a chronic HBV carrier (i.e., HBsAg- and anti-HBc–positive), were derived retrospectively several decades after the chimp titration studies. These results are different from the results obtained in our study, where the inocula was derived from the early ramp-up phase of viremia (HBsAg is positive but anti-HBc is negative) and the chimp titration and viral load analyses were performed prospectively. Hence, the minimum infectious dose defined as a single copy, proposed on a theoretical basis, would deserve revisiting in practical HBV infections. The window period of HBV infection changes with the size of the inoculum. The more copies of HBV inoculated therefore the shorter the incubation period in experimental transmission studies in chimps.[11] An inverse correlation is reported, also, between time before HBsAg appears in serum and the HBV dose in human beings.[21] In accordance with these reports, we also found that the NAT window was shorter in chimps receiving larger sizes of inocula both for genotypes A and C (Fig. 2). The NAT (<100 copies/mL) window period was approximately 1 week with an inoculum of 2.7×10^6 copies of genotype C, approximately 3 weeks with 6.9×10^4 copies of genotype A, 5 to 7 weeks when inoculating 35 to 46 copies of genotype C, and 8 to 11 weeks when inoculating 16 to 28 copies of HBV genotype A, while no infection was observed during 16 to 17 weeks of observation with an inocula of approximately 3 copies of genotype A or B. Theoretically, HBV infection might have become detectable after 17 weeks, but this is unlikely when extrapolating the data above. Inoculation with HBV in large amounts, as happens with transfusion with HBsAg-positive blood units, has been largely excluded since introduction of HBsAg testing in 1972. Barker and Murray[21] have shown that inoculation of lower infectious doses of HBV in the range of 10^4 to 10^7 diluted icteric plasma no longer caused clinical hepatitis in healthy individuals, while infection still occurred with up to a 10^7 diluted inoculum, as detected by an HBsAg complement fixation test. Our study showed that HBV DNA levels increase 6.5×10^3 to 2.2×10^5 copies per mL at the time of the first HBsAg-reactive sample in six chimpanzees in whom blood samples were taken at intervals of 2 to 7 days. These amounts are enough to cause clinical hepatitis B.[21] Indeed, Satake and coworkers[3] found that transmission of 5,000 to 50,000 copies of HBV by blood components with a low viral load in the pre-MP-NAT window phase could cause clinical hepatitis B. Transfusion-transmitted HBV after introduction of individual-donation or small-pool NAT (<10) is still possible, but would involve relatively low infectious doses of HBV of approximately 10 to 100 CID_{50}.

In the chimps inoculated with approximately 30 copies of HBV, the NAT window was determined by individual-donation NAT having a sensitivity of 10^2 copies per mL, while the HBsAg window was established by CLIA with the highest sensitivity presently available.[5,12] The NAT window was 55 to 76 days and HBsAg window was 69 to 97 days, respectively, in Chimp 279 and Chimp 280 who had been inoculated with approximately 30 copies of HBV genotype A. In contrast, the NAT window was 35 to 50 days and the HBsAg window was 50 to 64 days, respectively, for Chimp 269 and Chimp 285 inoculated with approximately 30 copies of HBV genotype C. Thus, neither

the NAT nor the HBsAg window phases overlapped between minimum-dose infections of HBV genotypes A and C; they were longer for genotype A than genotype C. It may be that the NAT window is longer for genotype A, prevalent in Western countries, than genotype C common in Japan. It cannot be excluded, however, that the results observed in our inoculation studies with a limited number of chimpanzees were influenced by the host rather than the genotype of the virus. The duration of the NAT and HBsAg windows are influenced at least by three factors: 1) the infectious dose, 2) individual variation among recipients, and 3) distinct HBV genotypes.

We found the replication velocity of HBV in the preacute phase of infection remarkably different between genotypes A and C. From three chimps infected with HBV genotype A, the doubling time was estimated to be 3.44 days (95% CI, 2.64-4.89 days) and the logarithmic time 11.42 days (95% CI, 8.80-16.26 days). From three chimps infected with HBV genotype C, the doubling time was estimated to be 1.90 days (95% CI, 1.63-2.27 days), and the logarithmic time 6.30 days (95% CI, 5.41-7.54 days). Also in chimeric mice with the liver replaced by human hepatocytes, genotype A was found to replicate much slower than genotype C in the initial weeks of HBV infection.[22]

The replication velocity of HBV in the circulation, indicated by the viral doubling time, is an important factor when calculating the window-period reduction provided by NAT screening systems. Biswas and colleagues[5] calculated a doubling time of 2.56 days (95% CI, 2.24-2.97 days) based on a seroconversion panel of 23 HBV infections. Yoshikawa et al.[4] followed 93 donors in preacute phase HBV infections who had been identified by the routine NAT screening program on 50-MPs at JRC Blood Centers. They estimated a median doubling time of HBV at 2.6 days (range: 1.3-15.2).

Kleinman and Busch[7] have assessed the HBsAg window period based on the HBV doubling time of 2.56 days documented by Biswas and colleagues.[5] They estimated an HBsAg window at 38.3 days (95% CI, 33.0-43.7 days) by the CLIA HBsAg seroconversion point at a concentration of 1650 copies per mL, while Minegishi and coworkers[12] determined the HBsAg seroconversion point at 2100 copies per mL. We found the HBsAg seroconversion with AxSYM occurred when the HBV DNA concentration reached a level of 6.5×10^3 to 2.2×10^5 in six chimpanzees. The differences in HBV levels at HBsAg seroconversion in CLIA may be related to the genotype, but also could reflect differences in the calibration of HBV quantitative assays in genome copies.

It is not known if the chimpanzee model is as susceptible for HBV infection as human beings. As a result, the minimum dose of HBV for transmitting infection to man is, in fact, not precisely known. Nevertheless, a minimum human infectious dose of approximately 10 HBV DNA copies, as indicated by our chimpanzee infectivity experiments, seems a reasonable assumption for modeling the HBV transmission risk in the pre-HBV-NAT window period.

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REFERENCES

1. Japanese Red Cross Non-A, Non-B Hepatitis Research Group. Effect of screening for hepatitis C virus antibody and hepatitis B virus core antibody on incidence of post-transfusion hepatitis. Lancet 1991;338:1040-1.
2. Kojima M, Shimizu M, Tsuchimochi T, Koyasu M, Tanaka S, Iizuka H, Tanaka T, Okamoto H, Tsuda F, Miyakawa Y, Mayumi M. Posttransfusion fulminant hepatitis B associated with precore-defective HBV mutants. Vox Sang 1991; 60:34-9.
3. Satake M, Taira R, Yugi H, Hino S, Kanemitsu K, Ikeda H, Tadokoro K. Infectivity of blood components with low HBV-DNA levels identified in a look back program. Transfusion 2007;47:1197-205.
4. Yoshikawa A, Gotanda Y, Itabashi M, Minegishi K, Kanemitsu K, Nishioka K. HBV NAT positive [corrected] blood donors in the early and late stages of HBV infection: analyses of the window period and kinetics of HBV DNA. Vox Sang 2005;88:77-86.
5. Biswas R, Tabor E, Hsia CC, Wright DJ, Laycock ME, Fiebig EW, Peddada L, Smith R, Schreiber GB, Epstein JS, Nemo GJ, Busch MP. Comparative sensitivity of HBV NATs and HBsAg assays for detection of acute HBV infection. Transfusion 2003;43:788-98.
6. Busch MP, Glynn SA, Stramer SL, Strong DM, Caglioti S, Wright DJ, Pappalardo B, Kleinman SH; NHLBI-REDS NAT Study Group. A new strategy for estimating risks of transfusion-transmitted viral infections based on rates of detection of recently infected donors. Transfusion 2005;45: 254-64.
7. Kleinman SH, Busch MP. Assessing the impact of HBV NAT on window period reduction and residual risk. J Clin Virol 2006;36 Suppl 1:S23-9.
8. Yugi H, Mizui M, Tanaka J, Yoshizawa H. Hepatitis B virus (HBV) screening strategy to ensure the safety of blood for transfusion through a combination of immunological testing and nucleic acid amplification testing—Japanese experience. J Clin Virol 2006;36 Suppl 1:S56-64.

9. Weusten JJ, van Drimmelen HA, Lelie PN. Mathematic modeling of the risk of HBV, HCV, and HIV transmission by window-phase donations not detected by NAT. Transfusion 2002;42:537-48.
10. Prince AM, Brotman B. Perspectives on hepatitis B studies with chimpanzees. ILAR J 2001;42:85-8.
11. Shikata T, Karasawa T, Abe K, Uzawa T, Suzuki H, Oda T, Imai M, Mayumi M, Moritsugu Y. Hepatitis B e antigen and infectivity of hepatitis B virus. J Infect Dis 1977;136: 571-6.
12. Minegishi K, Yoshikawa A, Kishimoto S, Yugi H, Yokoya N, Sakurada M, Kiyokawa H, Nishioka K. Superiority of minipool nucleic acid amplification technology for hepatitis B virus over chemiluminescence immunoassay for hepatitis B surface antigen screening. Vox Sang 2003;84: 287-91.
13. Ohtaki M, Satoh K, Kanda T, Fujikoshi Y. Local ridge estimate using random coefficient curve models for analyzing repeated measurements. J Jpn Stat Soc 2007;36:177-84.
14. Vonesh EF, Carter RL. Efficient inference for random-coefficient growth curve models with unbalanced data. Biometrics 1987;80:642-50.
15. Katayama K, Kumagai J, Komiya Y, Mizui M, Yugi H, Kishimoto S, Yamanaka R, Tamatsukuri S, Tomoguri T, Miyakawa Y, Tanaka J, Yoshizawa H. Titration of hepatitis C virus in chimpanzees for determining the copy number required for transmission. Intervirology 2004;47:57-64.
16. Tanaka J, Katayama K, Kumagai J, Komiya Y, Yugi H, Kishimoto S, Mizui M, Tomoguri T, Miyakawa Y, Yoshizawa H. Early dynamics of hepatitis C virus in the circulation of chimpanzees with experimental infection. Intervirology 2005;48:120-3.
17. Berninger M, Hammer M, Hoyer B, Gerin JL. An assay for the detection of the DNA genome of hepatitis B virus in serum. J Med Virol 1982;9:57-68.
18. Ulrich PP, Bhat RA, Seto B, Mack D, Sninski J, Vyas GN. Enzymatic amplification of hepatitis B virus DNA in serum compared with infectivity testing in chimpanzees. J Infect Dis 1989;160:37-43.
19. Prince AM, Stephan W, Brotman B. Beta-propiolactone/ ultraviolet irradiation: a review of its effectiveness for inactivation of viruses in blood derivatives. Rev Infect Dis 1983; 5:92-107.
20. Hsia CC, Purcell RH, Farshid M, Lachenbruch PA, Yu MY. Quantification of hepatitis B virus genomes and infectivity in human serum samples. Transfusion 2006;46:1829-35.
21. Barker LF, Murray R. Relationship of virus dose to incubation time of clinical hepatitis and time of appearance of hepatitis-associated antigen. Am J Med Sci 1972;263:27-33.
22. Sugiyama M, Tanaka Y, Kato T, Orito E, Ito K, Acharya SK, Gish RG, Kramvis A, Shimada T, Izumi N, Kaito M, Miyakawa Y, Mizokami M. Influence of hepatitis B virus genotypes on the intra- and extracellular expression of viral DNA and antigens. Hepatology 2006;44:915-24.

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識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2008 年 2 月 8 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	An international collaborative study to establish the 2nd World Health Organization International Standard for hepatitis B virus DNA nucleic acid amplification technology-based assays. Baylis, S. A. et al, Vox Sanguinis (2008) [Epub ahead of print]	公表国 英国	
販売名（企業名）						
研究報告の概要	1999 年，WHO は核酸増幅検査 (NAT) による B 型肝炎ウイルス (HBV) DNA 検出のための最初の国際標準用品（サンプル 1）を樹立し，10E6 IU/mL の力価を適用した。その当時，将来的に代替標準品となる可能性があるという発想から，同じ血漿から同じ凍結乾燥条件下で調製した別の DNA 検体（サンプル 2）が保存された。本試験の目的は，長期間保存したこれらサンプルの力価及び安定性を再評価することであった。サンプル 1 及び 2 は，6 ヵ所の分析機関に送付され，4 回に分けて質と量の両面から分析した。全ての分析機関で得られた結果から，これら検体の力価に有意な差は認められなかった。また，安定性試験では両検体ともに非常に安定しており，4℃又は 20℃で 51 ヵ月以上保存した後でも有意な変性は認められなかった。この結果を受けて，WHO は 2006 年 10 月，サンプル 2 を NAT 法による HBV DNA 検出のための新たな国際標準品として樹立した。					使用上の注意記載状況・その他参考事項等 BYL-2008-0308
報告企業の意見			今後の対応			
この試験は新たな国際標準品の樹立を報告するとともに，凍結乾燥により保存された HBV DNA が極めて安定であることが報告されている。			現時点で新たな安全対策上の措置を講じる必要はないと考える。			

Vox Sanguinis (2008)

REPORT

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An international collaborative study to establish the 2nd World Health Organization International Standard for hepatitis B virus DNA nucleic acid amplification technology-based assays

S. A. Baylis,[1] A. B. Heath,[1] M. Chudy,[2] G. Pisani,[3] A. Klotz,[4] S. Kerby[5] & W. Gerlich[6]

[1]National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, UK
[2]Paul Ehrlich Institute, Langen, Germany
[3]Instituto Superiore di Sanità, Rome, Italy
[4]Plasma Analytics, Baxter AG, Vienna, Austria
[5]Center for Biologics Evaluation and Research, Food and Drug Administration, Rockville, MD, USA
[6]Institute for Medical Virology, University of Giessen, Giessen, Germany

Vox Sanguinis

Background and Objectives The aim of this study was to replace the 1st World Health Organization International Standard for hepatitis B virus DNA for nucleic acid amplification technique (NAT)-based assays (code 97/746) with a new International Standard. Two lyophilized preparations freeze dried from the same bulk were evaluated in the original collaborative study (coded 97/746 and 97/750, and termed AA and BB, respectively, in the original study). This present study re-evaluates these two preparations in terms of potency and real-time stability.

Materials and Methods The 1st International Standard (97/746) and the second lyophilized preparation (97/750) were coded Samples 1 and 2, respectively, in the present study. The samples were distributed to six laboratories and assayed on four separate occasions. Accelerated thermal degradation samples of the two preparations were examined after long-term storage at 4 °C and 20 °C for more than 51 months.

Results Data were returned from a total of nine different NAT-based assays, five in qualitative format and four in quantitative format. The results of this study confirm the results of the original collaborative study, with no significant differences being found in estimated international units (IU)/ml or polymerase chain reaction-detectable units/ml for the 1st International Standard (Sample 1 in this study) and the proposed replacement preparation, Sample 2 (97/750). Real-time and accelerated degradation studies indicate that both samples are very stable. Storage of both preparations at 20 °C for more than 51 months resulted in no detectable degradation.

Conclusions On the basis of the data presented in this collaborative study, Sample 2 (code 97/750) was established as the 2nd International Standard for hepatitis B virus DNA for NAT-based assays with a potency of 10^6 IU/ml (500 000 IU/vial).

Key words: hepatitis B virus, International Standard, NAT.

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Correspondence: S. A. Baylis, Paul-Ehrlich-Institut, Paul-Ehrlich-Strasse 51–59, 63225 Langen, Germany
E-mail: baysa@pei.de

Introduction

The 1st International Standard (IS) for hepatitis B virus (HBV) DNA for nucleic acid amplification technique (NAT)-based assays (code 97/746) was established in 1999 by the

World Health Organization (WHO) Expert Committee on Biological Standardization (ECBS) [1]. This standard has been used in the calibration of secondary standards and working reagents, and has been used in the validation of assays for both the qualitative and quantitative detection of HBV DNA in serum and plasma. The standard has been used in the field of blood and blood product safety, as well as in the clinical investigation of HBV infection, both for diagnosis and for monitoring HBV loads in response to antiviral therapy.

In the original collaborative study, three preparations were evaluated. Two of the materials were lyophilized preparations formulated by dilution of R1, the Eurohep reference material [2] in pooled human plasma. This plasma bulk containing HBV was stored at −70 °C until processing. A single bulk material was lyophilized on two separate occasions, 2 weeks apart, using the same processing parameters [1]. These lyophilized preparations, coded 97/746 and 97/750, were termed AA and BB, respectively, in the original collaborative study. A third preparation, termed CC, was a liquid/frozen HBV plasma sample. No significant difference in potency was observed between AA and BB, which had been prepared from the same bulk material, but had been lyophilized on separate occasions. The 1st IS for HBV DNA for NAT-based assays was assigned a potency of 10^6 international units per ml (10^6 IU/ml). In the 50th report of the WHO ECBS [3], it was noted that 97/750 would be reserved as a potential replacement standard in the future. As 97/750 had been fully characterized in the original collaborative study, the WHO ECBS proposed examination of real-time stability data of the 1st IS and the candidate replacement standard 97/750.

In the present collaborative study, the potency and stability of the candidate replacement standard 97/750 is compared to the 1st IS for HBV DNA. The approach for the re-evaluation of 97/750 was agreed on at the 16th meeting of the International Scientific Working Group on the Standardization of Genome Amplification Techniques (SoGAT) in May 2005 [4].

Materials and methods

The 1st IS for HBV DNA for NAT-based assays (97/746) and the proposed replacement (97/750) were lyophilized from the same bulk starting material derived from a high-titre HBV genotype A2 (HBV surface antigen subtype *adw2*) sample (Eurohep R1), diluted in human plasma [1]. This HBV strain has a sequence characteristic of those circulating in central Europe [2].

Collaborative study

Six laboratories participated in the collaborative study and each was requested to assay the 1st IS for HBV DNA (97/746) concurrently with the candidate replacement standard (97/750). The participating laboratories were from five different countries and represented quality control laboratories, a manufacturer of plasma derivatives and an academic institution (a national reference laboratory for hepatitis B and D). Participants were sent four vials of the 1st IS (97/746) and four vials of the candidate replacement standard (97/750), these were coded Samples 1 and 2. The normal temperature for the long-term storage of 97/746 and 97/750 is −20 °C and participants were requested to store the samples under these conditions until analysis. The aim of the study was to determine whether there was any evidence of loss of potency of the two lyophilized preparations during normal storage conditions, since the time they were freeze dried. Participants were requested to test the samples on four separate occasions. The lyophilized samples were reconstituted with 0·5 ml of nuclease-free deionized water and the contents gently agitated for 20 min before analysis. In the case of qualitative assays, participants were requested to perform serial dilutions of the samples in four independent assay runs. In the first qualitative assay run, 10-fold dilutions were performed to determine the end-point for the detection of HBV DNA. In each of the subsequent three assay runs, a minimum of two half-$\log_{10}$ dilutions either side of the predetermined end-point were tested, and results reported as positive or negative. In the case of quantitative assays for HBV DNA, participants were requested to report results in IU/ml and to test the samples without dilution, or prepare dilutions of the samples as necessary, depending on the linear range of assays used. In addition, one laboratory analysed the Eurohep R1 reference in parallel, following continuous storage at −80 °C.

Stability studies

For accelerated thermal degradation studies, vials of 97/746 and 97/750 were incubated at 4 °C and 20 °C, for between 51 months and 56 months. The degradation samples were extracted as previously described [5] and analysed in parallel with samples of the two preparations that had been stored at −20 °C, to provide a baseline for analysis. One set of assay runs was performed using the Artus HBV LC PCR Kit (Qiagen GmbH, Hilden, Germany) and used in accordance with the manufacturer's instructions. A second set of assay runs was performed using previously published primers and probe sequences [5] and amplification reactions were performed using the LightCycler FastStart DNA Master Hybprobe kit (Roche Applied Science, Mannheim, Germany). Standard curves were prepared using serial 10-fold dilutions of the 1st IS for HBV DNA (97/746). The stability studies were performed by two different operators at the National Institute for Biological Standards and Control (NIBSC), UK.

Table 1 Laboratory codes and assay protocols used by participants

Laboratory code	Method	Type
1	Roche COBAS TaqMan HBV test with use of HPS viral nucleic acid kit	Quantitative
2A	Artus HBV LC PCR kit	Quantitative
2B	In-house real-time PCR	Quantitative
3A	Roche COBAS AmpliScreen HBV test	Qualitative
3B	In-house real-time PCR	Qualitative
3C	In-house real-time PCR	Qualitative
4	In-house real-time PCR	Quantitative
5	In-house PCR	Qualitative
6	Roche COBAS AmpliScreen HBV test	Qualitative

PCR, polymerase chain reaction; HBV, hepatitis B virus.
In-house assay details for the following laboratories; 2B, the assay was based on a previously published amplification method [6] targeting the HBs gene with detection using the Roche LightCycler; 3B, qualitative real-time PCR assay amplifying the core region of the HBV genome with detection using the Roche LightCycler; 3C as for 3B, with an initial ultracentrifugation step prior to extraction; 4 real-time PCR amplifying the X region of the HBV genome [7] and detection using the Roche LightCycler; 5, qualitative PCR assay amplifying the HBV core region and detection using capillary electrophoresis.

Table 2 Estimated IU/ml (log_{10}) from quantitative assays

Laboratory number	Sample	
	Sample 1	Sample 2
1	5·99	5·97
2A	6·08	5·99
2B	6·06	5·92
4	5·94	5·86
Mean[a]	6·00	5·93

[a]Results combined for laboratory 2 to give a single mean prior to calculating overall mean of laboratories.

Table 3 Estimated polymerase chain reaction (PCR)-detectable units/ml (log_{10}) for qualitative assays

Laboratory number	Sample	
	Sample 1	Sample 2
3A	6·48	6·58
3B	6·90	6·68
3C	6·56	6·35
5	6·49	6·25
6	6·51	6·59

Results

For the analysis of the results, a code number was allocated at random for each laboratory (Table 1), and does not reflect the numbers assigned to laboratories that participated in the original collaborative study to establish the 1st IS (97/746). Where individual laboratories returned data from more than one assay method, or repeat assays by different operators, the results were analysed separately, and referred to as, for example, laboratories 3A and 3B. Each participating laboratory performed four separate assay runs on the two preparations as requested in the study protocol. The types of assays used by participants are recorded in Table 1; these cover a range of in-house (n = 5) and commercially available tests (n = 4). Where they have been disclosed, details of the assay and region of the HBV genome amplified are indicated (Table 1). Three laboratories (1, 2A, 2B, and 4) returned data from quantitative assays, with results expressed in IU/ml. All calculations were based on the estimates of log_{10} IU/ml, to give overall mean figures for each laboratory. Three laboratories (3A, 3B, 5 and 6) returned data from end-point dilution series, produced using qualitative assays. These were analysed to determine the polymerase chain reaction (PCR)-detectable units/ml for each sample, using the statistical methods described in the publication of the original collaborative study to establish the 1st IS for HBV DNA [1].

The estimated IU/ml (log_{10}) from the quantitative assays and PCR-detectable units/ml (log_{10}) from the qualitative assays are shown in Tables 2 and 3, respectively. For both quantitative and qualitative assays, the results for Samples 1 and 2 are extremely close. For the quantitative assays, combining the results from laboratory 2 to give a single laboratory mean, the overall estimate for the 1st IS, Sample 1, is 6·00 log_{10} IU/ml, exactly the assigned unitage, and 5·93 log_{10} IU/ml for Sample 2. If the results of the assays from laboratory 2 are considered separately (2A and 2B), then the overall means are 6·02 and 5·94 log_{10} IU/ml for Samples 1 and 2, respectively. There is also very close agreement between the results from the individual laboratories. One set of results submitted by laboratory 3C was returned as crossing point (Ct) values; these were not included in the main analysis, as it was not possible to convert them to either IU or PCR-detectable units. However, these results were in line with all other assay methods (i.e. demonstrating equivalence of Samples 1 and 2). Calculating the pairwise difference in log_{10} estimates between Samples 1 and 2 for each laboratory that provided quantitative data, there was a small, but marginally significant (P = 0·044) difference of 0·08. When the results from laboratory 2 are combined to give a single laboratory mean, the difference between Samples 1 and 2 is similar (0·07), but no longer significant. Laboratory 4 also measured the Eurohep reference sample R1. Samples 1 and

Table 4 Estimated IU/ml (log_{10}) for accelerated degradation samples

Storage temperature	Sample	
	Sample 1	Sample 2
−20 °C	6·02	5·92
4 °C	5·92	5·91
20 °C	5·94	6·03

The accelerated thermal degradation samples were stored at 4 °C and 20 °C for a period of 51 and 56 months; these samples were compared to vials of 97/746 that were stored continuously at −20 °C. Four vials of each sample stored at 4 °C and 20 °C were analysed on four separate occasions, each sample extract was tested in triplicate on each occasion. The data were pooled for the two different storage times and mean values shown for the estimated IU/ml (log_{10}).

2 were originally prepared from R1 following a 1 in 500 dilution in human plasma. The titre of R1 was determined to be 8·73 log_{10} IU/ml, which is in very good agreement with the expected titre of 8·70 log_{10} IU/ml. The difference between Samples 1 and 2 was not significant when estimates from all laboratories were included. This was the case whether treating the different assay methods of laboratory 3 as three separate laboratories ($P = 0·099$) or combining their estimates into a single laboratory mean ($P = 0·124$).

Stability studies

A total of four separate assay runs were performed by a single laboratory. The overall mean estimated IU/ml (log_{10}) for the different samples and storage temperatures are shown in Table 4. From analysis of the raw data, no degradation was evident for any of the test samples when compared with baseline samples stored at −20 °C; as a consequence the results were combined for the samples stored for 51 months, and those stored for 56 months. The results summarized in Table 4 clearly demonstrate that no degradation has occurred. Performing a formal significance test, there was no significant difference in estimated IU/ml across the temperatures for either sample. It should be noted that the formal test allowed for any possible differences between the samples stored for 51 months and those stored for 56 months. It is not possible to obtain precise predictions of expected loss per year, because no observed degradation has taken place and, thus, it was not possible to apply the Arrhenius model of accelerated degradation [8,9]. However, if it were assumed that the degradation rate would double with every 10 °C increase in storage temperature, the lack of any detectable degradation at 20 °C for over 4 years would equate to no detectable degradation at −20 °C for 64 years. Real-time stability, of the 1st IS (Sample 1) and Sample 2, as effectively detemined in the present collaborative study, indicates no loss of potency of these two preparations since time of manufacture, as evidenced by the values reported by the participants.

Conclusions

The results of this collaborative study are in good agreement with the results of the original study [1]. Using only the results of the quantitative assays, which are expected to be more precise than the qualitative assays, there was a difference of around 0·07 to 0·08 log_{10} between the estimated IU/ml for the 1st IS and the candidate replacement, Sample 2. If assays from two differing methods used by laboratory 2 are treated as if from separate laboratories, this difference is marginally significant ($P = 0·044$). However, if the results for laboratory 2 are first combined, the difference is no longer significant. Including the results from all participants, using both quantitative and qualitative assays, there is no significant difference between the 1st IS and the candidate replacement, Sample 2. This lack of significant difference is in contrast to a recently completed study to establish the 3rd IS for hepatitis C virus (HCV) RNA [10]. Here two lyophilized preparations, derived from the same bulk, were evaluated by 33 laboratories that calibrated them against the 2nd HCV IS, using a wide range of commercial and in-house quantitative and qualitative assays. The relative potencies of the two new lyophilized HCV RNA preparations were 5·19 and 5·41 log_{10} IU/ml, while the unprocessed bulk material had a relative potency of 5·70 log_{10} IU/ml. These differences in relative potencies between the two lyophilized HCV RNA preparations were statistically significant ($P < 0·0001$), with a clear loss of potency on processing. This is in contrast to the HBV study presented here. From the original collaborative study and data from this new study, there is no significant difference between the potencies of the two HBV DNA Samples 1 and 2, nor was there any detectable loss of titre of the preparations following lyophilization [1].

The results of the accelerated degradation studies have also demonstrated that both Samples 1 and 2 are extremely stable and suitable for long-term use, with no detectable degradation for either preparation after storage at 20 °C for more than 4 years. This stability is in contrast to the 1st and 2nd IS for HCV RNA (96/790 and 96/798, respectively), which showed an average decrease of log_{10} 1·9 for samples stored at 20 °C for more than 5 years [11]. This difference in the observed stability may be due to the nature of the viral nucleic acid, which in the case of HBV is DNA, in contrast to the RNA genome of HCV, which is likely to be more unstable and susceptible to degradation. However, it is possible that further unknown factors influence the stability.

On the basis of this study, Sample 2 (97/750) was established as the 2nd IS for HBV DNA for NAT-based assays by the WHO

ECBS in October 2006. This preparation has a potency of 10^6 IU/ml. Each vial contains the equivalent of 0·5 ml of material, and the content of each vial is 5×10^5 IU per vial. Vials of 97/750 are available from NIBSC.

References

1 Saldanha J, Gerlich W, Lelie N, Dawson P, Heermann K, Heath A, the WHO Collaborative Study Group: an international collaborative study to establish a World Health Organization international standard for hepatitis B virus DNA nucleic acid amplification techniques. *Vox Sang* 2001; 80:63–71

2 Heermann KH, Gerlich WH, Chudy M, Schaefer S, Thomssen R, the Eurohep Pathobiology Group: Quantitative detection of hepatitis B virus DNA in two international reference plasma preparations. *J Clin Microbiol* 1999; 37:68–73

3 World Health Organization: *WHO Expert Committee on Biological Standardization*; technical report series, 904; Fiftieth report, 2002:23–24

4 Baylis SA, Davis C, Holmes H: Minutes of the eighteenth meeting of the Scientific Working Group on the Standardisation of Genome Amplification Techniques for the Safety Testing of Blood, Tissues and Organs (SoGAT). National Institutes of Health, Bethesda MD, USA, 24–25 May 2005

5 Baylis SA, Shah N, Minor PD: Evaluation of different assays for the detection of parvovirus B19 DNA in human plasma. *J Virol Methods* 2004; 121:7–16

6 Weinberger KM, Wiedenmann E, Böhm S, Jilg W: Sensitive and accurate quantitation of hepatitis B virus DNA using a kinetic fluorescence detection system (TaqMan PCR). *J Virol Methods* 2000; 85:75–82

7 Jursch CA, Gerlich WH, Glebe D, Schaefer S, Marie O, Thraenhart O: Molecular approaches to validate disinfectants against human hepatitis B virus: *Med Microbiol Immunol* 2002; 190:189–197

8 Kirkwood TBL: Predicting the stability of biological standards and products. *Biometrics* 1977; 33:736–742

9 Tydeman MS, Kirkwood TBL: Design and analysis of accelerated thermal degradation tests for the stability of biological standards I. Properties of maximum likelihood estimators. *J Biol Stand* 1984; 12:195–206

10 Baylis SA, Heath A, the collaborative Study Group: WHO collaborative study to establish a replacement WHO International Standard for hepatitis C virus RNA nucleic acid amplification technique (NAT)-based assays. WHO ECBS Report 2007; WHO/BS/07.2055

11 Saldanha J, Heath A, Aberham C, Albrecht J, Gentili G, Gessner M, Pisani G: World Health Organization collaborative study to establish a replacement WHO international standard for hepatitis C virus RNA nucleic acid amplification technology assays. *Vox Sang* 2005; 88:202–204

別紙様式第2-1

No. 34

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2008. 2. 18	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況	沼尾宏, 渡辺泰宏, 立花直樹. 第37回日本肝臓学会西部会; 2007 Dec 7-8; 長崎.	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)			日本

研究報告の概要

○輸血によりHBs抗体エスケープ変異株に感染した一例
【症例】40歳代女性。平成16年8月より発熱あり、白血病の疑いにて平成16年10月21日入院。混合型急性白血病として10月25日より化学療法を施行。入院時HBsAg-、HCVAb-であった。10月末より11月にかけてALT387IU/Lまで上昇した。11月10日にはHBsAg-、HBsAb+、HbcAb+、HBeAg-、HBeAb-、HBV-DNAポリメラーゼ0CPM。一時肝機能は正常化したものの、平成18年2月中旬より再びトランスアミナーゼの上昇を軽度認めた。白血病が血液学的寛解となり4月17日退院。外来で化学療法を施行していたが、5月30日AST 947IU/L、ALT 1683IU/Lと上昇し再度入院。HBeAg+、HBeAb+、HBcAb·IgM+で、他の肝炎ウイルスマーカーが陰性であったためHBV感染を疑い、6月5日よりラミブジンを100mg/日で投与開始した。6月6日HBV-PCR 3.4LC/mLであった。その後ALT 2357IU/L、T-Bil 7.41mg/dlまで上昇し、肝炎の改善傾向はなかった。ラミブジンの継続と肝庇護療法にて肝機能は改善した。6月23日の肝生検では小葉内肝細胞壊死を伴った高度の炎症細胞浸潤を認め、急性肝炎の所見であった。AST17IU/L、ALT27IU/Lとなり7月22日退院。HBV-PCR陽性となるまでに患者に投与されたすべての血液製剤について個別HBV-NATを実施した結果、平成16年11月に輸血したFFPがHBV陽性であった。製剤と患者のHBVはいずれもGenotype C/Subtype ayrでS抗原のN末端から145番目のアミノ酸がGlyからSerに置換しており、エスケープミュータントであった。また、両者のα領域(PreS/S領域を含むP領域の前半部)の塩基配列は一部の塩基の共存を除き、完全に一致した。HBV-DNAはいずれも定量限界(100copies/mL)未満であった。患者はその後もラミブジンの投与を継続し、骨髄移植を行った。肝炎の再燃は認めなかったが、白血病のため平成18年10月永眠された。【考察】核酸増幅検査を含む献血者のスクリーニングを行っているにもかかわらず、本邦では年間10-20例のHBV感染が報告されている。その原因の一つがHBs抗体エスケープミュータントであるが、本症例のように献血者、受血者ともに塩基配列の解析を行い感染が証明された例はきわめて稀と考えられ報告する。

報告企業の意見	今後の対応
輸血によりHBs抗体エスケープ変異株に感染し、献血者、受血者の塩基配列の解析を行って感染が証明された症例の報告である。	日本赤十字社では、HBs抗原検査及びHBc抗体検査を実施することに加えて、HBVについて20プールでスクリーニングNATを行い、陽性血液を排除している。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。また、これまでの凝集法と比べて、より感度の高い化学発光酵素免疫測定法(CLEIA)の導入を順次進めている。NATの精度向上についても評価・検討している。

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」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

自発報告:2006年3月16日付1-05000059

(3)

O-9　初診時よりHBs抗体陽性であったB型急性肝炎の一例

○石田素子，松本修一，筋浦立成
福岡徳洲会病院総合内科

【症例】33歳男性【主訴】全身関節痛【現病歴】来院5日前起床時に頚部痛を自覚し日毎に全身関節に拡大，症状の改善がなく当科外来を初診した．関節痛は時間帯，安静労作に関係なく，食欲低下を認める以外は発熱，かぜ症状などは認めない．【生活歴】MSM (men who have sex with men)で最終性交は半年前，パートナーは固定していない．刺青・輸血歴・海外渡航歴なし．常用薬なし．喫煙は20本/日．機会飲酒．【家族歴】肝疾患なし．【来院時現症】眼瞼結膜に黄疸なし．胸部異常所見なし．肝脾触知せず．疼痛のある関節に腫脹，発赤，熱感なし．【検査所見】AST 1430IU/l，ALT 2630 IU/l，PT50%，T.bil0.8mg/dl，IgM-HA抗体陰性，HCV-RNA陰性，HBs抗原(2000)陽性，HBs抗体(1000)陽性，IgMHBc抗体(31.5)陽性，HBV-DNA>7.6LGE/ml，HBVgenotypeA【経過】安静のみで採血データは徐々に改善し，入院時認めた食欲低下や関節痛もそれとともに軽快した．HBs抗体陽性ではあったが，IgMHBc抗体高濃度陽性でB型急性肝炎と診断した．感染経路としては性感染症と予想され，その他の感染症も検査した結果，2期梅毒を認めたが，HIVは陰性であった．【まとめ】診断初期よりHBs抗原抗体が共に高値を示しその判断に苦慮したB型急性肝炎を経験した．HBs抗原抗体の共存について文献的な報告を含め考察する．

O-10　輸血によりHBs抗体エスケープ変異株に感染した一例

○沼尾　宏[1]，渡辺泰宏[2]，立花直樹[3]
青森県立中央病院消化器内科・化学療法科[1]，わたなべ内科クリニック[2]，青森県立中央病院輸血部[3]

【症例】40歳代女性．主訴：肝機能障害．家族歴：特記すべきことなし．既往歴：平成元年切迫早産．現病歴：平成16年8月より発熱あり近医受診．白血病の疑いにて平成16年10月21日当院リウマチ・血液内科紹介入院．経過：混合型急性白血病として10月25日より化学療法を施行．入院時HBsAg-(0.00)，HCVAb-(0.1)であった．10月末より11月にかけてALT 387 IU/Lまでの上昇を認めた．11月10日の採血ではHBsAg-(<0.05IU/mL)，HBsAb+(69.0mIU/mL)，HbcAb+(1.82 S/CO)，HBeAg-(0.4)，HBeAb-(16%)，HBV-DNAポリメラーゼ0CPM．その後一時肝機能は正常化したものの平成18年2月中旬より再びトランスアミナーゼの上昇を軽度認めた．白血病が血液学的寛解となり4月17日退院．外来でプレドニゾロンを含む化学療法を施行していたが，5月30日AST 947 IU/L，ALT 1683IU/Lと上昇しリウマチ・血液内科入院．HBeAg+(1.52S/CO)，HBeAb+(65.2)，HBcAb-IgM+(20.9 S/CO)で，かつ他の肝炎ウイルスマーカーが陰性であったためHBV感染を疑い6月5日よりラミブジンを100mg/日で投与開始した．6月6日HBV-PCR 3.4LC/mLであった．その後ALT 2357IU/L，T-Bil 7.41mg/dlまで上昇し，肝炎の改善傾向がないため当科転科となった．ラミブジンの継続と肝庇護療法にて肝機能は改善した．6月23日の肝生検では小葉内肝細胞壊死を伴った高度の炎症細胞浸潤を認め，急性肝炎の所見であった．AST 17IU/L，ALT 27IU/Lとなり7月22日退院．HBV-PCR陽性となるまでに患者に投与されたすべての血液製剤について個別HBV-NATを実施した結果，平成16年11月に輸血したFFPがHBV陽性であった．この製剤中と患者のHBVはいずれもGenotype C/Subtype ayrでS抗原のN末端から145番目のアミノ酸がGlyからSerに置換しており，エスケープミュータントであった．また，両者のα領域(PreS/S領域を含むP領域の前半部)の塩基配列は一部の塩基の共存を除き，完全に一致した．HBV-DNAはいずれも定量限界(100copies/mL)未満であった．患者はその後もラミブジンの投与を継続し，骨髄移植を行った．肝炎の再燃は認めなかったが，白血病のため平成18年10月永眠された．【考察】核酸増幅検査を含む献血者のスクリーニングを行っているにもかかわらず，本邦では年間10~20例のHBV感染(occult HBVによる感染)が報告されている．その原因の一つがHBs抗体エスケープミュータントであるが，本症例のように献血者，受血者ともに塩基配列の解析を行い感染が証明された例はきわめて稀と考えられ報告する．

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医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2007. 11. 22	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	Dhalla S, Tenner CT, Aytaman A, Shukla NB, Villanueva G, Punla G, Patterson C, Comas J, Bini EJ. American Society for the Study of Liver Diseases; 2007 Nov 2-6; Boston.	公表国 米国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社) 合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)			

研究報告の概要

○入れ墨とC型肝炎ウイルス感染の強い相関:患者3871名による多施設共同研究

背景:HCV感染のリスク要因として静注薬物使用と1992年以前の輸血がよく知られてきたが、入れ墨に関しては議論があった。さらに、複数の先行する研究では、HCV感染のリスク要因としての入れ墨は静注薬物使用と混同されてきた可能性がある。本研究の目的は、典型的なHCV感染リスクのない患者多数における入れ墨とHCV感染の関係を明らかにすることである。

方法:慢性HCV感染の患者(HCV RNA陽性)とコントロール群(HCV抗体陰性)が3つの研究施設での外来診療時、詳細なアンケートに回答した。患者の人口統計学的データとHCVリスク要因を含むデータを収集した。

結果:合計3871名の患者のうち1930名が慢性HCV感染群、1941名が陰性のコントロール群だった。平均年齢(55.2±9.0 vs. 55.6±11.3年、p=0.34)と男女比(80.3% vs 81.4%、p=0.39)に有意差はなかった。しかし、感染群は人種的/民族的マイノリティが多かった(78.5% vs 56.5%、p<0.001)。予想されたとおり、静注薬物使用(65.9% vs 17.8%、p<0.001)と1992年以前の輸血(22.3% vs 11.1%、p<0.001)は、HCV感染群の方がコントロール群よりも多かった。感染群は2つ以上の入れ墨がある傾向が強く(35.2% vs 12.5%;OR=3.81;95% CI、3.24-4.49;p<0.001)、これは年齢、性別、人種/民族についての補正後も有意だった(OR=4.57;95% CI、3.83-5.45;p<0.001)。静注薬物使用歴と1992年以前の輸血歴を持つ患者を除外した後に残った1887名を分析した(466名が感染群、1421名がコントロール群)。陽性群の患者は入れ墨歴がある傾向が強く(34.1% vs. 11.9%;OR=3.84;95% CI、2.99-4.93;p<0.001)、年齢、性別、人種/民族についての補正後も統計的に有意だった(OR=4.47;95% CI, 3.42-5.83;p<0.001)。

結論:入れ墨は、静注薬物使用や1992年以前の輸血などの典型的なHCV感染リスク要因のない患者群においても、HCV感染と強く相関している。入れ墨のある患者にはHCV検査が推奨されるべきである。

報告企業の意見	今後の対応
入れ墨は、静注薬物使用や1992年以前の輸血などの典型的なHCV感染リスク要因のない患者群においても、HCV感染と強く相関しているとの報告である。	日本赤十字社は、輸血感染症対策として問診時に過去1年以内に入れ墨を入れた人は献血不適としている。今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-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

6

AASLD 2007

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ID# 136
Location: Auditorium (Hynes)
Time of Presentation: Nov 05 5:30 PM - 5:45 PM
Category: Q04. HCV: Epidemiology

Strong Association between Tattoos and Hepatitis C Virus Infection: A Multicenter Study of 3,871 Patients

E. J. Bini[5]; S. Dhalla[1]; C. T. Tenner[2]; A. Aytaman[3]; N. B. Shukla[4]; G. Villanueva[4]; G. Punla[5]; C. Patterson[4]; J. Comas[3]
1. Department of Medicine, NYU School of Medicine, New York, NY, USA.
2. Division of General Internal Medicine, VA Medical Center & NYU School of Medicine, New York, NY, USA.
3. Division of Gastroenterology, VA Medical Center, Brooklyn, NY, USA.
4. Division of Gastroenterology, Bellevue Hospital Center & NYU School of Medicine, New York, NY, USA.
5. Division of Gastroenterology, VA Medical Center & NYU School of Medicine, New York, NY, USA.

Background: Although injection drug use and blood transfusions prior to 1992 are well-accepted risk factors for hepatitis C virus (HCV) infection, the evidence for tattoos as a risk factor for HCV is conflicting. Furthermore, several prior studies that have evaluated tattoos as a risk factor for HCV infection were potentially confounded by injection drug use. The aim of this study was to determine the association between tattoos and HCV infection in a large population of patients without traditional risk factors for HCV infection.
Methods: Patients with chronic HCV infection (HCV RNA positive) and controls (HCV antibody negative) completed a detailed questionnaire at the time of their scheduled visit to the outpatient primary care or GI clinic at 3 study sites. Data collected included patient demographics and information on HCV risk factors.
Results: A total of 3,871 patients were enrolled, including 1,930 with chronic HCV infection and 1,941 HCV negative controls. There were no differences in the mean age (55.2 ± 9.0 vs. 55.6 ± 11.3 years, p = 0.34) or the proportion who were male (80.3% vs. 81.4%, p = 0.39) between HCV-infected patients and controls. However, HCV positive patients were more likely to be racial/ethnic minorities (78.5% vs. 56.5%, p <0.001). As expected, injection drug use (65.9% vs. 17.8%, p < 0.001) and blood transfusions prior to 1992 (22.3% vs. 11.1%, p <0.001) were more common in HCV-infected patients than in control subjects. Patients with HCV infection were significantly more likely to have had one or more tattoos (35.2% vs. 12.5%; OR = 3.81; 95% CI, 3.24 – 4.49; p <0.001) and this remained highly significant after adjustment for age, sex, and race/ethnicity (OR = 4.57; 95% CI, 3.83 – 5.45; p <0.001). After excluding all patients with a history of ever injecting drugs and those who have had a blood transfusion prior to 1992, a total of 1,887 subjects remained for analysis (466 HCV positive and 1,421 controls). Among these 1,887 patients without traditional risk factors for HCV infection, we found that HCV positive patients were still significantly more likely to have a history of tattoos (34.1% vs. 11.9%; OR = 3.84; 95% CI, 2.99 – 4.93; p <0.001) and this remained highly statistically significant after adjustment for age, sex, and race/ethnicity (OR = 4.47; 95% CI, 3.42 – 5.83; p <0.001).
Conclusions: Tattoos are strongly associated with HCV infection, even among those without traditional HCV risk factors such as injection drug use and blood transfusions. All patients with tattoos should be offered HCV testing.

別紙様式第2-1

No. 39

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研究報告の概要

○イタリアの血液透析施設におけるC型肝炎集団感染の分子疫学

血液透析患者は、C型肝炎ウイルス(HCV)感染のリスクが高い。本試験の目的は、疫学的、分子学的手法を用いて、血液透析施設におけるHCV集団感染について検討することである。2003年4月～2003年10月に、当該施設を利用する患者4名にHCV抗体セロコンバージョンが認められた。この4名を、当該施設来院時にすでにHCV抗体陽性であった患者10名に加え、14名全員の抗HCV抗体陽性患者のHCV RNAおよびHCV遺伝子型を検査した。HCV RNA 陽性患者のNS5BおよびHVR1/ E2遺伝子領域を増幅し、配列を決定し、系統発生解析を行った。さらに、患者全員から得られた臨床疫学的記録を調べた。新たに感染した患者4名はいずれも遺伝子型2cであった。来院時にすでにHCV抗体陽性であった患者10名のうち2名でも遺伝子型2cが検出された。系統発生解析は、新規HCV感染患者全員が、2c慢性感染患者2名中1名から検出された2c分離ウイルスと群生した分離ウイルスと近縁であることを示した。いずれのHCV-2c感染患者にも血液透析以外のリスク因子はなかった。新規HCV-2c感染患者4名中3名と当該集団感染に関与したHCV-2c慢性感染患者1名は、同日の同一シフト時に透析を受けたが、装置は別のものを使用していた。残りのHCV-2c新規感染患者と前述の3名中1名は、同日の別のシフト時に同一の装置を使用して透析を受けた。当該集団感染は、おそらく感染制御手段の不備によるものであると考えられるが、1症例においては関連装置による伝播が除外できない。

報告企業の意見	今後の対応
2003年4月～2003年10月に、イタリアの血液透析施設でHCVの集団感染が発生し、感染制御手段の不備と装置による伝播が疑われたとの報告である。	HCV感染の新たな伝播ルート等について、今後も情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-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

(1)

Journal of Medical Virology 80:261–267 (2008)

Molecular Epidemiology of a Hepatitis C Virus Outbreak in a Hemodialysis Unit in Italy

Enea Spada,[1]* Isabella Abbate,[2] Elvia Sicurezza,[3] Andrea Mariano,[4] Vincenza Parla,[3] Sebastiano Rinnone,[5] Mario Cuccia,[5] Maria Rosaria Capobianchi,[2] and Alfonso Mele[1]

[1]Istituto Superiore di Sanità, National Centre of Epidemiology, Surveillance and Health Promotion, Rome, Italy
[2]Istituto Nazionale di Malattie Infettive Lazzaro Spallanzani, Laboratory of Virology, Rome, Italy
[3]Azienda Ospedaliera Universitaria, Vittorio Emanuele II, Ferrarotto S. Bambino, Unità Operativa di Nefrologia-Dialisi, Catania, Italy
[4]Istituto Nazionale di Malattie Infettive Lazzaro Spallanzani, Clinical Research Department, Rome, Italy
[5]Azienda Unità Sanitaria Locale 3 Catania, Dipartimento di Prevenzione, Servizio Epidemiologia e Prevenzione, Catania, Italy

Hemodialysis patients are at increased risk of hepatitis C virus (HCV) infection. The aim of this study was to investigate a HCV outbreak in a hemodialysis unit using epidemiological and molecular methods. Between April 2003 and October 2003, anti-HCV seronconversion was detected in four patients attending the unit. These cases were added to 10 patients already anti-HCV positive upon admission in the unit. All 14 anti-HCV patients were tested for HCV RNA and HCV genotype. NS5B and HVR1/E2 genomic regions were amplified and sequenced in all HCV RNA positive patients and phylogenetic analysis was performed. Furthermore, clinical-epidemiological records obtained from all patients were examined. All four patients newly infected harbored genotype 2c. Genotype 2c was also detected in 2 of 10 patients already anti-HCV positive upon admission. Phylogenetic analysis showed that all newly HCV infected patients harbored very closely related viral isolates that clustered together with the 2c isolate found in one of the two 2c chronic infected patients. All HCV-2c infected patients had no other risk factors except hemodialysis. Three of four newly HCV-2c infected patients and the one HCV-2c chronically infected involved in the outbreak received dialysis on the same day and same shift but used different machines. The remaining HCV-2c newly infected patient and one of the above cited three received dialysis on the same day during different shifts but used the same machine. The outbreak was probably due to breaks of infection control procedures although a related-machine transmission cannot be excluded in one of the cases. ***J. Med. Virol. 80:261–267, 2008.***

KEY WORDS: epidemiological investigation; genotype; hemodialysis; hepatitis C virus; nosocomial infection; phylogenetic analysis

INTRODUCTION

Patients on hemodialysis are recognized as a group at increased risk of infection with hepatitis C virus (HCV). The prevalence and incidence of HCV infection among patients receiving hemodialysis varies widely between countries and also within the same country [Fabrizi et al., 2002]. In Italy, the prevalence of HCV among hemodialysis patients ranges between 10% and 50%, and the incidence is around 1–2 cases per 100 person-years [Petrosillo et al., 2001; Di Napoli et al., 2006].

The risk of HCV transmission by blood transfusion to patients receiving hemodialysis has been considerably reduced since screening of blood donors for HCV antibodies (anti-HCV) was introduced and recombinant erythropoietin for treatment of anemia became available [Di Napoli et al., 2006]. However, HCV transmission in hemodialysis units still occurs, and occasionally it is responsible for large outbreaks [Le Pogam et al., 1998; Delarocque-Astagneau et al., 2002; Fabrizi et al., 2002; Kokubo et al., 2002;

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*Correspondence to: Dr. Enea Spada, Istituto Superiore di Sanità, National Centre of Epidemiology, Surveillance and Health Promotion, Clinical Epidemiology Unit, Via Giano Della Bella, 34-00162 Rome, Italy. E-mail: enea.spada@iss.it

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Savey et al., 2005]. Several studies, by using molecular biology techniques, provided evidence of a nosocomial patient-to-patient mode of transmission in most of these HCV infection occurring in hemodialysis settings, despite rigorous preventive measures [Le Pogam et al., 1998; Delarocque-Astagneau et al., 2002; Kokubo et al., 2002; Savey et al., 2005]. Important risk factors for acquiring nosocomial HCV infection in patients on hemodialysis seems to be particularly a longer duration of hemodialysis, a high HCV prevalence in the unit and a low personnel/patient ratio (<1/3 or at least 1/4) [Petrosillo et al., 2001].

However, the exact mechanisms of the patient-to-patient transmission of HCV within hemodialysis units have not been clearly identified and they may be different in relation to the different policies followed in each hemodialysis unit for the management of patients [Petrosillo et al., 2001; Fabrizi et al., 2002]. Most authors currently believe that most cases of HCV patient-to-patients transmission can be attributed to lack of implementation of or breaks in infection control procedures [Le Pogam et al., 1998; Petrosillo et al., 2001; Delarocque-Astagneau et al., 2002; Fabrizi et al., 2002; Kokubo et al., 2002; Savey et al., 2005]. The possibility of HCV transmission between patients through the dialysis machines is controversial. However, this possibility cannot be entirely excluded especially in case of contamination of internal components of the machine not accessible to routine disinfection, and in the hemodialysis units in which the disinfection of the machines between treatments is not routinely performed or those in which dialysers and/or dialysis tubing sets are reused [Le Pogam et al., 1998; Delarocque-Astagneau et al., 2002; Fabrizi et al., 2002; Savey et al., 2005].

This study describes an outbreak of acute HCV type 2c infection involving four patients attending an outpatient hemodialysis unit in southern Italy. Molecular analysis of viral isolates in association with an epidemiological investigation was performed to trace the source and the possible routes of transmission of HCV during this outbreak.

PATIENTS AND METHODS

Hemodialysis Setting and Procedures

At the time of the outbreak, the unit consisted in a room with 8 dialysis machines in which 32 outpatients regularly underwent maintenance hemodialysis three times weekly (Monday-Wednesday-Friday or Tuesday-Thursday-Saturday) on 1 of the 2 shifts per day (either morning or evening shift). Thus, every machine was used by two persons per day. Normally patients were dialysed on the same shift, but not always on the same machine. No dedicated areas or machines or personnel were used for HCV infected patients. Hemodialysis was carried out using Hospal-INTEGRA® dialysis machine. The machines were disinfected with chlorine dioxide (ISTRUMET, Hospal®) between each shift and dialysers and tubing sets were disposable and were never reused. Two nurses took care of eight patients in each shift, but they could also move from patient to patient if needed. No multidose vials were used among patients.

HCV Infection Monitoring

HCV infection was monitored in all dialysis patients by performing testing for serum alanine aminotranserase (ALT) monthly and for anti-HCV upon admission and then every 6 months. Anti-HCV test was also performed in case of ALT elevation. Prior to the beginning of the outbreak, the prevalence of anti-HCV among the 32 patients attending the unit was 31.2% (10 patients).

Case Definition, Case Finding and Data Collection

During the routine screening for HCV infection conducted from April 2003 to October 2003 four incident cases of HCV infection were identified in the unit. That the four cases had occurred in a relatively brief period of time led to suspect a nosocomial outbreak. A potential outbreak case-patient was defined as any patient who had showed seroconversion between October 2002 and October 2003 and who had received dialysis in the unit at least 6 months before the detection of the first seroconversion case.

Since in the unit, at the time of the outbreak, the monitoring of HCV infection was based on the detection of anti-HCV only, to identify retrospectively other cases of new infections and the potential source of the outbreak on June 2004 blood samples for anti-HCV and HCV RNA testing were obtained from all the patients who had received dialysis in the unit since April 2002 and from all their household contacts. All healthcare workers employed in the unit underwent periodical testing for blood-borne viruses. A blood sample was also obtained from the one healthcare worker (a doctor) who was known to be anti-HCV positive.

From the medical records, kept constantly for all patients, data on medical and dialysis history, blood transfusion, recent surgical, or medical invasive procedures, intravenous drug use and other parenteral exposure, such as tattoos and piercing, were obtained. Furthermore, the dialysis schedule (day and shift) seating arrangements, type of vascular access, type of dialyser membrane, hemodialysis machine, bleeding episodes, nurse-patient assignment, dialysis equipment maintenance as well as infection control measures were all recorded.

Virological Analysis

The seroconversions of the patients involved in the outbreak were detected during the routine screening for anti-HCV performed in all patients attending the unit. In the unit, anti-HCV antibodies were detected by using a third generation enzyme immunoassay (Cobas Core Anti-HCV EIA II, Roche Diagnostic Systems, Basel,

Switzerland) and samples that resulted anti-HCV positive were then tested for HCV RNA (COBAS Amplicore, Roche Diagnostic Systems). An in-depth retrospective virological analysis was performed on serum samples obtained on June 2004 from all hemodialysis patients attending the unit, from the household contacts of the patients who had shown a seroconversion and from the only health care worker who was found to be anti-HCV positive.

All serum samples obtained were tested again for anti-HCV by a third generation enzyme immunoassay (Axsym HCV, version 3.0; Abbott Laboratories, Abbott Park, IL). The viral load in positive serum samples was determined by quantitative reverse transcription (RT)-PCR (HCV Amplicor Monitor, Roche Diagnostics, Milan, Italy), in accordance with the manufacturer's instructions. HCV genotyping was first performed with a commercial reverse-hybridization line probe assay (INNO-LiPA HCV II, Bayer Diagnostics, Milan, Italy) based on the 5'-noncoding region (NCR). The HCV RNA was extracted from serum samples collected in June 2004 using the QIAamp Viral RNA kit (QIAGEN, Hilden, Germany), underwent retrotranscription by random hexamer method and was used to perform molecular analysis. Two-strand direct sequencing was carried out on nested PCR products obtained from the NS5B region and from the hypervariable region 1 (HVR1) encompassing in the E2 gene, as previously reported [Faustini et al., 2005]. Sequencing was performed on ABI Prism 3100, using the BigDye Terminator cycle sequencing kit (Applied Biosystems, Warrington, UK). Sequences of the NS5 regions obtained were aligned by using BLAST with the *National Center for Biotechnology Information* (NCBI) database (U.S. National Library of Medicine, Bethesda, MD, www.ncbi.nlm.nih.gov) and were able to attribute to the genotype 2c all the HCV from patients who seroconverted. For the amplification of the HVR1 region 2 type specific primers were chosen [Faustini et al., 2005]. The sequences were then aligned with CLUSTAL W software (version 1.5). The mean genetic distance between nucleotide sequences was calculated using a Kimura 2-parameter distance matrix with a transition/transversion ratio of 2.0. Phylogenetic trees were constructed using the neighbor-joining method, including NS5B and HVR-1 reference sequences (see figure legends) and local epidemiological unrelated HCV 1, 2b, and 2c strains. Bootstrap analysis with 1,000 replications was performed to assess the significance of the nodes; values >85% were considered to be significant. All of the algorithms used were included in MEGA software (version 2.1).

RESULTS

In October 2002, the anti-HCV seroprevalence in our HD unit was 31.2% (10/32). Between April 2003 and October 2003, four new HCV seroconversions were detected; the first (CT6) in April 2003, second and third (CT2 and CT4, respectively) in August 2003 and the fourth (CT11) in October 2003; thus, raising the total number of anti-HCV positive patients to 14 in the HD unit (Table I).

In all four patients who seroconverted the infection was asymptomatic. In the first patient who showed a seroconversion (CT6), ALT levels that were normal on the date of seroconversion and had always been normal previously, showed an important increase only in the following month. In one of the two patients (CT2) who were found to be anti-HCV on August 2003, ALT levels showed a moderate increase only at the time of seroconversion and they had always been normal previously. The other newly infected patient (CT4) who tested anti-HCV positive on August 2003 had shown an important ALT levels increase on May 2003, but he tested anti-HCV negative in that date; however, his ALT levels had always been normal until April 2003. In these two patients anti-HCV test was performed, and repeated at monthly interval for patient CT 4, as consequence of the detected ALT levels elevation. The last newly infected patient (CT11), who was found to be

TABLE I. Anti-HCV Test Results, Genotype Determination and First Detection of Elevated Alanine Aminotransferase Levels and of HCV RNA in HCV Infected Patients Receiving Dialysis in the Unit (April 2002–October 2003)

Pt	April 2002	October 2002	April 2003	May 2003	June 2003	July 2003	August 2003	September 2003	October 2003
CT 7	Pos	Pos	Pos	NT	NT	NT	NT	NT	Pos
CT 14	Pos	Pos	Pos	NT	NT	NT	NT	NT	Pos
CT 1	Pos	Pos	Pos	NT	NT	NT	NT	NT	Pos
CT 10	Pos	Pos	Pos	NT	NT	NT	NT	NT	Pos
CT 13	Pos	Pos	Pos	NT	NT	NT	NT	NT	Pos
CT 8	Pos	Pos	Pos	NT	NT	NT	NT	NT	Pos
CT 3	Pos	Pos	Pos	NT	NT	NT	NT	NT	Pos
CT 5	Pos	Pos	Pos	NT	NT	NT	NT	NT	Pos
CT 9	Pos	Pos	Pos	NT	NT	NT	NT	NT	Pos
CT 12	Pos	Pos	Pos	NT	NT	NT	NT	NT	Pos
CT 6	Neg	Neg	**Pos**	* NT ↑	NT	NT	NT	NT	Pos
CT 2	Neg	Neg	Neg	Neg	Neg	Neg	**Pos** ↑	* NT	Pos
CT 4	Neg	Neg	Neg	Neg ↑	Neg	Neg	**Pos**	* NT	Pos
CT 11	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	* **Pos**

Pos, anti-HCV test positive; Neg, anti-HCV test negative; NT, not tested for anti-HCV; ↑ denotes the first detection of elevated ALT levels; * denotes the first HCV RNA detection. Pos in bold denotes the first anti-HCV positive test.

anti-HCV positive on October 2003 (on the occasion of the anti-HCV six-monthly screening), had always shown ALT normal values before seroconversion at the monthly screening routinely performed in the unit (Table I). All patients newly infected showed a positive HCV RNA test after the seroconversion.

No further cases of new HCV infection were detected when the serum samples drawn on June 2004 from all the patients receiving hemodialysis in the unit were tested for anti-HCV and HCV RNA. Genotyping performed by INNO-LiPA revealed that all the patients who had shown a new seroconversion were infected with HCV genotype 2c. Six out of the 10 dialysis patient with chronic HCV infection were infected with genotype 1b, 2 with genotype 2c (CT1 and CT3), and in 2 patients it was not possible to determine the infecting genotype (one refused to give a blood sample and another one deceased before June 2004). Serum HCV RNA levels ranged from 7,000 to 488,000 IU/ml for the four newly infected patients and from 43,000 to >500,000 IU/ml for patients with chronic HCV infection, for which a serum sample was available. The two chronically infected patients harboring genotype 2c had serum HCV RNA titres of 171,000 UI/ml (CT1) and >500,000 IU/ml (CT3). Since in June 2004 all newly infected patients still resulted HCV RNA positive, that is more than 6 months after the detection of their seroconversion, they must be considered as having all developed a persistent HCV infection. These patients continued to be viremic while attending the unit, although their ALT levels were constantly normal, and none of them underwent antiviral therapy.

Figure 1 shows the phylogenetic tree analysis of the NS5B region sequences isolated from all HCV RNA positive patients receiving hemodialysis in the unit (newly infected and chronically infected patients). All four newly infected patients harbored very closely related viral isolates that clustered together with the 2c isolate found in one of the two 2c chronically infected patients, which is consistent with the hypothesis that the outbreak had a single epidemiologic origin (patient CT3). Phylogenetic analysis also revealed that the other patient with chronic infection harboring genotype 2c (CT1) was not associated with the outbreak. The results of the phylogenetic analysis in the HVR1 region, using only genotype 2 sequences, with the majority being 2c collected from GenBank, were consistent with the findings in the NS5B region (Fig. 2).

The results of the epidemiological investigation were also consistent with a patient-to-patient mode of transmission of the infection during the outbreak. The four newly infected patients had never received transfusion of blood or blood products. Two of them (CT2 and CT6) had no exposure to surgical or medical invasive procedures outside the hemodialysis unit, while patient CT4 had undergone dental extraction 2 months before his seroconversion, and patient CT11 had dialysed outside the unit on summer holidays (July and August). None of the newly infected patients were known to have used intravenous drug and none of their household

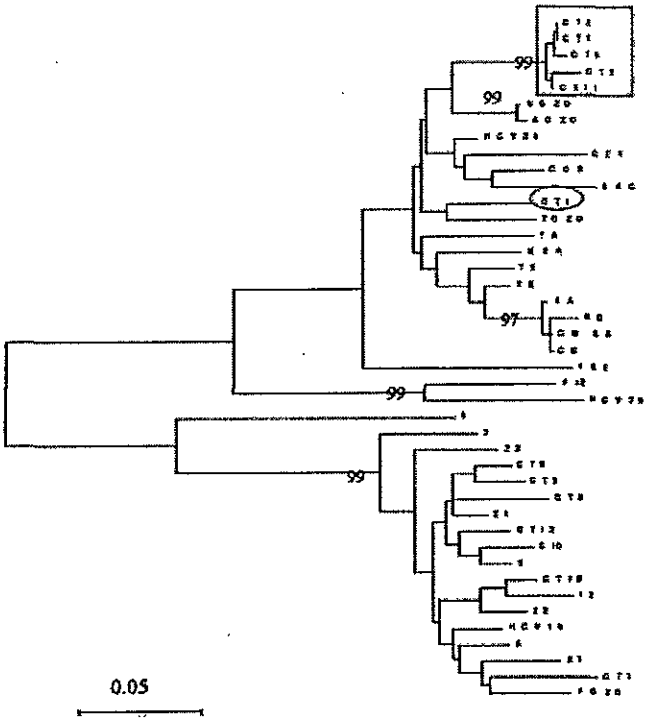

Fig. 1. Phylogenetic tree of NS5B region. The patients involved in the outbreak were indicated with the initials CT and numbered as in the table. Reference genotypes 2b, 2c, and 1b sequences from GenBank are indicated with their accession numbers (for HCV2c AJ291280; for HCV2b AB030907; for HCV 1b AY257435). Local nonrelated patient sequences, including patients with genotypes 2c, 2b, and 1b, are also included. Genetic distance is indicated by a horizontal bar. The numbers at the nodes indicate the frequency with which the node occurred in 1,000 bootstrap replicates; values greater than 95% are indicated.

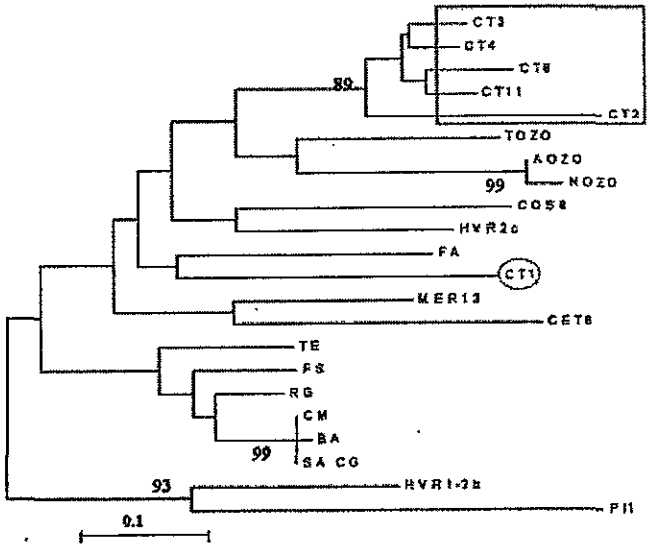

Fig. 2. Phylogenetic tree of HVR1/E2 region. Patients are indicated as in Figure 1. Reference genotypes 2c and 2b GenBank accession numbers are AF 237649 and AB030907, respectively. Local nonrelated patient sequences with genotypes 2c and 2b are also included. Genetic distance is indicated by a horizontal bar. The numbers at the nodes indicate the frequency with which the node occurred in 1,000 bootstrap replicates; values greater than 85% are indicated.

contacts were found to be anti-HCV positive. The only healthcare worker who was anti-HCV positive resulted negative when tested for HCV RNA. All patients infected by closely related genotype 2c isolates (new and old infections: patients CT2, CT3, CT4, CT6, and CT11) received dialysis on the same days (Monday-Wednesday-Friday). Patients CT2, CT3, CT4, and CT6 received dialysis on the morning shift (shift 1) and used different machines. Patients CT11 received dialysis on afternoon shifts but he had shared in several occasions the same machine with patient CT2.

The healthcare workers employed in the unit denied any violation of the standard infection control procedures. After the implementation of the infection control procedures and the use of dedicated machines for anti-HCV positive patients (but not dedicated rooms or personnel), no additional cases of new HCV infection were observed in the unit.

DISCUSSION

In this study, molecular analysis together with epidemiological investigation provided strong evidence for nosocomial patient-to-patient HCV transmission during an outbreak in a hemodialysis unit. Two potential routes of transmission were identified: horizontal transmission via healthcare workers and/or environmental contamination allowed by breaks in infection control procedures; vertical transmission via the dialysis apparatus. The occurrence of all but one cases of infection with closely related subtype 2c strains in patients who had received dialysis on the same days and the same shift (CT2, CT3, CT4, and CT6) suggests horizontal transmission during care due to breaks in infection control procedures. Several types of breaks in infection control procedures able to facilitate HCV transmission in hemodialysis setting have been suggested, such as absence of hand washing or inconstant glove use [Niu et al., 1992], failure to change gloves between patients [Fabrizi et al., 2002], sharing of multidose vials between patients [Kokubo et al., 2002], and lack of environmental disinfection (surfaces and instruments) [Delarocque-Astagneau et al., 2002; Savey et al., 2005]. Although the healthcare workers employed in the unit denied any violation of the standard infection control procedures, occasional or inadvertent mistakes might have occurred particularly during busy periods or an emergency with a patient. In an environment in which frequently performed percutaneous procedures may contribute through blood spillage to HCV contamination of surfaces and instruments [Caramelo et al., 1999], occasional mistakes could be enough to transmit the virus between patients, particularly if they are immunocompromised. Indeed, patients CT2, CT3, CT4, and CT6 had not always used the same machine, but in several occasions, two of them had shared a given machine with 36 hr of difference. However, vertical transmission via dialysis machines in this group of patients has to be considered very unlikely for two principal reasons: first, after these patients had received dialysis, each machine regularly underwent two complete cycles of disinfection during the following 36 hr (after the fist and second shift, respectively); second, none of patients receiving dialysis in the first shift on the following day had resulted to have acquired a new infection with genotype 2c or other genotypes. On the contrary, vertical transmission via dialysis apparatus between patient CT2 and CT11 could not be quite excluded. These two patients had never received dialysis on the same shift, but in several occasions have shared the same machine in the same day. Apart from patient CT2, none of the other patients infected with closely related 2c strains had had any kind of contact with patient CT11 (dialysis on the same shift or use of the same machines). Thus, even if patient CT11 had received dialysis in another unit on vacation, he had undoubtedly acquired HCV infection in the unit where the outbreak occurred, and with high probability patient CT2 had been the source of his infection. HCV transmission via the dialysis apparatus has been suggested to occur in case of dialysers reuse [dos Santos et al., 1996; Fabrizi et al., 2002] or by dialysate [Sampietro et al., 1994], when the dialysis fluid circuit was not disinfected after each session [Le Pogam et al., 1998]. Transmission has also been suggested to occur in case of potential contamination of internal components of the dialysis machine not accessible for routine disinfection. Some authors [Niu et al., 1992; Delarocque-Astagneau et al., 2002; Savey et al., 2005] have reported wetting of arterial and venous filters due to accidental blood backflow creating a potential contamination of the internal pressure sensing port, which is not accessible for routine disinfection. Thus, two successive episodes of blood backflow in the filters can contribute to the transmission of HCV to another patient. However there has been controversy in literature about the potential role of hemodialysis machines in HCV transmission: several other studies concluded that this route probably is a rare occurrence, playing a minor role or no role at all in the transmission of HCV in hemodialysis settings [Jadoul et al., 1998; Fabrizi et al., 2002; Barril and Traver, 2003]. In the unit where the outbreak occurred, dialysers and tubing sets were disposable and were never reused and disinfection of dialysis machines were performed after each shift. Furthermore episodes of accidental blood backflow into external filter were not reported on patient CT2 medical charts in the days in which he had shared the machine with patient CT11. However, it cannot be excluded that some incidents could have not been registered in the case that the nurses had considered the backflow not sufficient for concern. Alternatively, a break in infection control procedures can be supposed, such as an unsatisfactory environmental cleaning and disinfection between the first and second shift that had resulted in HCV transmission from CT2 to CT11 via contaminated environmental surfaces. A recent study suggests that HCV in dried plasma can cause infection in experimental animals when left at room temperature for $\geq$16 hr but not longer than 4 days [Krawczynski et al., 2003].

Since this investigation was retrospective it had some limitations. It was not possible to directly observe the

health care personnel employed in the unit during their day-to-day working in the period the outbreak occurred. Consequently, it was not allowed to verify if in that period there were evident breaks in universal standard precautions during tasks and procedures performed by personnel during the care of the patients and/or in environmental control procedures; thus it was necessary to rely on healthcare workers interview and medical chart review. Both two latter sources of information could have minimized the recognition and the impact of some events able to enhance the probability of HCV transmission. On the basis of the seroconversion data and epidemiological findings, assuming there were no substantial delays in the seroconversion times, the possible chain of transmission of the infection among the hemodialysis patient harboring closely related HCV 2c should have been depicted as that reported in Figure 3. Indeed, it is well known that uremic patients receiving hemodialysis may suffer a degree of immunosuppression and may have a delayed or disturbed HCV antibody response, which results in a prolonged seronegative window phase after infection [Le Pogam et al., 1998; Savey et al., 2005]. Since serum samples obtained from the patients attending the unit at various time points were not stored and thus were no longer available for HCV RNA detection, it was impossible to determine the time in which each patient involved in the outbreak could be considered actually infected and able to transmit the infection. In other words, it was impossible to establish with certainty which patient among CT2, CT4, and CT6 was infected first and consequently when patient CT2 transmitted the infection to patient CT11. Nevertheless, routine monitoring of HCV infection as was performed in the unit, that is screening ALT monthly plus anti-HCV testing upon the admission, then every 6 months and in case of ALT increase, had permitted the detection of the outbreak. However, because of the high risk of HCV transmission in hemodialysis units even through unrecognized cases, particularly in those units where the

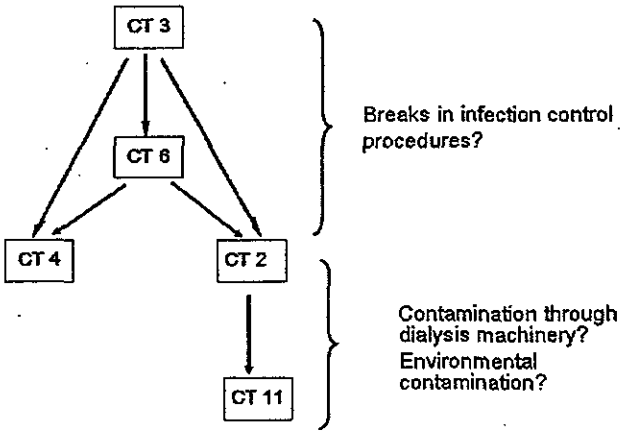

Fig. 3. Scheme of the possible chain and mechanisms of HCV transmission among genotype 2c infected patients involved in the outbreak, assuming there were no substantial delays in seroconversion times.

prevalence of HCV infected patients is high, and considering the high efficacy of an early anti-HCV therapy for newly infected patients [Gursoy et al., 2001], even this strategy may be not optimal. As suggested by other authors [Savey et al., 2005; Hmaied et al., 2006], it seems appropriate to test for HCV RNA any patient showing, at the monthly screening for ALT, a significant enzyme level increase (at least twice the baseline level of the patient) but a negative anti-HCV test and each new patient who enter the unit. To that end, it is necessary to archive monthly at least at −20°C, just for a brief time period, a serum sample for determination of HCV RNA in case of ALT elevation. However, for two of the newly infected patients (CT6 and CT11), monthly ALT screening did not help the detection of the infections, since their ALT levels had been normal until after seroconversion. Indeed, patient CT11 had dialysed outside the unit for 2 months on summer holidays (July and August 2003) and no data about his ALT levels were available for that period. On the contrary, for the two other newly infected patients (CT2 and CT4) the detection of increased ALT on monthly screening helped the diagnosis by inducing to perform and then to repeat at monthly interval (for patient CT4) the anti-HCV test. If on the occasion of the detection of an ALT levels increase in patient CT4 (May 2003) the detection of HCV RNA had been performed, the diagnosis of HCV infection probably could have been made 3–4 months early. While these facts underline uncertainty in depicting the chain of transmission in this outbreak, they also stress the importance of testing for HCV RNA whenever a significant ALT increase occurs.

After the implementation of the infection control procedures and the use of dedicated machines for anti-HCV positive patients (since January 2004), no additional cases of new HCV infection were observed in the unit. The decision of using dedicate machines for anti-HCV positive patients, that became operative from January 2004, was taken by the hospital managers according to published guidelines [Barril et al., 2004]. Isolation policy of HCV infected patients on maintenance hemodialysis by rooms, machines, and personnel, is controversial. At present, The Centers for Disease Control and Prevention does not recommend the use of dedicated machines or patient isolation [Anonymous, 2001], however in some European countries, including Italy, a good proportion of the hemodialysis units, particularly those with high prevalence of infection, currently adopt isolation strategy for HCV infected patients [EBPGEGH and ERA, 2002; Fabrizi et al., 2002; Barril and Traver, 2003; Barril et al., 2004; Di Napoli et al., 2006]. Notwithstanding the high prevalence of infection, before the outbreak occurred in the unit, no isolation measures were adopted for HCV positive patients. This was due to the lack of room for patients and to the unavailability of further hospital personnel. There are convincing arguments supporting a policy of isolation of HCV infected patients. Some prospective studies have clearly showed an important

decrease in the incidence of HCV infections by a complete isolation of the infected patients [Saxena et al., 2003]. Other studies reported a reduced number of seroconversions in unit in which all patients had a dedicated machine or some machine were dedicated for HCV infected patients [Shamshirsaz et al., 2004]. The use of dedicated machine for HCV infected patients, could be useful in units with high prevalence of HCV infection and with a low patient-personnel ratio [Barril and Traver, 2003]. On the other hand, several considerations oppose to the need to isolate HCV infected patients. HCV infectivity is lower than that of HBV. An effective isolation policy would include reliable diagnostic methods to detect HCV infected patients; this means that it would be necessary to routinely test all patients for HCV RNA. Furthermore, several investigators have been able to significantly reduce the number of seroconversion by only reinforcing infection control measures [Jadoul et al., 1998]. Finally, others authors showed the HCV transmission can occur despite the use of dedicated machine because of breaks in infection control procedures [Hmaied et al., 2006]. Even if the debate over the need for isolation policy is not resolved, there is a consensus that using dedicated machines for HCV infected patients does not exclude reinforcement of universal precautions [Delarocque-Astagneau et al., 2002; Barril and Traver, 2003].

In conclusion, molecular analysis and epidemiological investigation suggested a patient-to-patient HCV transmission in this outbreak mainly due to breaks in infection control procedures even if a related-machine transmission cannot be quite excluded in one case.

Universal infection control precautions remain the key stone in the prevention of nosocomial HCV transmission in hemodialysis units. They include avoidance of sharing equipment and devices, frequently hand washing and proper gloves use, cleaning and disinfection with virucidal agents of all the unit (instruments, machine, floor, surfaces). All these measures require continuous education, written procedures and adequate patient-personnel ratio.

REFERENCES

Anonymous. 2001. Recommendations for preventing infections among chronic hemodialysis patients. MMWR Recomm Rep 50:1–43.

Barril G, Traver JA. 2003. Decrease in the hepatitis C virus (HCV) prevalence in hemodialysis patients in Spain: Effect of time, initiating HCV prevalence studies and adoption of isolation measures. Antiviral Res 60:129–134.

Barril G, González -Parra E, Alcázar R, Arenas D, Campistol JM, Caramelo C, Carrasco M, Carreño V, Espinosa M, García Valdecasas J, Górriz JL, López MD, Martín L, Ruíz P, Terruel JL. 2004. Guía sobre enfermedades víricas en hemodiálisis. Nefrologia 24: 43–66.

Caramelo C, de Sequera P, Lopez MD, Ortiz A. 1999. Hand-borne mechanisms of dissemination of hepatitis C virus in dialysis units: Basis for new addenda to the present preventive strategies. Clin Nephrol 51:59–60.

Delarocque-Astagneau E, Baffoy N, Thiers V, Simon N, de Valk H, Laperche S, Courouce AM, Astagneau P, Buisson C, Desenclos JC. 2002. Outbreak of hepatitis C virus infection in a hemodialysis unit: potential transmission by the hemodialysis machine? Infect Control Hosp Epidemiol 23:328–334.

Di Napoli A, Pezzotti P, Di Lallo D, Petrosillo N, Trivelloni C, Di Giulio S. 2006. Epidemiology of hepatitis C virus among long-term dialysis patients: A 9-year study in an Italian region. Am J Kidney Dis 48:629–637.

dos Santos JP, Loureiro A, Cendoroglo Neto M, Pereira BJ. 1996. Impact of dialysis room and reuse strategies on the incidence of hepatitis C virus infection in haemodialysis units. Nephrol Dial Transplant 11:2017–2022.

European Best Practice Guidelines Expert Group on Hemodialysis, European Renal Association. 2002. Section VI. Haemodialysis-associated infection. Nephrol Dial Transplant 17:72–87.

Fabrizi F, Poordad FF, Martin P. 2002. Hepatitis C infection and the patient with end-stage renal disease. Hepatology 36:3–10.

Faustini A, Capobianchi MR, Martinelli M, Abbate I, Cappiello G, Perucci CA. 2005. A cluster of hepatitis C virus infections associated with ozone-enriched transfusion of autologous blood in Rome, Italy. Infect Control Hosp Epidemiol 26:762–767.

Gursoy M, Gur G, Arslan H, Ozdemir N, Boyacioglu S. 2001. Interferon therapy in haemodialysis patients with acute hepatitis C virus infection and factors that predict response to treatment. J Viral Hepat 8:70–77.

Hmaied F, Ben Mamou M, Saune-Sandres K, Rostaing L, Slim A, Arrouji Z, Ben Redjeb S, Izopet J. 2006. Hepatitis C virus infection among dialysis patients in Tunisia: Incidence and molecular evidence for nosocomial transmission. J Med Virol 78:185–191.

Jadoul M, Cornu C, van Ypersele de Strihou C. 1998. Universal precautions prevent hepatitis C virus transmission: A 54 month follow-up of the Belgian Multicenter Study. The Universitaires Cliniques St-Luc (UCL) Collaborative Group. Kidney Int 53:1022–1025.

Kokubo S, Horii T, Yonekawa O, Ozawa N, Mukaide M. 2002. A phylogenetic-tree analysis elucidating nosocomial transmission of hepatitis C virus in a haemodialysis unit. J Viral Hepat 9:450–454.

Krawczynski K, Alter MJ, Robertson BH, Lu L, Spelbring JE, McCaustland KA. 2003. Environmental stability of hepatitis C virus (HCV): Viability of dried/stored HCV in chimpanzee infectivity studies. In: Program and abstracts of the 54th annual meeting of the American Society for the study of liver disease. Philadelphia: WB Saunders [abstract 555].

Le Pogam S, Le Chapois D, Christen R, Dubois F, Barin F, Goudeau A. 1998. Hepatitis C in a hemodialysis unit: Molecular evidence for nosocomial transmission. J Clin Microbiol 36:3040–3043.

Niu MT, Alter MJ, Kristensen C, Margolis HS. 1992. Outbreak of hemodialysis-associated non-A, non-B hepatitis and correlation with antibody to hepatitis C virus. Am J Kidney Dis 19:345–352.

Petrosillo N, Gilli P, Serraino D, Dentico P, Mele A, Ragni P, Puro V, Casalino C, Ippolito G. 2001. Prevalence of infected patients and understaffing have a role in hepatitis C virus transmission in dialysis. Am J Kidney Dis 37:1052–1055.

Sampietro M, Graziani G, Badalamenti S, Salvadori S, Caldarelli R, Como G, Fiorelli G. 1994. Detection of hepatitis C virus in dialysate and in blood ultrafiltrate of HCV-positive patients. Nephron 68:140.

Savey A, Simon F, Izopet J, Lepoutre A, Fabry J, Desenclos JC. 2005. A large nosocomial outbreak of hepatitis C virus infections at a hemodialysis center. Infect Control Hosp Epidemiol 26:752–760.

Saxena AK, Panhotra BR, Sundaram DS, Naguib M, Venkateshappa CK, Uzzaman W, Mulhim KA. 2003. Impact of dedicated space, dialysis equipment, and nursing staff on the transmission of hepatitis C virus in a hemodialysis unit of the middle east. Am J Infect Control 31:26–33.

Shamshirsaz AA, Kamgar M, Bekheirnia MR, Ayazi F, Hashemi SR, Bouzari N, Habibzadeh MR, Pourzahedgilani N, Broumand V, Shamshirsaz AH, Moradi M, Borghei M, Haghighi NN, Broumand B. 2004. The role of hemodialysis machines dedication in reducing Hepatitis C transmission in the dialysis setting in Iran: A multicenter prospective interventional study. BMC Nephrol 5:13.

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No. 41

医薬品 研究報告 調査報告書

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販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社) 合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)			

研究報告の概要

○サウスハンプシャーにおける急性E型肝炎国内感染率の予想外の高さ:ルーチン検査を行う時期か？
先進国特有のE型肝炎とは、流行地域への渡航歴のない者に発現したE型肝炎である。近年、英国を含む経済的に発展した多くの国に新興する疾病と認識されている。しかし、E型肝炎は現在も稀な疾患と考えられており、E型肝炎のルーチンな臨床検査は実施されていない。英国サウスハンプシャーの単一施設において2005年6月から13ヶ月の期間に診断されたE型肝炎13例について報告する。これらの患者は、ルーチンのE型肝炎血清検査を導入した新規スクリーニング手順開始後に特定された。患者は中年～高齢者で、男性の方が多かった。4名(31%)は入院を要した。RT-PCR法にて確定された症例はいずれも、英国のブタに蔓延しているHEVと相同性の高いE型肝炎ウイルス(HEV)遺伝子型3を保有した。急性肝炎発症前の2ヶ月間によく加熱しない豚肉製品を食べたり、ブタと密接な接触を持った記憶のある患者はいなかった。これに対して、同一期間中、A型肝炎の診断は2例のみ、B型肝炎の診断は4例であった。これらのデータは、原因不明の急性肝疾患を発症し、関連する渡航歴のない患者全員にルーチンのE型肝炎検査を導入することの重要性を示している。ルーチン検査は、急性肝疾患患者の臨床管理を改善しながら、E型肝炎の疫学を明らかにすることができる。

報告企業の意見	今後の対応
英国サウスハンプシャーの単一施設において2005年6月から13ヶ月の期間にE型肝炎13例が発生し、同一期間中A型肝炎は2例、B型肝炎は4例であったことから、原因不明の急性肝疾患を発症し、関連する渡航歴のない患者全員にルーチンのE型肝炎検査を実施することの重要性が示されるとの報告である。	日本赤十字社では、厚生労働科学研究「E型肝炎の感染経路・宿主域・遺伝的多様性・感染防止・診断・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。北海道における輸血HEV感染報告を受け、試験的に北海道では研究的NATを行うなど安全対策を実施している。また、輸血による肝炎ウイルス感染防止のため、血液中のALT値61IU/L以上の血液を輸血用から排除している。今後もHEV感染の実態に関する情報の収集及び安全対策に努める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

MedDRA/J Ver.11.0J

Journal of Medical Virology 80:283–288 (2008)

Unexpectedly High Incidence of Indigenous Acute Hepatitis E Within South Hampshire: Time for Routine Testing?

Aminda N. De Silva,[1] Ajay K. Muddu,[1] John P. Iredale,[2] Nick Sheron,[1] Salim I. Khakoo,[1] and Emanuela Pelosi[3]*

[1]*Department of Gastroenterology, Southampton University Hospital NHS Trust, Southampton, UK*
[2]*Department of Hepatology, Royal Infirmary of Edinburgh, Edinburgh, UK*
[3]*Department of Microbiology and Virology, Health Protection Agency Southeast Regional Laboratory, Southampton University Hospital NHS Trust, Southampton, UK*

Hepatitis E indigenous to developed countries (hepatitis E^{IDC}) is a form of hepatitis E in persons with no travel history to highly endemic areas. It has been recognized recently as an emerging clinical entity in a significant number of economically developed countries including UK. However, it is still perceived as a rare disease and routine laboratory testing for hepatitis E is not performed. A series of 13 cases of hepatitis E^{IDC}, diagnosed in a 13-month period from June 2005 within a single center in South Hampshire, UK, is presented. These patients were identified after implementing a novel-screening algorithm that introduced routine hepatitis E serological investigations. Patients were middle aged or elderly and males were affected more commonly. Four patients (31%) required hospital admission. All reverse transcriptase-polymerase chain reaction (RT-PCR) confirmed cases carried hepatitis E virus (HEV) genotype-3, which bore close sequence homology to HEV circulating in UK pigs. None of these patients recalled eating undercooked pork products or close contact with pigs during the 2 months preceding the onset of acute hepatitis. In comparison, during the same period, only two cases of hepatitis A and five cases of acute hepatitis B were diagnosed. These data illustrate the importance of introducing routine hepatitis E testing in all patients with unexplained acute liver disease and absence of relevant travel history. Routine testing can clarify hepatitis E epidemiology whilst improving the clinical management of patients with acute liver disease. ***J. Med. Virol. 80:283–288, 2008.*** © 2007 Wiley-Liss, Inc.

KEY WORDS: non-travel associated hepatitis E; serology; RT-PCR

INTRODUCTION

Hepatitis E virus (HEV) is a small non-enveloped virus, with a single-stranded RNA genome of positive polarity. First documented as the cause of non-A, non-B enterically transmitted hepatitis in the eighties [Gandhi et al., 1982; Balayan et al., 1983; Bradley and Maynard, 1986], HEV was cloned and sequenced in the early nineties [Reyes et al., 1990; Tam et al., 1991] and classified as the sole member of the genus *Hepevirus*, family *Hepeviridae*, in 2004 [Emerson et al., 2004]. In developing countries, where sanitation is poor, HEV can cause epidemics of acute hepatitis E when the water supply is fecally contaminated [Tsega et al., 1991; Naik et al., 1992; Rab et al., 1997]. In this setting hepatitis E is generally a mild disease of young adults; however, pregnant women may suffer significant morbidity and mortality [Hussaini et al., 1997; Kumar et al., 2004; Boccia et al., 2006]. In developed countries, by contrast, hepatitis E is a sporadic disease identified predominantly in travelers returning from developing countries. More recently, a form of hepatitis E with no travel history to highly endemic areas has been identified and referred to as "hepatitis E indigenous to developed countries" or "hepatitis E^{IDC}" [Teo, 2006]. This form appears to affect predominantly elderly males [Sainokami et al., 2004; Ijaz et al., 2005].

Genotyping of HEV has given insights into the epidemiology of this infection. There are four main genotypes of HEV. Hepatitis E in developing countries is

*Correspondence to: Dr. Emanuela Pelosi, Department of Microbiology and Virology, South Laboratory Block, Southampton University Hospital NHS Trust, Tremona Rd, Southampton SO16 6YD UK. E-mail: emanuela.pelosi@suht.swest.nhs.uk

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caused by genotypes 1 and 2. However, hepatitis E^{IDC} is caused by genotype 3 in most countries and by genotypes 3 and 4 in Japan [Lu et al., 2006; Okamoto, 2007]. Genotype 4 causes hepatitis E in China where it appears to be increasingly common compared to genotype 1 [Li et al., 2006]. Critically, HEV genotypes 3 and 4 are known to infect a range of animals [Wang et al., 2002; Michitaka et al., 2007], particularly pigs [Banks et al., 2004; Zheng et al., 2006; Herremans et al., 2007], suggesting that exposure to animals or animal products may be the source of infection in humans. Indeed acquisition of hepatitis E^{IDC} by dietary consumption of wild boar, deer, and pig meat or viscera, contaminated with HEV, has been documented in Japan [Tei et al., 2003; Yazaki et al., 2003; Takahashi et al., 2004; Masuda et al., 2005].

Hepatitis E^{IDC} was first reported as a clinical entity in the United Kingdom (UK) 7 years ago, following a report of four cases [McCrudden et al., 2000]. Subsequent investigations of stored serum samples, together with enhanced prospective surveillance in several UK centers, have shown that hepatitis E^{IDC} is indeed an under-diagnosed disease in UK [Ijaz et al., 2005; Lewis et al., 2006; Dalton et al., 2007]. The relatively low sensitivity [Zhang et al., 2002; Mansuy et al., 2004; Myint et al., 2006] and high costs of currently available diagnostic tests have meant that they are not used routinely in the diagnosis of unexplained abnormal liver function tests. Therefore, to date, systematic testing for hepatitis E is not routinely performed in UK diagnostic laboratories, and the true incidence and the clinical impact of this disease remain to be fully clarified.

In order to address this, a novel diagnostic algorithm, introducing routine testing for antibodies to HEV, was defined and implemented. The experience of a single diagnostic center in Hampshire, UK, is presented.

METHODS

Patients and Samples

This work was performed at Southampton University Hospital NHS Trust. Between May 2005 and June 2006, 139 (70 females, 69 males) serum samples received at the Health Protection Agency (HPA) South East Regional laboratory of Southampton, which were negative for markers of acute infection by hepatitis viruses A, B, C, Epstein–Barr virus (EBV), and cytomegalovirus (CMV), and with an ALT level greater than 300 IU/L (normal range of 10–40 IU/L), were tested for HEV IgM and IgG.

All patients with laboratory data consistent with acute hepatitis E were investigated for travel history to highly endemic areas in the 2 months preceding symptoms onset. Whenever travel history was negative, patients were asked to complete a questionnaire, which assessed contacts with animals, including pigs, dietary habits, and exposure to other jaundiced individuals. The questionnaire, developed by the Center for Infections, HPA, London (www.hpa.org.uk), after the initial cases of hepatitis E^{IDC} had been detected in UK in 1999 [Mc Crudden et al., 2000], is part of an enhanced surveillance program for this infection, in England and Wales.

HEV Serology

HEV IgM and IgG serology was performed using the Gene Lab ELISA assays, two immune enzymatic commercial tests based on recombinant antigens from HEV genotypes 1 and 2 (Genelabs Diagnostics, Singapore). Both laboratory test results were interpreted according to the directions given by the manufacturer: all samples with an optical density greater than the cut-off was considered positive. A positive HEV serology was confirmed by additionally testing a follow-up sample.

HEV Reverse Transcriptase-Polymerase Chain Reaction Assay (RT-PCR) and Genotyping

Samples reactive by the HEV IgM and/or IgG assays were additionally tested for HEV RT-PCR and genotyped, if HEV-RNA was detected, at the Center for Infections, HPA, London [Ijaz et al., 2005].

Clinical Features and Laboratory Results

Fifteen cases of acute hepatitis E were identified between May 2005 and June 2006. Two cases were diagnosed in patients of Indo-Pakistani origin who had traveled to the Indian subcontinent in the recent past. Thirteen patients were British of white European ethnicity, resident in three urban areas within a 10-mile radius of Southampton, Hampshire, UK (Fig. 1) and had not traveled to highly endemic areas in the 2 months prior to identification of raised serum ALT. Eight of the 13 patients (62%) returned the contact-tracing questionnaire. Two patients ate shellfish and three ate liver pate of unspecified animal origin in the 2 months prior to the detection of acute hepatitis. It is not known whether this consumption was occasional or habitual. During the same period no patient had consumed undercooked pork meat or had been in contact with jaundiced individuals or farm animals, including pigs. Five patients (38%) were dog owners, but no disease was reported in their pets.

Table I summarizes the patients' clinical details and laboratory results of hepatitis E^{IDC} cases. The median age was 71 years (range of 46–85 years) with 6 (46%) being 75 years of age or older; 11 (85%) were male. Twelve of the 13 initial samples were collected at the peak of the ALT value and the 13th was collected 2 weeks after the onset of jaundice, when the ALT value had normalized. HEV RNA genotype 3 was detected in 8/12 (67%) patients in the acute phase of the disease. Two HEV RNA positive patients had atypical serological profiles: one had only a detectable IgM response, without a measurable anti-HEV IgG response in spite of repeat analyses several weeks later, while the other patient had only detectable IgG.

The clinical presentations were similar in most cases. Typical features were a 2–3 weeks prodrome of malaise,

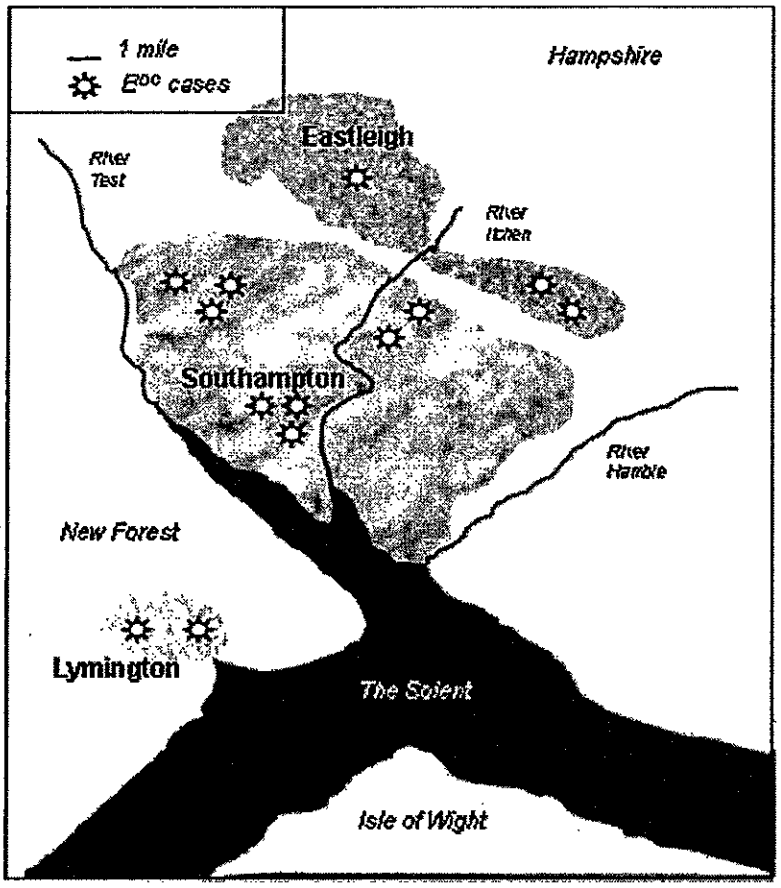

Fig. 1. South Hampshire. Proportion of people above state pension age (65 years for men and 60 years for women) according to 2001 population census data: Southampton 16.5%, Bastleigh 17.1%, Lymington New Forest 25.6%, England and Wales 18.4%).

fever, and anorexia before presenting to their general practitioner. Ten of 13 patients (77%) complained of jaundice and dark urine, suggestive of liver disease, whilst 3 (23%) had abdominal pain. Seven patients were referred to hospital and four (31%) were admitted. ALT levels varied between 300 and 6,777 IU/L (normal range of 10–40 IU/L). Liver synthetic function, as determined by international normalized ratio (INR) estimation, was impaired in two individuals (a third patient with raised INR was on concomitant warfarin therapy since the implantation of a prosthetic heart valve). The severity of the illnesses may have been contributed to by the co-morbidities that are prevalent in the elderly population. Four individuals had type II diabetes mellitus, three had hypertension, and one drank alcohol to levels above the UK Department of Health recommendations (www.dh.gov.uk/en/policyandguidance/healthandsocialcaretopics/alcoholmisus; version of 7.06.2007). Twelve out of 13 patients made a complete recovery after about 2 weeks, but one patient died 2 months after the acute illness from right lower lobe pneumonia. This death was most likely unrelated to his HEV infection.

In summary, by the use of a novel-testing algorithm, 15 cases of acute hepatitis E, of which 13 were not travel associated, have been identified in a 13-month period. By comparison, during the same period only two cases of acute hepatitis A and five cases of acute hepatitis B were identified, leading to the inference that hepatitis E^{IDC} is significantly under diagnosed.

DISCUSSION

In a 13-month period acute hepatitis E^{IDC} has been identified in 13 individuals resident in three towns of coastal Hampshire, UK, with a total population of about 340,000 inhabitants (Fig. 1). In the eight cases diagnosed during the viraemic phase of the disease, HEV genotype 3 was detected (Table I). This genotype, commonly circulating in pigs [Banks et al., 2004; Teo, 2006], has also been recognized in the other cases of hepatitis E^{IDC} reported in UK, summarized in Table II. A possible risk factor for acquiring hepatitis E^{IDC} was identified in two patients who ate shellfish [Mechnik et al., 2001; Koizumi et al., 2004; Ijaz et al., 2005] during the 2 months preceding the illness.

The majority of these patients were elderly males (85%, Table I). It is not clear if genotype 3 is attenuated in pathogenicity, thus causing preferentially overt disease in more susceptible hosts like elderly individuals, or if older people, particularly males, have a greater risk of exposure to HEV due to behavioral or environmental risk factors. This peculiar and puzzling

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TABLE I. Patients' Clinical Details and Laboratory Results of Hepatitis E^{IDC} Cases as Obtained From the First Serum Sample Tested

	Age (years)	Sex	Peak ALT[a] (IU/L)	INR	Bilirubin[b] (μmol/L)	Jaundice	Comorbid condition(s)	Hospital admission	HEV IgG	HEV IgM	HEV genotype
1	68	F	6,210	1.6	75	Yes	NIDDM and hypertension	Yes	+	+	3
2	61	M	951	1.1	28	No	None	No	+	+	3
3	71	M	1,037	1	10	No	NIDDM	No	+	+	ND
4	75	M	2,733	1	123	Yes	Bladder carcinoma, pharyngeal pouch	No	+	+	3
5	82	M	6,777	2.6	154	Yes	AF, IBS, THR	Yes	−	+	3
6	85	M	656	1	172	Yes	Aortic stenosis, hypertension	Yes	+	+	ND
7	76	M	1,705	>8[c]	320	Yes	AVR, CABG	Yes	+	+	3
8	80	F	945	1	94	Yes	Hypertension	No	+	+	3
9	47	M	630	1	28	No	None	No	+	+	ND
10	69	M	959	1.1	115	Yes	NIDDM, CABG, hypertension, hypercholesterolemia	No	+	−	3
11	83	M	3,554	1.2	115	Yes	NIDDM	No	+	+	3
12	56	M	300	NP[b]	68	Yes	Non-alcoholic fatty liver	No	+	+	NP
13	56	M	551	NP[b]	228	Yes	IHD	No	+	+	NP

NIDDM, non-insulin dependent diabetes mellitus; AF, atrial fibrillation; IBS, irritable bowel syndrome; THR, total hip replacement; AVR, aortic valve replacement; CABG, coronary artery by-pass graft; IHD, ischemic heart disease; INR, international normalized ratio; ND, not detected; NP, not performed.
[a]ALT normal value 10–40 IU/L.
[b]Bilirubin normal value 0–20 μmol/L.
[c]On warfarin since 1998.

TABLE II. Published Cases of Acute Hepatitis E^{IDC} in England and Wales

	Location	Period	Number of cases	Age (years)	Sex
Cases detected in single Centers	Southampton, Hampshire [McCrudden et al., 2000]	1999	4	41, 44, 70, 71	1 Male, 3 females
	Truro, Cornwall [Levine et al., 2000]	1999	1	61	Male
	Hull, East Yorkshire [Jary, 2005]	2005	1	54	Male
	Birmingham, Midlands [Sadler et al., 2006]	2005 (5-month period)	8[a]	Median age 60	4 Males, 3 females
	Cornwall and South-West Devon [Dalton et al., 2007]	March 1999–September 2005	21[b]	Median age 67	15 Males, 6 females
Cumulative data of England and Wales	National survey [Ijaz et al., 2005]	1997–2003	17[c]	Median age 70	13 Males, 4 females
	National survey [Lewis et al., 2006]	January–June 2005	24[d]	Median age 59	20 Males, 4 females

[a]2/8 Patients were RT-PCR positive, one patient with genotype 3 while the other with genotype 1 (the latter had been in recent contact with a jaundiced individual returning from Pakistan).
[b]HEV genotype 3 detected in 16/21 (76%) cases.
[c]HEV genotype 3 detected in 11/17 (65%) patients.
[d]10/25 (40%) cases were HEV RT-PCR positive, of which 9 were genotype 3.

demographic feature was previously documented in a nation-wide UK study [Ijaz et al., 2005] of 17 hepatitis E^{IDC} cases diagnosed between 1997 and 2003 in individuals, 14 of whom (82%) lived in coastal and estuarine areas, as are the ones found in our study in the South Hampshire region. Ijaz et al. [2005] pointed out the confounding effect of older age on the place of residence. This bias might not be relevant to this study where the elderly patients affected by acute hepatitis E belonged to a population which, on average, appears younger compared to that in the rest of England and Wales (www.statistics.gov.uk/census2001, Fig. 1).

Although documented in other European countries, Asia, and USA [van der Poel et al., 2001; Clemente-Casares et al., 2003; Widdowson et al., 2003; Buti et al., 2004; Amon et al., 2006; Peron et al., 2006], hepatitis E^{IDC} is still considered an uncommon disease. A recent report by Lewis et al. [2006] suggests that hepatitis E^{IDC} in UK is under diagnosed. However, implementation of routine serology for hepatitis E is hampered by the fact that currently available antibody assays, based on HEV genotypes 1 and 2, lack sensitivity [Lin et al., 2000; Myint et al., 2006]. This has been attributed to several factors of which the main one is likely to be that the currently available recombinant HEV proteins used in the assay systems may not include all relevant immunogenic B cell epitopes encoded within the HEV genome [Wang et al., 2001; Zhang et al., 2003; Zhou et al., 2004]. Additionally, the genetic diversity between HEV genotypes [Lu et al., 2006] warrants the inclusion of each HEV genotype in future diagnostic kits.

In spite of their limitations, currently available antibody assays have been capable of detecting a significant number of hepatitis E^{IDC} cases, leading to the recognition of this emerging disease. This consideration guided the decision to routinely include hepatitis E testing in our laboratory. Cases with a significantly deranged ALT value were tested, in order to target acute hepatitis of clinical importance. By adherence to this algorithm, a pick up rate of 9.3% was obtained.

In a situation of suboptimal performance of currently available antibody assays, RT-PCR represents a useful complementary diagnostic tool [Jothikumar et al., 2006]. Although the duration of viraemia is variable (from few days to few weeks) (1, 10) a serum sample collected at the peak of ALT values has a high chance to be RT-PCR positive thus clarifying cases of acute hepatitis E with atypical serological profiles, as found in two of our patients (Table II), including HEV seronegative cases [Lin et al., 2000; Mansuy et al., 2004].

The incidence of hepatitis E^{IDC} in our center exceeded the frequency of acute hepatitis A (two cases) and hepatitis B (five cases). In UK, where high standards of sanitation and vaccination programs have significantly reduced exposure to hepatitis A and B viruses, hepatitis E^{IDC} may emerge as a major cause of acute viral hepatitis [Lewis et al., 2006]. The high frequency observed in our uncontrolled series may in part be a reflection of a better ascertainment of hepatitis E^{IDC}, which had previously remained undiagnosed, as well as a true increase in incidence in recent time.

In conclusion, it is considered that these findings support the case for more widespread HEV testing according to clearly defined criteria and we propose an effective algorithm for this purpose. This is crucial not only for surveillance purposes and to clarify the epidemiology of HEV in UK, but also for the appropriate management of affected patients. In cases of acute hepatitis, where initial history and viral marker results are negative, autoimmune hepatitis, and idiosyncratic drug reactions are important to consider in the differential diagnosis, with implications for management and prognosis. Thus, in the absence of HEV testing, patients with unexplained raised transaminases may unnecessarily progress to liver biopsy, empirical trial of steroids, or withdrawal of presumed offending drugs. Consideration of HEV infection in individuals without travel-associated risk factors for acute hepatitis may have a major impact on clinical management.

REFERENCES

Amon JJ, Drobeniuc J, Bower WA, Magana JC, Escobedo MA, Williams IT, Bell BP, Armstrong GL. 2006. Locally acquired hepatitis E virus infection, El Paso, Texas. J Med Virol 78:741–746.

Balayan MS, Andjaparidze AG, Savinskaya SS, Ketiladze ES, Braginsky DM, Savinov AP, Poleschuk VF. 1983. Evidence for a virus in non-A, non-B hepatitis transmitted via the fecal-oral route. Intervirology 20:23–31.

Banks M, Heath GS, Grierson SS, King DP, Gresham A, Girones R, Widen F, Harrison TJ. 2004. Evidence for the presence of hepatitis E virus in pigs in the United Kingdom. Vet Rec 154:223–227.

Boccia D, Guthmann JP, Klovstad H, Hamid N, Tatay M, Ciglenecki I, Nizou JY, Nicand E, Guerin PJ. 2006. High mortality associated with an outbreak of hepatitis E among displaced persons in Darfur, Sudan. Clin Infect Dis 42:1679–1684.

Bradley DW, Maynard JE. 1986. Etiology and natural history of post-transfusion and enterically-transmitted non-A, non-B hepatitis. Semin Liver Dis 6:56–66.

Buti M, Clemente-Casares P, Jardi R, Formiga-Cruz M, Schaper M, Valdes A, Rodriguez-Frias F, Esteban R, Girones R. 2004. Sporadic cases of acute autochthonous hepatitis E in Spain. J Hepatol 41: 126–131.

Clemente-Casares P, Pina S, Buti M, Jardi R, MartIn M, Bofill-Mas S, Girones R. 2003. Hepatitis E virus epidemiology in industrialized countries. Emerg Infect Dis 9:448–454.

Dalton HR, Thurairajah PH, Fellows HJ, Hussaini HS, Mitchell J, Bendall R, Banks M, Ijaz S, Teo CG, Levine DF. 2007. Autochthonous hepatitis E in southwest England. J Viral Hepat 14:304–309.

Emerson SU, Anderson D, Arankalle A, Meng XJ, Purdy M, Schlauder GG, Tsarev SA. 2004. Hepevirus. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA, editors. Virus Taxonomy: viiith report of the ICTV. London: Elsevier/Academic Press. pp 851-855.

Gandhi BM, Joshi YK, Bijlani L, Tandon BN. 1982. Non-A non-B antigen and antibody detection by agar gel diffusion. Indian J Med Res 76:591–593.

Herremans M, Vennema H, Bakker J, van der Veer B, Duizer E, Benne CA, Waar K, Hendrixks B, Schneeberger P, Blaauw G, Kooiman M, Koopmans MP. 2007. Swine-like hepatitis E viruses are a cause of unexplained hepatitis in the Netherlands. J Viral Hepat 14:140–146.

Hussaini SH, Skidmore SJ, Richardson P, Sherratt LM, Cooper BT, O'Grady JG. 1997. Severe hepatitis E infection during pregnancy. J Viral Hepat 4:51–54.

Ijaz S, Arnold E, Banks M, Bendall RP, Cramp ME, Cunningham R, Dalton HR, Harrison TJ, Hill SF, Macfarlane L, Meigh RE, Shafi S, Sheppard MJ, Smithson J, Wilson MP, Teo CG. 2005. Non-travel-associated hepatitis E in England and Wales: demographic, clinical,

and molecular epidemiological characteristics. J Infect Dis 192: 1166–1172.

Jary C. 2005. Hepatitis E and meat carcasses. Br J Gen Pract 55:557–558.

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

Koizumi Y, Isoda N, Sato Y, Iwaki T, Ono K, Ido K, Sugano K, Takahashi M, Nishizawa T, Okamoto H. 2004. Infection of a Japanese patient by genotype 4 hepatitis E virus while traveling in Vietnam. J Clin Microbiol 42:3883–3885.

Kumar A, Beniwal M, Kar P, Sharma JB, Murthy NS. 2004. Hepatitis E in pregnancy. Int J Gynaecol Obstet 85:240–244.

Levine DF, Bendall RP, Teo CG. 2000. Hepatitis E acquired in the UK. Gut 47:740.

Lewis H, Morgan D, Ijaz S, Boxall E. 2006. Indigenous hepatitis E virus infection in England and Wales. BMJ 332:1509–1510.

Li RC, Ge SX, Li YP, Zheng YJ, Nong Y, Guo QS, Zhang J, Ng MH, Xia NS. 2006. Seroprevalence of hepatitis E virus infection, rural southern People's Republic of China. Emerg Infect Dis 12:1682–1688.

Lin CC, Wu JC, Chang TT, Chang WY, Yu ML, Tam AW, Wang SC, Huang YH, Chang FY, Lee SD. 2000. Diagnostic value of immunoglobulin G (IgG) and IgM anti-hepatitis E virus (HEV) tests based on HEV RNA in an area where hepatitis E is not endemic. J Clin Microbiol 38:3915–3918.

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

Mansuy JM, Peron JM, Bureau C, Alric L, Vinel JP, Izopet J. 2004. Immunologically silent autochthonous acute hepatitis E virus infection in France. J Clin Microbiol 42:912–913.

Masuda J, Yano K, Tamada Y, Takii Y, Ito M, Omagari K, Kohno S. 2005. Acute hepatitis E of a man who consumed wild boar meat prior to the onset of illness in Nagasaki, Japan. Hepatol Res 31:178–183.

McCrudden R, O'Connell S, Farrant T, Beaton S, Iredale JP, Fine D. 2000. Sporadic acute hepatitis E in the United Kingdom: An underdiagnosed phenomenon? Gut 46:732–733.

Mechnik L, Bergman N, Attali M, Beergabel M, Mosenkis B, Sokolowski N, Malnick S. 2001. Acute hepatitis E virus infection presenting as a prolonged cholestatic jaundice. J Clin Gastroenterol 33:421–422.

Michitaka K, Takahashi K, Furukawa S, Inoue G, Hiasa Y, Horiike N, Onji M, Abe N, Mishiro S. 2007. Prevalence of hepatitis E virus among wild boar in the Ehime area of western Japan. Hepatol Res 37:214–220.

Myint KS, Endy TP, Gibbons RV, Laras K, Mammen MP, Jr, Sedyaningsih ER, Seriwatana J, Glass JS, Narupiti S, Corwin AL. 2006. Evaluation of diagnostic assays for hepatitis E virus in outbreak settings. J Clin Microbiol 44:1581–1583.

Naik SR, Aggarwal R, Salunke PN, Mehrotra NN. 1992. A large waterborne viral hepatitis E epidemic in Kanpur, India. Bull World Health Organ 70:597–604.

Okamoto H. 2007. Genetic variability and evolution of hepatitis E virus. Virus Res.

Peron JM, Mansuy JM, Poirson H, Bureau C, Dupuis E, Alric L, Izopet J, Vinel JP. 2006. Hepatitis E is an autochthonous disease in industrialized countries. Analysis of 23 patients in South-West France over a 13-month period and comparison with hepatitis A. Gastroenterol Clin Biol 30:757–762.

Rab MA, Bile MK, Mubarik MM, Asghar H, Sami Z, Siddiqi S, Dil AS, Barzgar MA, Chaudhry MA, Burney MI. 1997. Water-borne hepatitis E virus epidemic in Islamabad, Pakistan: A common source outbreak traced to the malfunction of a modern water treatment plant. Am J Trop Med Hyg 57:151–157.

Reyes GR, Purdy MA, Kim JP, Luk KC, Young LM, Fry KE, Bradley DW. 1990. Isolation of a cDNA from the virus responsible for enterically transmitted non-A, non-B hepatitis. Science 247:1335–1339.

Sadler GJ, Mells GF, Shah NH, Chesner IM, Walt RP. 2006. UK acquired hepatitis E–An emerging problem? J Med Virol 78:473–475.

Sainokami S, Abe K, Kumagai I, Miyasaka A, Endo R, Takikawa Y, Suzuki K, Mizuo H, Sugai Y, Akahane Y, Koizumi Y, Yajima Y, Okamoto H. 2004. Epidemiological and clinical study of sporadic acute hepatitis E caused by indigenous strains of hepatitis E virus in Japan compared with acute hepatitis A. J Gastroenterol 39:640–648.

Takahashi K, Kitajima N, Abe N, Mishiro S. 2004. Complete or near-complete nucleotide sequences of hepatitis E virus genome recovered from a wild boar, a deer, and four patients who ate the deer. Virology 330:501–505.

Tam AW, Smith MM, Guerra ME, Huang CC, Bradley DW, Fry KE, Reyes GR. 1991. Hepatitis E virus (HEV): Molecular cloning and sequencing of the full-length viral genome. Virology 185:120–131.

Tei S, Kitajima N, Takahashi K, Mishiro S. 2003. Zoonotic transmission of hepatitis E virus from deer to human beings. Lancet 362:371–373.

Teo CG. 2006. Hepatitis E indigenous to economically developed countries: To what extent a zoonosis? Curr Opin Infect Dis 19:460–466.

Tsega E, Krawczynski K, Hansson BG, Nordenfelt E, Negusse Y, Alemu W, Bahru Y. 1991. Outbreak of acute hepatitis E virus infection among military personnel in northern Ethiopia. J Med Virol 34:232–236.

van der Poel WH, Verschoor F, van der Heide R, Herrera MI, Vivo A, Kooreman M, de Roda Husman AM. 2001. Hepatitis E virus sequences in swine related to sequences in humans, The Netherlands. Emerg Infect Dis 7:970–976.

Wang Y, Zhang H, Li Z, Gu W, Lan H, Hao W, Ling R, Li H, Harrison TJ. 2001. Detection of sporadic cases of hepatitis E virus (HEV) infection in China using immunoassays based on recombinant open reading frame 2 and 3 polypeptides from HEV genotype 4. J Clin Microbiol 39:4370–4379.

Wang YC, Zhang HY, Xia NS, Peng G, Lan HY, Zhuang H, Zhu YH, Li SW, Tian KG, Gu WJ, Lin JX, Wu X, Li HM, Harrison TJ. 2002. Prevalence, isolation, and partial sequence analysis of hepatitis E virus from domestic animals in China. J Med Virol 67:516–521.

Widdowson MA, Jaspers WJ, van der Poel WH, Verschoor F, de Roda Husman AM, Winter HL, Zaaijer HL, Koopmans M. 2003. Cluster of cases of acute hepatitis associated with hepatitis E virus infection acquired in the Netherlands. Clin Infect Dis 36:29–33.

Yazaki Y, Mizuo H, Takahashi M, Nishizawa T, Sasaki N, Gotanda Y, Okamoto H. 2003. Sporadic acute or fulminant hepatitis E in Hokkaido, Japan, may be food-borne, as suggested by the presence of hepatitis E virus in pig liver as food. J Gen Virol 84:2351–2357.

Zhang JZ, Im SW, Lau SH, Chau TN, Lai ST, Ng SP, Peiris M, Tse C, Ng TK, Ng MH. 2002. Occurrence of hepatitis E virus IgM, low avidity IgG serum antibodies, and viremia in sporadic cases of non-A, -B, and -C acute hepatitis. J Med Virol 66:40–48.

Zhang J, Ge SX, Huang GY, Li SW, He ZQ, Wang YB, Zheng YJ, Gu Y, Ng MH, Xia NS. 2003. Evaluation of antibody-based and nucleic acid-based assays for diagnosis of hepatitis E virus infection in a rhesus monkey model. J Med Virol 71:518–526.

Zheng Y, Ge S, Zhang J, Guo Q, Ng MH, Wang F, Xia N, Jiang Q. 2006. Swine as a principal reservoir of hepatitis E virus that infects humans in eastern China. J Infect Dis 193:1643–1649.

Zhou YH, Purcell RH, Emerson SU. 2004. An ELISA for putative neutralizing antibodies to hepatitis E virus detects antibodies to genotypes 1, 2, 3, and 4. Vaccine 22:2578–2585.

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一般的名称	①乾燥抗HBs人免疫グロブリン ②ポリエチレングリコール処理抗HBs人免疫グロブリン	研究報告の公表状況	The New England Journal of Medicine 2008; 358: 811-817	公表国 フランス
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研究報告の概要

HEVは急性肝炎の原因となる病原体であって、慢性肝炎に進展することはないと考えられている。我々は、HEV急性肝炎の14症例を確認したが、3名の患者は肝臓、9名の患者は腎臓、2名は腎臓と膵臓を移植されていた。患者は全員、血清HEV RNAが陽性であった。8名の患者が慢性肝炎になり、確認はアミノトランスフェラーゼ値上昇の持続、血清HEV RNA、慢性肝炎の組織学的特徴によって行われた。移植から診断までの時間は極めて短く、リンパ球数並びにCD2、CD3及びCD4 T細胞の数は、慢性肝炎に進展した患者では著しく低かった。

使用上の注意記載状況・その他参考事項等

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

報告企業の意見	今後の対応
少なくとも免疫抑制剤を投与されている臓器移植患者においては、HEV感染が慢性肝炎に進展し得るとの報告である。 本剤からHEVが伝播したとの報告はない。万一、原料血漿にHEVが混入したとしても、EMCおよびCPVをモデルウイルスとしたウイルスバリデーション試験成績から、本剤の製造工程において十分に不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

BRIEF REPORT

Hepatitis E Virus and Chronic Hepatitis in Organ-Transplant Recipients

Nassim Kamar, M.D., Ph.D., Janick Selves, M.D., Jean-Michel Mansuy, M.D., Leila Ouezzani, M.D., Jean-Marie Péron, M.D., Ph.D., Joëlle Guitard, M.D., Olivier Cointault, M.D., Laure Esposito, M.D., Florence Abravanel, Pharm.D., Marie Danjoux, M.D., Dominique Durand, M.D., Jean-Pierre Vinel, M.D., Jacques Izopet, Pharm.D., Ph.D., and Lionel Rostaing, M.D., Ph.D.

SUMMARY

Hepatitis E virus (HEV) is considered an agent responsible for acute hepatitis that does not progress to chronic hepatitis. We identified 14 cases of acute HEV infection in three patients receiving liver transplants, nine receiving kidney transplants, and two receiving kidney and pancreas transplants. All patients were positive for serum HEV RNA. Chronic hepatitis developed in eight patients, as confirmed by persistently elevated aminotransferase levels, serum HEV RNA, and histologic features of chronic hepatitis. The time from transplantation to diagnosis was significantly shorter and the total counts of lymphocytes and of CD2, CD3, and CD4 T cells were significantly lower in patients in whom chronic disease developed.

ACUTE HEPATITIS CAUSED BY THE HEPATITIS E VIRUS (HEV) IS ENDEMIC IN developing countries and appears to be an emerging disease in industrialized countries.[1,2] Seroprevalence studies have reported anti-HEV IgG antibodies in 6 to 16% of renal-transplant recipients.[3,4] This hepatotropic RNA virus is often not fully considered or routinely sought in cases of acute hepatitis in recipients of solid-organ transplants. Only three cases of acute HEV infection have been reported in organ-transplant recipients.[5-7] Even though two cases of persistent HEV infection have been reported,[8,9] HEV is considered an agent responsible for acute hepatitis that does not become chronic.[10]

We report here 14 cases of acute hepatitis E infection in organ-transplant recipients. We suggest that HEV infection may evolve to chronic hepatitis in immunocompromised patients.

From the Department of Nephrology, Dialysis, and Multiorgan Transplantation (N.K., L.O., J.G., O.C., L.E., D.D., L.R.) and INSERM Unité 858, IFR 31 (N.K., J.-M.P.), Centre Hospitalier Universitaire, Rangueil, France; and the Departments of Histopathology (J.S., M.D.), Virology (J.-M.M., F.A., J.I.), and Hepatology (J.-M.P., J.-P.V.), and INSERM Unité 563, IFR 30 (F.A., J.I., L.R.), Centre Hospitalier Universitaire, Purpan — all in Toulouse, France. Address reprint requests to Dr. Kamar at the Department of Nephrology, Dialysis, and Multiorgan Transplantation, CHU Rangueil, TSA 50032, 31059 Toulouse CEDEX 9, France, or at kamar.n@chu-toulouse.fr.

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PATIENTS AND METHODS

Between January 1, 2004, and December 31, 2006, all recipients of liver, kidney, or kidney and pancreas transplants attending our outpatient and inpatient clinics who presented with unexplained short-term elevations of liver-enzyme levels were screened for HEV infection by serologic and molecular tools. Patients chronically infected with hepatitis B, C, or D viruses were excluded from the study. Biliary-tract complications were ruled out by abdominal ultrasonography. Toxin- and drug-related causes of abnormal liver-function test results were ruled out by patient history. Fourteen of 217 patients (6.5%) tested positive for serum HEV RNA.

Anti-HEV status was determined by an enzyme immunoassay (HEV EIA, Abbott). HEV RNA in serum and stool was detected by real-time polymerase-chain-reaction (PCR) amplification (TaqMan, Applied Biosystems) of a 189-bp product located in the ORF2 region.[11] Strains were sequenced and compared with reference HEV strains (GenBank). The grades and stages of chronic hepatitis were assessed according to the Metavir classification.[12]

Proportions were compared by the chi-square test or Fisher's exact test. Quantitative variables were compared by the nonparametric Mann–Whitney, Friedman, and Wilcoxon tests. A P value of less than 0.05 was considered to indicate statistical significance.

RESULTS

PREVALENCE OF ANTI-HEV IgG

All patients who received a kidney transplant (241 recipients) or a liver transplant (86 recipients) between January 1, 2004, and December 31, 2006, in the department of nephrology, dialysis, and multiorgan transplantation were screened for HEV infection at the time of transplantation. The prevalence of anti-HEV IgG was 13.5% for all recipients, 14.5% for kidney recipients, and 10.4% for liver recipients.

CLINICAL AND BIOLOGIC PRESENTATION

We identified 14 patients with a solid-organ transplant (3 liver recipients, 9 kidney recipients, and

Table 1. Demographic Features of Transplant Recipients at Diagnosis of Acute HEV Infection.*

Patient No.	Organ Transplanted	HEV Infection†	Donor‡	Years of Age	Sex	Mo since Transplantation	Initial Organ Disease	Induction Therapy	Immunosuppressive Therapy
1	Liver	Chronic	Cadaver	57	M	6	Alcoholic cirrhosis	None	Tacrolimus/mycophenolate mofetil/steroid
2	Liver	Chronic	Cadaver	67	M	53	Alcoholic cirrhosis	Basiliximab	Tacrolimus/mycophenolate mofetil/steroid
3	Liver	Chronic	Cadaver	28	F	10	Wilson's disease	None	Tacrolimus/mycophenolate mofetil/steroid
4	Kidney	Chronic	Cadaver	49	M	10	Thrombotic microangiopathy	Basiliximab	Mycophenolate mofetil/steroid
5	Kidney	Resolving	Cadaver	34	M	90	Malformative uropathy	Rabbit antithymocyte globulins	Everolimus/mycophenolate mofetil/steroid
6	Kidney	Resolving	Living	33	M	57	Interstitial nephropathy	Basiliximab	Sirolimus/mycophenolate sodium/steroid
7	Kidney	Chronic	Cadaver	52	M	63	IgA nephropathy	None	Sirolimus/steroid
8	Kidney	Resolving	Cadaver	42	M	168	Crescentic glomerulonephritis	Rabbit antithymocyte globulins	Cyclosporin A/mycophenolate mofetil
9	Kidney	Chronic	Cadaver	30	M	48	Alport's disease	Rabbit antithymocyte globulins	Sirolimus/steroid
10	Kidney	Resolving	Cadaver	51	M	67	Interstitial nephropathy	Rabbit antithymocyte globulins	Cyclosporin A/mycophenolate mofetil/steroid
11	Kidney	Resolving	Cadaver	62	F	108	Chronic glomerulonephritis	Rabbit antithymocyte globulins	Cyclosporin A/steroid
12	Kidney	Resolving	Cadaver	28	M	25	IgA nephropathy	Rabbit antithymocyte globulins	Tacrolimus/mycophenolate mofetil/steroid
13	Kidney and pancreas	Chronic	Cadaver	55	F	60	Diabetes mellitus	Rabbit antithymocyte globulins	Tacrolimus/azathioprine/steroid
14	Kidney and pancreas	Chronic	Cadaver	58	M	27	Diabetes mellitus	Rabbit antithymocyte globulins	Tacrolimus/mycophenolate mofetil

* All patients were born in France. HEV denotes hepatitis E virus.
† Resolving indicates clearance of HEV RNA from serum and stools, and chronic indicates persisting elevated liver-enzyme levels and detectable RNA in the serum or stools at least 6 months after the acute phase.
‡ Cadaveric donors had a heartbeat.

2 kidney and pancreas recipients) in whom acute HEV infection developed (Table 1). The acute hepatitis episode was asymptomatic in 7 of the 14 patients; these 7 patients were tested for HEV after liver-enzyme abnormalities were detected during routine biologic examinations that are performed every 3 to 4 months after organ transplantation. The seven other patients presented with fatigue, diffuse arthralgias, and myalgias that had evolved over a period of 1 to 2 weeks. One of the symptomatic patients also had marked weight loss (approximately 8 kg [18 lb] during the month before the presenting symptoms appeared) and was icteric. The symptoms disappeared within 2 weeks after diagnosis. No abnormalities were detected during physical examination of any other patient. No patients were febrile, and none had traveled outside France during the year before their hepatitis episode. Only two patients reported having been in contact with animals: one patient with chickens and rabbits and the other with birds. No patients had had an acute rejection episode after undergoing transplantation. Immunosuppressive therapy had remained unchanged in all patients for at least 6 months before their acute episode. Liver-enzyme levels were significantly higher than the levels 3 to 4 months before the diagnosis of HEV infection (Table 2).

DIAGNOSIS OF HEV INFECTION

At admission, classic causes of hepatitis were ruled out (Table 1 of the Supplementary Appendix, available with the full text of this article at www.nejm.org). The ferritin level was 567 ng per milliliter (range, 110 to 2007; normal range, 30 to 380), and the ceruloplasmin level was 0.35 ng per milliliter (range, 0.24 to 0.47; normal range, 0.20 to 0.45). At diagnosis, HEV RNA was detected in the serum of all patients and in the stool of the three patients whose stool was examined. PCR-amplification products of the serum HEV of 12 patients were sequenced and analyzed. Phylogenetic analysis revealed that all the strains belonged to genotype 3 (GenBank accession numbers, EU220992 to EU221003) (Fig. 1 of the Supplementary Appendix). We tried but failed to sequence the strains of the remaining two patients. No correlation was found between HEV RNA concentration and either liver-enzyme levels or liver-activity scores at diagnosis.

LIVER HISTOLOGIC FINDINGS DURING THE ACUTE PHASE

In the acute phase, 9 of the 14 patients underwent a liver biopsy to evaluate the severity of the acute episode of hepatitis; the remaining 5 patients declined biopsy. In liver-transplant recipients, liver biopsy also was performed to detect acute rejection. The mean (±SD) Metavir activity and fibrosis scores were 1.3±1.0 and 0.9±0.6, respectively (for assessment of disease activity, a Metavir score of 0 indicates no activity, 1 mild activity, 2 moderate activity, and 3 severe activity; for assessment of fibrosis, a Metavir score of 0 indicates no fibrosis, 1 portal fibrosis without septa, 2 a few septa, 3 numerous septa without cirrhosis, and 4 cirrhosis). The dominant lesions were lobular, with inflammation but no ballooning, and with spotty necrosis that included acidophilic bodies. The portal tract was mildly or moderately expanded and included an inflammatory infiltrate composed mainly of lymphocytes. Mild piecemeal necrosis was observed in six patients.

COURSE OF HEV INFECTION

Immunosuppressive therapy and target immunosuppressive trough levels were not modified after the diagnosis of HEV infection (data not shown). HEV infection resolved in six patients (43%); serum and stool HEV RNA in these patients became undetectable within 6 months after diagnosis and remained undetectable until the last follow-up at a mean of 12 months (range, 5 to 36) (Table 2). However, in the eight other patients (57%), HEV infection evolved to chronic hepatitis, as indicated by persistently elevated liver-enzyme levels and detectable HEV RNA in the serum or stool for a mean of 15 months (range, 10 to 24) after the acute phase.

Among the patients with resolving HEV infection, the levels of aspartate aminotransferase and alanine aminotransferase returned to preinfection values within 1 month (five patients) or 3 months (one patient) after diagnosis. The levels of γ-glutamyltransferase and alkaline phosphate returned to baseline levels within 3 months after diagnosis. Among those with chronic HEV infection, liver-enzyme levels remained above the upper limit of normal at the last follow-up. In both groups, the total bilirubin levels rapidly returned to preinfection levels. In both groups, hematologic and re-

Table 2. Liver Function in Patients with HEV Infection.

Patient No.	Time of Measurement	Alanine Aminotransferase*	Aspartate Aminotransferase†	γ-Glutamyl-transferase‡	Bilirubin§	Liver Biopsy	
			units/liter		*mg/dl*	*Metavir activity score*¶	*Metavir fibrosis score*‖
1	Baseline	10	16	18	584		
	Diagnosis	69	37	40	584	0	1
	15-Mo follow-up	59	41	30	409	3	2
2**	Baseline	102	95	1164	584		
	Diagnosis	248	229	3482	2339		
	16-Mo follow-up	59	54	173	701	1	3
3	Baseline	49	23	35	584		
	Diagnosis	169	76	76	994	1	1
	17-Mo follow-up	85	47	35	701	1	1
4	Baseline	26	12	19	701		
	Diagnosis	166	47	167	760	1	1
	15-Mo follow-up	135	57	146	760	3	1
5	Baseline	41	26	73	584		
	Diagnosis	66	47	118	526	0	1
	5-Mo follow-up	52	35	148	584		
6	Baseline	26	25	26	608		
	Diagnosis	245	104	118	468		
	12-Mo follow-up	30	32	18	584		
7	Baseline	26	18	55	397		
	Diagnosis	874	436	669	701	1	0
	10-Mo follow-up	158	89	156	584		
8	Baseline	32	24	32	1286		
	Diagnosis	770	340	373	2514		
	36-Mo follow-up	22	22	19	1169		
9	Baseline	42	39	26	584		
	Diagnosis	310	160	92	643	2	2
	24-Mo follow-up	90	39	42	760	2	2
10	Baseline	37	30	26	584		
	Diagnosis	518	235	459	1286		
	36-Mo follow-up	28	27	109	1286		
11	Baseline	23	18	42	351		
	Diagnosis	255	154	1055	3041	3	1
	12-Mo follow-up	13	7	80	351		
12	Baseline	12	14	19	368		
	Diagnosis	298	71	216	818	2	1
	5-Mo follow-up	15	24	51	877		
13	Baseline	13	22	8	643		
	Diagnosis	156	115	47	935	2	0
	15-Mo follow-up	298	238	79	760		

Table 2. (Continued.)

Patient No.	Time of Measurement	Alanine Aminotransferase*	Aspartate Aminotransferase†	γ-Glutamyltransferase‡	Bilirubin§	Liver Biopsy	
			units/liter		*mg/dl*	*Metavir activity score*¶	*Metavir fibrosis score*‖
14	Baseline	14	23	30	1169		
	Diagnosis	143	106	132	877		
	13-Mo follow-up	126	118	585	994	1	3
Median							
	Baseline	26	23	32	584		
	Diagnosis††	248	115	167	818		
	Follow-up‡‡	59	40	79.5	731		

* Normal values for alanine aminotransferase range from 5 to 34 units per liter.
† Normal values for aspartate aminotransferase range from 3 to 30 units per liter.
‡ Normal values for γ-glutamyltransferase range from 7 to 38 units per liter.
§ To convert values for bilirubin to micromoles per liter, multiply by 17.1. Normal values range from 2 to 21 mg per deciliter.
¶ For assessment of disease activity, a Metavir score of 0 indicates no activity, 1 mild activity, 2 moderate activity, and 3 severe activity.
‖ For assessment of fibrosis, a Metavir score of 0 indicates no fibrosis, 1 portal fibrosis without septa, 2 a few septa, 3 numerous septa without cirrhosis, and 4 cirrhosis.
** Patient 2 had substantial alcohol consumption before the acute phase.
†† The differences between values at baseline and at diagnosis are significant for alanine aminotransferase, aspartate aminotransferase, and γ-glutamyltransferase (P=0.001) and for bilirubin (P=0.02).
‡‡ The differences between values at diagnosis and at last follow-up (median, 15 months) are significant for alanine aminotransferase (P=0.003), aspartate aminotransferase (P=0.02), and γ-glutamyltransferase (P=0.03).

nal measurements remained unchanged during the follow-up as compared with preinfection levels (data not shown). HEV seroconversion was observed in four patients with resolving HEV infection (two at 1 month and one each at 3 and 6 months after diagnosis) and seven patients with chronic infection (one at 3 months, two at 6 months, two at 12 months, and one each at 13 and 15 months after diagnosis).

Only six of the eight patients with chronic infection underwent a second liver biopsy (one at 10 months, two at 12 months, and one each at 13, 15, and 18 months after the diagnosis of acute HEV infection). The two remaining patients declined liver biopsy. The mean Metavir activity and fibrosis scores of the six patients who underwent biopsy were 2.0±1.0 and 1.8±0.8, respectively. All biopsy specimens showed features of chronic viral hepatitis, characterized by fibrosis and portal hepatitis, with dense lymphocytic infiltrate and variable degrees of piecemeal necrosis. Lobular hepatitis was mild to moderate in all cases. In the four patients who underwent a liver biopsy during both the acute phase and the chronic phase, the Metavir activity scores progressed from 1.0±0.8 to 2.2±0.9 and the fibrosis scores from 1.2±0.5 to 1.5±0.5.

RESOLVING VERSUS CHRONIC HEV INFECTION

During the acute phase, there were no significant differences between the patients with resolving HEV infection and those with chronic infection in median serum HEV RNA concentrations (5.97 $\log_{10}$ copies of RNA per milliliter [range, 5.79 to 6.44] and 6.18 $\log_{10}$ copies per milliliter [range, 4.92 to 7.28], respectively). There also were no significant differences between the groups in peak liver-enzyme levels. Hepatitis developed later after transplantation in patients with resolving HEV infection than in those in whom the infection progressed. Patients in whom chronic hepatitis developed had significantly lower serum creatinine levels at baseline and significantly lower counts of leukocytes, total lymphocytes, platelets, and CD2, CD3, and CD4 lymphocytes (Table 3). The percentages of patients who received induction therapy at transplantation or who received calcineurin inhibitors, mycophenolate mofetil or sodium, or inhibitors of the mammalian target of rapamycin (mTOR) were similar in the two groups. The dosage and trough levels of immunosuppressive drugs, as well as the proportions of patients with anti–hepatitis A virus, anticytomegalovirus, or IgG antibodies to Epstein–Barr virus, were similar in the two groups (data not shown).

Table 3. Patients with Resolving HEV Infection and Those in Whom the Infection Evolved to Chronic Hepatitis.

Variable	Patients with Resolving Infection (N=6)	Patients with Chronic Infection (N=8)	P Value
	median (range)		
At diagnosis			
Time since transplantation — mo	78.5 (25–168)	37.5 (6.0–63.0)	0.03
Leukocyte count — $\times 10^{-3}$/mm^3	8.85 (6–9.66)	4.31 (2.19–7.20)	0.004
Lymphocyte count — $\times 10^{-3}$/mm^3			
Total	1.73 (1.12–2.33)	0.75 (0.63–1.04)	0.004
CD2+	1.59 (0.84–2.25)	0.66 (0.58–0.92)	<0.001
CD3+	1.54 (0.70–1.88)	0.61 (0.49–0.79)	0.01
CD4+	0.93 (0.49–1.07)	0.22 (0.16–0.40)	0.004
Platelet count — $\times 10^{-3}$/mm^3	261 (190–285)	155.5 (75.0–250.0)	0.01
Serum creatinine — mg/dl*	2.15 (1.31–2.84)	1.33 (1.08–1.89)	0.01
At last follow-up			
Aspartate aminotransferase — IU/liter	25.5 (7–35)	55.5 (39.0–238.0)	0.002
Alanine aminotransferase — IU/liter	25 (13–45)	108.0 (59.0–298.0)	0.002

* To convert values for creatinine to micromoles per liter, multiply by 88.4.

DISCUSSION

HEV infection is transmitted by the fecal–oral route and may be a zoonosis in industrialized countries. It has a mortality rate of about 1% in the general population and 30% in pregnant women.[13] HEV-induced acute hepatitis may be fulminant,[14] but we are not aware that any cases of chronic hepatitis have previously been reported. Recently, the diagnosis of many cases of acute HEV hepatitis in nonimmunocompromised patients in southwest France[15] prompted us to look systematically for HEV in recipients of solid-organ transplants who had unexplained hepatitis. Of the 14 patients with acute HEV infection whom we report on here, 8 underwent progression to chronic hepatitis. In addition, in this issue of the *Journal*, Gérolami et al. report a case of HEV-related cirrhosis in a kidney-transplant recipient.[16]

After all other causes of hepatitis had been ruled out, the serum of 14 patients, none of whom had traveled outside France in the previous year, was found to be positive for HEV RNA. We did not identify any source of contamination. The peak aminotransferase levels were lower than in nonimmunocompromised patients.[17,18] Histologic lesions (mainly spotty lobular necrosis) that are characteristic of classic acute viral hepatitis were seen; these lesions were less severe than those typically seen in nonimmunocompromised patients. These findings could be related to the immunosuppressive therapy in transplant recipients.

HEV infection resolved in 6 of the 14 patients within 6 months after the end of the acute phase. In contrast, HEV infection in eight patients evolved to chronic hepatitis, as indicated by persistently elevated liver-enzyme levels and detectable serum HEV RNA at a median of 15 months (range, 10 to 24) after the end of the acute phase. Liver biopsies performed at a median of 12.5 months (range, 10 to 18) after the acute phase revealed signs of chronic viral hepatitis. The histologic lesions — dense lymphocytic portal infiltrate with constant piecemeal necrosis — were similar to those observed in patients chronically infected with hepatitis C virus. None of the patients received any specific therapy; in particular, none received antiviral therapy. Immunosuppressive therapy was not modified after the diagnosis of HEV. In the absence of available therapeutic recommendations for patients infected with HEV, we only performed close monitoring of liver-enzyme levels.

There were no significant differences between patients with resolving HEV infection and those with chronic HEV infection in demographic or clinical features, including treatment with immunosuppressive agents before the acute phase. However, the immunologic status of the patients may have had a role in the evolution to chronic dis-

ease. In patients in whom the infection became chronic, the time from transplantation to the development of infection was significantly shorter — and consequently, the total lymphocyte counts and the CD2, CD3, and CD4 lymphocyte counts were significantly lower — than in patients in whom HEV infection resolved. Hence, the T-cell response seems to have a role in HEV clearance, as does the B-cell response.

HEV seroconversion occurred later in patients with chronic infection than in those with resolving infection. This difference may be related to the reduction in the humoral immune response caused by treatment with mycophenolate, inhibitors of mTOR, or both. These drugs are known to decrease the synthesis of antibodies[19,20] and to inhibit the cell-cycle progression and differentiation of human B lymphocytes.[21] The humoral immune response is necessary to clear HEV and to prevent hepatitis. Bryan et al. have shown that antibodies to the HEV capsid can be protective against hepatitis E.[22] Passive immunoprophylaxis studies in cynomolgus monkeys have confirmed that the antibody to the HEV capsid may prevent HEV infection in humans.[23] Recently an HEV recombinant protein vaccine was found to be effective in preventing HEV infection.[24]

Further studies are required to determine the incidence of chronic HEV infection in transplant recipients who live in areas where the disease is not endemic. Vaccination against HEV could be proposed to patients before or after organ transplantation. However, the efficacy of vaccination in these populations should be addressed.

In conclusion, our data suggest that HEV should be considered an etiologic agent of hepatitis in organ-transplant recipients. We have demonstrated that HEV infection can evolve to chronic hepatitis, at least in organ-transplant recipients. A longer follow-up is required to assess the outcome of HEV infection in organ-transplant recipients.

No potential conflict of interest relevant to this article was reported.

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REFERENCES

1. Clemente-Casares P, Pina S, Buti M, et al. Hepatitis E virus epidemiology in industrialized countries. Emerg Infect Dis 2003;9:448-54.
2. Meng XJ, Wiseman B, Elvinger F, et al. Prevalence of antibodies to hepatitis E virus in veterinarians working with swine and in normal blood donors in the United States and other countries. J Clin Microbiol 2002;40:117-22.
3. Ibarra H, Riedemann S, Reinhardt G, Ardiles L, Calvo M, Siegel F. Anti-HEV in dialysis and renal transplant patients in an endemic region in Chile. Clin Nephrol 1998;50:267-8.
4. Buffet C, Laurent-Puig P, Chandot S, et al. A high hepatitis E virus seroprevalence among renal transplantation and haemophilia patient populations. J Hepatol 1996;24:122-5.
5. Sinha S, Jha R, Lakhtakia S, Narayan G. Acute pancreatitis following kidney transplantation — role of viral infections. Clin Transplant 2003;17:32-6.
6. Kamar N, Mansuy JM, Esposito L, et al. Acute hepatitis and renal function impairment related to infection by hepatitis E virus in a renal-allograft recipient. Am J Kidney Dis 2005;45:193-6.
7. Péron JM, Mansuy JM, Récher C, et al. Prolonged hepatitis E in an immunocompromised patient. J Gastroenterol Hepatol 2006;21:1223-4.
8. Mechnik L, Bergman N, Attali M, et al. Acute hepatitis B virus infection presenting as a prolonged cholestatic jaundice. J Clin Gastroenterol 2001;33:421-2.
9. Tamura A, Shimizu YK, Tanaka T, et al. Persistent infection of hepatitis E virus transmitted by blood transfusion in a patient with T-cell lymphoma. Hepatol Res 2007;37:113-20.
10. Emerson SU, Purcell RH. Hepatitis E virus. Rev Med Virol 2003;13:145-54.
11. Mansuy JM, Peron JM, Bureau C, Alric L, Vinel JP, Izopet J. Immunologically silent autochthonous acute hepatitis E virus infection in France. J Clin Microbiol 2004; 42:912-3.
12. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. Hepatology 1996;24:289-93.
13. Kumar A, Beniwal M, Kar P, Sharma JB, Murthy NS. Hepatitis E in pregnancy. Int J Gynaecol Obstet 2004;85:240-4.
14. Péron JM, Bureau C, Poirson H, et al. Fulminant liver failure from acute autochthonous hepatitis E in France: description of seven patients with acute hepatitis E and encephalopathy. J Viral Hepat 2007;14: 298-303.
15. Mansuy JM, Peron JM, Abravanel F, et al. Hepatitis E in the south west of France in individuals who have never visited an endemic area. J Med Virol 2004;74:419-24.
16. Gérolami R, Moal V, Colson P. Chronic hepatitis E with cirrhosis in a kidney-transplant recipient. N Engl J Med 2008; 859-60.
17. Péron JM, Mansuy JM, Poirson H, et al. Hepatitis E is an autochthonous disease in industrialized countries: analysis of 23 patients in South-West France over a 13-month period and comparison with hepatitis A. Gastroenterol Clin Biol 2006;30: 757-62.
18. Peron JM, Danjoux M, Kamar N, et al. Liver histology in patients with sporadic acute hepatitis E: a study of 11 patients from South-West France. Virchows Arch 2007;450:405-10.
19. Rentenaar RJ, van Diepen FN, Meijer RT, et al. Immune responsiveness in renal transplant recipients: mycophenolic acid severely depresses humoral immunity in vivo. Kidney Int 2002;62:319-28.
20. Luo H, Chen H, Daloze P, Chang JY, St-Louis G, Wu J. Inhibition of in vitro immunoglobulin production by rapamycin. Transplantation 1992;53:1071-6.
21. Aagaard-Tillery KM, Jelinek DF. Inhibition of human B lymphocyte cell cycle progression and differentiation by rapamycin. Cell Immunol 1994;156:493-507.
22. Bryan JP, Tsarev SA, Iqbal M, et al. Epidemic hepatitis E in Pakistan: patterns of serologic response and evidence that the antibody to hepatitis E virus protects against disease. J Infect Dis 1994;170:517-21.
23. Tsarev SA, Tsareva TS, Emerson SU, et al. Successful passive and active immunization of cynomolgus monkeys against hepatitis E. Proc Natl Acad Sci U S A 1994; 91:10198-202.
24. Shrestha MP, Scott RM, Joshi DM, et al. Safety and efficacy of a recombinant hepatitis E vaccine. N Engl J Med 2007; 356:895-903.

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研究報告の概要

○フランスの新規HIV診断例におけるHIV-2、HIV-1のグループO型の感染率:2003～2006年
新規HIV診断例のHIV血清型を同定するため、フランス国内で調査用に採取された乾燥血清spotsを用いた。2003年1月～2006年6月に、10,184の新規診断例が報告された。HIV-2、HIV-1のグループO型感染の割合は、それぞれ1.8、0.1%であった。これら症例のほとんどは、異性との接触により感染した流行地域出身の患者であった。HIV-2感染の3症例は、男性との性交渉を持つ非アフリカ系男性で報告された。

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
2003年1月～2006年6月に、フランスにおいて10,184の新規HIV診断例が報告され、HIV-2、HIV-1のグループO型感染の割合は、それぞれ1.8、0.1%であったとの報告である。	日本赤十字社では、HIV抗体検査に加えて20プールでスクリーニングNATを行い、陽性血液を排除している。また、これまでの凝集法と比べて、より感度の高い化学発光酵素免疫測定法(CLEIA)の導入を順次進めている。さらに、HIV-2及びHIVグループOの検出が可能な次世代NATの導入に向けた準備を進めている。

Prevalence of HIV-2 and HIV-1 group O infections among new HIV diagnoses in France: 2003–2006

Francis Barin[a], Françoise Cazein[b], Florence Lot[b], Josiane Pillonel[b], Sylvie Brunet[a], Damien Thierry[a], Florence Damond[c], Françoise Brun-Vézinet[c], Jean-Claude Desenclos[b] and Caroline Semaille[b]

French national surveillance of new HIV diagnoses included the collection of dried serum spots to identify HIV serotypes. Between January 2003 and June 2006, 10 184 new diagnoses were reported. The proportions of HIV-2 and HIV-1 group O infections were 1.8 and 0.1%, respectively. Most of these cases occurred in patients infected through heterosexual contact and originated from the corresponding endemic areas. Three cases of HIV-2 infections were reported in non-African men having sex with men.

HIV-2, first suspected by serological findings in west African residents, was isolated from patients with AIDS originating from Cape Verde and Guinea Bissau [1,2]. Although HIV-2 causes AIDS, it is clearly less pathogenic than HIV-1 [3,4]. The viral load is significantly lower in HIV-2-infected patients, and consequently HIV-2 is less transmissible [5,6]. The precise diagnosis of HIV-2 has implications, particularly for monitoring RNA levels, as no specifically dedicated commercial assays are currently available, and for the choice of antiretroviral treatment, because HIV-2 strains are naturally resistant to non-nucleoside reverse transcriptase inhibitors and fusion inhibitors, and are less sensitive *in vitro* to some protease inhibitors [7,8]. HIV-2 is endemic in west Africa. Most cases described outside Africa have been traced to contacts with individuals from this endemic region. This has been particularly observed in European countries with historical links with west Africa such as France, the United Kingdom and Portugal [9–11]. No extensive epidemiological surveys have, however, allowed the determination of the exact prevalence of HIV-2 in these European countries. Similarly, HIV-1 group O variants are restricted geographically, mainly to Cameroon and the surrounding areas [12]. Rare cases have been reported in industrialized countries, but the exact prevalence of these variants among HIV-1-infected patients is unknown. Similar to HIV-2, most of the commercially available assays for the quantification of HIV-1 RNA do not detect viral sequences from HIV-1 group O variants [13], and non-nucleoside reverse transcriptase inhibitors are inefficient at controlling HIV-1 group O replication [14].

Mandatory anonymous HIV case reporting was implemented in France in 2003, with which virological monitoring using dried serum spots was associated. The procedures and the first results of this surveillance system have been described elsewhere [15]. In brief, any HIV-positive serology confirmed for the first time by a clinical laboratory must be reported, with a unique anonymous code for each patient. Clinical and epidemiological details are supplied by the physicians in charge of the patients. For each case, the laboratory is asked to send dried serum spots collected on filter papers from the serum sample obtained for the original diagnosis to the National Reference Centre (NRC). Although HIV notification is mandatory, virological surveillance is based on volunteer participation by both microbiologists and patients. The patient's consent for virological surveillance is obtained by the reporting clinician through the HIV notification form. Serological identification of the type and group of HIV is performed by enzyme-linked immunosorbent assay at the NRC, as described [16]. Results from the NRC are then linked to the epidemiological data in the HIV national database using the patient's anonymous code. Any specific diagnosis of infection by either HIV-2 or HIV-1 group O implies transmission of the information to the clinical laboratory of origin in order to adapt the clinical, biological and therapeutic management of the patient.

Here we report the results of the HIV-2 and HIV-1 group O infections that were identified among new HIV diagnoses during the past 3 years. Between January 2003 and June 2006, 10 184 new diagnoses with participation in the virological surveillance were reported. Among these, 186 were from patients infected by HIV-2 [1.8%; 95% confidence interval (CI) 1.6–2.1], of which 164 (1.6%; 95% CI 1.4–1.9) were HIV-2 only and 22 (0.2%; 95% CI 0.1–0.3) were probable dual infections. The serological diagnosis of dual infection was based on similar high antibody binding to both the immunodominant epitope of gp41 and the V3 region of both HIV-1 and HIV-2 [16,17]. Such a stringent criteria was validated earlier [17], and more recently on a panel of samples for which single or dual infections were diagnosed by type-specific polymerase chain reaction (data not shown). Patients infected with HIV-2 were mostly citizens of a west African country (65%; $n=121$), mainly Côte d'Ivoire ($n=64$), Mali ($n=19$) and Senegal ($n=12$), but there were also 22 European individuals, 20 from France and two from Portugal (Fig. 1). The majority of cases was observed in women (63%; $n=118$). Although the risk factor was unknown for 26% ($n=48$) of cases, 72% ($n=134$) of HIV-2 infections were caused by heterosexual transmission. HIV-2 was, however, identified in three men who have sex with men (MSM), one from France and two from the Americas.

Twelve patients (0.1%; 95% CI 0.1–0.2) were infected with HIV-1 group O variants. Most of them originated from the sub-Saharan endemic area: nine from Cameroon and one from Chad (Fig. 1). Two of those patients had dual M/O infection; those two cases have been described in detail earlier [18]. The two other cases were French

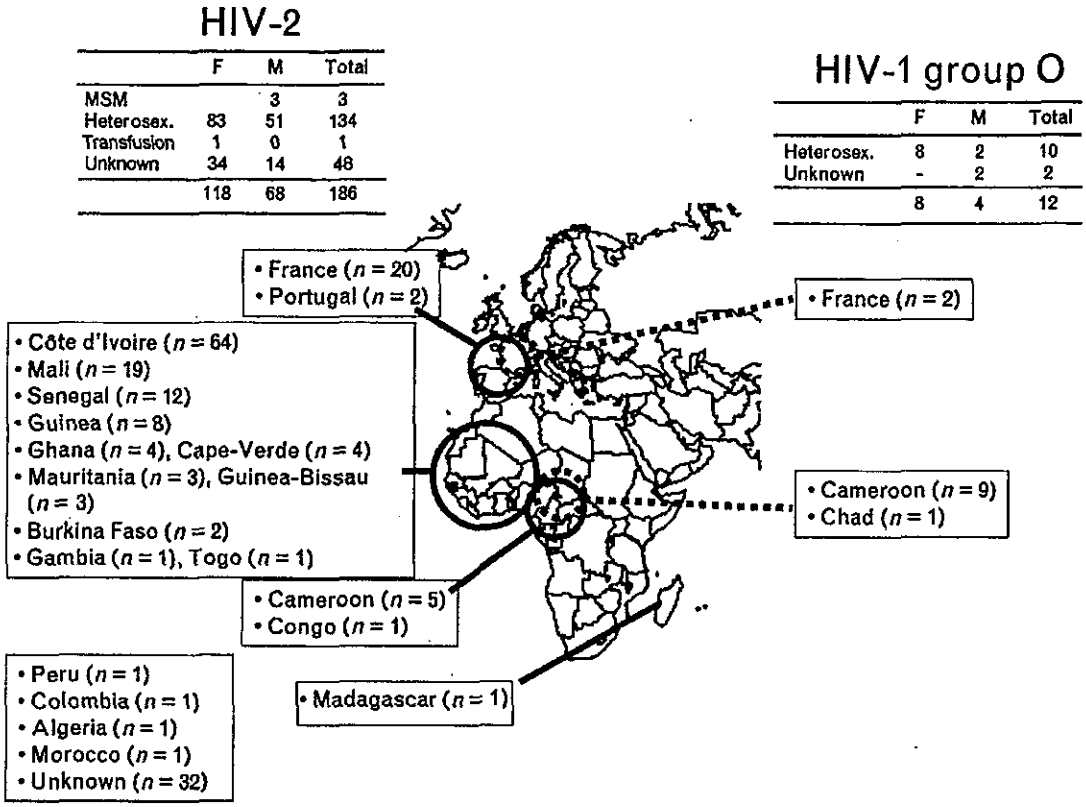

Fig. 1. Nationality and mode of transmission of patients identified as infected by HIV-2 (left) and HIV-1 group O (right) in France, 2003–2006. F, Female; Heterosex., heterosexual transmission; MSM, men who have sex with men; M, male.

citizens who had probably been infected through heterosexual intercourse.

A specific serological diagnosis of HIV-2 infection may be missed if adapted confirmation tools are not routinely used in clinical laboratories, a situation that is frequent in non-endemic areas. There is a frequent use of HIV-1 Western blots for confirmatory diagnosis, on which serum samples positive for antibodies to HIV-2 may cross-react, even on envelope glycoproteins, leading to a misclassification as anti-HIV-1 positives [19]. Similarly, HIV-1 group O infections are not systematically diagnosed as such, except if there are dissociations between clinical and biological findings in an HIV-1-positive patient; for example, AIDS stage with undetectable viral load. This is because there is no commercially available specific serological tool for this purpose. Therefore, there are no data that would provide estimates of the prevalence of these rare variants in western countries. The French national surveillance of new HIV diagnoses included the collection of dried serum spots to identify HIV serotypes with dedicated peptide immunoassays [16,17]. This allowed, for the first time, the provision of reliable estimates of the proportion of these rare variants in a European country. The results indicate that most of the cases diagnosed during this 3-year period still occurred in patients originating from the endemic areas, west Africa and Cameroon, for HIV-2 and HIV-1 group O, respectively. Three cases of HIV-2 infections were, however, reported in MSM, an observation that should deserve further attention because of the persistent high-risk behaviours in some individuals in the gay community.

[a]*Université François-Rabelais, Inserm ERI 19, Centre National de Référence du VIH, CHU Bretonneau, 37044 Tours cedex, France;* [b]*Institut de Veille Sanitaire, Saint-Maurice, France;* and [c]*Laboratoire de Virologie, Hôpital Bichat-Claude Bernard, Paris, France.*

Sponsorship: The National Reference Centre is funded by a grant from the Institut de Veille Sanitaire. The Institut de Veille Sanitaire is funded by the French Minister of Health. The enzyme-linked immunosorbent assays for serological discrimination between HIV variants were developed and validated through projects supported by the Agence Nationale de Recherche sur le Sida (ANRS, Paris, France). We thank all participants in the national surveillance programme, particularly the biologists, physicians and public health doctors.

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References

1. Barin F, M'Boup S, Denis F, Kanki P, Allan JS, Lee TH, *et al.* **Serological evidence for virus related to simian T-lymphotropic retrovirus III in residents of West Africa.** *Lancet* 1985; **2**:1387–1389.
2. Clavel F, Guetard D, Brun-Vézinet F, Chamaret S, Rey MA, Santos-Ferreira O, *et al.* **Isolation of a new human retrovirus from West African patients with AIDS.** *Science* 1986; **233**:343–346.
3. Marlink R, Kanki PJ, Thior I, Travers K, Eisen G, Siby T, *et al.* **Reduced rate of disease development after HIV-2 infection as compared to HIV-1.** *Science* 1994; **265**:1587–1590.
4. Matheron S, Pueyo S, Damond F, Simon F, Leprêtre A, Campa P, *et al.* **Factors associated with clinical progression in HIV-2 infected-patients: the French ANRS cohort.** *AIDS* 2003; **17**:2593–2601.
5. Berry N, Ariyoshi K, Jaffar S, Sabally S, Corrah T, Tedder R, *et al.* **Low peripheral blood viral HIV-2 RNA in individuals with high CD4 percentage differentiates HIV-2 from HIV-1 infection.** *J Hum Virol* 1998; **1**:457–468.
6. Kanki PJ, Travers K, M'Boup S, Hsieh CC, Marlink RG, Gueye-N'Diaye A, *et al.* **Slower heterosexual spread of HIV-2 than HIV-1.** *Lancet* 1994; **343**:943–946.
7. Reeves JD, Doms RW. **Human immunodeficiency virus type 2.** *J Gen Virol* 2002; **83**:1253–1265.
8. Damond F, Brun-Vézinet F, Matheron S, Peytavin G, Campa P, Pueyo S, *et al.* **Polymorphism of the human immunodeficiency virus type 2 (HIV-2) protease gene and selection of resistance mutations in HIV-2-infected patients treated with protease inhibitors.** *J Clin Microbiol* 2005; **43**:484–487.
9. Matheron S, Mendoza-Sassi G, Simon F, Olivares R, Coulaud JP, Brun-Vezinet F. **HIV-1 and HIV-2 AIDS in African patients living in Paris.** *AIDS* 1997; **11**:934–936.
10. Dougan S, Patel B, Tosswill JH, Sinka K. **Diagnoses of HIV-1 and HIV-2 in England, Wales, and Northrn Ireland associated with west Africa.** *Sex Transm Infect* 2005; **81**:338–341.
11. Soriano V, Gomes P, Heneine W, Holguin A, Doruana M, Antunes R, *et al.* **Human immunodeficiency virus type 2 (HIV-2) in Portugal: clinical spectrum, circulating subtypes, virus isolation, and plasma viral load.** *J Med Virol* 2000; **61**:111–116.
12. Roques P, Robertson DL, Souquiere S, Damond F, Ayouba A, Farfara I, *et al.* **Phylogenetic analysis of 49 newly derived HIV-1 group O strains: high viral diversity but no group M-like subtype structure.** *Virology* 2002; **302**:259–273.
13. Gueudin M, Plantier JC, Lemée V, Schmitt MP, Chartier L, Bourlet T, *et al.* **Evaluation of the Roche Cobas TaqMan and Abbott real time extraction-quantification systems for HIV-1 subtypes.** *J Acquir Immune Defic Syndr* 2007; **44**:500–505.
14. Descamps D, Collin G, Letourneur F, Apetrei C, Damond F, Loussert-Ajaka I, *et al.* **Susceptibility of human immunodeficiency virus type 1 group O isolates to antiretroviral agents: in vitro phenotypic and genotypic analyses.** *J Virol* 1997; **71**:8893–8898.
15. Semaille C, Barin F, Cazein F, Pillonel J, Lot F, Brand D, *et al.* **Monitoring the dynamics of the HIV epidemic using assays for recent infection and serotyping among new HIV diagnoses: experience after 2 years in France.** *J Infect Dis* 2007; **196**:377–383.
16. Barin F, Plantier JC, Brand D, Brunet S, Moreau A, Liandier B, *et al.* **Human immunodeficiency virus serotyping on dried serum spots as a screening tool for the surveillance of the AIDS epidemic.** *J Med Virol* 2006; **78 (Suppl 1)**:S13–S18.
17. Baillou A, Janvier B, Leonard G, Denis F, Goudeau A, Barin F. **Fine serotyping of human immunodeficiency virus serotype 1 (HIV-1) and HIV-2 infections by using synthetic oligopeptides representing an immunodominant domain of HIV-1 and HIV-2/simian immunodeficiency virus.** *J Clin Microbiol* 1991; **29**:1387–1391.
18. Brand D, Beby-Defaux A, Macé M, Brunet S, Moreau A, Godet C, *et al.* **First identification of HIV-1 groups M and O dual infections in Europe.** *AIDS* 2004; **18**:2425–2428.
19. Damond F, Apetrei C, Robertson DL, Souquière S, Lepretre A, Matheron S, *et al.* **Variability of human immunodeficiency virus type 2 infecting patients living in France.** *Virology* 2001; **280**:19–30.

別紙様式第2-1

No. 42

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2008. 1. 21	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	Iwanaga M, Chiyoda S, Kusaba E, Kamihira S. American Society of Hematology; 2007 Dec 8-11; Atlanta.	公表国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社) 合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)			日本

研究報告の概要

○1999～2006年の長崎における日本人献血者ヒトT細胞向性ウイルス1型 (HTLV-1) 感染率の傾向

HTLV-1の流行地域である日本の長崎では、1986年から献血者のルーチンの血清スクリーニング検査、1987年からウイルスキャリアの母親からの乳汁媒介伝播予防のために長崎県ATL ウイルス母子感染防止研究協力事業(APP)が実施されている。本試験では、1999年1月～2006年12月に献血を行った初回献血者の年齢別、出生年別、および期間別HTLV-1血清陽性率の傾向分析を行った。初回献血者55,668名(献血時年齢16～65歳、出生年1934～1990)のうち、718名はHTLV-1検査陽性であり、全体的な血清陽性率は1.29% (95%CI, 1.20-1.39)であった。陽性率は男性よりも女性の方が高かった(1.53%vs.1.13%; OR; 1.36, 95%CI; 1.17-1.57)。血清陽性率は、年齢が高くなるにつれ有意に上昇し、献血時年齢16～25歳では0.70%、献血時年齢56歳以上では7.34% (χ2乗検定、P<0.0001)であった。年間感染率は1999年が1.32、2002年が1.31、2006年が1.37であり、期間中に有意な経年傾向がないことが示された(P for trend=0.99)。献血時年齢の解析では、陽性率は56歳以上(P for trend=0.02) と16～25歳 (P for trend=0.0007)では有意に減少したが、出生年別解析では、1981～90年出生群で1999年の1.22%から2006年の0.44%へ減少(P for trend<0.0001)したことを除き、陽性率の経時的変化は見られなかった。1985～90年出生群の解析では、血清陽性率は1985～86年出生群の0.75% 、1987～88年出生群の0.31%から1989～90年出生群の0%に減少した(P for trend=0.0002)。HTLV-1血清陽性率は、APP開始後の1987～90年に生まれた献血者では、1985～86年に生まれた献血者と比較して有意に低かった。以上の結果は、ほとんどのウイルス伝播が幼児期に起こるため、HTLV-1陽性率の出生年解析が経年傾向の評価に適切であると考えられることを示し、ウイルスキャリアの母親の授乳を避けることを指導した県をあげての対応が、当該地域のHTLV-1血清陽性率の減少に貢献していることを示している。

報告企業の意見	今後の対応
1999～2006年の長崎における献血者のヒトT細胞向性ウイルス1型感染率は、1987～90年に生まれた献血者では1985～86年に生まれた献血者と比較して有意に低く、ウイルスキャリアの母親の授乳を避けることを指導した県をあげての対応が陽性率の低下に貢献していることが示されたとの報告である。	日本赤十字社では、HTLV-1のスクリーニング検査を行っている。今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-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

(二)

Basic Science and Clinical Practice in Blood Transfusion

Basic Science and Clinical Practice in Blood Transfusion

Trend in Prevalence of Human T-Lymphotropic Virus Type-1 (HTLV-1) Infection in Japanese Blood Donors, Nagasaki, 1999 to 2006.

Masako Iwanaga, MD, MPH[1,*], Shin Chiyoda, MD[2,*], Eisuke Kusaba, MD[3,*], Shimeru Kamihira, MD[4,*] (Intr. by Yasuaki Yamada)

[1] Department of Hematology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; [2] The Nagasaki Red Cross Blood Center, Nagasaki, Japan; [3] The Sasebo Red Cross Blood Center, Sasebo, Japan and [4] Department of Laboratory Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan.

Abstract

To aluate time-trend of HTLV-1 prevalence and the effect of preventative measure against the viral transmission are important in the virus endemic regions. In Nagasaki, Japan, an endemic area of HTLV-1, a routine serological virus screening for blood donors and a prefecture-wide intervention project (the ATL Prevention Program; APP) to prevent milk-borne transmission for the virus carrier mothers have been conducted since 1986 and 1987, respectively. However, the effects of both projects on the virus seroprevalence have not been well evaluated. In this study, we conducted trend analyses of age-specific, birth-year-specific, and period-specific seroprevalence of HTLV-1 for first-time blood donors who donated between January 1999 and December 2006. Among 55668 first-time donors (age at donation; 16–65 years, birth year; 1934–1990), 718 were test positive for HTLV-1, indicating that the overall seroprevalence was1.29% (95%CI, 1.20–1.39). Prevalence was significantly higher in women than men (1.53% vs. 1.13%; OR; 1.36, 95%CI; 1.17–1.57). Seroprevalence increased significantly with increasing age at donation from 0.70% at 16–25 years to 7.34% at over 56 years (Chi-square test, $P < 0.0001$). The annual prevalence was 1.32 in 1999, 1.31 in 2002, and 1.37 in 2006, indicating that there was no significant secular trend during 1999–2006 (P for trend=0.99). In analyses by age at donation, trends of HTLV-1 prevalence significantly de ied among age over 56 years (P for trend=0.02) and age 16–25 years (P for trend=0.0007), whereas in birth-year-specific analyses, there was no apparent change of the prevalence over time, except in birth year 1981–90 group in which the prevalence declined from 1.22% in 1999 to 0.44% in 2006 (P for trend < 0.0001). In analyses for limited birth year from 1985 to 1990, the seroprevalence declined from 0.75% in birth year 1985–86 group, 0.31% in 1987–88 group, to zero% in 1989–90 group (P for trend =0.0002). HTLV-1 seroprevalence was significantly lower among donors born in 1987–90 (after APP) than 1985–86 (before APP). These results indicate that a birth-year-specific analysis for HTLV-1 prevalence may be appropriate to evaluate secular trend since the virus mostly transmit during infancy, and that a prefecture-wide intervention, the refraining from breast-feeding by the virus carrier mothers, contributes a declining HTLV-1 seroprevalence in our region.

Footnotes

Disclosure: No relevant conflicts of interest to declare.

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2008. 3. 25	該当なし	

一般的名称	解凍人赤血球濃厚液	研究報告の公表状況	AABB Weekly Report. 2008 Feb 29.	公表国 米国
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)			

研究報告の概要

○インフルエンザパンデミックと血液供給に関するAABBの組織横断作業部会がパンデミック時に献血間隔の例外的な取り扱いを認めるようFDAに要求

インフルエンザパンデミックと血液供給に関するAABBの組織横断作業部会が、2月14日付で米国食品医薬品局に送付した文書である。

パンデミック時には、供血者が発症したり家族を看病したりするために、基準に合致する供血者の数が少なくなり、血液の安定供給に影響するという懸念が広がっている。

作業部会は、全血と赤血球採血の献血間隔を半分に短縮(8週間のところを4週間、16週間のところを8週間)することを提案した。これはパンデミック時の血液供給の問題を最小限にするためにFDAが取りうる手段としては最も効果的であると主張している。

また、作業部会の前回のミーティングでは、過去6ヵ月以内に血液が使用された供血者については、感染症検査の前に供給するという方法が紹介された。これに関してのFDAの意見を求めている。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
インフルエンザパンデミックと血液供給に関するAABBの組織横断作業部会はパンデミック時に献血間隔の例外的な取り扱いを認めるようFDAに求めているとの報告である。	日本赤十字社では家禽に高病原性トリインフルエンザの流行が認められた場合、当該飼養農場の関係者や防疫作業従事者の献血制限を行っている。新型インフルエンザが流行した場合、献血者減少につながることも予想される。今後も引き続き情報の収集に努める。

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IN THIS ISSUE

Advance Registration for 2008 Spring Conference Ends March 7

Only one week remains to register in advance for the 2008 AABB Spring Conference. After March 7, individuals who have not signed up for the conference can still attend but must register on-site. The conference, which will be held March 28-29 in Orlando, Fla., features educational sessions in four tracks — Blood Inventory Management, Cellular Therapy, Perioperative Blood Management and Tissue Management. Registration includes entrance to educational sessions in any of the four tracks, course materials, continental breakfast and access to the exhibits.

AABB Task Force Asks FDA for Exception to Interdonation Interval During Influenza Pandemic

The AABB Interorganizational Task Force on Influenza Pandemics and the Blood Supply sent a letter to the Food and Drug Administration on Feb. 14 asking the agency to review a template to be used by blood facilities to request an exception to the interdonation interval requirements in the event of an influenza pandemic. According to the letter, there is widespread concern that a pandemic would severely impact the availability of blood products by limiting the number of eligible donors. The task force stated that shortening the interdonation interval for whole blood and red blood cell collection is the most significant step the agency can take to minimize supply issues during a pandemic.

Report Finds Alternatives for Blood Irradiators, Other Radiation Sources to Improve National Security

EventCalendar

March 3-6 – The AIIM International Exposition & Conference read more »

March 4-5 – International Plasma Protein Congress read more »

March 5 – AABB Audioconference: Hematopoietic Cell Donation: Ensuring Safety for the Donor, Product and Recipient CT read more »

March 5-6 – Southeastern Area Blood Bankers Annual Meeting read more »

March 6-7 – President's Council on Bioethics Meeting read more »

March 19 – AABB Audioconference: Serological to Molecular Testing: Points to Consider for Successful Conversion read more »

Full Calendar read more »

New Web Resources

Updates to the Variances for Collection of Blood and Blood

Letter to the FDA on a Draft Template for a Shorter Inter-donation Interval During an Influenza Pandemic – 2/14/08

Alan Williams, PhD
Associate Director for Regulatory Affairs (HFM-300)
Food and Drug Administration
Center for Biologics Research and Review
Woodmont Office Center, Suite 400N
1401 Rockville Pike
Rockville, MD 20852

Dear Dr. Williams:

The AABB Interorganizational Task Force on Influenza Pandemics and the Blood Supply appreciates the Food and Drug Administration's (FDA) assistance in working to ensure that patients' blood needs can be met in the event of a severe influenza pandemic. As we have discussed, there is widespread concern that a severe pandemic will severely impact the availability of blood products as individuals who are sick or caring for sick loved ones will not be able to donate.

In an effort to prevent critical blood shortages, the Task Force has developed a template to request an exception for a shorter inter-donation interval for Whole Blood and Red Blood Cell donations in the event of a pandemic. Experience with donors with hemochromatosis has sho that donors can tolerate phlebotomy much more frequently than the standard eight-week requirement, so long as they have acceptable hemoglobin levels. The Task Force believes that allowance for a shorter inter-donation interval is the most significant step that FDA can take to alleviate shortages during a pandemic. We are asking FDA to acknowledge that the language proposed in the attached draft template is appropriate so that the Task Force can distribute it to blood collection facilities working to prepare for a severe pandemic.

In addition, during the last meeting of the Task Force, the concept of a "walking donor" pool – i.e., allowing blood collected from individuals who had successfully donated in the previous six months to be distributed prior to infectious disease testing, which would be completed subsequently – was introduced. The Task Force would welcome your expansion on how you see such an approach being applied. Would the donor examination or history be amended or truncated? Would testing requirements be relaxed? Please let us know your thoughts.

Thank you for your efforts to address blood supply issues relating to a possible influenza pandemic. The Task Force looks forward to your response to the above requests.

Sincerely,

Louis Katz, MD
Chair

Attachment

Cc: Elizabeth Callaghan

aft Request for Exception to Inter-Donation Interval in the Event of an Influenza Pandemic

Director, Division of Blood Applications (HFM-375)
Food and Drug Administration
Center for Biologics Research and Review
C/O Document Control Center (HFM-99)
Woodmont Office Center, Suite 200N
1401 Rockville Pike
Rockville, MD 20852-1448

n: (Blood Center/Bank Name)
(Mailing Address)
(License Number, if licensed, or Registration Number, if registered)

Request for Exception to Inter-Donation Interval in the Event of an Influenza Pandemic

uest: Our establishment requests an exception under 21 CFR 640.120 to allow, in the event of an influenza pandemic:

1. Single unit collection of Whole Blood and Red Blood Cells (RBCs) from donors who had donated a single unit less than eight weeks but at least four weeks previously, or a double unit of RBCs less than 16 weeks but at least eight weeks previously, provided the donor meets the other donor eligibility criteria in 21 CFR 640.3(b) and 640.3(c).
2. Double unit collection of Whole Blood and Red Blood Cells from donors who had donated less than sixteen weeks but at least eight weeks previously, provided the donor meets the other donor eligibility criteria in 21 CFR 640.3(b) and 640.3(c).

tification: There is widespread concern in the transfusion medicine community that a pandemic could severely impact the availability of od products in the United States since individuals who are sick or caring for sick loved ones will not be able to donate. This exception uld be granted to enhance the adequacy of the blood supply in the event of an influenza pandemic in the United States that the AABB *rorganizational Task Force on Domestic Disasters and Acts of Terrorism, with the concurrence of FDA, declares has or will negatively* ct the blood supply, either at a regional or national level.

re is substantial evidence that donors can tolerate a more frequent inter-donation frequency, as evidenced by the FDA guidance, *idance for Industry: Variance for Blood Collection from Individuals with Hereditary Hemochromatosis*", August 2001.

cerely,

sponsible Person

別紙様式第2-1

No. 16

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2008. 2. 18	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況	Robinson JL, Lee BE, Patel J, Bastien N, Grimsrud K, Seal RF, King R, Marshall F, Li Y. Emerg Infect Dis. 2007 Dec;13(12):1865-70.	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)			カナダ

研究報告の概要

○幼児におけるブタインフルエンザ(H3N2)感染および市中伝播の可能性:カナダ
ブタ起源と思われるインフルエンザ(H3N2)ウイルス(A/Canada/1158/2006)が、カナダの共同農場に居住する、7ヶ月齢の入院患児より分離された。当該患児は無事に回復した。A/Canada/1158/2006の赤血球凝集抑制反応を利用した血清学的検査を当該農場の農民90名のうち54名に実施した。初発患者およびその家族7名中4名、家族以外の農民46名中4名に血清陽性が示された;この前年に呼吸器疾患による入院歴を持つ者はいなかった。当該ブタインフルエンザ株の血清エビデンスは、当該農場のブタ10頭(12週齢～6ヶ月齢)中1頭に認められた。カナダではブタインフルエンザウイルスのヒトへの感染は、過少に評価されており、ウイルス株は農場に順応または再集合が可能であり、その結果、効率的なヒト-ヒト伝播にいたることから、パンデミックインフルエンザ対策の一環として、養豚業従事者の定期的な調査を検討すべきである。

報告企業の意見	今後の対応
ブタ起源と思われるインフルエンザH3N2ウイルスが、カナダの共同農場に居住する7ヶ月齢の入院患児より分離され、患児の家族7名中4名、家族以外の農民46名中4名と、当該農場のブタ10頭中1頭に血清陽性が認められたとの報告である。	新型インフルエンザが流行した場合、献血者減少につながることも予想される。今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
新鮮凍結血漿-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

(3)

Swine Influenza (H3N2) Infection in a Child and Possible Community Transmission, Canada

Joan L. Robinson,*† Bonita E. Lee,* Jagdish Patel,‡ Nathalie Bastien,§ Karen Grimsrud,¶ Robert F. Seal,† Robin King,‡ Frank Marshall,# and Yan Li†§

An influenza A virus (H3N2) of probable swine origin, designated A/Canada/1158/2006, was isolated from a 7-month-old hospitalized child who lived on a communal farm in Canada. The child recovered uneventfully. A serosurvey that used a hemagglutination-inhibition assay for A/Canada/1158/2006 was conducted on 54 of the 90 members of the farm. Seropositivity was demonstrated in the index patient, 4 of 7 household members, and 4 of 46 nonhousehold members; none had a history of hospital admission for respiratory illness in the preceding year. Serologic evidence for this strain of swine influenza was also found in 1 of 10 pigs (12 weeks–6 months of age) on the farm. Human infection with swine influenza virus is underrecognized in Canada, and because viral strains could adapt or reassort into a form that results in efficient human-to-human transmission, routine surveillance of swine workers should be considered as part of pandemic influenza preparedness.

Influenza A is endemic in a broad range of species, with avian and swine strains having the greatest potential for transmission to humans. Pandemics of influenza A occur when a major change occurs in the proteins of circulating strains of the virus. During the pandemics of the past century, this antigenic shift resulted from reassortment of human and avian strains or adaptation of avian viruses to facilitate person-to-person transmission (*1*). Avian influenza preferentially binds to sialic acid–galactose receptors with an α-2,3 linkage that is abundant on duck intestinal epithelium; human influenza preferentially binds to sialic acid–galactose receptors with an α-2,6 linkage that is abundant on human respiratory epithelium. The respiratory epithelium of swine contains both types of receptors and can potentially be simultaneously infected with avian and human influenza (*2*). Human infection with avian influenza subtype H5N1 is of great concern, with 194 deaths of 321 cases reported worldwide through August 16, 2007 (*3*). Swine infected with avian subtype H5N1 have been identified in Vietnam (*4*), raising the possibility that swine could act as the "mixing vessel" that allows avian influenza (H5N1) to reassort with a human influenza strain, resulting in a virus with high pathogenicity and a high potential for person-to-person spread.

Another theoretical mechanism for the origin of an influenza pandemic would be the adaptation of a swine strain that results in efficient person-to-person transmission, although cross-protection by antibodies to recently circulating human strains may prevent this from occurring with swine influenza virus (SIV) H1 and H3 strains. Infection of humans with SIV was first recognized in 1974 with an H1N1 strain (*5*); the solitary outbreak occurred in military recruits at Fort Dix, New Jersey, USA, in 1976 (*6*). Human infection with SIV subtype H3N2 was first described in Europe in 1993 (*7*). The first reported case of probable infection of a person in North America with a non-H1N1 subtype of SIV occurred in Ontario, Canada, in 2005 with an H3N2 strain detected in the respiratory tract of an adult with no serologic evidence of infection (*8*). We describe a case of SIV (H3N2) infection in a Canadian infant, confirmed by viral isolation and serologic testing.

*Public Health and Provincial Laboratory (Microbiology), Edmonton, Alberta, Canada; †University of Alberta, Edmonton, Alberta, Canada; ‡Alberta Agriculture and Food, Edmonton, Alberta, Canada; §National Medical Laboratory, Winnipeg, Manitoba, Canada; ¶Alberta Health and Wellness, Edmonton, Alberta, Canada; and #Marshall Swine Health Service, Camrose, Alberta, Canada

Case Report

A 7-month-old boy was admitted to the hospital on September 10, 2006, with a 3-day history of fever, rhinitis, and cough. He had had no previous contact with ill persons. The child was born at term and was hospitalized for 21 days at 5 weeks of age when he received ventilation for 6 days for pneumonia due to respiratory syncytial virus. He lived on a communal farm (90 occupants) with horses, cows, swine, sheep, dogs, cats, turkeys, geese, ducks, and chickens but had no direct contact with the animals. The swine were contained in barns and did not mix with the other animals. His household contacts did not work directly with animals, but his father occasionally spent time in the barns, and his uncle, who lived next door, worked in the swine barns.

On admission, the child was afebrile with a heart rate of 120 beats/min, respiratory rate 56/min, and oxygen saturation of 85% on room air. Diffuse wheeze was noted. Chest radiograph results were unremarkable. Direct fluorescent antibody testing on a nasopharyngeal aspirate was positive for influenza A, and the virus was isolated in rhesus monkey cell culture. The isolate was sent to the National Microbiology Laboratory for influenza subtyping as a requirement of the Canadian influenza surveillance program, where it was subsequently designated A/Canada/1158/2006. The child stayed in the hospital for 2 days and then made an uneventful recovery at home. A cough and rhinitis developed in his 19-month-old brother on the day the index patient was admitted to the hospital, but the brother was not assessed by a physician.

Methods

Antigenic Analysis

For the antigenic characterization of A/Canada/1158/2006, hemagglutination-inhibition (HI) assay was performed by using 4 hemagglutination units of virus, 0.7% v/v guinea pig erythrocytes, and postinfection fowl serum specimens for the currently circulating human strains (A/New Caledonia/20/99 [H1N1], A/Wisconsin/67/2005 [H3N2]), past circulating human strains (A/Panama/2007/99 and A/Nanchang/933/95), and swine serum for A/Swine/Texas/4199–2/98 (H3N2) treated with receptor-destroying enzyme (*9*).

Molecular Characterization

All 8 RNA segments of A/Canada/1158/2006 were amplified by reverse transcriptase–PCR (RT-PCR) and sequenced. A universal primer set for the full-length amplification of all influenza A viruses was used for the RT-PCR (*10*). Viral RNA was extracted from 100 μL of tissue culture fluid with the RNeasy Mini Kit (QIAGEN, Mississauga, Ontario, Canada). Viral RNA was amplified in a OneStep RT-PCR reaction (QIAGEN) following the manufacturer's recommendations. Briefly, 5 μL RNA was added to the RT-PCR mixture containing 2 μL QIAGEN OneStep RT-PCR enzyme mix, 10 μL 5× QIAGEN OneStep RT-PCR buffer, 400 μmol/L dNTP, 0.6 μmol/L of each primer, and 10 μL Q-solution in a final volume of 50 μL. The conditions used for the Gene Amp 97700 (Applied Biosystems, Streetsville, Ontario, Canada) thermocycler were as follows: 50°C for 30 min for reverse transcription, 95°C for 15 min for the activation of the HotStart DNA polymerase; then 35 cycles of 94°C for 20 s, 58°C for 30 s, 72°C for 4 min, followed by an extension of 10 min at 72°C. The PCR products were purified by using QIAquick PCR purification kit (QIAGEN) and sequenced on an ABI 377 Sequencer, using a fluorescent dye-terminator kit (Applied Biosystems). The DNA sequences were assembled and analyzed with SEQMAN, EDITSEQ, and MEGALIGN programs in Lasergene (DNASTAR, Madison, WI, USA). Phylogenetic trees were generated by the neighbor-joining method using the MEGA program (*11*).

Serologic Testing

Once it became evident that A/Canada/1158/2006 was closely related to swine influenza viruses, HI was performed on serum specimens collected from the index patient, the symptomatic sibling, and both parents 29 days after the hospitalization. To further investigate the spread of SIV to humans, approval was then granted by the Health Research Ethics Board of the University of Alberta to obtain information and serum specimens from other members of the communal farm. The study team visited the farm 3 months after the hospitalization of the index patient and explained the study to the occupants. Serum specimens were then collected from the other 4 siblings of the index patient and 46 other occupants who lived in a total of 17 households. Participants provided the following data: age, exposure to swine (none, <1 hour/week, or ≥1 hour/week), and history of influenza-like illnesses (ILI; defined as cough and fever) in the preceding year. Serum samples were tested by using an HI assay against the currently circulating human strains A/New Caledonia/20/99 (H1N1), A/Wisconsin/67/2005 (H3N2), and the isolate from the index patient, A/Canada/1158/2006. HI titers were defined as the reciprocal of the highest dilution of serum that completely inhibited hemagglutination of a 0.7% solution of guinea pig erythrocytes. Specimens were considered seropositive for influenza virus at a titer of ≥32.

Swine Investigation

The purpose of these investigations was to determine the extent of recent swine influenza in swine on the farm and to look for evidence of infection with the SIV strain isolated from the index child. The history of influenza or unexpected respiratory illness in the swine on the farm was

obtained. Nasal swabs were obtained from grower pigs (4 to 16 weeks of age) and processed by RT-PCR for influenza A matrix gene. Serologic testing for influenza, using an ELISA for H1N1 and H3N2 strains and HI for A/Canada/1158/06, was performed on samples from grower-finisher pigs (12 weeks to 6 months of age). Five grower pigs that were doing poorly were killed and pulmonary autopsies were performed. All swine used in these investigations were on the farm at the time the index child was ill.

Results

Antigenic and Molecular Characterization of A/Canada/1158/06

Initial HI testing showed that the isolate was not inhibited by antiserum against recent (A/Wisconsin/77/2005 and A/New Caledonia/20/99) and past (A/Panama/2007/99 and A/Nanchang/933/95) human influenza A strains but was inhibited by antiserum against A/swine/Texas/4199–2/98 (H3N2) virus with HI titer of 128. These findings indicate that the A/Canada/1158/06 virus was antigenically related to SIV (Table 1). The results also indicate that the assay is specific because no cross-reactivity was observed between the human reference strain antiserum and the swine influenza viruses (Table 1). Nucleotide sequences of the full-length coding regions of all 8 RNA segments of the isolate further determined that it was most closely related to A/swine/Ontario/33853/2005 (H3N2) virus, which shares the same human/classic swine/avian triple reassortant genotype as the H3N2 subtype viruses that emerged in swine in the United States in 1998 (*8*). Sequence analysis showed that nucleic acid homology between A/Canada/1158/2006 and A/swine/Ontario/33853/2005 ranges from 98.4% (HA) to 100% (M1), and that amino acid (aa) identities range from 97.9% (HA) to 100% (NP, NS2, M1). A deletion of 4 aa at position 156–159 was observed in the HA1 region of the A/Canada/1158/2006 HA protein. Amino acid substitutions were found in the HA (HA1 domain: G7, K142, S162; HA2 domain: T77, Q139, M149, E150, N160), neuraminadase (NA) protein (P45, K74, N150, M349, L354), NS1 (M112), PB1 (K211, D738), PB2 (K368, S661, T722), and PA (V44, R99, I42) proteins. Phylogenetic analysis showed that all of the genes of A/Canada/1158/06 clustered with Canadian swine isolates from 2005 (*9*) (data not shown). Nucleic acid identity between the HA and NA genes of A/Canada/1158/06 and the current vaccine strain A/Wisconsin/67/05 was 90.9% and 94.6%, and the aa identities were 90.2% and 94.5%, respectively.

Serologic Testing

Seropositivity (HI titer ≥32) to A/Canada/1158/2006 was demonstrated in the index patient, the symptomatic sibling, 1 asymptomatic sibling, and both parents (Table 2, household A). Three other siblings were seronegative. Four children from 2 other households were also seropositive (Table 2, households B and C); the father from household B, 1 other child from household B, and the mother from household C were seronegative. The father from household C worked in the swine barn but was unavailable for testing. History of ILI within the preceding 12 months in seropositive participants was reported only for the index patient and for a 3-year-old girl from household C who was not hospitalized or tested for influenza virus during her illness. Seronegative results were obtained from another 20 adults (14 women and 6 men) and 19 children (8 girls and 11 boys) from 14 different households. For these households, swine exposure was reported as none for 9 adults and 7 children, <1 hour/week for 11 adults and 8 children, and ≥1 hour/week for 4 children including 3 teenagers who worked in the swine barns. When serum samples from the 54 participants in the study were tested for HA-specific antibodies to the current human influenza A virus H3N2 and H1N1 subtypes, one of the patients who was seropositive for SIV at a titer of 32 had an identical titer for A/Wisconsin/67/2005 (H3N2) (Table 2), and one of the adults who was seronegative for SIV had a titer of 32 for A/New Caledonia/20/99 (H1N1) (data not shown). All other persons tested were seronegative for the 2 human strains of influenza.

Table 1. Hemagglutination-inhibition reaction of A/Canada/1158/2006 isolates with reference antiserum against currently circulating human and swine viruses

	Antiserum (titers)				
Antigen	A/New Caledonia/20/99 (human H1N1)	A/Wisconsin/67/2005 (human H3N2)	A/Panama/2007/99 (human H3N2)	A/Nanchang/933/95 (human H3N2)	A/Swine/Texas/4199–2/98 (swine H3N2)
Control					
A/New Caledonia/20/99 (human H1N1)	320	<4	<4	<4	<4
A/Wisconsin/67/2005 (human H3N2)	<4	320	64	8	8
A/Ontario/RV1273/2005 (swine H3N2)	<4	<4	<4	<4	256
Patient					
A/Canada/1158/2006	<4	<4	<4	<4	128

Table 2. Clinical features and hemagglutination-inhibition reaction of positive antiserum from 9 members of 3 different households of a communal farm with recently circulating swine influenza (H3N2) virus A/Canada/1158/2006*

Household	Age, y	Sex	A/Wisconsin/ 67/2005 titer	A/New Caledonia/ 20/99 titer	A/Canada/ 1158/2006 titer	Swine exposure	Clinical features
A (index patient)	0.6	M	<4	<4	256	None	Hospitalization with ILI and isolation of swine influenza
A†	1	M	<4	<4	256	None	None (URI coincident with ILI in index case)
A	35	F	<4	<4	32	None	None
A	38	M	8	<4	32	<1 h/wk	None
A	8	M	<4	<4	64	<1 h/week	None
B	7	M	32	<4	32	<1 h/wk	None
C	8	M	4	<4	64	≥1 h/week	None
C	5	M	<4	<4	128	<1 h/week	
C	3	F	<4	<4	128	None	ILI 1 mo before index case

*URI, upper respiratory illness; ILI, influenza-like illness.
†Symptomatic sibling.

Swine Investigation

Influenza (H3N2) was last documented in the swine herd in September 2005. The herd received breeding animals from a Manitoba herd, where swine influenza of an unknown subtype had recently been documented. Nasal swabs collected from 25 grower pigs ≈3 weeks after the index child was ill were negative for SIV. Serum specimens obtained from 10 grower-finisher pigs were all negative by ELISA for swine influenza (H1N1), but 4 were positive for swine influenza (H3N2) strains, with 1 of these 4 strains being seropositive for A/Canada/1158/2006 by HI assay (HI titer 32). Results of the lung autopsies all showed evidence of subacute bronchointerstitial pneumonia, varying from mild to moderate. Lesions typical for swine influenza were not noted, but an initial insult due to SIV could not be excluded.

Discussion

We describe an infant with virologic and serologic evidence of infection with SIV (H3N2) and an ILI. Serologic evidence of infection with the same strain was found in 4 of 7 household members and in 3 of 46 nonhousehold contacts, with only 1 of the seropositive patients having a history of an ILI within the preceding year, which demonstrated unrecognized human infection with SIV. This relatively high seroprevalence is in contrast to a recent outbreak of avian influenza (H7N3) in which seropositivity was not documented in 91 persons exposed to infected poultry, including 2 poultry workers from whom the virus was isolated (*12*). The difference in the apparent incidence of infection may be explained in part by the fact that culling of infected poultry occurred immediately; in our study, infection of swine was not recognized and long-term human exposure may have occurred.

Infection of swine with human influenza viruses has been recognized for decades (*2*); in a recent US study, 22.8% of pigs were seropositive for human influenza viruses, although some may have had vaccine-induced immunity (*13*). Swine influenza (H3N2) emerged in 1998 in the United States, where subtype H1N1 viruses had predominated for 60 years (*2*). The isolate from this current study is closely related to triple reassorting genotype viruses that spread rapidly throughout the US swine population and have HA, NA, and RNA polymerase (PB1) genes of human influenza virus lineage; nucleoprotein, matrix, and nonstructural genes of classic swine influenza (H1N1) lineage; and RNA polymerase (PA and PB2) genes of North American avian virus lineage (*8*). However, triple reassortant SIV was not documented in swine in Canada until 2005 (*8*), which makes it unlikely that human cases occurred before that year and that seroreversion had occurred in any of the persons in the current serosurvey.

A previous study showed cross-reactivity in HI assay between the vaccine strain A/Panama/2007/99 reference antiserum and the triple reassortant A/swine/Minnesota/593/99, which is not unexpected since the HA gene of the triple reassortant viruses is a descendant of human viruses that circulated in 1995 (*14,15*). However, no cross-reactivity was observed between the reference human strain antiserum and the isolate from this study, which suggests that the seroconversion observed was indeed due to infection with swine influenza (H3N2) and not to cross-reactive antibody to human influenza (H3N2) infection. The low rate of seropositivity to recently circulating strains of human influenza in the study is likely explained by the fact that the farm is a relatively closed community. The child who was seropositive for both human and swine influenza viruses was likely exposed to both viruses. The HA protein of A/Canada/1158/2006 diverges significantly from the one of A/Wisconsin/67/2005, and antiserum against A/Wisconsin/67/2005 does not inhibit A/Canada/1158/2006 in HI assay.

Swine influenza (H3N2) has recently reassorted with H1N1 strains to produce H1N2 subtypes and has spread to turkeys in the United States (*16*) and Canada (*8*). A 4-aa deletion was found in the HA protein of A/Canada/1158/2006 when compared with similar swine influenza (H3N2)

strains currently circulating in North America. This region of the protein has been assigned to antigenic sites (*17*) and has been associated with adaptation to growth in eggs (*18*). Phylogenetic analysis showed that each of the 8 viral genes of A/Canada/1158/2006 clustered with A/swine/Ontario/33853/2005 (H3N2) and other swine/turkey Canadian isolates from 2005. Although the HA gene of these isolates were shown to be closely related to American viruses that were first isolated from pigs in 1999, they represent a new distinct cluster (*2*). The NA genes are phylogenetically distinct from the US swine isolates and are represented by human influenza (H3N2) isolates from Asuncion, Paraguay (2001), and New York (2003) (*2*).

A recent review described 50 cases of symptomatic human infection with SIV, documented in the literature through April 2006; 46 cases were infected with subtype H1N1 and 4 were infected with subtype H3N2 (*19*). The spectrum of pathogenicity of SIV infection ranges from asymptomatic infection (*6*) to death; 7 of these 50 patients died (*5,20–24*). Laboratory-confirmed swine influenza in humans may be "the tip of the iceberg." Diagnosis of the current case was serendipitous because typing was performed only because the case occurred outside of influenza season.

The mode of spread of SIV in humans is not established. Because of his young age, the index patient was not likely to have had unrecognized direct contact with swine. That aerosolization of influenza virus occurs is increasingly recognized (*25*), but the child was reportedly never in the barns that housed the swine. However, other members of the farm reported that infants were sometimes taken for walks through the barn. The child also may have acquired the virus from person-to-person spread or from fomites. All 13 patients in the Fort Dix outbreak and 15 of 37 previously reported civilian case-patients also had no swine contact (*19,20*).

The Fort Dix outbreak of SIV in humans lasted only 21 days and never spread outside the military base. The calculated basic reproductive rate (R_0) was only 1.1 to 1.2. This suggests that person-to-person spread of the implicated H1N1 strain was not efficient enough to produce a major epidemic (*26*). However, future strains of SIV could have a higher R_0, and documentation of a case of swine influenza (H3N2) in a child with unrecognized transmission within the community adds another possible mechanism by which major epidemics of influenza could arise. Swine influenza infection in humans most commonly results in either no symptoms or a self-limited illness (*6*). However, routine surveillance for cases among swine workers may enable early detection of a strain with the potential for person-to-person transmission, prompting institution of infection control measures and vaccine development.

Dr Robinson is a pediatric infectious diseases physician at the Stollery Children's Hospital with an appointment at the Public Health and Provincial Laboratory (Microbiology) in Edmonton, Alberta, Canada. Her research interests center around the clinical features and prevention of viral respiratory infections.

References

1. Belshe RB. The origins of pandemic influenza—lessons from the 1918 virus. N Engl J Med. 2005;353:2209–11.
2. Olsen CW. The emergence of novel swine influenza viruses in North America. Virus Res. 2002;85:199–210.
3. World Health Organization. Confirmed human cases of avian influenza A (H5N1) [cited 2007 Aug 21]. Available from http://www.who.int/csr/disease/avian_influenza/country/en
4. Choi YK, Nguyen TD, Ozaki H, Webby RJ, Puthavathana P, Buranathal C, et al. Studies of H5N1 influenza virus infection of pigs by using viruses isolated in Vietnam and Thailand in 2004. J Virol. 2005;79:10821–5.
5. Smith TF, Burgert EO Jr, Dowdle WR, Noble GR, Campbell RJ, Van Scoy RE. Isolation of swine influenza virus from autopsy lung tissue of man. N Engl J Med. 1976;294:708–10.
6. Top FH Jr, Russell PK. Swine influenza A at Fort Dix, New Jersey (January–February 1976). IV. Summary and speculation. J Infect Dis. 1977;136:S376–80.
7. Claas EC, Kawaoka Y, de Jong JC, Masurel N, Webster RG. Infection of children with avian-human reassortant influenza virus from pigs in Europe. Virology. 1994;204:453–7.
8. Olsen CW, Karasin AI, Carman S, Li Y, Bastien N, Ojkic D, et al. Triple reassortant H3N2 influenza A viruses, Canada, 2005. Emerg Infect Dis. 2006;12:1132–5.
9. Kendal AP, Pereira MS, Skehel JJ. Concepts and procedures for laboratory-based influenza surveillance. Geneva: World Health Organization; 1982.
10. Hoffmann E, Stech J, Guan Y, Webster RG, Perez DR. Universal primer set for the full-length amplification of all influenza A viruses. Arch Virol. 2001;146:2275–89.
11. Kumar S, Tamura K, Jakobsen IB, Nei M. MEGA2: molecular evolutionary genetics analysis software. Bioinformatics. 2001;17:1244–5.
12. Skowronski DM, Li Y, Tweed SA, Tam TW, Petric M, David ST, et al. Protective measures and human antibody response during an avian influenza H7N3 outbreak in poultry in British Columbia, Canada. CMAJ. 2007;176:47–53.
13. Choi YK, Goyal SM, Joo HS. Prevalence of swine influenza virus subtypes on swine farms in the United States. Arch Virol. 2002;147:1209–20.
14. Zhou NN, Senne DA, Landgraf JS, Swenson SL, Erickson G, Rossow K, et al. Genetic reassortment of avian, swine, and human influenza A viruses in American pigs. J Virol. 1999;73:8851–6.
15. Myers KP, Olsen CW, Setterquist SF, Capuano AW, Donham KJ, Thacker EL, et al. Are swine workers in the United States at increased risk of infection with zoonotic influenza virus? Clin Infect Dis. 2006;42:14–20.
16. Choi YK, Lee JH, Erickson G, Goyal SM, Soo HS, Webster RG, et al. H3N2 influenza virus transmission from swine to turkeys, United States. Emerg Infect Dis. 2004;10:2156–60.
17. Wiley DC, Wilson IA, Skehel JJ. Structural identification of the antibody-binding sites of Hong Kong influenza haemagglutinin and their involvement in antigenic variation. Nature. 1981;289:373–8.
18. Katz JM, Wang M, Webster RG. Direct sequencing of the HA gene of influenza (H3N2) virus in original clinical samples reveals sequence identity with mammalian cell-grown virus. J Virol. 1990;64:1808–11.

19. Myers KP, Olsen CW, Gray GC. Cases of swine influenza in humans: a review of the literature. Clin Infect Dis. 2007;44:1084–8.
20. Gaydos JC, Top FH, Hodder RA, Russell PK. Swine influenza A outbreak, Fort Dix, New Jersey, 1976. Emerg Infect Dis. 2006;12: 23–8.
21. Patriarca PA, Kendal AP, Zakowski PC, Cox NJ, Trautman MS, Cherry JD, et al. Lack of significant person-to-person spread of swine influenza-like virus following fatal infection in an immunocompromised child. Am J Epidemiol. 1984;119:152–8.
22. Wells DL, Hopfensperger DJ, Arden NH, Harmon MW, Davis JP, Tipple MA, et al. Swine influenza infections. Transmission from ill pigs to humans at a Wisconsin agricultural fair and subsequent probable person-to-person transmission. JAMA. 1991;265:478–81.
23. Wentworth DE, Thompson BL, Xu X, Regnery HL, Cooley AJ, McGregor MW, et al. An influenza A (H1N1) virus, closely related to swine influenza virus, responsible for a fatal case of human influenza. J Virol. 1994;68:2051–8.
24. Kimura K, Adlakha A, Simon PM. Fatal case of swine influenza virus in an immunocompetent host. Mayo Clin Proc. 1998;73:243–5.
25. Tellier R. Review of aerosol transmission of influenza A virus. Emerg Infect Dis. 2006;12:1657–62.
26. Krause R. The swine flu episode and the fog of epidemics. Emerg Infect Dis. 2006;12:40–3.

Address for correspondence: Joan L. Robinson, Room 8213, Aberhart Centre 1, 11402 University Ave, NW, University of Alberta, Edmonton, Alberta T6G 2J3, Canada; email: jr3@ualberta.ca

別紙様式第2

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医薬品　研究報告　調査報告書

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一般的名称			研究報告の公表状況	Clinical and virological characterization of persistent human infection with simian foamy viruses. AIDS research and human retroviruses, Nov 2007, 23	公表国 米国	
販売名（企業名）	タココンブ（CSL ベーリング株式会社）					

研究報告の概要 91

問題点（SFV 感染者は輸血や移植）

HIV と HTLV の病原性のレトロウイルスは、非ヒト霊長類から SIV と STLV のヒトへの感染が定着し、人から人への感染が拡大した。simian foamy viruses (SFV) 感染は、捕獲された非ヒト霊長類間でかなり伝播している。職業上非ヒト霊長類やその体液と接触したり、岐まれたり、引掻かれた人達に SFV 感染が確認されてきた。遺伝子配列を解析すると、人への SFV 感染はチンパンジー、ヒヒ、アフリカサバンナモンキーなど多様な非ヒト霊長類を起源としていることが分かっている。

筆者らは、SFV 感染した人 13 名の中で 7 名を長期追跡調査した。遺伝子配列を解析すると、SFV は参加者 2 名がヒヒ由来であり、他の 5 名はチンパンジー由来であった。

参加者 7 名は、非ヒト霊長類やその唾液、尿などの体液に接する機会があり、体液が参加者の皮膚と粘膜と暴露したり、皮膚損傷があると報告している。

全ての参加者の抹消血単核球（PBMC）から SFV DNA が検出され、口腔の検体、尿、精液からも検出された。

自己申告による健康状態を検討したが、SFV 感染と関連がある共通した臨床的な症候群は示唆できなかった。

臨床検査を調べ最も興味ある事は、参加者 3 名で好酸球減少、血小板減少の軽度の異常があったことである。しかし臨床上重要ではない。

参加者 3 名の妻を SAV 感染者との接触者として、ウエスタンブロット法と PCR 法で調べたが陰性であった。少人数での限られた追跡調査期間での観察のため、SFV 感染に関連する病状や人の二次的 SFV 感染は特定できなかった。

筆者らは、人における感染は医学的に重要性が解明されていないので、SFV 感染者は輸血や移植を控えるべきであると警告している。

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
SFV 感染の人での病態や二次感染について明確になっていない。SFV はレトロウイルスであることから、本剤の製造工程で不活化できると考えられる。	今後とも情報収集に努める所存である。

(4)

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Clinical and Virological Characterization of Persistent Human Infection with Simian Foamy Viruses

ROUMIANA S. BONEVA,[1,*] WILLIAM M. SWITZER,[1,†] THOMAS J. SPIRA,[2,‡] VINOD B. BHULLAR,[1,§] VEDAPURI SHANMUGAM,[1,‡] MIAN-ER CONG,[1,†] LEE LAM,[2,**] WALID HENEINE,[1,†] THOMAS M. FOLKS,[1,†] and LOUISA E. CHAPMAN[1,††]

ABSTRACT

Persons occupationally exposed to nonhuman primates (NHPs) can be persistently infected with simian foamy virus (SFV). The clinical significance and person-to-person transmissibility of zoonotic SFV infection is unclear. Seven SFV-infected men responded to annual structured interviews and provided whole blood, oral, and urogenital specimens for study. Wives were tested for SFV infection. Proviral DNA was consistently detected by PCR in PBMCs of infected men and inconsistently in oral or urogenital samples. SFV was infrequently cultured from their PBMCs and throat swabs. Despite this and a long period of intimate exposure (median 20 years), wives were SFV negative. Most participants reported nonspecific symptoms and diseases common to aging. However, one of two persons with mild thrombocytopenia had clinically asymptomatic nonprogressive, monoclonal natural killer cell lymphocytosis of unclear relationship to SFV. All participants worked with NHPs before 1988 using mucocutaneous protection inconsistently; 57% described percutaneous injuries involving the infecting NHP species. SFV likely transmits to humans through both percutaneous and mucocutaneous exposures to NHP body fluids. Limited follow-up has not identified SFV-associated illness and secondary transmission among humans.

INTRODUCTION

TWO PATHOGENIC RETROVIRUSES, human immunodeficiency virus (HIV) and human T-lymphotropic virus (HTLV), established endemicity in human populations following infection of individual humans with simian immunodeficiency viruses (SIV) and simian T cell lymphotropic viruses (STLV) from nonhuman primate (NHP) reservoirs, respectively, and viral adaptation facilitating human-to-human spread.[1] Continued direct contact between human and NHPs in occupational and other settings provides an ongoing opportunity for introduction of additional simian retroviruses across species into human populations.

Foamy viruses (FVs), retroviruses in the *Spumavirus* genus,[2,3] establish persistent infections endemic to many mammalian species. Simian FV (SFV) infection is highly prevalent among captive NHPs.[4–6] Despite SFV coevolving with primates for at least 30 million years,[7] endemically infected human populations have not been identified.[6] A prototype FV (PFV), previously termed "human" FV (HFV) because it was isolated from a nasopharyngeal carcinoma (NPC) from a Kenyan man in 1971,[8] is now known to be of chimpanzee origin.[9,10] SFV infections have been identified in persons exposed directly to NHPs and their body fluids occupationally or through hunting, butchering, or habitat sharing.[10–16] Sequence analysis suggests

[1]HIV and Retrovirology Branch, Division of AIDS, STD and TB Laboratory Research (DASTLR), National Center for Infectious Diseases (NCID), Centers for Disease Control and Prevention (CDC), Atlanta, Georgia 30333.
[2]Immunology Branch, DASTLR, NCID, CDC, Atlanta, Georgia 30333.
*Present address: Chronic Viral Diseases Branch, Division of Viral and Rickettsial Diseases, National Center for Zoonotic, Vector-Borne and Enteric Diseases, Coordinating Center for Infectious Diseases (CCID), Centers for Disease Control and Prevention (CDC), Atlanta, Georgia 30333.
†Present address: Laboratory Branch, Division of HIV/AIDS Prevention, National Center for HIV, Hepatitis, STD, and TB Prevention (NCHHSTP), CCID, CDC, Atlanta, Georgia 30333.
‡Present address: International Laboratory Branch, Global AIDS Program, NCHHSTP, CCID, CDC, Atlanta, Georgia 30333.
§Present address: National Center for Prevention, Detection and Containment of Infectious Diseases, CCID, CDC, Atlanta, Georgia 30333.
**Present address: Office of the Director, NCHHSTP, CCID, CDC, Atlanta, Georgia 30333.
††Present address: National Center for Immunizations and Respiratory Diseases, CCID, CDC, Atlanta, Georgia 30333.

that these human SFV infections originated from multiple NHP species, including chimpanzee, baboon, African green monkey, macaque, De Brazza's guenon, mandrill, and gorilla.[4,6,10] Putative associations between FV infection and various human diseases, including NPC, have not been supported in well-designed studies.[6,17,18]

Unlike HIV and HTLV, the clinical significance and secondary transmissibility of human infections with SFV are unknown. SFV is strongly cytopathic in human and NHP cells *in vitro*,[6] but is not recognized to be associated with disease in any natural host, though this has not been systematically evaluated. SFVs are easily transmitted between NHPs, mostly by contact with oral secretions during grooming or biting.[5,6]

Surveillance for simian retrovirus infection in persons occupationally exposed to NHPs at research centers and zoos in North America identified 14 persons with serological evidence of SFV infection.[10,12] Using archived sera, most workers were shown to have long-standing zoonotic infection with unclear exposure risks or public health significance [10,12] We report preliminary results from the first prospective cohort study of persons persistently infected with SFV. We characterize the potential for secondary transmission by determining the presence of virus in various body fluids and by longitudinal testing of intimate contacts, evaluate the health status of humans persistently infected with SFV, and assess possible occupational risk exposures leading to infection.

MATERIALS AND METHODS

Study design and enrollment

Persons with documented SFV infection identified through surveillance of occupationally exposed workers were eligible to provide informed consent and enroll as primary participants in a prospective cohort study approved by the Centers for Disease Control and Prevention (CDC) Institutional Review Board.[10,12] Documented SFV infection was defined as seroreactivity to SFV antigens by Western blot (WB) combined with evidence of proviral DNA sequences in peripheral blood mononuclear cells (PBMCs) by polymerase chain reaction (PCR) and/or isolation of SFV from PBMCs.[10,12]

Spouses/partners and children of SFV-infected humans or other persons living in the same household were also eligible to enroll as contact participants. Informed consent was obtained from all participants before enrollment. All enrollees were offered follow-up for a minimum of 5 years.

Participant interviews

Participants were interviewed by telephone at enrollment and annually for 5 years using a standard questionnaire. Information was collected on demographics and health status at the time of enrollment. Participants were asked about their general health status including personal health observations and conditions diagnosed by physicians and about symptoms known to be associated with retroviral infections such as malignancies or lymphoproliferative, inflammatory, and neurological diseases.[3] To evaluate exposure opportunity, information was collected on the duration of occupational exposure to specific NHP species, work activities and practices including use of protective equipment, past injuries, and specific exposures to NHP blood or body fluids. To identify opportunities for secondary transmission, participants were questioned about sexual contacts, practices, and other activities that may result in intimate exchange of body fluids, including donation of blood or other living biological material. Participants were counseled regarding current knowledge about human SFV infection and provided the opportunity to ask questions.

Specimen collection and preparation

Whole blood, parotid saliva, swabs of saliva and the posterior oropharynx ("throat swabs"), urine, and semen (all primary participants were male) were requested from participants annually for clinical, virological and immunological testing. Parotid saliva was collected in intraoral (Schaefer) cups and immediately transferred with a pipette to cryovials.[19] Throat and saliva swabs were collected with viral culturettes (Becton/Dickinson). Nonblood specimens were shipped to the CDC immediately after collection on wet ice; whole blood was shipped at room temperature.

Upon arrival at the CDC parotid saliva was centrifuged for 2 min at $1000 \times g$ and cell pellets and supernatant were aliquoted and frozen at $-80°C$ until tested. Throat and saliva swabs were placed in 2 ml phosphate-buffered saline (PBS), vortexed, and then centrifuged for 5 min at $1000 \times g$ to pellet any cells present. The cell pellet was washed twice with PBS, then divided equally for PCR testing and for tissue culture for some participants. Urine and, when available, semen samples were centrifuged for 10 min at $800 \times g$ to pellet any cells present and washed twice with PBS and stored at $-80°C$. Urine and semen supernatants were aliquoted and stored at $-80°C$ for mucosal immunity studies.[19]

Clinical laboratory testing

Clinical testing was performed by a commercial diagnostic laboratory. Clinical testing included complete blood counts (CBC) with differential analysis of white blood cells, testing of serum for electrolytes, glucose, creatinine, blood urea nitrogen, uric acid, total protein, albumin and globulin, total bilirubin, adenosine phosphatase, lactate dehydrogenase (LDH), serum aspartate aminotransferase (AST), and serum alanine aminotransferase (ALT).

Virological and immunological analysis

DNA lysates were prepared from PBMCs and from pelleted cells from parotid saliva, mixed saliva, throat swabs, and urine and tested for SFV polymerase (*pol*) proviral sequences using nested PCR.[10,12] DNA quality was confirmed by β-actin PCR as previously described.[10,12] Virus isolation was attempted from selected participant's PBMCs, throat, and saliva samples. Specimens were cultured on canine thymocytes and/or *Mus dunni* fibroblasts and monitored biweekly for up to 40 days for cytopathic effect, reverse transcriptase (RT) activity, and proviral *pol* sequences.[10,12]

Plasma was tested for SFV antibodies using a WB assay that can detect both monkey and ape SFV as described in detail elsewhere.[4,10,19,20] Saliva and urine were tested for the presence of anti-SFV IgG and IgA by WB analysis.[19]

To perform mononuclear cell phenotyping, 20 μl of well-mixed MultiTEST four-color reagent (CD3/CD8/CD45/CD4) (Becton Dickinson Biosciences, San Jose, CA) and 50-μl aliquots of EDTA-anticoagulated whole blood were added to a TruCOUNT tube (Becton Dickinson Biosciences, San Jose, CA) containing a known concentration of beads. The mixture was incubated for 20 min at room temperature in the dark before 450 μl of FACS lysing solution (Becton Dickinson Biosciences, San Jose, CA) was added. After 15 min of incubation, the lyse/no-wash-stained samples were analyzed with the FACSort flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA). Data were analyzed using MultiSET software (Becton Dickinson Immunocytometry Systems, San Jose, CA).

Contact enrollments

Contacts of SFV-infected participants were evaluated annually for evidence of SFV infection by WB testing of serum or plasma and PCR testing of PBMC DNA, but are not interviewed.

RESULTS

Primary participants

Seven of 13 persons eligible to participate enrolled for long-term follow-up. We refer to these participants as cases 2, 3, 6, 7, 9, 10, and 12 to be consistent with previous reports and the chronological order in which they were identified as SFV-infected.[10,12] Sequence analysis indicates that the virus infecting cases 2 and 3 originated from baboons while virus infecting the remaining five participants all originated from chimpanzees.[10,12] Case 11 dropped out of the initial surveillance study and was not available for further study.[10]

Table 1 summarizes demographic and exposure data of SFV-infected persons by enrollment status. Participants are not entirely representative of the eligible population, limiting interpretation of results. Male gender, higher level job status, and longer duration of both occupational exposure and SFV seropositivity are overrepresented among study participants.

All participants are male compared to four/six (67%) eligible persons who did not enroll ("nonparticipants"). Two of five (40%) animal caretakers, both animal care supervisors, the sole research associate, and two/four (50%) veterinarians participated. At the time SFV infection was confirmed, the median age of participants (median 56, range 41–62 years) was similar to that of three nonparticipants for whom age was available (median 57, range 49–58). Participants enrolled a median of 2 years (range 1–5) after infection was confirmed. Participants were exposed to NHPs longer prior to confirmation of infection than were five nonparticipants for whom adequate data were available (median 26, range 8–37 years versus median 19, range 10–29 years, respectively).

The availability of stored serum allowed determination of a minimal duration of seropositivity for six/seven participants and four/six nonparticipants. Prior to documentation of SFV infection,

TABLE 1. DEMOGRAPHIC AND EXPOSURE CHARACTERISTICS OF ELIGIBLE AND ENROLLED PARTICIPANTS

Case	*Sex*[a]	*SFV species origin*	*Occupation*	*Age when SFV infection documented (years)*	*Year SFV infection documented*	*Year first positive sera archived*	*Minimal duration (years) SFV infection when identified*	*Duration of NHP exposure (years) when SFV infection identified*
				Enrolled participants				
2	M	Baboon	Research associate	56	1996	1978	18	29
3	M	Baboon	Animal care supervisor	57	1996	1988	8	37
6	M	Chimpanzee	Veterinarian	60	1998	1981	17	19
7	M	Chimpanzee	Veterinarian	41	1999	1990	9	23
9	M	Chimpanzee	Animal caretaker	41	1999	1980	19	8
10	M	Chimpanzee	Animal care supervisor	50	1999	1976	23	26
12	M	Chimpanzee	Animal caretaker	62	2001	NA[b]	NA	32
				Eligible nonparticipants				
1	M	AGM[c]	Animal caretaker	57	1996	1995	1	19
4	M	Baboon	Veterinarian	49	1997	1994	3	20
5	M	Baboon	Veterinarian	58	1998	1979	19	29
8	M	Chimpanzee	Animal caretaker	NA	1999	1985	14	>14
13	F[d]	Chimpanzee	Veterinary technician	NA	2002	NA	NA	11
14	F	Chimpanzee	Animal caretaker	NA	2002	NA	NA	10
				Ineligible nonparticipants				
11	M	Chimpanzee-like[d]	Research technician	NA	1999	NA	NA	10

[a]M, male; F, female.
[b]NA, not available.
[c]AGM, African green monkey.
[d]Based on SFV-type-specific WB.

participants with available stored sera were seropositive a median 9 years longer than were nonparticipants (median 17.5, range 8–23 years versus median 8.5, range 1–19 years, respectively). Initial clinical testing of participants under this protocol occurred after a median of 19 years (range 2–24) of documented infection.

Exposure history and use of protective equipment

All seven participants reported direct, frequent, and intimate opportunity for exposure to NHPs and their biological fluids including animal saliva, urine, feces, and blood. All seven workers reported histories of both mucocutaneous exposures to NHP body fluids and skin-penetrating injuries. Five of seven participants (71%) described NHP bite wounds, five/seven (71%) described scratch wounds, and six/seven (86%) described percutaneous exposure to NHP body fluids via skin penetrating sharp injuries. However, only four/seven (57%) participants (cases 3, 6, 7, and 9) described a percutaneous injury that was associated with the NHP species from which sequence analysis suggests their infecting virus arose.

All participants reported currently wearing leather or latex gloves when handling NHPs. However, these seven participants worked with NHPs for a median of 16 years (range 5–29) prior to 1988 when universal precaution guidelines were established. Five workers consistently and two inconsistently wore gloves prior to 1988. All reported historically inconsistent use of face shields and goggles transitioning to more regular use in recent years. Two participants denied use of goggles or face shields for mucocutaneous protection; four reported wearing them 25–75% of the time for specific tasks such as working with chemicals or infected animals or cleaning cages. One wore face shields consistently "when required." The use of protective equipment was rare prior to 1988, and workers noted that current use of face shields did not always protect them from mucocutaneous exposure to NHP saliva and other body fluids.

Body distribution of SFV

The distribution of SFV in human body fluids is summarized in Table 2. SFV DNA sequences were found in PBMCs from all seven persons and in all of their 19 serial samples tested. Virus culture was attempted at least once on PBMCs from six/seven participants. SFV was isolated from only one/two PBMC samples from each of cases 6 and 10, representing 33% of persons and 20% of specimens from which virus culture was attempted.

SFV DNA was detected in oral cavity specimens from three/seven (43%) participants; overall, 6/16 (38%) throat swab samples from these seven participants were SFV DNA positive. Saliva specimens from two/seven (29%) persons were positive for SFV DNA; 6/23 (26%) serial samples were positive. Interestingly, SFV was isolated from throat swab samples from case 6, for whom six/seven (86%) oral cavity specimens were positive for SFV DNA. SFV was not isolated from saliva available from the other five participants (seven samples).

Although all subjects provided urine specimens, most (13/18, 72%) samples had insufficient cellular DNA present to support SFV PCR testing. Of five samples from four persons with sufficient cellular DNA, two persons (cases 3 and 6) were positive for SFV DNA. Two participants (cases 2 and 12) each provided two semen samples; a third (case 6) provided a single specimen. Specimens from case 2 (who had received a vasectomy more than 20 years ago) and case 12 tested negative for B-actin sequences, indicating the absence of cellular DNA or the presence of PCR inhibitors. Thus these semen specimens were not suitable for further PCR testing. The semen specimen from case 6, who had benign hemospermia, was positive for SFV DNA. Insufficient material was available to attempt virus isolation from semen or urine specimens.

SFV-specific immunoglobulin G (IgG) antibodies against structural (Gag) and accessory (Bet) proteins were detected by WB in plasma, saliva, and urine from participants. Immunoglobulin A antibodies were not detected in any specimens, as previously reported.[19]

Clinical status

Self-reported medical histories identified chronic conditions, nonspecific symptoms, and diseases of aging common in the U.S. population. In addition, case 9 reported congenital heart disease and mild thrombocytopenia since 1997 and case 3 underwent aortic valve replacement during this observational pe-

TABLE 2. PRESENCE OF SFV IN BODY COMPARTMENTS

	Number of specimens positive/number of specimens tested (%)					
	Peripheral blood mononuclear cells		*Oral cavity*[a]			
Enrollee	*SFV DNA (%)*	*SFV isolation (%)*	*SFV DNA (%)*	*SFV isolation (%)*	*Urine*[b] *SFV DNA (%)*	*Semen*[b] *SFV DNA (%)*
Case 2	2/2 (100)	0/1 (0)	0/6 (0)	0/2 (0)	0/1 (0)	NT[c]
Case 3	4/4 (100)	0/2 (0)	0/4 (0)	0/4 (0)	1/1 (100)	NT
Case 6	4/4 (100)	1/2 (50)	6/7 (86)	1/4 (25)	1/2 (50)	1/1 (100)
Case 7	4/4 (100)	0/2 (0)	0/9 (0)	0/4 (0)	0/1 (0)	NT
Case 9	1/1 (100)	0/1 (0)	1/2 (50)	0/2 (0)	NT[c]	NT
Case 10	3/3 (100)	1/2 (50)	0/6 (0)	0/2 (0)	NT	NT
Case 12	1/1 (100)	NT	3/5 (60)	NT	NT	NT
Total	19/19 (100)	2/10 (20)	10/39 (26)	1/18 (6)	2/5 (40)	1/1 (100)

[a]Includes throat and saliva swabs and parotid saliva.
[b]Specimen quantity insufficient for SFV isolation.
[c]NT, not tested.

riod. No symptom or diagnostic patterns suggested a common clinical syndrome associated with SFV infection.

Results of clinical laboratory testing for each participant are summarized in Table 3. Repeated clinical laboratory testing was within normal limits for cases 2, 10, and 12. Testing identified unremarkable patterns of mildly abnormal glucose and renal function tests compatible with a three decade history of diabetes (case 3) and of fluctuating mild liver transaminase elevations (case 7).

Clinical laboratory testing identified hematological abnormalities for three participants. Three times on annual testing case 6 had eosinophil counts at the lower limit of or below normal range; his eosinophil count was within normal limits on subsequent testing in May 2002. Mild thrombocytopenia without other blood count abnormalities was confirmed in case 9. In addition to mild laboratory abnormalities expected to accompany long standing diabetes, case 3 had the unexpected findings of intermittent mild thrombocytopenia accompanied by natural killer (NK) cell lymphocytosis (NKCL). NK cells [$CD3^-CD16^+56^+$ cells] at three time points were 44% (780/μl), 39% (854/μl), and 38% (840/μl), respectively (upper normal limit 25% or 480/μl). Additional hematological tests performed by a specialized laboratory on specimens collected 9 months apart (personal communication, W.G. Morice, II and A. Tefferi, Mayo Clinic) repeatedly showed that 40% of lymphocytes were CD16-positive, CD3-negative NK cells, expressing CD56 and CD57, and CD161 with partial loss of CD94. These findings along with other tested markers indicated monoclonal NKCL consistent with a chronic NK-large granular lymphocytosis (indolent large granular lymphocytosis) of unclear clinical significance. The patient has no related clinical symptoms.

Secondary transmission

Wives of cases 2, 3, and 9 were tested for SFV infections as contact participants. All remained WB and PCR negative despite a collective minimum of 57 person-years of intimate exposure to an SFV-infected partner (median 20, range 13–24 years). The wives of three additional nonparticipants also tested SFV negative.[10,12] All participants reported intimate marriages that provided frequent opportunity for exposure to saliva and other body fluids. All denied the routine use of barrier precautions or other practices documented to minimize sexual transmission of infections.

Case 2 ceased regular blood donation in 1969. A serum collected in 1967 was WB negative; the next available specimen collected in 1978 was WB seroreactive. Cases 6, 7, and 10 ceased regular blood donation when notified of SFV infection; retrospective testing of archived sera confirmed earlier seropositivity. As previously reported elsewhere, four recipients of leuko-reduced blood products from one SFVcpz-infected donor (case 6) showed no evidence of SFV infection.[20]

DISCUSSION

This is the first longitudinal description of the clinical, immunological, and virological status of humans infected with SFV. After a minimum median of 26 years (range 6–31) of documented infection as of 2007, participants continue to demonstrate strong antibody response by WB testing. Viral DNA can be consistently detected in all subjects' PBMCs but inconsistently from other body sites. SFV was successfully cultured from the throat swab and PBMCs of one participant and from the PBMCs of a second. SFV DNA was also detected in the urogenital tract of two persons. Despite this, no secondary transmission among humans via intimate exposure or blood product transfusion from one SFV-infected donor was identified.[20] Our data support a persistent infection in humans consistent with the demonstrated nature of endemic infections in NHPs, but suggest that the presence of detectable viral DNA in human body fluids does not correlate with transmissibility.

Repeated exposure to SFV DNA in body fluids may be insufficient for human-to-human transmission due to a limited viral load, the persistent presence of virus in a latent noninfectious state, or other mechanisms. SFV isolations were most frequent from case 6, the only participant whose specimens were received and processed within 8 h of collection. This may suggest that time elapsed from collection to processing influenced virus recovery, or may reflect higher viral loads in case 6 than other participants. Quantitative PCR testing for viral DNA and RNA levels in blood and other body compartments might provide further insight on the relationship between variations in viral load and inconsistencies between detection of SFV DNA in, and isolation of SFV from, body fluids. Fluctuations in viral load may also explain the variable detection of SFV in some body fluids.

Six of seven retrovirus genera are associated with hematological, neurological, dermatological, arthritic, or oncogenic diseases affecting nearly all vertebrate species, typically after long incubation periods.[3] Despite being highly cytopathic in cell culture, FVs have not been associated with any *in vivo* disease.[6] Our preliminary observations on humans with prolonged infection, while limited, are reassuring. Most clinical laboratory results were within normal limits or explained by the presence of conditions common in human populations of comparable age.

The most intriguing clinical observations were mild hematological abnormalities in three participants. The fluctuating, inconsistent and mild eosinopenia of case 6 appears to be clinically insignificant. The mild thrombocytopenia in two/seven participants deserves attention, but also does not appear to be clinically significant.

Although the total lymphocyte counts in all participants were normal, the NKCL in one participant with minimal thrombocytopenia is a notable finding. NKCL is a rare condition of unknown etiology, accompanied by thrombocytopenia in 12% of subjects in one series.[21] Persistent viral infection (e.g., Epstein–Barr, hepatitis B or C viruses)[22] or persistent immunological stimulation[23] has been hypothesized to play an etiological role. With the caveat that we do not know whether case 3 has other persistent infections, we may speculate that persistent SFV infection and, possibly, the presence of long-term diabetes mellitus[24] might contribute to case 3's NKCL. Future studies quantifying SFV integration in specific blood cells or cell lines may cast light on whether SFV plays a role in the observed hematological abnormalities.

SFV transmits naturally among NHPs via casual exposure to oral and respiratory secretions, and has been experimentally

TABLE 3. ABNORMAL CLINICAL LABORATORY TEST RESULTS OVER THE PERIOD OF FOLLOW-UP

Participant	*Years of age, Year 1*	*Known duration of infection (years), Year 1*	*Year 1*	*Year 2*	*Year 3*	*Year 4*	*Year 5*
Case 2	62	23	WNL[a]	WNL	WNL	NA[b]	NA
Case 3	62	13	Glucose 111 mg/dl (65–109)[c]; creatinine 1.7 mg/dl (0.5–1.4); BUN[d] 33 mg/dl (7–25); NK[e] cells 780/μl, 44% (68–482, 4–25%)	NK cells 854/μl, 39%; uric acid 8.6 (1.7–8.2); platelets 138,000 (140,000–400,000)	Glucose 131 mg/dl; creatinine 1.4; ALT[f] 116 U/liter (5–35); NK cells 38%	NA	NA
Case 6	62	19	Eosinophils 34/mcl (50–500)	Eosinophils 17/mcl	Eosinophils 56; platelets 122,000	Eosinophils 33 (15–500)	WNL
Case 7	42	10	AST[g] 48 IU (0–42); ALT 70 IU (0–48)	ALT 67	WNL	NA	WNL
Case 9	41	20	Platelets 107,000	NA	NA	NA	NA
Case 10	50	24	WNL	WNL	WNL	NA	NA
Case 12	62	2	WNL	NA	NA	NA	NA

[a]WNL, within normal limits.
[b]NA, not available.
[c]Limits of normal are shown in parentheses.
[d]BUN, blood urea nitrogen.
[e]NK, natural killer.
[f]ALT, alanine aminotransferase.
[g]AST, aspartate aminotransferase.

transmitted among NHP through fresh whole blood.[5,6,25,26] Previous reports have stressed the role of skin penetrating injuries in human acquisition of infection.[10–12] The absence of a discernible history of percutaneous injury associated with the species from which the infecting virus strain arose for 43% of participants raises the possibility that human infection with SFV may be acquired through mucocutaneous exposure to SFV-containing NHP body fluids without injury, similar to the routes of transmission of simian herpes viruses.[27] Thus, it is prudent for persons occupationally exposed to NHPs to take precautions to avoid exposure to primate saliva and other body fluids through either percutaneous injuries or mucocutaneous exposures.

Limited observations have not identified infection-associated pathology or secondary SFV transmission among humans through either intimate contact or transfusion of blood products. However, the small number of observed individuals and the limited duration of follow-up restrict our ability to draw definitive conclusions about the clinical significance of human infection with SFV and the ability of SFV to transmit secondarily. Like HTLV, the incidence of disease may be low or may follow long latency periods. It is also unknown what effect, if any, immunosuppression may have on clinical outcomes of human infection with SFV. For example, SFV replication was recently shown to expand to the small intestinal jejunum of SIV-immunosuppressed macaques, a site for significant $CD4^+$ T cell depletion and inflammation in these animals, suggesting that SFV may play a role in the gut-associated pathology observed during progression to simian AIDS.[28] We caution SFV-infected persons to refrain from donation of biological materials for transfusion or transplantation pending a better understanding of the significance of human infection.[12,29] Additional observations will be necessary to further define the public health significance of zoonotic SFV infection.

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REFERENCES

1. Wolfe ND, Switzer WM, and Heneine W: Emergence of novel retroviruses. In: *Emerging Infections*, Vol. 7 (Scheld WM, Craig WA, and Hughes JM, eds.). ASM Press, Washington, DC, 2006, pp. 139–152.
2. Linial ML, Fan H, Hahn B, *et al.*: Retroviridae. In: *Virus Taxonomy, Seventh Report of the International Committee on Taxonomy of Viruses* (Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA, eds.). Elsevier/Academic Press, London, 2004, pp. 421–440, available at www.virustaxonomyonline.com/.
3. Goff, SP: Retroviridae: The retroviruses and their replication. In: *Fields Virology*, 4th ed., Vol. 2 (Knipe DM, Howley PM, Driffin DE, *et al.*, eds.). Raven Press, New York, 2001, pp. 1871–1941.
4. Hussain AI, Shanmugam V, Bhullar VB, *et al.*: Screening for simian foamy virus infection by using a combined antigen Western blot assay: Evidence for a wide distribution among Old World primates and identification of four new divergent viruses. Virology 2003;309:248–257.
5. Blewett EL, Black DH, Lerche NW, White G, and Eberle R: Simian foamy virus infections in a baboon breeding colony. Virology 2000;278:183–193.
6. Meiering C and Linial ML: Historical perspective of foamy virus epidemiology and infection. Clin Microbiol Rev 2001;14:165–176.
7. Switzer WM, Salemi M, Shanmugam V, *et al.*: Ancient co-speciation of simian foamy viruses and primates. Nature 2005;434: 376–380.
8. Achong B, Mansell PWA, Epstein MA, *et al.*: An unusual virus in cultures from a human nasopharyngeal carcinoma. J Natl Cancer Inst 1971;46:299–307.
9. Herchenroder O, Renne R, Loncar D, *et al.*: Isolating, cloning and sequencing of simian foamy viruses from chimpanzees (SFVcpz): High homology to human foamy virus (HFV). Virology 1994;201: 187–199.
10. Switzer WM, Bhullar V, Shanmugam V, *et al.*: Frequent simian foamy virus infection in persons occupationally exposed to nonhuman primates. J Virol 2004;78:2780–2789.
11. Schweizer M, Falcone V, Gange J, Turek R, and Neumann-Haefelin D: Simian foamy virus isolated from an accidentally infected human individual. J Virol 1997;71:4821–4824.
12. Heneine W, Switzer WM, Sandstrom P, *et al.*: Identification of human population infected with simian foamy viruses. Nat Med 1998;4:403–407.
13. Sandstrom PA, Phan KO, Switzer WM, *et al.*: Simian foamy virus infection among zoo keepers. Lancet 2000;355:551–552.
14. Wolfe ND, Switzer WM, Carr JK, *et al.*: Naturally acquired simian retrovirus infections in central African hunters. Lancet 2004;363: 932–937.
15. Brooks JI, Rud EW, Pilon RG, Smith JM, Switzer WM, and Sandstrom PA: Cross-species retroviral transmission from macaques to human beings. Lancet 2002;360:387–388.
16. Jones-Engel L, Engel GA, Schillaci MA, *et al.*: Primate-to-human retroviral transmission in Asia. Emerg Infect Dis 2005;11: 1028–1035.
17. Schweizer M, Turek R, Hahn H, *et al.*: Markers of foamy virus infections in monkeys, apes, and accidentally infected humans: Appropriate testing fails to confirm suspected foamy virus prevalence in humans. AIDS Res Hum Retroviruses 1995;11:161–170.

18. Ali M, Taylor GP, Pitman RJ, *et al.*: No evidence of antibody to human foamy virus in widespread human populations. AIDS Res Hum Retroviruses 1996;12:1473–1483.
19. Cummins JE Jr, Boneva RS, Switzer WM, *et al.*: Mucosal and systemic antibody responses in humans infected with simian foamy virus. J Virol 2005;79:13186–13189.
20. Boneva RS, Grindon AJ, Orton S, *et al.*: Infection of a blood donor with Simian foamy virus. Transfusion 2002;42:886–891.
21. Rabbani GR, Phyliky RL, and Tefferi A: A long-term study of patients with chronic natural killer cell lymphocytosis. Br J Haematol 1999;106:960–966.
22. Zambello R, Loughran TP Jr, Trentin L, *et al.*: Serologic and molecular evidence for a possible pathogenetic role of viral infection in CD3-negative natural killer-type lymphoproliferative disease of granular lymphocytes. Leukemia 1995;9:1207–1211.
23. Granjo E, Lima M, Fraga M, *et al.*: Abnormal NK cell lymphocytosis detected after splenectomy: Association with repeated infection, relapsing neutropenia, and persistent polyclonal B-cell proliferation. Int J Hematol 2002;75:484–488.
24. Hoffmann JJ and Breed WP: Two patients with chronic lymphocytosis of large granular lymphocytes; benign or premalignant? Ned Tijdschr Geneeskd 2000;144:1323–1327.
25. Khan AS and Kumar D: Simian foamy virus infection by whole-blood transfer in rhesus macaques: Potential for transfusion transmission in humans. Transfusion 2006;46:1352–1359.
26. Brooks JI, Merks HM, Fournier J, Boneva R, Folks TM, and Sandstrom PA: Characterization of blood-borne transmission of simian foamy virus. Transfusion 2007;47:162–170.
27. Centers for Disease Control and Prevention: Fatal Cercopithecine herpesvirus 1 (B virus) infection following a mucocutaneous exposure and interim recommendations for worker protection. MMWR Morb Mortal Wkly Rep 1998;47:1073–1076.
28. Murray SM, Picker LJ, Axthelm MK, and Linial ML: Expanded tissue targets for foamy virus replication with simian immunodeficiency virus-induced immunosuppression. J Virol 2006;80:663–670.
29. Centers for Disease Control and Prevention: Nonhuman primate spumavirus infections among persons with occupational exposure–United States, 1996. MMWR Morb Mortal Wkly Rep 1997; 46:129–131.

Address reprint requests to:
William M. Switzer
CDC, Mailstop G-45
1600 Clifton Road
Atlanta, Georgia 30333

E-mail: bis3@cdc.gov

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
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一般的名称	(製造承認書に記載なし)	研究報告の公表状況		公表国
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研究報告の概要

○アデノウイルス血清型14に関連した米国4州の急性呼吸器疾患(2006～2007年の報告)
アデノウイルス血清型14型(Ad14)は、希にしか報告されないが新興しているアデノウイルスの血清型株で、健常若年成人を含め全ての年齢層の患者に、重症で時に致死性の呼吸器疾患を惹起する可能性がある。2006年5月に、ニューヨーク州で生後12日目の乳児が、Ad14が原因の呼吸器疾患により死亡した。2007年3月～6月の間に、オレゴン州、ワシントン州の介護施設、およびテキサス州の空軍基地で発生した小集積事例において、合計で140名のAd14感染患者が確認された。このうち53名(38%)が入院し、24名(17%)はICUで治療を受け、9名(5%)が死亡した。全4州の患者から分離されたAd14株は、hexonおよびfiber遺伝子全長の塩基配列データは同一であったが、1955年以来のAd14レファレンス株とは区別された。このことから、米国で新たなAd14変異株が新興し感染拡大したことが示唆される。州および各地公衆衛生当局は、Ad14が原因の集団感染発生可能性に警戒すべきである。
アデノウイルスは1950年代に初めて記録され、結膜炎、発熱性上気道疾患、肺炎および胃腸疾患などの広範囲な臨床症状に関連している。新生児や高齢患者、基礎疾患のある患者では重症化の可能性があるが、健常成人では一般的に致死性感染とはならない。本報告は、米国内に感染拡大した新規病原性Ad14変異株の新興を示唆している点で異例である。Ad14感染は1955年に初めて記録され、1969年にはヨーロッパの新兵での流行性急性呼吸器疾患と関連したが、それ以降はあまり検出されていなかった。Ad14のより広域での感染循環は数年前から発生している可能性もある。

報告企業の意見	今後の対応
2006～2007年に、米国ニューヨーク州、オレゴン州、ワシントン州、テキサス州で合計140名のアデノウイルス血清型14感染患者が確認され、新たなAd14変異株が新興し感染拡大した可能性が示唆されるとの報告である。	日本赤十字社は、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、問診で呼吸器疾患などの体調不良者を献血不可としている。今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-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

MMWR
Weekly
November 16, 2007 / 56(45);1181-1184

Acute Respiratory Disease Associated with Adenovirus Serotype 14 --- Four States, 2006--2007

Adenovirus serotype 14 (Ad14) is a rarely reported but emerging serotype of adenovirus that can cause severe and sometimes fatal respiratory illness in patients of all ages, including healthy young adults. In May 2006, an infant in New York aged 12 days died from respiratory illness caused by Ad14. During March--June 2007, a total of 140 additional cases of confirmed Ad14 respiratory illness were identified in clusters of patients in Oregon, Washington, and Texas. Fifty-three (38%) of these patients were hospitalized, including 24 (17%) who were admitted to intensive care units (ICUs); nine (5%) patients died. Ad14 isolates from all four states were identical by sequence data from the full hexon and fiber genes. However, the isolates were distinct from the Ad14 reference strain from 1955, suggesting the emergence and spread of a new Ad14 variant in the United States. No epidemiologic evidence of direct transmission linking the New York case or any of the clusters was identified. This report summarizes the investigation of these Ad14 cases by state and city health authorities, the U.S. Air Force, and CDC. State and local public health departments should be alert to the possibility of outbreaks caused by Ad14.

New York

In May 2006, a fatal case of Ad14 illness occurred in New York City in an infant girl aged 12 days. The infant was born after a full-term pregnancy and uncomplicated delivery. She was found dead in bed, where she had been sleeping. The infant had been examined 3 days after birth and noted to have lost weight but was otherwise healthy. The next week she had decreased tears with crying, suggesting early dehydration. Physical activity and feeding progressively decreased during the week before her death.

Postmortem tracheal and gastric swabs from the infant were sent to the Wadsworth Center laboratory of the New York State Department of Health, where adenovirus was detected by polymerase chain reaction (PCR). Adenovirus also was isolated by culture, confirmed by immunofluorescence assay (IFA), and typed as Ad14 by antibody neutralization assay. Analysis at CDC identified the same unique genetic sequences in this isolate as were later identified in the Ad14 isolates from the three 2007 clusters.

Autopsy and histologic findings at the Office of the Chief Medical Examiner in New York City included presence in the lung of chronic inflammatory cells with intranuclear inclusions, consistent with adenoviral bronchiolitis and acute respiratory distress syndrome. Investigation by the New York City Department of Health and Mental Hygiene has not identified any other local cases of Ad14 illness.

Oregon

In early April 2007, a clinician alerted the Oregon Public Health Division (OPHD) regarding multiple patients at a single hospital who had been admitted with a diagnosis of severe pneumonia during March 3--April 6. A total of 17 specimens were obtained from patients; 15 (88%) yielded isolates that were identified by CDC as Ad14. Through retrospective examination of laboratory reports from the three clinical laboratories in the state that have virology capacity and the Oregon State Public Health Laboratory (OSPHL), OPHD identified 68 persons who tested positive (by culture, PCR, or IFA) for adenovirus during November 1, 2006--April 30, 2007. Isolates from 50 (74%) of these patients were available for further adenovirus typing at either CDC or OSPHL. Of the 50 patient isolates, 31 (62%) were identified as Ad14, and 15 (30%) were identified as another adenovirus type

Figure); four (8%) did not test positive for adenovirus.

Among 30 Ad14 patients (i.e., all but one) whose medical charts were reviewed, 22 (73%) were male; median age was 53.4 years (range: 2 weeks--82 years). Five cases (17%) occurred in patients aged <5 years, and the remaining 0 (83%) occurred in patients aged >18 years. Twenty-two patients (73%) required hospitalization, sixteen (53%) required intensive care, and seven (23%) died, all from severe pneumonia. Median age of the patients who died was 63.6 years; five (71%) were male. One death occurred in an infant aged 1 month. Of the 30 Ad14 cases with patient residence information available, 28 (93%) occurred in residents of seven Oregon counties, and two cases occurred in residents of two Washington counties. No link was identified in hospitals or the community to explain transmission of Ad14 from one patient to another.

In comparison with the Ad14 patients, among the 12 adenovirus non-type 14 patients (i.e., all but three) whose medical charts were reviewed, nine (75%) were male. Median age was 1.1 years, and 11 (92%) patients were aged 5 years. Two (17%) adenovirus non-type14 patients required hospitalization; no ICU admissions or deaths were reported in this group.

Washington

On May 16, 2007, the Tacoma-Pierce County Health Department notified the Washington State Department of Health (WADOH) of four residents housed in one unit of a residential-care facility who had been hospitalized recently for pneumonia of unknown etiology. The patients were aged 40--62 years; three of the four were female. One patient had acquired immunodeficiency syndrome (AIDS); the three others had chronic obstructive pulmonary disease. All four were smokers.

The patients had initial symptoms of cough, fever, or shortness of breath during April 22--May 8, 2007. Three patients required intensive care and mechanical ventilation for severe pneumonia. After 8 days of hospitalization, one patient with AIDS died; the other patients recovered. Respiratory specimens from all four patients tested positive for adenovirus by PCR at the WADOH laboratory; isolates were available from three patients, and all three isolates were identified as Ad14 by CDC. Ad14 had last been identified in an isolate from a patient from Washington in May 2006, marking the first identification of Ad14 in the state since 2004. Active surveillance among facility residents and staff did not identify any other cases of Ad14 illness.

Texas

Since February 2007, an outbreak of cases of febrile respiratory infection* associated with adenovirus infection has been reported among basic military trainees at Lackland Air Force Base (LAFB). During an initial investigation, conducted from February 3 to June 23, out of 423 respiratory specimens collected and tested, 268 (63%) tested positive for adenovirus; 118 (44%) of the 268 were serotyped, and 106 (90%) of those serotyped were Ad14. Before this outbreak, the only identification of an Ad14 isolate at LAFB occurred in May 2006 (*1*).

During February 3--June 23, 2007, a total of 27 patients were hospitalized with pneumonia (median hospitalization: 3 days), including five who required admission to the ICU. One ICU patient required extracorporeal membrane oxygenation for approximately 3 weeks and ultimately died. All 16 hospitalized patients from whom throat swabs were collected, including the five patients admitted to the ICU, tested positive for Ad14. Fifteen of these hospitalized patients tested negative for other respiratory pathogens, and one patient had a sputum culture that was positive for *Haemophilus influenzae*.

All health-care workers from hospital units where trainees had been admitted were offered testing for Ad14, regardless of history of respiratory illness. Of 218 health-care workers tested by PCR, six (3%) were positive for Ad14; five of the six reported direct contact with hospitalized Ad14 patients.

Prevention measures implemented during the outbreak included increasing the number of hand-sanitizing stations, widespread sanitizing of surfaces and equipment with appropriate disinfectants, increasing awareness of Ad14 among trainees and staff members, and taking contact and droplet precautions for hospitalized patients with Ad14. Beginning on May 26, trainees with febrile respiratory illness were confined to one dormitory and both patients and staff members were required to wear surgical masks.

Cases reported postinvestigation. Since the investigation, new cases of febrile respiratory illness have continued to occur at LAFB, but the weekly incidence has declined from a peak of 74 cases with onset during the week of May 27--June 2, to 55 cases with onset during the week of September 23--29 (the most recent period for which data were available). In addition, during March--September 2007, three other military bases in Texas that received trainees from LAFB reported a total of 220 cases of Ad14 illness (Air Force Institute for Operational Health, personal communication, 2007). However, whether Ad14 spread from LAFB to these three bases has not been determined. Ad14 also was detected in April in an eye culture from an outpatient in the surrounding community who had respiratory symptoms and conjunctivitis. No link between this case and the LAFB cases was identified.

Reported by: *Oregon Dept of Human Svcs. Washington State Dept of Health Communicable Diseases. 37th Training Wing, 59th Hospital Wing, Air Force Institute for Operational Health, Epidemic and Outbreak Surveillance, US Air Force. Naval Health Research Center, US Navy. Texas Dept of State Health Svcs. New York City Dept of Health and Mental Hygiene. Div of Viral Diseases, National Center for Immunization and Respiratory Diseases; Div of Healthcare Quality Promotion, National Center for Preparedness, Detection, and Control of Infectious Diseases; Career Development Div, Office of Workforce and Career Development, CDC.*

Editorial Note:

Adenoviruses were first described in the 1950s and are associated with a broad spectrum of clinical illness, including conjunctivitis, febrile upper respiratory illness, pneumonia, and gastrointestinal disease. Severe illness c occur in newborn or elderly patients or in patients with underlying medical conditions but is generally not life-threatening in otherwise healthy adults. Adenoviruses are known to cause outbreaks of disease, including keratoconjunctivitis, and tracheobronchitis and other respiratory diseases among military recruits (*2,3*). Although adenovirus outbreaks in military recruits are well-recognized (*3*), infection usually does not require hospitalization and rarely requires admission to an ICU. Beyond the neonatal period, deaths associated with community-acquired adenovirus infection in persons who are not immunodeficient are uncommon and usually sporadic.

Fifty-one adenovirus serotypes have been identified (*4*). The cases described in this report are unusual because they suggest the emergence of a new and virulent Ad14 variant that has spread within the United States. Ad14 infection was described initially in 1955 (*5*) and was associated with epidemic acute respiratory disease in military recruits in Europe in 1969 (*6*) but has since been detected infrequently. For example, during 2001--2002, Ad14 was associated with approximately 8% of respiratory adenoviral infections in the pediatric ward of a Taiwan hospital, with approximately 40% of Ad14 cases in children aged 4--8 years manifesting as lower airway disease (*7*).

The National Surveillance for Emerging Adenovirus Infections system includes military and civilian laboratories at 15 sites. During 2004--2007, this surveillance system detected 17 isolates of Ad14 from seven sites (*8*). Ten of t 17 isolates (60%) were collected from three military bases (*8*). Despite this surveillance, adenovirus infections often go undetected, because few laboratories routinely test for adenovirus and even fewer do serotyping. Wider circulation of Ad14 might have occurred in recent years and might still be occurring.

Further work is needed to understand the natural history of Ad14, risk factors for severe Ad14 disease, and how Ad14 transmission can be prevented effectively. Vaccines against adenovirus serotypes four and seven (i.e., Ad4 and Ad7) were used among military recruits during 1971--1999, before vaccines were no longer available. Adenoviral disease among U.S. military recruits subsequently increased (*9*). Ad4 and Ad7 oral vaccines have been redeveloped and are being evaluated in clinical trials. Work is ongoing to determine whether the new Ad4 and Ad7 vaccines will protect against Ad14 infection. Management of adenoviral infections is largely supportive. A number of antiviral drugs, including ribavirin, vidarabine, and cidofovir, have been used to treat adenoviral infections such as Ad14, but none have shown definitive efficacy against adenoviruses (*2*).

Control of adenovirus outbreaks can be challenging because these viruses can be shed in both respiratory secretions and feces and can persist for weeks on environmental surfaces. Guidelines for the care of patients with pneumonia (*10*) should be followed in cases of suspected adenoviral pneumonia.

Clinicians with questions related to testing of patients for adenovirus or Ad14 infection should contact their state health departments, which can provide assistance. State health departments and military facilities should contact CDC to report unusual clusters of severe adenoviral disease or cases of Ad14 or to obtain additional information

garding laboratory testing.

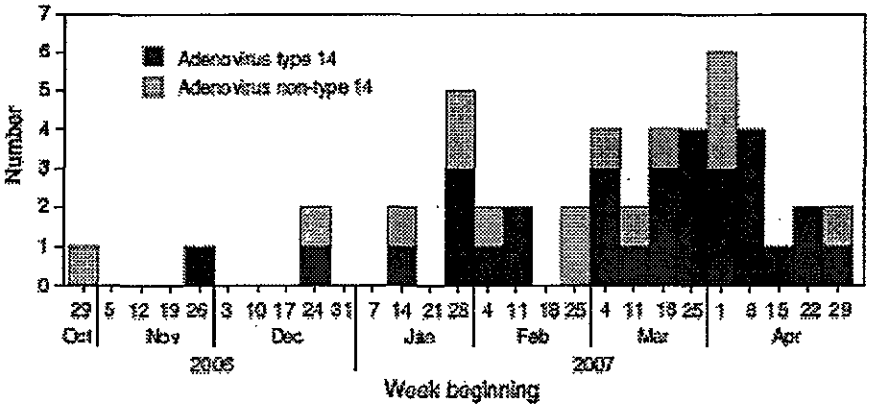
eferences

1. Metzgar D, Osuna M, Kajon AE. Abrupt emergence of diverse species B1 and B2 adenoviruses in US military recruit training centers. J Infect Dis. In press.
2. Adenovirus. In: Mandell GL, Bennett JE, Dolin R, eds. Principles and practice of infectious disease. 6th edition. Philadelphia, PA: Churchill Livingstone; 2004.
3. Dingle JH, Langmuir AD. Epidemiology of acute, respiratory disease in military recruits. Am Rev Respir Dis 1968;97(Suppl):1--65.
4. Kajon AE, Moseley JM, Metzgar D, et al. Molecular epidemiology of adenovirus type 4 infections in US military recruits in the postvaccination era (1997--2003). J Infect Dis 2007;196:67--75.
5. Van der Veen J, Kok G. Isolation and typing of adenoviruses recovered from military recruits with acute respiratory disease in The Netherlands. Am J Hyg 1957;65:119--29.
6. Hierholzer JC, Pumarola A. Antigenic characterization of intermediate adenovirus 14-11 strains associated with upper respiratory illness in a military camp. Infect Immun 1976;13:354--9.
7. Chen H, Chiou S, Hsiao H, et al. Respiratory adenoviral infections in children: a study of hospitalized cases in southern Taiwan in 2001--2002. J Trop Pediatr 2002;50:279--84.
8. National Surveillance for Emerging Adenovirus Infections. Available at http://www.public-health.uiowa.edu/adv.
9. Russell KL, Hawksworth AW, Ryan MA, et al. Vaccine-preventable adenoviral respiratory illness in US military recruits, 1999--2004. Vaccine 2006;24:2835--42.
10. CDC. Guidelines for preventing health-care--associated pneumonia, 2003. Recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee. MMWR 2004;53(No. RR-3).

Defined as 1) fever ≥100.5°F (≥38.1°C) plus at least one other sign or symptom of respiratory illness or 2) diagnosis of pneumonia.

igure

FIGURE. Number of cases of laboratory-confirmed adenovirus (type 14 and non-type 14*), by week of illness onset — Oregon, November 1, 2006–April 30, 2007



*Confirmatory typing performed at Oregon State Public Health Laboratory or CDC.

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別紙様式第2

別紙(3)-3

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日 2008年1月29日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①～③人血清ｱﾙﾌﾞﾐﾝ④人血液凝固第Ⅹ Ⅲ因子 ⑤⑥ﾌｨﾌﾞﾘﾉｹﾞﾝ加第Ⅹ Ⅲ因子		研究報告の公表状況	Clinical outcome of frequent exposure to Torque Teno virus (TTV) through blood transfusion in thalassemia patients with or without hepatitis C virus (HCV) infection Journal of Medical Virology 1, 2008, 80/2 (365-371)	公表国 米国	
販売名（企業名）	①アルブミン－ベーリング②アルブミナー5% ③アルブミナー25%④フィブロガミン P⑤ベリプラスト⑥ベリプラスト P コンビセット（CSL ベーリング株式会社）					

研究報告の概要

問題点（サラセミア患者の輸血による TTV 感染）

サラセミア患者は、頻繁にウイルスに暴露された輸血に依存していて肝不全や肝障害を頻繁に合併している。HBV と HCV 検査によるスクリーニング導入以来、この患者グループでの輸血関連の肝炎は激減した。しかしながら既知の肝炎に感染していないサラセミア患者の 37%がいまだに ALT 異常値を示しているが、原因は特定できていない。

TTV は一般の人々の間で高率で広く分布しているが、特にサラセミア患者などの頻繁に輸血を受ける患者では 80%以上が複数の TTV の遺伝子型を保有している。

著者らはアラブ首長国連邦で定期的に輸血を受ける（年間 13-18 回）サラセミア患者 197 名の TTV の遺伝子型およびフェリチン、AST、ALT レベルを検査した。

フェリチン、AST、ALT レベルは TTV 陽性患者群が、陰性患者群より有意に高かったが、HCV と TTV 共に感染した患者群は、TTV 単独感染患者群に比べ ALT が有意に高かった。

TTV 陽性群において ALT 異常値率は、年齢による差はなく、年間の投与間隔や投与数に起因していないことが示唆された。

TTV ウイルスは 27 遺伝子型から成る少なくとも 5 グループに分類される。TTV DNA を RD プライマー、TT6/7/8/9 プライマーおよび NG プライマーの 3 種を用いて増幅して、遺伝子型を解析したが、TTV 感染患者のほとんどが複数の遺伝子型を保持しているので、フェリチン、AST、ALT を上昇に関与する遺伝子を特定できなかった。

筆者らは、TTV 感染が HCV 感染患者の肝疾患を重篤にさせるとは結論できないとしている。重篤な肝疾患の進展には TTV よりも HCV の方が重要な役割を果たしている。

報告企業の意見	今後の対応
現在まで TTV の病原性は十分解明されていないので、当社製品の原料血漿の段階で TTV に対しての検査は実施されていない。 TTV と ALT に関する CPMP の見解（Plasma-derived medicinal products : position paper on ALT testing : CPMP/BWP/385/99）は、TTV DNA 値と ALT 値との間には明確な相関関係はないことを示唆しているとしている。 TTV 検査は実施していないが、本剤の製造工程（60℃10 時間の液状加熱）で不活化されると考えられる。 今後とも情報収集に努める。	今後とも情報収集に努める所存である。

使用上の注意記載状況・その他参考事項等

Journal of Medical Virology 80:365–371 (2008)

Clinical Outcome of Frequent Exposure to Torque Teno Virus (TTV) Through Blood Transfusion in Thalassemia Patients With or Without Hepatitis C Virus (HCV) Infection

Yu-Wen Hu,[1,2]* Moslih I. Al-Moslih,[3] Mahmoud Talib Al Ali,[4] Samra Uzicanin,[1] Heather Perkins,[1] Qi-Long Yi,[1] Shabnam Rahimi Khameneh,[1] Jun Wu,[5] and Earl G. Brown[2]

[1]*Canadian Blood Services, Ottawa, Ontario, Canada*
[2]*Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada*
[3]*College of Health Sciences, University of Sharjah, Sharjah, UAE*
[4]*Thalassemia and Genetic Center, Al Wasal Hospital, Dubai, UAE*
[5]*Bloodborne Pathogens and Blood Safety Surveillance Division, Centre for Infectious, Disease Prevention and Control, Public Health Agency of Canada, Ottawa, Ontario, Canada*

As a consequence of the high prevalence of TorqueTeno virus (TTV) in blood donors, thalassemia patients frequently acquire various genotypes of this virus through therapeutic blood transfusions. At present, the clinical consequences of TTV infection remain indeterminate for these patients. Here, several hundred thalassemia patients were tested for the presence of TTV and its genotypes using a combination of PCR and clone-based DNA sequencing. Approximately 10% (12/118) of the patients aged 2–20 years remained negative for TTV including eight genotypes of SENV. Ferritin, aspartate-aminotransferase (AST) and alanine-aminotransferase (ALT) levels were invariably lower in TTV-negative patients ($P=0.02$, <0.01, and 0.06, respectively) than in TTV-positive patients. Patients with TTV–HCV co-infection showed elevated ferritin and ALT levels compared with patients with TTV infection alone ($P<0.02$ and $P<0.01$). AST and ALT levels were within the normal range for all TTV-negative patients, whereas abnormal levels of AST and ALT were seen in a significant proportion of TTV-positive patients (30.7% and 33.6%, respectively) and patients with TTV–HCV co-infections (70.0% and 56.6%, respectively). Only TTV-positive patients (28.0%) and patients with TTV–HCV co-infections (36.3%) had hyper-ferritin levels (≥3,000 ng/ml). The genotype(s) of TTV responsible for the liver dysfunction could not be determined. However, high levels of AST and ALT were found to be correlated with detection of a higher number of TTV genotypes in the patients. The data suggests that frequent and persistent TTV infection through blood transfusion is associated with hepatic dysfunction and/or damage in transfusion dependent thalassemia patients. ***J. Med. Virol. 80:365–371, 2008.*** © 2007 Wiley-Liss, Inc.

KEY WORDS: TTV; HCV thalassemia patient; liver disease

INTRODUCTION

Hepatic dysfunction and/or damage are frequent complications in thalassemia patients who depend on blood transfusions that are associated with a high frequency of exposure to viruses. Accordingly, the incidence of transfusion related hepatitis for this group of patients has been markedly reduced since the implementation of blood screening for hepatitis B virus (HBV) and hepatitis C virus (HCV) nucleic acid and antibodies. However, over one third (37%) of thalassemia patients without infection by known hepatitis viruses still have an abnormal alanine-aminotransferase (ALT) pattern [Chen et al., 1999]. The exact cause of the ALT abnormality in those patients remains unknown [Okamoto and Mayumi, 2001].

A virus with a small single-stranded DNA genome was identified by Nishizawa et al. [1997] in Japan from patients with non-A-E transfusion acquired hepatitis in

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*Correspondence to: Yu-Wen Hu, Canadian Blood Services, 1800 Alta Vista Drive, Ottawa, Ontario, Canada K1G 4J5. E-mail: yu-wen.hu@blood.ca

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1997. With reference to the index patient, the virus was originally named TT virus however it is currently renamed as Torque Teno virus (TTV) the type species of the genus Anellovirus, in an unassigned family that is most closely related to the Circoviridae [Hino, 2002]. TTV is widely distributed geographically with a high rate of viremia within the general population [Simmonds, 1998; Cossart, 2000]. Mixed genotype infections are therefore common, particularly in frequently transfused patients such as thalassemia patients where 80% of them carry more than one genotype of TTV [Chen et al., 1999; Okamoto et al., 1999b]. This is presumably due to the high frequency of viral transmission through blood transfusion and the persistent nature of TTV viral infections [Chen et al., 1999; Gallian et al., 1999; Kanda et al., 1999; Kobayashi et al., 1999; Matsumoto et al., 1999; Oguchi et al., 1999; Prati et al., 1999]. TTV viruses have been classified into at least five groups consisting of more than 27 genotypes as a result of the extremely wide range of sequence divergence observed among TTV isolates [Okamoto et al., 1998; Tanaka et al., 1998; Takayama et al., 1999; Worobey, 2000; Okamoto and Mayumi, 2001]. Early studies indicate that TTV might cause some forms of cryptogenetic hepatitis, post transfusion hepatitis and/or other diseases, however these observations have not been confirmed in most subsequent studies although it has been suggested that certain TTV groups or genotypes (e.g., group 4, genotype 1 and genotype 21) might be especially pathogenic and associated with liver or other diseases [Simons et al., 1995; Okamura et al., 2000; Sugiyama et al., 2000; Bendinelli et al., 2001; Maggi et al., 2003; Pifferi et al., 2005; Szladek et al., 2005]. The clinical significance of TTV infection thus remains controversial. [Okamura et al., 2000; Sugiyama et al., 2000; Maggi et al., 2003; Pifferi et al., 2005; Szladek et al., 2005]. Considering that thalassemia patients frequently acquire multiple genotypes of TTV through repeated blood transfusion administered throughout their lifetime, the role of this virus in the development of clinical disease in this group of patients cannot be excluded [Chen et al., 1999]. In addition to TTV, a substantial proportion of thalassemia patients have acquired HCV infection through blood transfusion. It remains uncertain if HCV–TTV co-infections result in more severe biochemical and histological changes compared to TTV infection alone [Charlton et al., 1998; Watanabe et al., 1999; Yuki et al., 1999; Zein et al., 1999; Cleavinger et al., 2000; Meng et al., 2001; Tokita et al., 2002].

Since TTV cannot be cultivated in vitro, PCR is the only available tool for detection of TTV. It has been difficult to determine the clinical significance of TTV infections because diagnostic systems using one or two sets primers for PCR are unable to detect the entire spectrum of TTV genotypes and their variants that exist in individuals [Okamoto et al., 1999b, 2000; Maggi et al., 2003]. Obviously, this has impeded a proper assessment of TTV viral pathogenesis. We recently found that all TTV genotypes (except genotype 21), and all SENV genotypes (A–H) can be detected using three TTV primer sets [Hu et al., 2005]. This finding has provided a more accurate and efficient tool for TTV diagnosis. In this study, we used this efficient primer system combined with clone-based DNA sequencing to investigate the prevalence of various genotypes of TTV with respect to clinical outcome in various age groups of blood transfusion dependent thalassemia patients with or without HCV co-infection.

MATERIALS AND METHODS

Study Groups

A total of 197 thalassemia patients from the United Arab Emirates (UAE) who had received regular blood transfusions (13–18/year) were enrolled in this study, including 54 with hepatitis C virus (HCV) infection. Among these patients a younger group of 118 thalassemia patients (49 female and 69 male) ranging in age from 2 to 20 years (with a median age of 10.8 years) was tested for blood ferritin, AST and ALT levels to study the clinical outcome of TTV and HCV infections. The remaining thalassemia patients (n = 79: aged 21–53 years) were only tested for ALT in this study. All samples were negative in standard donor-screening tests including HIV, Human T Cell Leukemia Viruses, and hepatitis A, B and G.

Isolation of Viral DNA

Plasma (100 μl) was used for isolation of viral DNA with a silica gel based membrane while using microspin technology as described in the QIAamp blood kit (QIAGEN, Inc., Mississauga, Ontario).

Amplification and Detection of TTV DNA

Purified TTV DNA was amplified using three sets of nested primers derived from the conserved regions in 5′UTR and ORFs of the TTV genome following the procedure as previously described [Okamoto et al., 1999b; Hu et al., 2005]. They include RD037-038 plus RD051-052 [Okamoto et al., 1998], TT6-7 plus TT8-9 [Hohne et al., 1998], and NG 5′UTR based nested primers (NG054-147 plus NG133-132) [Okamoto et al., 1999a]. TTMV (TTV-like mini virus) was also tested in the thalassemia patients using two sets of TTMV specific primers as described previously [Hu et al., 2005]. The sensitivity of PCR used in this study had previously been evaluated by both DNA dilution and real-time PCR methods. The sensitivity was determined to be $\leq$10 copies/ml. To confirm that thalassemia patients testing negative for TTV were truly negative, the 12 TTV negative samples were subjected to additional PCR reaction conditions including different and lower stringency primer annealing temperatures and additional reaction cycles. PCR amplified TTV DNA was detected on a 1.5% agarose gel using ethidium bromide staining.

Direct DNA Sequencing

Direct DNA sequencing was performed using an automated DNA sequencer (Visible Genetics). A 5′

Cy5.5 labeled sense primer, RD051, was used to produce a sequence of approximately 150 bases from RD primer set PCR fragments. For the PCR products amplified using TT primers, a 5′ Cy5.5 labeled sense primer, TT8, was used to produce a sequence of approximately 150 bases in length. 5′UTR based primers NG133F and NG132R were used for sequencing the PCR products amplified by 5′UTR based primers.

Cloning Based DNA Sequencing

Clonal based DNA sequencing was performed using TOPO- TA™ vector (Amersham Pharmacia Biotech, USA) for cloning of purified PCR products. The TTV sequence was amplified from colonies of *Escherichia coli* using M13 primers (Amersham Pharmacia Biotech, USA). Fifteen clones from each individual were sequenced. Re-amplified PCR products were sequenced using the same procedure as for direct DNA sequencing.

Genotyping and Computer Analysis of Nucleotide Sequences

DNA sequences derived from DNA sequencing were compared using an on line database for the best possible match using the Basic Local Alignment Search Tool (BLAST) program at the National Center for Biotechnology Information home page (http://www.ncbi.nlm.nih.gov/). All further sequence analyses and comparisons were performed using the DNA-STAR Lasergene '99 software package (DNAStar, Madison, WI).

Statistical Analysis

The mean differences in AST, ALT and ferritin between three clinical groups were compared using Student's *t*-tests. Since the sample size for both TTV-negative and TTV+HCV positive groups is small compared to that of the TTV positive group, we performed a matching comparison to increase the comparison power. Each case in the TTV-negative group (or TTV + HCV positive group) was matched to a case of the same age and gender in the TTV-positive group. Paired *t*-test was performed for the matching comparison. In addition, we categorized the AST, ALT and ferritin into normal and abnormal groups using the clinical normal range as the cut-off value, and compared the abnormal rates in the three clinical groups. Chi-square tests were used in the comparison of rates. The SAS software package was used for the statistical analyses.

RESULTS

Clinical Significance of TTV Infection

Ferritin, AST and ALT levels. For a thorough analysis of the clinical data, we first investigated the impact of age and gender on the levels of clinical parameters (Table I). Ferritin, AST and ALT levels were found to be higher in patients aged 11–20 years than in younger patients aged 2–10 years. However, the difference did not reach statistical significance ($P = 0.13$, 0.10 and 0.10 for ferritin, AST and ALT, respectively). All three parameters were slightly increased in male relative to female patients, but again no significant difference was found between different genders ($P = 0.46$, 0.19 and 0.43 for ferritin, AST and ALT, respectively). The data suggest that age and gender are not major factors that significantly contributed to liver disease in thalassemia patients aged 2–20 years. However, since all three parameters were slightly higher in older patients and male patients in comparison with younger patients and female patients, a minor impact of age and gender on liver dysfunction could not be completely excluded. To improve the accuracy of data analysis, we therefore decided to use both age-sex matching and non-age-sex matching methods for evaluating the clinical significance of TTV infection in thalassemia patients.

The results in Table II indicate that by using an exact age-sex matching method, the ferritin, AST and ALT levels were found to be invariably lower in TTV-negative patients compared to the TTV-positive patients ($P = 0.02$, $P < 0.01$ and $P < 0.06$, respectively). The trends of the results generated by non-age-sex matching were, in general, consistent with those derived using the age-sex matching approach, where ferritin, AST and ALT levels were shown to be higher in TTV-positive patients than in TTV-negative patients ($P < 0.02$, $P > 0.05$ and $P > 0.05$, respectively), but in this case only ferritin was significantly elevated. The clinical data regarding TTMV (TTV-like mini virus) negative samples (8.5%, 10/118) was also analyzed and compared to the TTMV positive samples by age-sex matching method. The levels of ALT, AST and ferritin were not significantly different between TTMV-negative and TTMV-positive patients (data not shown). Of the TTV negative patients, 91.7% (11/12) were positive for TTMV. These data further support the observation that TTV, but not TTMV causes liver dysfunction and/or damage. Using age-sex matched groups, the levels of both ferritin and

TABLE I. Ferritin, AST and ALT Levels in Different Age and Gender Groups of TTV Infected Thalassemia Patients

Patients (no.)	Ferritin (ng/ml) (*P*)	AST (IU/L) (*P*)	ALT (IU/L) (*P*)
Age			
2–10 (45)	2290.9 ± 1331.2	35.1 ± 20.7	37.3 ± 47.2
11–20 (47)	2706.3 ± 1274.5 (0.13)	42.9 ± 24.2 (0.10)	53.2 ± 44.8 (0.10)
Gender			
Male (52)	2566.4 ± 131318.5	42.2 ± 24.4	48.8 ± 47.4
Female (40)	2364.1 ± 1296.5 (0.46)	35.9 ± 21.4 (0.19)	41.2. ± 43.8 (0.43)

TABLE II. Comparison of Ferritin, AST and ALT Levels Between TTV Negative Patients, Patients With TTV Infection Alone (TTV+) and With TTV–HCV Co-Infections (TTV + HCV+)

Patients (no.)	Age	Ferritin (ng/ml) (*P*)	AST (U/L) (*P*)	ALT (U/L) (*P*)
Age-sex matching				
TTV– (12)	9.5 ± 5.6[a]	1470.3 ± 514.7	27.1 ± 7.1	22.1 ± 12.2
TTV+ (12)	9.5 ± 5.6	2340.0 ± 733.5 (0.02)	38.8 ± 18.1 (<0.01)	49.3 ± 45.3 (0.06)
No age-sex matching				
TTV– (12)	9.5 ± 5.6	1470.3 ± 514.7	27.1 ± 7.1	22.1 ± 12.2
TTV+ (92)	10.8 ± 4.6	2477.7 ± 1305.6 (0.02)	39.5 ± 23.3 (0.08)	45.5 ± 45.8 (0.11)
Age-sex matching				
TTV+ alone (11)	13.2 ± 4.4	2340.0 ± 733.5	36.2 ± 22.0	27.6 ± 17.0
TTV + HCV+ (11)	13.2 ± 4.4	3112.9 ± 1477.8 (0.02)	53.1 ± 27.6 (0.13)	55.4 ± 27.6 (<0.01)
No age-sex matching				
TTV+ alone (92)	10.8 ± 4.6	2477.7 ± 1305.6	39.5 ± 23.3	45.5 ± 45.8
TTV + HCV+ (11)	13.2 ± 4.4	3112.9 ± 1477.8 (0.08)	53.1 ± 27.6 (>0.05)	55.4 ± 27.6 (>0.05)

[a]Mean ± SD; significant differences are in bold.

ALT were found to be higher in patients with HCV–TTV co-infections than in patients with TTV infection alone ($P = 0.02$ and $P < 0.01$). It was expected that patients with TTV–HCV co-infections would have more severe liver disease than TTV alone as HCV is a serious liver pathogen. Non-age-sex matched analysis showed that all three clinical parameters were higher in patients with TTV–HCV co-infections than in patients with TTV infection alone, but no significant difference was found between the two groups of patients. This suggests that the use of age-sex matching approach is necessary for the clinical data analysis.

Abnormality rate of ferritin, AST and ALT. We found that all 12 TTV-negative patients aged 2–20 years had normal AST (≤40 IU/L ranging from 14 to 40 IU/L) and ALT (≤50 IU/L, ranging from 10 to 48 IU/L) levels. However, AST and ALT levels were elevated in nearly one-third of TTV-positive patients aged 2–20 years (30.7%, 27/88 for AST; and 33.6%, 48/143 for ALT; Fig. 1). Abnormal levels of AST (70%, 7/10) and ALT (56.6%, 30/53) was even higher in patients with TTV–HCV co-infections than patients with TTV infection alone. Data resulting from analysis of abnormalities in three clinical parameters agreed completely with those obtained from the assessment of actual differences in ferritin, AST and ALT levels. Although the ferritin levels were significantly higher ($P = 0.02$) with respect to TTV and HCV positivity for matched patient groups, the ferritin baseline level of the patient group is high due to transfusion treatments. The ferritin level in TTV-negative thalassemia patients was five times higher (1470.3 ± 514 ng/ml; Table II) than normal (10–300 ng/ml). Therefore, we used the hyper-ferritin level value, which is ten times the normal limit (3,000 ng/ml) to calculate the abnormality rate in thalassemia patients. None of the TTV-negative patients had ferritin levels over 3,000 ng/ml with the highest level being 2,440 ng/ml. However, about one third of patients with TTV infection alone (28.0%, 26/93) and patients with HCV–TTV co-infections (36.4%, 4/11) had hyper-ferritin levels (3,000 ng/ml ranging up to 6,250 ng/ml).

One might expect that the ALT abnormality rate should be highest in older patients due to the greater duration of transfusion treatment. In fact, the ALT abnormality rate was the highest (33.6%, 48/143) in young patients (11–20 years) compared to the youngest patients aged 2–10 years (21.2%, 14/66) and the older patient group, between the ages of 21 and 53 (20.9%, 9/43; Fig. 2). This indicates that ALT elevation in thalassemia patients was not dependent or directly attributable to the time span or number of transfusions alone.

TTV Prevalence and Pathogenesis of Various Genotypes

As reported previously [Hu et al., 2005], RD primers are known to specifically amplify genotype 1 (1a and 1b) and TT6/7/8/9 primers detect genotypes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 14, 15, and 16; mainly in genogroups 1 and 3. TT6/7 primers specifically detect TTV genotypes 2 and 3. NG primers can detect almost all known TTV genotypes except genotype 21, including eight genotypes (A–H) of SENV. Use of the three nested sets of TTV primers

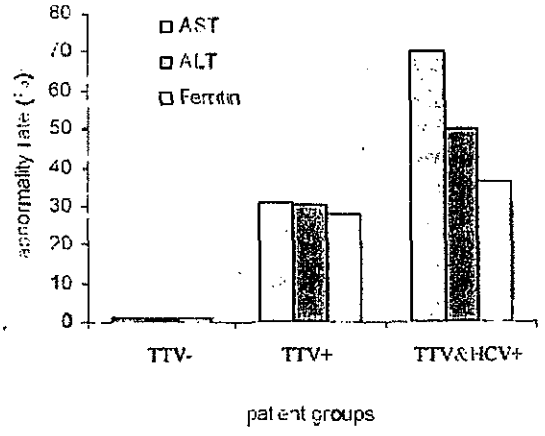

Fig. 1. Abnormality rate of ferritin, aspartate-aminotransferase (AST) and alanine-aminotransferase (ALT) levels in TTV-negative patients (TTV–), TTV-positive patients (TTV+) and patients with TTV–HCV co-infections (TTV and HCV+).

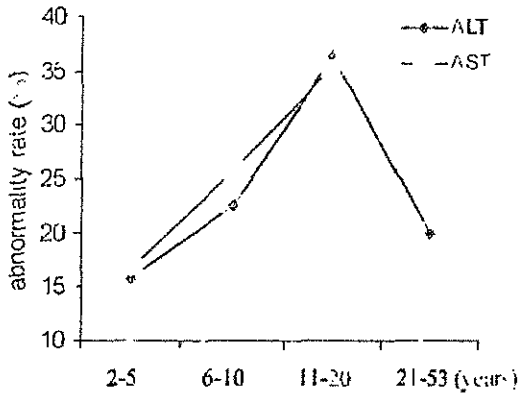

Fig. 2. Abnormality rate of AST (■) and ALT (◆) at different ages in TTV-positive thalassemia patients.

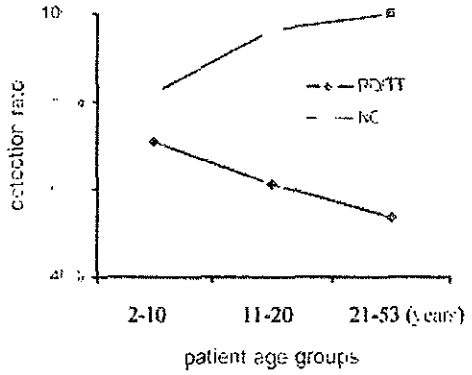

Fig. 3. TTV-positive rates of various genotypes in different age groups of thalassemia patients. The graphs indicate the viremia rates (%) of NG primer-specific genotypes (■), RD/TT6/7/8/9-specific genotypes (◆). The ages of patient groups (2–10, 11–20 and 21–53 years) are plotted against rate of infection.

allowed us to assess the prevalence of various genotypes in different age groups of thalassemia patients. Figure 3 shows that the positivity rate for the genotypes detected by NG primers increased significantly with age from younger (2–10 years) to older groups of patients (ages 11–20 years as well as 21–53 years) with detection rates of 81.8%, 45/55; 96.2%, 50/52; and 100.0%, 30/30, respectively (trend test $P < 0.0001$). In contrast, the detection frequency for the most prevalent genotypes that are detectable by RD/TT6/7/8/9 primers (i.e., genotypes 1–10 and 14–16) declined significantly from younger to older groups. The TTV-positive rate was 71.0%, 27/38; 61.5%, 32/52; and 53.6%, 30/56; for the younger and the two older groups of patients, respectively (trend test $P < 0.001$). The genotypes of amplified TTV viruses were confirmed by clone-based sequencing (see Materials and Methods Section, data not shown). The shifting of predominant TTV genotypes over a patient's lifetime suggests that younger patients have a greater susceptibility to the most prevalent genotypes relative to older patients. Presumably this is due to the fact that some of the older patients may become immune due to prior exposure and subsequent clearance of infection. As a result, these patients are protected from re-infection by some genotypes such as the genotypes detectable by RD/TT6/7/8/9 primers. However, in addition to being exposed to more TTV genotypes, the older patients have also been exposed to less prevalent TTV genotypes over time because of increased exposure due to repeated transfusions. Overall, the TTV viremia rate is higher in older patients. The genotype shifting during a patient's life-time provides strong evidence to indicate that both self-limited and chronic TTV infections exist and that persistence of the virus (at least certain genotypes) may not be life-long in thalassemia patients. The declining prevalence of some genotypes in older patients was paralleled by a decrease in the ALT abnormality rate (see Figs. 2 and 3). This suggests that the most prevalent genotypes such as 1, 2 and 3 may play a more important role in the development of liver disease in thalassemia patients. However, the exact genotype(s) responsible for the elevation of ferritin, AST and ALT levels could not be determined because the majority of TTV infected patients carried more than one genotype.

DISCUSSION

The role of TTV infection in liver disease has been the subject of much debate since the first TT virus was identified in 1997. In our present study, the data suggest that frequent and persistent TTV infection through blood transfusion is associated with abnormal AST, ALT and ferritin levels (seen in about one third of blood-dependent thalassemia patients). This supports the hypothesis that certain genotypes or variants of TTV cause disease when individuals are exposed to these genotypes or their variants for the first time and/or re-exposed to partially cross-reactive or non-cross reactive TTV strains [Bendinelli et al., 2001]. To further assess whether a specific genotype or group of TTV is associated with elevated ALT levels in thalassemiacs, we compared the genotypes infecting patients with abnormal ALT levels (>100 IU/L) and patients with normal ALT levels (<50 IU/L). A total of 480 clones from 32 samples (15 clones for each sample) were genotyped by DNA sequencing. We found that the majority (78.1%, 25/32) of the thalassemia patients were infected with more than one genotype. It was therefore difficult to judge which genotype was responsible for the elevation of ALT levels in patients with mixed genotype infections (data not shown). However, the number of mixed genotypes was found to be significantly increased in patients with abnormal ALT levels (three vs. two genotypes per patient, $P = 0.01$). It appears that ALT elevation is associated with a higher frequency of TTV mixed genotype infections, including transient and persistent infections through blood transfusion.

It is reasonable to propose that multiply transfused thalassemia patients are at a much greater risk of being infected by new and more pathogenic genotypes or strains than blood donors. In the UAE thalassemia patients average over 15 transfusion per year, where over one third of blood donors carry the virus. It would be expected that transfusion dependent thalassemia

patients could be infected by TTV at least six times per year or 60 times during the first 10 years of blood transfusion therapy [Al Moslih et al., 2004]. Thus, patients with a 10-year transfusion history could have been infected or re-infected by all genotypes existing in the UAE. In addition, the extent of virus replication in thalassemia patients may be higher due to the large viral inocula injected directly into the blood stream through transfusion. This is obviously different from the small amount of virus acquired through infection via the oral route in normal blood donors.

It was not possible to conclude that TTV infection enhances the severity of liver disease in HCV infected patients because very few patients infected with HCV alone were available for comparison with patients co-infected with TTV and HCV. It is obvious that HCV plays a more important role than TTV in the development of severe liver disease.

It is well known that TTV infections are persistent. Consequently, the presence of TTV-negative thalassemia patients was unexpected. We do not yet have an explanation for this observation. Perhaps TTV host dependent genetic factors play an important role in determining the resistance or outcome of TTV infection among patients.

Follow-up studies of TTV infection and clearance in TTV-negative and TTV-positive thalassemia patients will eventually provide clues to understanding the natural history and pathogenesis of TTV. Of equal importance, a thorough understanding of the immune response to TTV infection, including viral persistence, quasipecies evolution, and viral immune escape, is needed to characterize the disease causing potential of this new group of viruses.

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REFERENCES

Al Moslih MI, Abuodeh RO, Hu YW. 2004. Detection and genotyping of TT virus in healthy and subjects with HBV or HCV in different populations in the United Arab Emirates. J Med Virol 72:502–508.

Bendinelli M, Pistello M, Maggi F, Fornai C, Freer G, Vatteroni ML. 2001. Molecular properties, biology, and clinical implications of TT virus, a recently identified widespread infectious agent of humans. Clin Microbiol Rev 14:98–113.

Charlton M, Adjei P, Poterucha J, Zein N, Moore B, Therneau T, Krom R, Wiesner R. 1998. TT-virus infection in North American blood donors, patients with fulminant hepatic failure, and cryptogenic cirrhosis. Hepatology 28:839–842.

Chen BP, Rumi MG, Colombo M, Lin YH, Ramaswamy L, Luna J, Liu JK, Prati D, Mannucci PM. 1999. TT virus is present in a high frequency of Italian hemophilic patients transfused with plasma-derived clotting factor concentrates. Blood 94:4333–4336.

Cleavinger PJ, Persing DH, Li H, Moore SB, Charlton MR, Sievers C, Therneau TM, Zein NN. 2000. Prevalence of TT virus infection in blood donors with elevated ALT in the absence of known hepatitis markers. Am J Gastroenterol 95:772–776.

Cossart Y. 2000. TTV—A virus searching for a disease. J Clin Virol 17:1–3.

Gallian P, Berland Y, Olmer M, Raccah D, de MP, Biagini P, Simon S, Bouchouareb D, Mourey C, Roubicek C, Touinssi M, Cantaloube JF, Dussol B, de LX. 1999. TT virus infection in French hemodialysis patients: Study of prevalence and risk factors. J Clin Microbiol 37:2538–2542.

Hino S. 2002. TTV, a new human virus with single stranded circular DNA genome. Rev Med Virol 12:151–158.

Hohne M, Berg T, Muller AR, Schreier E. 1998. Detection of sequences of TT virus, a novel DNA virus, in German patients. J Gen Virol 79:2761–2764.

Hu YW, Al Moslih MI, Al Ali MT, Khameneh SR, Perkins H, Diaz-Mitoma F, Roy JN, Uzicanin S, Brown EG. 2005. Molecular detection method for all known genotypes of TT virus (TTV) and TTV-like viruses in thalassemia patients and healthy individuals. J Clin Microbiol 43:3747–3754.

Kanda Y, Chiba S, Tanaka Y, Kami M, Saito T, Asai T, Izutsu K, Yuji K, Ogawa S, Honda H, Mitani K, Yazaki Y, Hirai H. 1999. TT virus in frequently transfused patients. Am J Med 106:116–117.

Kobayashi M, Chayama K, Arase Y, Tsubota A, Suzuki Y, Koida I, Saitoh S, Murashima N, Ikeda K, Koike H, Hashimoto M, Kumada H. 1999. Prevalence of TT virus before and after blood transfusion in patients with chronic liver disease treated surgically for hepatocellular carcinoma. J Gastroenterol Hepatol 14:358–363.

Maggi F, Pifferi M, Fornai C, Andreoli E, Tempestini E, Vatteroni M, Presciuttini S, Marchi S, Pietrobelli A, Boner A, Pistello M, Bendinelli M. 2003. TT virus in the nasal secretions of children with acute respiratory diseases: Relations to viremia and disease severity. J Virol 77:2418–2425.

Matsumoto A, Yeo AE, Shih JW, Tanaka E, Kiyosawa K, Alter HJ. 1999. Transfusion-associated TT virus infection and its relationship to liver disease. Hepatology 30:283–288.

Meng XW, Komatsu M, Goto T, Nakane K, Ohshima S, Yoneyama K, Lin JG, Watanabe S. 2001. Clinical significance of TT virus in chronic hepatitis C. J Gastroenterol Hepatol 16:202–208.

Nishizawa T, Okamoto H, Konishi K, Yoshizawa H, Miyakawa Y, Mayumi M. 1997. A novel DNA virus (TTV) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology. Biochem Biophys Res Commun 241:92–97.

Oguchi T, Tanaka E, Orii K, Kobayashi M, Hora K, Kiyosawa K. 1999. Transmission of and liver injury by TT virus in patients on maintenance hemodialysis. J Gastroenterol 34:234–240.

Okamoto H, Mayumi M. 2001. TT virus: Virological and genomic characteristics and disease association. J Gastrenterol 36:519–529.

Okamoto H, Nishizawa T, Kato N, Ukita M, Ikeda H, Iizuka H, Miyakawa Y, Mayumi M. 1998. Molecular cloning and characterization of a novel DNA virus (TTV) associated with posttransfusion hepatitis of unknown etiology. Hepatol Res 10:1–16.

Okamoto H, Nishizawa T, Ukita M, Takahashi M, Fukuda M, Iizuka H, Miyakawa Y, Mayumi M. 1999a. The entire nucleotide sequence of a TT virus isolate from the United States (TUS01): Comparison with reported isolates and phylogenetic analysis. Virology 259:437–448.

Okamoto H, Takahashi M, Nishizawa T, Ukita M, Fukuda M, Tsuda F, Miyakawa Y, Mayumi M. 1999b. Marked genomic heterogeneity and frequent mixed infection of TT virus demonstrated by PCR with primers from coding and noncoding regions. Virology 259: 428–436.

Okamoto H, Takahashi M, Kato N, Fukuda S, Tanaka T, Miyakawa Y, Mayumi M. 2000. Sequestration of TT virus of restricted genotypes in peripheral blood mononuclear cells. J Virol 74:10236–10239.

Okamura A, Yoshioka M, Kikuta H, Kubota M, Ma X, Hayashi A, Ishiko H, Kobayashi K. 2000. Detection of TT virus sequences in children with liver disease of unknown etiology. J Med Virol 62:104–108.

Pifferi M, Maggi F, Andreoli E, Lanini L, Marco ED, Fornai C, Vatteroni ML, Pistello M, Ragazzo V, Macchia P, Boner A, Bendinelli M. 2005. Associations between nasal torquetenovirus load and spirometric indices in children with asthma. J Infect Dis 192:1141–1148.

Prati D, Lin YH, De MC, Liu JK, Farma E, Ramaswamy L, Zanella A, Lee H, Rebulla P, Allain JP, Sirchia G, Chen B. 1999. A prospective study on TT virus infection in transfusion-dependent patients with beta-thalassemia. Blood 93:1502–1505.

Simmonds P. 1998. Transfusion virology: Progress and challenges. Blood Rev 12:171–177.

Simons JN, Leary TP, Dawson GJ, Pilot-Matias TJ, Muerhoff AS, Schlauder GG, Desai SM, Mushahwar IK. 1995. Isolation of novel virus-like sequences associated with human hepatitis. Nat Med 1:564–569.

Sugiyama K, Goto K, Ando T, Mizutani F, Terabe K, Yokoyama T, Wada Y. 2000. TT virus infection in Japanese children: Isolates from genotype 1 are overrepresented in patients with hepatic dysfunction of unknown etiology. Tohoku J Exp Med 191:233–239.

Szladek G, Juhasz A, Kardos G, Szoke K, Major T, Sziklai I, Tar I, Marton I, Konya J, Gergely L, Szarka K. 2005. High co-prevalence of genogroup 1 TT virus and human papillomavirus is associated with poor clinical outcome of laryngeal carcinoma. J Clin Pathol 58:402–405.

Takayama S, Yamazaki S, Matsuo S, Sugii S. 1999. Multiple infection of TT virus (TTV) with different genotypes in Japanese hemophiliacs. Biochem Biophys Res Commun 256:208–211.

Tanaka H, Okamoto H, Luengrojanakul P, Chainuvati T, Tsuda F, Tanaka T, Miyakawa Y, Mayumi M. 1998. Infection with an unenveloped DNA virus (TTV) associated with posttransfusion non-A to G hepatitis in hepatitis patients and healthy blood donors in Thailand. J Med Virol 56:234–238.

Tokita H, Murai S, Kamitsukasa H, Yagura M, Harada H, Takahashi M, Okamoto H. 2002. High TT virus load as an independent factor associated with the occurrence of hepatocellular carcinoma among patients with hepatitis C virus-related chronic liver disease. J Med Virol 67:501–509.

Watanabe H, Shinzawa H, Shao L, Saito T, Takahashi T. 1999. Relationship of TT virus infection with prevalence of hepatitis C virus infection and elevated alanine aminotransferase levels. J Med Virol 58:235–238.

Worobey M. 2000. Extensive homologous recombination among widely divergent TT viruses. J Virol 74:7666–7670.

Yuki N, Kato M, Masuzawa M, Ishida H, Inoue T, Tabata T, Matsushita Y, Kishimoto H, Sasaki Y, Hayashi N, Hori M. 1999. Clinical implications of coinfection with a novel DNA virus (TTV) in hepatitis C virus carriers on maintenance hemodialysis. J Med Virol 59:431–436.

Zein NN, Arslan M, Li H, Charlton MR, Gross JBJ, Poterucha JJ, Therneau TM, Kolbert CP, Persing DH. 1999. Clinical significance of TT virus infection in patients with chronic hepatitis C. Am J Gastroenterol 94:3020–3027.

別紙様式第2-1

No. 32

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2008. 2. 22	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況	ProMED 20080218.0645, 2008 Feb 18. 情報源:[1]G1 Globo.com, 2008 Feb 13. [2]Milenio.com, 2008 Feb 17.	公表国 [1]ブラジル[2]パラグアイ
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)			

研究報告の概要

○南米における黄熱のアウトブレイク
[1]ブラジル
2008年1月21日、32歳の男性が黄熱のため死亡した。これは、ブラジルで発生した15人目の黄熱死亡患者である。保健当局の発表によると、この男性は2月13日に感染が確認されており、首都ブラジリア近郊のソブランディーノの病院で死亡した。ブラジリアで感染したと見られている。また、Mato Grossoでも1名の感染と死亡が確認された。
[2]パラグアイ
保健当局は2月16日に、首都アスンシオンの病院で集中治療を受けていた39歳の女性が死亡したと発表した。パラグアイではこれまでに、少なくとも6名が黄熱によって死亡した。多くの市民がワクチン投与を求めて病院に殺到している。政府は944,000人分のワクチンをブラジルから輸入した。その大半はブラジル政府から寄付されたものである。ドゥアルテ大統領は15日、黄熱感染対応のため非常事態宣言を発令した。

報告企業の意見	今後の対応
南米で黄熱の流行が拡大し、パラグアイで6名、ブラジルで15名の黄熱死亡患者が発生したの報告である。	日本赤十字社は、輸血感染症対策として献血時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
新鮮凍結血漿-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

Archive Number 20080218.0645
Published Date 18-FEB-2008
Subject PRO/AH/EDR> Yellow fever - South America (02): Paraguay, Brazil

YELLOW FEVER - SOUTH AMERICA (02): PARAGUAY, BRAZIL
****
A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail is a program of the
International Society for Infectious Diseases
<http://www.isid.org>

[1] Brazil
[2] Paraguay

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[1] Brazil
Date: Wed 13 Feb 2008
Source: G1 Globo.com [in Portuguese, trans. Mod. TY, edited]
<http://g1.globo.com/Noticias/Brasil/0,,MUL297999-5598,00.html>

A 32-year-old man died in Brazilia of yellow fever (YF) on 21 Jan 2008. With this death, the number of deaths in the country due to this disease has increased to 15.

The Secretary of Health of the Federal District (DF) confirmed this additional death from yellow fever on Wednesday [13 Feb 2008]. The man died at the hospital in Sobradinho, a satellite city of Brasilia. The report confirming the cause of death was issued this past Wednesday [13 Feb 2008].

According to the Ministry of Health, the likely location of infection of the man was in the Federal District. That contradicts what the health authorities in Brasilia have previously expressed. According to them, prior to this announcement, the people who died of YF in the DF had all been infected in Goias [state]. Of the cases reported in the DF, 11 were confirmed, 3 are being investigated and 2 were discarded [based on] clinical [grounds] and laboratory [results].

Mato Grosso
The Ministry of Health, also confirmed on Wednesday [13 Feb 2008], the 1st YF case in Mato Grosso (MT). Laboratory tests performed by the Evandro Chagas Institute, in Para, indicated that a farmer from Novo Sao Joaquim, MT died of the disease.

According to the Ministry of Health, the state of Mato Grosso has 2 other suspected cases of the disease which are still under investigation.

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

[This worrisome report indicated that the man who died of YF acquired his infection in a satellite city of the DF, suggesting possible urban transmission. ProMED-mail requests more information concerning the probable location of infection and the travel history of the above mentioned fatality (in the DF), in order to have a better idea if this was another sylvan (jungle or forest) YF case or was truly a case of urban YF virus transmission. The Mato Grosso death is very likely a sylvan YF case.

An interactive ProMED health map of Brazil showing the location of Goias and Mato Grosso states and the Federal District can be accessed at: <http://healthmap.org/promed?v=-10.8,-53.1,4>. - Mod.TY]]

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[2] Paraguay
Date: Sun 17 Feb 2008
Source: Milenio.com [in Spanish, trans. & summ. Mod. TY, edited]
<http://www.milenio.com:80/index.php/2008/02/17/194717/>

Health authorities reported this Sunday [17 Feb 2008] that a 39-year-old woman died Saturday night [16 Feb 2008], after a week of intensive therapy in a hospital in the capital [Asuncion].

At least 6 people have died in Paraguay as a result of the yellow fever (YF) outbreak which has the entire population on alert, and responding with a massive [influx going to] vaccination centers, the government announced. Thousands of citizens went to the health centers in the capital where massive vaccination is taking place.

This weekend, the country received 944 000 doses of [YF] vaccine from Brazil, of which 800 000 were donated by the government of the neighboring country and 144 000 were furnished by the Panamerican Health Organization.

Nicanor Duarte, the President of Paraguay, this past Friday [15 Feb 2008] declared a national state of emergency to address the YF outbreak, so that the [governmental] authorities can deal with this health emergency.

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

[Given the massive vaccination campaign in the capital city, it appears that the previous urban YF cases that were acquired there have generated considerable concern (panic?) on the part of both the government and the citizens. ProMED-mail would be interested to know if similar vaccination campaigns are being carried out in other areas of Paraguay. Brazil, which had embargoed the export of the YF vaccine produced there, has shown remarkable public health citizenship by providing vaccine to Paraguay in a very timely way, despite continuing YF cases in Brazil.

A map of Paraguay can be accessed at:
<http://www.lib.utexas.edu/maps/americas/paraguay_pol98.jpg>. - Mod.TY]

[see also:
Yellow fever - South America: Paraguay, Brazil 20080217.0627
Yellow fever - Paraguay (03): (San Pedro): corr. 20080209.0533
Yellow fever - Paraguay (03): (San Pedro) 20080208.0511
Yellow fever - Paraguay (02): (San Pedro) alert 20080206.0475
Yellow fever - Paraguay (San Pedro) 20080205.0467
Yellow fever, monkeys - Argentina (02): conf. 20080212.0568
Yellow fever - Brazil (10): 20080205.0461
Yellow fever, monkeys - Argentina: (Misiones), susp. 20080205.0459
Yellow fever - Brazil (09): 20080203.0439
Yellow fever - Brazil (08): 20080124.0293
Yellow fever - Brazil (07): 20080119.0240
Yellow fever - Brazil (06): 20080116.0203
Yellow fever - Brazil (05): conf. 20080115.0194
Yellow fever - Brazil (04): susp. 20080111.0147
Yellow fever - Brazil (03) 20080110.0139
Yellow fever - Brazil (02): alert 20080109.0107
Yellow fever - Brazil: (Goias) susp. 2007 20080105.0056
2007

Yellow fever, monkeys - Brazil: (Goias), susp., RFI corr. 20071231.4196
Yellow fever, monkeys - Brazil: (Goias, Fed. Distr.): conf. 20071229.4173
Yellow fever, human, monkey - Brazil, Bolivia: 2007 20071224.4126
Yellow fever, monkey - Brazil (PI): susp 20071222.4119
Yellow fever, monkeys - Brazil (Goias): susp., RFI 20071217.4052
Yellow fever, monkeys - Brazil (RS): alert 20070910.2979
Yellow fever, human, monkey - Brazil (MG): not 20070508.1486
Yellow fever - Brazil (G[illegible]) alert 20070424.1335

Yellow fever, human, monkey - Brazil (MG) 20070421.1304]
.....mpp/ty/mpp

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2007. 11. 22	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	Ziemann M, Krueger S, Maier AB, Unmack A, Goerg S, Hennig H. Transfusion. 2007 Nov;47(11):1972-83.	公表国 米国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社) 合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)			

研究報告の概要

○セロコンバージョンと関連した供血者血漿検体中のサイトメガロウイルスDNAの高頻度陽性

背景:ヒトサイトメガロウイルス(CMV)は、血液細胞に潜伏感染すると考えられている。免疫不全患者の輸血感染(TT-CMV)は、CMV-血清反応陰性成分または白血球除去成分を使用しても発現する。

試験デザインおよび方法:過去にCMV血清反応陰性で、初めて抗CMV IgG陽性を示した供血者82名、1年以上血清反応陽性である供血者598名、血清反応陰性供血者150名を対象として、血漿中のCMV DNA陽性率を検討した。本試験後半では、供血血液31,745に基づく供血血液全体のCMV DNA陽性率を評価した。

結果:CMV DNAは、新たに血清反応陽性となった供血者の血漿検体の44%に反復的に検出された(直近前回の血清反応陰性成分供血までの期間に応じて12%～62%の範囲)。継続的な血清反応陽性または血清反応陰性供血者はいずれも、CMV DNA陰性であった。セロコンバージョンに関連したCMV DNAの検出は、ネオプテリンの有意な増加、ALT増加、白血球数減少と関連付けられたが、これら代替マーカーの感度はわずか71%であった。CMV初感染供血者による血液製剤中のCMV DNAの全体的な陽性率は0.13%以上であった。

結論:白血球除去の実施にもかかわらず、新規血清反応陽性供血者のウイルス血症はTT-CMV残存リスクの重大原因であると考えられる。本試験ではウインドウ期が検出可能で、再燃は検出できなかったため、血清反応陰性供血者由来の白血球除去血液の輸血には、1年以上血清反応陽性である供血者由来白血球除去血液の輸血と比較して、TT-CMVの高いリスクが示される可能性が考えられた。

報告企業の意見	今後の対応
新規CMV血清反応陽性供血者は血漿中のCMV DNA陽性率が高く、白血球除去を実施していてもTT-CMV残存リスクの重大原因であると考えられるとの報告である。	CMV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。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-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

High prevalence of cytomegalovirus DNA in plasma samples of blood donors in connection with seroconversion

Malte Ziemann, Sabine Krueger, Andrea B. Maier, Alexander Unmack, Siegfried Goerg, and Holger Hennig

BACKGROUND: Human cytomegalovirus (CMV) is considered to latently infect blood cells. Transfusion-transmitted infection (TT-CMV) of immunocompromised patients occurs despite the use of CMV-seronegative or leukoreduced units.

STUDY DESIGN AND METHODS: The prevalence of CMV DNA in plasma was investigated in 82 blood donors who had previously been seronegative for CMV and showed anti-CMV immunoglobulin G for the first time, 598 blood donors who were seropositive for at least 1 year, and 150 seronegative blood donors. In a second part of the study, the overall prevalence of CMV DNA in blood donations was assessed based on 31,745 donations.

RESULTS: CMV DNA was repeatedly detected in plasma samples of 44 percent of newly seropositive donors (12%-62%, depending on the interval to the last seronegative donation). All steadily seropositive or seronegative donors were negative for the presence of CMV DNA. Detection of CMV DNA in connection with seroconversion was accompanied by significantly increased neopterin, increased alanine aminotransferase, and reduced white blood cell counts, but the sensitivity of these surrogate markers was only 71 percent. The overall prevalence of CMV DNA in blood products due to primary CMV infection of donors was at least 0.13 percent.

CONCLUSION: Viremia of newly seropositive donors may be an important reason for the residual risk of TT-CMV despite leukoreduction. Furthermore, transfusion of WBC-reduced blood components from seronegative donors could imply a greater risk of TT-CMV than transfusion of WBC-reduced blood from donors who have been seropositive for at least 1 year, because window-phase donations but no reactivation could be detected in this study.

Human cytomegalovirus (CMV) is an ubiquitous β-herpesvirus causing mostly asymptomatic or mild mononucleosislike infections in immunocompetent subjects[1] with a prevalence of between 40 and 100 percent in adult populations.[2] Contrarily, infection of immunocompromised patients with CMV is a significant cause of morbidity and mortality. Symptoms of CMV infection in these patients cover a broad range from direct manifestations of viral replication like fever, leukopenia, thrombocytopenia, hepatitis, enteritis, and pneumonia to indirect sequelae like an elevated risk for renal allograft rejection or an impaired cellular immune response.[3,4]

An important route of infection for risk groups like seronegative recipients of marrow transplants or newborns is assumed to be transmission of CMV by blood products from latently infected blood donors (so called transfusion-transmitted CMV infection [TT-CMV]). Even if the exact sites and mechanisms of latency still remain to be clarified, CMV DNA has repeatedly been found in peripheral blood white blood cells (WBCs) of healthy, CMV-seropositive individuals, especially in cells of the myeloid lineage.[5,6]

Consequently, leukodepletion of blood products and inventories of seronegative blood donors have been employed to reduce the rates of TT-CMV since the 1980s.[7,8] Even after implementation of these strategies, however,

ABBREVIATIONS: CRP = C-reactive protein; TT-CMV = transfusion-transmitted cytomegalovirus.

From the Institute of Immunology and Transfusion Medicine, University of Lübeck, Lübeck, Germany.

Address reprint requests to: Holger Hennig, Institute of Immunology and Transfusion Medicine, University of Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany; e-mail: hennig@immu.mu-luebeck.de.

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TRANSFUSION 2007;47:1972-1983.

"break-through" infections persist with rates as high as 1 to 3 percent of transfused high-risk patients.[9-15] The impact of active CMV infection of donors (both primary and reactivated) with transmission of infectious virus as a reason for these break-through infections is discussed controversially.[16-18]

To our knowledge, infectious virus has never directly been detected in leukodepleted blood components. This may be due to the relatively poor sensitivity of commonly applied viral cultures or shell vial assays[19] even if more sensitive methods have been described recently.[20] CMV DNA in serum or plasma, on the other hand, is associated with active CMV infection[21] and used routinely for diagnosis and monitoring of CMV infections in risk groups like transplant recipients[22] or acquired immune deficiency syndrome (AIDS) patients.[23]

Drew and coworkers[17] found CMV DNA in the last seronegative sample of 1 of 192 donors (0.5%) and in the first seropositive sample of 2 donors (1.0%), both of whom were excluded from donation because of elevated alanine aminotransferase (ALT). This contrasts with the findings of Zanghellini and colleagues[24] who detected CMV DNA in plasma of 4 of 5 adolescents with CMV seroconversion, but so far no further study has addressed the prevalence of CMV DNA associated with seroconversion of donors.

Because the actual date of seroconversion can be any point between the last seronegative and the first seropositive donation, the prevalence of CMV DNA in the plasma of first-time seropositive donors would be expected to be higher if the interval since the last seronegative sample is relatively short. Nevertheless, there are no data about the correlation between interdonation interval and prevalence of CMV DNA in plasma of newly seroconverted donors or the variations in prevalences of CMV DNA between different donor collectives.

Therefore, we conducted a prospective study, grouping newly seropositive donors according to the interval since their last seronegative sample and measuring CMV DNA in plasma samples before and after seroconversion. Another objective of our study was to determine the sensitivity of surrogate markers for viral infections, like neopterin, ALT, or WBC count for the detection of CMV DNA in plasma in connection with seroconversion.

MATERIALS AND METHODS

Blood donors

Between August 2000 and June 2004, approximately 12,800 volunteer regular blood donors (47% female, 53% male) donated approximately 34,000 whole-blood donations per year (41% by female and 59% by male donors). They were between 18 and 67 years old and healthy and gave informed consent before the donation. Out of this blood donor collective, we investigated 82 well-defined CMV seroconversion cases, whereas the total number of CMV seroconversions during this period has not been determined. Donors were grouped according to the interval since the last seronegative sample, with intervals of less than 120, 120 to 729, and 730 days or more.

Additionally, 598 latently infected blood donors who had been seropositive for at least 1 year were included in this study, 148 of whom had been excluded from donation because of elevated ALT (more than 73 U/L or 112 U/L for female and male donors, respectively). Sampling dates from latently infected donors were distributed evenly throughout the year considering potential seasonal reactivations.[16] A total of 150 CMV-seronegative donors were tested for CMV DNA as controls.

In a further part of the study, all available samples from previously seronegative donors who were repeatedly reactive in the recombinant CMV immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA) between January and December 2006 were tested by polymerase chain reaction (PCR) to determine the minimum rate of CMV DNA–positive donations due to primary CMV infection of donors in our donor population.

Blood specimens

Whole-blood samples were collected in 5.5-mL tubes containing potassium-ethylenediaminetetraacetate (EDTA) at a concentration of 1.6 mg EDTA per milliliter of blood (Monovette, Sarstedt, Nümbrecht, Germany). Such samples were centrifuged at $3291 \times g$ for 4 minutes and EDTA plasma was separated within 24 hours. Plasma specimens were stored at 4 to 8°C for no longer than 72 hours or at less than –30°C until further processing.

Because of the impossibility of determining the actual seroconversion date, the date of the first seropositive sample from a previously seronegative donor was assumed to be the date of seroconversion.

Standard and control specimens

Human CMV quantitated viral DNA control, AD169 strain, Lot 110-018 (Advanced Biotechnologies Inc., Columbia, MD) was used to determine the detection limit of the CMV PCR (TaqMan, Applied Biosystems, Foster City, CA) described below and to quantify CMV DNA–positive samples. Lyophilized CMV DNA–positive cells from an external proficiency testing program (Instand e.V., Düsseldorf, Germany) were used as positive samples for the development and optimization of the TaqMan CMV PCR.

CMV serology

Anti-CMV screening was performed with an automated enzyme immunoassay to detect IgG antibodies against the autologous fusion proteins CG1 and CG2 (Biotest Anti-CMV recombinant IgG ELISA, Biotest AG, Dreieich,

Germany). Reactive samples were retested in duplicate and considered to be repeatedly reactive if at least one of the two repetitions also gave a positive result. In the first part of the study, repeatedly reactive samples were further confirmed by an automated *ELISA* with AD169-coated microparticles (AxSYM CMV IgG, Abbott GmbH, Wiesbaden, Germany).

Nucleic acid isolation

DNA from 1 mL of EDTA-plasma was prepared using the Extractor (NucliSens™, bioMérieux Deutschland GmbH, Nürtingen, Germany) according to the manufacturer's protocol for pooled plasma or serum samples up to 2.0 mL. To increase the nucleic acid yield, we added a first incubation step of samples together with the lysis buffer, which is based on guanidine thiocyanate at 60°C with horizontal shaking at 110 r.p.m. for 30 minutes. Total nucleic acids from 1 mL of plasma were eluted in 50 µL of elution buffer of which 20 µL was investigated in one PCR experiment to detect CMV DNA.

During the last part of the study between January and December 2006, DNA from 1 mL of EDTA-plasma was isolated with magnetic extraction reagents (NucliSens, bioMérieux, Boxtel, the Netherlands) according to the manufacturer's instructions.

TaqMan PCR

For amplification and simultaneous detection of PCR products, we developed a novel approach based on a quantitative PCR core kit (qPCR, Eurogentec, Seraing, Belgium) on a sequence detection system (ABI Prism 7700 SDS, Applied Biosystems). Primers and fluorogenic TaqMan probe for CMV DNA detection were chosen after comparative analysis of 68 sequences containing the glycoprotein B region of the CMV genome, which were available from the GenBank Nucleotide Database with computer software (OMIGA, Version 2.0, Oxford Molecular, Oxford, UK). In addition, we performed a nucleotide-nucleotide BLAST search via the Internet at http://www.ncbi.nlm.nih.gov/BLAST/ for the chosen oligonucleotides. For the forward primer, TaqMan probe, and reverse primer, we found 84, 80, and 84 hits, respectively, to CMV sequences that had been submitted to various databases. The sequence alignments ensured that the primers were homologous at the last 12 nucleotides at the 3′ end in all of the sequences and showed a maximum of one single-nucleotide polymorphism at the upper sequence. The TaqMan probe showed 100 percent homology or only one mismatch to all of the CMV hits. Seventeen non-CMV BLAST hits each to only one of the three CMV oligonucleotides ensured that no other organism could be detected with this method.

A sequence from the human C-reactive protein (CRP) gene, which was found to be detectable in human plasma was coamplified in each reaction as internal control.[25] The CMV probe was labeled with FAM as reporter and TAMRA as quencher and the CRP probe with VIC and TAMRA dyes. The CMV primers and probe were custom-synthesized by Eurogentec (Liege, Belgium), the CRP probe by Applied Biosystems (Weiterstadt, Germany), and the CRP primers by TIB Molbiol (Berlin, Germany). The sequences of all the oligonucleotides are provided in Table 1.

PCR experiments were carried out in special optical tubes (MicroAmp optical tubes/caps, PE Applied Biosystems, Foster City, CA) in a total volume of 50 µL. Concentrations of $MgCl_2$, CMV probe and primers were optimized by means of chessboard titrations. Final concentrations were 3.5 mmol per L for $MgCl_2$, 150 nmol per L for CMV forward primer, 300 nmol per L for the respective reverse primer, and 250 nmol per L for the CMV probe. The concentration of the CRP probe was 100 nmol per L, whereas those of the CRP primers were limited to 40 nmol per L each. Thermal cycler conditions were 10 minutes at 95°C followed by 40 cycles of 15 seconds at 95°C and 1 minute at 60°C. Threshold values were calculated as the upper 10-fold standard deviation (SD) of the background fluorescence signal measured over the baseline from Cycle 3 to Cycle 30. Results were interpreted as follows: a C_T of less than 40 is positive; a C_T of equal to 40 is negative.

To determine the 95 percent detection limit of the TaqMan CMV PCR, we investigated semilogarithmic dilutions of the CMV quantitated viral DNA control containing between 10^2 and $10^{-0.5}$ genome equivalents per µL (geq/µL) of CMV strain AD169. Twenty-eight samples of each concentration were processed in four consecutive TaqMan PCR procedures according to our protocol for

TABLE 1. Primer and probe sequences

Oligonucleotide	Sequence 5′→3′	Melting temperature (°C)
CMV		
Forward primer	CCCTCAAGTATGGAGATGTGGTG	59
TaqMan probe	FAM-AACACCACCAAGTACCCCTATCGCGTG-TAMRA	69
Reverse primer	AGCGAATAAGATCCGTACCCTG	58
CRP		
Forward primer	CCTGACCAGCCTCTCTCATGC	61
TaqMan probe	VIC-TTTGGCCAGACAGGTAAGGGCCACC-TAMRA	70
Reverse primer	TGCAGTCTTAGACCCCACCC	59

plasma samples. The 95 percent detection limit was calculated by means of probit analysis. Quantification of CMV DNA–positive samples was carried out by means of a standard curve derived from these validation experiments.

Diagnosis of CMV DNAemia

All samples were analyzed by TaqMan PCR in duplicate. Samples with invalid internal control or diverging results were retested twice. DNAemia was diagnosed by reproducibly positive results.

Surrogate markers for viral infections

As part of the routine blood donor screening, ALT levels were determined by the standard IFCC method at 37°C (GPT ALAT liquid IFCC, Medizintechnik Guder, Bad Oeynhausen, Germany) with an automated analyzer (COBAS Mira plus CC, Roche Diagnostics Instruments Center, Rotkreuz, Switzerland). ALT screening was mandatory in Germany until 2004 with limits for donor admission of no more than 73 and 112 U per L for female and male donors, respectively. WBC counts were measured with an automated hematology analyzer (Coulter Gen S, Beckman Coulter, Krefeld, Germany).

In a subgroup of 56 samples, the neopterin concentration was analyzed by an ELISA (neopterin ELISA RE59349, IBL Immuno Biological Laboratories, Hamburg, Germany). The cutoff value for elevated neopterin was set at 10 nmol per L representing the 98th percentile of a healthy asymptomatic population.[26]

Statistical analysis

Unless stated otherwise, means are calculated as arithmetic means ± SD. Confidence intervals (CIs) were calculated with a p value of 0.05. Differences between groups were examined with the U test. Calculations were assisted by database and statistical programs (Excel, Microsoft Corp., Redmond, WA; SPSS, SPSS Inc., Chicago, IL). The probability of appearance of CMV DNA in plasma of latently infected blood donors was calculated with the upper limits of $1 - \alpha$ confidence intervals of the binomical distribution for an α level of 0.05.

The sensitivity of surrogate markers for detection of CMV DNA–positive donations was calculated as the number of CMV DNA–positive donations with elevated markers related to the total number of CMV DNA–positive donations tested for this marker. For neopterin, for instance, this results in the formula

Sensitivity = (Number of CMV DNA–positive donations with elevated neopterin)/(Number of CMV DNA–positive donations tested for neopterin).

The percentage of patients potentially transfused with CMV DNA–positive blood components due to primary CMV infection of donors was calculated according to the formula

$$\%\text{Patients} = 100 \times [1 - (1 - p)^{\text{number of units transfused}}].$$

In this formula, p denotes the proportion of CMV DNA–positive donations related to all donations. Therefore, $(1 - p)$ is the probability of donations being negative for the presence of CMV DNA, and $(1 - p)^n$ is the probability of n units of blood from different donations all being negative for CMV DNA. So $1 - (1 - p)^n$ equals the probability of n units blood containing at least one CMV DNA–positive unit.

RESULTS

TaqMan PCR

Of 1055 plasma samples tested by TaqMan PCR, 1042 (98.8%) were clearly positive or negative, whereas only 13 (1.2%) showed ambiguous results. These were due to insufficient sample volume for repeated testing (6 samples) or equivocal results even of repeated testing (7 samples with 2 positive and 2 negative results each). All samples with ambiguous results were excluded from analysis.

The 95 percent detection limit of the TaqMan PCR was calculated to be 4.88 geq per PCR procedure (3.66-8.22 geq/PCR) with semilogarithmic dilutions of CMV quantitated viral DNA control (Table 2). For 1-mL plasma specimens, it would correspond to approximately 13.5 geq per mL, if an efficacy of DNA isolation of 90 percent is assumed. The mean CMV DNA concentration in positive samples was 166 geq per mL (SD, 395 geq/mL), with a maximum of approximately 3200 geq per mL.

CMV DNA in connection with seroconversion of blood donors

Eighty-two blood donors who were previously tested negative for the presence of CMV IgG antibodies at the

TABLE 2. Observed frequencies in TaqMan CMV PCR

Standard (geq/PCR procedure)	Number of subjects	Observed responses	Probit
100	28	28	1.000
31.6	28	28	1.000
10	28	28	0.999
3.16	28	21	0.784
1	28	15	0.384
0.316	27*	4	0.263

* One subject was excluded due to negative results for CRP DNA.

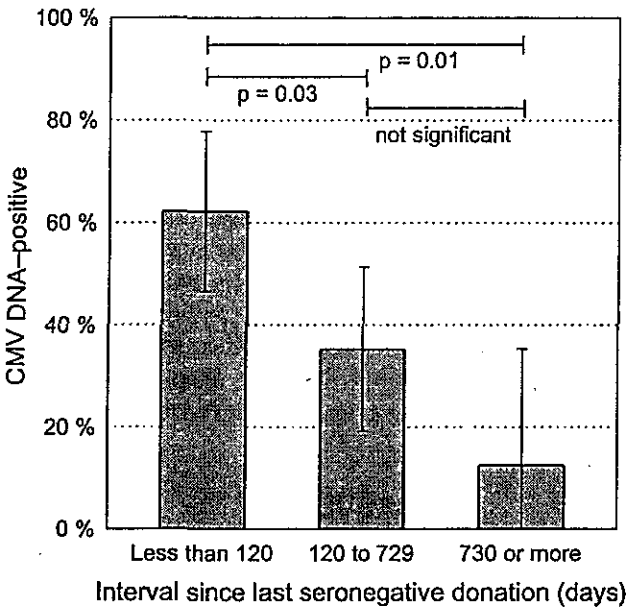

Fig. 1. Prevalence of CMV DNA in plasma of newly seropositive donors ($n = 79$) in dependence on the interval since the last seronegative sample. Prevalences are shown as percentages with 95 percent CI. The percentage of CMV DNA–positive donors was significantly greater after intervals of less than 120 days.

time of their last donation were repeatedly reactive in both anti-CMV assays and further investigated for the presence of CMV DNA. Thirty-six of 82 newly seropositive samples (44%) were repeatedly positive for the presence of CMV DNA by TaqMan PCR. The prevalence of CMV DNA was significantly higher if the interval since the last seronegative donation was less than 120 days ($p < 0.01$), whereas further differences between donors with longer intervals were not significant (Fig. 1).

In 68 (83%) of 82 seroconversion cases, we investigated the last CMV-seronegative donation before seroconversion. This way, we identified 2 reproducibly CMV DNA–positive window-phase donations (2.9%), 68 and 98 days before the first CMV-seropositive donation.

A second seropositive sample was available from 71 (87%) of 82 donors. Sixty-two of these samples (76%) were drawn within 1 year after the first seropositive sample. Samples from 4 donors were reproducibly CMV DNA–positive on Days 3, 5, 20, and 84 after the first seropositive sample, respectively (Table 3). Further plasma samples were available from only 2 of these donors. Both tested CMV DNA–negative on Day 97 and 207, respectively. So CMV DNA–negative samples were available from 59 of 82 donors (72%) within 1 year after the first seropositive sample, whereas no sample tested CMV DNA–positive 1 year or more after the first seropositive sample.

CMV DNA in latently infected and seronegative blood donors

All plasma samples of 150 seronegative and 450 latently infected donors who had been seropositive for at least 1 year were tested negative for the presence of CMV DNA. Additionally, 148 samples of latently infected donors with elevated ALT were available with mean ALT levels of 113 U per L (range, 76-906 U/L). These samples, too, all tested negative for the presence of CMV DNA. Based on the sample size, the proportion of CMV DNA–positive donors related to the total donor population (95% CI) was estimated to be less than 0.5 percent for latently infected donors and no more than 2 percent for latently infected donors with elevated ALT or seronegative donors.

Overall prevalence of CMV DNA in blood donations

In 2006, 102 previously seronegative donors tested repeatedly reactive in the recombinant IgG ELISA. This corresponds to an annual seroconversion rate of 0.8 percent relative to the total donor population.

Thirty-six donations from newly seropositive donors (41% of available samples) tested repeatedly positive for CMV DNA. Assuming the prevalence of CMV DNA in the first seropositive donation to be 41 percent for all 102 seroconversion cases results in a minimum rate of CMV DNA–positive units of 42 of 15,094 seropositive units (0.28%) or 42 of 31,745 units (0.13%), if the CMV serostatus is not taken into consideration (Fig. 2). These rates underestimate the actual prevalence of CMV DNA–positive units, because both window-phase donations and further seropositive donations containing CMV DNA have been ignored.

Surrogate markers for CMV DNAemia

The three common surrogate markers for subclinical viral infections, neopterin, ALT, and WBC count, have been tested in comparison to CMV DNA. Newly seropositive donors with CMV DNAemia had significantly higher neopterin and ALT values, as well as significantly lower WBC counts compared to newly seropositive donors without detectable CMV DNA in plasma. The best sensitivity was achieved by the neopterin ELISA, which detected 61 percent of CMV DNA–positive samples. The sensitivity of ALT was 42 percent if any values outside the normal range were considered. ALT values above the former German national limits for donor admission (>73 or 112 U/L for female and male donors, respectively) were detected in only 4 of 36 CMV DNA–positive donors and in no CMV DNA–negative donor. This equals a sensitivity of 11 percent.

WBC counts were slightly low in 6 of 36 CMV DNA–positive subjects (between 3.4×10^9 and 3.9×10^9/L),

TABLE 3. CMV DNA in plasma samples*

Donor status	Number of samples	CMV DNA		Excluded
		Positive	Negative	
Seronegative donors	150	0 (0%)	150 (100%)	0 (0%)
Donors with seroconversion				
Last seronegative sample	68	2† (3%)	64‡ (94%)	2§ (3%)
First seropositive sample	82	36 (44%)	43 (52%)	3‖ (4%)
Second seropositive sample	71	4¶ (6%)	66** (93%)	1†† (1%)
Donors who were seropositive for at least 1 year	450	0 (0%)	450 (100%)	0 (0%)
Donors with elevated ALT‡‡ who were seropositive for at least 1 year	148	0 (0%)	148 (100%)	0 (0%)

* Data are reported as number (%).
† Drawn 68 and 98 days before the first seropositive sample (median, 83 days).
‡ Drawn 15 to 1513 days before the first seropositive sample (median, 192 days).
§ One due to ambiguous PCR results and one due to insufficient sample volume for repeated testing after a positive PCR result. Drawn 35 and 105 days before the first seropositive sample (median, 70 days).
‖ One due to ambiguous PCR results and two due to insufficient sample volume for repeated testing after a positive PCR result.
¶ Drawn 3 to 84 days after the first seropositive sample (median, 12.5 days).
** Drawn 15 to 798 days after the first seropositive sample (median, 131 days). Fifty-seven of 66 samples (86%) were drawn after an interval of no more than 365 days.
†† Due to insufficient sample volume for repeated testing after a positive PCR result. Drawn 39 days after the first seropositive sample.
‡‡ 76 U/L or more.

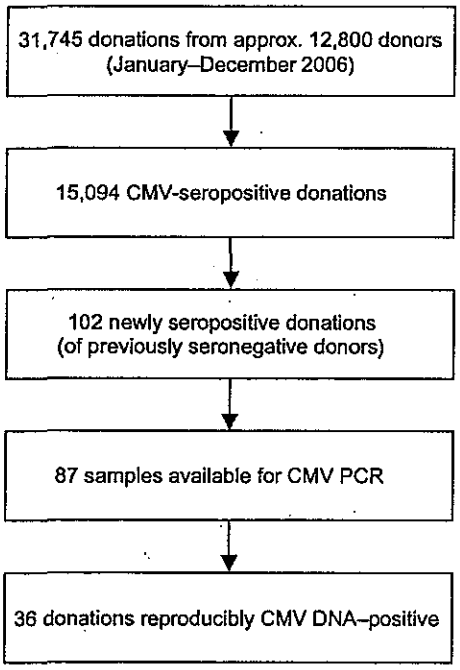

Fig. 2. Prevalence of CMV DNA in plasma of blood donors due to primary CMV-infections. Between January and December 2006, 36 out of 87 donations from newly seropositive donors contained CMV DNA (41%). 44 samples tested DNA-negative and 7 samples were excluded (5 because of ambiguous results and 2 due to insufficient sample volume for repeated testing after a positive PCR result). Assuming the prevalence of CMV DNA in the first seropositive donation to be 41 percent for all 102 seroconversion cases results in a minimum rate of CMV DNA–positive units of 42 out of 15,094 seropositive units (0.28%) or 42 out of 31,745 units (0.13%), if the CMV-serostatus is not considered.

resulting in a sensitivity of 17 percent. A combined screening with neopterin, ALT, and WBC counts would have a sensitivity of 71 percent, if all values outside the normal range are considered (Table 4).

DISCUSSION

The prevalence of CMV DNA in plasma of newly seropositive donors was 44 percent in our study. To our knowledge, the only other study examining CMV DNA in connection with seroconversion of blood donors is the study of Drew and colleagues[17] who detected CMV DNA in only 2 of 192 first-time seropositive donors (1%).

One possible reason for this marked difference is the lower limit of detection of the TaqMan PCR (~13.5 geq/mL) in comparison to the PCR applied by Drew and coworkers (400 geq/mL). In the first part of our study, only 4 of 82 newly seropositive donors (5%) had CMV DNA levels of 400 geq per mL or more in their first seropositive sample. Furthermore, the interval to the last seronegative donation is given as "8 weeks to years" in the study of Drew and coworkers without any mean or medium interval given. A high proportion of donors with long interdonation intervals could have led to a lower number of CMV DNA–positive donors, as the prevalence of CMV DNA in our study was significantly higher after short interdonation intervals. Even an influence of the different target sequence used by Drew and coworkers (pol instead of gB) cannot be excluded.

Zanghellini and colleagues[24] reported approximately 45 seronegative adolescents, who were screened for development of CMV antibodies at monthly intervals. They detected 6 seroconversion cases and tested plasma samples from 5 seroconverted adolescents by CMV PCR finding CMV DNA in samples from 4 subjects (80%). This

TABLE 4. Neopterin concentration, ALT level, and WBC count as surrogate markers for CMV DNA in plasma of newly seropositive donors

Surrogate marker	CMV DNA–positive donors	CMV DNA–negative donors
Neopterin concentration (n = 56)*	13.6 ± 9.1†	6.8 ± 2.8†
<10 nmol/L	11	24
≥10 nmol/L	17	4
Donor sensitivity (%)	61 (17/28)	
ALT level (n = 79)	43.9 ± 35.7†	23.2 ± 11.2†
≤30 or 40 U/L‡	21	37
>30 or 40 U/L‡	15	6
Donor sensitivity (%)	42 (15/36)	
WBC count (n = 78)§	5.2 ± 1.1†	6.3 ± 1.9†
$<4 \times 10^9$/L	6	3
Between 4 and 10×10^9/L	30	38
$>10 \times 10^9$/L	0	1
Donor sensitivity (%)	17 (6/36)	
Combined screening (n = 56)*		
All tests normal	8	21
Any test positive	20	7
Donor sensitivity (%)	71 (20/28)	

* Neopterin was measured in a subset of 59 donors, of whom 3 had ambiguous PCR results.
† p = 0.001, p = 0.002, and p = 0.004 for differences in neopterin concentrations, ALT levels, or WBC counts between DNA-positive and DNA-negative donors, respectively.
‡ Values for female and male donors, respectively.
§ WBC counts for one CMV DNA–negative donor are missing.

high prevalence of CMV DNA must be interpreted with caution owing to the low number of subjects studied, but it could be caused by the short screening interval, which would be in accordance with our results.

In a study of 420 blood donors conducted by Glock and coworkers,[18] CMV DNA in serum was detected solely in an IgG-positive donor with equivocal results for IgM, but not in 185 IgM-negative and IgG-positive donors. No information about the date of seroconversion is supplied by the authors, however.

Detection of CMV DNA in serum or plasma correlates well with presence of infectious virus in transplant recipients[21] and patients with AIDS.[23] Even if assays for detection of viable CMV with detection limits corresponding to 27 geq CMV DNA per mL have been described[20] most viral cultures or shell vial assays have relatively low sensitivities.[19,27] Therefore, detection of CMV DNA in plasma or serum is routinely used for diagnosis and monitoring of CMV infections in transplant recipients.[22] Consequently, CMV PCR has recently been suggested for screening of cord blood samples used for transplantation.[28]

The concerns of some authors[29] about lacking infectivity of CMV DNA–positive blood donations are based on a single study of three renal transplant recipients with active CMV infection showing CMV DNA in plasma to be highly fragmented.[30] But even the authors of this study conclude that, "It is beyond doubt that CMV DNA load measurements are important for prediction and diagnosis of CMV disease." Neutralizing antibodies against CMV could reduce the infectivity of seropositive CMV DNA–positive donations, but they are not expected to achieve complete neutralization as studies of convalescent sera showed neutralization capacities not exceeding 50 percent.[31]

Early studies of CMV DNA showed inconsistent results with some reports of high prevalences even in seronegative donors,[32-34] which could not be reproduced by validated PCR assays.[35] Therefore, the need for appropriate validation of PCR assays was stressed by Roback and associates.[36] Our TaqMan PCR was carefully designed to detect CMV genome with high sensitivity without cross-reaction with other organisms' DNA. Additionally, samples from all study populations were processed in arbitrary order, whereby CMV DNA was detected in connection with seroconversion, but not in plasma of 150 seronegative donors or of 598 donors who had been seropositive for at least 1 year, 148 of whom even had elevated ALT. Also ambiguous results of the TaqMan PCR were detected only in connection with seroconversion. Those results may represent CMV DNA concentrations below the 95 percent detection limit, but to ensure a conservative interpretation of the data, they had been excluded from analysis. The presence of active infection in newly seropositive donors with detection of CMV DNA in plasma is confirmed by a significantly higher percentage of donors with elevated levels of neopterin and ALT compared to seroconverted donors without detection of CMV DNA.

CMV DNA in plasma of seronegative donors during the "window period" of CMV infections was rare in our study, because CMV DNA was detected in the last seronegative sample of only 2 of 68 donors (3%). This is confirmed by Drew and colleagues[17] who studied the last seronegative sample of 192 seroconverting donors, finding only 1 DNA-positive sample (0.5%). In contrast, 2 donors in our study were CMV DNA–positive for at least 84 or 98 days, respectively. Further studies are necessary to determine the duration of CMV DNAemia in asymptomatic immunocompetent persons and to calculate the risk of window-period donations.

Even if the percentage of donors with abnormal surrogate markers like elevated ALT, elevated neopterin, or low WBC count was significantly higher in CMV DNA–positive donors compared to CMV DNA–negative donors, the sensitivity for detection of CMV DNA–positive donations with all these unspecific markers for infectious diseases was no more than 71 percent. Usually, cutoff limits for surrogate markers are set beyond the normal range to

prevent the exclusion of unnecessarily high numbers of donors.[37] This would further decrease the sensitivity of ALT and WBCs, especially, with for example only 11 percent of CMV DNA–positive donors having ALT values outside the former German limits for blood donation. This is in contrast to the results of Drew and coworkers[17] who reported both seroconverted donors with CMV DNA as being excluded from donation because of elevated ALT (92 and 117 U/mL, respectively). As reported by others[38] neopterin could be an option for blood donor screening with a sensitivity of 61 percent in our study. More effective prevention of CMV transmission could be achieved by transient exclusion of newly seroconverted donors. This was already suggested by Beneke and coworkers[39] who found a correlation between anti-CMV IgM-positive donors and TT-CMV. Lamberson and coworkers[40] confirmed that IgM-positive donors were responsible for TT-CMV in 7 of 70 seronegative neonates, whereas the only case of TT-CMV in 87 seronegative neonates after transfusion of anti-CMV IgG-positive and IgM-negative blood was explained by a false-negative result of the IgM assay.

The period of exclusion is difficult to determine on the basis of our data. The last CMV DNA–positive sample was drawn 84 days after the first seropositive donation, but additional samples were only available for 2 of 4 donors who tested CMV DNA–positive in their second seropositive sample. Thus, we cannot determine the possible duration of CMV DNAemia after seroconversion. In contrast, DNA-negative samples were available from 59 of 82 newly seropositive donors earlier than 1 year after the first seropositive sample. Furthermore, 598 donors (148 of whom even had elevated ALT), who had been seropositive for at least 1 year, tested negative for the presence of CMV DNA. This results in a 95 percent CI for the prevalence of CMV DNA–positive donors of less than 0.5 percent in this donor population. So exclusion of newly seroconverted donors for a period of 1 year after seroconversion seems to be sufficient to avoid TT-CMV.

A seasonal reactivation of latent CMV infections reported by Dumont and colleagues[16] could not be found in our donors. CMV reactivation in response to environmental allergens, which was suggested by Dumont and colleagues, could account for the differences, because a correlation between reactivation and pine tree pollen season was reported and those pollen are not a relevant allergen in Germany. A simpler explanation would be the use of systemic steroids in hay fever therapy,[41] which is very rare in Germany, but no data are available on this.

Given that 1 of every 1,000 or 10,000 peripheral blood monocytes from healthy CMV-positive individuals is supposed to be latently infected with a range of 2 to 13 geq per cell,[42,43] the number of latently infected monocytes in WBC-depleted red blood cell (RBC) units was estimated to be up to 50 and the CMV DNA concentration equals approximately 10^2 to 10^3 geq per unit.[44] Our study yielded comparable results for the mean CMV DNA concentration in connection with seroconversion ranging from 10^3 geq per unit in plasma-reduced RBC units to 10^4 geq per unit in fresh-frozen plasma or platelet (PLT) concentrates.

The seroconversion rate of 0.8 percent in our donor population corresponds well to the results of others, who found a seroconversion rate among healthy blood donors of between 0.2 and 1.2 percent.[38,45,46] In a recent meta-analysis Vamvakas[13] reports the risk of TT-CMV after transfusion of WBC-reduced components as being 2.73 percent versus 1.45 percent after transfusion of components from seronegative donors. He concludes that CMV-seronegative blood components are more efficacious in preventing TT-CMV than WBC-reduced components. Reviewing the included studies as well as other studies about TT-CMV after transfusion of WBC-reduced blood components shows great variations both in the rate of TT-CMV and in the amount of blood products transfused (Table 5).

Under the assumption that the prevalence of CMV DNA in blood products due to primary CMV infections was equal in the studies' donor populations to the minimum prevalence calculated for our institution (0.13%), the percentage of patients who had been transfused with CMV DNA–positive blood can be calculated with the previously explained equation:

$$\%\text{Patients} = 100 \times [1 - (1 - 0.0013)^{\text{number of units transfused}}].$$

With the exception of the study of Ohto and colleagues,[47] this percentage is about equal to or even higher than the percentage of patients actually developing TT-CMV. There is no correlation between the rate of TT-CMV and the proportion of patients potentially transfused with CMV DNA–positive blood. This may be due to differences between patient populations as well as donor populations, because no study provides information about the rate of CMV DNA–positive blood components or about factors influencing it such as, for example, the proportion of newly seroconverted donors relative to all seropositive donors or the length of interdonation intervals of first-time seropositive donors.

The study of Ohto and coworkers[47] differs from the other studies, because 94 percent of the studied neonates were fed with milk from their seropositive mothers. Therefore, the authors conclude that the observed CMV infections are probably unrelated to transfusions.[47]

In the retrospective study of Nichols and associates,[12] only 6 percent of the transfused units had been WBC reduced of CMV-seropositive donors, whereas 94 percent were unfiltered units of seronegative donors. A multivariate analysis identified only filtered RBCs from seropositive donors associated with an elevated risk for TT-CMV of a 32 percent relative or about 1 percent (0.2%-2%) absolute

TABLE 5. CMV infection after transfusion of WBC-depleted blood products and patients potentially transfused with CMV DNA–positive blood due to primary CMV infections*

Report (year)	Sample size	Mean number of transfused units	Percent with CMV infection	Percentage of patients potentially transfused with CMV DNA–positive blood†
Murphy et al. (1988)[48]	11	43	0	5.6
Bowden et al. (1989)[49]	17	153	0	18.4
De Graan-Hantzen et al. (1989)[50]	59	69	0	8.8
Gilbert et al. (1989)[6]	30	2	0	0.3
De Witte et al. (1990)[51]	28	52	0	6.7
Bowden et al. (1991)[52]	35	189‡	0	22.2
Eisenfeld et al. (1992)[53]	48	9	0	1.2
Van Prooijen et al. (1994)[54]	60	65	0	8.3
Bowden et al. (1995)[9]	250	102	1.2-2.4§	12.7
Narvios et al. (1998)[14]	45	141‖	2.2	17.1
Ohto et al. (1999)[47]	33	3	9.1¶	0.4
Pamphilon et al. (1999)[55]	62	Not specified	0	Not applicable
Nichols et al. (2003)[12]	807	24**	3.0	3.1
Narvios et al. (2005)[15]	72	55-77††	2.8	7.1-9.7

* This table summarizes studies included in the meta-analysis of Vamvakas[13] as well as two additional studies.[14,52]
† Estimated according to the mean number of units blood transfused in the respective study and to an assumed prevalence of CMV DNA in the whole donor population due to primary CMV infections of 0.13 percent, with the previously explained formula: %patients = $100 \times [1 - (1 - 0.0013)^{\text{number of units}}]$.
‡ 164 units of WBC-reduced PLTs and 25 unfiltered RBC units from seronegative donors.
§ Dependent on whether infections occurring between Day 0 and Day 20 are counted.
‖ Approximately 30 percent from unscreened donors and 70 percent from seronegative donors.
¶ 94 percent of neonates were fed with milk from CMV–seropositive mothers.
** 0.3 WBC reduced units from CMV-seropositive donors and 23.7 unfiltered units from seronegative donors.
†† Dependent on the number of donations pooled for random PLT concentrates.

increase per RBC unit. This is comparable with the rate of units from newly seropositive donors containing CMV DNA estimated for our donor population (0.28%). Because free CMV cannot be removed efficiently by WBC reduction, transmission of cell-free virus from newly seroconverted donors could be an explanation for at least some of the cases of TT-CMV after transfusion of WBC-reduced components.

We did not analyze whether residual WBCs in WBC-depleted blood components of newly seroconverted or latently infected donors contained CMV DNA. Nevertheless, it cannot be ruled out that the residual risk of TT-CMV with WBC-depleted blood components is mainly due to viremia in connection with seroconversion of blood donors. In this instance, transfusion of WBC-reduced blood components from seronegative donors would imply a greater risk of TT-CMV than transfusion of WBC-reduced blood from donors who have been seropositive for at least 1 year, because window-phase donations but no reactivation could be detected in our study. Therefore, there is an urgent need for further studies comparing the risk of TT-CMV after transfusion of WBC-reduced blood from seronegative donors and donors who have been seropositive for at least 1 year.

In conclusion, the detection of CMV DNA was closely related to the first detection of CMV IgG antibodies in up to 62 percent of our newly seroconverted donors, depending on the interval to the last seronegative donation. Otherwise, the probability of detection of CMV DNA in plasma of blood donors at least 1 year after seroconversion was lower than 0.5 percent. Window-phase donations occurred in only 3 percent of seroconversion cases. On the whole, the main source of blood products containing free CMV DNA were newly seroconverted donors. Thus, it is necessary to discuss whether those donors should be excluded transiently from blood donations. Furthermore, transfusion of WBC-reduced blood components from seronegative donors could imply a greater risk of TT-CMV than transfusion of WBC-reduced blood from donors who have been seropositive for at least 1 year, because window-phase donations but no reactivation could be detected in our study.

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REFERENCES

1. Klemola E, Kaariainen L. Cytomegalovirus as a possible cause of a disease resembling infectious mononucleosis. Br Med 1965;2:1099-102.
2. De Jong MD, Galasso GJ, Gazzard B, Griffiths PD, Jabs DA, Kern ER, Spector SA. Summary of the II International Sym-

posium on cytomegalovirus. Antiviral Res 1998;39: 141-62.
3. Fishman JA, Rubin RH. Infection in organ-transplant recipients. N Engl J Med 1998;338:1741-51.
4. Grigoleit U, Riegler S, Einsele H, Laib SK, Jahn G, Hebart H, Brossart P, Frank F, Sinzger C. Human cytomegalovirus induces a direct inhibitory effect on antigen presentation by monocyte-derived immature dendritic cells. Br J Haematol 2002;119:189-98.
5. Sinclair J, Sissons P. Latency and reactivation of human cytomegalovirus. J Gen Virol 2006;87:1763-79.
6. Larsson S, Söderberg-Naucler C, Moller E. Productive cytomegalovirus (CMV) infection exclusively in CD13-positive peripheral blood mononuclear cells from CMV-infected individuals. implications for prevention of CMV transmission. Transplantation 1998;65:411-5.
7. Yeager AS, Grumet FC, Hafleigh EB, Arvin AM, Bradley JS, Prober CG. Prevention of transfusion-acquired cytomegalovirus infections in newborn infants. J Pediatr 1981;98: 281-7.
8. Gilbert GL, Hayes K, Hudson IL, James J. Prevention of transfusion-acquired cytomegalovirus infection in infants by blood filtration to remove leucocytes. Neonatal Cytomegalovirus Infection Study Group. Lancet 1989;1:1228-31.
9. Bowden RA, Slichter SJ, Sayers M, Weisdorf D, Cays M, Schoch G, Banaji M, Haake R, Welk K, Fisher L, McCullough J, Miller W. A comparison of filtered leukocyte-reduced and cytomegalovirus (CMV) seronegative blood products for the prevention of transfusion-associated CMV infection after marrow transplant. Blood 1995;86:3598-603.
10. Bowden RA, Sayers M, Flournoy N, Newton B, Banaji M, Thomas ED, Meyers JD. Cytomegalovirus immune globulin and seronegative blood products to prevent primary cytomegalovirus infection after marrow transplantation. N Engl J Med 1986;314:1006-10.
11. Miller WJ, McCullough J, Balfour HH Jr, Haake RJ, Ramsay NK, Goldman A, Bowman R, Kersey J. Prevention of cytomegalovirus infection following bone marrow transplantation: a randomized trial of blood product screening. Bone Marrow Transplant 1991;7:227-34.
12. Nichols WG, Price TH, Gooley T, Corey L, Boeckh M. Transfusion-transmitted cytomegalovirus infection after receipt of leukoreduced blood products. Blood 2003;101: 4195-200.
13. Vamvakas EC. Is white blood cell reduction equivalent to antibody screening in preventing transmission of cytomegalovirus by transfusion? A review of the literature and meta-analysis. Transfus Med 2005;19:181-99.
14. Narvios AB, Przepiorka D, Tarrand J, Chan KW, Champlin R, Lichtiger B. Transfusion support using filtered unscreened blood products for cytomegalovirus-negative allogeneic marrow transplant recipients. Bone Marrow Transplant 1998;22:575-7.
15. Narvios AB, Shah H, Lichtiger B. Transfusion of leukoreduced cellular blood components from cytomegalovirus-unscreened donors in allogeneic hematopoietic transplant recipients: analysis of 72 recipients. Bone Marrow Transplant 2005;36:499-501.
16. Dumont LJ, Luka J, VandenBroeke T, Whitley P, Ambruso DR, Elfath MD. The effect of leukocyte-reduction method on the amount of human cytomegalovirus in blood products: a comparison of apheresis and filtration methods. Blood 2001;97:3640-7.
17. Drew WL, Tegtmeier G, Alter HJ, Laycock ME, Miner RC, Busch MP. Frequency and duration of plasma CMV viremia in seroconverting blood donors and recipients. Transfusion 2003;43:309-13.
18. Glock B, Schistal E, Mayr WR. CMV DNA in blood donors with IgM and IgG CMV antibodies. Transfusion 2003;43: 1493-4.
19. Boeckh M, Gallez-Hawkins GM, Myerson D, Zaia JA, Bowden RA. Plasma polymerase chain reaction for cytomegalovirus DNA after allogeneic marrow transplantation: comparison with polymerase chain reaction using peripheral blood leukocytes, pp65 antigenemia, and viral culture. Transplantation 1997;64:108-13.
20. Jayarama V, Marcello J, Ohagen A, Gibaja V, Lunderville D, Horrigan J, Chapman J, Lazo A. Development of models and detection methods for different forms of cytomegalovirus for the evaluation of viral inactivation agents. Transfusion 2006;46:1580-8.
21. Brytting M, Xu W, Wahren B, Sundqvist VA. Cytomegalovirus DNA detection in sera from patients with active cytomegalovirus infections. J Clin Microbiol 1992;30:1937-41.
22. Einsele H, Ehninger G, Hebart H, Wittkowski KM, Schuler U, Jahn G, Mackes P, Herter M, Klingebiel T, Loffler J, Wagner S, Müller CA. Polymerase chain reaction monitoring reduces the incidence of cytomegalovirus disease and the duration and side effects of antiviral therapy after bone marrow transplantation. Blood 1995;86:2815-20.
23. Spector SA, Merrill R, Wolf D, Dankner WM. Detection of human cytomegalovirus in plasma of AIDS patients during acute visceral disease by DNA amplification. J Clin Microbiol 1992;30:2359-65.
24. Zanghellini F, Boppana SB, Emery VC, Griffiths PD, Pass RF. Asymptomatic primary cytomegalovirus infection: virologic and immunologic features. J Infect Dis 1999;180: 702-7.
25. Wandinger K, Jabs W, Siekhaus A, Bubel S, Trillenberg P, Wagner H, Wessel K, Kirchner H, Hennig H. Association between clinical disease activity and Epstein-Barr virus reactivation in MS. Neurology 2000;55:178-84.
26. Westermann J, Thiemann F, Gerstner L, Tatzber F, Kozak I, Bertsch T, Kruger C. Evaluation of a new simple and rapid enzyme-linked immunosorbent assay kit for neopterin determination. Clin Chem Lab Med 2000;38:345-53.
27. Revello MG, Zavattoni M, Sarasini A, Percivalle E, Simoncini L, Gerna G. Human cytomegalovirus in blood of immunocompetent persons during primary

infection: prognostic implications for pregnancy. J Infect Dis 1998;177:1170-5.
28. Albano MS, Taylor P, Pass RF, Scaradavou A, Ciubotariu R, Carrier C, Dobrila L, Rubinstein P, Stevens CE. Umbilical cord blood transplantation and cytomegalovirus: post-transplant infection and donor screening. Blood 2006;108: 4275-82.
29. Preiksaitis JK. Prevention of transfusion-acquired CMV infection: is there a role for NAT? Transfusion 2003;43: 302-5.
30. Boom R, Sol CJ, Schuurman T, Van Breda A, Weel JF, Beld M, Wertheim-Van Dillen PM, De Jong MD. Human cytomegalovirus DNA in plasma and serum specimens of renal transplant recipients is highly fragmented. J Clin Microbiol 2002;40:4105-13.
31. Speckner A, Glykofrydes D, Ohlin M, Mach M. Antigenic domain 1 of human cytomegalovirus glycoprotein B induces a multitude of different antibodies which, when combined, results in incomplete virus neutralization. J Gen Virol 1999;80:2183-91.
32. Larsson S, Söderberg-Naucler C, Wang FZ, Moller E. Cytomegalovirus DNA can be detected in peripheral blood mononuclear cells from all seropositive and most seronegative healthy blood donors over time. Transfusion 1998; 38:271-8.
33. Nitsche A, Steuer N, Schmidt CA, Landt O, Ellerbrok H, Pauli G, Siegert W. Detection of human cytomegalovirus DNA by real-time quantitative PCR. J Clin Microbiol 2000; 38:2734-7.
34. Zhang LJ, Hanff P, Rutherford C, Churchill WH, Crumpacker CS. Detection of human cytomegalovirus DNA, RNA, and antibody in normal donor blood. J Infect Dis 1995;171:1002-6.
35. Roback JD, Drew WL, Laycock ME, Todd D, Hillyer CD, Busch MP. CMV DNA is rarely detected in healthy blood donors using validated PCR assays. Transfusion 2003;43: 314-21.
36. Roback JD, Hillyer CD, Drew WL, Laycock ME, Luka J, Mocarski ES, Slobedman B, Smith JW, Soderberg-Naucler C, Todd DS, Woxenius S, Busch MP. Multicenter evaluation of PCR methods for detecting CMV DNA in blood donors. Transfusion 2001;41:1249-57.
37. Brinkmann T, Dreier J, Diekmann J, Gotting C, Klauke R, Schumann G, Kleesiek K. Alanine aminotransferase cut-off values for blood donor screening using the new International Federation of Clinical Chemistry reference method at 37 degrees C. Vox Sang 2003;85:159-64.
38. Schennach H, Hessenberger G, Mayersbach P, Schönitzer D, Fuchs D. Acute cytomegalovirus infections in blood donors are indicated by increased serum neopterin concentrations. Med Microbiol Immunol 2002;191:115-8.
39. Beneke JS, Tegtmeier GE, Alter HJ, Luetkemeyer RB, Solomon R, Bayer WL. Relation of titers of antibodies to CMV in blood donors to the transmission of cytomegalovirus infection. J Infect Dis 1984;150:883-8.
40. Lamberson HV Jr, McMillian JA, Weiner LB, Williams ML, Clark DA, McMahon CA, Lentz EB, Higgins AP, Dock NL. Prevention of transfusion-associated cytomegalovirus (CMV) infection in neonates by screening blood donors for IgM to CMV. J Infect Dis 1988;157:820-3.
41. Ostergaard MS, Ostrem A, Soderstrom M. Hay fever and a single intramuscular injection of corticosteroid: a systematic review. Prim Care Respir J 2005;14:124-30.
42. Soderberg-Naucler C, Fish KN, Nelson JA. Interferon-gamma and tumor necrosis factor-alpha specifically induce formation of cytomegalovirus-permissive monocyte-derived macrophages that are refractory to the antiviral activity of these cytokines. J Clin Invest 1997;100: 3154-63.
43. Soderberg-Naucler C, Fish KN, Nelson JA. Reactivation of latent human cytomegalovirus by allogeneic stimulation of blood cells from healthy donors. Cell 1997;91:119-26.
44. Roback JD, Bray RA, Hillyer CD. Longitudinal monitoring of WBC subsets in packed RBC units after filtration: implications for transfusion transmission of infections. Transfusion 2000;40:500-6.
45. Galea G, Urbaniak SJ. Cytomegalovirus studies on blood donors in north-east Scotland and a review of UK data. Vox Sang 1993;64:24-30.
46. Hecker M, Qiu D, Marquardt K, Bein G, Hackstein H. Continuous cytomegalovirus seroconversion in a large group of healthy blood donors. Vox Sang 2004;86:41-4.
47. Ohto H, Ujiie N, Hirai K. Lack of difference in cytomegalovirus transmission via the transfusion of filtered-irradiated and nonfiltered-irradiated blood to newborn infants in an endemic area. Transfusion 1999;39: 201-5.
48. Murphy MF, Grint PC, Hardiman AE, Lister TA, Waters AH. Use of leucocyte-poor blood components to prevent primary cytomegalovirus (CMV) infection in patients with acute leukaemia. Br J Haematol 1988;70:253-4.
49. Bowden RA, Sayers M, Cays M, Slichter SJ. The role of blood product filtration in the prevention of transfusion associated cytomegalovirus (CMV) infection after marrow transplant. Transfusion 1989;29(Suppl):57S.
50. de Graan-Hentzen YC, Gratama JW, Mudde GC, Verdonck LF, Houbiers JG, Brand A, Sebens FW, van Loon AM, The TH, Willemze R, et al. Prevention of primary cytomegalovirus infection in patients with hematologic malignancies by intensive white cell depletion of blood products. Transfusion 1989;29:757-60.
51. De Witte T, Schattenberg A, Van Dijk BA, Galama J, Olthuis H, Van der Meer JW, Kunst VA. Prevention of primary cytomegalovirus infection after allogeneic bone marrow transplantation by using leukocyte-poor random blood products from cytomegalovirus-unscreened blood-bank donors. Transplantation 1990;50:964-8.
52. Bowden RA, Slichter SJ, Sayers MH, Mori M, Cays MJ, Meyers JD. Use of leukocyte-depleted platelets and cytomegalovirus-seronegative red blood cells for preven-

tion of primary cytomegalovirus infection after marrow transplant. Blood 1991;78:246-50.
53. Eisenfeld L, Silver H, McLaughlin J, Klevjer-Anderson P, Mayo D, Anderson J, Herson V, Krause P, Savidakis J, Lazar A, et al. Prevention of transfusion-associated cytomegalovirus infection in neonatal patients by the removal of white cells from blood. Transfusion 1992;32:205-9.
54. van Prooijen HC, Visser JJ, van Oostendorp WR, de Gast GC, Verdonck LF. Prevention of primary transfusion-associated cytomegalovirus infection in bone marrow transplant recipients by the removal of white cells from blood components with high-affinity filters. Br J Haematol 1994;87:144-7.
55. Pamphilon DH, Foot ABM, Adeodu A, Steward CG, Cornish JM, Marks DI, Oakhill A. Prophylaxis and prevention of CMV infection in BM allograft recipients: Leukodepleted platelets are equivalent to those from CMV-seronegative donors. Bone Marrow Transplant 1999;23(Suppl 1):S66. ◘

別紙様式第2

別紙(3)-3

医薬品　研究報告　調査報告書

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研究報告の概要 137

問題点（WNV の献血への混入）

本研究プロジェクトは、ドイツの Robert-Koch 研究所、Bernhard-Nocht 研究所と Paul-Ehrlich 研究所の共同で実施され、WNV がドイツにおいて国民の健康と血液供給にとって脅威となりうるかを調査した。

9976 名のドイツの健康なドナー、78 名のドイツの薬物使用者及び 198 名の米国の抗 WNV 抗体陽性患者から採取された検体を用いて WNV-RNA を NAT で測定した結果、全て陰性であった。

欧州で採取された血漿分画製剤用の原料血漿 96 件及び東アジアで採取された血漿分画製剤用の原料血漿 51 件は全て陰性であったが、米国で採取された原料血漿は 174 件中 32 件が陽性であった。

さらに本研究プロジェクトは、パスツリゼーションの WNV の不活化を実験室レベルで評価した。人血清アルブミンに安定剤を加え、WNV をスパイクした各々の溶液を 60℃で 1-10 時間加熱し、ウイルス不活化を $TCID_{50}$ で評価したところ、同実験系で測定した SFV と BVDV に似た不活化過程が確認された。

血漿分画製剤の不活化で良く用いられるパスツリゼーション処理、S/D 処理、低 pH 処理は、原料血漿のエンベロープで覆われたウイルスを不活化するのに十分であることが過去の研究で証明されている。

血漿分画製剤の製造工程にウイルス不活化工程が含まれていれば、血漿プール中に WNV が混入していても、感染リスクは無いと結論している。

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
本剤はドイツや米国の原料血漿を使用している。万一、原料血漿に WNV が混入していても、本論文のとおり本剤のパスツリゼーション処理により不活化されると考えられる。	今後とも情報収集に努める所存である。

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West Nile Virus and Blood Product Safety in Germany

Christa Pfleiderer,[1] Johannes Blümel,[1] Michael Schmidt,[2] W. Kurth Roth,[2] M. Kai Houfar,[2] Jana Eckert,[2] Michael Chudy,[1] Eva Menichetti,[3] Sigrid Lechner,[4] and C. Micha Nübling[1]*

[1]*Paul-Ehrlich-Institut, Abteilung Virologie, Langen, Germany*
[2]*Institut für Transfusionsmedizin und Immunhämatologie, Deutsches Rotes Kreuz, J.-W. Goethe Universität, Frankfurt (Main), Germany*
[3]*Österreichisches Rotes Kreuz, Institut Wien, Wien, Austria*
[4]*Österreichisches Rotes Kreuz, Institut Klagenfurt, Klagenfurt, Austria*

West Nile Virus (WNV) is a mosquito-transmitted flavivirus, widely distributed throughout Africa, Asia and the Middle East. WNV may cause epidemics of human meningoencephalitis. The unexpected emergence of WNV (New York, 1999) and its rapid spread throughout North America during the following years caused a number of blood transfusion- and organ transplant-associated transmissions of WNV. In order to estimate the potential WNV threat for Central Europe, we analyzed the anti-WNV prevalence and WNV-RNA incidence among 14,437 and 9,976 blood donors from Germany. There was a high rate of initially anti-WNV reactives (5.9%), but only a few cases (0.03%) were confirmed as anti-WNV positive by neutralization assay. No WNV-RNA positive blood donor was identified in this study. Whereas WNV-RNA was frequently detected in manufacturing plasma pools from the US, none was detected in pools of European or Asian origin. Virus inactivation steps integrated into the manufacturing process of plasma derivatives were shown to be sufficient to assure the WNV safety of plasma derivatives. A well-characterized WNV reference material was prepared, showing 340 WNV-RNA copies per infectious dose. ***J. Med. Virol. 80:557–563, 2008.***

KEY WORDS: West Nile Virus; WNV neutralization; anti-WNV IgG ELISAs; blood safety; plasma derivatives; virus inactivation

INTRODUCTION

West Nile Virus (WNV) was identified in 1937 in Uganda and is widely distributed throughout Africa, Asia, the Middle East and parts of Europe [Solomon et al., 2003]. This enveloped virus is classified under the virus family *Flaviviridae* in the genus *Flavivirus*, which includes more than 100 members that all are characterised by a complex replication cycle involving both insects and at least one additional animal species. This is why flaviviruses had been included in the previous taxonomic group of arboviruses (arthropod-borne viruses). The natural WNV replication cycle involves *culicine* mosquitoes and different bird species. Humans, horses and other mammalian species are so-called "dead-end" hosts characterised by WNV infections with potential clinical symptoms, but transient and low virus levels that are insufficient to establish a mosquito-mammalian WNV replication cycle. The vast majority of WNV infections in humans undergo an asymptomatic course. Approximately 20% of infected humans develop West Nile fever, a febrile illness of sudden onset, often associated with a long recovery period. Only a few cases (<0.2%) develop a neuroinvasive disease resulting in more serious symptoms, including meningitis or encephalitis, sometimes with fatal outcome [Petersen and Marfin, 2002]. The rate of serious outcome of WNV infection is much higher in immune-compromised patients, a status more frequently found in elderly persons or in recipients of blood transfusions.

After a flavivirus infection, the diagnostic differentiation of specific antibodies is complicated by a high rate of cross-reactivity between different members of the genus Flavivirus, for example, Dengue virus (DenV), tick-borne encephalitis virus (TBEV), and WNV [Allwinn et al., 2002; Koraka et al., 2002]. Cross-reactive antibodies are mainly directed against an envelope protein of flaviviruses, the E-protein.

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*Correspondence to: C. Micha Nübling, Division of Virology, Paul-Ehrlich-Institut, Paul-Ehrlich-Strasse 51-59, D-63225 Langen, Germany. E-mail: nuemi@pei.de

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The unexpected emergence of WNV in New York in 1999 was associated with an encephalitis outbreak. The viral strain responsible for this epidemic was a lineage 1 virus already known from previous epidemics in other parts of the world, such as in Israel [Lanciotti et al., 1999]. In the following years, WNV spread across North America from East to West, causing the largest arbovirus epidemic in recorded history with more than 23,000 human infection cases until December 2006, including 893 deaths [Centers for Disease Control and Prevention, 2006]. During this WNV epidemic, new transmission routes, including breast-feeding [MMWR, 2002], organ transplantation [Iwamoto et al., 2003] and transfusion of blood components [Biggerstaff and Petersen, 2002; Hollinger and Kleinman, 2003], were recorded. To assure the safety of the US blood supply, the screening of all blood donations by nucleic acid amplification techniques (NATs) was recommended by the FDA beginning in July 2003. This measure resulted in the detection of WNV-RNA in more than 1,000 blood donations until the middle of 2005, which would otherwise have been used for transfusion of non-inactivated cellular blood components (red cells, platelets) or therapeutic plasma [Busch et al., 2005a; Stramer et al., 2005].

Despite some reports about sporadic WNV infections in humans and horses across Europe, for example, in France, Italy, and Romania [Zeller et al., 2004], no WNV epidemiology data were available for Germany. With the US epidemic still ongoing, the question arose as to whether a similar scenario could also affect Europe. The German Ministry of Health initiated an investigation into the prevalence and incidence of WNV infections in Germany. This publication relates to the assessment of WNV safety of blood and plasma products used in Germany.

MATERIALS AND METHODS

Human Serum and Plasma Samples

For the evaluation of WNV prevalence, 14,437 plasma or serum samples were collected during Summer 2004 from healthy blood donors from central Germany (Hesse) and 928 samples from Austrian blood donors (Carinthia and Vienna).

For the determination of WNV incidence, plasma samples from 9,978 healthy blood donors from central Germany (Hesse) were collected during the 2005 summer mosquito season, combined in pools of 8 using a Tecan robot, and tested for the presence of WNV-RNA.

Serum samples from German intravenous drug users (IVDUs) who tested positive for other blood-borne viruses (HIV and/or HCV) were collected during May 2002 and January 2004 at University Hospital Frankfurt/Main.

Anti-WNV IgG-positive plasma samples were obtained from confirmed clinical cases in the US. These plasma samples were obtained from BBI (West Bridgewater, MA) or kindly provided by H. Hofmann (Genzyme Virotech GmbH, Mainz, Germany).

One panel of WNV RNA-positive plasma samples from clinical cases in the US was provided by L. Tobler from the Blood Systems Research Institute, San Francisco.

Panels of anti-DenV and anti-TBEV positive specimens were designed with materials obtained from the respective clinical cases in Germany, which were kindly provided by Universität München, Abt. für Infektions und Tropenmedizin (T. Löscher), Universität Freiburg, Institut für Medizinische Mikrobiologie & Hygiene (D. Neumann-Haefelin), Universität Heidelberg, Hygiene Institut (P. Schnitzler) and Universität Frankfurt, Zentrum für Hygiene (R. Allwinn).

Aliquots of plasma pools collected for the fractionation of plasma derivatives were obtained from different plasma manufacturers. The respective plasma units had been collected in the US, in Europe or in Asia during the years 2004 and 2005.

ELISA Tests

The following ELISAs were performed strictly following the instructions given in the package insert: "Flavivirus IgG indirect ELISA" (PANBIO Brisbane, Australia), "Anti-West-Nile-Virus-ELISA IgG" (prototype version, Euroimmun, Lübeck, Germany), "West Nile Virus IgG DxSelect™" (Focus Diagnostics, Cypress, CA), and "Enzygnost anti-TBE Virus IgG and IgM" (DADE Behring, Marburg, Germany).

WNV NATs

Human plasma or serum and cell culture-derived samples were tested for WNV RNA using the "Procleix WNV assay" (Chiron Corporation, Blood Testing Division, Emeryville, CA). This test system is a qualitative nucleic acid amplification technique (NAT) based on TMA (transcription mediated amplification) technology. This NAT was performed after passing a training seminar organized by the manufacturer and strictly following the manufacturer's instructions.

WNV-RNA was quantified with the Artus Real Art™ WNV LC RT RCR Kit (QIAGEN, Hilden, Germany) using the QIAamp Viral RNA Mini Kit (QIAGEN) for viral RNA extraction. Quantification standards were provided with the test kit and run in parallel.

Viruses and Cells

Bovine viral diarrhea virus (BVDV), strain Osloss, was obtained from G. Pauli, Robert Koch Institut, Berlin, Germany, and was propagated and titered in MDBK cells (ATTC CCL-22). SFV was provided by J. Thiel (Institut für Virologie, Universität Giessen, Germany), and was propagated and titered in Vero cells (ATTC CCL-81). WNV (1999 New York isolate) was received from T.R. Kreil (Baxter, Vienna, Austria), and was propagated and titered in Vero cells in a biosafety level 3 laboratory.

Virus Titration

Virus infectivity was quantified by estimation of the tissue culture infectious dose ($TCID_{50}$) using standard cell culture conditions. Briefly, Vero and MDBK cells were grown to confluence in 96-well microtiter plates (MTPs). Threefold serial dilutions of the samples in

DMEM medium were prepared, and eight replicates per dilution were assayed by inoculation of a 50 µl sample per well. The cytopathic effects of WNV, Semliki Forest Virus (SFV), and BVDV were checked on days 3–6 post-infection. The $TCID_{50}$ was calculated according to the Maximum Likelihood statistical tool. If no virus was detected, the limit of detection was calculated according to the Poisson distribution.

WNV Neutralization Assay

For the WNV neutralization assay, 50 µl of a 1:3 serial dilution (1:10 to 1:270) of inactivated (56°C, 30 min) serum was mixed in eight replicates in MTP wells with 50 µl of 20 $TCID_{50}$ units of virus. After incubation for 60 min at 37°C, 50 µl of each well was transferred to another well containing 60% confluent (6,000–7,000) Vero cells. MTPs were incubated for 5–6 days at 37°C, and wells containing evidence of viral cytopathic activity were scored. The log ND_{50} was calculated according to the Maximum Likelihood function [Kundi, 1999]. For a high throughput version of the neutralization assay, only one serum dilution (1:30) was tested under the same conditions described above.

Virus Inactivation Studies

Pasteurization was performed at laboratory scale to assess this virus inactivation step. Stabilizer (1.1 g/ml saccharose, 0.3 g/ml glycine, 0.0162 g/ml $CaCl_2$ dihydrate) was added to 50 ml of a commercial 5% human normal immunoglobulin preparation. The solution was spiked with virus and heated to 60°C. Samples were taken after time intervals as indicated (0–10 hr) and immediately cooled. Cooled samples were subsequently titered. Before application on indicator cells, samples were diluted 1:100 in order to avoid cytotoxic and interfering effects of the test material. Absence of cytotoxic effects was verified by microscopic examination of control cells inoculated with non-spiked 1:100 diluted test material. Absence of interfering effects was verified by positive detection of virus from diluted (1:100) test material that had been spiked with a known amount of virus (100 $TCID_{50}$ per well).

RESULTS

Sensitivity and Specificity of Anti-WNV IgG ELISAs

To assess the relative diagnostic sensitivity and specificity of three anti-WNV IgG ELISAs, we used sera from well-characterized flavivirus-infected patients: 26 WNV-infected, 39 TBEV-infected, and 13 DenV-infected individuals.

Two assays (PANBIO, Focus) recognized all of the 26 anti-WNV-positive sera originating from U.S. patients. The third ELISA, the Euroimmun prototype ELISA version, missed three specimens, resulting in a relative sensitivity of 88% in this study (Table I).

The cross-reactivity rates with related flaviviruses were determined for the anti-WNV ELISAs using specimens from DenV or TBEV-infected patients. The highest cross-reactivity rate (lowest specificity) with these specimens was obtained for the PANBIO assay (92.3% and 79.5%, respectively) followed by the Focus ELISA (92.3% and 56.4%, respectively) and the Euroimmun prototype ELISA, which displayed cross-reactivity rates of 35.7% and 17.9%, respectively.

Based on this analysis of the relative sensitivity and specificity of the different WNV ELISA assays, the following test algorithm for determination of the WNV prevalence was chosen: after screening donor blood with the sensitive Focus ELISA test, a further analysis of all reactive specimens was performed with the more specific Euroimmun prototype WNV ELISA. Those samples that were reactive (positive or borderline) in the second anti-WNV ELISA were then tested in a WNV neutralization assay as a confirmatory test. Additionally, we characterised all samples reactive in the Focus WNV ELISA with an anti-TBEV IgG ELISA. However, this assay displayed 42% cross-reactivity when anti-WNV-positive specimens were tested.

WNV Prevalence

More than 14,000 healthy blood donors from Hesse/Germany were screened for anti-WNV specific IgG antibodies. 5.9% (852/14,437) of the tested donors were reactive in the Focus anti-WNV ELISA test. To estimate the potential impact of TBEV vaccination on the test results, more than 900 blood donors from Austria (where TBEV vaccination is a general public health measure) were also screened for anti-WNV. Seventy-two percent of these blood donors (669/928) were reactive in the anti-WNV ELISA.

All anti-WNV reactive samples from German blood donors were re-tested in the second anti-WNV ELISA and in the anti-TBEV IgG assay. Thirty-four percent of these samples were reactive in the second anti-WNV and 15% were non-reactive in the anti-TBEV ELISA, whereas 9.7% were reactive in the WNV-ELISA and non-reactive in the TBEV-ELISA.

TABLE I. Sensitivity and Cross-Reactivity of Different Anti-WNV IgG ELISAs (pos = Positive; React = Reactive)

		Anti-WNV IgG ELSA		
	Sera	PANBIO	Focus	Euroimmun
Sensitivity	Anti-WNV IgG positive	100% (26/26)	100% (26/26)	88% (23/26)
Cross-reactivity	Anti-dengue IgG positive	92.3% (12/13)	92.3% (12/13)	35.7% (5/14)
	Anti-TBE IgG positive	79.5% (31/39)	56.4% (22/39)	17.9% (7/39)

Two hundred two of the pre-selected anti-WNV suspicious blood donor samples were tested in the WNV neutralization assay. Most of these plasma samples (148/202; 73%) had no or very low WNV neutralization activity (log $ND_{50} < 1$), while 50 specimens (25%) neutralized WNV infection, with log ND_{50} titers between 1 and 2. Only four plasma samples from our blood donors exhibited relatively high neutralization titers (log $ND_{50} \geq 2$) equivalent to those titers observed with the anti-WNV IgG sera from US patients. To check whether any anti-WNV positive might have been missed by our test algorithm, 388 additional anti-WNV reactive specimens were tested in a high throughput neutralization assay. No further specimens with titers of log $ND_{50} > 2$ were identified by this approach.

WNV Incidence

The WNV incidence in a population of healthy German blood donors was investigated during the mosquito season of Summer 2005. In total, 9,976 blood donors were tested for the presence of detectable WNV-RNA using the Procleix WNV NAT assay in minipools of eight. All of the 1,247 minipools tested WNV-RNA negative, with the exception of one initially reactive test result for one minipool. This result was not confirmed on retesting and on testing of the individual plasmas. Furthermore, 198 of the anti-WNV reactive blood donor samples (see above) were tested as individual specimens, with negative test results. Plasma or serum samples collected from a population of German intravenous drug users (IVDU, n = 78), representing a population with increased risk for blood-borne pathogens also tested negative for WNV-RNA (Table II).

Plasma Derivatives

Plasma pools for manufacturing of plasma derivatives with the source plasma collected in the US, East Asia, or Europe were analyzed for the presence of WNV RNA using the Chiron TMA assay. All plasma pools from East Asia (n = 51) and Europe (n = 96) tested negative, while 32 out of 174 (18%) plasma pools from the US tested WNV RNA-positive in this qualitative WNV-NAT (Table II). These pools had been collected in different regions of the US during the years 2004 and 2005. Since the viral load in most pools was too low for accurate results in the quantitative WNV-NAT, viral load was analyzed after concentration of WNV particles by ultracentrifugation and subsequent extraction of the viral nucleic acids. Viral loads were calculated as ranging from 57 to 837 copies WNV-RNA/ml plasma, with 351 copies WNV-RNA/ml as an average value for the TMA-positive pools.

TABLE II. Detection of WNV RNA in Blood Specimens and Plasma Pools Using the Procleix WNV Assay (Chiron)

	Tested	WNV-RNA positive
Blood donors (pools of n = 8)	9,976	0
IVDUs	78	0
Anti-WNV reactive blood donors	198	0
Plasma pools (Europe)	96	0
Plasma pools (USA)	174	32
Plasma pools (East-Asia)	51	0

Virus inactivation steps are included in the manufacturing process of different plasma derivatives. For the production of human-derived medicinal products, a 10 hr heating step of a liquid product intermediate at 60°C (pasteurization) is often performed to inactivate a wide range of potential virus contaminants. The inactivation kinetics of WNV upon pasteurization of a sucrose-stabilized immunoglobulin preparation was compared with the inactivation kinetics of other commonly used model enveloped viruses. BVDV is a pestivirus that frequently serves as a model virus for hepatitis C virus and other members of the Flaviviridae. The inactivation kinetics of WNV were similar to the inactivation kinetics of BVDV. The inactivation kinetics (Fig. 1) confirm that WNV is effectively inactivated by this commonly used manufacturing step.

WNV Reference Preparation

We established and characterized a WNV reference preparation that may be useful for standardization and control of WNV-NATs and WNV-neutralization assays. Supernatant from WNV-infected Vero cells was harvested and characterized for its infectivity titer ($TCID_{50}$) and WNV-RNA content. WNV-RNA concentration was determined by replicate (24 per concentration) testing of limiting dilutions (factor of 2) using the qualitative WNV-NAT followed by calculation of the 95% cut-off concentration using Probit analysis. WNV-RNA concentration was also determined using the quantitative NAT test.

Both NAT approaches revealed a WNV-RNA concentration for the stock material of 6.5×10^9 or 8.1×10^9 copies/ml. Titration in Vero cells gave an infectivity titer of log 7.33 TCID/ml, correlating to approximately 340 copies WNV-RNA per infectious dose.

DISCUSSION

New emerging pathogens may be a threat to public health, not only because of their impact on the population, but also because of their potential to contaminate the blood or plasma supply and to be transmitted to recipients of blood products. Therefore, a research project was initiated by the German Ministry of Health after the huge WNV epidemic that followed the introduction of the virus to the New World in 1999. This research project was performed in cooperation among the Robert-Koch-Institut (RKI; Berlin), the Bernhard-Nocht-Institut (BNI; Hamburg), and the Paul-Ehrlich-Institut (PEI; Langen). In this study, we investigated whether WNV is or could become a threat to public health and the blood supply in Germany. Here, we focus on the prevalence and incidence of WNV among healthy blood donors and the potential for the transmission of the pathogen via plasma derivatives.

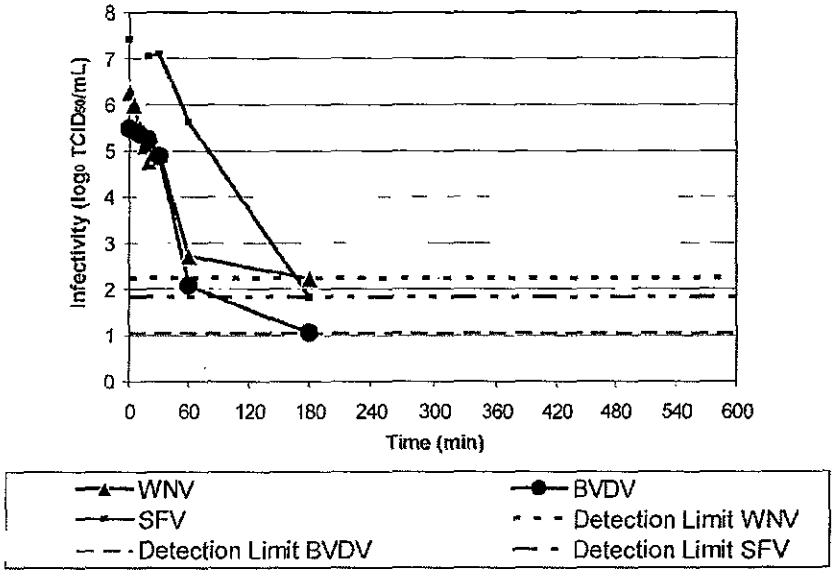

Fig. 1. Virus inactivation through heat inactivation (pasteurization). A sucrose-stabilized human immunoglobulin preparation (5%) was spiked with BVDV, SFV or WNV. After incubation at 60°C, samples were taken after time intervals as indicated (0–10 hr) and cooled immediately. Virus infectivity was quantified by calculation of the tissue culture infectious dose ($TCID_{50}$).

WNV Prevalence

First, we wanted to qualify the anti-WNV screening tests and define an appropriate test algorithm. We decided to use anti-WNV positive specimens from the US as decisive specimens for testing sensitivity for different reasons. First, the respective materials from clinically ill patients were easily available from the US. Second, WNV infections had been confirmed by the clinical course, the seasonal occurrence of the infection and the results of different diagnostic assays performed on individual follow-ups. Furthermore, TBEV, the human flavivirus most frequently found in Europe, is not yet present on the American continent. Therefore, cross-reacting TBEV antibodies should not be an issue for these samples.

The sensitivity was 100% for two anti-WNV assays and 88% for the third assay using such an anti-WNV positive serum panel. However, further panels composed of either anti-Dengue or anti-TBEV positive specimens showed high rates of cross-reactivity with the more sensitive anti-WNV assays and low cross-reactivity with the third assay. We decided to include an anti-TBEV assay as a further diagnostic tool because all of the anti-WNV positive specimens from the US that were missed by the third assay tested as anti-TBE negative, and only a few members of the entire US anti-WNV panel cross-reacted in the anti-TBEV assay. We aimed to preferentially choose anti-WNV reactive/anti-TBEV negative specimens for entering the "gold standard" WNV neutralization test. However, even with this standard, we experienced a high rate of cross-reactivity with anti-DenV-positive specimens. Fortunately, anti-TBEV-positive specimens, which are much more frequent in our region, showed cross-neutralization only at a low level. In conclusion, serological flavivirus diagnostics show high rates of cross-reactivity, and correct interpretation of test results requires extreme caution.

To determine the extent of past WNV infections, samples from more than 14,000 German blood donors were screened with the Focus anti-WNV IgG ELISA. Nearly 6% of the German blood donors were anti-WNV reactive in this assay. Many of the reactive test results were probably caused by cross-reactive antibodies originating from a related flavivirus infection or from vaccination. Though some parts of Southern Germany are TBE risk areas (as the clinical cases show), no reliable TBE incidence or prevalence data are available for Germany.

The TBE vaccination coverage of the Austrian population is in the range of 80%. Sera from Austrian blood donors had a similar reactivity rate in our anti-WNV screening assay. This illustrates the high rate of anti-TBEV cross-reactivity and confirms the similar results obtained in our test qualification study (see above).

Therefore, the anti-WNV reactive rate for blood donors in Hesse (5.9%) may primarily reflect the TBEV vaccination and/or infection level in donors from our region.

To narrow down the number of samples that had to be tested in a WNV neutralization assay, all blood donations that were reactive in the Focus ELISA were re-tested in the more specific prototype anti-WNV IgG ELISA test and in an anti-TBEV IgG assay.

Blood samples that were non-reactive in the anti-TBEV ELISA and reactive in the pre-market anti-WNV

ELISA were tested in the WNV neutralization assay, the "gold standard" for antibody detection, to confirm a past infection. Most of the pre-selected specimens did not inhibit WNV infection in cell culture at all or did so only at a low level (log $TCID_{50} < 1.5$). The titer of 13 additional samples was between ≥ 1.5 and <2.

Only four blood donor samples exhibited a log titer of >2, which was similar to those titers we determined for different clinically confirmed WNV positive sera from the US (Table I). We interviewed the four anti-WNV positive blood donors to evaluate if these putative WNV infections occurred in Germany or Europe or if they might be associated with WNV-endemic countries. One of the four donors had never left Europe, but had visited South and East Europe; another donor had only stayed outside Germany in Turkey. The other two donors had traveled abroad to the USA and Africa. We therefore cannot exclude that singular sporadic and asymptomatic WNV infections may have occurred in Europe and the Middle East. However, WNV prevalence among German blood donors is overall very low; in our study, only 0.03% (95% CI: 0.01–0.07%) of blood donors that were tested were confirmed positive for anti-WNV antibodies by our approach.

WNV-RNA Incidence

For WNV infections, a diagnostic window of only few days (until detection of anti-WNV) has been described during which viral RNA may be detectable. With a WNV-RNA concentration range described from <50 copies/ml up to 10^5 copies/ml [Busch et al., 2005b], the short viremia is at a moderate level when compared to the respective figures for other blood-borne viral infections like HCV (3 months, 10^5–10^8 IU/ml) or HIV (4 weeks, 10^2–10^6 IU/ml) [Lelie et al., 2002].

WNV-RNA incidence in Germany was analyzed in minipools comprised of eight blood donations collected during Summer 2005 and using the highly sensitive Chiron Procleix WNV NAT [Gallian et al., 2005]. Since different WNV strains were described as circulating in Central Europe [Bakonyi et al., 2005, 2006], we investigated whether the Procleix assay detects all available WNV strains. A panel from an external quality assurance study [Niedrig et al., 2006] was retrospectively analyzed. The result confirmed the capability of the Procleix assay to detect WNV lineage 1 and 2 with high sensitivity (data not shown).

No minipools were confirmed positive for WNV-RNA after screening of 1,247 minipools (equal to 9,976 donations). Negative NAT results were also obtained for all individually tested samples from a population that was at increased risk for blood-transmitted infections [i.e., intravenous drug users (IVDUs, n = 78)], or for plasma samples of serologically anti-WNV-reactive blood donors (n = 198).

This outcome is in accordance with the recent WNV-RNA screening results of more than 60,000 Dutch blood donors [Koppelman et al., 2006]. Regardless, blood donors who had just recently visited a WNV endemic country (e.g., the USA) are excluded from the actual donation as a precautionary measure in German blood banks.

Plasma Derivatives

Plasma derivatives (e.g., immunoglobulin preparations, albumin, factor concentrates) may enter the European market with source materials collected in different parts of the world. We were interested in the question of whether WNV-RNA is detectable in aliquots of manufacturing plasma pools.

Some of the plasma pools with plasma of US origin tested positive for WNV RNA by the qualitative NAT assay. The viral load in these plasma pools was determined in the range between 57 and 837 copies/ml, with a median of 351 copies/ml. Each of these pools was composed of several thousand plasma units. Assuming that WNV input in these pools originates from only few donors, WNV concentration of 10^5–10^6 copies/ml are calculated for some individual plasma units. Such high WNV-RNA concentrations have been described for the diagnostic window phase in few cases. However, WNV input into plasma pools from lower viremia cases may be not detected due to the dilution effect though they are expected to be much more frequent and representative for the early infection phase.

Several studies proved that inactivation steps commonly used during the manufacture of plasma derivatives, such as pasteurization for human albumin, S/D treatment for IVIG and FVIII inhibitor-bypassing activity, and incubation at low pH for IVIG, should be sufficient to inactivate enveloped viruses present in source materials [Kreil et al., 2003]. We comparatively investigated the efficacy of pasteurization with regards to WNV and model viruses for enveloped viruses. In these experiments WNV inactivation kinetics were similar between WNV and the two model viruses, BVDV and SFV. This result confirmed the validity of predictions based on model viruses if chosen appropriately. It allowed the conclusion that moderate WNV concentrations in plasma pools should not pose an infection risk to recipients if virus inactivation procedures are included in the manufacturing process of these biological medicinal products.

CONCLUSION

There is currently no indication that WNV could cause an epidemic in Europe similar to that in the USA during the recent years, although temporally and regionally limited outbreaks of WNV infections in humans and horses have been observed in Europe since the 1950s. In contrast to North America, Europe has had direct vector contact with WNV endemic areas in Africa for a long time, via migratory birds, for example. This may have resulted in natural herd immunity in birds. In America, a highly pathogenic WNV strain was imported in 1999 into a virgin territory, meeting a bird population without herd immunity. Environmental factors, such as climate change or global warming and the increasing

mobility of people, may enhance the emergence of new viruses. Therefore, continuous surveillance is an important tool to protect public health and the safety of the blood supply.

ACKNOWLEDGMENTS

We thank Claudia König for excellent technical assistance, Gerd Sutter for support and helpful comments throughout the project, and Ina Plumbaum for critically reading the manuscript. We are grateful to diagnostic companies for their valuable cooperation: Euroimmun (for providing prototype anti-WNV ELISA) and Chiron (for providing the Procleix WNV system).

REFERENCES

Allwinn R, Doerr HW, Emmerich P, Schmitz H, Preiser W. 2002. Cross-reactivity in flavivirus serology: New implications of an old finding? Med Microbiol Immunol 190:199–202.

Bakonyi T, Hubalek Z, Rudolf I, Nowotny N. 2005. Novel flavivirus or new lineage of West Nile virus, central Europe. Emerg Infect Dis 11:225–231.

Bakonyi T, Ivanics E, Erdelyi K, Ursu K, Ferenczi E, Weissenbock H, Nowotny N. 2006. Lineage 1 and 2 strains of encephalitic West Nile virus, central Europe. Emerg Infect Dis 12:618–623.

Biggerstaff BJ, Petersen LR. 2002. Estimated risk of West Nile transmission through blood transfusion during an epidemic in Queens, New York City. Transfusion 42:1019–1026.

Busch MP, Caglioti S, Robertson EF, McAuley JD, Tobler LH, Kamel H, Linnen JM, Shyamala V, Tomasulo P, Kleinman SH. 2005. Screening the blood supply for West Nile virus RNA by nucleic acid amplification testing. N Engl J Med 353:460–467.

Busch MP, Tobler LH, Saldana J, Caglioti S, Shyamala V, Linnen JM, Phleps B, Smith RI, Drebot M, Kleinman SH. 2005. Analytical and clinical sensitivity of West Nile virus RNA screening and supplemental assays available in 2003. Transfusion 45:492–499.

Centers for Disease Control and Prevention (CDC). 2006. http://www.cdc.gov/ncidod/dvbid/westnile/index.htm, accessed at October 23, 2006.

Gallian P, De Micco P, de Lamballerie X, Levayer T, Levacon F, Guntz P, Mercier B, Dupond I, Cornillot C, Andreu G. 2005. Prevention of West Nile virus Transmission by blood transfusion: A comparison of NAT Screening assays. Transfusion 45:1540–1541.

Hollinger FB, Kleinman S. 2003. Transfusion transmission of West Nile virus: A merging of historical and contemporary perspectives. Review. Transfusion 43:992–997.

Iwamoto M, Jernigan DB, Guasch A, Trepka MJ, Blackmore CG, Hellinger WC, Pham SM, Zaki S, Lanciotti RS, Lance-Parker SE, DiazGranados CA, Winquist AG, Perlino CA, Wiersma S, Hillyer KL, Goodman JL, Marfin AA, Chamberland ME, Peterson LR. 2003. Transmission of West Nile virus from an organ donor to four transplant recipients. N Engl J Med 348:2196–2203.

Koppelman MH, Sjerps MS, de Waal M, Reesink HW, Cuypers HT. 2006. No evidence of West Nile virus infection in Dutch blood donors. Vox Sang 90:166–169.

Koraka P, Zeller H, Niedrig M, Osterhaus ADME, Groen J. 2002. Reactivity of serum samples from patients with a flavivirus infection measured by immunofiorescence assay and ELISA. Microbes Infec 4:1206–1215.

Kreil TR, Berting A, Kistner O, Kindermann J. 2003. West Nile virus and the safety of plasma derivatives: Verification of high safety margins, and the validity of predictions based on model virus data. Transfusion 43:1023–1028.

Kundi M. 1999. One-hit models for virus inactivation studies. Antiviral Res 41:145–152.

Lanciotti RS, Roehrig JT, Deubel V, Smith J, Parker M, Steele K, Crise B, Volpe KE, Crabtree MB, Scherret JH, Hall RA, MacKenzie JS, Cropp CB, Panigrahy B, Ostlund E, Schmitt B, Malkinson M, Banet C, Weissman J, Komar N, Savage HM, Stone W, McNamara T, Gubler DJ. 1999. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. Science 286:2333–2337.

Lelie PN, van Drimmelen HA, Cuypers HT, Best SJ, Stramer SL, Hyland C, Allain JP, Moncharmont P, Defer C, Nubling M, Glauser A, da Silva Cardoso M, Viret JF, Lankinen MH, Grillner L, Wirthmuller U, Coste J, Schottstedt V, Masecar B, Dax EM. 2002. Sensitivity of HCV RNA and HIV RNA blood screening assays. Transfusion 42:527–536.

MMWR. 2002. Possible West Nile virus transmission to an infant through breast feeding, Michigan. MMWR 51:877–878.

Niedrig M, Linke S, Zeller H, Drosten C. 2006. First international proficiency study on West Nile virus molecular detection. Clin Chem 52:1851–1854.

Petersen LR, Marfin AA. 2002. West Nile virus: A primer for clinician. Ann Intern Med 137:173–179.

Solomon T, Ooi MH, Beasley DW, Mallewa M. 2003. West Nile encephalitis. BMJ 326:865–869.

Stramer SL, Fang CT, Foster GA, Wagner AG, Brodsky JP, Dodd RY. 2005. West Nile virus among blood donors in the United States, 2003 and 2004. N Engl J Med 353:451–459.

Zeller HG, Schuffenecker I. 2004. West Nile Virus. An overview of its spread in Europe and in the Mediterranean Basin in contrast to its spread in the Americas. Eur J Clin Microbiol Infect Dis 23:147–156.

別紙様式第2-1

No. 27

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2007. 12. 4	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	ProMED 20071130-3869, 2007 Nov 30. 情報源:[1]World Health Organisaation (WHO), CSR, Disease Outbreak News, 2007 Nov 30. [2]Agence France Press (AFP) report, 2007 Nov 30.	公表国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社) 合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)			ウガンダ

研究報告の概要

○ウガンダにおけるエボラ出血熱アウトブレイク
[1]ウガンダの保健省は西部のBundibugyo地区でのエボラ出血熱アウトブレイクを確認した。2007年11月28日時点で疑い症例51例、うち死亡例16例が報告されている。報告例には医療関係者の感染も3例含まれており、このうち1例は死亡している。国立研究所および米国疾病対策予防センター(CDC)の実施した臨床検査により、患者検体からエボラウイルスの新種の存在が確認された。現地調査によると、アウトブレイクは2007年9月から始まっていた可能性がある。同国保健省の対策委員会、WHOや他の国際機関は協力して対応に当たっていく。
[2]2007年11月30日、ウガンダ保健省は、同国西部で51人が感染し、少なくとも16人が死亡したエボラウイルスは未知のウイルス株であると発表した。CDCの検査施設に送られた患者の血液及び組織検体を分析したところ、これまでウガンダの他の地区やコンゴ民主共和国で流行していたエボラウイルスの株とは異なった性質が見られた。専門家によると、これまでの株は血管の内膜を破壊することで出血を引き起こし、ショックによって患者を死に至らしめるが、新しい株では出血はそれほど多くなく、患者は高熱を発症後に死亡するとのことである。当局は疫学やウイルス学の専門家を集めて同地区の疾患を監視し、高熱や腹痛、嘔吐、紅斑を発症した人に注意している。

報告企業の意見	今後の対応
ウガンダ西部のBundibugyo地区でエボラ出血熱の集団発生が見られ、エボラウイルスの新種の存在が確認されたとの報告である。	日本赤十字社は、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
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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

Back

Archive Number 20071130.3869
Published Date 30-NOV-2007
Subject PRO/AH/EDR> Ebola hemorrhagic fever - Uganda: (Bundibugyo), WHO

EBOLA HEMORRHAGIC FEVER - UGANDA: (BUNDIBUGYO), WHO
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A ProMED-mail post
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International Society for Infectious Diseases
<http://www.isid.org>

[1]
Date: Fri 30 Nov 2007
Source: World Health Organisaation (WHO), CSR, Disease Outbreak News [edited]
<http://www.who.int/csr/don/2007_11_30a/en/index.html>

Ebola haemorrhagic fever in Uganda

The Ministry of Health (MoH), Uganda, has confirmed an outbreak of Ebola haemorrhagic fever, in Bundibugyo District, western Uganda. As of Thu 28 Nov 2007, 51 suspected cases, including 16 deaths have been reported. Among the reported cases, 3 health care workers were also infected, including one fatality. The patients are being hospitalized at Kikyo and Bundibugyo.

Laboratory analysis undertaken at the National Reference Laboratories and the Centres for Disease Control and Prevention (CDC), Atlanta, USA, has confirmed the presence of a new species of Ebola virus in samples taken from cases associated with the outbreak.

Based on initial field investigations, the MoH/WHO Country office has reported that the outbreak might have been ongoing since September 2007. A national task force comprising MoH, WHO and other international partners in the field, is coordinating the response to this outbreak. WHO Country office is assisting the MOH national field team and the District health officials.

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Communicated by:
ProMED-mail Rapporteur Marianne Hopp

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[2]
Date: Fri 30 Nov 2007
Source: Agence France Press (AFP) report [edited]
<http://afp.google.com/article/ALeqM5j8JhykvCqpuWN9lWD4ZjGnYZeA_g>

Uganda's Ebola outbreak is new strain

A lethal Ebola virus that has killed at least 16 people and infected 51 others in western Uganda is a previously unknown strain, health authorities said Friday [30 Nov 2007]. Analysis on victims' blood and tissue samples sent to the Atlanta-based Centers for Disease Control's pathogens laboratory behaved differently from previous known strains of Ebola, they said. "It is a new type of strain. It is different from the one we suffered in Gulu and also different from the one that was reported in the Democratic Republic of Congo," said Sam Okware, who chairs Uganda's national hemorrhagic fever task force. The 1st Ebola case was reported on 10 Nov 2007 in Bundibugyo district on the border with the Democratic Republic of Congo, where 3

patients are currently in an isolation ward.

Virologists say previous strains destroyed the linings of blood capillaries and vessels, prompting fluids to drain out of the circulatory system through the body's orifices and pores, killing the victim through shock. But there is not much bleeding in the new strain that appears to kill its victims after provoking a high fever, they say.

Authorities have assembled epidemiologists and virologists in the affected district to monitor the disease. "We have put our people on alert for anyone who is complaining of fever, abdominal pain, vomiting and has developed rashes," Okware said, referring to the early symptoms of the disease caused by the new strain.

An outbreak of Ebola, a highly contagious disease that can have fatality rates as high as 90 percent, killed at least 170 people in northern Uganda's Gulu district in 2000. A similar outbreak has killed at least 26 people in the West Kasai region of the Democratic Republic of Congo in recent weeks, according to the country's Health Minister Victor Makwenge Kaput.

It spreads by direct human contact, especially through infected blood. The Ebola virus was first identified in 1976 in Sudan and in a nearby region of Democratic Republic of the Congo (then Zaire). Outbreaks of Ebola have also occurred in the Ivory Coast and Gabon.

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[The difference in symptoms of the disease associated with this new strain of Ebola virus, and its less hemorrhagic presentation, may account in part for the delay in identifying the causes of the outbreak. On the basis of the WHO report it must be presumed that the disease was present in the region for several weeks prior to recognition of the 1st case on 10 Nov 2007.

An interactive map of the Bundibugyo region of Western Uganda is available at: <http://www.maplandia.com/uganda/bundibugyo/>. - Mod.CP]

[see also:
Hemorrhagic fever - Uganda (04): (Bundibugyo), Ebola confirmed 20071130.3859
Hemorrhagic fever - Uganda (03): (Bundibugyo) 20071121.3775
Hemorrhagic fever - Uganda (02): (Bundibugyo), Marburg NOT 20071116.3718
Hemorrhagic fever - Uganda (Bundibugyo): Marburg susp., RFI 20071114.3697
Marburg hemorrhagic fever - Uganda (06): new case 20071002.3257
Marburg hemorrhagic fever - Uganda (05) 20070817.2697
Marburg hemorrhagic fever - Uganda (04), WHO 20070814.2656
Marburg hemorrhagic fever - Uganda (03) 20070810.2609
Marburg hemorrhagic fever - Uganda (02) 20070804.2533
Marburg hemorrhagic fever - Uganda 20070803.2514
Viral hemorrhagic fever - Uganda (Kamwenge): Marburg conf. 20070801.2490
Viral hemorrhagic fever - Uganda (Kamwenge): Marburg susp., RFI 20070801.2475
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Ebola hemorrhagic fever - Uganda: postscript 20011019.2576]
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医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2007. 12. 17	該当なし	
一般的名称	(製造承認書に記載なし)		研究報告の公表状況	Rezza G, Nicoletti L, Angelini R, Romi R, Finarelli AC, Panning M, Cordioli P, Fortuna C, Boros S, Magurano F, Silvi G, Angelini P, Dottori M, Ciufolini MG, Majori GC, Cassone A; CHIKV study group. Lancet. 2007 Dec 1;370(9602):1840-6.	公表国 イタリア	
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社) 合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)					

研究報告の概要

○イタリアでのチクングニヤウイルス感染症:温帯地域におけるアウトブレイク

背景:ヤブカ類(Aedes spp.)によって伝播されるチクングニヤウイルス(CHIKV)は、近年インド洋諸島やインド亜大陸において複数のアウトブレイクを引き起こした。ここではイタリアでのアウトブレイクを報告する。

方法:イタリア北東部の隣りあった2つの村で原因不明の発熱性疾患が多数報告された後、主要感染源と伝播形態を特定するためのアウトブレイク調査を実施した。能動的サーベイランスシステムも導入した。臨床症例定義は、発熱と関節痛の発症とした。血液検体を採取、PCRと血清学的検査を行って病原体を特定した。現地で採取した蚊にもPCRを実施した。CHIKV E1領域の系統発生学的解析を行った。

知見:ヒトおよび蚊由来検体の分析により、当該アウトブレイクはCHIKVによるものと判明した。2007年7月4日から9月27日までにCHIKV感染症例205例が特定された。推定初発症例は、当該の村に親類を訪ねた際に発症したインドの男性とされた。系統発生学的解析では、イタリアで特定された株とこれより前にインド洋諸島のアウトブレイク時に特定された株との間に高い相同性が示された。ほぼ全例とも症状はかなり軽度で、死亡報告は1例のみであった。

考察:非熱帯地域における今回のCHIKV感染症アウトブレイクは、ある意味予期せぬ事態であり、グローバル化時代における新興感染症の脅威に対する準備と対策の必要性が強く示唆される。

報告企業の意見	今後の対応
2007年7月4日から9月27日までにイタリアにおいてチクングニヤウイルス感染症例205例が集団発生し、グローバル化時代における新興感染症の脅威に対する準備と対策の必要があるとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。国内でチクングニヤ熱が確認されたため、渡航歴確認の徹底を図っている。また、チクングニヤ熱の既往歴がある場合、治癒後6ヵ月間は献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

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vCJD等の伝播のリスク

Infection with chikungunya virus in Italy: an outbreak in a temperate region

G Rezza*, L Nicoletti*, R Angelini, R Romi, A C Finarelli, M Panning, P Cordioli, C Fortuna, S Boros, F Magurano, G Silvi, P Angelini, M Dottori, M G Ciufolini, G C Majori, A Cassone, for the CHIKV study group†

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*GR and LN contributed equally
†Members listed at end of paper
Department of Infectious, Parasitic, and Immunomediated Diseases, Istituto Superiore di Sanità, Rome, Italy (G Rezza MD, L Nicoletti PhD, R Romi BSc, C Fortuna BSc, S Boros SA, F Magurano BSc, M G Ciufolini BSc, G C Majori MD, Prof A Cassone MD); Local Health Unit, Ravenna, Italy (R Angelini MD, G Silvi MD); Regional Health Authority, Emilia-Romagna, Italy (A C Finarelli MD, P Angelini BSc); Clinical Virology, Bernhard-Nocht-Institute for Tropical Medicine, Hamburg, Germany (M Panning PhD); and Istituto Zooprofilattico, Brescia, Italy (P Cordioli MV, M Dottori MV)
Correspondence to: Prof A Cassone, Department of Infectious, Parasitic, and Immunomediated Diseases, Istituto Superiore di Sanità, Viale Regina Elena, 299, 00161 Roma, Italy
cassone@iss.it

Summary

Background Chikungunya virus (CHIKV), which is transmitted by *Aedes* spp mosquitoes, has recently caused several outbreaks on islands in the Indian Ocean and on the Indian subcontinent. We report on an outbreak in Italy.

Methods After reports of a large number of cases of febrile illness of unknown origin in two contiguous villages in northeastern Italy, an outbreak investigation was done to identify the primary source of infection and modes of transmission. An active surveillance system was also implemented. The clinical case definition was presentation with fever and joint pain. Blood samples were gathered and analysed by PCR and serological assays to identify the causal agent. Locally captured mosquitoes were also tested by PCR. Phylogenetic analysis of the CHIKV E1 region was done.

Findings Analysis of samples from human beings and from mosquitoes showed that the outbreak was caused by CHIKV. We identified 205 cases of infection with CHIKV between July 4 and Sept 27, 2007. The presumed index case was a man from India who developed symptoms while visiting relatives in one of the villages. Phylogenetic analysis showed a high similarity between the strains found in Italy and those identified during an earlier outbreak on islands in the Indian Ocean. The disease was fairly mild in nearly all cases, with only one reported death.

Interpretation This outbreak of CHIKV disease in a non-tropical area was to some extent unexpected and emphasises the need for preparedness and response to emerging infectious threats in the era of globalisation.

Introduction

Chikungunya virus (CHIKV) is an arthropod-borne virus transmitted to human beings by *Aedes* spp mosquitoes. After the isolation of the virus in Tanzania in 1953,[1] sporadic cases and a number of outbreaks of infection with CHIKV have been reported in several African countries, on the Indian subcontinent, and in southeast Asia.[2] In the past few years, a series of outbreaks have been reported over a large geographical area that includes African islands in the Indian Ocean and the Indian subcontinent. The first of the outbreaks occurred in Kenya in 2004, followed by outbreaks on the Comoros Islands, the island of La Réunion, and other islands in the southwest Indian Ocean in early 2005, and by a large outbreak in India in 2005–06.[3,4] According to the molecular analysis of the strains isolated on islands in the Indian Ocean and in India, the epidemic was caused by a variant of the central/east African genotype of CHIKV.[5]

During the outbreak on islands in the Indian Ocean, a large number of travellers from industrialised countries with a temperate climate became infected with CHIKV and were still infected on returning home.[6–9] In some of these industrialised countries, *Aedes albopictus*—a vector of CHIKV—was introduced a number of years ago and is now widespread,[10] with an especially high population density in Italy.[11] This situation is particularly threatening because it has been suggested that the strain of CHIKV in the Indian Ocean has better adapted to *A albopictus* than it has to other *Aedes* spp.[4] Nonetheless, to date, no outbreaks due to the local transmission of CHIKV have been reported in these countries. Here, we report on a large outbreak of CHIKV infection that occurred in two neighbouring villages in Italy.[12]

Methods

Patients

In July and August, 2007, the local health unit of the province of Ravenna (region of Emilia Romagna, northeastern Italy) detected an unusually high number of cases of febrile illness in Castiglione di Cervia and Castiglione di Ravenna, two small villages divided by a river. In the second week of August, the local health unit implemented an active surveillance system to identify, both prospectively and retrospectively, all individuals with febrile illness, on the basis of reports provided by general practitioners and hospital emergency units. Patient data were collected with a standardised questionnaire and included age, sex, place of residence, countries visited, travel dates, and date of onset of symptoms. In late August, an outbreak investigation was done to identify the agent and the source of the infection and to better understand the dynamics of the epidemic of febrile illness.

Early in the outbreak investigation, infection with CHIKV was suspected because of clinical symptoms and the fact that the first patient with febrile illness was a man from a country affected by an outbreak. Furthermore, the presence of *A albopictus* in the area was known. A case of CHIKV infection was defined as the presence of

high fever (>38·5°C) and joint pain and living in, or having visited, one of the two villages; for this definition, laboratory confirmation was not required. Individuals with fever but no joint pain and those with these symptoms who did not live in the villages or who had not visited them were deemed to be cases only if laboratory confirmation was obtained (ie, positivity to either haemagglutination inhibition or PCR).

Procedures

Blood and serum samples were stored at –80°C before being tested. Samples were tested for antibodies to CHIKV by haemagglutination inhibition and initially confirmed by plaque reduction neutralisation assays in 12 cases who were haemagglutination inhibition-positive; both tests were done at the Istituto Superiore di Sanità, Rome.[11] Thereafter, only the haemagglutination inhibition test was used. Samples were also tested for antibodies to dengue virus and yellow fever, also with the haemagglutination inhibition test.

To detect the presence of viral genomic RNA in human samples, real-time RT-PCR targeting the *nsp1* gene of CHIKV was done. The assay was based on the Qiagen One Step RT-PCR kit, and a 25 µL reaction volume included 3 µL RNA extract (Qiagen Viral RNA Mini kit), 40 ng/µL bovine serum albumin, 400 µmol/L of each dNTP, 600 nmol/L CHIKV sense (tgatcccgactcaaccatcct), 600 nmol/L CHIKV anti-sense (ggcaaacgcagtggtacttcct) primers, and 200 nmol/L probe ChikP (FAM-tccgac-atcatcctccttgctggc-Black Hole Quencher 1). Amplification was done in a Roche Light Cycler (Indianopolis, IN, USA) and involved the following steps: 50°C for 30 min, 95°C for 15 min, and 45 repetitions of 95°C for 15 s then 58°C for 30 s.

PCR was also used to detect CHIKV in specimens of *A albopictus* that were captured locally during the outbreak. Total RNA was extracted from the supernatant of an homogenate of mosquitoes in minimal essential medium, using TRIzol LS (Invitrogen, Carlsbad, CA, USA)). The RNA was retrotranscribed to cDNA with SuperScript II (Invitrogen) and random primers. Two different PCR protocols were used on the same samples: an RT nested PCR[14] and a real-time PCR with Taqman probe.[15]

Two pairs of primers (CHIKV 10264F/CHIKV 11300R and CHIKV 10564F/CHIKV 11081R)[16] were used to

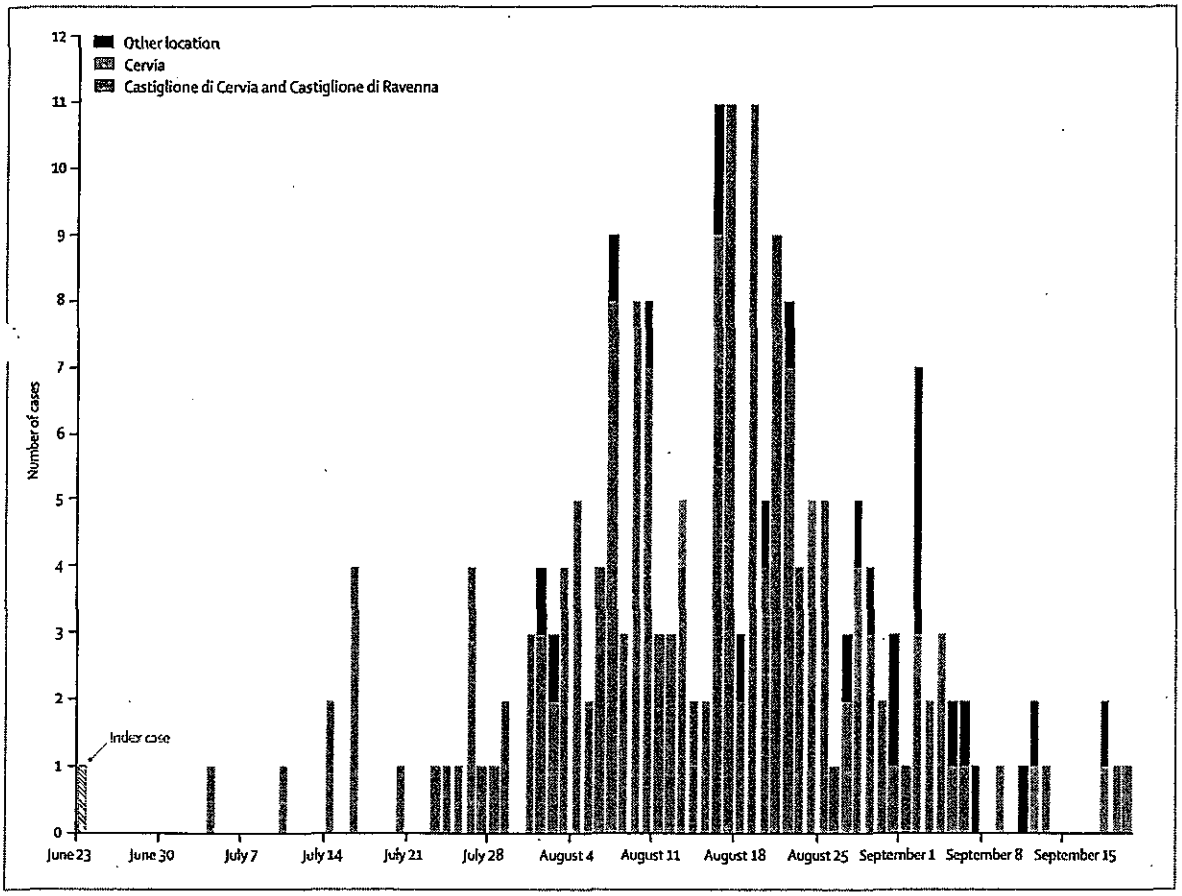

Figure 1: **Epidemic curve**
Distribution of dates of onset of symptoms for CHIKV cases by presumed place of infection (ie, Castiglione di Cervia and Castiglione di Ravenna, Cervia, or other/unknown location).

amplify part of the E1 gene directly from the extracted RNA from RT-PCR-positive samples. Nucleotide sequences were assembled with BioEdit software (version 7·0·9·0) and were aligned with Clustal W software (version 1.6). Phylogenetic analysis based on the available partial E1 gene sequences of CHIKV and tree reconstructions were done with MEGA version 4. For the construction of phylogenetic trees, the neighbour-joining algorithm and the Kimura two-parameter distance model were used. The reliability of the analysis was assessed by a bootstrap test with 1000 replications.

Statistical analysis

In the present analysis, we considered those cases identified between July 4 and Sept 27, 2007. The dates of the onset of symptoms of the cases were plotted to fit the epidemic curve. The frequency distribution of the cases' main characteristics and their signs and symptoms were calculated. Attack rates (both overall and stratified by age and sex) were calculated for the two villages that were affected. Risk ratios (RR) and their 95% CI were also estimated. Age-adjusted attack rates were also calculated separately by sex. The origin and spread of CHIKV cases in the two initially affected villages were mapped. For each case, the address of the individual and the date of the onset of symptoms were entered into Microsoft Access 2003 and linked to the locations on georeferenced maps in the geographic information system (ArcView 8·3, ESRI, Redlands, CA, USA).

Role of the funding source

There was no funding source for this study. All authors had full access to all the data. The corresponding author had final responsibility for the decision to submit for publication.

Results

205 cases of CHIKV infection occurred between July 4 to Sept 27, 2007, in Ravenna (figure 1). There were several waves of cases, with the number peaking in the third week of August. Up to the time of this peak, most cases had occurred in Castiglione di Cervia and Castiglione di Ravenna. Afterwards, and after the first mosquito control measures in the area that was mainly affected had been implemented (on Aug 18), a new wave of cases was observed, most of which occurred outside the two villages.

The distribution of cases by age, sex, residence, and place where the infection was presumably acquired is shown in table 1. The median age was 60 (range 1–95) years; 58 (1–92) years for male cases and 62 (3–95) years for female cases. Most patients reported that they lived in or had visited one of the two villages. The others were scattered throughout the province, although a cluster of 13 cases due to local transmission was reported in Cervia, a town of 8606 inhabitants located about 9 km from the villages. This cluster occurred in a restricted area of the town, near a public gathering place frequented by people coming from—or who had visited—Castiglione di Cervia and who had developed the disease.

	Number of cases (%)
Age (years)	
0–19	12 (6%)
20–39	26 (13%)
40–59	62 (30%)
60–70	78 (38%)
≥80	27 (13%)
Sex	
Male	99 (48%)
Female	106 (52%)
Presumed place of infection	
Castiglione di Cervia or Castiglione di Ravenna	171 (83%)
Cervia	13 (6%)
Other/unknown	21 (10%)
Classification of cases	
Laboratory confirmed	175 (85%)
Clinically defined (untested)	30 (15%)

Table 1: Demographic characteristics of the 205 individuals infected with CHIKV

The first identified case, a man of Indian origin living in Castiglione di Cervia, reported that he had not been abroad during the previous year. However, a relative of his, who had arrived in Italy on June 21 from Kerala, India (an area affected by the CHIKV epidemic), visited the man and became feverish on the afternoon of June 23, when he was in Castiglione di Cervia. A serum sample that had been collected in early September from this man, who was assumed to be the index case, showed high antibody titres against CHIKV (>1:1280). This individual was excluded from the further data analyses.

The spatial-temporal spread of CHIKV in the primarily affected area and the rest of the province is shown in figure 2. After the first cases, which occurred in Castiglione di Cervia, the infection spread both by contiguity, as an expansion of the primary cluster (figure 2 A, B, and C), and by jumping from place to place in both villages, with cases developing more than 2 km away from the primary cluster (figure 2 B and C). Sporadic cases and clusters occurring outside the villages are shown in figure 2 D.

The attack rate was 5·4% in Castiglione di Cervia (115 resident cases out of 2134 inhabitants) and 2·5% in Castiglione di Ravenna (46/1834). The attack rate did not differ between female and male individuals (4·5% of 81 females *vs* 4·0% of 80 males; RR 1·13, 95% CI 0·81–1·57). The rate of attack increased with age: 1·6% of 27 people under 40 years of age, 4·5% of 52 individuals aged 40–59 years, 7·0% of 57 aged 60–79 years, and 8·8% of 25 aged 80 years or older were affected (RR 2·78, 95% CI 1·75–4·39 for the 40–59 years age-group; 4·21,

Figure 2: **Geographical origin and spatial-temporal diffusion of CHIKV cases**
Number of cases in Castiglione di Cervia and Castiglione di Ravenna between days 0–15 (A), between days 0–45 (B), cumulatively (C), and in the province of Ravenna (D).

2·86–6·61 for the 60–79 years age-group; and 5·20, 3·08–8·83 for those aged 80 years or more, all relative to the under 40 years age-group; χ^2 for trend p<0·0001). There was no difference in attack rate between those aged 0–19 years and those aged 20–39 years (1·6% [10/631 individuals] *vs* 1·6% [17/1082]). The age-adjusted attack rates for male and female individuals were much the same (4·2% *vs* 4·1%).

The frequency of clinical symptoms is shown in table 2. All patients presented with high fever (median maximum temperature 39·5°C, 25–75th percentile 39–39·8°C), and most of them had pain in multiple joints. About half the cases developed skin rash, in some cases with itching. Clinical disease was mild and self-limiting in most cases. One 83-year-old man died, although this man had severe underlying conditions.

Laboratory confirmation was obtained for 175 cases: 32 were PCR-positive only; 135 were haemagglutination inhibition-positive only; and eight were positive for both PCR and haemagglutination inhibition. The median time between the onset of symptoms and obtaining positive results was 2 days for PCR (maximum 7 days) and 15 days for haemagglutination inhibition. 30 cases who met the clinical and epidemiological criteria

	Number of cases (%)
Fever	205 (100%)
Joint paint	199 (97%)
Fatigue	190 (93%)
Skin rash	106 (52%)
Headache	105 (51%)
Muscle pain	94 (46%)
Diarrhoea	48 (23%)
Itching	42 (20%)
Vomiting	40 (19%)
Photophobia	31 (15%)
Conjunctivitis	7 (3%)

†Mandatory in the case definition, ‡Not mandatory if diagnosis is laboratory confirmed.

Table 2: **Distribution of symptoms**

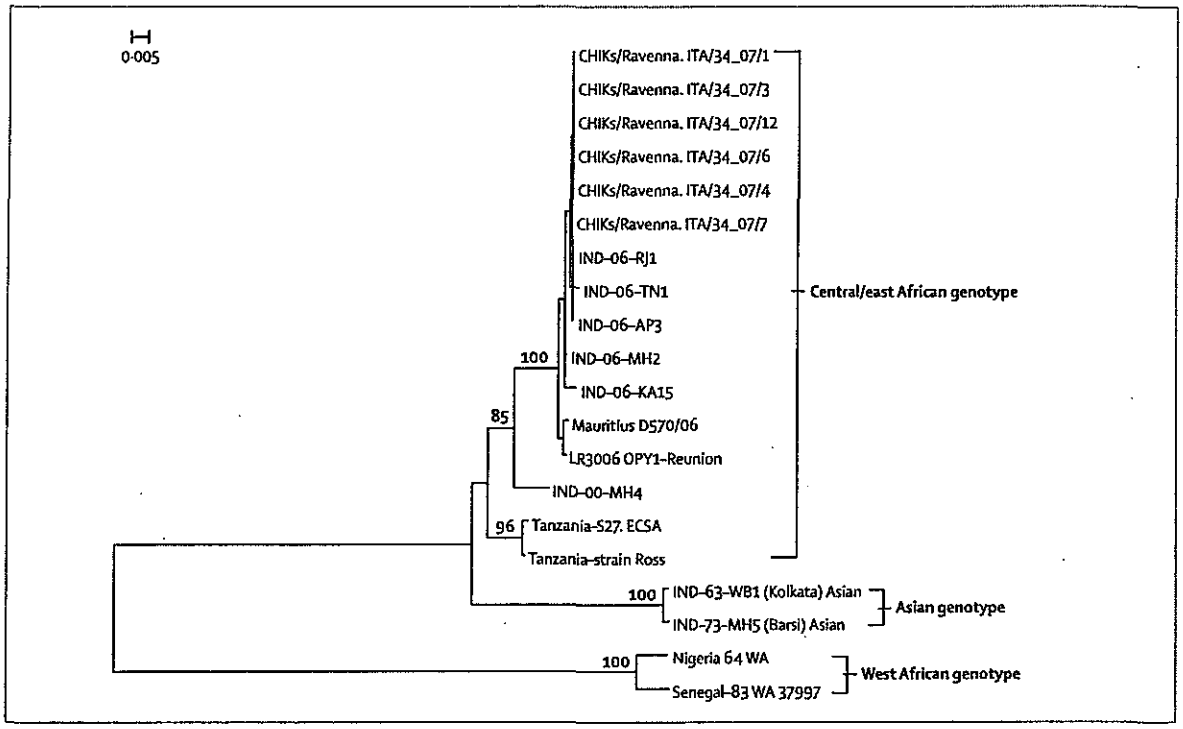

Figure 3: **Phylogenetic analysis of the partial nucleotide sequence (1011 nucleotides) of the E1 gene of CHIKV strains identified in Italy and in different parts of the world**

remained untested because no blood sample was available or because it was inadequate for testing (table 1).

CHIKV sequences were detected by PCR in *A albopictus* mosquitoes captured during the outbreak. Positive results were obtained from pools of 125 mosquitoes from Castiglione di Ravenna and and 90 from Castiglione di Cervia. The results of the phylogenetic analysis are shown in figure 3. Human and mosquito strains clustered with Indian strains, and they contained a change (Ala226Val) in the membrane fusion glycoprotein E1 that had also been found in the Indian Ocean variant of the African genotype of CHIKV.

Discussion

This outbreak of CHIKV infection, outside a tropical country, was probably begun by a man from India, who developed a febrile syndrome 2 days after his arrival in Italy. He had high titres of antibodies against CHIKV at the time of examination (early September) and was probably highly viraemic when visiting his relatives (late June) in the village where the epidemic began. The phylogenetic analysis showed that the strain that caused this outbreak was similar to the strains detected on the Indian subcontinent[5] and that it contained the same mutation found in a variant in the Indian Ocean islands,[7,17] which is thought to be better adapted to *A albopictus* than are other variants. The hypothesis that this variant has a high virus-vector fitness seems to be confirmed by both the successful introduction and rapid spread of the infection from one infected human host and by the further occurrence of other smaller clusters in different localities in the same province yet located several kilometres from the two villages initially affected.

Samples of *A albopictus* mosquitoes from the villages were found to be positive for CHIKV sequences. The high density of the vector at the time of arrival of the index case, as anecdotally reported by villagers, was probably a major determinant of the outbreak. Actually, the population of *A albopictus* was already well established in scattered foci in Ravenna province (an average of >80% positive ovitraps), but had only recently enlarged its peripheral area to include these villages, which might explain the high vector density (ie, before control measures had been implemented). The presence of *A albopictus* in Italy is not surprising. The mosquito was first documented in Genoa (northwestern Italy) in 1990,[18] and the presence of a breeding population was first reported near Padua (northeastern Italy) in 1991.[19] The source of infestation was identified as a warehouse of a tyre retreading company that had imported used tyres infested with mosquito eggs from Georgia, USA.[20] Unfortunately, despite efforts made to control the spread of *A albopictus* mosquitoes, they rapidly colonised almost the entire country,[21,22] showing a high degree of fitness.

The peak of the outbreak occurred during the third week of August, more than 6 weeks after the onset of symptoms in the first locally acquired case, and 8–9 weeks after the onset of symptoms in the presumed

index case. The occurrence of new cases in the initially affected area started to decrease a few days after vector control measures were first implemented. The infection seemed to spread both by contiguity within the initially affected villages and by jumping from place to place within and from the initial outbreak area to the other locations. A small cluster, caused by local transmission, was reported in the town of Cervia, where the infection was probably introduced through population movement from Castiglione, although passive transport of infected mosquitoes cannot be ruled out completely.

The attack rate in Castiglione di Cervia—the most affected village—was 5·4%, much lower than the 34% reported in La Réunion.[4] This difference might be due to early intervention in Italy, although the role of different background vector density or climate-dependent vector behaviour cannot be excluded. Moreover, we cannot rule out under-reporting, which could have occurred if our surveillance system had a low sensitivity in the first month or if there was an excess of asymptomatic cases compared with those found in La Réunion.[23] The attack rates by sex and age, calculated for Castiglione di Cervia and Castiglione di Ravenna, were stably low for people under 40 years of age but tended to increase for older ages, with the highest rates in the oldest group. Whether this trend was due to behavioural factors leading to differential exposure to mosquitoes or to biological factors, implying a different host response with a different proportion of asymptomatic cases, needs to be investigated further.

The clinical course of the disease was fairly mild. The case-fatality rate was less than 0·5%, consistent with the rate of one death per 1000 clinical cases reported in La Réunion.[4] Almost all patients reported joint pain, which was often severe and persistent, and which seems to be strongly indicative of CHIKV disease. Similar findings were reported in La Réunion,[24] whereas a lower proportion of cases with joint pain (78%) was found in Malaysia in 1998.[25] About half the patients presented with skin rash, similar to previous findings.[2]

85% of the cases were confirmed by either serology or PCR. No viral sequences were detected in 31 samples collected more than 7 days after the onset of symptoms, suggesting that the viraemic phase is fairly short, as found in previous reports.[26]

Measures for controlling the population of *A albopictus* were implemented in all areas where cases were reported, beginning on Aug 18. These measures included the use of fast-acting insecticides (synergised pyrethrins) for 3 days consecutively, applied with a truck-mounted atomiser in public spaces and a backpack mist blower in private spaces. Antilarval measures, using formulations of insect growth regulators and *Bacillus thuringiensis* var *israeliensis* were also implemented. House-to-house interventions were done to eliminate breeding places, and community participation was encouraged. For each suspected case of infection, these control measures were done within a radius of 100 m of the individual's residence; for clusters, the control measures were done within a 300-m radius of the most external case. Since Sept 27, 2007—the date at which the present analysis was censored—sporadic cases have continued to occur in Ravenna; two small clusters outside Ravenna (in Cesena and Rimini) have also been identified. Whether transovarial transmission of CHIKV might result in a reappearance of the infection in spring, 2008, is being considered carefully.

The occurrence of an outbreak of CHIKV infection in a country with a temperate climate emphasises that the predicted globalisation of human beings and vectors[4,7] has become a reality. To promptly identify new potential threats that were previously restricted to tropical areas, clinical and diagnostic capacities have to be developed in countries with a temperate climate and in which vectors of exotic diseases already circulate.

Contributors

GR was responsible for the clinical and epidemiological investigation and for writing the manuscript. LN was responsible for laboratory diagnosis and contributed to writing the manuscript. CF, FM, and MGC did laboratory tests on human and mosquito samples, and phylogenetic analysis; PC and MD identified viral sequences in the mosquitoes. MP developed the PCR used in this investigation and contributed to writing the manuscript. RR, GM, and PA were responsible for the entomological investigation and contributed to writing the manuscript. ACF supervised the field activities that were implemented by RA and GS, who also contributed to data analysis. SB was responsible for data management and analysis. AC supervised and coordinated all of the activities and revised the manuscript.

CHIKV study group

P Macini, R Bellini, G Venturi, C Fiorentini, A Marchi, E Benedetti, P Bucci, P Bonilauri, C Venturelli, M Montanari, F Severini, B M Borrini, L Droghini, G Frasca, E Massimiliani, A Mattivi, M L Moro, L Moschella, K Petropulacos, C Po, L Bevilacqua, F Ghiselli, T Zauli, T Samorè, L Salizzato, F Toni, V Sambri, M L Ciofi degli Atti, C Rizzo, T Seyler, S Salmaso, M G Pompa.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

1 Ross RW. The Newala epidemic. III. The virus: isolation, pathogenic properties and relationship to the epidemic. *J Hyg (Lond)* 1956; 54: 177–91.
2 Pialoux G, Gaüzère B-A, Jauréguiberry S, Strobel M. Chigungunya, an epidemic arbovirosis. *Lancet Infect Dis* 2007; 7: 319–27.
3 World Health Organization. Chikungunya and dengue, south-west Indian Ocean. *Wkly Epidemiol Rec* 2006; 81: 105–16.
4 Charrel RN, de Lamballerie X, Raoult D. Chikungunya outbreaks—the globalization of vectorborne diseases. *N Engl J Med* 2007; 356: 769–71.
5 Yergolkar PN, Tandale BV, Arankalle VA, et al. Chikungunya outbreaks caused by African genotype, India. *Emerging Infect Dis* 2006; 12: 1580–83.
6 Hochedez P, Jaureguiberry S, Debruyne M, et al. Chikungunya infection in travellers. *Emerging Infect Dis* 2006; 12: 1565–67.
7 Parola P, de Lamballerie X, Jourdan J, et al. Novel chikungunya virus variant in travelers returning from Indian Ocean islands. *Emerg Infect Dis* 2006; 12: 1483–99.
8 Lanciotti RS, Kosoy OL, Laven JJ, et al. Chikungunya virus in US travelers returning from India, 2006. *Emerg Infect Dis* 2007; 13: 764–67.

9 Beltrame A, Angheben A, Bisoffi Z, et al. Imported chikungunya infection in Italy. Report of 17 consecutive cases in travellers. *Emerg Infect Dis* 2007; 13: 1264–66.

10 Gratz NG. Critical review of the vector status of *Aedes albopictus*. *Med Vet Entomol* 2004; 18: 215–17.

11 Knudsen AB, Romi R, Majori G. Occurrence and spread in Italy of *Aedes albopictus*, with implications for its introduction into other parts of Europe. *J Am Mosq Control Assoc* 1996; 12: 177–83.

12 Angelini R, Finarelli AC, Angelini P, et al. An outbreak of chikungunya fever in the province of Ravenna, Italy. *Eurosurveillance* 2007; 12 (9). http://www.eurosurveillance.org/ew/2007/070906.asp (accessed Nov 6, 2007).

13 Peragallo MS, Nicoletti L, Lista F, D'Amelio R, on behalf of the East Timor Dengue Study Group. Probable dengue virus infection among Italian troops, East Timor, 1999–2000. *Emerg Infect Dis* 2003; 9: 876–80.

14 Pfeffer M, Linssen B, Parke MD, Kinney RM. Specific detection of chikungunya virus using a RT-PCR/nested PCR combination. *J Vet Med B Infect Dis Vet Public Health* 2002; 49: 49–54.

15 Pastorino B, Bessaud M, Grandadam M, Murri S, Tolou HJ, Peyrefitte CN. Development of a TaqMan RT-PCR assay without RNA extraction step for the detection and quantification of African chikungunya viruses. *J Virol Methods* 2005; 124: 65–71.

16 Edwards CJ, Welch SR, Chamberlain J, et al. Molecular diagnosis and analysis of chikungunya virus. *J Clin Virol* 2007; 39: 271–75.

17 Schuffenecker I, Iteman I, Michault A, et al. Genome microevolution of chikungunya viruses causing the Indian Ocean outbreak. *PLoS Med* 2006; 3: e263.

18 Sabatini A, Raineri V, Trovato G, Coluzzi M. *Aedes albopictus* in Italy and possible diffusion of the species into the Mediterranean area. *Parassitologia* 1990; 32: 301–04.

19 Dalla Pozza G,Majori G. First record of *Aedes albopictus* establishment in Italy. *J Am Mosq Control Assoc* 1992; 8: 318–20.

20 Dalla Pozza GL, Romi R, Severini C. Source and spread of *Aedes albopictus* in the Veneto region of Italy. *J Am Mosq Control Assoc* 1994; 10: 589–92.

21 Romi R. History and updating of the spread of *Aedes albopictus* in Italy. *Parassitologia* 1995. 37: 99–103.

22 Romi R, Di Luca M, Majori G. Current status of *Aedes albopictus* and *Aedes atropalpus* in Italy. *J Am Mosq Control Assoc* 1999; 15: 425–27.

23 Ramful D, Carbonnier M, Pasquet M, et al. Mother-to-child transmission of chikungunya virus infection. *Pediatr Infect Dis J* 2007; 26: 811–15.

24 Staikowsky F, Le Roux K, Schuffenecker I, et al. Retrospective survey of chikungunya disease in Réunion Island hospital staff. *Epidemiol Infect* 2007; published online Apr 16. DOI:10.1017/S0950268807008424.

25 Lam SK, Chua KB, Hooi PS, et al. Chikungunya infection—an emerging disease in Malaysia. *Southeast Asian J Trop Med Public Health* 2001; 32: 447–51.

26 Taubitz W, Cramer JP, Kapaun A, et al. Chikungunya fever in travelers: clinical presentation and course. *Clin Infect Dis* 2007; 45: e1–e4.

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No. 8

医薬品 研究報告 調査報告書

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研究報告の概要

○輸血によるシャーガス病:感染受血者および供血者の寄生虫学的、血清学的モニタリング

シャーガス病はラテンアメリカの風土病であるが、人の移動により分布が拡大している。スペインでは、2005年からラテンアメリカ出身の供血者に対して*T. cruzi*抗体検査を実施している。

本報は、血液製剤の輸血によりシャーガス病に感染し、死亡したスペイン人患者の寄生虫学的、血清学的疾患経過、ならびに供血者の調査の報告である。患者は25歳男性で白血病の既往があり、少なくとも176名の供血者由来の血液製剤を輸血されていた。2005年1月(輸血後45日)に原因不明の発熱を発症し、抗菌薬による治療を行った。臍帯血移植後も発熱と神経障害を発症し、多臓器不全で7月上旬に死亡した(輸血後212日)。患者血清中に*T. cruzi* DNAがPCRで確認された。過去の検体を調べたところ、輸血後48日にはDNAが検出されていた。抗体はIFATとELISAで輸血後159日で陽性になり、204日で陰性化していた。輸血された製剤の供血者の血清学検査では、58歳のブラジル出身の女性供血者が抗体陽性であったことが判明した。彼女は2004年12月上旬に供血を行い、血小板製剤が患者に輸血されていた。追加調査時のPCRでは、血中に寄生虫は検出されなかったが、1ヵ月後シャーガス病の精密検査を行った際の血液からはPCRで検出された。

抗体価の動態から、患者はシャーガス病の急性期であったことが示唆された。移植のための免疫抑制状態で、寄生虫が血液脳関門を通過して神経系に感染したことが、CSF検体中の*T. cruzi* DNAから確認された。供血者は無症候の状態であったことから、患者の免疫状態が発症に関連したことが考えられる。複数回輸血患者は、免疫抑制剤治療実施前に、抗*T. cruzi*抗体のスクリーニングを受けるべきである。

報告企業の意見	今後の対応
輸血によりシャーガス病に感染し、死亡したスペイン人患者の寄生虫学的、血清学的疾患経過、ならびに供血者の調査についての報告である。	日本赤十字社は、輸血感染症対策として献血時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、シャーガス病の既往がある場合には献血不適としている。日本在住の中南米出身献血者については、国と協議しつつ対応を検討中である。今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

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BRIEF REPORT

Transfusional Chagas Disease: Parasitological and Serological Monitoring of an Infected Recipient and Blood Donor

María Flores-Chávez,[1] Begoña Fernández,[2] Sabino Puente,[3] Pilar Torres,[2] Mercedes Rodríguez,[1] Carolina Monedero,[1] Israel Cruz,[1] Teresa Gárate,[1] and Carmen Cañavate[1]

[1]Servicio de Parasitología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, and [2]Centro de Transfusión de la Comunidad de Madrid, and [3]Unidad de Enfermedades Tropicales, Hospital Carlos III, Madrid, Spain

Chagas disease is endemic to Latin America, but human migration is extending its distribution. This report describes the parasitological and serological course of disease in a Spanish patient fatally infected via a blood product transfusion, as well as the monitoring of the donor. Before undergoing immunosuppression, multitransfused patients should be screened for anti–*Trypanosoma cruzi* antibodies.

Chagas disease, or American trypanosomiasis, is endemic to Latin America. However, the recent changes in human patterns of migration have prompted the appearance of cases in areas where the vector of the disease is not found [1, 2]. The natural progress of infection involves an acute and a chronic phase. In areas of endemicity both forms are seen, whereas in nonendemic areas, the great majority of infections are diagnosed in the chronic phase, although 70% of infected persons remain asymptomatic. Despite technological advances, there is no reference standard laboratory technique for diagnosing Chagas disease [3]. In the acute phase, parasitological diagnostic methods are the most reliable. However, in the chronic, phase there may be little or no parasitemia, and diagnosis is made mainly on the basis of results of tests for anti–*Trypanosoma cruzi* antibodies. In recent years, Spain has become one of the favorite destination countries for South American emigrants. These citizens achieve a good degree of social integration in Spain, and they often voluntarily and altruistically support blood donation programs. Thus, since 2005, Spanish blood donation legislation has required donors from Latin America to be serologically screened for anti–*T. cruzi* antibodies (Royal Decree 1088/2005) [4]. The present work describes the retrospective laboratory evaluation of a Spanish patient with leukemia who died of Chagas disease contracted via a transfusion with contaminated blood, the retrospective study to identify the source of infection, and the monitoring of the donor.

Methods and materials. Anti–*T. cruzi* antibodies were sought in serum samples collected at different times before the patient's death; these samples were stored at −80°C in the serum library of the Centro Nacional de Microbiología (National Microbiology Center [Madrid]). Parasite DNA was also sought in these samples, in CSF (also collected before death), and in lung, kidney, and liver necropsy samples.

For the retrospective study, serum samples of 176 donors whose blood derivatives had been transfused into the patient were examined. Of these, 168 lived in Madrid (159 were of Spanish origin, 1 was Brazilian, 1 was Ecuadorian, 2 were Colombian, 3 were French, 1 was Polish, and 1 was German), 5 lived in Albacete (southeastern Spain), and 3 lived in Jaén (southern Spain). Samples belonging to all of the Madrid donors were preserved at the serum library of the Centro de Transfusión de Madrid (Madrid Transfusion Center); new samples were collected from the donors living in Albacete and Jaén once they had been traced. Serum and blood samples were collected from the infected blood donor to confirm the results of the retrospective study and to monitor the development of the infection after treatment.

Anti–*T. cruzi* antibodies were detected by the indirect immunofluorescent antibody test (IFAT) and by ELISA with modifications introduced by the Department of Parasitology at the Centro Nacional de Microbiología [5, 6]. *T. cruzi* DNA was detected by PCR with use of oligonucleotides 121–122 and Tcz1–Tcz2, which amplify the variable region of the kinetoplast DNA minicircle (330 bp) and a repetitive sequence of satellite DNA (195 bp), respectively [7, 8]. All assays were performed in duplicate with negative and positive controls.

Results. The Spanish patient was a 25–year-old man who had a history of leukemia [9] that eventually required a cord blood transplant; he received blood derivatives from at least 176 persons who donated blood at different transfusion centers. In January 2005, 45 days after infection onset, the patient was examined for fever of unknown origin. None of the infectious agents that commonly cause this problem in this kind of patient

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Reprints or correspondence: Dr. Carmen Cañavate, Servicio de Parasitología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Ctra. Pozuelo-Majadahonda, Km 2 28220 Majadahonda, Madrid, Spain (ccanave@isciii.es).

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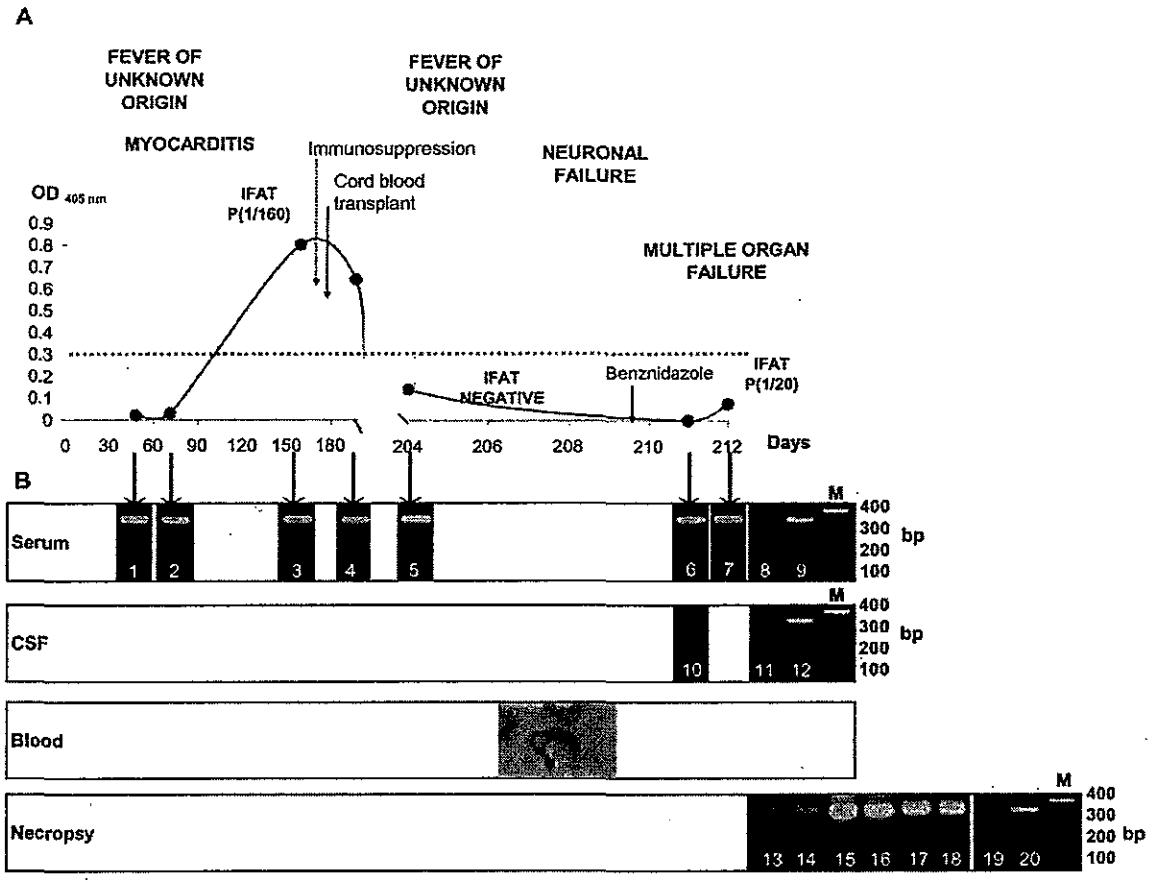

Figure 1. Parasitological and serological changes according to the clinical status of the patient. The day the patient received the platelet concentrate was defined as day 0. *A,* Changes in anti–*Trypanosoma cruzi* antibody levels according to indirect immunofluorescent antibody test (IFAT) and ELISA. *The last serum dilution with a positive (P) reaction is shown. B, Presence of parasites as determined by microscopy and PCR. Lanes 8, 11, and 19,* Negative controls. *Lanes 9, 12,* and *20,* Positive controls. *Lanes 13–14, 15–16,*and *17–18,* Duplicate samples of *T. cruzi* DNA amplified from kidney, liver, and lung tissues, respectively. The PCR results obtained using the oligonucleotides 121–122 confirmed those obtained with Tcz1–Tcz2. *Dotted line,* Threshold; OD, optical density.

(lymphotropic viruses, exanthema-causing viruses, adenoviruses, influenza virus, *Mycoplasma pneumoniae,* or *Toxoplasma gondii,* among others) were detected. After treatment with itraconazole, the symptoms receded, and the patient was assessed and treated in preparation for cord blood transplantation as described by Forés et al. [9]. In the first week of July 2005 (day 211 after infection onset), the Department of Parasitology at the Centro Nacional de Microbiología received several serum and CSF samples obtained from the patient, as well as the supernatants of cell cultures used in the identification of flagellates by microscopy and in diagnostic tests. Microscopy revealed the presence of trypomastigotes, and PCR identified DNA of *T. cruzi,* indicating infection by this pathogen. Tests for anti–*T. cruzi* antibodies, however, yielded negative results.

The patient died of multiorgan failure (day 212 after infection onset), and a retrospective evaluation was undertaken to determine the source of infection. Patient serum samples that were sent to the Centro Nacional de Microbiología for the diagnosis of problems other than Chagas disease and that were preserved at our center's serum library were analyzed by IFAT, ELISA, and PCR (figure 1). PCR showed *T. cruzi* to have first appeared in the patient's serum 48 days after he received a transfusion of platelets. IFAT and ELISA confirmed positive seroconversion on day 159 after infection onset, followed by a negative seroconversion on day 204 after infection onset.

At the same time, the donors whose blood products had been given to the patient were screened for anti–*T. cruzi* antibodies (figure 2*A*). This analysis ruled out all of the donors from Albacete and Jaén and 167 of the donors from Madrid as potential sources of infection. IFAT and ELISA yielded positive results for the remaining Madrid-based donor. This person made a blood donation at the beginning of December 2004 (figure 2*B*); the patient received a concentrate of platelets prepared from this blood (day 0).

The donor was a 58–year-old woman originally from Alto Parnaiba, in the Brazilian state of Maranhão. She was asked to

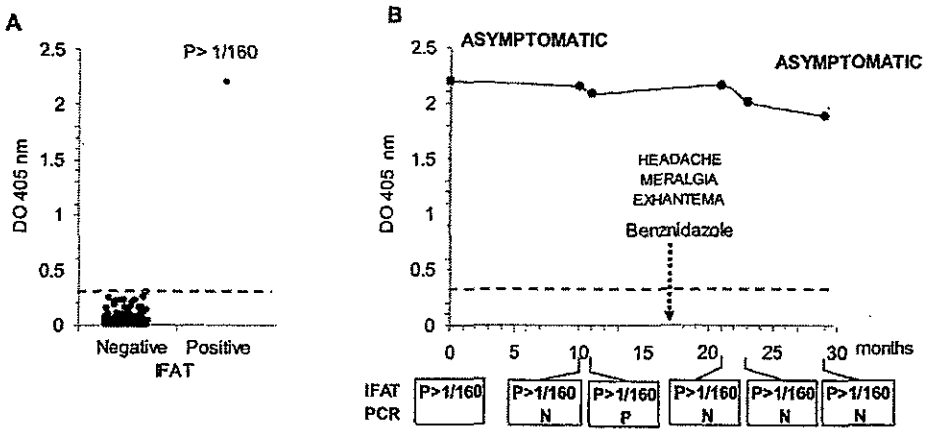

Figure 2. *A,* Determination of anti–*Trypanosoma cruzi* antibodies by indirect immunofluorescent antibody test (IFAT) and ELISA in serum of the different blood donors. The last serum dilution with a positive (P) reaction is shown. *B,* Serological and PCR monitoring of the infected donor. The month in which the infected donor made a blood donation was defined as month 0. *Dotted line,* Threshold. N, negative; OD, optical density.

attend an appointment to confirm the results obtained in the retrospective investigation. At that time, no parasites were detected in her blood by PCR. She was then referred to the Tropical Diseases Unit at the Hospital Carlos III in Madrid, where she underwent a clinical examination, chest radiography, electrocardiography, and echocardiography, all of which yielded normal results. No other signs or symptoms of interest were noted except for constipation, which the donor had experienced for some 8 years (defecation once every 2–3 days). On this occasion (1 month after the first appointment), however, PCR did detect parasites in the blood. In March 2006, treatment with benznidazole (6 mg/kg/day) was begun, but this was suspended after 24 days because of the appearance of intense headaches, meralgia paresthesica of the femorocutaneous nerve, and generalized macular exanthema. No hematologic toxicity was recorded. Following this treatment, test results for blood parasites remained negative, although anti–*T. cruzi* antibodies remained detectable (figure 2*B*).

Discussion. Figure 1 shows that anti–*T. cruzi* antibodies were detectable in the patient only before the start of the immunosuppressive protocol associated with the cord blood transplant (day 159 after infection onset). In the absence of an immune response, the parasites crossed the blood-brain barrier and infected the nervous system. This was confirmed by the presence of *T. cruzi* DNA in the CSF sample. Given the general condition of the patient, treatment with benznidazole had no immediate effect on the parasite load, although IFAT detected a slight increase in the antibody titer (1/20).

The detection of *T. cruzi* in the necropsy samples agrees with the systemic distribution of the parasite and the multiorgan failure that caused the patient's death. The kinetics of the antibody titer can be explained in terms of an acute, recently acquired infection. The detection of *T. cruzi* by PCR since January 2005 (day 48 after infection onset) agrees with the date when the patient received the infected blood products. Thus, the results of the parasitological and serological investigations agree with the patient's clinical signs and symptoms and suggest that he was in the acute phase of Chagas disease. Acute transfusional Chagas disease can last from 1 to 6 months after the entry of the parasite [3].

The discrepant PCR results (1 positive and 1 negative) obtained for the infected donor before benznidazole treatment was begun agree with the low-level parasitemia typical of the chronic phase of *T. cruzi* infection [10]. These results could also be because the first analysis involved a 5-mL blood sample and the second a 10-mL sample. When blood parasite concentrations are low, detection is more likely in larger blood volumes [11]. Similarly, at blood donation units, collecting as much as 450 mL of blood from donors increases the risk of contamination with small numbers of parasites.

Although, for successful blood culturing and artificial xenodiagnosis, it is recommended that blood samples be processed within 4 h of collection to ensure parasite viability [12]; in the present case, the parasites remained viable over the entire platelet conservation period, because the maintenance temperature (22°C; range, 20°C–24°C) is close to that used for culturing *T. cruzi* (25°C–27°C). The recipient's immunodepression caused by his leukemia and the immunosuppression induced before cord blood transplantation appear to have been of maximum importance in the development of the infection, because the parasite caused no appreciable symptoms in the donor. This highlights the role of the host immune system in protection from and the development of infection. In immunodepressed patients, infection may be severe and have fatal consequences. It is therefore recommended that higher-risk organ donors be screened for anti–*T. cruzi* antibodies, as should

multitransfused candidates for transplantation—irrespective of their origin—if they are to undergo immunosuppression protocols.

It should be stressed that before October 2005, Spanish blood donation legislation permanently excluded donors with Chagas disease. It did not, however, contemplate the use of a reliable screening test for the detection of healthy *T. cruzi* carriers. In the present case, the donor did not know of her trypanosome infection status, and no risks were detected during the predonation assessment interview. Her blood donation was therefore accepted in December 2004. In contrast, the current legislation (October 2005) outlines new technical requirements for blood donation [4] and establishes the use of a *T. cruzi* diagnostic assay to assess the eligibility of donors from areas where Chagas disease is endemic, as well as those with risk factors for infection. Under this legislation, the present donor would have been excluded.

In Spain, the supply of blood is a permanent problem, and the Latin American population—~1.5 million residents—has already became an important source of potential donors. A preliminary *T. cruzi* seroprevalence survey of immigrants from areas of endemicity returned positive estimates of close to 1% [13]. Because blood transfusion is the main route for *T. cruzi* transmission in Spain, the new legislation guarantees the quality of blood and blood component transfusions for recipients and allows the inclusion of immigrants from the Americas in the pool of potential blood donors.

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Potential conflicts of interest. All authors: no conflicts.

References

1. Gascon J, Grupo de Trabajo del Taller Enfermedad de Chagas Importada: Un nuevoreto de salud publica? Diagnosis and treatment of imported Chagas disease [in Spanish]. Med Clin (Barc) **2005**; 125:230–5.
2. Wendel S, Gonzaga AL. Chagas' disease and blood transfusion: a New World problem? Vox Sang **1993**; 64:1–12.
3. World Health Organization. Control of Chagas disease. Geneva: World Health Organization Press, **2002**:1–124.
4. Royal Decree 1088/2005. Technical requirements and minimum conditions of the blood donation and of the transfusion centers and services [in Spanish]. Official Spanish Gazette **2005**; 225:31288–304.
5. Camargo ME. Fluorescent antibody test for the serodiagnosis of American trypanosomiasis: technical modification employing preserved culture forms of *Trypanosoma cruzi* in a slide test. Rev Inst Med Trop Sao Paulo **1966**; 8:227–35.
6. Scott P, Pearce E, Natovitz P, Sher A. Vaccination against cutaneous leishmaniasis in a murine model. I. Induction of protective immunity with a soluble extract of promastigotes. J Immunol **1987**; 139:221–7.
7. Wincker P, Britto C, Pereira JB, Cardoso MA, Oelemann W, Morel CM. Use of a simplified polymerase chain reaction procedure to detect *Trypanosoma cruzi* in blood samples from chronic chagasic patients in a rural endemic area. Am J Trop Med Hyg **1994**; 51:771–7.
8. Moser DR, Kirchhoff LV, Donelson JE. Detection of *Trypanosoma cruzi* by DNA amplification using the polymerase chain reaction. J Clin Microbiol **1989**; 27:1477–82.
9. Forés R, Sanjuan I, Portero F, et al. Chagas disease in a recipient of cord blood transplantation. Bone Marrow Transplant **2007**; 39:127–8.
10. Castro C, Prata A. Absence of both circadian rhythm and *Trypanosoma cruzi* periodicity with xenodiagnosis in chronic chagasic individuals. Rev Soc Bras Med Trop **2000**; 33:427–30.
11. Junqueira AC, Chiari E, Wincker P. Comparison of the polymerase chain reaction with two classical parasitological methods for the diagnosis of Chagas disease in an endemic region of north-eastern Brazil. Trans R Soc Trop Med Hyg **1996**; 90:129–32.
12. Castro C, Santos MC, Silveira CA. Comparative study between artificial xenodiagnosis performed immediately and four hours after venous punch. Rev Soc Bras Med Trop **2004**; 37:128–30.
13. Castro E. Blood transfusion and Chagas disease: initiatives in Spanish transfusion centers [in Spanish]. Enferm Emerg **2006**; 8 (Suppl. 1): 48–50.

別紙様式第 2-1

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-5

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2007 年 10 月 11 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	Blood donor screening for parvovirus B19 in Germany and Austria. Schmidt, M. et al, Transfusion, 47, 1775-1782 (2007).	公表国 ドイツ	
販売名（企業名）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
ドイツ及びオーストリアで 4 年間総計 280 万の献血検体に対して行なわれた B19 ウイルススクリーニングの結果が報告された。測定期間中，2004 年 5 月から 2006 年 1 月におけるウイルス検出頻度が最も高かった。しかし，その B19 DNA 陽性の頻度は 0.274%と低く，B19 ウイルス 1 型のみが検出された。B19 DNA が 10E5 IU/mL を超えた 50 人のドナーからは，初回献血時（T0）から 3 及び 6 ヵ月後の 2 回採血が実施された。詳細な分析の結果，ウイルス価については，T0 時点の中央値が 4.85×10E7 IU/mL から 3 ヵ月後に 4.6×10E2 IU/mL へ有意に減少し，その後 6 ヵ月時点までそのまま推移した検体と，さらに減少した検体が認められた。同時に実施された B19 ウイルス抗体分析では，3 及び 6 ヵ月後の 50 人の全ての検体から，構造蛋白 VP2 に対する中和抗体（IgG）が認められた。従って，この抗体がウイルスを中和していると考えられた。本結果から，本試験に参加中の献血業者の出荷手順を以下のように変更した。 ・10E5 IU/mL を超える高濃度の B19 DNA が検出された献血検体は廃棄とした。しかし，ドナーはその後も献血を行うことができることとした。 ・B19 DNA が 10E5 IU/mL 未満である献血検体は中和抗体を含むため安全と考えられ，輸血された。 ・特殊なリスクを有する患者（小児，妊婦及び免疫が低下した患者）に対しては依然として B19 DNA 陰性の血液製剤が推奨された。	BYL-2008-0301

報告企業の意見	今後の対応
B19 ウイルスの検出頻度は測定の時期及び方法によって，1:260 から 1:50000 まで報告に幅がある。本論文では，4 年間で計 280 万サンプルを測定しており，B19 ウイルスの検出頻度を考慮する上で，信頼性の高いデータを示したと考えられる。また，10E5 IU/ml 以下の B19 ウイルスを含有する検体では相対的に高濃度となる中和抗体が存在し，安全であることが示され，感染リスクを考慮するために重要な情報が提供されていると考えられる。 弊社のポリグロビン N の製造に使用されるミニプール血漿においては，ヒトパルボウイルス B19 に対する NAT を実施しており，10E5 IU/mL 以上が確認された場合は，そのミニプール血漿は製造工程から除去している。現在の科学水準では，ヒトパルボウイルス B19 を確実に不活化する方法は存在しないため，感染リスクを完全に排除することはできないが，伝播の可能性は非常に低いと考える。	ウイルス検出及び安全性に関する閾値に関しては今後とも情報収集に努める。

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Blood donor screening for parvovirus B19 in Germany and Austria

Michael Schmidt, Anna Themann, Camilla Drexler, Michaela Bayer, Gerhard Lanzer, Eva Menichetti, Sigrid Lechner, Dietmar Wessin, Barbara Prokopl, Jean-Pierre Allain, Erhard Seifried, and Michael Kai Hourfar

BACKGROUND: Although the main transmission pathway of parvovirus B19 (B19) is typically via the respiratory route, several transfusion-transmitted infections have been reported. To increase blood safety, all blood donations to our blood donor service have been screened by a B19 minipool real-time nucleic acid testing (NAT) since April 2000. Additional customers have been screened since the summer of 2003.

STUDY DESIGN AND METHODS: In total, 2.8 million donations from Germany and Austria were screened for B19 by real-time minipool NAT. A subgroup of 50 B19 DNA–positive donors was screened for B19 immunoglobulin G (IgG) and IgM antibodies and B19 DNA over a 6-month period. Results were compared to those of 100 B19 DNA–negative donors.

RESULTS: Data accumulated over the past 6 years indicate a high incidence period from May 2004 to January 2006. In total, the incidence was 12.7 and 261.5 per 100,000 donations with high virus loads equal to or above 10^5 and below 10^5 IU per mL, respectively. Median virus concentration in the case group was 4.85×10^7 IU per mL at Time Point T0 and was reduced to 4×10^2 IU per mL at the time of the next donation (3 months later). Neutralizing antibodies (VP2) were detected in all donations if virus load was reduced to less than 10^5 IU per mL.

CONCLUSION: The release of B19 DNA–positive blood products with a concentration of less than 10^5 IU per mL is thought to be safe due to the high level of neutralizing VP2 antibodies and is currently examined in a donor recipient infectivity study. In contrast, blood products with a high B19 DNA concentration ($\geq 10^5$ IU/mL), some of which did not contain neutralizing antibodies, were discarded to protect at risk individuals.

Parvovirus B19 (B19) was detected for the first time in 1975 in a blood product from a healthy donor.[1-3] During the onset of B19 infection, virus concentration can increase up to 10^{14} virions per mL.[4-6] Because B19 is a non–lipid-enveloped viral pathogen, inactivation methods like solvent/detergent treatment are ineffective for reduction of virus concentration in plasma. Most infections occur in childhood and result in a mild rash and formation of protective antibodies.[7-13] Infection normally results in seroconversion with neutralizing immunoglobulin G (IgG) antibodies affording lifelong protection from reinfection in most cases.[14] Chronic infection, however, may be associated with a poor antibody response.[15,16]

Screening for B19 DNA by minipool real-time nucleic acid amplification technology (NAT; testing in donor pools up to 96 samples per pool) was introduced into our blood donor screening protocol in 2000. NAT amplification was analyzed in a semiquantitative manner. Blood

ABBREVIATIONS: B19 = parvovirus B19; C_t = cycle threshold.

From the German Red Cross, Institute Frankfurt, Frankfurt, Germany; the Department of Blood Group Serology and Transfusion Medicine and Immunohematology, Medical University of Graz, Graz; the Austrian Red Cross, Institute Vienna, Vienna; the Austrian Red Cross, Institute Klagenfurt, Klagenfurt; the Austrian Red Cross, Institute Feldkirch, Feldkirch, Austria; the German Military Forces, Institute for Transfusion Medicine, Koblenz, Germany; and the University of Cambridge, Cambridge, United Kingdom.

Address reprint requests to: Michael Schmidt, MD, Institute of Transfusion Medicine and Immunohematology, Johann Wolfgang Goethe University, German Red Cross, Sandhofstrasse 1, 60528 Frankfurt am Main, Germany; e-mail: mschmidt@blutspende.de.

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products with B19 DNA virus load equal to or higher than 10^5 IU per mL were discarded. In contrast, minipools with B19 DNA virus load below 10^5 IU per mL were not resolved, and all blood products contained were released. In any case, donors were not informed about their B19 infection and were allowed to give subsequent donations.

This study provides results for 4 years of NAT screening, including a case-controlled study for B19 antibodies performed over a 6-month period to monitor the development of structural (VP-1 and VP-2) and nonstructural (NS-1) antibodies.

MATERIALS AND METHODS

Incidence studies

Donations from six different sites were involved in the study. In Germany samples from the German Red Cross Institute Frankfurt (1,732,355 samples, Area 1) and from the German Armed Forces (99,176 samples, Area 2) were included in the study. In contrast, Austrian samples from four test areas including the Medical University of Graz (203,880 samples, Area 3), Austrian Red Cross Institute Klagenfurt (85,811 samples, Area 4), Austrian Red Cross Institute Feldkirch (51,041 samples, Area 5), and Austrian Red Cross Institute of Vienna (626,373 samples, Area 6) were included in the study (Table 1). All donations for the German Red Cross were screened by B19 real-time NAT beginning in April 2000 and in August 2003 for all other institutes. All donations were tested at the GRC Institute in Frankfurt. The screening procedure was not modified during the study period. Donations with B19 virus concentrations of at least 10^5 IU per mL were discarded, whereas minipools that contained donations with a virus load of not more than 10^5 IU per mL were not resolved. All products included in these minipools were designated as being weakly B19 DNA–positive and were released for transfusion. This procedure is in accordance with the requirements of the plasma industry, where the release level per individual donation is 10^5 IU per mL, as well as the German transfusion law, the German authorities (Paul Ehrlich Institute) and the local ethics commission, which approved of this study.

TABLE 1. B19 questionnaire and characteristics of the case and control group*

Characteristic	Group		Significance
	Case	Control	
Total number	50	50	Not done
Men/women	27/23	26/24	0.50
Age (years)	39.0 ± 10.9	44.4 ± 15.1	0.06
Chronic diseases	8/50	12/50	0.23
Tiredness	12/50	11/50	0.50
Joint pains	11/50	9/50	0.40
Neurologic symptoms	1/50	1/50	0.75
Fever, flulike symptoms	1/50	1/50	0.75
Pregnancy	12/23	12/24	0.55
Complications during pregnancy	6/12	2/12	0.10
Disease in childhood			
B19 infection	3/50	2/50	0.50
Rubella	12/50	14/50	0.41
Mumps	10/50	11/50	0.50
Chicken pox	10/50	15/50	0.18
Bordetella pertussis	1/50	3/50	0.31

* Donors of both groups were matched with regard to sex and age and were interviewed about B19-specific clinical symptoms. All women were asked about pregnancies and complications during pregnancies.

Donor substudy (case-control study)

A group of 50 B19 DNA–positive blood donors with a virus concentration of at least 10^5 IU per mL at the index donation (Time Point T0, high-virus-load group) was analyzed in a prospective study involving two subsequent blood draws (with the first occurring approximately 12 weeks after the index donation), for B19 DNA concentration as well as B19 antibodies. The 50 donors were randomly selected from all B19 DNA–positive donors ($\geq 10^5$ IU/mL) residing in Area 1.

In addition, 100 B19 DNA NAT-negative donors were screened for B19 antibodies as a control group. Both the case and the control groups were comparable with regard to age and sex (Table 1). All donors positive for the presence of B19 DNA ($\geq 10^5$ IU/mL) at the index donation (case group) and 50 randomly selected members of the control group were interviewed by standard questionnaire within 4 weeks after the donation about clinical symptoms of a B19 infection (Table 1).

B19 screening techniques

Routine testing. An aliquot of 100 µL plasma of each blood donation was pooled overnight into minipools containing up to 96 samples per pool. The complete pool of up to 9.6 mL was centrifuged at 58,000 × *g* for 60 minutes at 4°C. Supernatants were discarded and pellets were subjected to nucleic acid extraction with a viral RNA kit (QIAamp, Qiagen, Hilden, Germany). Five-microliter aliquots of the total eluted volume of 75 µL were subjected to polymerase chain reaction (PCR) amplification for B19 DNA. Two positive controls and at least three quantitative standards (10^6, 10^5, and 10^4 IU/mL) were included in each PCR procedure.[17-19]

Resolving of B19 DNA–positive minipools. All samples achieving a positive B19 DNA minipool NAT result with a virus concentration of less than 10^5 IU per mL were released as weakly positive B19 DNA donations without resolving the minipool. In contrast, all

minipools that yielded a B19 DNA concentration higher than 10^5 IU per mL were resolved by creating subpools from archive plates. Next the identified B19 DNA–positive samples were discarded and all negative or weakly positive B19 DNA samples included in the minipool were released for transfusion.

NAT. Real-time quantitative amplification of B19 DNA was performed with a CE labeled B19 PCR kit (DRK Baden-Württemberg-Hessen, Frankfurt, Germany) according to the manufacturers' instructions with a thermocycler (ABI PRISM 7000, 7700, 7300, or 7900HT; Applied Biosystems, Foster City, CA). Five microliters of extract was analyzed in a total volume of 25 µL. The assay contains reagents and enzymes for the specific amplification of the VP1-capsid protein gene of B19. Thermal cycling was as follows: 50°C for 2 minutes, 95°C for 15 minutes, 10 cycles of 95°C for 10 seconds and 62°C for 30 seconds, 40 cycles of 93°C for 10 seconds, and 56°C for 40 seconds.

Data analysis was performed with the computer software (sequence detection software, Version 1.6.3, Applied Biosystems). A positive real-time PCR result is reflected by an increase in the fluorescence intensity of a reporter dye. After PCR, the number of PCR cycles necessary to reach a defined fluorescence threshold in each sample was defined as the cycle threshold (C_t). The C_t value is related to the amount of PCR product and therefore to the original amount of target present in the PCR procedure. Low C_t values indicate a high initial target amount and high C_t values indicate the opposite.

Sensitivity and specificity of the DRK B19 PCR kit. Sensitivity was analyzed in accordance with the directive of European Commission 98/79/EC. Probit analysis was done on at least 24 replicates of each dilution from a dilution series containing at least six steps. The calculation was performed on nonlog converted data. Specificity was tested with 200 negative plasma samples. Additionally, the amplification efficiency of different B19 genotypes (Genotype 1, Genotype 2 [Subtype A6], and Genotype 3 [Subtype V9]) was evaluated. Genotype 3 was obtained from a Ghanaian blood donor service.[20-22]

Precautions to prevent B19 DNA cross-contamination. All steps of NAT (pooling, enrichment by centrifugation and extraction, master mix preparation, and amplification) were performed in separate rooms. All rooms were equipped with ultraviolet light and were decontaminated once per week. The daily decontamination procedure included decontamination of all workbenches, pipettes, and centrifuges with a disinfectant (Bacillol Plus, Bode, Hamburg, Germany) and sodium hypochlorite (Roth, Karlsruhe, Germany). All PCR procedures were monitored by the addition of at least six negative controls. PCR procedures were only valid if all negative controls gave a negative test result. All personnel performing nucleic acid extraction and resolution of highly B19 DNA–positive pools have been thoroughly trained to be competent in performing these procedures without cross-contamination.

Screening for B19 antibodies. Samples were screened with two assays for IgM and IgG antibody detection. A parvovirus IgG and IgM assay (recomLine, Mikrogen, Neuried, Germany) was used to analyze antibodies against VP-2, VP-N, VP-1S, VP-2r, VP-C, and NS-1 epitopes. Band intensities were compared with a control band and were scored as –, +/–, 1+, 2+, 3+, or 4+. Additionally all samples were screened with the microtiter plate–based B19 enzyme immunoassay (EIA; Biotrin, Dublin, Ireland) for IgG and IgM antibodies. All antibody assays were performed according to the manufacturers' instructions.

Antibody adsorption. Eight samples with a B19 DNA concentration of more than 10^5 IU per mL and eight samples with a B19 DNA load below 10^5 IU per mL were analyzed for B19 IgG antibodies by use of the recomLine assay. In these samples, virus load was determined by real-time NAT before and after treatment with a protein G column (MAb Trap kit, Amersham, Uppsala, Sweden). One-hundred microliters of each sample was filtered through a protein G column and washed with 5 mL of binding buffer. The flowthrough of the binding step was centrifuged at 58,000 × *g* for 1 hour at 4°C followed by a standard minipool extraction protocol.

B19 sequence analysis. Sequence analysis was performed as described in detail by Hokynar and colleagues.[23] Overlapping amplicons of 1000 bp that spanned the entire protein coding region of the genome were used. Primers (NSofwd and NSirev, NSsfwd and NSorev, p6 and p3, p9 and rtsrev, and rt1 and VP2orev) were used for sequencing plus and minus strands. Amplification products were sequenced directly with a cycle sequencing ready reaction kit (BigDye Terminator, Applied Biosystems, Darmstadt, Germany) and a DNA sequencer (ABI PRISM 310, Applied Biosystems).

Statistical analysis

The sensitivity, standard deviation (SD), and coefficient of variation (CV) of the real-time PCR test were calculated with computer software (Excel 2000, Microsoft Corp., Redmond, WA). For the Probit analysis, another computer program (SPSS 12.0, SPSS, Chicago, IL) was used. Comparison between the case and control groups was calculated with Fisher's exact test or the t test. Statistical significance was assumed if p values were less than 0.05.

RESULTS

Incidence of B19 in different areas

B19 incidence between 2003 and 2006 was demonstrated for six different areas (Fig. 1). There was a high incidence

period of B19 from May 2004 to January 2006 in all screened regions. The highest incidence was found in Areas 4 and 5, although the incidence of B19 DNA–positive donors with a high virus load (B19 DNA concentration $\geq 10^5$ IU/mL) as well as with low B19 DNA virus loads (B19 DNA concentration $<10^5$ IU/mL) did not differ significantly between the areas (Table 2).

Sequence analysis of the 50 B19 NAT–positive blood donors included in the substudy identified only Genotype 1 strains. Sequence analyses of all B19 DNA–positive samples are currently being processed to better understand the genotype distribution in our donor population.

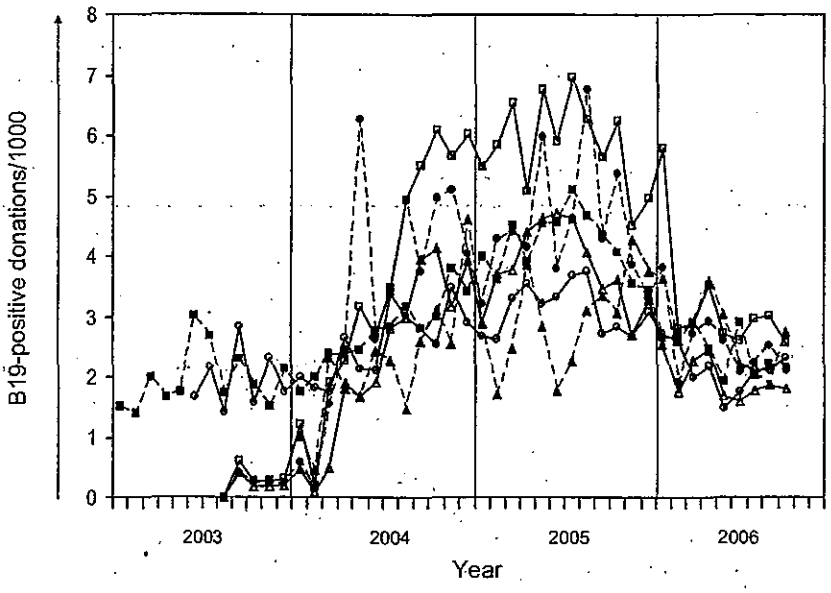

Fig. 1. Incidence of B19 virus infections between 2003 and 2006. Donations were screened for B19 by real-time minipool NAT. Incidence was increased between May 2004 and January 2006, especially in Areas 4 and 5. (■) Area 1 = GRC Institute Frankfurt; (▲) Area 2 = German Armed Forces; (△) Area 3 = Medical University of Graz; (□) Area 4 = Austrian Red Cross Institute Klagenfurt; (●) Area 5 = Austrian Red Cross Institute Feldkirch; and (○) Area 6 = Austrian Red Cross Institute Vienna.

Monitoring of B19 DNA–positive blood donors

In the substudy, two additional donations were taken from 50 B19 DNA–positive multiple-time donors (high-virus-load group) randomly selected from all B19 DNA–positive samples in Test Area 1 to determine B19 DNA concentration and the course of antibody development to B19.

All donors included in the substudy were B19 DNA–positive with a virus load of more than 10^5 IU per mL at the index donation (Time Point T0). The virus load was significantly reduced within 12 weeks from a median of 4.85×10^7 IU per mL (T0; SD) to 4.6×10^2 IU per mL (SD; T1; Fig. 2) and either remained at this level or declined further at Time Point T2. Additional follow-up in a subset of these donors beyond Time Point T2 revealed that B19 DNA concentration was stable around the NAT detection level for up to 1 year (range, 100 and 1500 IU/mL; data not shown). All samples from donors of the case group were below the release level of 10^5 IU per mL at Time Point T1.

B19 antibody levels were investigated with an enzyme-linked immunosorbent assay and a line probe assay. Both commercially available B19 antibody assays gave comparable results for B19 IgM (Table 3) and IgG (Table 4) antibodies. At each time point, IgM antibodies were detected more frequently ($p < 0.05$) in the case group compared with the control group, and antibody titers generally showed an increase from Time Point T0 to Time Point T1 followed by a decrease at Time Point T2. IgM antibodies against the nonstructural protein (NS-1) were not detected at any time point. In contrast, neutralizing IgG antibodies against VP-2 were detected in all samples of the high-virus-load group

TABLE 2. Incidence of B19 virus infections in different areas per 100,000 donations*

	B19 DNA virus load (IU/mL)													
	Area 1		Area 2		Area 3		Area 4		Area 5		Area 6		All	
Year	$>10^5$	$<10^5$	$>10^5$	$<10^5$	$>10^5$	$<10^5$	$>10^5$	$<10^5$	$>10^5$	$<10^5$	$>10^5$	$<10^5$	$>10^5$	$<10^5$
2003	9.3	185.2	1.0	200.3	0.0	19.6	0.0	29.9	0.0	23.9	ND†	ND	6.3	172.0
2004	17.9	254.3	13.6	239.4	9.3	217.0	25.1	340.3	50.0	279.1	11.5	212.9	16.3	247.7
2005	25.0	395.1	12.6	300.6	36.0	345.8	3.9	580.9	6.2	434.7	2.7	295.7	19.4	362.8
2006	5.7	237.1	3.7	209.9	6.0	170.8	0.0	317.1	0.0	255.4	3.6	268.9	4.5	227.6
All	15.0	269.8	9.1	245.0	15.7	221.3	9.3	366.1	17.6	289.7	6.1	259.1	12.7	261.5

* Donations were tested from six different areas in Germany and Austria. Incidence was demonstrated in two groups: 1) donations with high B19 DNA virus load over 10^5 IU/mL and 2) donations with low B19 DNA virus load below 10^5 IU per mL. Incidence increases were observed in all areas in 2004 and 2005 for both groups.
† ND = not done.

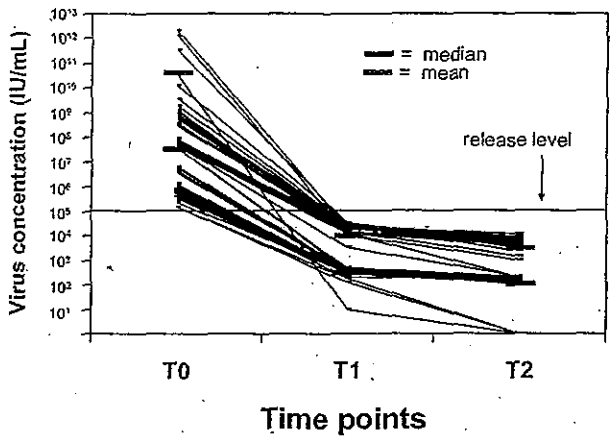

Fig. 2. Virus load during the 6-month study period. All donors of the case group were B19 DNA–positive at the index donation with a value of more than 10^5 IU per mL (highest concentration was 2.1×10^{12} IU/mL). The black bar represents the median virus concentration and the gray bar indicates the mean virus concentration of all donations for each time point. Virus load was significantly reduced from Time Point T0 to Time Point T1. The SDs were 3.5×10^{11}, 1.0×10^4, and 3.5×10^3 for T0, T1, and T2, respectively.

(case group) at Time Point T1 and T2 without any exception. Both antibody assays and titers were significantly higher in the case group compared to the control group. Likewise, antibodies against nonstructural antigens of NS-1 increased up to 92.3 percent from Time Point T0 to Time Point T2.

In one experiment, plasma from donors with B19 DNA concentrations of more than 10^5 IU per mL and with B19 DNA concentrations of less than 10^5 IU per mL was filtered through protein G columns. The viral load was determined before and after IgG absorption. Reduction of the B19 virus concentration was significantly higher in samples with low virus load and high IgG antibodies titers as shown in Table 5. In two of eight samples (viral load, $<10^5$ IU/mL), no virus was detectable after column filtration. In the other six samples, low virus concentrations were detected (mean C_t value, 30.6; virus concentration, <100 IU/mL).

All donors included in the case-control substudy were matched by age and sex and were interviewed with a standard B19 questionnaire about clinical symptoms (Table 1). Typical clinical symptoms for B19 infections such as tiredness, joint pain, or complications between pregnancies did not significantly differ between groups.

TABLE 3. IgM antibodies in the case group and the control group*

	Biotrin EIA	Mikrogen immunoblot					
Group	VP2 (%)	VP-2p (%)	VP-N (%)	VP-1S (%)	VP-2r (%)	VP-C (%)	NS-1 (%)
Case							
T0†	42.9	42.9	42.9	57.1	42.9	35.7	0.0
T1	71.4	71.4	85.7	85.7	50.0	35.7	0.0
T2	23.1	46.2	69.2	69.2	38.5	30.8	0.0
Control							
T0	1.8	9.2	4.6	6.1	1.5	1.5	0.0

* Fifty B19 DNA–positive donors were screened for IgM antibodies by two different assays to detect antibodies against structural (neutralizing) and nonstructural antigens at three time points, and the data were compared to results from 100 B19 DNA–negative blood donors (control group).
† T0 = index donation.

TABLE 4. IgG antibodies in the case and control groups*

	Biotrin EIA	Mikrogen immunoblot					
Group	VP2 (%)	VP-2p (%)	VP-N (%)	VP-1S (%)	VP-2r (%)	VP-C (%)	NS-1 (%)
Case							
T0†	35.7	35.7	28.6	28.6	28.6	21.4	0.0
T1	100	100	100	100	100	85.7	57.1
T2	100	100	100	100	100	76.9	92.3
Control							
T0	74.8	73.3	71.0	68.7	46.6	16.0	14.5

* Fifty B19 DNA–positive donors were screened for IgG antibodies by two different assays to detect antibodies against structural (neutralizing) and nonstructural antigens at three time points, and the data were compared to results from 100 B19 DNA–negative blood donors (control group).
† T0 = index donation.

TABLE 5. IgG adsorption by protein G columns*

Sample B19 virus load (IU/mL)	Total number	Mean C_t Before column absorption	Mean C_t After column absorption	Mikrogen immunoblot mean reactivity ΔC_t	VP-2p	VP-N	VP-1S	VP-2r	VP-C	NS-1
$>10^5$	8	7.5	8.0	0.5	0.4	0.5	0.5	0.8	0.4	0.0
$<10^5$	8	25	30.6	5.6†	2.0	2.9	2.9	3.1	1.8	2.4

* Samples with B19 virus load of more than 10^5 IU per mL and less than 10^6 IU per mL were filtered through protein G columns. The virus load was determined before and after filtering. Additionally, B19 IgG antibodies were analyzed by immunoblots. Band intensities were compared with a control band and were scored as –, +/–, 1+, 2+, 3+, or 4+. For each sample, the mean reactivity is given. Samples with high levels of neutralizing antibodies (higher mean reactivity values) showed significantly higher virus reduction after filtering (higher ΔC_t).
† $p < 0.01$.

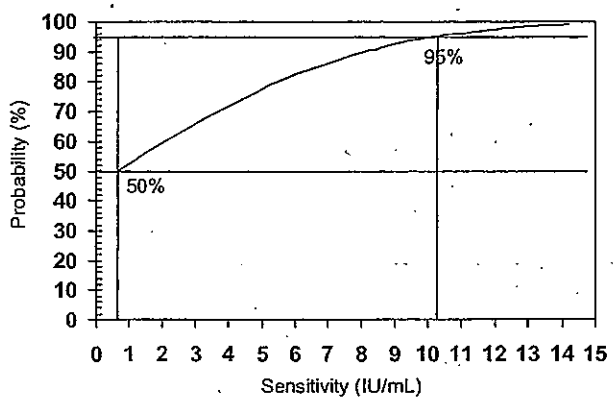

Fig. 3. Sensitivity of B19 NAT kit. The WHO standard (99/800) was diluted into six concentrations. Each standard concentration was tested in 24 replicates. Probit analysis was performed with SPSS Version 12.0 on nonlog converted data. Sensitivity of the minipool NAT was 10.2 IU per mL at 95 percent probability (CI, 7.5 and 18.8 IU/mL) and 0.65 IU per mL at 50 percent probability (CI, –4.1 to 2.47).

Sensitivity and specificity of the B19 PCR kit

As demonstrated in Fig. 3, the 95 percent detection probability of the NAT assay was 10.2 IU per mL (confidence interval [CI], 7.5-18.8 IU/mL) per processed volume. Sensitivity for an individual donation present in a minipool was 982 IU per mL (CI, 724-1811 IU/mL). Specificity was 100 percent as 200 of 200 negative samples gave a negative test result. The amplification efficiency of the DRK B19 PCR kit was comparable for all three genotypes (data not shown). Precision is defined as the degree of scattering within a series of analyses. It is expressed as the SD and the percent CV (%CV). SD and %CV were 0.6, 0.58, and 0.67 and 2.47, 2.37, and 4.20 for intraassay variability, interassay variability, and interbatch variability, respectively.

DISCUSSION

The frequency of B19 viremia in voluntary blood donors has been estimated to range from 1:260 to 1:50,000 and to depend on both the sensitivity of the screening method and the season.[24-26] Here we report results from screening blood donors over a period of more than 4 years with a sensitive real-time NAT method. The mean frequency of DNA-positive blood donors was 274 per 100,000 donations, which was within the range previously reported.[27,28]

Although the incidence of B19 DNA–positive blood products is high, transfusion-transmitted infections have rarely been reported when compared to other transfusion-relevant virus infections like human immunodeficiency virus-1, hepatitis C virus, or hepatitis B virus. This could be explained by the fact that most recipients already have B19 antibodies due to previous infections and that many B19 DNA–positive blood products were also positive for the presence of B19 VP-1 or VP-2 antibodies, resulting in neutralization of the virus. Another possible explanation is that B19 infections were underreported because most recipients get only mild or no clinical symptoms.[29] In the present study, we analyzed the development of anti-B19 and the decrease of B19 DNA in 50 blood donors and compared the data to a control group. In accordance with the literature,[30,31] VP-2 IgG antibodies already existed in the majority (75%) of B19-negative donors (control group). Without exception, all donors in the high-virus-load group (case group) were anti-VP-2 IgG–positive at Time Points T1 and T2. The increase in VP-2 antibodies correlated directly with a significant decrease in B19 virus load. The obvious explanation for this is that the antibodies neutralize the virus.[32]

Although antibodies persist for a long period of time, however, B19 DNA was detectable by real-time NAT for more than 1 year. The question is whether blood products with low levels of B19 DNA and B19 antibodies are infectious. This question is controversial in the literature and is currently being examined by a retrospective donor-recipient study.

Since B19 screening was initiated, the following release procedure was used in our blood donor service. Donations with high B19 DNA concentrations (equal or higher than 10^5 IU/mL) were discarded, but donors were permitted to make subsequent donations and were not informed about their infection. Blood products with B19 DNA concentrations less than 10^5 IU per mL are thought

to contain neutralizing antibodies. Therefore, minipools containing low B19 DNA–positive blood products were not dissolved, and all products contained in those minipools were transfused. The in vitro experiment with a protein G column indicates a significantly higher virus reduction in samples containing low viral loads and high levels of neutralizing antibodies compared to samples containing high viral loads and low B19 antibodies. This observation supports our release procedure, because it suggests that these samples are unlikely to be infectious (Table 5).

Nonetheless, for special-risk patients (immunocompromised patients, young children, or pregnant women), our blood donor service offers B19 DNA–negative blood products. The opportunity to obtain blood testing negative for the presence of B19 DNA has been available since summer 2003. Until now, however, less than 10 B19-negative blood products have been ordered, which demonstrates that physicians are relatively unaware of this infection.

In summary, all blood products have been screened for B19 by a real-time minipool PCR since 2000. A high-incidence period was observed between May 2004 and January 2006. Transfusion of blood products with a low virus concentration seems to be safe because of the coexistence of neutralizing antibodies, whereas blood products with high virus concentrations may pose a risk for transfusion recipients. Therefore, these donations were discarded at our blood donor service. Transfusion-transmitted B19 infections might be underreported and should be examined in donor-recipient studies.

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REFERENCES

1. Cossart YE, Field AM, Cant B, Widdows D. Parvovirus-like particles in human sera. Lancet 1975;1:72-3.
2. Young NS, Brown KE. Parvovirus B19. N Engl J Med 2004; 350:586-97.
3. Chisaka H, Morita E, Yaegashi N, Sugamura K. Parvovirus B19 and the pathogenesis of anaemia. Rev Med Virol 2003; 13:347-59.
4. Koppelman MH, Cuypers HT, Emrich T, Zaaijer HL. Quantitative real-time detection of parvovirus B19 DNA in plasma. Transfusion 2004;44:97-103.
5. Anderson LJ, Tsou C, Parker RA, Chorba TL, Wulff H, Tattersall P, Mortimer PP. Detection of antibodies and antigens of human parvovirus B19 by enzyme-linked immunosorbent assay. J Clin Microbiol 1986;24:522-6.
6. Prowse C, Ludlam CA, Yap PL. Human parvovirus B19 and blood products. Vox Sang 1997;72:1-10.
7. Enders M, Weidner A, Enders G. Current epidemiological aspects of human parvovirus B19 infection during pregnancy and childhood in the western part of Germany. Epidemiol Infect 2007;135:563-9.
8. Pattison JR. Human parvovirus B19. BMJ 1994;308:149-50.
9. Heegaard ED, Hornsleth A. Parvovirus: the expanding spectrum of disease. Acta Paediatr 1995;84:109-17.
10. Hartman KR, Brown KE, Green SW, Young NS. Lack of evidence for parvovirus B19 viraemia in children with chronic neutropenia. Br J Haematol 1994;88:895-6.
11. Muir KW, Fitzsimons E. Human parvovirus infection and transient erythroblastopenia of childhood. Br J Haematol 1992;81:622.
12. Shirono K, Tsuda H. Parvovirus B19-associated haemophagocytic syndrome in healthy adults. Br J Haematol 1995; 89:923-6.
13. Nagai K, Morohoshi T, Kudoh T, Yoto Y, Suzuki N, Matsunaga Y. Transient erythroblastopenia of childhood with megakaryocytopenia associated with human parvovirus B19 infection. Br J Haematol 1992;80:131-2.
14. Anderson MJ, Higgins PG, Davis LR, Willman JS, Jones SE, Kidd IM, Pattison JR, Tyrrell DA. Experimental parvoviral infection in humans. J Infect Dis 1985;152:257-65.
15. Kurtzman G, Frickhofen N, Kimball J, Jenkins DW, Nienhuis AW, Young NS. Pure red-cell aplasia of 10 years' duration due to persistent parvovirus B19 infection and its cure with immunoglobulin therapy. N Engl J Med 1989; 321:519-23.
16. Prospective study of human parvovirus (B19) infection in pregnancy. Public Health Laboratory Service Working Party on Fifth Disease. BMJ 1990;300:1166-70.
17. Roth WK, Seifried E. The German experience with NAT. Transfus Med 2002;12:255-8.
18. Roth WK, Buhr S, Drosten C, Seifried E. NAT and viral safety in blood transfusion. Vox Sang 2000;78(Suppl 2): 257-9.
19. Roth WK, Weber M, Seifried E. Feasibility and efficacy of routine PCR screening of blood donations for hepatitis C virus, hepatitis B virus, and HIV-1 in a blood-bank setting. Lancet 1999;353:359-63.
20. Candotti D, Danso K, Parsyan A, Dompreh A, Allain JP. Maternal-fetal transmission of human parvovirus B19 genotype 3. J Infect Dis 2006;194:608-11.
21. Parsyan A, Addo-Yobo E, Owusu-Ofori S, Akpene H, Sarkodie F, Allain JP. Effects of transfusion on human erythrovirus B19-susceptible or -infected pediatric recipients in a genotype 3-endemic area. Transfusion 2006;46: 1593-600.
22. Parsyan A, Kerr S, Owusu-Ofori S, Elliott G, Allain JP. Reactivity of genotype-specific recombinant proteins of human erythrovirus B19 with plasmas from areas where genotype 1 or 3 is endemic. J Clin Microbiol 2006;44:1367-75.
23. Hokynar K, Brunstein J, Soderlund-Venermo M, Kiviluoto

O, Partio EK, Konttinen Y, Hedman K. Integrity and full coding sequence of B19 virus DNA persisting in human synovial tissue. J Gen Virol 2000;81:1017-25.
24. McOmish F, Yap PL, Jordan A, Cohen BJ, Simmonds P. Detection of parvovirus B19 in donated blood: a model system for screening by polymerase chain reaction. J Clin Microbiol 1993;31:323-8.
25. Cohen BJ, Field AM, Gudnadottir S, Beard S, Barbara JA. Blood donor screening for parvovirus B19. J Virol Methods 1990;30:233-8.
26. Tsujimura M, Matsushita K, Shiraki H, Sato H, Okochi K, Maeda Y. Human parvovirus B19 infection in blood donors. Vox Sang 1995;69:206-12.
27. Kelly HA, Siebert D, Hammond R, Leydon J, Kiely P, Maskill W. The age-specific prevalence of human parvovirus immunity in Victoria, Australia compared with other parts of the world. Epidemiol Infect 2000;124: 449-57.
28. Zaaijer HL, Koppelman MH, Farrington CP. Parvovirus B19 viraemia in Dutch blood donors. Epidemiol Infect 2004; 132:1161-6.
29. Modrow S. Parvovirus B19: the causative agent of dilated cardiomyopathy or a harmless passenger of the human myocard? Ernst Schering Res Found Workshop 2006;55: 63-82.
30. Manaresi E, Gallinella G, Morselli Labate AM, Zucchelli P, Zaccarelli D, Ambretti S, Delbarba S, Zerbini M, Musiani M. Seroprevalence of IgG against conformational and linear capsid antigens of parvovirus B19 in Italian blood donors. Epidemiol Infect 2004;132:857-62.
31. Manaresi E, Gallinella G, Venturoli S, Zerbini M, Musiani M. Detection of parvovirus B19 IgG: choice of antigens and serological tests. J Clin Virol 2004;29:51-3.
32. Cohen BJ, Buckley MM. The prevalence of antibody to human parvovirus B19 in England and Wales. J Med Microbiol 1988;25:151-3.

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研究報告の概要

○血漿および静注用免疫グロブリン製剤によるヒトパルボウイルスB19の中和

背景:ヒトパルボウイルスB19(B19V)は広く蔓延する病原体であり、血漿由来製剤原料血漿プールは、B19V抗体(B19V免疫グロブリンG[IgG])を含有することが示されている。

試験デザインおよび方法:巨核芽球細胞株UT7/Epo-S1はB19V 遺伝子型1に感染し、また、本試験において、免疫組織学的方法、ウエスタンブロット法、B19V特異的mRNAのRT-PCR法を用いて示したとおり、最近発見された遺伝子型2にも感染する。感染UT7/Epo-S1細胞のB19V RT-PCR解析に基づき、感染実験を確立し、B19V中和検査を実施した。B19V IgG力価に関係したB19V中和抗体の役割を検討するため、製造血漿プール1000以上について酵素免疫測定法による検査を実施した。

結果:血漿プールは、B19V IgG力価:平均33±9IU/mL(最小値11IU/mL)を含有することが判明した。これらの11IU/mLのB19V IgGは、B19V遺伝子型1の感染性を4.6 log、遺伝子型2の感染性を3.9 log以上を中和した。このため、このようなプール由来の10%静注用免疫グロブリン製剤(IVIG)は、さらに高いB19V中和活性を含有することが分かった。

結論:分画用血漿プールにおけるB19遺伝子型1、2中和活性の高さは、当該プールのB19V IgG力価が一貫して高いために備わった特徴であることが示された。検討した10%IVIG製剤において、B19V IgGの中和活性を維持することが示された。

報告企業の意見	今後の対応
血漿由来製剤原料血漿プールにおけるヒトパルボウイルスB19中和活性の高さは、当該プールのB19V IgG力価が一貫して高いために備わった特徴であり、検討した10%IVIG製剤において、B19V IgGの中和活性を維持することが示されたとの報告である。	本製剤は現在製造・供給しておらず、当面特別な対応を必要としない。

使用上の注意記載状況・その他参考事項等

人免疫グロブリン「日赤」

ウイルス等の感染性

Neutralization of human parvovirus B19 by plasma and intravenous immunoglobulins

Jens Modrof, Andreas Berting, Björn Tille, Andreas Klotz, Christina Forstner, Sandra Rieger, Claudia Aberham, Matthias Gessner, and Thomas R. Kreil

BACKGROUND: Human parvovirus B19 (B19V) is a highly prevalent pathogen, and plasma pools for manufacturing of plasma-derived products have been shown to contain antibodies against B19V (B19V immunoglobulin G [IgG]).

STUDY DESIGN AND METHODS: The megakaryoblastic cell line UT7/Epo-S1 can be infected with B19V Genotype 1 and as demonstrated here by immunocytochemistry, Western blot, and reverse transcription–polymerase chain reaction (RT-PCR) of B19V-specific mRNA, also with the more recently discovered Genotype 2. Based on B19V RT-PCR analysis of infected UT7/Epo-S1 cells, an infectivity assay was established and implemented for a B19V neutralization assay. To investigate the role of B19V neutralization in relation to B19V IgG titers, more than 1000 manufacturing plasma pools were tested by enzyme-linked immunosorbent assay.

RESULTS: Plasma pools were found to contain a mean B19V IgG titer of 33 ± 9 IU per mL, with the lowest titer at 11 IU per mL. These 11 IU per mL B19V IgG neutralized 4.6 log B19V Genotype 1 and greater than 3.9 log Genotype 2 infectivity. Accordingly, a 10 percent intravenous immunoglobulin (IVIG) product prepared from such pools was found to contain an even higher B19V neutralization capacity.

CONCLUSION: A high capacity of B19V Genotypes 1 and 2 neutralization was demonstrated in plasma pools for fractionation, an inherent feature based on the constantly high titer of B19V IgG in these pools. The neutralizing activity of B19V IgG was shown to be maintained in the 10 percent IVIG product tested.

Human parvovirus B19 (B19V) belongs to the genus *Erythrovirus* (family *Parvoviridae*), which has recently been reclassified to contain three different B19V genotypes (1-3).[1] Although B19V Genotype 1 is by far the most prevalent, Genotype 2 has also been sporadically detected in Europe and was shown to occur in plasma pools for manufacturing into plasma derivatives.[2,3] B19V Genotype 3 appears to be mostly restricted to West Africa.[4]

Soon after its initial identification,[5] B19V was recognized to cause fifth disease in children (erythema infectiosum), whereas more serious clinical manifestations of B19V infection were only recently understood to include arthropathy, transient aplastic crises, persistent anemia, and hydrops fetalis.[6] In addition, an association of B19V with inflammatory heart disease in adults has lately been suggested.[7,8] The only treatment option available for B19V infection so far is intravenous immunoglobulin (IVIG), although based on anecdotal evidence rather than, for example, established dose–response correlations.[9]

Owing to the global prevalence of B19V Genotype 1, 30 to 60 percent of adults carry antibodies against B19V (B19V immunoglobulin G [IgG]), with a good correlation between antibody prevalence and age.[10] Consequently, the presence of B19V IgG was found in all of the few plasma pools for manufacturing so far investigated.[11-14] Mostly due to the lack of a widely available B19V

ABBREVIATIONS: B19V = parvovirus B19; MOI = multiplicity of infection; NC_{50} = 50 percent B19V neutralization capacity; $TCID_{50}$ = 50 percent tissue culture–infectious dose.

From the Global Pathogen Safety and Plasma Control, Baxter BioScience, Vienna, Austria.

Address reprint requests to: Thomas R. Kreil, PhD, Baxter BioScience, Benatzkygasse 2-6, A-1220 Vienna, Austria; e-mail: thomas_kreil@baxter.com.

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infectivity assay, however, no information was available with respect to antibody function, that is, B19V neutralization, and whether this potentially clinically relevant variable would correlate with the presence of B19V antibodies detected by, for example, enzyme-linked immunosorbent assay (ELISA).

Although still to date a continuous cell culture system that would allow detection of B19V infectivity by determination of a classical cytopathic effect does not exist, B19V replication has been demonstrated in a few specialized cell lines mostly utilizing the detection of de novo transcribed B19V-specific mRNA after infection of and virus replication in these cells.[15-19] Particularly the erythroid progenitor cell line KU812Ep6 was used to detect B19V infectivity and was found to be sensitive to both B19V Genotypes 1 and 2.[18,20] These cells are, however, not widely available. Alternatively, use of the megakaryoblastic cell line UT7/Epo-S1 has also allowed accurate quantification of B19V Genotype 1 infectivity over several orders of magnitude.[15,16,21]

With an *internally controlled reverse transcription–*polymerase chain reaction (RT-PCR) system (TaqMan, Applied Biosystems, Foster City, CA), an infectivity assay for both B19V Genotypes 1 and 2 in UT7/Epo-S1 cells was established. The presence of B19V IgG antibodies in plasma pools for further manufacturing was confirmed, and the titers of B19V IgG were quantified. With our infectivity assay, a correlation between B19V antibody presence as determined by ELISA and function as determined by neutralization of B19V infectivity was established for manufacturing plasma pools. Given the clinical relevance of B19V antibodies in IVIG as the only treatment option for human B19V infection, particularly given the recent recognition of more severe disease associations[7,8] and emerging B19V variants,[22] the level of B19V IgG function was also determined for a commercially available IVIG preparation.

MATERIALS AND METHODS

Cells, B19V, and plasma

UT7/Epo-S1 cells were provided by K.E. Brown (Health Protection Agency, London, UK; with permission of K. Sugamura, Tohoku University, Sendai, Japan). Cells were maintained in Iscove's modified Dulbecco's medium, containing 10 percent fetal calf serum (JRH Biosciences, Lenexa, CA), 1 percent L-glutamine, 1 percent gentamicin sulfate, and 2 IU per mL erythropoietin (Janssen-Cilag, Neuss, Germany) at 37°C with 5 percent CO_2.

Plasma donations containing high titers of B19V as detected by the routine B19V PCR donor screening program of Baxter BioScience (Plasma Analytics Department, Vienna, Austria) were used as the source of infectious B19V. Plasma Donation 990237 contained B19V Genotype 1 (titer, 11.8 log IU/mL) and Donation IM 81 contained B19V Genotype 2 (titer, 11.4 log IU/mL).

Infection of UT7/Epo-S1 cells with B19V and isolation of mRNA

UT7/Epo-S1 cells (10^5 per six-well) were infected with B19V at multiplicity of infection (MOI) of 10^{-3} to 10^6 and incubated for 7 days or mock infected with buffer for negative controls. Seven days after infection, mRNA was extracted with a direct mRNA miniprep kit (GenElute, Sigma-Aldrich, Vienna, Austria) according to the manufacturer's protocol.

Immunocytochemical staining and Western blot of B19V capsid proteins

For immunocytochemical staining, infected cells were pelleted and fixed on glass slides coated with 70 to 150 kDa poly-L-lysine (0.01 mg/mL in phosphate-buffered saline [PBS]) with 4 percent paraformaldehyde. After permeabilization with 0.5 percent Triton X-100, cells were incubated for 1 hour with a monoclonal antibody (10 µg/mL) specific for B19V capsid proteins VP1 and VP2 (R92F6/MAB8293, against amino acids 328-344 of VP2; Chemicon, Chandlers Ford, UK).[23] After washing (PBS), an anti-mouse–horseradish peroxidase polymer conjugate (SuperPicTure polymer detection kit, Zymed, Vienna, Austria) was applied, and the signal was developed by 15-minute incubation with 3,3'-diaminobenzidine chromogen solution before mounting slides with aqueous mounting medium. To determine the intracellular localization of B19V structural proteins, cells were counterstained with methyl green (Vector, Burlingame, CA) according to the supplier's instructions.

For Western blotting, approximately $10^{5.6}$ cells were collected, 2 to 7 days after infection and washed in Tris-buffered saline (TBS). Proteins were then separated by Bis-Tris sodium dodecyl sulfate (SDS)–4 to 12 percent polyacrylamide gel electrophoresis and blotted onto polyvinylidene difluoride membranes with a "semidry" discontinuous protein transfer chamber (Immobilon P, Bio-Rad, Munich, Germany). After blocking, membranes were incubated with the primary antibody R92F6 diluted 1:2000. Membranes were washed in 0.5× TBS containing (vol/vol) 0.2 percent Triton X-100 and incubated for 1 hour with polyclonal rabbit α-mouse immune *globulins–alkaline phosphatase* (at 1:5000; Dako, Glostrup, Denmark). Bound antibodies were detected with a color reaction (5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium solution, Sigma-Aldrich).

RT-PCR of spliced B19V transcripts

Real-time RT-PCR (TaqMan) was performed with a sequence detection system (ABI Prism 7900HT PE Applied

Biosystems) with a RT-PCR core reagent kit (TaqMan EZ, PE Applied Biosystems). The B19V mRNA primers amplify a region from nucleotide position nucleotide 365 to nucleotide 1978, spanning splice donor site nucleotide 406 to splice acceptor site nucleotide 1910 (reference sequence PVBAUA, NCBI GeneBank, Accession Number M13178), that is, effectively preventing B19V DNA amplification by use of an intron-spanning probe. As control for mRNA quality and to exclude false-negative B19V results, all samples were tested in parallel for the expression of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) with a predeveloped TaqMan assay reagents human GAPDH kit (PE Applied Biosystems).

Amplification of B19V mRNA was performed with 640 nmol per L each primer (PA3F, 5′-TTTCCTGGAC TTTCTTGCTGTT-3′; PA3R, 5′-CACCACCACTGCTGCTGA TACT-3′), 160 nmol per L the intron-spanning probe (PA3P, 5′-VIC-TTTGTGAGCTAACTAACAGATGCCCTCCAC CCAGAC-TAMRA-3′), and 10 ng of mRNA. Appropriate reagent controls and 200 copies of in vitro transcribed control RNA (10 copies/μL) in duplicate were included on each microtiter plate. Primers and probe for B19V PCR (synthesized by Ingenetix, Vienna, Austria) were high-performance liquid chromatography purified to a purity of greater than 90 percent.

The amplification program for GAPDH and B19V was composed of reverse transcription of mRNA into cDNA (50°C for 2 min and 60°C for 20 min) and subsequent PCR amplification with an initial denaturation step at 95°C for 5 minutes and 45 cycles of denaturation at 94°C for 20 seconds and annealing and extension at 57°C for 1 minute. Analysis of data generated was performed with the TaqMan software (SDS software Version 2.0, PE Applied Biosystems) with a threshold of 0.07 ΔRn fluorescent signal (label, VIC).

B19V neutralization with plasma pools or IVIG

To investigate B19V neutralization at B19V IgG titers of 11 IU per mL or below, two single plasma donations that had been tested nonreactive for B19V IgG were blended with plasma from manufacturing pools (cryorich plasma) to obtain defined titers between 0.4 and 11 IU per mL B19V IgG. The actual B19V IgG titers of these plasma pool blends (4 and 11 IU/mL) were confirmed by ELISA (Novagnost Parvovirus B19 IgG-ELISA; NovaTec, Dietzenbach, Germany).

A quantity of 450 μL of plasma pool blends or original plasma pool samples (cryorich plasma) were mixed with 50 μL of cell culture medium containing between 10^2 and 10^{10} IU B19V. After incubation for 1 hour at 37°C, the entire 500-μL mixture was incubated on 10^5 UT7/Epo-S1 cells for 7 days, before testing B19V infectivity by RT-PCR as described above. To control for any potential complement influence, neutralization experiments were also performed after incubation of B19V IgG containing plasma samples at 56°C for 30 minutes before use. As a control, B19V titers between 10^2 and 10^{10} IU were incubated with the two single plasma donations (both tested nonreactive for B19V IgG and B19V) instead of the B19V IgG containing plasma pool blends or original plasma pools for each experiment.

For each B19V IgG concentration used for neutralization tests at least four (0.4, 4, and 11 IU/mL) or two (1 and 36 IU/mL) independent titrations were performed. A human IVIG product (for Europe, KIOVIG [Baxter Healthcare Corporation, Westlake Village, CA]; for the United States, Gammagard liquid [Baxter Healthcare Corporation]) was used in neutralization tests as described. Because the original IVIG preparation had a B19V IgG titer of 562 IU per mL (manufactured from plasma pools with a mean B19V IgG titer of 40 IU/mL), dilutions ranging in B19V IgG titer from 0.1 to 25 IU were prepared in 0.2 mol per L glycine, the medium of the IVIG preparation.

The results obtained by duplicate TaqMan RT-PCR runs for each sample were analyzed qualitatively, that is, crossing of the TaqMan RT-PCR threshold of 0.07 ΔRn VIC was scored positive while not exceeding that background threshold was scored negative.

Statistical analysis

Statistical evaluation of B19V IgG titer frequencies in manufacturing plasma pools (containing a few thousand donations each), the calculation of B19V titers that equaled 50 percent infectivity and unpaired t tests were calculated with computer software (GraphPad Prism 4, GraphPad Software, San Diego, CA).

RESULTS

Infection of UT7/Epo-S1 cells with B19V Genotype 1 and 2

Although it had been shown that B19V Genotype 1 infectivity can be quantified by RT-PCR analysis of mRNA isolated from infected UT7/Epo-S1 cells, it was suggested that this same assay might not work for B19V Genotype 2.[22] To reinvestigate the susceptibility of UT7/Epo-S1 cells for infection with B19V Genotype 2, cells were in parallel exposed to either B19V Genotype 1 or B19V Genotype 2, and the following events were examined by immunocytochemical staining and Western blot (Fig. 1).

Immunocytochemistry of cells prepared on Day 1 after B19V infection served as negative control that confirmed that inoculum virus was sufficiently removed. Cells prepared on Day 3 after infection, however, revealed the presence of newly synthesized B19V capsid proteins within infected UT7/Epo-S1 cells, after exposure to both B19V Genotype 1 or B19V Genotype 2 (Fig. 1A). As sug-

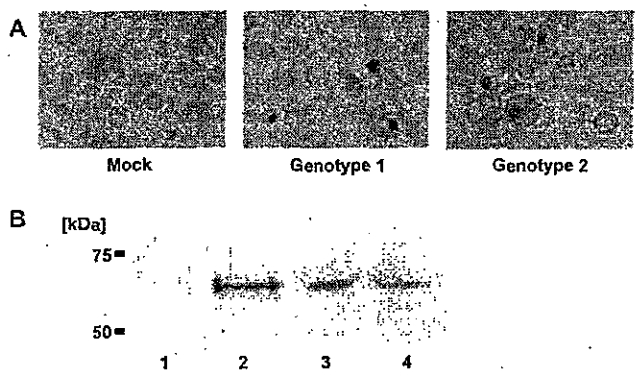

Fig. 1. Infection of UT7/Epo-S1 cells with B19V Genotypes 1 and 2. (A) Immunocytochemical staining of UT7/Epo-S1 cells that were mock-infected, infected with B19V Genotype 1 (MOI, 10^6) or B19V Genotype 2 (MOI, $10^{5.6}$) for 7 days. Original magnification, ×40. (B) Western blot of cell lysates from UT7/Epo-S1 cells. Lane 1 = negative control, mock-infected cells; Lanes 2 through 4 = B19V-infected cells; Lane 2 = Genotype 1 (MOI, 10^5); Lane 3 = Genotype 1 (MOI, 10^4); Lane 4 = Genotype 2 (MOI, 10^5).

gested by Fig. 1A, and confirmed by counterstaining of infected cells with methyl green (not shown), the VP1/VP2 staining was primarily confined to the nucleus. Quantitatively, approximately 10 percent cells infected with either Genotype 1 or Genotype 2 were positive on Day 3 after infection. Seven days after infection UT7/Epo-S1 cells had multiplied to an approximately 10-fold higher cell number, yet the percentage of infected cells was still at approximately 10 percent (data not shown).

The detection of B19V capsid proteins by Western blot also confirmed the susceptibility of UT7/Epo-S1 cells to infection with both B19V Genotype 1 and B19V Genotype 2 (Fig. 1B). The predominant B19V capsid protein splice variant VP2 was clearly detectable for both B19V genotypes at 7 days postinfection, although the signal for Genotype 1 appeared somewhat stronger compared to the signal for Genotype 2. This effect became more apparent when the MOI used for Genotype 1 was 10 times lower than that for Genotype 2 (Fig. 1B, Lanes 3 and 4). Cell homogenates obtained during Days 1 through 4 after infection did not result in detectable Western blot signals, confirming de novo synthesis of B19V proteins rather than detection of residual inoculum virus particles. The detection limits of B19V infectivity determined by immunocytochemical staining and Western blot were for both approximately 10^9 IU B19V.

The presence of B19V proteins in infected UT7/Epo-S1 cells as now demonstrated for both B19V Genotype 1 and B19V Genotype 2 would, as a prerequisite, require production of B19V-specific mRNAs, spliced exactly as during infection of humans.[24] The presence of these spliced mRNA species would then provide the basis for detection of B19V infectivity by TaqMan RT-PCR as described under Material and Methods.

As expected, RT-PCR confirmed infectivity of B19V Genotypes 1 and 2 for UT7/Epo-S1 cells. For an accurate calculation of the B19V PCR titer that corresponds to one 50 percent tissue culture–infectious dose ($TCID_{50}$), at least eight replicates of the B19V titers that did not result in all negative or in all positive RT-PCR results were analyzed (Table 1). The respective $TCID_{50}$ values obtained were 3.7 log IU for B19V Genotype 1 versus 6.1 log IU for B19V Genotype 2.

Neutralization of B19V

Plasma manufacturing pools consist of typically several thousand individual donations and at a B19V IgG seroprevalence of approximately 30 to 60 percent in the plasma-donating population[10] statistically rather evenly distributed B19V antibody levels should be expected to occur in plasma pools. To establish a statistically meaningful estimate of the B19V antibody concentrations present in plasma manufacturing pools, samples were obtained from a total of 1174 pools, representing a few million donations collected in either Europe or the United States over the course of 2 years, to be tested for B19V antibodies by ELISA.

As expected from the high seroprevalence of B19V in the human population, the analysis revealed a rather high mean ELISA titer of 33 ± 9 IU per mL (mean ± SD) among all the plasma pools tested, with a high of 71 IU per mL and a low of 11 IU per mL (Fig. 2A). Although thus the presence of a varying yet significant level of B19V antibodies had been confirmed for a statistically relevant number of plasma pools by ELISA, we sought to measure antibody function—rather than presence—considering this clinically more meaningful.

Neutralization of B19V infectivity in plasma pool specimens was thus investigated, utilizing the detection of B19V infectivity by TaqMan RT-PCR, after incubation in the presence or absence of defined concentrations of B19V IgG as determined by ELISA. Of potentially the most significant clinical importance, the B19V neutralization capacity at the lowest ELISA titer ever found for a plasma manufacturing pool in our investigation, that is, 11 IU per mL, was investigated (Fig. 2A). The test material was derived by blending plasma manufacturing pool specimens of higher B19V antibody titer (36-40 IU/mL B19V IgG) with plasma donations individually screened to be nonreactive for B19V IgG and B19V, to reach the target concentration of 11 IU per mL B19V IgG.

The comparison of B19V Genotype 1 infectivity after incubation with either B19V antibody–negative plasma (control) or the plasma pool sample blended to contain 11 IU per mL B19V IgG can be seen in Fig. 2B. For each data point given in Fig. 2B, at least five infectivity tests

TABLE 1. Infectivity of B19V Genotypes 1 and 2, detected by mRNA TaqMan RT-PCR*

	Detection of B19V infectivity by mRNA RT-PCR	
B19V (IU) for infection	Genotype 1	Genotype 2
10^{10}	2+	8+
10^{9}	2+	12+
10^{8}	2+	7+/1–
10^{7}	2+	10+/2–
10^{6}	18+	5+/5–
10^{5}	12+	6–
10^{4}	9+/3–	NT
10^{3}	1+/9–	NT
10^{2}	10–	NT
$TCID_{50}$ (log)	3.7	6.1

* Seven days after infection of UT7/Epo-S1 cells with different B19V concentrations, mRNA was isolated and subjected to TaqMan RT-PCR. From the numbers of positive (+) and negative (–) results obtained at certain virus dilutions, the virus titer corresponding to 50 percent infectivity ($TCID_{50}$) was calculated.
NT = not tested.

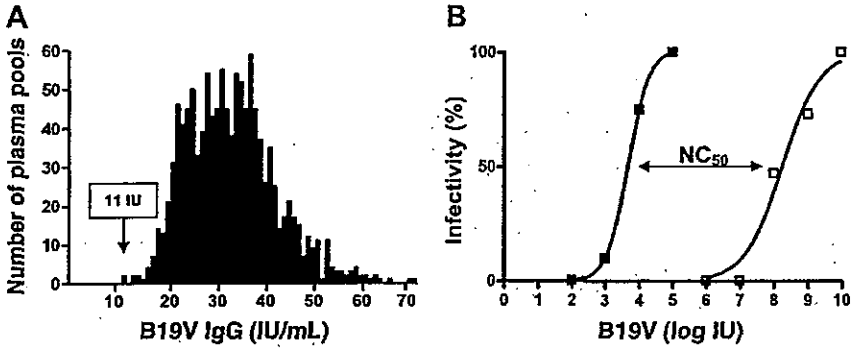

Fig. 2. Neutralization of B19V by plasma manufacturing pools. (A) B19V IgG antibody titers of 1174 plasma manufacturing pools as determined by ELISA (IU/mL). (B) Infectivity (%, detected by TaqMan mRNA RT-PCR) of B19V samples on UT7/Epo-S1 cells that were inoculated with 10^2 to 10^6 IU B19V (■) or 10^5 to 10^{10} IU B19V incubated with 11 IU per mL B19V IgG before infection of the cells (□). The difference in B19V concentrations that corresponds to 50 percent B19V infectivity between the two sets of samples represents the NC_{50} of B19V IgG at 11 IU per mL.

have been performed to determine the percentage of infectivity from the respective number of positive and negative TaqMan RT-PCR results. Compared to the $TCID_{50}$ of 3.7 log IU per mL for B19V Genotype 1 in the presence of control plasma, incubation with the plasma containing 11 IU per mL B19V IgG increased the $TCID_{50}$ to a corresponding B19V concentration of 8.2 log IU per mL. From these results, the 50 percent B19V neutralization capacity (NC_{50}) for plasma containing 11 IU per mL B19V IgG could be calculated, that is, the difference of both 50 percent infectivity calculations, as 4.6 log B19V IU per mL.

To substantiate the relevance of this result that was obtained by use of blended plasma specimens, samples from the two original plasma manufacturing pools from 2004 and 2005, each containing only 11 IU B19V IgG per mL, were also investigated in the same neutralization assay setup. Although the available sample volumes from these two specific plasma manufacturing pools were limited, only three neutralization assays could be performed with the original pool samples, demonstrating a mean NC_{50} neutralization capacity of 4.6 log B19V IU per mL, thus confirming the results earlier obtained with the blended plasma samples.

To establish a dose–response relationship between the B19V neutralization capacity and the presence of B19V antibodies as determined by ELISA, samples at B19V IgG concentrations higher than the 11 IU per mL or even lower, that is, concentrations that never occur in plasma manufacturing pools, were generated with the blending approach described. These samples, at B19V antibody concentrations between 0.4 and 36 IU per mL B19V IgG, were subsequently tested for their B19V neutralization capacity (Fig. 3).

The NC_{50} obtained for a plasma sample containing an artificially low 0.4 IU per mL B19V antibody titer was 1.8 log B19V. At 1 and 4 IU per mL B19V IgG, that is, still well below the lowest B19V IgG concentrations ever observed in a plasma manufacturing pool, 3.1 log and 4.4 log B19V IU per mL were neutralized, respectively. At B19V antibody concentrations higher than the earlier tested 11 IU, the demonstrable B19V neutralization did just marginally increase, showing a NC_{50} of 4.9 log B19V at 36 IU per mL B19V IgG.

Neutralization experiments with B19V Genotype 2 were somehow limited by the significantly higher limit of detection for Genotype 2 compared to Genotype 1 (Table 1). Specifically, already at 11 IU per mL B19V IgG the NC_{50} was beyond the limit of detection for the method, and the corresponding result was neutralization of greater than 3.9 log for B19V Genotype 2. This level of neutralization, however, was well comparable to the one earlier observed for B19V Genotype 1.

Because IVIG products are indicated for the treatment of severe B19V infections, information about the functional B19V neutralization capacity of such products would be clinically relevant. Consequently, the neutralization capacity of IVIG samples was tested. Because the original IVIG preparation contained 562 IU per mL B19V IgG (corresponding to 5.62 IU B19V IgG/mg IgG), predilution to contain B19V IgG titers between 0.1 and 25 IU was

required for evaluation by the neutralization assay. Coherent with the results earlier obtained for plasma samples, 2.2 log infectious B19V were already neutralized by an IVIG sample at 0.3 IU B19V antibody, and 5.2 log infectious B19V were neutralized by an IVIG specimen diluted to 8.4 IU B19V IgG.

Anti B19V titers in different manufacturing pools

Comparing the B19V IgG content of different US plasma manufacturing pools (pooled between January 5, 2004, to November 18, 2005), prepared from either source plasma, that is, collected by plasmapheresis, or recovered plasma, that is, derived from whole-blood donations, we found significantly different B19V IgG titers depending on the plasma source: while recovered plasma pools ($n = 48$) contained a mean ± SEM titer of 43 ± 1.5 IU per mL (range 22-71 IU/mL), source plasma pools ($n = 630$) only had 31 ± 0.3 IU per mL (range, 11-53 IU/mL). These differences were significant, as shown by analysis of the B19V antibody titers between source and recovered plasma pools by unpaired t test ($p < 0.0001$; Fig. 4A).

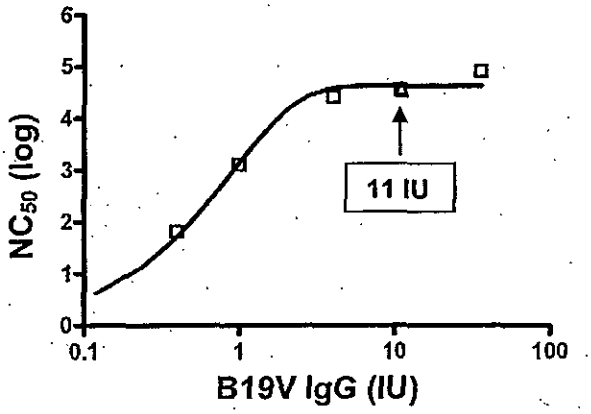

Fig. 3. Dose–response relation for neutralization of B19V. Relation of B19V infectivity NC_{50} (see Fig. 2) and concentrations of B19V IgG (IU).

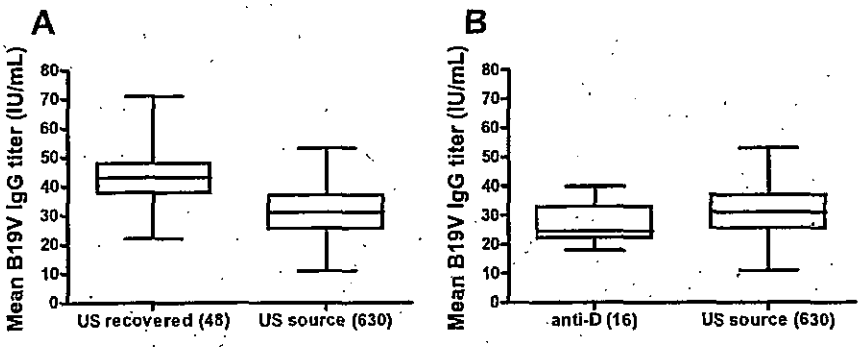

Fig. 4. Comparison of mean B19V IgG titers in plasma manufacturing pools, produced in 2004 and 2005, by unpaired t test. (A) US source ($n = 630$) versus US recovered ($n = 48$), $p < 0.0001$. (B) Anti-D ($n = 16$) versus US source ($n = 630$), $p = 0.0694$.

Whereas for the plasma pools so far tested for B19V antibodies an industry voluntary standard[25] required limiting the presence of B19V by PCR pretesting to less than 10^5 IU per mL, a limit of less than 10^4 IU per mL B19V has been mandatory for pools of anti-D plasma.[26] Reaching this lower limit requires interdiction of additional donations that contain B19V. As such donations might be expected to also contain B19V antibodies,[6] it is conceivable that the different PCR testing strategies applied for regular versus anti-D plasma might also result in somewhat different B19V IgG concentrations.

Comparing the ELISA B19V IgG results, however, for anti-D plasma ($n = 16$) and for regular US source plasma ($n = 630$), that is, plasma types that with respect to B19V only differ in the respective cutoff limits for PCR testing, they were not significantly different ($p = 0.0694$; Fig. 4B).

DISCUSSION

With the emergence of the A6 and V9 erythroviruses,[22,27] that is, viruses that have now been reclassified as B19V genotypes, a useful assay for quantification of B19V infectivity should also be capable of quantifying these genotypes or at least Genotype 1 as the by far most prevalent and Genotype 2 that has been shown to—more rarely—also occur in plasma for fractionation. In contrast to an earlier suggestion that UT7/Epo-S1 cells might not be susceptible to B19V Genotype 2,[22] infection of UT7/Epo-S1 cells by both B19V Genotype 1 and Genotype 2 is demonstrated in the current work. This apparent discrepancy with earlier results is likely due to human immunodeficiency virus coinfection of the earlier used Genotype 2 B19V specimen and its heat inactivation before use in the B19V study, a procedure that possibly would inactivate B19V infectivity.[18] Even in our study, however, the detection of B19V Genotype 2 by Western blot was approximately 10 times less sensitive compared to Genotype 1, and with the TaqMan RT-PCR B19V the limit of detection for Genotype 1 was approximately 100 times lower than for Genotype 2 (Table 1). For the RT-PCR assay, this difference was initially considered to be the consequence of a few mismatches of the primers used for B19V Genotype 2. Another set of primers was therefore designed and tested, specifically designed for the sequence of the B19V Genotype 2 sample used.[20] Even with those, however, the same detection limit was determined as with the Genotype 1 specific primers, suggesting that B19V Genotype 2 infects UT7/Epo-S1 cells somewhat less efficiently compared to B19V Genotype 1, a possible explana-

tion also for the Western blot discrepancy between the two genotypes. Genotype 1 B19V was detected with one $TCID_{50}$ corresponding to 3.7 log IU, that is, well in agreement with results from other investigations.[15,16,28]

Owing to the high prevalence of B19V in the population, the titer of anti-B19V in plasma pools for manufacturing was anticipated to be high and rather constant.[11] Data on B19V IgG in plasma pools available so far, however, were rather limited, with only 20 to 66 plasma pools investigated with mean (± SD) B19V IgG contents ranging from 29.8 ± 17.2 to 64.7 ± 17.5 IU per mL.[11-14] Here a total of 1174 plasma pools were analyzed, representing a few million donations collected over a period of 2 years in both the United States and the European Union, which revealed a mean (± SD) B19V IgG titer of 33 ± 9 IU per mL. Of all these pools, the minimum B19V IgG titer ever found was 11 IU per mL. In the following series of neutralization experiments, correlating the presence of B19V IgG (ELISA) to functional capacity and/or neutralization, B19V Genotype 1 neutralization by B19V IgG was found to be dose-dependent with a neutralization capacity greater than 4 log B19V already at only 4 IU per mL, that is, an artificially low antibody concentration compared to the naturally occurring minimal concentration in plasma pools (11 IU/mL B19V IgG). A neutralization capacity of greater than 4 log has also been shown for higher B19V IgG concentrations, at up to 36 IU per mL. Neutralization of B19V Genotype 2 with 11 IU per mL B19V IgG was likewise shown to be greater than 3.9 log, although a more exact quantification was limited by the higher limit of detection for B19V Genotype 2 in the assay used. In agreement with this, other neutralization experiments with the KU812Ep6 cell line also showed neutralization of more than 4 log B19V infectivity by less than 3.5 IU per mL B19V IgG.[20] In contrast to our study, however, only single plasma donations instead of plasma manufacturing pools were investigated. Even for these individual samples, Blumel and coworkers[20] have indicated B19V antibody cross-reactivity for different B19V genotypes. Based on the contribution of many thousand donors for any plasma manufacturing pool, it can be expected that an even broader range of different B19V genotypes can be neutralized with B19V IgG from plasma pools, a perspective of particular importance for the B19V neutralization capacity of IVIG products produced from these pools. An experimental comparison of the neutralization capacity of plasma manufacturing pools and IVIG showed that with only 8 IU B19V IgG contained within an IVIG sample greater than 5 log B19V were neutralized. A similar degree of B19V neutralization can also be extracted from recent work by another group.[29]

Although several reports have described the transmission of B19V through plasma derived products, the amount of B19V that represents an infectious dose in humans is not yet clear. In one anecdotal B19V transmission episode, however, solvent/detergent-treated plasma that contained $10^{7.5}$ genome equivalents (geq) B19V per mL in the presence of at least 8.8 IU per mL B19V IgG transmitted B19V, while no transmission occurred at a B19V load of $10^{3.5}$ geq per mL or less.[30,31] This observation is in good agreement with our neutralization experiments that have shown neutralization of more than 10^4 IU per mL B19V by even only 4 IU per mL B19V IgG (Fig. 3; note that according to an internal validation, for the B19V Genotype 1–positive donation used in this study, virus quantification in IU is equivalent to geq).

The even higher B19V neutralization capacity as shown for an IVIG product compared to plasma pools would also support the use of IVIG as treatment for fulminant B19V infections, based on the potent neutralization efficacy.[9] To extrapolate our data from in vitro neutralization of B19V by IVIG to the clinical situation, we made a comparison with the amount routinely administered during common variable immune deficiency treatment, that is, 100 to 400 mg immunoglobulins per kilogram of human body weight. With a typical volume of 84.4 mL blood per kilogram of body weight,[32] the IVIG preparation (containing immunoglobulins at 10%) is diluted 1:84 to 1:21 during common variable immune deficiency treatment. Because the IVIG preparation investigated in this study contained 562 IU per mL B19V IgG, the concentration of B19V IgG in the human blood after administration would be 7 to 27 IU per mL. We have shown in vitro neutralization of more than 5 log infective B19V with 8 IU anti-B19V IgG, thus indeed indicating efficient protection from infection with B19V conferred by IVIG.

Evidence exists to suggest that the prevalence of B19V IgG in the population constantly increases with age.[10] In this context, our analysis of B19V IgG titers in plasma pools from recovered and source plasma that revealed a significant difference of mean B19V IgG titers may be caused by a difference in the age structure of plasma donors and blood donors. A B19V nucleic acid test (NAT) limit of less than 10^5 IU per mL B19V in plasma pools has been implemented by manufacturers of plasma derivatives as a voluntary standard some years ago.[25] Since the introduction of that test limit, no B19V transmission by plasma-derived products manufactured according to the standard has been observed, to our knowledge. The recent tendency toward tightening the B19V PCR test limit to less than 10^4 IU per mL, which would require interdicting donations that contain lower levels of B19V but may also contain B19V IgG, carries the possibility of also resulting in lower concentrations of B19V IgG in plasma for fractionation.[6] A direct comparison of plasma pools for which a B19V NAT test limit of 10^4 IU per mL has been implemented by regulatory requirement (anti-D plasma[26]) with plasma pools tested to comply with the Plasma Protein Therapeutics Association voluntary B19V PCR testing standard of less than 10^5 IU per mL (US source plasma)

did, however, not reveal any significant difference in B19V IgG titers. This suggests that lowering the B19V PCR test limit to 10^4 IU per mL would not impair the B19V neutralization capacity contained in plasma pools and thus also IVIG products. Also, although our data show that even at the lowest B19V IgG concentrations determined for a plasma pool of greater than 10^4 IU per mL B19V are neutralized, the combination between uncompromised B19V antibody levels and further reduced B19V loads in plasma manufacturing pools might even enhance the safety margins of plasma products as primarily afforded by the virus reduction capacity of their manufacturing processes, particularly for IVIG that has already enjoyed a long-standing history of safety with respect to B19V transmission[33] and other antibody-containing products.

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REFERENCES

1. Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA. Virus taxonomy: classification and nomenclature of viruses—eighth report of the International Committee on the Taxonomy of Viruses. San Diego: Elsevier Academic Press; 2005.
2. Liefeldt L, Plentz A, Klempa B, Kershaw O, Endres AS, Raab U, Neumayer HH, Meisel H, Modrow S. Recurrent high level parvovirus B19/genotype 2 viremia in a renal transplant recipient analyzed by real-time PCR for simultaneous detection of genotypes 1-3. J Med Virol 2005;75:161-9.
3. Schneider B, Becker M, Brackmann HH, Eis-Hubinger AM. Contamination of coagulation factor concentrates with human parvovirus B19 genotype 1 and 2. Thromb Haemost 2004;92:838-45.
4. Candotti D, Etiz N, Parsyan A, Allain JP. Identification and characterization of persistent human erythrovirus infection in blood donor samples. J Virol 2004;78:12169-78.
5. Cossart YE, Field AM, Cant B, Widdows D. Parvovirus-like particles in human sera. Lancet 1975;1(7898):72-3.
6. Young NS, Brown KE. Parvovirus B19. N Engl J Med 2004; 350:586-97.
7. Pankuweit S, Ruppert V, Eckhardt H, Strache D, Maisch B. Pathophysiology and aetiological diagnosis of inflammatory myocardial diseases with a special focus on parvovirus B19. J Vet Med B Infect Dis Vet Public Health 2005;52:344-7.
8. Eid AJ, Brown RA, Patel R, Razonable RR. Parvovirus B19 infection after transplantation: a review of 98 cases. Clin Infect Dis 2006;43:40-8.
9. Red book: report of the Committee on Infectious Diseases. 27th ed. Elk Grove Village (IL): American Academy of Pediatrics; 2006.
10. Heegaard ED, Brown KE. Human parvovirus B19. Clin Microbiol Rev 2002;15:485-505.
11. Solheim BG, Rollag H, Svennevig JL, Arafa O, Fosse E, Bergerud U. Viral safety of solvent/detergent-treated plasma. Transfusion 2000;40:84-90.
12. Bonvicini F, Gallinella G, Cricca M, Ambretti S, Delbarba S, Musiani M, Zerbini M. Molecular testing for detection of in vitro infectivity of plasma pools contaminated with B19 virus. J Med Virol 2004;74:272-6.
13. Daly P, Corcoran A, Mahon BP, Doyle S. High-sensitivity PCR detection of parvovirus B19 in plasma. J Clin Microbiol 2002;40:1958-62.
14. Schmidt I, Blumel J, Seitz H, Willkommen H, Lower J. Parvovirus B19 DNA in plasma pools and plasma derivatives. Vox Sang 2001;81:228-35.
15. Wong S, Brown KE. Development of an improved method of detection of infectious parvovirus B19. J Clin Virol 2006; 35:407-13.
16. Prikhod'ko GG, Vasilyeva I, Reyes H, Wong S, Brown KE, Jameson T, Busby TF. Evaluation of a new LightCycler reverse transcription-polymerase chain reaction infectivity assay for detection of human parvovirus B19 in dry-heat inactivation studies. Transfusion 2005;45:1011-9.
17. Caillet-Fauquet PG, Draps ML, de Hougardy VL, Laub R. An assay for parvovirus B19 neutralizing antibodies based on human hepatocarcinoma cell lines. Transfusion 2004; 44:1340-3.
18. Blumel J, Schmidt I, Willkommen H, Lower J. Inactivation of parvovirus B19 during pasteurization of human serum albumin. Transfusion 2002;42:1011-8.
19. Yunoki M, Tsujikawa M, Urayama T, Sasaki Y, Morita M, Tanaka H, Hattori S, Takechi K, Ikuta K. Heat sensitivity of human parvovirus B19. Vox Sang 2003;84:164-9.
20. Blumel J, Eis-Hubinger AM, Stuhler A, Bonsch C, Gessner M, Lower J. Characterization of Parvovirus B19 genotype 2 in KU812Ep6 cells. J Virol 2005;79:14197-206.
21. Shimomura S, Komatsu N, Frickhofen N, Anderson S, Kajigaya S, Young NS. First continuous propagation of B19 parvovirus in a cell line. Blood 1992;79: 18-24.
22. Nguyen QT, Wong S, Heegaard ED, Brown KE. Identification and characterization of a second novel human erythrovirus variant, A6. Virology 2002;301:374-80.
23. Kerr JR, O'Neill HJ, Deleys R, Wright C, Coyle PV. Design and production of a target-specific monoclonal antibody

to parvovirus B19 capsid proteins. J Immunol Methods 1995;180:101-6.
24. Ozawa K, Ayub J, Hao YS, Kurtzman G, Shimada T, Young N. Novel transcription map for the B19 (human) pathogenic parvovirus. J Virol 1987;61:2395-406.
25. PPTA. PPTA voluntary standard parvovirus B19. Report 017677. Annapolis (MD): Plasma Protein Therapeutics Association; 2001 Feb 8. p. 1-2.
26. European Pharmacopoeia Expert Group. Human anti-D immunoglobulin. Pharmeurope 2002;14:81.
27. Nguyen QT, Sifer C, Schneider V, Allaume X, Servant A, Bernaudin F, Auguste V, Garbarg-Chenon A. Novel human erythrovirus associated with transient aplastic anemia. J Clin Microbiol 1999;37:2483-7.
28. Miyagawa E, Yoshida T, Takahashi H, Yamaguchi K, Nagano T, Kiriyama Y, Okochi K, Sato H. Infection of the erythroid cell line, KU812Ep6 with human parvovirus B19 and its application to titration of B19 infectivity. J Virol Methods 1999;83:45-54.
29. Yunoki M, Urayama T, Tsujikawa M, Sasaki Y, Abe S, Takechi K, Ikuta K. Inactivation of parvovirus B19 by liquid heating incorporated in the manufacturing process of human intravenous immunoglobulin preparations. Br J Haematol 2005;128:401-4.
30. Davenport RJ, Geohas G, Cohen S, Beach K, Lazo A, Lucchesi K, Pehta J. Phase IV Study of Plas+SD: hepatitis A (HAV) and parvovirus B19 (B19) safety results. Blood 2000;96 Suppl 1:451a.
31. Brown KE, Young NS, Alving BM, Barbosa LH. Parvovirus B19: implications for transfusion medicine. Summary of a workshop. Transfusion 2001;41:130-5.
32. Feldschuh J, Enson Y. Prediction of the normal blood volume relation of blood volume to body habitus. Circulation 1977;56:605-12.
33. Farrugia A. Risk of parvovirus infection by immunoglobulin. Br J Haematol 2003;121:955-6. ◘

別紙様式第 2-1

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-1

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2007 年 10 月 11 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	Parvovirus B19 genotypes 1 and 2 detection with real-time polymerase chain reaction assays. Koppelman, M.H.G.M. et al, Vox Sanguinis, 93, 208-215 (2007).	公表国 オランダ	
販売名（企業名）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
2005 年 3 月から 2007 年 3 月の間にオランダで実施された，260 万の血漿成分献血検体を対象とした B19 ウイルス（B19V）のスクリーニング試験の結果を報告した。献血検体は，B19 の遺伝子 1 型のみを検出する Roche 社製の市販の DNA 定量アッセイと，3 種すべての遺伝子型を検出できる社内アッセイ（Sanquin，オランダ）の 2 種類の PCR アッセイで測定した。本試験では 10^6 IU/mL を超えるウイルス価を示す検体を B19V 陽性と判断した。両アッセイの検出限界は 100 IU/mL 前後と同等であった。480 検体からなるテスト用プール，及び製造用プールを測定し，5000 IU/ml 超を示したプールに関して，さらに詳細に測定した。その結果，232 検体（11000 検体につき 1 検体）で B19V が確認（10^6 IU/mL 以上）された。これら B19 陽性検体の大多数ではアッセイ間で一致が見られたが，3 検体（1.3%）では不一致が認められた。ジェノタピング及び各アッセイで用いたプライマー及びプローブの結合領域の配列解析により，2 検体は B19 遺伝子 1 型に分類され，残りの 1 検体は遺伝子 2 型に分類されることを明らかとした。従って，遺伝子 2 型及び 3 型の保有率はヨーロッパ人ドナーにおいては極めて低いと考えられる。今回の試験は別のグループによる過去の知見を裏付けている。	BYL-2008-0297

報告企業の意見	今後の対応
オランダにおける献血検体中 B19 ウイルスのスクリーニング結果が報告された。異なる PCR アッセイを組み合わせることで，3 種類の B19 ウイルスアイソフォームの検出を可能としている点は，新規性が高く，今後の応用が期待される。大規模スクリーニングの結果，ヨーロッパにおける献血では，11000 検体に 1 検体の割合で B19 ウイルスが検出され，1 検体を除き遺伝子 1 型に分類された。ヨーロッパ人ドナーにおいて 2 型及び 3 型の保有率は極めて低いと考えられた。 弊社のポリグロビン N の製造に使用されるミニプール血漿においては，ヒトパルボウイルス B19 に対する NAT を実施しており，10E5 IU/mL 以上が確認された場合は，そのミニプール血漿は製造工程から除去している。現在の科学水準では，ヒトパルボウイルス B19 を確実に不活化する方法は存在しないため，感染リスクを完全に排除することはできないが，伝播の可能性は非常に低いと考える。	現時点で新たな安全対策上の措置を講じる必要はないと考える。

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Parvovirus B19 genotypes 1 and 2 detection with real-time polymerase chain reaction assays

M. H. G. M. Koppelman,[1] I. G. H. Rood,[1] J. F. Fryer,[2] S. A. Baylis[2] & H. T. M. Cuypers[1]

[1]Sanquin, Diagnostic Services Division, Amsterdam, The Netherlands
[2]Division of Virology, National Institute for Biological Standards and Control (NIBSC), Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QG, UK

Vox Sanguinis

Background and Objectives Parvovirus B19 (B19V) DNA screening has been introduced to comply with European regulations for certain plasma products. Current commercial and some in-house B19V DNA assays fail to detect or under-quantify the recently identified genotypes 2 and 3. In this report, we describe 2-year experience with B19V DNA screening using the commercial assay from Roche (detecting only genotype 1) combined with an in-house assay (detecting genotypes 1, 2 and 3). This dual testing approach enables the identification of molecular variants of B19V.

Materials and Methods Between 2005 and 2007, approximately 2·6 million plasma donations were screened for B19V DNA loads exceeding 10^6 IU/ml using the Roche and the in-house real-time polymerase chain reaction assay.

Results A total of 232 plasma units were identified with B19V DNA loads above 10^6 IU/ml. Concordant results were observed for the majority of B19V positive samples; however, three of these showed discrepant results between the two assay systems. One was a B19V genotype 2 strain not detected by the Roche assay; another was a B19V genotype 1 strain with a mismatch in the 3′-end of the reverse primer and therefore under-quantified by the Roche assay; and the third one was also a B19V genotype 1 strain that gave an unusual amplification plot in the in-house assay due to a mismatch in the probe-binding site.

Conclusions New, high viral load, B19V genotypes 2 and 3 infections are rare in blood donors tested by Sanquin. One case was found while testing 2·6 million donations. The prevalence of B19V genotype 1 variants not detected by commercial or in-house assays might be in the same range or even higher than the prevalence of B19V genotype 2 viruses, which remain undetected.

Key words: B19V genotype 2, parvovirus B19 DNA, screening, real-time PCR.

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Introduction

In 2004, European regulations came into force to limit the potential parvovirus B19 (B19V) burden in plasma pools for the manufacture of human anti-D immunoglobulin and pooled human plasma treated for virus inactivation [1]. The level of B19V DNA in these manufacturing pools should not exceed a threshold concentration of 10 000 IU/ml. To comply with these requirements, the plasma fractionation industry set up a screening system to prevent plasma units with high B19V DNA loads from entering large manufacturing pools. Most of the industry have introduced systems in which donations are prescreened in test pools of 480–960 donations [2–4]. When the B19V DNA level in a test pool exceeds the defined exclusion limit, the index donation is traced using a break-down protocol to smaller test pools.

Correspondence: M. H. G. M. Koppelman, Sanquin Diagnostic Services Division, Plesmanlaan 125, 1066 CX, Amsterdam, The Netherlands
E-mail: m.koppelman@sanquin.nl

Donor screening for B19V DNA requires quantitative nucleic acid amplification tests (qNAT) because donations exceeding a defined exclusion limit must be identified and subsequently removed. Several in-house and commercially available qNAT assays have been developed for this purpose between 1999 and 2004 [2,5,6]. Two commercial assays from Artus (RealArt Parvovirus B19 LightCycler PCR kit) and Roche (LightCycler Parvovirus B19 DNA quantification kit) are currently on the market for quantitative detection of B19V DNA.

In parallel with the development of qNAT assays for B19V DNA, several virus strains have been identified that show greater sequence diversity than that was previously recognized for B19V [7–10]. Phylogenetic analysis of B19V and these related variants showed that the viruses fall into three genotypes [10]. The prototypical sequences for B19V fall into genotype 1; genotype 2 viruses include A6 [7] and LaLi [9], while genotype 3 viruses include V9 [8] and D91·1 [10]. In the eighth report from the International Committee on the Taxonomy of Viruses (ICTV), A6, LaLi and V9 have all been classified as strains of B19V [11]. The consequence of this official classification is that detection of these two new genotypes of B19V is now mandatory according to the European regulations for 'in process testing' of manufacturing pools for B19V DNA.

Detection of these recently classified B19V genotypes 2 and 3 with commercial assays and in-house assays is limited. Several publications and the proficiency testing studies (PTSs) organized by European Directorate for the Quality of Medicines (EDQM) showed that commercial B19V DNA assays and several in-house assays have issues with the detection and/or quantification of B19V genotypes 2 and 3 strains. The Artus B19V DNA assay reliably quantified B19V genotypes 1 and 2 and some genotype 3 subtypes. However, one of the genotype 3 B19V subtypes is under-quantified by at least 3 logs [5,12,13]. The Roche assay reliably quantifies B19V genotype 1, but fails to detect genotypes 2 and 3 [2,5,12,14]. These findings are also reflected in the recent PTSs [12]. In the study performed in 2004 (PTS052), 56% of the laboratories that participated missed the B19V genotype 2 sample. The study organized in 2005 (PTS064) showed that 41% of the participants missed the B19V genotype 2 sample. In the latter study, 25% of laboratories using in-house assays were unable to detect the B19V genotype 2.

The Roche B19V DNA assay has been used for screening all plasma, in test pools of 480 donations [2]. As the Roche assay fails to detect genotypes 2 and 3 of B19V, an additional assay able to detect and quantify all three genotypes of B19V was introduced in 2005 [5]. Currently, all donations are tested in parallel with these two B19V DNA assays. This study reports the results of 2-year experience using the dual testing approach on more than 2·6 million donations.

Materials and methods

B19V DNA testing of plasma

Between March 2005 and March 2007, Sanquin tested approximately 2·6 million blood donations for B19V DNA load. Plasma was tested in test pools of 480 donations and in manufacturing pools. Test pools with B19V DNA loads above 5000 IU/ml were subjected to further testing to track down the index donation(s).

Commercial and in-house B19V DNA real-time polymerase chain reaction amplification

Nucleic acid from manufacturing pools, test pools and individual donations (0·1–1·0 ml plasma input) was isolated using the NucliSens extractor (NucliSens, bioMerieux, Boxtel, The Netherlands) [15].

Two real-time polymerase chain reaction (PCR) assays were performed with the LightCycler instrument (Roche Diagnostics, Mannheim, Germany) on the same nucleic acid extract. The first assay was the B19V DNA quantification assay from Roche. This assay amplifies a fragment within the non-structural protein (NS1) gene [2]. The amplicons are detected with hybridization probes. The second assay was an in-house developed B19V DNA assay with primers (EVF and EVR; see Table 1) and TaqMan probes in the NS1 region. This assay was adapted from Baylis et al. [5] and reliably detects and quantifies B19V genotypes 1, 2 and 3. To improve the robustness of the in-house TaqMan assay, a modified hydrolysis probe was included. The modified probe had an identical DNA sequence; however, locked nucleic acid (LNA) bases were incorporated at specific sites [16,17]. The sequence of the modified TaqMan probe is as follows, with LNA bases shown underlined: 5′ (FAM)-AAC.CCC.GCG.CTC.TAG.TAC-(BBQ3) 3′. The sensitivity (95% detection limit) was similar for both B19V DNA assays and was approximately 100 IU/ml (data not shown).

B19V DNA sequence analysis

Purified PCR products were sequenced with the ABI PRISM BigDye terminator cycle sequencing ready reaction kit (version 3·0) according to the manufacturer's instructions (Applied Biosystems/Hitachi, Nieuwerkerk a/d IJssel, The Netherlands). Sequence analysis was performed on the ABI PRISM 3130xl Genetic Analyser. Table 1 shows the panel of primers used for sequencing in this study.

Determination of target and probe-binding regions in the Roche B19V DNA assay

In order to determine the region in the B19V that is targeted by the Roche assay, the assay was performed using the 10 000 IU/ml

Table 1 Panel of primers for sequencing of B19V (NS1–VPu region)

Primer (forward or reverse)	Sequence (5′→3′)	Nucleotide position in AF162273	Reference
P2 (f)	AAA.CTA.GCA.ATT.TAT.AAA.GC	1392–1411	This study
P3 (f)	TGG.ATT.GAT.AAA.AAA.TGT.GG	1551–1570	This study
P4 (f)	TTG.GTG.GTC.TGG.GAT.GAA.GG	1716–1735	This study
PV8-3 (f)	ATA.AAC.TAC.ACT.TTT.GAT.TTC.CCT.G	2052–2076	[20]
EVF (f)	AAT.GCA.GAT.GCC.CTC.CAC	2082–2099	[5]
EVR (r)	ATG.ATT.CTC.CTG.AAC.TGG.TCC	2254–2274	[5]
PV-1 (f)	GGA.CCA.GTT.CAG.GAG.AAT.CAT	2254–2274	This study
PV-2 (f)	GCT.TGG.TAT.AAT.GGA.TGG.AA	2481–2500	This study
PV-3 (r)	CCA.GAC.AGG.TAA.GCA.CAT.TT	2583–2602	This study
PV-4 (f)	TTT.GAC.TTA.GTT.GCT.CG	2800–2816	This study
PV-5 (r)	TGA.AAA.TGA.TGA.CTA.TAT.A	2849–2867	This study
B19SR (r)	CCA.GGC.TTG.TGT.AAG.TCT.TC	2959–2978	[20]

B19V run control. In this instance, the internal control from the kit was not added prior to extraction. DNA sequence analysis was performed to identify the location of the B19V amplicon in the viral genome. The sequence of the probe-binding region for the B19V was determined by comparing the sequence of the B19V amplicon with the sequence of the internal control amplicon (amplified in the absence of B19V DNA).

B19V genotyping

Viral DNA for genotyping was obtained by PCR amplification of a 1587-bp fragment spanning the NS1–VP1u junction in the B19V genome with primers P2f and B19SR (see Table 1). This fragment overlaps the PCR fragments amplified by the Roche and the in-house assay. Both DNA strands were sequenced with the panel of 12 sense and antisense oligonucleotides.

Phylogenetic analysis was performed using the Vector NTI 10·1·1 software package (Invitrogen, Carlsbad, CA, USA) and the Molecular Evolutionary Genetics Analysis software (MEGA2·1: Arizona State University, Tempe, AZ, USA). Neighbour-joining phylogenetic analysis was performed on a 1536-bp fragment (nucleotides 1436–2971 in AF162273). Nucleotide distances were calculated using the Kimura 2-parameter model using the bootstrap test with 1000 replicates.

The following B19V sequences from GenBank were used as reference sequences: *B19V genotype 1*, AF161226, AF162273, AY504945, DQ293995, M24682, M13178; *B19V genotype 2*, AJ717293, AY064476, AY064475, AY044266, AY903437, DQ333426, EF216869; *B19V genotype 3*, AJ249437, AY582125, AY647977, AY083234, AX003421, DQ234769, DQ234779, DQ408305, NC-004295.

Nucleotide sequence accession numbers

The GenBank accession numbers of the nucleotide sequences of the B19V variants analysed in this study are EF151136 (strain 163429), EF151137 (strain 903321), EF151138 (strain 207458) and EF216868 (strain F8-87-A).

Results

Identification of B19V genotypes 1 and 2 strains in plasma samples

Between March 2005 and March 2007, 2·6 million donations were prescreened for B19V DNA using dual assay testing approach. B19V DNA screening of test pools aims to identify donations with B19V DNA loads above 10^6 IU/ml. During the 2-year study period, 232 donations were identified with B19V DNA levels exceeding 10^6 IU/ml. Thus, donations with loads above the exclusion level occur with a frequency of about 1 in 11 000 in this donor population. In three cases (1·3%), discrepant results between the Roche and the in-house B19V DNA test were found (Table 2). Plasma sample 207458 is a donation undetectable by the Roche assay; however, it is found to have a viral load of 3×10^7 IU/ml in the in-house assay. The second sample, 163429, is a donation containing 10^8 IU/ml B19V DNA in the Roche assay. In contrast, this donation was hardly detectable by the in-house assay in the original test pool of 480 donations. Also at the individual

Table 2 Discrepant cases between the Roche and the in-house parvovirus B19 (B19V) DNA assay

Sample	Viral load (Roche assay)	Viral load (in-house assay)
207458	Not detectable	3×10^7 IU/ml
163429	1×10^8 IU/ml	Hardly detectable and not quantifiable[a]
903321	7×10^2 IU/ml	3×10^4 IU/ml

[a]Individual donation testing revealed a shallow amplification curve (see Fig. 1a).

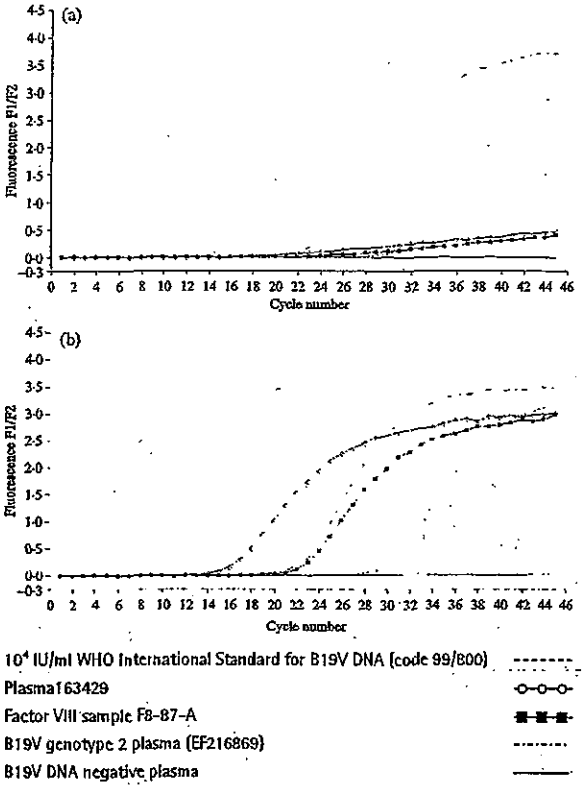

Fig. 1 Amplification plots obtained with the in-house assay under normal conditions using a TaqMan probe labelled with FAM/TAMRA and used at 0·2 μM [5] (a) and the assay performed under normal conditions using a locked nucleic acid (LNA) probe labelled with FAM/BBQ3 and used at 0·02 μM (b).

donation level, it was hardly detectable when the routine procedure (fit-points method) was used for calculation of the crossing-point. When the crossing-point was calculated with an alternative method (second derivative maximum method), the value of the crossing-point was comparable to the one obtained with the Roche assay. The amplification plot of sample 163429 obtained with the in-house assay showed a very shallow amplification signal (Fig. 1a). The third case, 903321, is a plasma sample with an almost 100-fold difference in load between the Roche and the in-house assays.

B19V genotyping

In each case, the sequence of a 1536-bp fragment overlapping the NS1–VP1 region of B19V was determined and aligned with B19V genotype 1, 2 and 3 sequences from GenBank. This alignment was used to construct a phylogenetic tree as shown in Fig. 2. The tree clearly shows that cases 163429 (not detected by the in-house assay) and 903321 (not detected by the Roche assay) are strains of B19V genotype 1 and that case 207458 (not detected by the Roche assay) is a B19V genotype 2 strain.

Molecular basis of the discrepant results

Initially, it was necessary to determine the region of the B19V genome targeted by the Roche assay. The amplified B19V product from the Roche assay was sequenced and found to correspond to a 177-bp region of the B19V NS1 gene (nucleotides 1552–1708 of the reference strain HV; accession number AF162273). It was assumed that the primers used in the Roche assay were 25 bp in length. In order to identify the probe-binding region for the Roche assay, the DNA sequence of the internal control amplicon from the Roche assay was determined. This revealed that the internal control corresponded to the wild-type B19V sequence with the exception of a 52-bp insert derived from the human telomerase RNA gene (nucleotides 881–932; accession number AF047386). This insert is four nucleotides longer than the corresponding wild-type B19V sequence it has replaced (i.e. nucleotides 48–94 of the wild-type B19V PCR fragment). This 46-bp sequence is considered to represent the hybridization probe-binding region of the Roche B19V DNA assay.

Figure 3 shows sequence alignments of the relevant regions of the PCR fragments (primer and probe-binding sites) of the Roche assay (a) and the in-house assay (b).

With respect to the Roche assay, there are a considerable number of mismatches in both the primer and the probe-binding regions of B19V genotypes 2 and 3 sequences (Fig. 3a). The forward primer contains three mismatches. The reverse primer contains one mismatch in the B19V genotype 3 strain and two mismatches in the B19V genotype 2 strain. Notably, one mismatch (C→T) in the B19V genotype 2 reverse primer sequence is located at or near the 3′-end. Mutations at the 3′-end of a primer may result in no amplification. This is the most likely reason why sample 903321 is not detected by the Roche assay. Although sample 903321 is a B19V genotype 1 strain, it harbours the B19V genotype 2 typical C→T mismatch in the reverse primer. In this case, the mismatch probably leads to inefficient amplification rather than no amplification at all. This has been reported for the A6 genotype 2 B19V strain [5]. Inefficient amplification could explain the 100-fold difference between the Roche and the in-house assay. In order to investigate this further, sample 903321 containing 30 000 IU/ml (in-house assay) and the run control containing 10 000 IU/ml were amplified, using the Roche assay in the absence of the internal control. Both amplicons were analysed by agarose gel electrophoresis and staining with SYBR green. While it was clear that amplification had occurred, the stained band of sample 903321 was of reduced intensity compared to the run control, suggesting that the C→T mutation caused inefficient amplification (data not shown).

The probe-binding region of the Roche assay contains six mismatches in the B19V genotypes 2 and 3 sequences (Fig. 3a). These six mismatches only partly explain the detection

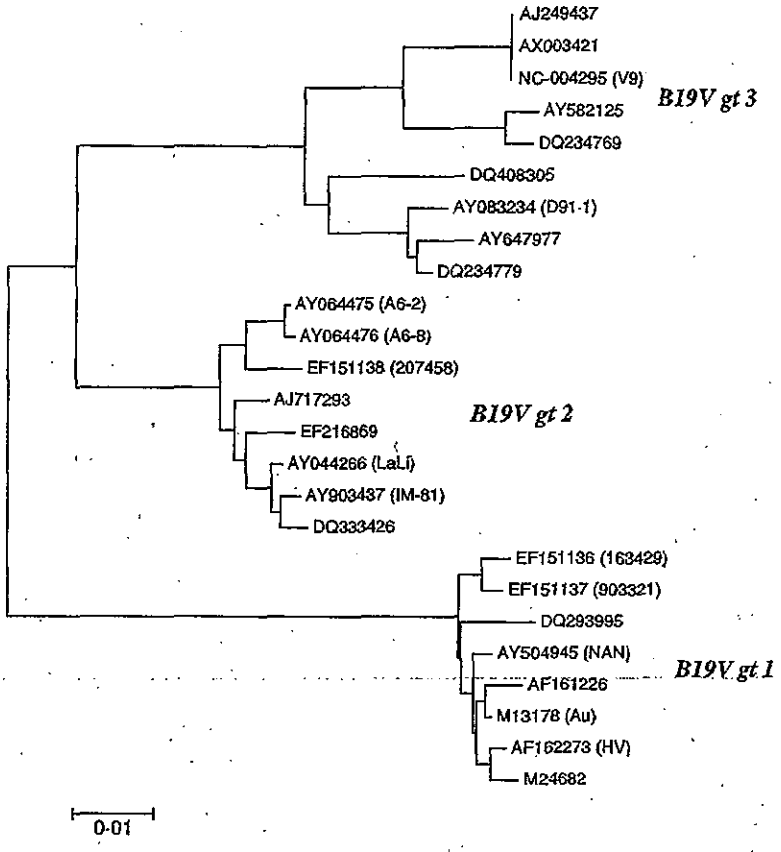

Fig. 2 Phylogenetic tree of parvovirus B19 (B19V) DNA sequences from three B19V variants (903321, 163429 and 207458) and B19V DNA sequences with published genotypes from GenBank. The sequence of a 1536-bp fragment corresponding to part of the NS1–VP1 region was used to create the tree.

(a) Roche B19V DNA assay

	FORWARD PRIMER	PROBE BINDING REGION	REVERSE PRIMER
B19V GENOTYPE 1	GGGGCAGCATGTGTTAAAGTGGATT	TACACTGTGGTTTTATGGGCCGCCAAGTACAGGAAAAACAAACTTG	ACTTTCCATTTAATGATGTAGCAGG
B19V run control	----------	----------	----------
903321	----------	----------	-T--------
163429	----------	----------	---------G---
B19V GENOTYPE 2	---T--A-----T----------	C--C---------C-----C--------T----------T---	-T---------G--
207458	---T--A-----T----------	C--C---------C-----T--------T----------T---	-T---------G--
B19V GENOTYPE 3	---T--A----------G-------	C--C---------C-----A--------T----------T---	---------G--

(b) In-house B19V DNA assay

	FORWARD PRIMER	PROBE BINDING REGION	REVERSE PRIMER
B19V GENOTYPE 1	AATGCAGATGCCCTCCAC	AACCCCGCGCTCTAGTAC	GGACCAGTTCAGGAGAATCAT
B19V run control	----------	----------	----------
903321	----------	----------	----------
163429	----------	-----T----------	----------
B19V GENOTYPE 2	----------	----------	----------
207458	----------	----------	----------
B19V GENOTYPE 3	----------	----------	----------

Fig. 3 Sequence of primers and probe-binding region of the Roche (a) and the in-house (b) B19V DNA assays. The B19V genotype 1 sequence AF162273 was used for reference. B19V sequences of samples 903321, 163429 and 207458 were aligned with the corresponding regions from B19V genotype 2 (A6 strain; AY064476), B19V genotype 3 (V9 strain; AX003421), and the B19V DNA run control. The length of both primers of the Roche test is assumed to be 25 bp. Identical nucleotides as compared to the reference sequence are indicated (-).

failure of the B19V genotype 2 sample 207458. In addition, the sequence of 207458 harbours three mismatches in the forward primer and the already mentioned C→T mismatch in the reverse primer. Of note, Fig. 3a also shows that the six mismatches in the probe-binding region are probably the main reason why the Roche assay is unable to detect B19V genotype 3 strains.

With respect to the in-house assay, sequence analysis of sample 163429 (B19V genotype 1) revealed a mismatch (C→T) in the probe-binding region (Fig. 3b). This mismatch appears to have led to a dramatic destabilization and a decreased hybridization temperature of the TaqMan probe. Indeed, the amplification signal of this sample could be partly restored when the standard annealing temperature of 60 °C

was lowered to 58 or 56 °C (data not shown). The same polymorphism was identified in a commercial factor VIII preparation (coded F8-87-A) produced in the USA with an expiry date of 1987. It was found to give a very shallow amplification plot very similar to that observed for the plasma 163429 (Fig. 1a).

Evaluation of a modified version of the in-house TaqMan assay

The in-house B19V DNA assay was performed using two different versions of the TaqMan probe. The original probe [5] was compared with a modified version containing LNA bases. In the modified probe, LNA bases were incorporated away from the site of the C→T polymorphism observed in strains 163429 and F8-87-A. The LNA bases were included to enhance hybridization to the target sequence by increasing thermal duplex stability and resulting in improvement of the amplification plot. Figure 1a,b shows the results where the two versions of the probe are compared. These amplification plots for strains 163429 and F8-87-A now appear very similar to the wild-type samples. There were no differences observed in the amplification and detection of the B19V controls whether the original version of the TaqMan probe was used or the one containing LNA bases.

Discussion

We applied a commercial (Roche) and an in-house B19V NAT assay for the prescreening of more than 2·6 million donations. The Roche assay was developed prior to the identification of B19V genotypes 2 and 3 and therefore only detects B19V genotype 1. The in-house assay was designed to include genotypes 2 and 3. Three high load B19V DNA samples were identified that gave discordant results between the two B19V DNA assays. Two of these samples (903321 and 163429) were classified as strains of B19V genotype 1. One of them (207458) was classified as B19V genotype 2 strain. To our knowledge, this is the second publication on B19V genotype 2 DNA in a donation of European origin. The first report came from Germany [18]. The conclusion from our study is that new, high viral load B19V genotype 2 infections are rare among blood donors tested by Sanquin, with only a single case identified in 2·6 million donations. This study confirmed the results from an earlier study, in which 321 manufacturing pools (representing more than 950 000 donations from The Netherlands) were tested with a genotype 2- and 3-specific PCR assay and no reactive pools were found (data not shown).

Several previous studies indicated that the prevalence of B19V genotypes 2 and 3 is very low among blood donors from Europe. Heegaard *et al.* [19] found no B19V genotype 3 sequences in 100 000 Danish blood donations. Hokynar *et al.* [14] analysed 140 160 Finnish blood donations and did not reveal any B19V genotype 2 or 3 positive donations. Candotti *et al.* [20] screened donations from the UK and sub-Saharan Africa for the presence of B19V genotypes. Genotype 3 B19V was found to be prevalent in donations from Ghana. Donations originating from the UK, Malawi and South Africa only harboured B19V genotype 1 sequences. Baylis *et al.* [5] tested 52 plasma pools from nine different manufacturers and did not detect any B19V genotype 2 or 3 sequences. These manufacturing pools were sourced from donations collected in Europe and North America. The study of Gierman *et al.* [21] representing a total of 1·5 million donations for US source plasma did also not reveal any B19V genotypes 2 and 3 sequences.

B19V genotype 2 sequences have been sporadically found in final container plasma products. Schneider *et al.* [22] reported B19V genotype 2 sequences in five out of 202 (2·5%) batches of clotting factor concentrates. Recent studies in looking at the persistence of B19V in tissue samples collected in Europe have suggested that in those people born before 1950, either genotype 1 or 2 B19V were found to be present, while those born after this date were predominantly infected with genotype 1 B19V [23]. This may explain why genotype 1 B19V is found so widely in the current blood donor population. This study focused on high load B19V infections rather than low load persistent infections. As the prevalence of low loads of B19V DNA in blood donors is around 1% [20], our study cannot exclude that there might be a significant number of B19V genotype 2 persistent infections, especially in older blood donors.

From the previously published studies [5,12,14], it was already known that the Roche assay was unable to detect B19V genotypes 2 and 3 variants. This study unravels the molecular reasons for this detection failure. With respect to both genotypes 2 and 3 B19V, there are three mismatches in the region of the forward primer. In the case of the reverse primer, there is a single mutation in genotype 3, while there are two mutations for genotype 2; one of these mutations is located at or very near the 3′-end of the primer region. This accounts for observations made in our previous study and in this present one, where there is a reduction in the amplification of genotype 2, when analysed by gel electrophoresis and compared to genotypes 1 and 3 [5]. While all three genotypes are amplified in the PCR, genotypes 2 and 3 are not detected in the real-time assay format, generating no amplification plots. This failure is a consequence of six mismatches found to be present in the region bound by the hybridization probes with these virus genotypes.

Two discrepant samples were classified as B19V genotype 1. Sample 903321 was under-quantified by 2 logs in the Roche assay due to one mismatch at or near the 3′-end of the reverse primer. The other sample 163429 was not detected by the in-house assay because of one mismatch (C→T) in the probe-binding region. This B19V polymorphism was also

detected in a clotting factor VIII concentrate manufactured in the 1980s. Recently, Baylis *et al.* [24] showed the effects of certain mutations in the binding site for TaqMan hydrolysis probes. The conclusion of this study is that the amplification signal correlates with the number of mismatches present in the hydrolysis probe. A single mismatch (G→A) in the wild-type probe-binding region only had a minor effect on the amplification signal. Where four mismatches were present, no amplification signal was observed. Interestingly, none of the described mismatches were C→T changes. This might explain the more dramatic results of our B19V variant where a single mismatch (C→T) caused an unexpectedly dramatic effect. Detection problems due to one C→T mismatch in the TaqMan probe have been described by Teupser *et al.* [25]. The C→T mismatch found in this study led to the misclassification of a polymorphism in the cholesteryl ester transfer protein. It appears that this particular mismatch can lead to dramatic destabilization and decreased hybridization temperature of the TaqMan probe. It is likely that the position of the mismatch within the probe and the adjacent nucleotides also plays a role. The in-house assay was designed to a region within the NS1 gene conserved between all known genotypes of B19V [5]. The identification of a polymorphism within this conserved region was unexpected. In order to improve the robustness of the in-house assay, the TaqMan probe was modified to incorporate LNA bases that counter for the effect of the C→T mutation by increasing the thermal duplex stability. In preliminary studies, the specificity and dynamic range of the test appear not to be impaired by the introduction of these modified nucleotides and more extensive validation studies are in progress.

Our study also shows that amplification curves generated with real-time PCR assays should be interpreted with great care. Sequencing analysis should be performed where unusual amplification patterns are observed.

A systematic approach to find molecular variants of B19V, undetectable or under-quantified with an established PCR assay can be achieved by using a second independent PCR assay. This study of B19V variants uses a generic extraction of nucleic acid. Subsequently, two different parts of the NS1 region are amplified to detect and quantify B19V.

It has recently been shown that the variation within the B19V genome is greater than that was previously believed [26]. Indeed, it was found that B19V had a surprisingly high rate of evolutionary change, at approximately 10^{-4} nucleotide substitutions per site per year. These observations, together with the data presented in this study, indicate that the variation in the B19V genome should be carefully monitored. Constant monitoring of B19V sequences in the population will help to ensure that primers and probes, based upon conserved sequences, are still applicable when variant viruses are identified. The nature of the genetic variation ranges from the identification of new genotypes, through to single nucleotide polymorphisms that can affect assay performance. Where new viral variants are identified, and this extends beyond B19V, kit manufacturers are faced with validation and regulatory challenges to vary existing tests or introduce new ones. Such changes impact upon the end-users implementing the tests. Prevalence studies of virus variants may be useful to determine whether it is necessary to broaden the scope of a particular test.

In summary, we identified one B19V genotype 2 strain and two B19V genotype 1 strains that were under-quantified or not detected at all by a commercial and an in-house B19V DNA assay while screening more than 2·6 million blood donations in plasma pools. As compared to B19V genotype 2 strains, the prevalence of B19V genotype 1 variants not detected by commercial or in-house assays might be in the same range or even higher.

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References

1 Anonymous: Human anti-D Immunoglobulin, monograph 0557 (since 1/1/2004); Human anti-D Immunoglobulin for intravenous administration, monograph 1527 (since 1/1/2004); Human plasma (pooled and treated for virus inactivation), monograph 1646 (since 1/7/2004); in *Ph Europe*, 6th edn, Strasbourg, France, Council of Europe, 2006

2 Koppelman MH, Cuypers HT, Emrich T, Zaaijer HL: Quantitative real-time detection of parvovirus B19 DNA in plasma. *Transfusion* 2004; 44:97–103

3 Weimer T, Streichert S, Watson C, Groner A: High-titer screening PCR: a successful strategy for reducing the parvovirus B19 load in plasma pools for fractionation. *Transfusion* 2001; 41:1500–1504

4 Aubin JT, Defer C, Vidaud M, Maniez MM, Flan B: Large-scale screening for human parvovirus B19 DNA by PCR: application to the quality control of plasma for fractionation. *Vox Sang* 2000; 78:7–12

5 Baylis SA, Shah N, Minor PD: Evaluation of different assays for the detection of parvovirus B19 DNA in human plasma. *J Virol Methods* 2004; 121:7–16

6 Aberham C, Pendl C, Gross P, Zerlauth G, Gessner M: A quantitative, internally controlled real-time PCR Assay for the detection of parvovirus B19 DNA. *J Virol Methods* 2001; 92:183–191

7 Nguyen QT, Wong S, Heegaard ED, Brown KE: Identification and characterization of a second novel human erythrovirus variant, A6. *Virology* 2002; 301:374–380

8 Nguyen QT, Sifer C, Schneider V, Allaume X, Servant A, Bernaudin F, Auguste V, Garbarg-Chenon A: Novel human erythrovirus associated with transient aplastic anemia. *J Clin Microbiol* 1999; 37:2483–2487

9 Hokynar K, Soderlund-Venermo M, Pesonen M, Ranki A, Kiviluoto O, Partio EK, Hedman K: A new parvovirus genotype persistent in human skin. *Virology* 2002; 302:224–228
10 Servant A, Laperche S, Lallemand F, Marinho V, De Saint MG, Meritet JF, Garbarg-Chenon A: Genetic diversity within human erythroviruses: identification of three genotypes. *J Virol* 2002; 76:9124–9134
11 Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (eds): *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses (ICTV)*. London, UK, Elsevier, 2005:360–361
12 Nubling M, Buchheit K-H: Results from recent EDQM/OMCL PTS studies (PTS052 and PTS064) on B19 DNA NAT testing of plasma pools; Presentation at the SoGAT meeting, 14 June 2006, Bern, Switzerland
13 Cohen BJ, Gandhi J, Clewley JP: Genetic variants of parvovirus B19 identified in the United Kingdom: implications for diagnostic testing. *J Clin Virol* 2006; 36:152–155
14 Hokynar K, Norja P, Laitinen H, Palomaki P, Garbarg-Chenon A, Ranki A, Hedman K, Soderlund-Venermo M: Detection and differentiation of human parvovirus variants by commercial quantitative real-time PCR tests. *J Clin Microbiol* 2004; 42:2013–2019
15 Boom R, Sol CJA, Salimans MMM, Jansen CL, Wertheim-van-Dillen PME, van der Noordaa J: Rapid and simple method for purification of nucleic acids. *J Clin Microbiol* 1990; 28:495–503
16 Johnson MP, Haupt LM, Griffiths LR: Locked nucleic acid (LNA) single nucleotide polymorphism (SNP) genotype analysis and validation using real-time PCR. *Nucleic Acids Res* 2004; 32:e55
17 Latorra D, Arar K, Hurley JM: Design considerations and effects of LNA in PCR primers. *Mol Cell Probes* 2003; 17:253–259
18 Blumel J, Eis-Hubinger AM, Stuhler A, Bonsch C, Gessner M, Lower J: Characterization of Parvovirus B19 genotype 2 in KU812Ep6 cells. *J Virol* 2005; 79:14197–14206
19 Heegaard ED, Panum JI, Christensen J: Novel PCR assay for differential detection and screening of erythrovirus B19 and erythrovirus V9. *J Med Virol* 2001; 65:362–367
20 Candotti D, Etiz N, Parsyan A, Allain JP: Identification and characterization of persistent human erythrovirus infection in blood donor samples. *J Virol* 2004; 78:12169–12178
21 Gierman TM: Industry experience with B19 virus reagents, test kits, and in-process testing of plasma donor samples. EDQM B19 virus test kit meeting, 9 November 2006, Strasbourg
22 Schneider B, Becker M, Brackmann HH, Eis-Hubinger AM: Contamination of coagulation factor concentrates with human parvovirus B19 genotype 1 and 2. *Thromb Haemost* 2004; 92:838–845
23 Norja P, Hokynar K, Aaltonen LM, Chen R, Ranki A, Partio EK, Kiviluoto O, Davidkin I, Leivo T, Eis-Hubinger AM, Schneider B, Fischer HP, Tolba R, Vapalahti O, Vaheri A, Soderlund-Venermo M, Hedman K: Bioportfolio: lifelong persistence of variant and prototypic erythrovirus DNA genomes in human tissue. *Proc Natl Acad Sci USA* 2006; 103:7450–7453
24 Baylis SA, Fryer JF, Grabarczyk P: Effects of probe binding mutations in an assay designed to detect parvovirus B19: implications for the quantitation of different virus genotypes. *J Virol Methods* 2007; 139:97–99
25 Teupser D, Rupprecht W, Lohse P, Thiery J: Fluorescence-based detection of the CETP TaqIB polymorphism: false positives with the TaqMan-based exonuclease assay attributable to a previously unknown gene variant. *Clin Chem* 2001; 47:852–857
26 Shackelton LA, Holmes EC: Phylogenetic evidence for the rapid evolution of human B19 erythrovirus. *J Virol* 2006; 80:3666–3669

別紙様式第 2-1

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-2

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2007 年 10 月 11 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	Improved detection of acute parvovirus B19 infection by immunoglobulin M EIA combined with a novel antigen EIA. Corcoran, A. et al, Vox Sanguinis, 93, 216-222 (2007).	公表国 アイルランド	
販売名（企業名）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
本稿では，ヒト血漿中における B19 ウイルス抗原を直接検出する酵素免疫測定法（EIA）について報告した。アッセイの検出限界は 10 pg/mL の組換え VP2 カプシドタンパク質であり，理論的には 1ml 中に 1.9x10E6 個の B19V 粒子を検出できることに相当する。当該アッセイを用いて 2003 年 2 月から 2004 年 7 月の間にオランダにおいて無症候ドナーから採取した 70 のウイルス血症性の献血検体(B19 DNA の濃度が 10E6 IU/mL を超える）を検査した。これらの検体は，低 pH の状態では B19 検出が大幅に増加することがわかった。興味深いことに，B19 抗原の検出は B19 の抗体（IgM 又は IgG）が共存することによって左右されなかった。さらに，本アッセイではヒトパルボウイルスの遺伝子型 1，2 及び 3 を同等に検出した。また，B19 抗原の EIA 法及び B19 IgM の EIA 法を合わせることで，B19V 感染初期と思われる（IgM が検出される）検体の 91%を検出した。B19 IgM 検出と B19 抗原検出を組み合わせた EIA 法は PCR に替わる最近の B19 感染の有効な検出法となると思われる。	BYL-2008-0298

報告企業の意見	今後の対応
本論文では，ヒト血漿中における B19 ウイルス抗原を直接検出する酵素免疫測定法（EIA）について報告した。抗原には P2 カプシドタンパク質を用いた。特に，B19 IgM 検出を組み合わせることで，効果的に感染初期のサンプルを検出可能であることを示している。本方法は，B19 ウイルス 1，2 及び 3 型を検出可能であり，測定感度も十分に高く，PCR に変わる測定方法として期待される。 弊社のポリグロビン N の製造に使用されるミニプール血漿においては，ヒトパルボウイルス B19 に対する NAT を実施しており，10E5 IU/mL 以上が確認された場合は，そのミニプール血漿は製造工程から除去している。現在の科学水準では，ヒトパルボウイルス B19 を確実に不活化する方法は存在しないため，感染リスクを完全に排除することはできないが，伝播の可能性は非常に低いと考える。	本稿で報告されたような，大規模試験に利用可能な測定法に関して今後とも情報収集に努める。

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ORIGINAL PAPER

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Improved detection of acute parvovirus B19 infection by immunoglobulin M EIA in combination with a novel antigen EIA

A. Corcoran,[1] S. Kerr,[1] G. Elliott,[1] M. Koppelman[2] & S. Doyle[3]

[1]Biotrin International, Dublin, Ireland
[2]Sanquin Diagnostic Services, Plesmanlaan 125, Amsterdam, The Netherlands
[3]National Institute for Cellular Biotechnology, National University of Ireland Maynooth, Co. Kildare, Ireland

Vox Sanguinis

Background and Objectives Although parvovirus B19 is a significant blood product contaminant, few methods other than polymerase chain reaction (PCR) have been developed to detect the presence of the virus.

Material and Methods A B19 antigen enzyme immunoassay (EIA) has been developed and the sensitivity of detection is ascertained using dilutions of the B19 capsid protein VP2 and 10-fold dilutions of B19 viraemic serum. Once the assay cut-off was established, a panel of viraemic donations (n = 70) was screened by the antigen EIA. The B19 immunoglobulin M (IgM) and IgG status of these specimens was also determined. During screening of blood donor units by quantitative PCR, 70 individuals were identified with levels of B19 DNA greater than 10^6 IU/ml at the time of blood donation.

Results The sensitivity of the B19 antigen EIA was estimated to be equivalent to between 10^8 and 10^9 IU/ml B19 DNA or 1–10 pg/ml of recombinant capsid protein. B19 detection was significantly enhanced when viraemic specimens were pretreated with a low pH proprietary reagent. Unlike other virus-detection assays, detection of the B19 antigen was not affected by the presence of B19 IgM or IgG antibodies. In addition, the assay was capable of detecting all three genotypes of human erythrovirus. Combined specimen analysis by the B19 antigen assay and a B19 IgM assay facilitated the detection of 91% of acute B19 infections in the test population.

Conclusion In combination with B19 IgM detection, application of the B19 antigen EIA is a flexible and efficient method of detecting recent B19 infection and can be used as an alternative to PCR.

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Introduction

Parvovirus B19 (B19V) infection of immunocompromised patients may result in severe morbidity and mortality [1,2]. Moreover, B19 infection of pregnant women may lead to fetal death [3]. The recent implementation of minipool polymerase chain reaction (PCR) screening procedures for pooled plasma, combined with mandatory European guidelines on acceptable B19 contamination of human immunoglobulin preparations (< 10 000 IU/ml B19 DNA), will minimize B19 contamination and improve the safety of pooled blood products [4,5]. However, the extremely high levels of B19 viraemia in recently infected individuals (10^{13} IU B19 DNA/ml) [6], asymptomatic B19 infections and the resilience of the virus to many of the virus-inactivation procedures mean that

Correspondence: Amanda Corcoran, Biotrin International, 93 The Rise, Mount Merrion, Co. Dublin, Ireland
E-mail: amanda.corcoran@biotrin.ie

B19 screening and elimination are still problematic [7,8]. Although PCR is currently the method of choice, contamination issues during screening [9], accurate erythrovirus genotype detection [10] and lack of individual donation screening necessitate continual evaluation of emerging technologies to ensure blood product safety.

Currently, B19 viral capsid protein production *in vivo* is detected by immunofluorescent staining and receptor-mediated haemagglutination (RHA) assays whereas viral DNA production is detected by PCR, dot blot hybridization and quantitative PCR (qPCR) [11–15]. RHA does not detect the B19 antigen at the required sensitivity in specimens that contain B19 IgG/M [11,15]. This is unacceptable especially when B19 IgG positive solvent/detergent-treated plasma, contaminated with B19 DNA, has been shown to transmit infection [16].

B19 antigen detection by enzyme immunoassay (EIA) is an alternative strategy for individual donor screening but may also be confounded by low assay sensitivity, differential reactivity between VP2 capsid and native B19 antigen detection and B19 antibody presence [17,18]. The B19 antigen assay described by Lowin *et al.* [18] has an apparent sensitivity of detection for recombinant VP2 capsids of 10^8 particles per ml; however, application of the assay to native B19 antigen detection was not demonstrated.

Using a Food and Drug Administration (FDA)-cleared B19 IgM EIA [19], Beersma *et al.* [20] have shown that in sera with B19 DNA levels greater than 10^6 per ml, B19 IgM reactivity always exceeds 3·0 (EIA cut-off = 1·0). Thus, it is clear that the presence of B19 VP2 IgM antibodies in sera is predictive for the presence of B19 DNA. This observation represents the first data unambiguously correlating B19 viral load with IgM antibody levels. Importantly, it also provides for an alternative strategy, employing simultaneous B19 IgM and antigen detection, to overcome the sensitivity issues pertaining to B19 antigen detection in individual donor units. Here, we show that such a strategy facilitates detection of B19 antigen levels in plasma donations.

Materials and methods

B19 antigen EIA optimization

Recombinant B19 VP2 capsids were expressed and purified as previously described [21] and were used for sheep and rabbit immunization. Affinity-purified sheep IgG (anti-B19 VP2) was coated onto microtitre plates (Nunc Maxisorp, Roskilde, Denmark) and the rabbit IgG (anti-B19 VP2) was conjugated to horseradish peroxidase (HRP), as described by Hermanson [22], and was used to detect captured B19 antigen.

Optimal IgG (anti-B19 VP2) plate-coating concentration (4 μg/ml) and conjugate dilution (1/4000 dilution) were established by testing B19-viraemic and non-viraemic plasma specimens. Dilutions of B19 VP2 capsids from 0·01 to 10 000 ng/ml were also analysed by the antigen EIA to determine the limit of detection in terms of protein concentration. The mean absorbance of the negative control for each batch of VP2 plus three standard deviations was used to set the assay cut-off value (COV).

To determine sensitivity in terms of B19 viral antigen detection, viraemic plasma was evaluated (qPCR testing was performed at the National Genetics Institute, CA, USA and results were reported in copies/ml). The mean absorbance of a panel of 201 non-viraemic human plasma samples plus three standard deviations was used to set the assay COV. This was matched to a dilution of a B19-viraemic plasma, which was used in all subsequent assays as a cut-off calibrator and facilitated determination of the positive or negative status of specimens tested on the antigen EIA.

Specimen preparation and final assay procedure

Test plasma and control specimens were diluted (1/5) in a low pH proprietary diluent (citrate buffer-containing detergents; available from Biotrin International Ltd., Dublin, Ireland) and were added to IgG (anti-B19 VP2) sensitized microwells (100 μl per well) for 1 h. Following a wash step, the rabbit IgG (anti-B19 VP2)–HRP conjugate was incubated in the wells for 30 min. Tetramethylbenzidine substrate (BioFX Laboratories Inc., Owings Mills, MD, USA) was added to the wells for 30 min. The reaction was terminated using 1 N sulphuric acid and the absorbance was measured at 450/630 nm. The presence of B19 antigen in a sample was determined by the absorbance ratio of specimen sample to cut-off calibrator sample (index value; IV). Specimens yielding index values ≥ 1·0 were classed positive while those < 1·0 were deemed negative.

Parvovirus B19 IgM and IgG

All specimens in this study were screened for B19 IgM and B19 IgG using commercial assays (Biotrin) as described previously [21].

Donor screening by B19 qPCR

The blood donor population in The Netherlands was screened for B19V over an 18-month period (February 2003–July 2004) using qPCR analysis as described previously [12]. Test pools of 480 were made from smaller pools of 48 donations. A pool identified with > 10^4 IU/ml B19 DNA was resolved via test pools of 48 donations and subsequently eight donations to trace the viraemic donor(s). Identified viraemic donations (n = 70) were then used to evaluate the B19 antigen EIA [12]. Results were expressed in IU/ml [23]. The copies-to-IU conversion factor has been calculated previously to be 3·34 [14].

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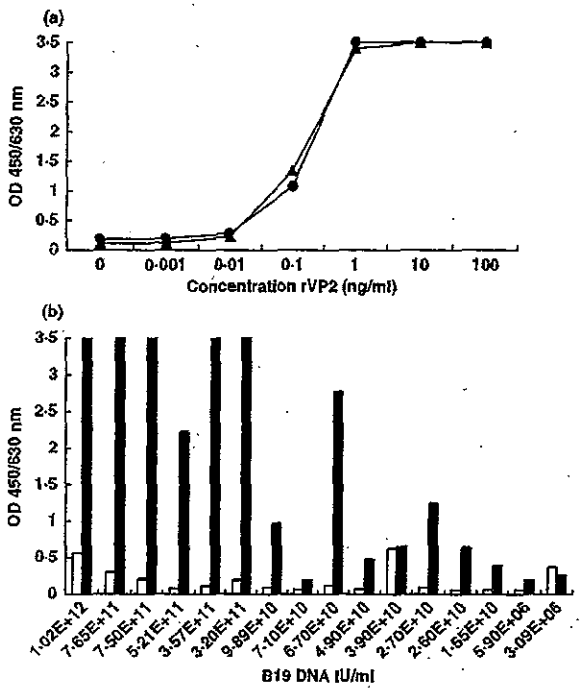

Fig. 1 Determination of B19 antigen enzyme immunoassay (EIA) assay sensitivity. (a) Two independent batches of recombinant capsid VP2 (rVP2), V056 (circles) and V057 (triangles) were decimally diluted to determine assay sensitivity. (b) Comparison of specimen diluents used in the detection of B19 viral capsids. Specimens were diluted in either Tris-buffered saline Tween-20 (TBST) (clear boxes) or a low pH proprietary reagent (filled boxes). Error bars represent the standard deviation from the mean.

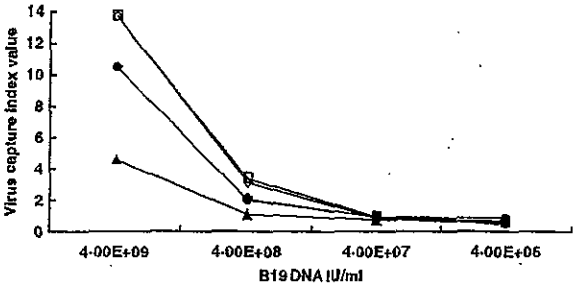

Fig. 2 Determination of antigen assay sensitivity using titrations of polymerase chain reaction (PCR)-quantified viraemic specimens. Viraemic plasma Bt72 (diamonds), Bt73 (squares), Bt80 (triangles) and genotype 2 Bt81 (circles) were decimally diluted in B19 negative serum to determine assay cut-off.

Results

Assay optimization and validation

Figure 1a shows identical standard curves [absorbance$_{450/630\,nm}$ vs. B19 recombinant VP2 capsid concentration (ng/ml)] generated from two independent batches of recombinant VP2 capsids in the B19 antigen EIA. These standard curves show that the minimal detectable level of B19 VP2 capsid detectable was 0·01 ng/ml, which theoretically equates to $1·9 \times 10^6$ viral particles per ml.

However, detection of B19 viraemic plasma in the same assay format required the implementation of an alternative specimen diluent (Fig. 1b). Here, dilution of viraemic specimens (n = 16) in a low pH, proprietary diluent, compared to using Tris-buffered saline Tween-20 (TBST), facilitated a considerable increase in virus capture in the majority of specimens (0- to 30-fold). Only one specimen ($3·9 \times 10^{10}$ IU/ml B19 DNA) that was negative for B19 IgM did not display a significant signal increase post-treatment, but did remain positive. Interestingly, the two specimens with the highest absorbance values in the assay without low pH pretreatment were IgM negative.

Non-viraemic plasma remained unreactive when subjected to the same pretreatment (data not shown). Assay specificity was determined by screening non-viraemic plasma (n = 20), all of which were unreactive in the antigen EIA based on the cut-off calibrator sample (data not shown).

The assay sensitivity (limit of detection) was estimated using dilutions of viraemic specimens and was shown to be approximately between 4×10^7 and 4×10^8 copies per ml B19 DNA (Fig. 2). However, the cut-off calibrator used in the EIA contained 10^9 copies per ml B19 DNA as determined by qPCR, which equates to 2×10^7 copies B19 DNA per microwell. To further define the limit of detection, plasma specimens (n = 17), containing a range of B19 DNA concentrations and B19 IgM/G reactivity, were subsequently screened in the antigen EIA. Table 1 shows that 53% (9/17) of specimens, all of which contained greater than $1·4 \times 10^{11}$ copies per ml B19 DNA, were also detectable in the antigen EIA. One specimen containing $7·2 \times 10^8$ copies per ml B19 DNA, which was B19 IgM reactive, tested borderline positive (IV = 1·0) in the antigen EIA. All remaining specimens, which contained less than $1·9 \times 10^7$ copies per ml B19 DNA and either B19 IgM or IgG or both, were unreactive in the antigen EIA.

Detection of the B19 antigen in the presence of specimen-derived B19-specific IgG or IgM is essential to avoid false negativity. Table 2 clearly illustrates that specimen-derived B19 antigen is detectable in the presence of both B19 IgG and IgM (n = 8), IgM only (n = 2) or IgG only (n = 3). Furthermore, B19 antigen is also detectable in specimens Bt72 and Bt73, which contained B19 IgM (Fig. 2). It is clear, therefore, that only B19 levels greater than 4×10^7 B19 DNA copies per ml are detectable in the antigen EIA and that the presence or absence of IgM or IgG in the specimen does not affect detection of the B19 antigen (Fig. 2 and Table 2). A specimen containing erythrovirus genotype 2 (specimen Bt81) was detected as well as erythrovirus genotype 1 (specimens Bt72, Bt73 and Bt80) in the antigen EIA (Fig. 2). Furthermore, erythrovirus genotype

Table 1 Parvovirus B19 detection by antigen enzyme immunoassay (EIA) and serological analysis (B19 IgM and IgG) of specimens previously quantified by polymerase chain reaction (PCR) (copies per ml). For the antigen EIA an index value (IV) ≥ 1·0 is positive (+) and < 1·0 is deemed negative (–). For both the B19 IgM and IgG EIA IV > 1·1 is positive; IV < 0·9 is negative; and IV between < 1·1 and IV > 0·9 is deemed equivocal (*eq*)

Sample identifier	IgM EIA	IV	IgG EIA	IV	qPCR (copies per ml)	Antigen EIA	IV
Cut-off calibrator	6·77	+	0·99	*eq*	$1·3 \times 10^{9}$	1·00	+
W P	0·80	–	0·14	–	$6·9 \times 10^{11}$	18·7	+
C4	0·26	–	0·06	–	$6·0 \times 10^{11}$	> 3·0	+
PL19	0·59	–	0·07	–	$5·6 \times 10^{11}$	> 3·0	+
C7	0·58	–	0·06	–	$5·5 \times 10^{11}$	> 3·0	+
C1	0·13	–	0·04	–	$4·8 \times 10^{11}$	> 3·0	+
C2	0·08	–	0·06	–	$4·6 \times 10^{11}$	> 3·0	+
C6	0·24	–	0·05	–	$3·3 \times 10^{11}$	> 3·0	+
C3	0·08	–	0·09	–	$3·9 \times 10^{11}$	> 3·0	+
PL9	0·11	–	0·06	–	$1·4 \times 10^{11}$	> 11·0	+
C5	2·02	+	0·17	–	$7·2 \times 10^{8}$	1·0	+
E R	3·0	+	8·1	+	$1·9 \times 10^{7}$	0·03	–
PL1	6·3	+	1·95	+	$1·6 \times 10^{7}$	0·39	–
C8	0·15	–	2·56	+	$2·6 \times 10^{4}$	0·04	–
D T	2·3	+	6·2	+	$7·4 \times 10^{3}$	0·07	–
R S	6·6	+	6·8	+	$8·9 \times 10^{3}$	0·42	–
PL20	0·11	–	4·78	+	550	0·42	–
PL16	0·2	–	4·80	+	200	0·39	–

Table 2 Effect of B19 IgM and IgG in plasma on the detection of B19 antigen. B19 antigen enzyme immunoassay (EIA) and serology results for plasma from patients with suspected B19 infection. For the antigen EIA an index value (IV) ≥ 1·0 is positive (+) and < 1·0 is deemed negative (–). For both the B19 IgM and IgG EIA an IV > 1·1 is positive; IV < 0·9 is negative; and IV between < 1·1 and > 0·9 is deemed equivocal (*eq*)

Sample Identifier	IgM EIA	IV	IgG EIA	IV	Antigen EIA IV
Cut-off calibrator	6·77	+	0·99	*eq*	1
931	0·14	–	0·70	–	18·6
420	0·16	–	0·90	*eq*	18·3
981	1·73	+	1·50	+	18·1
410	0·25	–	0·90	*eq*	18·1
375	0·14	–	0·70	–	18·1
939	0·30	–	0·80	–	18·0
889	4·99	+	1·70	+	17·9
976	0·17	–	1·20	+	17·8
441	3·40	+	0·80	–	17·6
973	0·28	–	1·28	+	17·3
966	1·92	+	1·46	+	17·3
936	1·21	+	1·40	+	16·3
444	0·86	–	1·00	*eq*	15·4
980	0·71	–	1·70	+	12·0
427	2·06	+	0·80	–	11·9
929	2·74	+	1·40	+	11·2
888	0·25	–	1·10	+	8·2
925	1·32	+	1·50	+	6·76
416	6·89	+	2·80	+	1·3
895	6·02	+	1·90	+	1·0

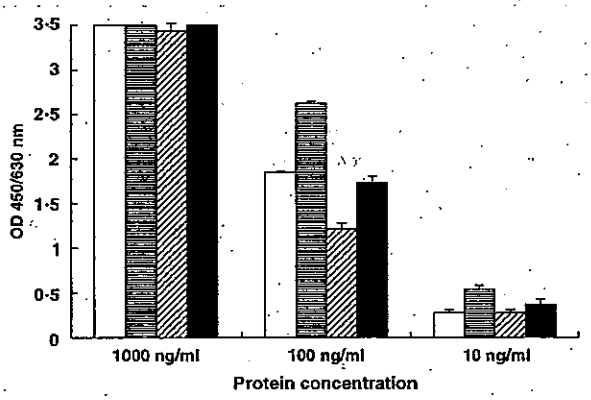

Fig. 3 Comparison of erythrovirus genotype 1 and 3 VP2 reactivity in the antigen enzyme immunoassay (EIA). Genotype 1 (clear and horizontal lined bars) and genotype 3 (diagonal lined and filled bars) recombinant VP2 was decimally diluted in either Tris-buffered saline Tween-20 (TBST) (clear and diagonal lined bars) or the proprietary low pH buffer (horizontal lined and filled bars). Error bars represent the standard deviation from the mean.

3 recombinant VP2 capsids exhibit indistinguishable reactivity in the assay to genotype 1 recombinant VP2 (Fig. 3).

Donor sample evaluation

During an 18-month period, approximately 1·4 million donations were tested for B19 DNA in The Netherlands [14], and 70 cases of asymptomatic donors (0·005%) with levels of B19 DNA greater than 10^{6} IU/ml were identified. Of these, 49/70 (70%) tested positive on the antigen EIA assay for B19

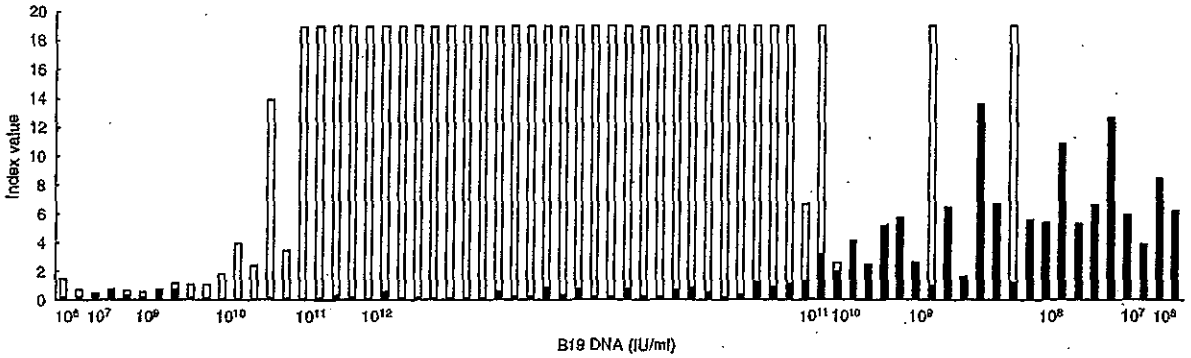

Fig. 4. A summary of the B19 antigen enzyme immunoassay (EIA) and immunoglobulin M (IgM) EIA reactivity of the panel of viraemic donors. An index value (IV) > 1·1 (denoted by line) is considered positive on both the B19 IgM EIA (filled bars) and antigen EIA (clear bars). The *y*-axis was truncated for clarity.

(range: $3{\cdot}1 \times 10^6$–$3{\cdot}2 \times 10^{12}$ IU/ml; mean: $1{\cdot}1 \times 10^{12}$ IU/ml, median: $1{\cdot}2 \times 10^{12}$ IU/ml B19 DNA) (Fig. 4). Thus, Fig. 4 depicts the combined B19 IgM and antigen EIA data of the 70 viraemic specimens, and the *x*-axis is arranged to show the rise (10^6–10^{12} IU/ml) and subsequent drop in viraemia with the development of B19-specific IgM antibodies (10^{12}–10^6 IU/ml). Testing further revealed that the panel of viraemic specimens was either pre- or early antibody seroconversion as none contained B19 IgG (data not shown).

There was a positive correlation (correlation coefficient $r = 0{\cdot}81$) between the level of B19 DNA (qPCR) and the level of B19 antigenemia (antigen EIA), but this relationship was not directly proportional. Concordance between qPCR and the antigen EIA was highest when viraemia titres were high ($> 1 \times 10^{11}$ IU/ml). Of the viraemic donor specimens, 27 (38·6%) tested positive (IV > 1·1) or borderline positive (two specimens were equivocal: IV ≤ 1·1, IV ≥ 0·9) for B19 IgM (Fig. 4). The specimens that were equivocal for IgM reactivity reacted strongly in the antigen EIA (IV > 19). The overlap between the two groups was considerable and 17% of the specimens tested positive for both B19 IgM and antigen (Fig. 4). Significantly, 91% of the viraemic donors were positive for either B19 IgM or antigen. Thus, these data clearly demonstrate that the combined implementation of a screening algorithm for B19 IgM and antigen readily facilitates the detection of specimens containing greater than 10^6 IU/ml B19 DNA equivalents.

Discussion

Here we describe a B19 antigen EIA for the direct detection of B19 antigen in human plasma. The detection limit of the assay was 0·01 ng/ml of purified recombinant VP2 capsids (which theoretically corresponds to $1{\cdot}9 \times 10^6$ viral particles per ml). Using dilutions of viraemic serum, the sensitivity was estimated at between 4×10^7 and 10^8 copies per ml B19 DNA equivalents. The antigen EIA was capable of detecting both erythrovirus genotypes 2 (virus) and 3 (recombinant capsids). When the antigen assay was used to test B19 viraemic donations, 70% tested positive of which had viral loads between $3{\cdot}1 \times 10^6$ and $3{\cdot}2 \times 10^{12}$ IU/ml.

B19 detection in plasma was greatly enhanced by specimen acidification. The low pH conditions may act by disrupting the viral capsid into its structural subunits, making it more accessible to the capture antibody. Although it was previously thought that B19V was highly resistant to physicochemical treatments, more recent work has shown the susceptibility of B19V to low pH treatment [24]. Boschetti *et al.* [24] showed that B19V was inactivated by greater than 5 logs after 2 h at pH 4 and that infectivity also decreased.

When the antigen assay was performed at physiological pH, the specimens that gave the highest absorbance values were B19 IgM negative, implying immune complexes hinder detection. However, when specimens were prepared in low pH conditions, neither the presence of IgM nor IgG, even at high levels, affected the detection of B19 (Table 2). It is probable that acidification caused the dissociation of any immune complexes present. False-negative results due to immunocomplexes present a problem for B19 RHA assays, which exploit the binding of a B19V receptor to red blood cells [11]. Hence, the RHA assay is ineffective for antigen detection in specimens that have seroconverted a problem resolved by the B19 antigen EIA.

B19 detection by PCR has a greater sensitivity, but such assays have many disadvantages (e.g. potential cross-contamination) not shared with an EIA. First, although erythrovirus genotypes may diverge significantly at the genomic level [25,26], requiring primer optimization [13], there does not appear to be any antigenic or immunological differences between the genotypes. The antigen EIA could identify genotype 2 erythrovirus and genotype 3 recombinant VP2 capsids at the same sensitivity as genotype 1. This is supported by the fact that all three erythrovirus genotypes can haemagglutinate human red blood cells and also infect myeloid cells with equal efficiency [27]. Second, the significance of DNA in plasma postviraemia

is unclear as low levels of B19 DNA can persist for several years post-infection, even after IgM is lost and IgG reactivity has been established [28]. A virus detection assay, however, allows simultaneous testing of hundreds of specimens, is suitable for large-scale screening, is more economical and has a shorter time to result.

Combined B19 antigen and IgM EIA analysis of the viraemic donor specimens revealed that 91% of the donor specimens could be diagnosed as acute infection using this screening algorithm. Previously, clinical samples taken from individuals with a suspected B19 infection, which had a level of B19 DNA greater than 10^5 IU/ml, were shown to be positive for specific IgM also [20]. This was not the case with the Dutch donor specimens herein, as this panel was from asymptomatic individuals whose infection was detected due to routine screening. Donor specimens, therefore, would be from all stages post-infection including the preseroconversion stage. Experimental infection has shown that B19 infection has two phases [29], characterized by symptom-free initial high viraemia (~10^{11} copies per ml serum) followed by detectable IgM antibody and appearance of symptoms such as rash and arthralgia. IgM seroconversion causes a rapid decline of viral titre. The 70 viraemic specimens identified in this study showed a typical viraemia and IgM seroconversion pattern (Fig. 4), confirming that the donor samples are representative of all stages of acute infection.

It is important to confirm the diagnosis of acute B19 infection in a public health setting where an outbreak could lead to serious medical consequences, especially for pregnant women and immunocompromised patients. In addition, B19 screening of blood donors prior to donation would avoid the risk of contaminating blood products. The B19 antigen EIA in conjunction with specific B19 IgM detection offers an effective method of detecting acute infection.

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References

1 Kurtzman GJ, Cohen BJ, Meyers P, Amunullah A, Young NS: Persistent B19 parvovirus infection as a cause of severe chronic anemia in children with acute lymphocytic leukaemia. *Lancet* 1988; 2:1159–1162

2 Wildig J, Michon P, Siba P, Mellombo M, Ura A, Mueller I, Cossart Y: Parvovirus B19 infection contributes to severe anemia in young children in Papua New Guinea. *J Infect Dis* 2006; 194:146–153

3 Enders M, Weidner A, Zoellner I, Searle K, Enders G: Fetal morbidity and mortality after acute human parvovirus B19 infection in pregnancy: prospective evaluation of 1018 cases. *Prenat Diagn* 2004; 24:513–518

4 European Pharmacopoeia: *European Pharmacopoeia Monograph of Human anti-D Immunoglobulin*. European Pharmacopoeia, Strasbourg, France, 2004; Document 01:0557

5 Groeneveld K, van der Noordaa J: Blood products and parvovirus B19. *Neth J Med* 2003; 61:154–156

6 Prowse C, Ludlam CA, Yap PL: Human parvovirus B19 and blood products. *Vox Sang* 1997; 72:1–10

7 Santagostino E, Mannucci PM, Gringeri A, Azzi A, Morfini M, Musso R, Santoro R, Schiavoni M: Transmission of parvovirus B19 by coagulation factor concentrates exposed to 100 degrees C heat after lyophilization. *Transfusion* 1997; 37:517–522

8 Laub R, Stengers P: Parvovirus B19 and blood products. *Pathol Biol* 2002; 50:339–348

9 Hausler M, Scheithauer S, Ritter K, Kleines M: Molecular diagnosis of Epstein-Barr virus. *Expert Rev Mol Diagn* 2003; 3:81–92

10 Baylis SA, Shah N, Minor PD: Evaluation of different assays for the detection of parvovirus B19 DNA in human plasma. *J Virol Methods* 2004; 121:7–16

11 Sakata H, Ihara H, Sato S, Kato T, Ikeda H, Sekiguchi S: Efficiency of donor screening for human parvovirus B19 by the receptor-mediated hemagglutination assay method. *Vox Sang* 1999; 77:197–203

12 Koppelman M, Cuypers T, Emrich T, Zaaijer H: Quantitative real-time detection of parvovirus B19 DNA in plasma. *Transfusion* 2004; 44:97–103

13 Aubin JT, Defer C, Vidaud M, Maniez Montreuil M, Flan B: Large-scale screening for human parvovirus B19 DNA by PCR: application to the quality control of plasma for fractionation. *Vox Sang* 2000; 78:7–12

14 Wong S, Brown KE: Development of an improved method of detection of infectious parvovirus B19. *J Clin Virol* 2006; 35:407–413

15 Takeda Y, Wakisaka A, Noguchi K, Murozuka T, Katsubayashi Y, Matsumoto S, Tomono T, Nishioka K: Receptor-mediated haemagglutination screening and reduction in the viral load of parvovirus B19 DNA in immunopurified Factor VIII concentrate (Cross Eight M). *Vox Sang* 2001; 81:266–268

16 Doyle S, Corcoran A: The immune response to parvovirus B19 exposure in previously seronegative and seropositive individuals. *J Infect Dis* 2006; 194:154–158

17 O'Keeffe S, O'Leary D, Doyle S, Kilty C, Kerr S: The detection of parvovirus B19 in human sera using antigen-capture EIA. Poster presented at the Society for General Microbiology (Irish Branch), National University of Ireland, Maynooth, Co. Kildare, Ireland, 24–25 April 2003

18 Lowin T, Raab U, Schroeder J, Franssila R, Modrow S: Parvovirus B19 VP2-proteins produced in *Saccharomyces cerevisiae*: comparison with VP2-particles produced by baculovirus-derived vectors. *J Vet Med B Infect Dis Vet Public Health* 2005; 52:348–352

19 Doyle S, Kerr S, O'Keeffe G, O'Carroll D, Daly P, Kilty C: Detection of parvovirus B19 IgM by antibody capture enzyme immunoassay: receiver operating characteristic analysis. *J Virol Methods* 2000; 90:143–152

20 Beersma MF, Claas EC, Sopaheluakan T, Kroes AC: Parvovirus B19 viral loads in relation to VP1 and VP2 antibody responses in diagnostic blood samples. *J Clin Virol* 2005; 34:71–75

21 Kerr S, O'Keeffe G, Kilty C, Doyle S: Undenatured parvovirus B19 antigens are essential for the accurate detection of parvovirus IgG. *J Med Virol* 1999; 57:179–185

22 Hermanson GT: Heterobifunctional Cross-Linkers; in *Bioconjugate Techniques.* San Diego, Academic Press, 1996:228–286

23 Saldanha J, Lelie N, Yu MW, Heath A: B19 Collaborative Study Group: Establishment of the first World Health Organization International Standard for human parvovirus B19 DNA nucleic acid amplification techniques. *Vox Sang* 2002; 82:24–31

24 Boschetti N, Niederhauser I, Kempf C, Stuhler A, Lower J, Blumel J: Different susceptibility of B19 virus and mice minute virus to low pH treatment. *Transfusion* 2004; 44:1079–1086

25 Nguyen QT, Wong S, Heegaard ED, Brown K: Identification and characterisation of a second novel human erythrovirus variant, A6. *Virology* 2002; 301:374–380

26 Servant A, Laperche S, Lallemand F, Marinho V, De Saint Maur G, Meritet JF, Garbarg-Chenon A: Genetic diversity within human erythroviruses: identification of three genotypes. *J Virol* 2002; 76:9124–9134

27 Hokynar K, Ekman A, Kantola K, Kakkola L, Hedman L, Bonden H, Aberham C, Gessner M, Garbarg-Chenon A, Hedman K, Soderlund-Venermo M: Biological and immunological relation of the three variants of human parvovirus B19. Poster presented at the XIth Parvovirus Conference, Les Diablerets, Switzerland, 27–31 August 2006

28 Cassinotti P, Siegl G: Quantitative evidence for persistence of human parvovirus B19 DNA in an immunocompetent individual. *Eur J Clin Microbiol Infect Dis* 2000; 19:886–887

29 Anderson MJ, Higgins PG, Davis LR, Willman JS, Jones SE, Kidd IM, Pattison JR, Tyrrell DA: Experimental parvovirus infection in humans. *J Infect Dis* 1985; 152:257–265

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研究報告の概要

ヒト血液製剤のパルボウイルス B19 汚染に関するゲノム増幅法の標準化についての国際ワーキンググループ会議（2007 年 3 月開催）で協議された議題の要約である。
パルボウイルス B19 の新規の遺伝子型が発見されていることから，規制基準を満たすためには核酸増幅法（NAT）による血漿プールのスクリーニング手順を更新する必要がある。そのため，本会議は，管理分析機関及び血漿分画製剤の製造者間でのパルボウイルス B19 の種々の遺伝子型の検出及び定量結果を統一する方法を見いだす目的で開催された。パルボウイルス B19 の全 3 種の遺伝子型は極めて似通っており，in vitro 試験で感染性の差は認められなかった。遺伝子型 1 及び 2 は，熱又は低 pH 条件に対し同等に不活化されることが知られている。さらに，先に示した文献［BYL-2008-0297］に記載の結果も本会議で提示された；米国人及びヨーロッパ人の血漿ドナーにおける遺伝子型 2 及び 3 の保有率は非常に低く，ガーナにおけるパルボウイルス B19 感染は大部分が遺伝子型 3 に起因していた。
本会議では，特性が十分に明らかになっている標準物質を用いたアッセイの標準化について合意が得られた。これにより，パルボウイルス B19 の種々の遺伝子型を示す血漿検体パネルが作成されることが示唆される。また，会議中，パルボウイルス B19 株の新規 DNA 配列がある場合はデータベースに蓄積し，閲覧可能な状態にしておくべきであることも強調されていた。

報告企業の意見	今後の対応
パルボウイルス B19 の新規の遺伝子型が発見されており，規制基準に対応するためには，パルボウイルス B19 の検出アッセイを新たに開発するか，又は高感度，或いは特異的なアッセイにする必要があると考えられる。 弊社のポリグロビン N の製造に使用されるミニプール血漿においては，ヒトパルボウイルス B19 に対する NAT を実施しており，10E5 IU/mL 以上が確認された場合は，そのミニプール血漿は製造工程から除去している。現在の科学水準では，ヒトパルボウイルス B19 を確実に不活化する方法は存在しないため，感染リスクを完全に排除することはできないが，伝播の可能性は非常に低いと考える。	引き続き，パルボウイルス B19 の亜型の検出や疫学に関する研究の進展の情報収集に努める。

使用上の注意記載状況・その他参考事項等
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Standardization of nucleic acid amplification technique (NAT)-based assays for different genotypes of parvovirus B19: a meeting summary

S. A. Baylis

Division of Virology, National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire, UK

Vox Sanguinis

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An extraordinary meeting of the International Working Group on the Standardization of Genome Amplification Techniques for the safety testing of blood, tissues and organs for blood borne pathogens was held on 2 March 2007, at the National Institute for Biological Standards and Control. The aim of the meeting was to investigate ways to harmonize results obtained for the detection and quantification of different genotypes of parvovirus B19 (B19V) DNA by control laboratories and manufacturers of plasma derivatives. The meeting explored issues of B19V such as the classification of B19V strains, the prevalence and distribution of different genotypes, the clinical and biological significance of different genotypes, the detection of different genotypes in plasma-derived products, and their susceptibility to virus-inactivation procedures. At this meeting and through subsequent studies, high titre, high volume samples have been identified representing different genotypes of B19V, which will be evaluated by collaborative study to prepare reference panels for the purposes of assay validation.

Key words: erythrovirus B19V, plasma screening, B19V variants.

Introduction

Since 2004, European regulatory requirements have meant that plasma used in the production of anti-D immunoglobulin and pooled human plasma treated for virus inactivation must be screened to ensure that levels of parvovirus B19 (B19V) DNA do not exceed 10 IU/μl [1–3]. Plasma donations containing high titres of B19V are removed by the manufacturers of plasma derivatives, and the appropriate pools are tested by a group of European Official Medicines Control Laboratories (OMCLs) for subsequent batch release. Screening is performed using nucleic acid amplification technique (NAT)-based assays for B19V DNA. The introduction of these regulatory requirements was underpinned by the establishment of the first World Health Organization (WHO) International Standard for B19V DNA (NIBSC code 99/800) [4]. The discovery that B19V was more genetically diverse than was originally thought, forming three genotypes [5] has led to a review of testing procedures. Strains, representing each of the two more recently identified genotypes, have now been formally classified as B19V by the International Committee on the Taxonomy of Viruses (ICTV) [6]. This classification has led to regulatory issues. The guidelines for validation of quantitiative NAT assays for B19V, due to be published in the European Pharmacopoeia (Ph. Eur.), recommend that all genotypes of B19V should be detected. Recent Proficiency Testing Schemes (PTS), run by the European Directorate for the Quality of Medicines (EDQM), who coordinate the OMCL network, have highlighted discrepant results, when samples representing different genotypes of B19V have been included in the panels [7]. This was discussed further at a meeting held at the EDQM in Strasbourg on 9 November 2006, which focused on some of the issues with the types of commercial NAT assays available for the detection and quantification of B19V DNA. In an effort to harmonize results obtained by control laboratories and plasma fractionators, an extraordinary meeting of Standardization of Genome Amplification Techniques (SoGAT) was held at National Institute for Biological Standards and Control (NIBSC) on 2 March 2007. The aim of the meeting was to identify ways to provide appropriate reference materials, to support the

Correspondence: Sally A. Baylis, Division of Virology, National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QG, UK
E-mail: sbaylis@nibsc.ac.uk

implementation of these regulations and to discuss how best to respond to changes in the molecular epidemiology of viruses.

Classification of B19V

Dr K. Brown [Health Protection Agency (HPA), UK] described the criteria used by the ICTV to classify viruses. Classification by the ICTV does not extend beyond species and no consideration is given to either genotypes or clades. In the case of B19V, it is classified as a member of the *Parvoviridae* family, belonging to the erythrovirus genus. While sequence comparisons are becoming increasingly important in classification, other criteria are considered including mode of replication, virus structure, genomic organization, transcriptional and biological properties. The ICTV has classified the recently identified variant viruses, specifically V9, originally identified in France [8], A6 [9] and LaLi [10] as strains of B19V [6]. The genetic diversity of the B19V strains falls into three well-recognized genotypes [5], which can be confirmed by pairwise sequence identity profiles. Nucleotide divergence is approximately 10–15% between the different genotypes. These B19V strains are clearly distinct from other erythroviruses, such as the primate parvoviruses viruses [pig-tailed macaque parvovirus (PmPV), rhesus macaque parvovirus (RmPV) and simian parvovirus (SPV)], and more distant, tentative members such as bovine parvovirus 3 and chipmunk parvovirus. Dr Brown mentioned that the two recently identified human parvoviruses, that is, human parvovirus PARV4 [11,12] and human bocavirus [13], are quite distinct from B19V and would not be discussed further during the meeting.

Regulatory issues

Dr J.-M. Spieser (EDQM, Strasbourg, France) summarized the B19V test kit meeting held at EDQM on 9 November 2006, in response to differences in the ability of laboratories to detect genotype 2 B19V. Genotype 2 B19V has been identified in plasma pools that are undergoing batch release in Europe. The assay available from Roche is suitable only for the detection of genotype 1 B19V. The Artus (Qiagen, Hamburg, Germany) assay detects genotype 2 and some of the genotype 3 viruses. Both companies are addressing the shortfalls in the current assay kits. Currently, the Official Control Authority Batch Release (OCABR) guidelines require the detection of genotype 1 B19V, and recommend the detection of viruses such as A6 and V9 [14]. The batch release advisory group have endorsed the proposal that the guideline for B19V should be updated to reflect the requirement for the detection of different virus genotypes and be mandated in the Ph. Eur.

Dr M. W. Yu (Center for Biologics Evaluation and Research; CBER, Bethesda, MD, USA) reviewed the US Food and Drug Administration's (FDA) previous discussions on NAT testing for B19V in the USA. Most source plasma fractionators perform in-process B19V NAT testing, excluding high-titre donations following mini-pool testing. Blood collection establishments voluntarily retrieve and discard in-date components from donors with high titres of B19V DNA, to prevent their use in transfusion recipients. An infusion of a coagulation factor VIII product devoid of any anti-B19V, which was derived from plasma unscreened for B19V by NAT in a mini-pool format, with an overall load of B19V DNA as low as 2×10^4 IU, has been shown to transmit in a seronegative recipient [15]. For manufacturing pool B19V NAT testing, the FDA is currently proposing a limit of $\leq 10^4$ IU/ml for all plasma-derived products. The FDA has reviewed and approved some in-house B19V NAT procedures, for mini-pools and manufacturing pools under the Biologics Licensing Applications or their supplements for plasma derivatives. B19V NAT assays are required to be validated as analytical procedures and should be capable of detecting all virus genotypes. In the future, the FDA may consider B19V testing as donor screening, because of known risks in individuals with chronic anaemia, those who are pregnant or immunocompromised. Such screening would be dependent upon the availability of suitable commercial kits and sufficient resolution time.

Prevalence and clinical properties of different genotypes of B19V

Ms K. Hokynar (Haartman Institute, Helsinki, Finland) described studies where B19V DNA was identified in skin biopsies. Sequence analysis identified more divergent viruses, now recognized as genotype 2 B19V [10]. Analysis of tissue samples from North West Europe failed to identify genotype 3 B19V; however, genotypes 1 and 2 were both readily identified individuals born prior to 1950, while those born after this date were predominantly infected with genotype 1 [16]. *In vitro* studies of the three genotypes showed no differences in infectivity or in the activity of the p6 promoter, which is most efficient in cells permissive for B19V infection, and enhanced by the expression of NS1 [17]. Serological cross-reactivity is observed between B19V genotypes 1 and 2 using recombinant antigens and sera from individuals infected with specific genotypes [17]. All three genotypes of B19V are extremely similar, constituting a single serotype, with amino acid divergence for VP1 no greater than 4%.

Professor S. Modrow (University of Regensburg, Germany) described a clinical case, where a renal transplant recipient developed transient anaemia and arthritis and was diagnosed with B19V. Detailed molecular analysis revealed that the patient was infected with a genotype 2 B19V. A review of the original assays performed showed that there was differential sensitivity for the different genotypes of B19V. The patient showed persistent, high levels of B19V DNA [$> 10^{11}$ genome equivalents (geq)/ml] and episodes of severe anaemia [18]. Treatment with intravenous immunoglobulin (IVIG) lowered

viral loads and resolved anaemia. After 4 years, B19V DNA and anti-B19V IgM antibodies were still detectable. The patient subsequently started to develop anti-B19V IgG antibodies. Both IgG reactivity and avidity were comparable in sera from genotypes 1 and 2 B19V-infected individuals, when challenged by enzyme-linked immunosorbent assay (ELISA) using antigen from the VP1-unique region from all three genotypes. It was noted that this case presented in a very similar way to ones seen with a genotype 1 B19V infection.

Epidemiological studies of B19V infection in blood donors, pregnant women and children in Ghana were described by Dr D. Candotti (University of Cambridge, UK). In Ghana, approximately 8% of children have anti-B19V IgG, rising to 80% in adults. Viral loads and levels of anti-B19V IgM are higher in children. The rate of persistent infection is ~1·4%. One of the most striking observations of B19V infection in Ghana is that the circulating viruses are almost all genotype 3 [19]. DNA sequence analysis has revealed that the genotype 3 viruses can be divided into two subtypes or clusters that differ by more than 5% nucleotide identity [20]. These have been termed 3a and 3b, and the clustering is independent of the region of the B19V genome analysed. The nucleotide substitution rates were examined for B19V in Ghana and compared with V9, the prototype genotype 3 virus, identified nearly 10 years ago [8]. It was found that like genotype 1 B19V and canine parvoviruses, the genotype 3 viruses have an unexpectedly high rate of evolutionary change [21,22]. It would appear that the type 3a and 3b clusters were derived from a common ancestor approximately 500 years ago; however, there is a wide interval around this date.

Presence of different B19V genotypes in plasma products and susceptibility to inactivation

Professor A.-M. Eis-Hübinger (University of Bonn, Germany) reviewed studies performed on factor VIII and factor IX concentrates to determine the frequency of contamination of these products with genotypes 1 and 2 of B19V. A total of 202 different lots of clotting factor concentrates were examined. Older products used until the early 1980s that had not undergone viral inactivation procedures (21 lots, representing eight different products) were compared with more recent batches in use between 2000 and 2003 (181 lots, representing 13 different products). In the factor VIIIs, 81% were contaminated with genotype 1 B19V, and 14% were contaminated with genotype 2. In the more recent factor VIIIs, 46% were contaminated with genotype 1 B19V and 1·6% were contaminated with genotype 2 (two products were co-contaminated with genotype 1). The highest loads of genotype 1 and genotype 2 B19V were ~10^7 and ~10^5 geq/ml, respectively. It was suggested that the much lower frequency of detection of genotype 2 B19V is due to generally lower prevalence compared to genotype 1 [23].

Dr M. W. Yu (CBER) described a study looking at factor VIII concentrates using a consensus polymerase chain reaction (PCR) for genotypes 1–3 for B19V, followed by specific restriction endonuclease digestion of the product to discriminate genotype 1 from genotypes 2 and 3. A range of products (n = 202 lots) of differing purity produced before 1984 until 2004 were analysed. Of these, 79 lots were positive in the initial screening assay, and a single lot, from 1997, was positive for B19V genotype 2. DNA sequence analysis confirmed the genotype of this virus. This final product did not contain any genotype 1 B19V, and the load for genotype 2 was 10^3 geq/ml. In the case of more recent lots, contamination with B19V was generally less frequent, reflecting the implementation of NAT screening by the manufacturers [24].

Dr M. Nübling (Paul Ehrlich Institute; PEI, Langen, Germany) presented data on behalf of Dr J. Blümel (PEI) comparing the biological and physicochemical properties of B19V genotypes 1 and 2 (isolate IM-81) [25]. Infection of the cell line KU812Ep6 with the two B19V genotypes revealed that there were no differences in expression of the capsid at either the mRNA or protein level. Thermal inactivation occurs through the disintegration of the capsid proteins, with no differences observed between the two genotypes. Virions were heated and subjected to DNAse treatment prior to Southern blotting to analyse the integrity of the viral genomic DNA. Treatment of 5% albumin solution, spiked with B19V and heated to 56 °C, resulted in the same temporal inactivation kinetics, regardless of virus genotype. Similar inactivation profiles were observed for genotype 2 B19V, as had been shown previously for genotype 1 B19V virus, when subjected to low pH conditions [26].

Professor Jean-Pierre Allain (Cambridge) outlined a PCR inhibition method, utilizing a preamplification step to quantify B19V inactivation by photochemical treatment using amotosalen (S59). This molecular approach to measuring the inactivation effects of S59 on B19V has been established as an alternative to *in vitro* culture of the virus [27].

Experience with commercial and in-house assays for the detection and quantification of B19V DNA

The performance of two commercially available kits for the quantification of B19V DNA was reviewed by Dr S. Baylis (NIBSC, UK). The first kit, the Roche parvovirus B19 quantification kit for the LightCycler, only detects genotype 1 B19V. When equivalent copy number (10^5) were analysed for the three genotypes, no amplification plots were observed for genotypes 2 and 3 B19Vs in this real-time assay. However, analysis of amplification products by gel electrophoresis revealed that all three genotypes were amplified, with a much reduced signal for genotype 2 B19V, suggesting mismatches in primer and probe sequences. In the case of the Artus *RealArt* Parvo

LC kit, good amplification plots were observed for genotypes 1, 2 and 3a of B19V, while the genotype 3b virus was under quantified by approximately 2–3 logs generating much later threshold cycle (Ct) values, which could have an impact on the threshold concentration of 10 IU/ml applying to certain plasma pools [28–30]. Primer and probe sequences are of critical importance in the detection of variant viruses, this is further complicated with requirements to perform quantitative assays.

Dr T. Cuypers (Sanquin, Amsterdam, the Netherlands) described the experience of running two assays concurrently for B19V in a screening centre. The assays included the commercially available Roche LightCycler assay and a previously published consensus assay [28], validated in-house. Screening assays, performed during the previous 2 years, identified three instances where discrepant results occurred between the two tests. Molecular characterization was performed to identify the reasons for the discrepant results. One sample, not detected in the Roche assay was found to be a genotype 2 B19V, containing mutations in the primer and probe binding regions. A genotype 1 sample was under quantified by ~2 $\log_{10}$ in the Roche assay compared with the in-house assay, with a mutation at or near the end of the reverse primer binding region in the Roche test. In a third case, there was a single point mutation in the probe binding site of the in-house assay, which resulted in a failure to detect a genotype 1 B19V in the plasma sample [31]. Genotypes 2 and 3 for B19V appear to be very rare in Dutch and Belgian donors.

Dr Marta José (Grifols, Barcelona, Spain) described the validation of both in-house qualitative and quantitative consensus B19V assays, for the detection of all three genotypes. Validation was performed according to current guidelines. Particular attention was paid to B19V assay specificity, with no cross-reactivity observed with other blood borne viruses. A variety of genotype 2 and genotype 3 B19V-positive plasma samples were analysed, and good correlations were found with previously determined titres from other laboratories and the ones determined by the in-house quantitative assays.

Dr T. Gierman (Talecris, Raleigh, NC, USA) was unable to attend the meeting and his presentation on experience in testing for B19V genotypes was summarized by Dr Zerlauth.[1]

Three tests are utilized to reduce B19V viral loads in plasma fractionation pools: a qualitative donor sample test for testing mini-pools; a separate qualitative test for the QC of fractionation pools; and a quantitative test using dual-labelled fluorogenic detection probes for quality and technical operations investigations. As part of on-going efforts to assess the performance of this test system, the potential frequency of occurrence of variant B19V genotypes in US source plasma was examined. Archived sample pools created from 'non-elevated' plasma samples (samples containing B19V genotype 1 titres > 2×10^5 IU/ml and genotypes 2 and 3 titres > 5×10^6 copies/ml would have been excluded as a result of screening with the donor sample test) were retested using a fluorogenic detection probe capable of differentially detecting B19V genotypes 2 or 3. The testing of 242 large-scale sample pools (3840 samples) and 609 intermediate-scale pools (960 samples) failed to identify the presence of B19V genotypes 2 and 3. PCR analysis of 340 individual 'elevated' samples also failed to identify B19V genotypes 2 or 3 among them. The inability to detect B19V genotypes 2 and 3 in material representing approximately 1·5 million source plasma donations, which suggests that the prevalence of these genotypes within the US source plasma donor population is very low.

Provision of plasmid clones to OMCL network and manufacturers

Dr J. Fryer (NIBSC, UK) discussed how plasmid clones representing the main B19V genotypes would be distributed through the next EDQM PTS for B19V DNA later in 2007. High-titre DNA stocks have been prepared for near full-length plasmid clones, representing genotypes 1, 2, 3a and 3b of B19V (N8, A6, V9 and D91·1, respectively). Dilution to equal copy number gave equivalent results using a consensus in-house TaqMan assay for B19V DNA [28]. These plasmid clones will be distributed as a validation panel only for use in the PTS, until a plasma reference panel becomes available.

Availability of B19V viraemic plasma for reference panel development

Genotype 2

Several plasma units were identified that contained high titres of genotype 2 B19V. Dr M. Gessner (Baxter, Vienna, Austria) described the B19V plasma samples termed IM-81 and IM-82. IM-81 was a high-titre (~11·3 $\log_{10}$ IU/ml) genotype 2 plasma sample, which had been sequenced and characterized previously [25] and shown to be cross-neutralized by genotype 1 sera. IM-82 represents a subsequent bleed (4 days later) from the same donor with a titre of 7·4 $\log_{10}$ IU/ml. Dr S. Baylis (NIBSC) described a plasma pool, sourced in the

[1]Dr G. Zerlauth (Baxter, Vienna, Austria) summarized this special meeting at SoGAT XX, held in Warsaw, Poland, on 12–13 June 2007. At the same meeting, Drs L. Rinckel and T. Gierman (Talecris, Raleigh, NA, USA) reported that they have identified a high-titre, high-volume genotype 3 B19V plasma. Thus, in order to harmonize results obtained by control laboratories and plasma fractionators, a genotype panel containing each of the three genotypes of B19V will be jointly formulated by NIBSC and CBER. The panel will be evaluated together with additional genotype 2 samples and be calibrated against the current WHO International Standard for B19V DNA (99/800) in an international collaborative study. The presentations from the extraordinary SoGAT meeting are available at the following link: http://www.nibsc.ac.uk/partners/SoGAT/March_2007_Presentations.html.

USA containing a genotype 2 B19V. The pool was identified due to discrepant results, using different B19V NAT assays. This pool contains 6·2 $\log_{10}$ IU/ml of genotype 2 B19V DNA. The virus was not infectious in culture, and was likely to be neutralized by anti-B19V present in the pool. Despite the plasma being pooled, there was no genotype 1 B19V present. Dr M. Koppelman (Sanquin, Amsterdam, the Netherlands) described the identification of a genotype 2 B19V plasma sample (207458), with a titre of ~7 $\log_{10}$ IU/m. Sequence analysis indicated that this B19V was most closely related to the A6 virus [31]. Dr José (Grifols) described another genotype 2 B19V plasma sample. This sample was identified by using two different assays: the first specific for genotype 1 B19V and the second, a consensus assay described in her earlier presentation. The plasma gave negative results in the genotype 1 B19V assay, but was positive in the consensus assay. This led to further characterization of the plasma sample, which was found to have a titre of 7·3 $\log_{10}$ IU/ml. The sample was negative for a range of other virus markers, and was also negative for anti-B19V IgG and IgM, and likely to represent the early ramp-up phase.

Genotype 3

While several high-titre, high-volume plasma samples have been identified for genotype 2 B19V, there is limited material available for genotype 3. Dr D. Candotti (University of Cambridge) summarized a series of clinical samples, comprising both genotypes 3a and 3b viruses. None of the available samples exceeded ~6 $\log_{10}$ IU/ml of B19V DNA. It was proposed that B19V samples might be sought prospectively, by identifying persistent infections in blood donors (which may have titres as high as 4–5 $\log_{10}$ IU/ml). However, several thousand donations would have to be screened. Additional sources of genotypes 2 and 3 B19V have been examined and these include the screening of anti-B19V IgM-positive sera from Brazilian patients presenting with rash-like illness by Dr K. Brown (HPA). In a recently published study from Brazil [32], clinical samples from patients with B19V-like symptoms were tested for B19V DNA and the virus genotype determined. All three genotypes of B19V were identified. Dr K. Brown outlined the approach taken in his study using biotinylated PCR products and pyrosequencing to determine the genotype of each B19V-positive sample. The method was validated using previously identified variant viruses [30]. Of 50 B19V IgM-positive samples studied by this approach, 29 were positive for B19V DNA by PCR, ranging in concentration from 10^2 to 10^{10} geq/ml. These PCR-positive viruses were all genotype 1, with three unique point mutations being identified. A small study was presented by Dr S. Baylis on behalf of Dr D. York (Molecular Diagnostic Services Pty Ltd, South Africa) and Mr D. Stubbings (National Bioproducts Institute, South Africa). High-titre B19V plasma donations (n = 9) were genotyped and in contrast to the findings on the West Coast of Africa, these B19V-positive samples were all genotype 1.

Conclusions and recommendations

Overall, based upon the classification by the ICTV and in terms of what is currently know about the biological and serological properties of the different genotypes of B19V, these genotypes clearly represent strains of the same virus. The more recently identified variants appear not to be so well represented in Europe and North America as genotype 1 B19V. However, different genotypes of B19V have been found in donor plasma that has led to batch release issues and based upon recent PTS studies, some assays have proved ineffective in detecting genotype 2 B19V DNA [7]. In order to harmonize the results obtained for the detection and quantification of B19V DNA between control laboratories and the manufacturers of plasma derivatives, it was agreed that standardization of assays using well-characterized reference materials would be the way forward.

The consensus opinion at the meeting was to produce a genotype panel of plasma samples representing the different genotypes of B19V. As B19V DNA testing has a quantitative limit (10 IU/ml), any reference panel would be required to reflect the need for accuracy around this threshold concentration. Future collaborative studies used to evaluate candidate plasma samples for a reference panel would need to be calibrated against the WHO International Standard for B19V DNA [4]. In the absence of sufficient genotype 3 B19V material, it was felt that cloned DNAs may be suitable for preparing a panel, until a plasma reference panel becomes available. The European common technical specifications for *in vitro* diagnostic medical devices permits the use of materials such as cloned DNAs (independently quantified by spectrophotometry) where a suitable source of native material is absent. What was emphasized throughout the meeting is the importance of depositing DNA sequence for B19V strains in the databases, to ensure that as much information is available as possible to enable good assay design. However, genetic variation was to be expected in the future, including genotype 1 B19V variants, and robust assay design is essential to deal with inevitable genetic changes.

References

1 Anonymous: Council of Europe: Human anti-D immunoglobulin, monograph 0557; in *European Pharmacopoeia*, 6th edn. Strasbourg, France, Council of Europe, 2006

2 Anonymous: Council of Europe Human anti-D immunoglobulin for intravenous administration, monograph 1527; in *European Pharmacopoeia*, 6th edn. Strasbourg, France, Council of Europe, 2006

3 Anonymous: Council of Europe Human plasma (pooled and treated for virus inactivation), monograph 1646; in *European Pharmacopoeia*, 6th edn. Strasbourg, France, Council of Europe, 2006
4 Saldanha J, Lelie N, Yu MW, Heath A; and the B19 Collaborative Study Group: Establishment of the first World Health Organization International Standard for human parvovirus B19 DNA nucleic acid amplification techniques. *Vox Sang* 2002; 82:24–31
5 Servant A, Laperche S, Lallemand F, Marinho V, De Saint Maur G, Meritet JF, Garbarg-Chenon A: Genetic diversity within human erythroviruses: identification of three genotypes. *J Virol* 2002; 76:9124–9134
6 Tattersall P, Bergoin M, Bloom ME, Brown KE, Linden RM, Muzyczka N, Parrish CR, Tijssen P: Parvoviridae; in Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (eds): *Virus Taxonomy: Eighth report of the International Committee on Taxonomy of Viruses*. New York, Academic Press, 2005;353–369
7 Nübling M, Buchheit K-H: Results from recent EDQM/OMCL PTS studies (PTS052 and PTS064) on B19 DNA NAT testing of plasma pools; Presentation at the XIX SoGAT meeting, 14 June 2006, Bern, Switzerland
8 Nguyen QT, Sifer C, Schneider V, Allaume X, Servant A, Bernaudin F, Auguste V, Garbarg-Chenon A: Novel human erythrovirus associated with transient aplastic anemia. *J Clin Microbiol* 1999; 37:2483–2487
9 Nguyen QT, Wong S, Heegaard ED, Brown KE: Identification and characterization of a second novel human erythrovirus variant, A6. *Virology* 2002; 301:374–380
10 Hokynar K, Söderlund-Venermo M, Pesonen M, Ranki A, Kiviluoto O, Partio EK, Hedman K: A new parvovirus genotype persistent in human skin. *Virology* 2002; 302:224–228
11 Jones MS, Kapoor A, Lukashov VV, Simmonds P, Hecht F, Delwart E: New DNA viruses identified in patients with acute viral infection syndrome. *J Virol* 2005; 79:8230–8236
12 Fryer JF, Delwart E, Bernardin F, Tuke PW, Lukashov VV, Baylis SA: Analysis of two human parvovirus PARV4 genotypes identified in human plasma for fractionation. *J Gen Virol* 2007; 88:2162–2167
13 Allander T, Tammi M, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Andersson B: Cloning of a human parvovirus by molecular screening of respiratory tract samples. *Proc Natl Acad Sci USA* 2005; 102:12891–12896
14 PA/PH/OMCL (03) 38 DEF: OMCL Guideline for validation of nucleic acid amplification techniques (NAT) for quantitiation of B19 virus DNA in plasma pools; in *Biological Substances Submitted to the Official Control Authority Batch Release*. Strasbourg, France, Council of Europe; 2006
15 Wu C, Mason B, Jong J, Erdman D, McKernan L, Oakley M, Soucie M, Evatt B, Yu MW: Parvovirus B19 transmission by a high-purity factor VIII concentrate. *Transfusion* 2005; 45:1003–1010
16 Norja P, Hokynar K, Aaltonen LM, Chen R, Ranki A, Partio EK, Kiviluoto O, Davidkin I, Leivo T, Eis-Hübinger AM, Schneider B, Fischer HP, Tolba R, Vapalahti O, Vaheri A, Söderlund-Venermo M, Hedman K: Bioportfolio: lifelong persistence of variant and prototypic erythrovirus DNA genomes in human tissue. *Proc Natl Acad Sci USA* 2006; 103:7450–7453
17 Ekman A, Hokynar K, Kakkola L, Kantola K, Hedman L, Bondén H, Gessner M, Aberham C, Norja P, Miettinen S, Hedman K, Söderlund-Venermo M: Biological and immunological relations among human parvovirus B19 genotypes 1 to 3. *J Virol* 2007; 81:6927–6935
18 Liefeldt L, Plentz A, Klempa B, Kershaw O, Endres AS, Raab U, Neumayer HH, Meisel H, Modrow S: Recurrent high level parvovirus B19/genotype 2 viremia in a renal transplant recipient analyzed by real-time PCR for simultaneous detection of genotypes 1 to 3. *J Med Virol* 2005; 75:161–169
19 Candotti D, Etiz N, Parsyan A, Allain JP: Identification and characterization of persistent human erythrovirus infection in blood donor samples. *J Virol* 2004; 78:12169–12178
20 Parsyan A, Szmaragd C, Allain JP, Candotti D: Identification and genetic diversity of two human parvovirus B19 genotype 3 subtypes. *J Gen Virol* 2007; 88:428–431
21 Shackelton LA, Parrish CR, Truyen U, Holmes EC: High rate of viral evolution associated with the emergence of carnivore parvovirus. *Proc Natl Acad Sci USA* 2005; 102:379–384
22 Shackelton LA, Holmes EC: Phylogenetic evidence for the rapid evolution of human B19 erythrovirus. *J Virol* 2006; 80:3666–3669
23 Schneider B, Becker M, Brackmann HH, Eis-Hübinger AM: Contamination of coagulation factor concentrates with human parvovirus B19 genotype 1 and 2. *Thromb Haemost* 2004; 92:838–845
24 Geng Y, Wu C, Bhattacharyya SP, Tan D, Guo Z, Yu MW: Parvovirus B19 DNA in factor VIII concentrates: effects of manufacturing procedures and B19 NAT screening. *Transfusion* 2007; 47:883–889
25 Blümel J, Eis-Hübinger AM, Stühler A, Bönsch C, Gessner M, Löwer J: Characterization of parvovirus B19 genotype 2 in KU812Ep6 cells. *J Virol* 2005; 79:14197–14206
26 Boschetti N, Niederhauser I, Kempf C, Stühler A, Löwer J, Blümel J: Different susceptibility of B19 virus and mice minute virus to low pH treatment. *Transfusion* 2004; 44:1079–1086
27 Allain JP, Hsu J, Pranmeth M, Hanson D, Stassinopoulos A, Fischetti L, Corash L, Lin L: Quantification of viral inactivation by photochemical treatment with amotosalen and UV A light, using a novel polymerase chain reaction inhibition method with preamplification. *J Infect Dis* 2006; 194:1737–1744
28 Baylis SA, Shah N, Minor PD: Evaluation of different assays for the detection of parvovirus B19 DNA in human plasma. *J Virol Methods* 2004; 121:7–16
29 Hokynar K, Norja P, Laitinen H, Palomäki P, Garbarg-Chenon A, Ranki A, Hedman K, Söderlund-Venermo M: Detection and differentiation of human parvovirus variants by commercial quantitative real-time PCR tests. *J Clin Microbiol* 2004; 42:2013–2019
30 Cohen BJ, Gandhi J, Clewley JP: Genetic variants of parvovirus B19 identified in the United Kingdom: implications for diagnostic testing. *J Clin Virol* 2006; 36:152–155
31 Koppelman MHGM, Rood IGH, Fryer JF, Baylis SA, Cuypers HTM: Parvovirus B19 genotype 1 and 2 detection with real-time PCR assays. *Vox Sang* 2007; 93:208–215
32 Sanabani S, Neto WK, Pereira J, Sabino EC: Sequence variability of human erythroviruses present in bone marrow of Brazilian patients with various parvovirus B19-related hematological symptoms. *J Clin Microbiol* 2006; 44:604–606

Appendix

The meeting participants were from regulatory/research/reference laboratories, kit manufacturers, and plasma derivative manufacturers. The following is a list of speakers at the meeting: Dr G. Zerlauth, Baxter, Austria; Dr K. Brown, HPA, UK; Dr J.-M. Spieser, EDQM, France; Dr M. W. Yu, CBER, Bethesda, MD, USA; Ms K. Hokynar, Haartman Institute, Finland; Professor S. Modrow, University of Regensburg, Germany; Dr D. Candotti, University of Cambridge, UK; Professor A.-M. Eis-Hübinger, University of Bonn, Germany; Dr M. Nübling, Paul Ehrlich Institute, Germany; Professor J.-P. Allain, University of Cambridge, UK; Dr S. Baylis, NIBSC, UK; Dr T. Cuijpers, Sanquin, the Netherlands; Dr M. José, Grifols, Barcelona, Spain; Dr J. Fryer, NIBSC, UK; Dr M. Gessner, Baxter, Austria; Dr M. Koppelman, Sanquin, the Netherlands. The meeting was chaired by Dr P. Minor (NIBSC, UK).

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研究報告の概要

メルケル細胞癌（Merkel cell carcinoma: MCC）は、稀ではあるが進行の早いヒトの皮膚がんであり、主に高齢者や免疫抑制者にみられる。われわれは今回、digital transcriptome subtraction (DTS)法を用いてMCC検体について調べ、新種のポリオーマウイルスを同定し、メルケル細胞ポリオーマウイルス（MCVまたはMCPyV）と命名した。このウイルスは、MCC腫瘍の10検体のうち8検体（80%）で検出されたが、体内のさまざまな部位から採取した対照組織では59検体中5検体（8%）、対照皮膚組織では25検体中4検体（16%）でしか検出されなかった。MCV陽性であった8MCC検体のうち6検体では、ウイルスDNAは腫瘍ゲノム内に組み込まれており、そのパターンより、MCVの感染と組み込みにより腫瘍細胞のクローン増殖となったことが示唆された。したがって、MCVはMCCの発生に寄与する因子であると考えられる。

報告企業の意見	今後の対応
メルケル細胞癌の発生に寄与すると考えられる新規のポリオーマウイルスを同定したとの報告である。 ポリオーマウイルスは、直径40nmのエンベロープを有しないDNAウイルスである。 静注用ヘブスブリン-IHについては、万一本剤の原料血漿にポリオーマウイルスが混入したとしても、EMC及びCPVをモデルウイルスとしたウイルスバリデーション試験成績から、本剤の製造工程において十分に不活化・除去されると考えている。 ヘブスブリンについては、EMC及びCPVをモデルウイルスとしたウイルスバリデーション試験成績からは、製造工程において不活化・除去が十分であるとは説明困難であるため、ポリオーマウイルスの原血漿への混入が判明した場合にはその事実を総合機構及び厚生労働省に報告する。	ポリオーマウイルスに関連する情報については、今後も注視することとする。

使用上の注意記載状況・その他参考事項等

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

mains of TRF1 and TRF2, these structural variations emphasize that the TRFH domain is a versatile framework for interactions with different proteins.

The crystal structure of the $TRF2_{TRFH}$-$Apollo_{TBM}$ complex is corroborated by mutagenesis. Mutations of the conserved hydrophobic residues of Apollo (F504, L506, and P508) or TRF2 (*F120*) completely abolished the interaction both in vitro and in vivo (Fig. 4, F and G). We further assayed the cellular localization of wild-type and mutant Apollo by expressing hemagglutinin (HA)–tagged proteins in human telomerase reverse transcriptase (hTERT)–immortalized human BJ fibroblasts. Although wild-type Apollo showed the expected telomere localization, the L506E/P508A double mutant was distributed throughout the nucleoplasm with no obvious accumulation at telomeres (Fig. 4H). This result confirms the structural information and indicates that the binding of Apollo to the TRFH domain of TRF2 is required for the telomeric localization of Apollo.

We next asked whether other shelterin-associated proteins might contain the F/Y-X-L-X-P motif suggestive of an interaction with the TRFH domain of TRF1 or TRF2. We identified this motif in PinX1, originally identified as a TRF1-interacting protein in a yeast two-hybrid screen (*6*). An 11-residue fragment of PinX1 (R287-D-F-T-L-K-P-K-K-R-R297), referred to as $PinX1_{TBM}$, closely resembles $TIN2_{TBM}$ (fig. S12A), suggesting that it may bind to $TRF1_{TRFH}$ in the same fashion as does $TIN2_{TBM}$. ITC data confirmed the $TRF1_{TRFH}$-$PinX1_{TBM}$ interaction, whereas no measurable interaction was observed between $TRF2_{TRFH}$ and $PinX1_{TBM}$ (fig. S12B). Mutagenesis studies showed that PinX1-L291 and TRF1-*F142* are critical for the interaction, whereas PinX1-P293 is not (fig. S12C). These results are consistent with those of the $TRF1_{TRFH}$-$TIN2_{TBM}$ interaction (Fig. 2D) and indicate that PinX1, like TIN2, binds the TRFH domain of TRF1 but not TRF2. Protein sequence database searches showed many instances of telomere-associated proteins containing the F/Y-X-L-X-P motif (fig. S13). Future studies are needed to address whether this motif mediates the TRF1/TRF2 binding of these telomere-associated proteins in vivo.

Our results indicate that binding to the TRFH docking site involves the sequence F/Y-X-L-X-P in shelterin-associated proteins, which contacts the same molecular recognition surface of the TRFH domains of TRF1 and TRF2 with distinct specificities. Because TRF1 and TRF2 play different roles in telomere length homeostasis and telomere protection (*1*), we propose that the TRFH domains of TRF1 and TRF2 function as telomeric protein docking sites that recruit different shelterin-associated factors with distinct functions to the chromosome ends.

References and Notes

1. T. de Lange, *Genes Dev.* **19**, 2100 (2005).
2. M. van Overbeek, T. de Lange, *Curr. Biol.* **16**, 1295 (2006).
3. X. D. Zhu, B. Kuster, M. Mann, J. H. Petrini, T. de Lange, *Nat. Genet.* **25**, 347 (2000).
4. X. D. Zhu *et al.*, *Mol. Cell* **12**, 1489 (2003).
5. S. Smith, I. Giriat, A. Schmitt, T. de Lange, *Science* **282**, 1484 (1998).
6. X. Z. Zhou, K. P. Lu, *Cell* **107**, 347 (2001).
7. T. Nishikawa *et al.*, *Structure* **9**, 1237 (2001).
8. R. Court, L. Chapman, L. Fairall, D. Rhodes, *EMBO Rep.* **6**, 39 (2005).
9. B. Li, S. Oestreich, T. de Lange, *Cell* **101**, 471 (2000).
10. A. Bianchi, S. Smith, L. Chong, P. Elias, T. de Lange, *EMBO J.* **16**, 1785 (1997).
11. L. Fairall, L. Chapman, H. Moss, T. de Lange, R. Rhodes, *Mol. Cell* **8**, 351 (2001).
12. S. H. Kim, P. Kaminker, J. Campisi, *Nat. Genet.* **23**, 405 (1999).
13. C. Lenain *et al.*, *Curr. Biol.* **16**, 1303 (2006).
14. T. H. Lee, K. Perrem, J. W. Harper, K. P. Lu, X. Z. Zhou, *J. Biol. Chem.* **281**, 759 (2006).
15. S. H. Kim *et al.*, *J. Biol. Chem.* **279**, 43799 (2004).
16. J. Z. Ye *et al.*, *J. Biol. Chem.* **279**, 47264 (2004).
17. Materials and methods are available as supporting material on *Science* Online.
18. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
19. P. Fotiadou, O. Henegariu, J. B. Sweasy, *Cancer Res.* **64**, 3830 (2004).
20. Coordinates and structure factor amplitudes have been deposited in the Protein Data Bank with access numbers 3BQO ($TRF1_{TRFH}$-$TIN2_{TBM}$), 3BU8 ($TRF2_{TRFH}$-$TIN2_{TBM}$), and 3BUA ($TRF2_{TRFH}$-$Apollo_{TBM}$). We thank F. Wang and K. Wan for assistance. Work was supported by an NIH grant (to T.de L.) and an American Cancer Society Research Scholar grant and a Sidney Kimmel Scholar award (to M.L.). Use of Life Sciences Collaborative Access Team Sector 21 was supported by the Michigan Economic Development Corporation and the Michigan Technology Tri-Corridor (grant 085P1000817). Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under contract no. DE-AC02-06CH11357.

Supporting Online Material

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Materials and Methods
SOM Text
Figs. S1 to S14
Table S1
References

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Clonal Integration of a Polyomavirus in Human Merkel Cell Carcinoma

Huichen Feng, Masahiro Shuda, Yuan Chang,* Patrick S. Moore*

Merkel cell carcinoma (MCC) is a rare but aggressive human skin cancer that typically affects elderly and immunosuppressed individuals, a feature suggestive of an infectious origin. We studied MCC samples by digital transcriptome subtraction and detected a fusion transcript between a previously undescribed virus T antigen and a human receptor tyrosine phosphatase. Further investigation led to identification and sequence analysis of the 5387–base-pair genome of a previously unknown polyomavirus that we call Merkel cell polyomavirus (MCV or MCPyV). MCV sequences were detected in 8 of 10 (80%) MCC tumors but only 5 of 59 (8%) control tissues from various body sites and 4 of 25 (16%) control skin tissues. In six of eight MCV-positive MCCs, viral DNA was integrated within the tumor genome in a clonal pattern, suggesting that MCV infection and integration preceded clonal expansion of the tumor cells. Thus, MCV may be a contributing factor in the pathogenesis of MCC.

Polyomaviruses have been suspected as potential etiologic agents in human cancer since the discovery of murine polyoma virus (MuPyV) by Gross in 1953 (*1*). However, although polyomavirus infections can produce tumors in animal models, there is no conclusive evidence that they play a role in human cancers (*2*). These small double-stranded DNA viruses [~5200 base pairs (bp)] encode a variably spliced oncoprotein, the tumor (T) antigen (*3*, *4*), and are divided into three genetically distinct groups: (i) avian polyomaviruses, (ii) mammalian viruses related to MuPyV, and (iii) mammalian polyomaviruses related to simian virus 40 (SV40) (*5*). All four known human polyomaviruses [BK virus (BKV), JCV, KIV, and WUV (*6*, *7*)] belong to the SV40 subgroup. In animals, integration of polyomavirus DNA into the host genome often precedes tumor formation (*8*).

Merkel cell carcinoma (MCC) is a neuroectodermal tumor arising from mechanoreceptor Merkel cells (Fig. 1A). MCC is rare, but its incidence has tripled over the past 2 decades in the United States to 1500 cases per year (*9*). It is one of the most aggressive forms of skin cancer; about 50% of advanced MCC patients

Molecular Virology Program, University of Pittsburgh Cancer Institute, University of Pittsburgh, 5117 Centre Avenue, Suite 1.8, Pittsburgh, PA 15213, USA.

*These authors contributed equally to this work. To whom correspondence should be addressed. E-mail: yc70@pitt.edu (Y.C.); psm9@pitt.edu (P.S.M.)

live 9 months or less. Gene expression profiling studies indicate that MCC may comprise two or more clinically similar diseases with distinct etiologies (*10*). Like Kaposi's sarcoma (KS), MCC occurs more frequently than expected among immunosuppressed transplant and AIDS patients (*11*). These similarities to KS, an immune-related tumor caused by KS-associated herpesvirus (*12*), raise the possibility that MCC may also have an infectious origin.

To search for viral sequences in MCC, we used digital transcriptome subtraction (DTS), a methodology we developed that can identify foreign transcripts by using human high-throughput cDNA sequencing data (*13*). We generated two cDNA libraries from a total of four anonymized MCC tumors. One library was prepared with the use of mRNA from a single tumor (MCC347), and the other was prepared with mRNA pooled from three tumors (MCC337, 343, and 346) to increase the likelihood of detecting rare viral sequences (table S1).

From these two libraries, we respectively pyrosequenced 216,599 and 179,135 cDNA sequences (~150 to 200 bp). These 395,734 cDNA sequences were trimmed with LUCY stringency equivalent to PHRED scores of 20 or higher (*14*). Copolymers of adenine or thymidine [poly(A) and poly (T), respectively], dust (low-complexity), human repeat, and primer adaptor sequences were then removed, leaving 382,747 sequences to form a high-fidelity (HiFi) data set. Of these, 380,352 (99.4%) aligned to human RefSeq RNA, mitochondrial, assembled chromosomes, or immunoglobulin sequences in National Center for Biotechnology Information (NCBI) databases. Of the remaining 2395 HiFi candidate sequences, one transcript (DTS1) from MCC347 cDNA aligned with high homology to African green monkey (AGM) lymphotropic polyomavirus (LPyV) and to human BK polyomavirus T antigen sequences. A second DTS transcript (DTS2) had no homology to deposited polyomavirus sequences but was subsequently identified by aligning HiFi candidates to the full-length viral genome (see below). These two sequences define a previously unknown human polyomavirus that we call Merkel cell polyomavirus (MCV or MCPyV) because of its close association with MCC.

Rapid amplification of cDNA ends (3′-RACE) extended DTS1 to three different cDNAs (Fig. 1B). One transcript terminated at a poly(A) site in the T antigen sequence, and two cDNAs read through this weak poly(A) site to form different length fusions with intron 1 of the human receptor tyrosine phosphatase type G gene (*PTPRG*) (GenBank:18860897) on chromosome 3p14.2. Viral integration at this site was confirmed by sequencing DNA polymerase chain reaction (PCR) products with the use of a viral primer and a human *PTPRG* primer. The same three RACE products were independently cloned from MCC348, a lymph node metastasis from the MCC347 primary tumor, indicating that this tumor was seeded from a single tumor cell already positive for the T antigen–PTPRG fusion transcript.

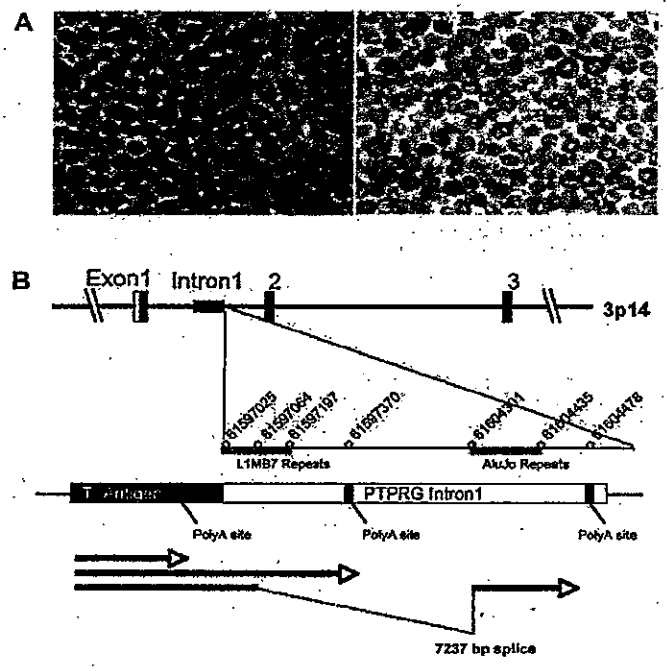

Fig. 1. (**A**) MCC is an aggressive skin cancer derived from Merkel mechanoreceptor cells that expresses neuroendocrine and perinuclear cytokeratin 20 markers, distinguishing it from other small round cell tumors (MCC349, left, hematoxylin and eosin; right, cytokeratin 20 staining, 40×. Scale bar represents 10 μm). (**B**) Discovery of Merkel cell polyomavirus transcripts in (MCC). 3′-RACE mapping of an MCC fusion transcript between the MCV T antigen and human *PTPRG*. A cDNA corresponding to a polyomavirus-like T antigen transcript was found by DTS analysis of MCC. This T antigen cDNA was extended by 3′-RACE to map three mRNA sequences (arrows), one of which terminates at a viral polyadenylation site and two of which extend into flanking human sequence and terminate in intron 1 of the human *PTPRG* gene on chromosome 3p14, indicative of viral DNA integration into the tumor cell genome. The two viral-human chimeric transcripts were generated by read-through of a weak polyadenylation signal in the viral T antigen gene. Identical RACE products were also sequenced from a lymph node metastasis of this primary tumor.

By viral genome walking, we sequenced the complete closed circular genome of MCV (5387 bp, prototype) from tumor MCC350. A second genome, MCV339 (5201 bp), was then sequenced by using MCV-specific primers. The sequences of MCV350 and MCV339 have GenBank accession numbers EU375803 and EU375804, respectively. Both viruses encode sequences with high homology to polyomavirus T antigen, VP1, VP2/3, and replication origin sequences (Fig. 2A). MCV has an early gene expression region [196 to 3080 nucleotides (nt)] containing the T antigen locus with large T and small T open reading frames and a late gene region containing VP1 and VP2/3 open reading frames between 3156 and 5118 nt. The T antigen locus has features conserved with other polyomavirus T antigens, including cr1, DnaJ, pRB1-binding Leu-X-Cys-X-Glu (LXCXE) motif, origin-binding, and helicase/adenosine triphosphatase (ATPase) domains. Mutations in the C terminus of MCV350 and 339 large T open reading frames are predicted to truncate large T protein but are unlikely to affect small T antigen protein expression. The replication origin is highly conserved with that of other polyomaviruses and includes features such as a poly(T) tract and conserved T antigen binding boxes (fig. S1). MCV has highest homology to viruses belonging to the MuPyV subgroup and is most closely related to AGM LPyV (Fig. 2B) (*15*). It is more distantly related to known human polyomaviruses and SV40. The principal differences between MCV350 and MCV339 are a 191-bp (1994 to 2184 nt) deletion in the MCV339 T antigen gene and a 5-bp (5216 to 5220 nt) insertion in the MCV339 late promoter. Excluding these sites, only 41 (0.8%) nucleotides differ between MCV350 and 339.

To investigate the association between MCV infection and MCC, we compared tumors from 10 MCC patients to two tissue control groups. The first control group was composed of unselected tissues from various body sites (including nine skin samples) from 59 patients without MCC (table S2). These samples were taken consecutively on a single surgical day and tested for MCV positivity with two PCR primer sets in the T antigen locus (LT1 and LT3) and one in the VP1 gene (VP1). These primers do not amplify cloned human BKV or JCV genomic DNA or SV40 genome from COS-7 cells. A second control group composed of skin and skin tumor samples from 25 immunocompetent and immunosuppressed patients without MCC were tested with LT1 and VP1 primers (table S2). Samples were randomized and tested in a blinded fashion. Southern blotting of PCR products was performed to increase sensitivity (fig. S2).

Of the 10 MCC tumors from different patients, 8 (80%) were positive for MCV sequences by PCR (Table 1 and table S1). Seven tumors showed robust amplification, and one tumor was positive only after PCR–Southern hybridization. MCC348 (metastasis from MCC347) and

MCC338 (infiltrating tumor from MCC339) were also positive. Two tumors, MCC343 and 346, remained negative after testing with 13 PCR primer pairs spanning the MCV genome. None of the 59 control tissues, including nine skin samples, was positive by PCR alone, but five gastrointestinal tract tissues tested weakly positive after PCR–Southern hybridization (8%, $P < 0.0001$, table S2). Viral T antigen sequences were recovered from three of these samples, confirming low copy number infection. Similarly, only 4 of 25 (16%, $P = 0.0007$, table S2) additional skin and non-MCC skin tumor samples from immunocompetent and immunosuppressed patients tested positive for MCV sequences (Table 2 and table S2).

To determine whether MCV DNA was integrated into the tumor genome, we examined MCC samples by direct Southern blotting without PCR amplification. When MCV DNA in MCC tumor is digested by single-cutter restriction endonucleases, such as EcoRI or BamHI, and probed with viral sequence, four possible patterns are predicted to occur: (i) if the viral DNA exists as freely replicating circular episomes, then a ~5.4 kilobase (kb) band will be present (integrated-concatenated virus will also generate a ~5.4 kb band); (ii) if MCV DNA integrates polyclonally, as might occur during secondary infection of the tumor if MCV is a passenger virus, then diffuse hybridization from different band sizes is expected; (iii) if MCV DNA integrates at one or a few chromosomal sites, then the tumors will have identical or near-identical non-5.4-kb banding patterns; or (iv) if MCV DNA integrates at different chromosomal sites before clonal expansion of the tumor cells, then distinct bands of different sizes will be present (monoclonal viral integration).

Eight of 11 MCC DNA samples (including MCC348 metastasis from MCC347) digested with either BamHI or EcoRI showed robust MCV hybridization, and these corresponded to the same tumors positive by PCR analysis with multiple primers (Fig. 3A and fig. S3). Monoclonal viral integration (pattern iv) was evident with one or both enzymes in six tumors: MCC339, 345, 347, 348, 349, and 352 (solid arrowheads). EcoRI digestion of MCC339, for example, produced two distinct 7.5- and 12.2-kb bands that would arise only if MCV is integrated at a single site in the majority of tumor cells. MCC344 and 350 bands have episomal or integrated-concatemeric bands (open arrowhead, pattern i). MCC352 has a monoclonal integration pattern (solid arrowheads, pattern iv) on BamHI digestion as well as an intense 5.4-kb band (open arrowhead), consistent with an integrated concatemer. All three tumors negative by PCR with ethidium bromide staining (MCC337, 343, and 346) were also negative by direct Southern blotting.

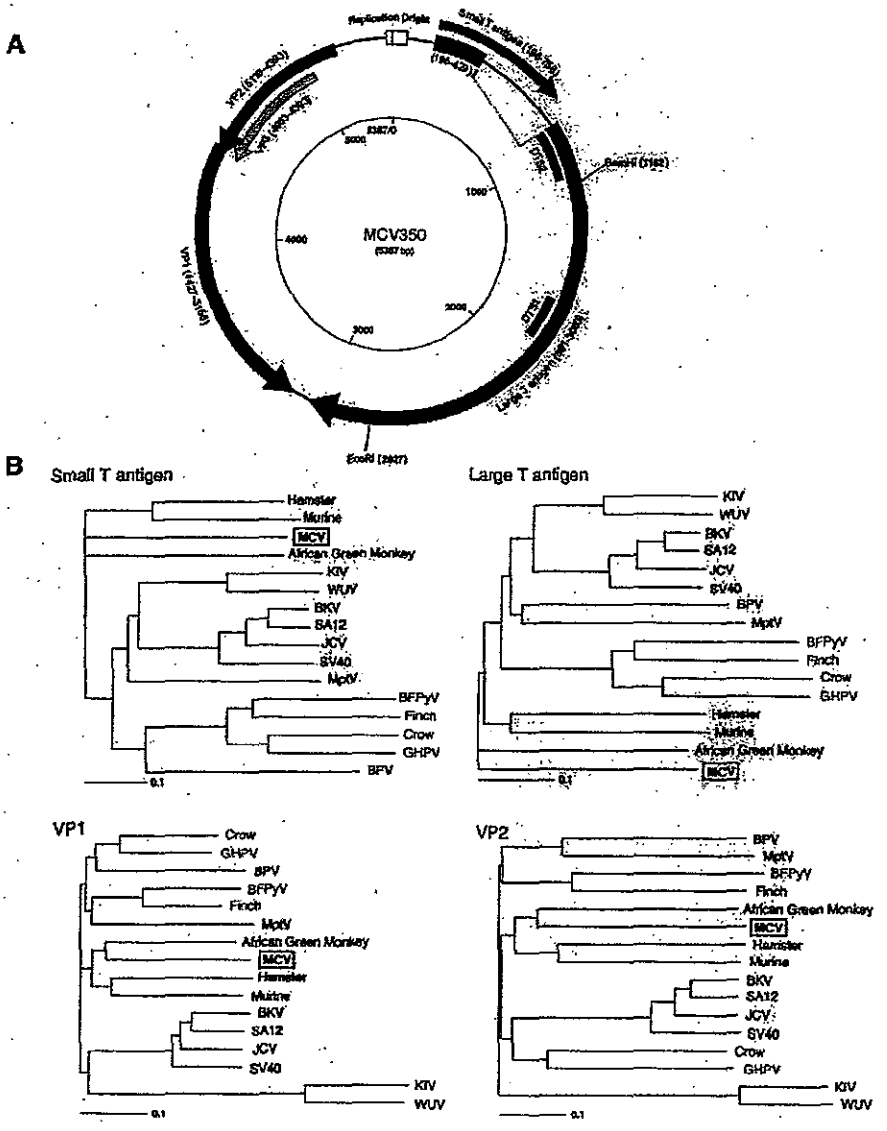

Fig. 2. (A) Schematic of MCV genome. Genome walking was used to clone the full MCV genome from tumor MCC350. The genome encodes typical features of a polyomavirus, including large T (purple) and small T (blue) open reading frames. Also shown are predicted VP1 (green) and overlapping VP2 (orange) and VP3 (yellow) genes. DTS1 and DTS2 (red) represent cDNA fragments originally identified by DTS screening. The former was used to identify MCV, and the latter is a spliced transcript with no homology to known polyomavirus sequences. (B) Neighbor-joining trees for putative MCV large T, small T, VP1, and VP2 proteins. The four known human polyomaviruses (BKV, JCV, KIV, and WUV) cluster together in the SV40 subgroup (blue), whereas MCV is most closely related to MuPyV subgroup viruses (red). Both subgroups are distinct from the avian polyomavirus subgroup (orange). Scale bars indicate an evolutionary distance of 0.1 amino acid substitutions per position in the sequence.

Table 1. PCR for MCV DNA in MCC tissues. A plus symbol indicates that the sample was strongly positive by ethidium bromide staining only with one or more primers. A minus symbol indicates that the tissue was negative for all primers. Entries with both plus and minus symbols indicate that the sample was negative by ethidium bromide staining but positive after Southern hybridization of PCR products.

	MCC cases (n = 10)	
Patient	Tissue ID	MCV positivity
1	MCC337	−/+
2†	MCC338	+
2	MCC339	+
3	MCC343	−
4	MCC344	+
5	MCC345	+
6	MCC346	−
7	MCC347	+
7‡	MCC348	+
8	MCC349	+
9	MCC350	+
10	MCC352	+
	Total (%)	8/10 (80)

†MCC338 was from an infiltrating tumor in skin tissue adjacent to MCC339 tumor. ‡MCC348 taken from a metastatic lymph node from MCC347.

The Southern blot banding patterns (Fig. 3A) were identical for MCC347 and its metastasis, MCC348, in line with 3′-RACE results (Fig. 1B) and confirming that MCC348 arose as a metastatic clone of MCC347. Because the genomic integration site (the *PTPRG* locus on chromosome 3p14) is mapped for these tumors, we performed Southern blotting with flanking human sequence probes to examine cellular monoclonal integration. NheI-SacI digestion of MCC347 and 348 is predicted to generate a 3.1-kb fragment from the wild-type allele and a 3.9-kb fragment from the allele containing the integrated MCV DNA. Hybridization with a flanking human *PTPRG* sequence probe revealed that the 3.9-kb allele was present in MCC347 and 348 DNA but not in control tissue DNA (Fig. 3B). As predicted, the same fragment hybridized to a MCV T antigen sequence probe, consistent with both cellular and viral monoclonality in this tumor. These results provide evidence that MCV infection and genome integration occurred in this tumor before clonal expansion of tumor cells. MCV in MCC may have some parallels to high-risk human papillomavirus (HPV), which causes cervical cancer mainly after viral episome disruption and integration into the cervical epithelial cell genome (*16*).

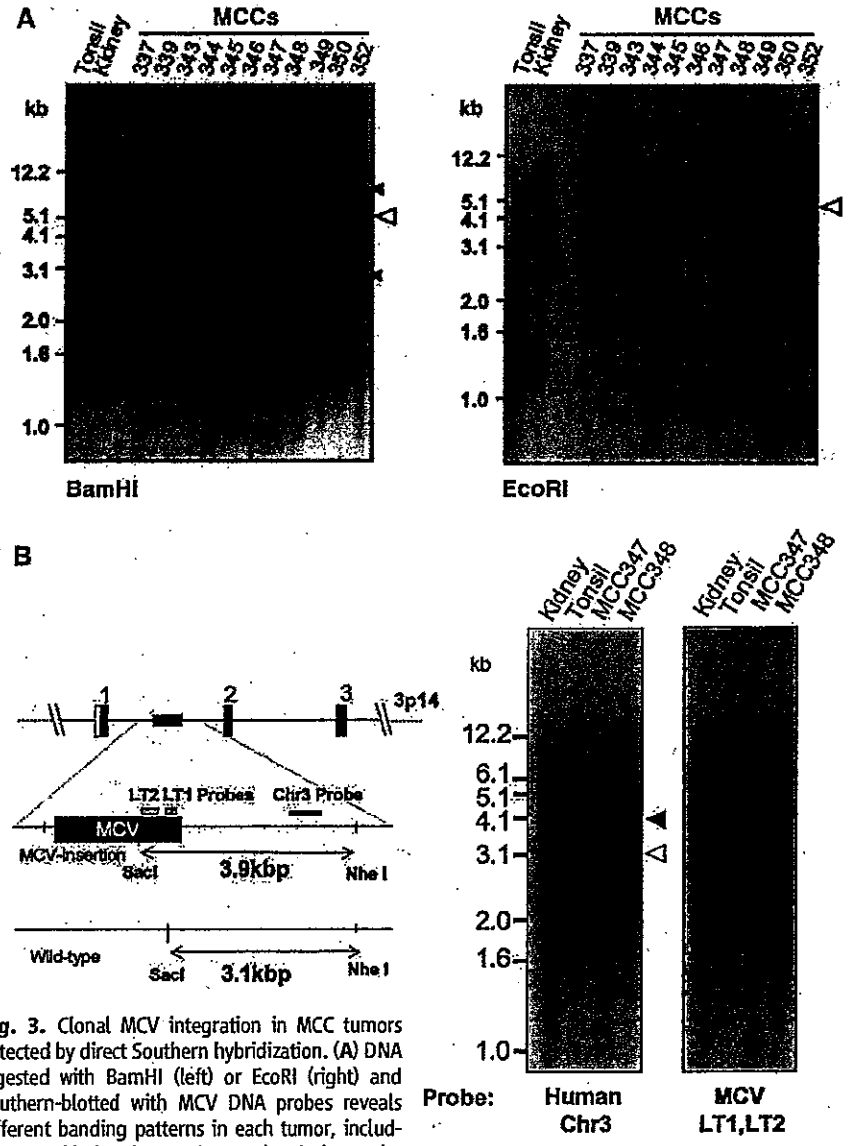

Fig. 3. Clonal MCV integration in MCC tumors detected by direct Southern hybridization. (**A**) DNA digested with BamHI (left) or EcoRI (right) and Southern-blotted with MCV DNA probes reveals different banding patterns in each tumor, including >5.4-kb bands. Open arrowhead shows the expected position for MCV episomal or concatenated-integrated genome (5.4 kb) with corresponding bands present in tumors MCC344 and 350. Tumors MCC339, 345, 347, 348, and 349 have different band sizes and doublet bands (solid arrowheads), consistent with genomic monoclonal integration. MCC352 has a prominent 5.4-kb band as well as higher and lower molecular weight monoclonal integration bands (BamHI), consistent with an integrated concatemer. Tumors MCC337, 343, and 346 have no MCV DNA detected by Southern blotting [bands at 1.5 kb (kidney) and 1.2 kb (MCC346) are artifacts]. (**B**) Viral and cellular monoclonality in MCC347 and 348. Tumor MCC347 and its metastasis MCC348 were digested with SacI and NheI and Southern-blotted with unique human flanking sequence probe [Chr3 (red), left] or viral probes [LT1 and LT2 (yellow), right]. The wild-type human allele is present in all samples at 3.1 kb (left). The MCC tumors, however, have an additional 3.9-kb allelic band formed by MCV DNA insertion into chromosome 3p14. Hybridization with probes for MCV T antigen sequence (yellow, right) generates an identical band.

If MCV plays a causal role in tumorigenesis, it could conceivably do so by several mechanisms, including T antigen expression, insertional mutagenesis, or both. Our DTS results show tumor expression of MCV T antigen, which has conserved DnaJ (*4*), pocket protein-binding LXCXE (*17*), and pp2A-binding (*18*, *19*) domains previously shown to play roles in polyomavirus-induced cell transformation. Mutational disruption of the *PTPRG* gene, which is suspected to be a tumor suppressor (*20*), could also play a role in MCC, although our Southern blot data suggest that MCV integration occurs at various genomic sites in different MCC tumors.

Our study validates the utility of DTS for the discovery of cryptic human viruses, but it has also revealed some limitations of the approach. Of the four tumors we sampled, only one (MCC347) was infected at high copy number. MCV transcripts in this tumor were present at 10 transcripts per million or about 5 transcripts per tumor cell. In future searches for other directly transforming tumor viruses (*21*), DTS should be used on multiple highly uniform samples sequenced to a depth of 200,000 transcripts or greater. Because DTS is quantitative, it is less likely to be useful in its current form for discovery of low-abundance viruses in autoimmune disorders or other chronic infectious diseases. Discovery of MCV by DTS nonetheless shows that DTS and related approaches (*22*) are promising methods to identify previously unknown human tumor viruses.

References and Notes

1. L. Gross, *Proc. Soc. Exp. Biol. Med.* **83**, 414 (1953).
2. D. L. Poulin, J. A. DeCaprio, *J. Clin. Oncol.* **24**, 4356 (2006).
3. S. M. Dilworth, *Nat. Rev. Cancer* **2**, 951 (2002).
4. J. M. Pipas, *J. Virol.* **66**, 3979 (1992).
5. K. A. Crandall, M. Perez-Losada, R. G. Christensen, D. A. McClellan, R. P. Viscidi, *Adv. Exp. Med. Biol.* **577**, 46 (2006).
6. T. Allander *et al.*, *J. Virol.* **81**, 4130 (2007).
7. A. M. Gaynor *et al.*, *PLoS Pathog.* **3**, e64 (2007).
8. D. Hollanderova, H. Raslova, D. Blangy, J. Forstova, M. Berebbi, *Int. J. Oncol.* **23**, 333 (2003).
9. B. Lemos, P. Nghiem, *J. Investig. Dermatol.* **127**, 2100 (2007).
10. M. Van Gele *et al.*, *Oncogene* **23**, 2732 (2004).
11. E. A. Engels, M. Frisch, J. J. Goedert, R. J. Biggar, R. W. Miller, *Lancet* **359**, 497 (2002).
12. Y. Chang *et al.*, *Science* **266**, 1865 (1994).
13. H. Feng *et al.*, *J. Virol.* **81**, 11332 (2007).
14. H. H. Chou, M. H. Holmes, *Bioinformatics* **17**, 1093 (2001).
15. M. Pawlita, A. Clad, H. zur Hausen, *Virology* **143**, 196 (1985).
16. M. Durst, L. Gissmann, H. Ikenberg, H. zur Hausen, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 3812 (1983).
17. J. A. DeCaprio *et al.*, *Cell* **54**, 275 (1988).
18. D. C. Pallas *et al.*, *Cell* **60**, 167 (1990).
19. G. Walter, R. Ruediger, C. Slaughter, M. Mumby, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 2521 (1990).
20. D. M. Pitterle, E. M. Jolicoeur, G. Bepler, *In Vivo (Athens)* **12**, 643 (1998).

Table 2. PCR for MCV DNA in comparison control tissues ($n = 84$). For detailed description of tissues and tissue sites, see table S2. MCV positivities marked with plus and minus symbols together are as in Table 1. For the various body site tissues, there were 59 samples; for the skin and skin tumor tissues, the sample size was 25 (table S2).

	MCV positivity
Various body site tissues	
Total MCV negative (%)	54/59 (92)
Total MCV positive (%)	5/59 (8)
Appendix control 1	−/+
Appendix control 2	−/+
Gall bladder	−/+
Bowel	−/+
Hemorrhoid	−/+
Skin and skin tumor tissues	
Total MCV negative (%)	21/25 (84)
Total MCV positive (%)	4/25 (16)
Skin	−/+
KS skin tumor 1	−/+
KS skin tumor 2	−/+
KS skin tumor 3	−/+

21. J. Parsonnet, in *Microbes and Malignancy*, J. Parsonnet, Ed. (Oxford Univ. Press, New York, 1999), pp. 3–18.
22. Y. Xu *et al.*, *Genomics* 81, 329 (2003).

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Materials and Methods
Figs. S1 to S3
Tables S1 to S5
References

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Worldwide Human Relationships Inferred from Genome-Wide Patterns of Variation

Jun Z. Li,[1,2]*† Devin M. Absher,[1,2]* Hua Tang,[1] Audrey M. Southwick,[1,2] Amanda M. Casto,[1] Sohini Ramachandran,[4] Howard M. Cann,[5] Gregory S. Barsh,[1,3] Marcus Feldman,[4]‡ Luigi L. Cavalli-Sforza,[1]‡ Richard M. Myers[1,2]‡

Human genetic diversity is shaped by both demographic and biological factors and has fundamental implications for understanding the genetic basis of diseases. We studied 938 unrelated individuals from 51 populations of the Human Genome Diversity Panel at 650,000 common single-nucleotide polymorphism loci. Individual ancestry and population substructure were detectable with very high resolution. The relationship between haplotype heterozygosity and geography was consistent with the hypothesis of a serial founder effect with a single origin in sub-Saharan Africa. In addition, we observed a pattern of ancestral allele frequency distributions that reflects variation in population dynamics among geographic regions. This data set allows the most comprehensive characterization to date of human genetic variation.

In the past 30 years, the ability to study DNA sequence variation has dramatically increased our knowledge of the relationships among and history of human populations. Analyses of mitochondrial, Y chromosomal, and autosomal markers have revealed geographical structuring of human populations at the continental level (*1–3*) and suggest that a small group of individuals migrated out of eastern Africa and their descendants subsequently expanded into most of today's populations (*3–6*). Despite this progress, these studies were limited to a small fraction of the genome, to limited populations, or both, and yield an incomplete picture of the relative importance of mutation, recombination, migration, demography, selection, and random drift (*7–10*). To substantially increase the genomic and population coverage of past studies (e.g., the HapMap Project), we have examined more than 650,000 single-nucleotide polymorphisms (SNPs) in samples from the Human Genome Diversity Panel (HGDP-CEPH), which represents 1064 fully consenting individuals from 51 populations from sub-Saharan Africa, North Africa, Europe, the Middle East, South/Central Asia, East Asia, Oceania, and the Americas (*11*). This data set is freely available (*12*) and allows a detailed characterization of worldwide genetic variation.

We first studied genetic ancestry of each individual without using his/her population identity. This analysis considers each person's genome as having originated from K ancestral but unobserved populations whose contributions are described by K coefficients that sum to 1 for each individual. To increase computational efficiency, we developed new software, *frappe*, that implements a maximum likelihood method (*13*) to analyze all 642,690 autosomal SNPs in 938 unrelated and successfully genotyped HGDP-CEPH individuals (*14*). Figure 1A shows the results for $K = 7$; those for $K = 2$ through 6 are in fig. S1. At $K = 5$, the 938 individuals segregate into five continental groups, similar to those re-

[1]Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305–5120, USA. [2]Stanford Human Genome Center, Stanford University School of Medicine, Stanford, CA 94305–5120, USA. [3]Department of Pediatrics, Stanford University School of Medicine, Stanford, CA 94305–5120, USA. [4]Department of Biological Sciences, Stanford University, Stanford, CA 94305–5120, USA. [5]Foundation Jean Dausset-Centre d'Etude du Polymorphisme Humain (CEPH), 75010 Paris, France.

*These authors contributed equally to this work.
†Present address: Department of Human Genetics, University of Michigan, 5789A MS II, Ann Arbor, MI 48109–5618, USA.
‡To whom correspondence should be addressed. E-mail: marc@charles.stanford.edu (M.F.); cavalli@stanford.edu (L.L.C.S.); myers@shgc.stanford.edu (R.M.M.)

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研究報告の概要

○海外帰国者とその妻におけるブルセラ症:*Brucella melitensis*のヒトーヒト感染の可能性

ブルセラ症は世界では一般的な人獣共通感染症で、年間50万例以上のヒト感染症例がある。ブルセラ症の一次感染は、*Brucella*種に汚染された殺菌処理されていない乳製品の摂取によって起こるが、複数の報告で男性から女性パートナーへブルセラ症が伝播した可能性が示唆されており、それらの症例は性交渉による感染と考えられてきた。

症例1:64歳の日本人男性が、6週間続く発熱で1998年6月2日に都内の病院に入院した。過去1週間の激しい腰痛も訴えた。入院時の血液培養からグラム陰性桿菌が検出され、*Brucella melitensis*バイオタイプ2と同定された。ブルセラ菌抗体価は800IUで、骨髄と肝生検からブルセラ症と確定された。患者は同年3月にイラクのバグダッドに10日間の滞在歴があり、滞在中にヒツジのチーズを摂取したことが判明した。抗生物質の投与によって症状は治まり、4ヵ月の投薬で完全に回復した。

症例2:患者1の妻で60歳の日本人女性が、1998年5月31日から発熱と左胸鎖関節の痛みを訴え始めた。血液と関節液の培養で*B melitensis*が生育した。ブルセラ菌抗体価は800IUであったが、抗生物質の投与によって回復した。患者はイラクへの渡航歴はなく、ブルセラ症に関する他のリスク要因もなかった。

考察:イラクを含め中東ではブルセラ症の発生数は多いが、日本では稀なことから、患者1は海外滞在中にブルセラ症に感染したと考えられる。2人の患者の発症には1ヵ月程度の間隔があり、標準的なブルセラ症の潜伏期間と一致する。患者1はイラクから日本に乳製品を持ち込んでおらず、患者2とブルセラ症との疫学的関連はない。患者1は疾患初期に患者2と性交渉があったことを報告しており、おそらく患者1から患者2への性感染が起こったと考えられる。同様に性感染と考えられる症例は過去にも報告されている。

報告企業の意見	今後の対応
イラクからの帰国者からその妻へ、ブルセラ症が性感染した可能性があるとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、問診で発熱などの体調不良者を献血不可としている。今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-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

Brucellosis in a Returned Traveler and His Wife: Probable Person-To-Person Transmission of *Brucella melitensis*

Yasuyuki Kato, MD, Gohta Masuda, MD, Ichiro Itoda, MD, Akifumi Imamura, MD, Atushi Ajisawa, MD, and Masayoshi Negishi, MD

Department of Infectious Diseases, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan

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Brucellosis is the most common zoonosis worldwide, with more than 500,000 new human cases annually. Although brucellosis is primarily transmitted to humans through the consumption of unpasteurized dairy products contaminated with *Brucella* species, several reports have indicated that brucellosis may be transmitted from a man to his female partner. It has been suggested that sexual intercourse is a means of transmission in these cases. Here, we describe an additional case of probable person-to-person transmission of *Brucella melitensis* in an elderly couple.

Case Report 1

A previously healthy 64-year-old Japanese man with a 6-week history of febrile illness was admitted to hospital in Tokyo, Japan, on June 2, 1998, following a 10-day visit to Baghdad, Iraq, on March 8, 1998. He also complained of severe lower back pain for 1 week. Findings on admission were fever (maximum temperature, 39.5°C) and normal pulse rate (80 beats/min). Neither heart murmurs nor adventitious breath sounds was heard. The liver was palpable 2 cm below the right costal margin; yet, the spleen was not palpated. He had tenderness of the lumber spine without abnormal neurological findings. He had no signs of epididymoorchitis. The white blood cell count was 8,400/μL and hemoglobin concentration 12.5 g/dL. Liver function tests showed elevation of alkaline phosphatase (378 IU/L) and alanine aminotransferase (67 IU/L). The erythrocyte sedimentation rate was 67 mm/h. Urinalysis findings were normal. Chest X-ray showed no opacities. T1-weighted magnetic resonance imaging of the spine revealed decreased signal intensity in the L3, L4, and L5 vertebral bodies and adjacent epidural space. These findings indicated that the patient had spondylitis, complicated by an epidural abscess.

The Gram-negative bacilli yielded by the blood culture at admission were subsequently confirmed as *Brucella melitensis* biotype 2. The *Brucella* antibody titer by the tube agglutination test was 800 IU. In addition, bone marrow and liver biopsy specimens showed evidence of granulomas consistent with brucellosis. A detailed travel history revealed that he had consumed sheep's cheese during his stay in Iraq. After confirmation of brucellosis, he was treated with intramuscular streptomycin (1 g daily), oral doxycycline (100 mg twice daily), and rifampicin (600 mg daily) for 1 month, and the fever and lower back pain gradually subsided. This treatment was followed by oral rifampicin (600 mg daily), trimethoprim–sulfamethoxazole (two standard-strength tablets twice daily), and tosufloxacin (200 mg thrice daily) for 4 months, with complete resolution.

Case Report 2

The wife of patient 1, a previously healthy 60-year-old Japanese woman, began to complain of fever and pain in the left sternoclavicular joint on May 31, 1998. Cultures of blood and the joint fluid grew *B melitensis* biotype 2. The *Brucella* antibody titer by the tube agglutination test was 400 IU. She was successfully treated with oral rifampicin (600 mg daily) and doxycycline (100 mg twice daily) for 6 weeks in combination with intramuscular streptomycin (750 mg daily) for the first 3 weeks. She did not visit Iraq with her husband and had no other risk factors for brucellosis.

Corresponding Author: Yasuyuki Kato, MD, Disease Control and Prevention Center, International Medical Center of Japan, 1-21-1 Toyama, Shinjuku, Tokyo 162-8655, Japan. E-mail: ykato@imcj.hosp.go.jp

Discussion

The Middle East, including Iraq, has the highest incidence of brucellosis in the world, whereas Japan is considered to be a brucellosis-free country.[1] Brucellosis is one of the reportable infectious diseases in Japan. According to the national surveillance data, only three cases of human brucellosis and two of livestock brucellosis were reported between 1999 and 2005 in Japan. No outbreaks of animal or human brucellosis were reported in Japan in 1998. Considering the incubation period of brucellosis (usually 2–4 wk, up to several months), his consumption of sheep's cheese in a brucellosis-endemic country, Iraq, and the rarity of brucellosis in his residential country, Japan, it is likely that patient 1 contracted brucellosis during his stay abroad.

The serial interval of the disease onset between patient 1 and patient 2 was approximately 1 month, which is similar to the mean incubation period of human brucellosis. Although the incubation period of brucellosis varies widely, it is difficult to argue that a common source exposure, such as food poisoning, occurred in these two patients, since patient 1 did not bring any dairy products or animals into Japan from Iraq. Furthermore, patient 2 had no other epidemiological links to brucellosis. Therefore, it is strongly suggested that the disease was transmitted from patient 1 to patient 2.

Through a PubMed search (1966–2005), we found six case reports of probable person-to-person transmission, excluding cases associated with blood transfusion, bone marrow transplantation, and breast-feeding (Table 1). Two of them are associated with international travel. In summary, it seems that men with symptoms of brucellosis are able to transmit the disease to their female partners. It is speculated that sexual transmission occurred in these cases since this is well known in animals. Interestingly, Mantur and colleagues reported that *B melitensis* was isolated from the semen, urine, and saliva of a man with epididymoorchitis, who transmitted the disease to his wife.[6] However, the presence of epididymoorchitis does not seem to be related to the transmissibility of human brucellosis. Furthermore, another report described that *B melitensis* was isolated from the sperm of one patient.[8] Patient 1 reported that he had intercourse with patient 2 during the initial stages of the disease. Therefore, we consider that person-to-person transmission, probably sexual transmission, of *B melitensis* occurred in our case.

Table 1 Published case reports of probable person-to-person transmission of brucellosis between men and women (English literature only)

Case reports	Goossens et al[2]	Stantic-Pavlinic et al[3]	Ruben et al[4]	Lindberg et al[5]	Mantur et al[6]	Thalhammer et al[7]	Present case
Age (y), sex, risk factor of primary case	25, male, laboratory exposure	34, male, laboratory exposure	61, male, laboratory exposure	35, male, travel to endemic area	30, male, animal exposure	25, male, travel to endemic area	65, male, travel to endemic area
Epididymoorchitis	Absent	Absent	Absent	Present	Present	Absent	Absent
Country where primary case was infected	Belgium	Yugoslavia	United States	Spain	India	Syria	Iraq
Age (y), sex, relationship of secondary case	21, female, fiancée	30, female, spouse	61, female, spouse	30, female, girlfriend	22, female, spouse	ND, female, girlfriend	60, female, spouse
Serial interval between two cases	3 mo	3 mo	8 mo	5 mo	1 mo	2 mo	1 mo
Isolated *Brucella* species and biotype	*Brucella melitensis* biotype 3	*B melitensis* biotype 2	*B melitensis* biotype 3	*B melitensis* biotype ND	*B melitensis* biotype 1	*Brucella abortus* biotype ND	*B melitensis* biotype 2
Suspected transmission route	Sexual intercourse	Sexual intercourse	Sexual intercourse	Sexual intercourse	Sexual intercourse	Sexual intercourse	Sexual intercourse

ND = not determined.

Although it has a little role in the epidemiology of brucellosis, person-to-person transmission is rather important in areas where brucellosis is not endemic such as most of developed countries; brucellosis has become a common imported disease in these areas.[9] Febrile-returned travelers should be educated to abstain from sexual intercourse because they could transmit the diseases to their partners. We would like to add brucellosis to the list of travel-related infections that are transmissible through sexual intercourse. This unusual mode of transmission of a common zoonosis requires special attention.

Acknowledgments

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Declaration of Interests

The authors state that they have no conflicts of interest.

References

1. Pappas G, Papadimitriou P, Akritidis N, et al. The new global map of human brucellosis. Lancet Infect Dis 2006; 6:91–99.
2. Goossens H, Marcelis L, Dekeyser P, Butzler JP. *Brucella melitensis*: person-to-person transmission? Lancet 1983; 1:773.
3. Stantic-Pavlinic M, Cec V, Mehle J. Brucellosis in spouses and the possibility of interhuman infection. Infection 1983; 11:313–314.
4. Ruben B, Band JD, Wong P, Colville J. Person-to-person transmission of *Brucella melitensis*. Lancet 1991; 337:14–15.
5. Lindberg J, Larsson P. Transmission of *Brucella melitensis*. Lancet 1991; 337:848–849.
6. Mantur BG, Mangalgi SS, Mulimani M. *Brucella melitensis*—a sexually transmissible agent? Lancet 1996; 347:1763.
7. Thalhammer F, Eberl G, Kopetzki-Kogler U. Unusual route of transmission for *Brucella abortus*. Clin Infect Dis 1998; 26:763–764.
8. Vandercam B, Zech F, de Cooman S, et al. Isolation of *Brucella melitensis* from human sperm. Eur J Clin Microbiol Infect Dis 1990; 9:303–304.
9. Pappas G, Akritidis N, Bosilkovski M, Tsianos E. Brucellosis. N Engl J Med 2005; 352:2325–2336.

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
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一般的名称	新鮮凍結人血漿	研究報告の公表状況	Bertherat E, Bekhoucha S, Chougrani S, Razik F, Duchemin JB, Houti L, Deharib L, Fayolle C, Makrerougrass B, Dali-Yahia R, Bellal R, Belhabri L, Chaieb A, Tikhomirov E, Carniel E. Emerg Infect Dis. 2007 Oct;13(10):1459-1462.	公表国 アルジェリア
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)			

研究報告の概要

○2003年のアルジェリアにおける50年ぶりのペスト再興
2003年6月から7月にアルジェリアOran地区においてペストの集団感染が発生した。アルジェリアでは、この疾患が50年以上報告されていなかった。腺ペスト症例18名が特定され、*Yersinia pestis*が6名から分離された。初発患者を除き、全員が回復した。標的予防的化学療法、公衆衛生、ベクターコントロールが、感染制御上重要な役割を果たした。疫学的、分子生物学的な知見から、当該期間中、現地の保菌動物の存在が強く示唆されたが、その起源(再興または再持ち込み)については特定できなかった。主要な貿易港における、今回の突然かつ予期せぬペスト再興は、国際的に重要な意味を持つ公衆衛生問題の典型的な例である。また、今回の再興は、ペスト再興の危険性が現在確認されているnatural foci(げっ歯類がペスト菌を保有する地区)に限られるものではないことも示している。

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」

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
2003年6月から7月にアルジェリアOran地区において、50年ぶりに腺ペストの集団感染が発生したとの報告である。	日本赤十字社は、輸血感染症対策として献血時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。今後も引き続き情報の収集に努める。

Plague Reappearance in Algeria after 50 Years, 2003

Eric Bertherat,* Souad Bekhoucha,† Saada Chougrani,‡ Fathia Razik,† Jean B. Duchemin,§ Leila Houti,‡ Larbi Deharib,‡ Corinne Fayolle,¶ Banaouda Makrerougrass,‡ Radia Dali-Yahia,† Ramdan Bellal,† Leila Belhabri,# Amina Chaieb,* Evgueni Tikhomirov,* and Elisabeth Carniel¶

An outbreak of plague occurred in the region of Oran, Algeria, from June to July 2003. Algeria had not reported this disease for >50 years. Eighteen bubonic cases were identified, and *Yersinia pestis* was isolated from 6 patients. Except for the index case-patient, all patients recovered. Targeted chemoprophylaxis, sanitation, and vector control played a crucial role in controlling the outbreak. Epidemiologic and biomolecular findings strongly suggested the existence of a local animal reservoir during this period, but its origin (resurgence or re-importation) could not be determined. This sudden and unexpected reemergence of plague, close to an important commercial seaport, is a textbook illustration of a public health event of international importance. It also demonstrates that the danger of plague reoccurrence is not limited to the currently indexed natural foci.

Plague is primarily a bacterial zoonosis affecting rodents. It is caused by *Yersinia pestis* and is transmitted from animal to animal by fleas. Humans usually become infected through the bite of an infected rodent flea. Bubonic plague, a severe infectious disease which, in the absence of appropriate antimicrobial drug therapy, can evolve to a rapidly fatal septicemia or pneumonia, can develop. A pneumonia form, which enables direct transmission to contacts, can be responsible for highly lethal outbreaks.

Currently, plague natural foci persist in Asia, the Americas, and Africa (where most human cases occur) (*1*). Plague foci have previously existed in the northern part of Africa but gradually disappeared in the last century, for unknown reasons. Libya is the only north African country that has experienced human cases in the past 40 years (*2*). In Algeria, archives report epidemics of plague as far back as the 14th century. These epidemics mainly affected ports, particularly that of Oran in 1556 and 1678 (3,000 deaths). In 1899, after an absence of nearly 100 years, plague reappeared in the port of Philippeville (now Skikda). Three large epidemics were subsequently reported in 1921 (185 cases), 1931 (76 cases), and 1944 (95 cases) as well as 158 sporadic cases. All but 2 cases occurred in ports (*3*,*4*). No natural focus of plague had ever been described in Algeria (*5*). We describe an outbreak of bubonic plague that occurred in 2003 in Algeria, where the last reported human case occurred in Oran in 1946 (*6*).

Methods

During June 9–18, 2003, several patients with signs of severe infection and painful inflammatory adenopathy were admitted to the University Hospital of Oran. All came from Kehailia (35°29′N, 0°32′E), a village of 1,300 inhabitants 25 km south of Oran. After eliminating all other possible differential diagnoses, clinicians suspected plague. The diagnosis was confirmed on June 18 by results of analysis of a bubo (lymph node) aspirate. A technical crisis committee was set up, and a case definition was adopted (Table). Any patient with a febrile syndrome and adenopathy who resided in the prefecture of Oran was hospitalized.

Clinical samples collected from patients (blood, bubo aspirate, cerebrospinal fluid) were sent to the Microbiology Department, University Hospital, Oran. Several of the initial cases were first diagnosed with the rapid diagnostic test (RDT) for plague developed by the Institut Pasteur (*7*); however, all samples were also examined with standard bacteriologic methods. Direct examination of smears was performed after Wayson and Gram staining. Blood samples were cultured in Castaneda medium for at least 10 days

*World Health Organization, Geneva, Switzerland; †University Hospital, Oran, Algeria; ‡University of Medicine, Oran, Algeria; §Centre d'Etude et de Recherche pour les Meningocoques et les Schistosomiases, Niamey, Niger; ¶Institut Pasteur, Paris, France; and #Institut Pasteur, Oran, Algeria

Table. Plague case definition adopted by technical crisis committee, 2003 plague outbreak, Oran region, Algeria*

Case definition	Criteria
Suspected	Clinical and epidemiologic characteristics compatible with plague; or, observation of suspect microorganisms on direct examination of clinical samples
Probable	Suspected case with anti-F1 antibodies in patient's blood; or, suspected case with a positive RDT without isolation of *Yersinia pestis* or in the absence of other cases reported in a radius of 10 km around the case
Confirmed	Culture positive for *Y. pestis*; or, RDT positive and *Y. pestis* isolated from patients living in a radius of 10 km around the case

*RDT, rapid diagnostic test.

at 28°C and examined daily. Suspected samples were inoculated into brain heart infusion and peptone broth and streaked on blood agar and cefsulodin-irgasan-novobiocin (Merck, Rahway, NJ, USA) plates. All media were incubated at 28°C. Bacterial identification was conducted with API 20 E strips (Analytab Products, Syosset, NY, USA) or individual tests in tubes. The biovar was determined (*8*). Antimicrobial drug susceptibility testing (ampicillin, amoxicillin-clavulanic acid, cefazolin, cefotaxime, gentamicin, amikacin, sulfamethoxazole, doxycycline) was conducted according to the technique of the Clinical and Laboratory Standards Institute (www.clsi.org). The serodiagnosis was determined by the ELISA-F1 technique (*9*). Serum samples from 30 study participants who had not contracted the disease but lived in the same area as the patients were used to determine the positive threshold of the technique. A serum was regarded as negative if its optical density at 490 nm (OD_{490}) was lower than a threshold defined as the mean (M) OD_{490} value of normal sera + 3 standard deviations (SD): $OD_{490} < M + 3$ SD. Sera with OD higher than this threshold were regarded as weak when the ratio $R = OD_{490}/(M + 3SD)$ was <2 and positive if R was ≥2.

Results

On June 9, 2003, a 19-year-old shepherd living in Kehailia was hospitalized with signs of septic shock (patient no. 2) (online Appendix Table, available from www.cdc.gov/EID/content/13/10/1459-appT.htm). He had been treated at home unsuccessfully with cephalosporins for inguinal adenopathy and fever during the previous 8 days. In the same village, 6 similar cases (nos. 3–8) occurred in the following days, until the diagnosis of plague was suspected and confirmed on June 18, first by RDT and then by isolation of a bacterium that had all the characteristics of *Y. pestis* biovar Orientalis and was susceptible to the antimicrobial agents tested. The epidemiologic investigation uncovered the index patient (no. 1), an 11-year-old child from Kehailia who was a cousin of case-patient 2. On June 2, an inguinal adenopathy with fever developed, and patient 2 was transferred to the hospital. He died 3 hours later, without a precise diagnosis.

Following the sanitation measures (reduction of rodent harborage, garbage removal, and vector control) implemented in Kehailia, no new cases of plague were reported in this locality after June 17. On June 19, a woman living in the suburbs of Oran (Hai Oussama) was hospitalized with bubonic plague (patient 9). The investigation showed that she had gone to Kehailia in the preceding days to consult a healer. Five cases of bubonic plague (nos. 10, 11, 14, 15, and 17) subsequently occurred from June 21 to July 16 among persons living in villages around Kehailia.

On June 28, a farmer and his wife (patients 12 and 13) who resided in Ain Temouchent, 50 km west of Kehailia (Figure), were hospitalized in Oran for symptoms suggestive of plague. The patients reported that they had not left their farm during the weeks preceding their illness. On July 1, a child from Beni Saf, on the Mediterranean coast 100 km southwest of Kehailia (Figure), had clinical signs of bubonic plague and a positive RDT result (patient 16). Neither he, nor his parents, had gone to the area of Kehailia or Ain Temouchent during the previous days. The last case (patient 18) occurred on July 22. The patient, a hunter who lived in Oran, had walked in the forest of M'sila, 30 km northwest of Kehailia, a few days before onset of his clinical signs.

Altogether, 18 cases were identified June 4–July 22, 2003: 10 confirmed, 3 probable, and 5 suspected (or 12 confirmed, 2 probable, and 4 suspected, according to the new World Health Organization case definition [*1*]). Most of the patients lived in unsanitary conditions, in close contact with livestock, and in the vicinity of storage areas of grain and fodder. In Kehailia, all the case-patients resided in different dwellings located within a 200-m radius. None of them reported direct contact with rodents. Sixteen of the 18 patients had an inguinal bubo, indicative of a flea bite on the leg. A septicemic form of plague developed in patients 1 and 2. Patient 1 died very soon after hospital admission. Patient 2 was admitted with a severe fever and neurologic syndrome and fell into a deep coma, despite broad-spectrum antimicrobial drug treatment that included vancomycin, cefotaxime, and gentamicin. He recovered from the coma 48 hours after treatment with ciprofloxacin (500 mg 2×/d for 30 days) was completed (F. Razik et al., unpub. data). No case of secondary pulmonary dissemination was observed. Other plague patients were treated with either doxycycline for adults (200 mg/d for 10 days) or cotrimoxazole for children (40 mg/kg/d for 10 days). All recovered without sequelae.

On the whole, 60 bubo aspirates, 143 blood samples, 6 sputum samples, and 2 cerebrospinal fluid samples were analyzed. In 5 samples, smear stains suggested infection

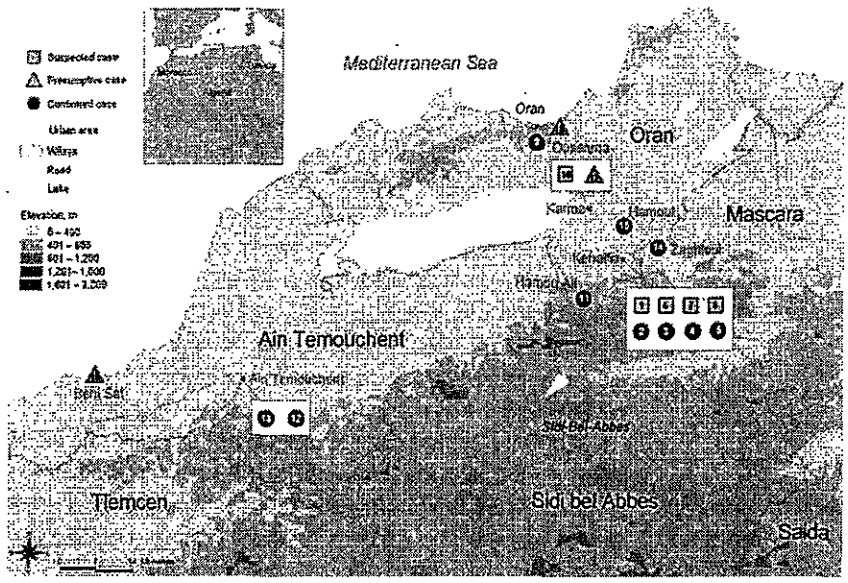

Figure. Geographic distribution of plague cases, Oran region, Algeria, June–July 2003. Boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization (WHO) concerning the legal status of any country, territory, city, or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on *maps represent approximate border lines for* which there may not yet be full agreement. Data source: Ministry of Health Algeria. Map production: Public Health Mapping and GIS, Communicable Diseases, WHO. Copyright WHO, 2006. Used with permission.

with *Y. pestis* (online Appendix Table). Among the 18 patients, 12 had a positive RDT result, but *Y. pestis* was isolated from only 6 patients: 5 from bubo aspirates and 1 from the blood culture of a patient whose bubo was too small to be punctured (patient 13). Results of ELISA-F1 serologic test conducted on the serum samples from 15 of the 18 patients were strongly positive 3 times and slightly positive 3 times (online Appendix Table).

Discussion

Epidemiologic investigation did not identify any other plague patients before patient 1. It is unlikely that other cases occurred and remained undetected during this period since plague, even in its bubonic form, is a severe infection with high fatality rates.

For the first time, the RDT was used in an epidemic situation outside of Madagascar, where it was developed. The case definition had to take into account this particularity. The bacteriologic diagnosis is a long procedure (at least 4 days) and, in this epidemic context, RDT contributed to the effectiveness of the response. Of the 44 RDTs that were conducted, 12 had positive results; by contrast, culture was positive only for 6. Among the 15 patients for whom a serologic test was conducted (online Appendix Table), a specific antibody response developed only in 6. This absence of specific antibodies can be explained by the fact that serum specimens were taken before the appearance of anti-F1 immunoglobulin G, or by a rapid administration of antimicrobial drugs, which stopped development of an immune response. The 3 clearly seropositive patients were those from whom a positive culture was obtained.

The outbreak occurred in a poor rural settlement, with inadequate sanitation. The residents observed an increase in the population of commensal rodents, which is often associated with the harvesting period, but no unusual rodent mortality was noted during the weeks preceding the outbreak. The appearance during the same week of 2 new cases in Ain Temouchent (50 km west of Kehailia) and then 1 case in Beni Saf (100 km southwest of Kehailia) could not be explained. Nonetheless, the fact that the *Y. pestis* strains isolated in Kehailia and Ain Temouchent had identical pulsotypes (V. Chenal-Francisque et al., unpub. data) argues for a single focus and not for independent foci that emerged simultaneously.

A crisis committee designed and supervised a control strategy based on standardized case management, prophylactic treatment and follow-up of contacts sharing the same dwelling as plague patients, and vector control. Environmental sanitation measures in Kehailia contributed to reduction in the occurrence of new cases in this village. Intra- and peridomestic spraying with permethrin was conducted. Deltamethrin was dusted on the tracks and around the burrows of rodents located in a radius of 10 km around the dwelling of the patients. Uncontrolled killing of rats was prohibited.

No natural focus of plague had ever been described in Algeria. Past cases were always regarded as imported through the ports. The reappearance of human cases in this area can be explained in 2 ways: a recent importation of infected animals or a sudden manifestation of a natural focus that had remained silent for decades. It is noteworthy that Kehailia, the epicenter of the outbreak, is in the vicinity of flour mills built 4 years before the outbreak. These mills are supplied regularly with cereals by trucks arriving from the port of Oran. A part of this traffic was still run by railway a year before the outbreak, and a marshalling yard was installed a few kilometers from Kehailia. In 1919, this mode of importation was responsible for the plague outbreak that

occurred 75 km south of the port of Skikda (*10*). The hypothesis of recent importation of the plague bacillus in Kehailia is therefore tempting but is tempered by the fact that 1) the grain is primarily imported from Europe, which is not affected by plague, and from North America where natural foci exist but have very limited areas of overlap with those regions where cereal grains are grown, 2) no higher mortality rate in the murine population of the port was noted, 3) no human cases occurred in this sector of the city, and 4) a 3IS–restriction fragment length polymorphism (*11*) analysis grouped these strains in a cluster clearly distinct from the strains isolated from Africa and America (V. Chenal-Francisque et al., unpub. data).

The geographic concentration of the cases in 2 foci, both contiguous in the mountainous area of Tessala, suggested the existence of a natural focus in this area. Moreover, *Meriones* are present in Tessala, and these rodents are a well-known potential reservoir of *Y. pestis* (*12*). The outbreak occurred at harvest time, and it is possible that the abrupt reduction in the source of food pushed the wild rodents to approach houses in which grain was stored.

The current challenge in terms of public health is to determine if this animal reservoir has disappeared or if it is well established in the ecosystem. The capture of 3 seropositive small mammals (2 *Mus musculus* and 1 *Aleterix algerius*) in July 2004 (J.L. Soares et al., unpub. data) and the identification of several *Y. pestis* infected fleas in the same area (*13*) favor the second option.

Beyond the local problem, the proximity of a possible natural reservoir of plague to Oran, a large international commercial port, raises the possibility of the risk for an urban outbreak. At the time of the investigation, the sanitation in the city and port were poor and rodents proliferated. These urban rodents could come in contact with infected rodents from rural areas in the uncontrolled dumps at the periphery or through a dry riverbed that penetrates as far as the city center. Because of Oran's population density and the commercial activities of its seaport, a plague outbreak would have international implications.

This outbreak is a textbook illustration of the unexpected and sudden reemergence of an infectious disease epidemic that is potentially highly lethal. It also demonstrates that the danger of a plague outbreak is not limited to the currently indexed natural foci.

Acknowledgments

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Dr Bertherat is a medical officer at the Department of Epidemic and Pandemic Alert and Response, Communicable Diseases, World Health Organization, Geneva. His activities focus on plague and epidemic meningitis.

References

1. World Health Organization. International meeting on preventing and controlling plague: the old calamity still has a future. Wkly Epidemiol Rec. 2006;81:278–84.
2. Misonne X. A natural focus of plague in Libya. Ann Soc Belg Med Trop. 1977;57:163–8.
3. Bulletin sanitaire. Gouvernorat General de l'Algerie. 1909–1941. p. 94–524.
4. Mafart B, Brisou P, Bertherat E. Epidémiologie et prise en charge des épidémies de peste en Méditerranée au cours de la Seconde Guerre Mondiale. Bull Soc Pathol Exot. 2004;97:306–10.
5. Pollitzer R. Plague. WHO Monograph Series. 1954;22:233–50.
6. Roux AH, Mercier C. Sur cinq cas de peste pulmonaire primitive dont trois suivis de guérison, observés à l'hôpital civil d'Oran. Bull Soc Pathol Exot. 1946;39:173–8.
7. Chanteau S, Rahalison L, Ralafiarisoa L, Foulon J, Ratsitorahina M, Ratsifasoamanana L, et al. A. Development and testing of a rapid diagnostic test for bubonic and pneumonic plague. Lancet. 2003;361:211–6.
8. Devignat R. Variétés de l'espèce *Pasteurella pestis*. nouvelle hypothèse. Bull World Health Organ. 1951;4:247–63.
9. Chanteau S, Rahalison L, Ratsitorahina M, Mahafaly M, Rasolomaharo M, Boisier P, et al. Early diagnosis of bubonic plague using F1 antigen capture ELISA assay and rapid immunogold dipstick. Int J Med Microbiol. 2000;290:279–83.
10. Raynaud L. Epidemie de peste à forme septicémique. Revue d'hygiène et police sanitaire. Aug. 1919.
11. Torrea G, Chenal-Francisque V, Leclercq A, Carniel E. Efficient tracing of global isolates of *Yersinia pestis* by restriction fragment length polymorphism analysis using three insertion sequences as probes. J Clin Microbiol. 2006;44:2084–92.
12. Baltazard M, Bahmanyar M, Mofidi C, Seydian B. Kurdistan plague focus [in undetermined language]. Bull World Health Organ. 1952;5:441.
13. Bitam I, Baziz B, Rolain JM, Belkaid M, Raoult D. Zoonotic focus of plague, Algeria. Emerg Infect Dis. 2006;12:1975–7.

Address for correspondence: Eric Bertherat, Communicable Diseases, EPR/ERI, World Health Organization, 20 av. Appia, CH-1211 Geneva 27, Switzerland; email: bertherate@who.int

"So many scientists think that once they figure it out, that's all they have to do, and writing it up is just a chore. I never saw it that way; part of the art of any kind of total scholarship is to say it well."

—Stephen Jay Gould

別紙様式第2-1

No. 21

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2007. 12. 17	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況		公表国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社) 合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)		Mead S, Joiner S, Desbruslais M, Beck JA, O'Donoghue M, Lantos P, Wadsworth JD, Collinge J. Arch Neurol. 2007 Dec;64(12):1780-4.	英国

研究報告の概要

○英国の若年女性におけるクロイツフェルト・ヤコブ病、プリオンタンパク質遺伝子コドン129VV、および新規PrP^{Sc}型

背景:変異型クロイツフェルト・ヤコブ病(vCJD)は、ウシ海綿状脳症と因果関係のある後天性プリオン疾患であり、若い成人に多く発現する。調査した臨床例は全て、プリオンタンパク遺伝子(PRNP)のコドン129がメチオニンホモ接合体であり、典型的な神経病理所見を伴い、分子学的系統は典型的なPrP^{Sc}タイプ4であった。トランスジェニックマウスのモデル試験では、他のPRNP遺伝子型もウシ海綿状脳症に感染しやすいことが示されているが、特徴的な表現型を発現すると考えられる。

目的:PRNP コドン129がバリンホモ接合である非定型孤発型CJDの若年英国人女性の組織病理学的、分子学的検討。

デザイン:症例報告、剖検、分子学的解析。

設定・施設:neurology referral centerおよびMRC(医学研究審議会)プリオン部門の研究所。

結果:剖検所見は非定型孤発型CJDであり、灰白質と白質の変性が顕著で、プリオンタンパク質(PrP)の広域な沈着があった。解析用のリンパ網内系組織は得られなかった。小脳組織由来のPrP^{Sc}(PrPのスクレイピーアイソフォーム)の分子解析は、vCJDで見られるものと同等の新規PrP^{Sc}型を示した(PrP^{Sc}タイプ4)。しかし、金属イオンキレート剤EDTA存在下においてプロテアーゼ切断部位が変化したことにより、典型的なvCJD パターンと区別することができた。

結論:本患者に見られたプリオン系統の特徴を明らかにし、ウシ海綿状脳症との因果関係を検討するには、さらに試験が必要である。本症例は、PrP^{Sc}のプロテアーゼ切断パターンの金属イオン依存性を検討するため、EDTAによるプリオン疾患の分子解析の重要性を明らかにしている。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-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
PRNP コドン129がバリンホモ接合である非定型孤発型CJDの若年英国人女性の症例報告である。	日本赤十字社は、vCJDの血液を介する感染防止の目的から、献血時に過去の海外渡航歴(旅行及び居住)を確認し、欧州36ヶ国に一定期間滞在したドナーを無期限に献血延期としている。また、英国滞在歴を有するvCJD患者が国内で発生したことから、平成17年6月1日より1980～96年に1日以上の英国滞在歴のある方からの献血を制限している。今後もCJD等プリオン病に関する新たな知見及び情報の収集に努める。

OBSERVATION

Creutzfeldt-Jakob Disease, Prion Protein Gene Codon 129VV, and a Novel PrP^{Sc} Type in a Young British Woman

Simon Mead, PhD, MRCP; Susan Joiner, MSc; Melanie Desbruslais, BSc; Jonathan A. Beck, BSc; Michael O'Donoghue, PhD; Peter Lantos, FRCP; Jonathan D. F. Wadsworth, PhD; John Collinge, FRS

Background: Variant Creutzfeldt-Jakob disease (vCJD) is an acquired prion disease causally related to bovine spongiform encephalopathy that has occurred predominantly in young adults. All clinical cases studied have been methionine homozygotes at codon 129 of the prion protein gene (*PRNP*) with distinctive neuropathological findings and molecular strain type (PrP^{Sc} type 4). Modeling studies in transgenic mice suggest that other *PRNP* genotypes will also be susceptible to infection with bovine spongiform encephalopathy prions but may develop distinctive phenotypes.

Objective: To describe the histopathologic and molecular investigation in a young British woman with atypical sporadic CJD and valine homozygosity at *PRNP* codon 129.

Design: Case report, autopsy, and molecular analysis.

Setting: Specialist neurology referral center, together with the laboratory services of the MRC [Medical Research Council] Prion Unit.

Subject: Single hospitalized patient.

Main Outcome Measures: Autopsy findings and molecular investigation results.

Results: Autopsy findings were atypical of sporadic CJD, with marked gray and white matter degeneration and widespread prion protein (PrP) deposition. Lymphoreticular tissue was not available for analysis. Molecular analysis of PrP^{Sc} (the scrapie isoform of PrP) from cerebellar tissue demonstrated a novel PrP^{Sc} type similar to that seen in vCJD (PrP^{Sc} type 4). However, this could be distinguished from the typical vCJD pattern by an altered protease cleavage site in the presence of the metal ion chelator EDTA.

Conclusions: Further studies will be required to characterize the prion strain seen in this patient and to investigate its etiologic relationship with bovine spongiform encephalopathy. This case illustrates the importance of molecular analysis of prion disease, including the use of EDTA to investigate the metal dependence of protease cleavage patterns of PrP^{Sc}.

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Author Affiliations: MRC [Medical Research Council] Prion Unit and Department of Neurodegenerative Disease, Institute of Neurology, University College London, National Hospital for Neurology and Neurosurgery, London, England (Drs Mead, Wadsworth, and Collinge; Mss Joiner and Desbruslais; and Mr Beck); and Institute of Psychiatry, King's College London (Dr Lantos). Dr O'Donoghue is now with the Department of Clinical Neurology, Nottingham University Hospitals NHS [National Health Service] Trust, Nottingham, England.

THE ORIGINAL RECOGNITION of variant Creutzfeldt-Jakob disease (vCJD) was based on a case series of young patients with rapidly progressive dementia, a geographic and temporal association with bovine spongiform encephalopathy (BSE), and novel neuropathological findings consisting of abundant florid prion protein (PrP) plaques.[1] Molecular strain typing allowed identification of a unique type of PrP^{Sc} (the scrapie isoform of PrP) (type 4) in the brain that was distinct from those seen in classic (sporadic or iatrogenic) CJD and similar to that seen in BSE prion infection of cattle and other species.[2] Subsequent biological strain typing in both conventional and transgenic mice confirmed that vCJD and BSE were caused by the same prion strain.[3,4]

Variant CJD also differs markedly from classic CJD in having prominent and consistent involvement of lymphoreticular tissue, allowing its diagnosis by tonsil biopsy findings.[5-7] To date, more than 160 individuals have died of vCJD in the United Kingdom; the number infected by BSE prions and who may develop prion disease in the years ahead is unknown because human prion incubation periods may exceed 50 years.[8]

All clinical cases of vCJD studied have had a methionine-homozygous (MM) genotype at polymorphic codon 129 of the prion protein gene (*PRNP*).[9] The extension of BSE prion-related disease to individuals with valine-homozygous (VV) or heterozygous (MV) genotypes at *PRNP* codon 129 has been predicted by comparison with other acquired human prion diseases[10,11] and by transgenic mouse

models.[12-14] These models also predict that infection of VV and MV genotypes with BSE or vCJD prions may result in propagation of distinct prion strain types and that patients with VV or MV genotypes might present with clinical, pathological, and molecular phenotypes distinct from that of vCJD.[12-14]

To date, we know of no reported cases of clinical vCJD occurring in the VV or MV genotypes. However, PrP^{Sc} has been reported in lymphoid tissues, but not in the brain, of a patient with *PRNP* 129 MV who had received blood from a person with preclinical vCJD and who died of an unrelated cause.[15] In addition, abnormal PrP immunoreactivity has been reported in anonymous archived lymphoid tissue from 2 individuals with *PRNP* 129 VV.[16] It is unknown whether the individual with the MV genotype would have gone on (or if those with VV will go on) to develop clinical disease and, if so, whether the phenotype will fit the case definition of vCJD.

METHODS

Brain homogenates (10% w/v) were prepared in Dulbecco phosphate buffered saline lacking Ca^{2+} or Mg^{2+} ions. Aliquots were analyzed with or without proteinase K digestion (50 µg/mL final protease concentration, 1 hour, 37°C) by immunoblotting with anti–PrP monoclonal antibody 3F4[17] as described previously.[7,18] Metal ion–dependent conformations of PrP were determined as previously described.[19] Genomic DNA was extracted from peripheral blood, and the entire *PRNP* open reading frame was amplified by polymerase chain reaction and sequenced as described previously.[20]

REPORT OF A CASE

A 39-year-old woman presented to an optician in January 1999 with episodes of blurred vision and photophobia, but no abnormality was found. Two months later, she noted memory impairment, diplopia, dysarthria, and an unsteady gait of fluctuating severity. Five months after onset, the gait and limb ataxia had progressed, although walking was still possible, and the memory loss became more profound. The patient then developed paranoid ideation, aggression, restless nocturnal behavior, anorexia, and mood disturbance. By 5½ months after onset, she could not walk and was unsteady sitting, and limb movements were clumsy.

Examination showed dysarthria, broken pursuit eye movements without nystagmus, impaired upgaze, and stereotyped involuntary movements of the legs. However, limb power, vibration, proprioception, tendon reflexes, and plantar responses were normal. During the ensuing 4 weeks, speech ceased and incontinence and jerky involuntary limb movements became evident. Eight months after onset, the patient was mute but could follow some commands. She was able to visually fixate and follow moving objects but also had abnormal, spontaneous horizontal roving eye movements with a supranuclear vertical gaze palsy. Her face was impassive with occasional twitching movements, brisk facial reflexes, and trismus. There were prominent jerking movements of all limbs brought out by use; power was relatively preserved and the plantar responses were extensor.

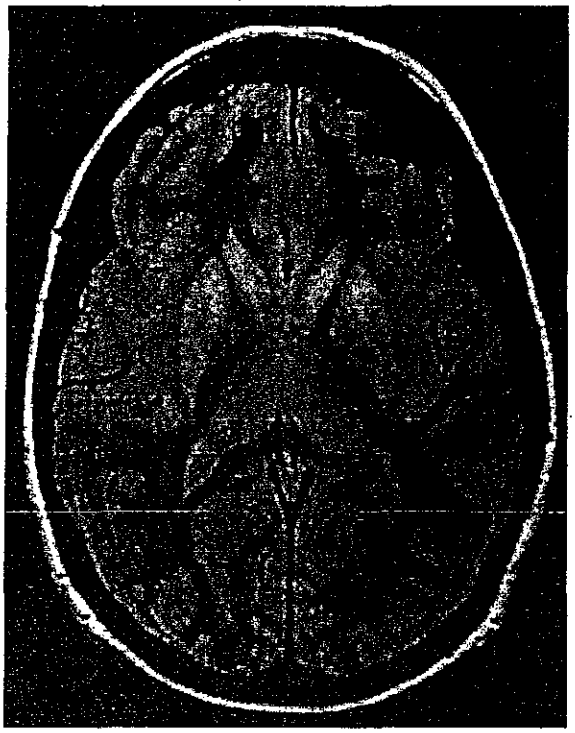

Figure 1. T2-weighted axial magnetic resonance image showing diffuse increased signal within both caudate nuclei and each putamen.

There was a strong family history of late-onset cerebellar ataxia consistent with autosomal dominant inheritance. A polyglutamine expansion in ataxin 3 associated with spinocerebellar ataxia type 3 was found in a symptomatic family member, but our patient did not share this expansion.

Normal results of the following investigations were found: complete blood cell count, erythrocyte sedimentation rate, C-reactive protein, electrolytes, liver function, thyroid function, enzyme-linked immunosorbent assay for syphilis, vitamin B_{12}, folate, ferritin, vitamin E, and serum ceruloplasmin. Tests for antinuclear, antineuronal, anti–Purkinje cell, and antiganglioside antibodies were negative. Nerve conduction studies showed no evidence of a peripheral neuropathy. The electroencephalogram 6 months after onset was reported as normal, but at 7 and 8 months electroencephalograms showed diffuse slow-wave activity, without epileptiform changes or periodic discharges typical of CJD. Cerebrospinal fluid examination showed a normal cell count, protein level, and glucose level, and oligoclonal immunoglobulin bands were absent. The protein S100b level of 4.39 ng/mL (reference cutoff, <0.38 ng/mL), neuron-specific enolase level of 98 ng/mL (reference cutoff, <20 ng/mL), and 14-3-3 protein were all abnormal values.

A magnetic resonance image of the brain (**Figure 1**) showed diffuse cerebellar atrophy and diffuse increased signal within both caudate nuclei and each putamen. Tonsil biopsy was not possible because of a previous tonsillectomy from which little tissue remained. Genetic testing for mutations associated with spinocerebellar ataxia 1, 2, 3, 6, and 7 and Friedreich ataxia gave negative re-

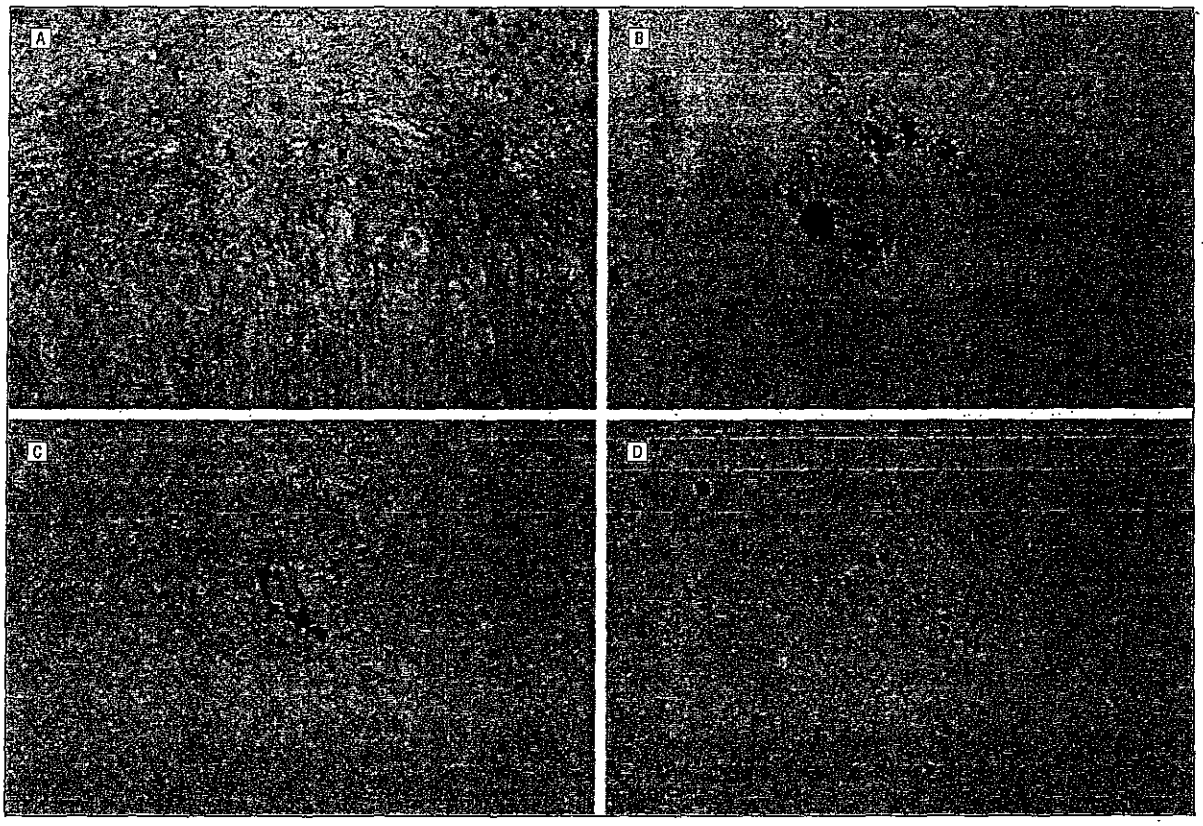

Figure 2. Immunohistochemical analysis of brain sections from the patient. A, Glial fibrillary acidic protein immunohistochemistry of the molecular and granule cell layers of the cerebellum showing neuronal loss and Bergmann astrocytosis (original magnification ×20). B, Granular prion protein staining in the cerebellum (original magnification ×40). C, Perineuronal prion protein staining in the temporal lobe (original magnification ×20). D, Prion protein plaques in the temporal lobe (original magnification ×20).

sults. Sequencing of the *PRNP* open reading frame was normal on 2 separate occasions. A polymerase chain reaction performed with primers designed to amplify the octapeptide repeat region of *PRNP* did not demonstrate an insertion mutation. The codon 129 polymorphism was homozygous for valine.

Fourteen months after onset, the patient died and an autopsy was performed.

AUTOPSY FINDINGS

Histopathologic examination was limited to the brain and spinal cord (**Figure 2**). The findings were atypical of sporadic CJD in the severity of white matter degeneration and the extensive nature of PrP deposition in the cortex and white matter. The frontal cortex showed extremely severe neuronal loss with striking astrocytosis and prominent spongiform vacuolation. There was severe overall loss of white matter, in places reminiscent of infarction. Deposition of PrP was extensive throughout the cortex and white matter. In places this was a diffuse punctate deposition similar to the recognized synaptic pattern. Occasionally, individual cells, mainly pyramidal neurons, were outlined by PrP deposition and had a fine granular intracellular deposition. More dense deposits, similar to plaques, were seen in the cortex. Also in the white matter, PrP deposits were seen ranging from a couple of micrometers to much larger plaquelike deposits, although these were not florid.

Temporal, parietal, and occipital lobes showed histologic features similar to those described in the frontal lobe, the occipital lobe being most severe. The hippocampus was relatively well preserved. In the caudate, putamen, and amygdala there was neuronal loss, astrocytosis, and microglial activation. The thalamus, midbrain, and substantia nigra showed mild to moderate spongiform change, neuronal loss, and astrocytosis with intraneuronal and extracellular punctate deposits. The pons and medulla were less severely affected than the midbrain with punctate PrP deposits. The cerebral peduncles were severely affected, with nearly complete loss of myelin. The cerebellum was very severely affected, with a dramatic loss of Purkinje and granule cells accompanied by vacuolation and astrocytosis. The cerebellar white matter showed severe white matter loss similar to incipient infarcts. Deposition of PrP in the cerebellum was marked with accumulation of punctate deposits resembling plaques, most commonly in the granule cell layer. In the white matter the deposits were denser still, occasionally plaquelike or forming irregular linear deposits.

PrP^{Sc} TYPING STUDIES

Western blot analysis was performed on fresh frozen cerebellar tissue from the patient. Identical results were ob-

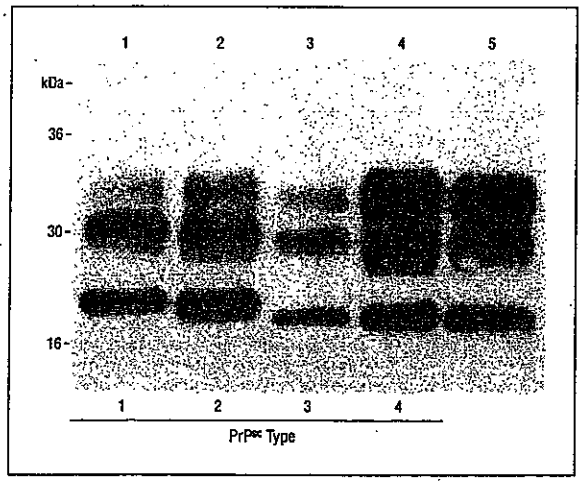

Figure 3. Immunoblotting of 10% brain homogenate after limited proteinase K digestion using anti–prion protein (PrP) monoclonal antibody 3F4. Lanes 1, 2, and 3 show 3 types of PrP^{Sc} (the scrapie isoform of PrP) seen in sporadic and iatrogenic cases of Creutzfeldt-Jakob disease; lane 4 shows PrP^{Sc} type 4, which is uniquely seen in brain tissue from patients with variant Creutzfeldt-Jakob disease.[21] Lane 5 shows PrP^{Sc} from the cerebellum of our patient demonstrating the same predominance of the high-molecular-mass diglycosylated PrP glycoform and a molecular mass of all PrP fragments similar to those of PrP^{Sc} type 4.

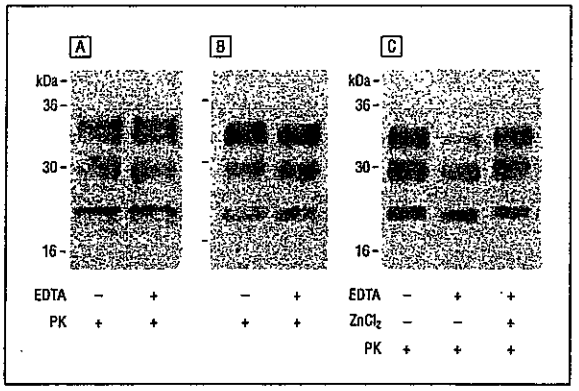

Figure 4. Immunoblotting of 10% brain homogenate after limited proteinase K (PK) digestion using anti–prion protein (PrP) monoclonal antibody 3F4. A, Cerebellum from a patient with variant Creutzfeldt-Jakob disease demonstrating a doublet of low-molecular-mass nonglycosylated bands of PrP^{Sc} (the scrapie isoform of PrP) with an identical pattern of PrP fragments observed after proteolysis in the presence of 25mM EDTA. B, Cerebellum from our patient demonstrating a doublet of low-molecular-mass nonglycosylated PrP^{Sc} bands. All bands migrate with lower apparent molecular mass following proteolysis in the presence of 25mM EDTA. C, Aliquots of cerebellum homogenate from our patient digested directly with proteinase K or after treatment with 25mM EDTA and sequential washing of insoluble pellets with *N*-ethyl morpholine buffer either lacking (–) or containing (+) 20μM zinc chloride ($ZnCl_2$).[19]

tained from separately analyzed tissue samples from opposite poles of the cerebellum. The glycoform ratio and fragment sizes resembled PrP^{Sc} type 4 seen in vCJD (**Figure 3**). The nonglycosylated band was seen as a doublet, as is seen for PrP^{Sc} in the cerebellum in vCJD (**Figure 4**). The effect of adding the metal ion chelator EDTA to the cerebellum homogenate before proteinase K cleavage was to reduce the apparent molecular weight of PrP^{Sc} fragments. This reflects the involvement of metal ions (most likely copper and zinc) in the conformation of PrP and determination of accessible protease cleavage sites.[19] This deduction was verified by showing that application of zinc ions to EDTA-treated samples before proteolysis resulted in preservation of the original PrP^{Sc} fragment size (Figure 4C). Although similar dependence on metal ions is observed for some PrP^{Sc} conformers associated with sporadic CJD,[19,21] this is not observed with PrP^{Sc} type 4 propagated in vCJD[19,21] (Figure 4). Therefore, these findings reflect a novel PrP^{Sc} type when compared with the diversity we and others have so far documented.[21,22]

COMMENT

Does the PrP^{Sc} typing suggest a BSE-related cause, or can our findings be accommodated by the spectrum seen in sporadic CJD cases worldwide? The molecular strain typing of the patient's brain material demonstrated a novel PrP^{Sc} type when compared with our archived cases.[21] There is as yet no internationally agreed-on classification of PrP^{Sc} type. Parchi and colleagues[23] identified 2 PrP^{Sc} types in sporadic CJD. However, Hill et al[21] described 3 PrP^{Sc} types associated with sporadic and iatrogenic CJD (types 1-3) and PrP^{Sc} type 4 associated with vCJD. The PrP^{Sc} type 5 has, to our knowledge, been observed only in mice expressing human PrP 129V inoculated with vCJD.[3,12] Hill et al[21] recently described a novel PrP^{Sc} type 6 in sporadic CJD.

The PrP^{Sc} type from our case has features similar to PrP^{Sc} type 4 (vCJD) in the predominance of the diglycosylated band; however, it is distinct from PrP^{Sc} type 4 in the dependence of the protease cleavage pattern of PrP^{Sc} on metal ions, suggesting a distinct PrP^{Sc} conformation. Unfortunately, only cerebellum was available for Western blotting in this case, although in vCJD cases from which whole brain was available we have not found evidence of any regional variation in PrP^{Sc} type. Others have reported coexistence of Gambetti PrP^{Sc} type 1 in the brain from patients with vCJD as a minority component.[24] It would also have been interesting to look for peripheral lymphoreticular PrP deposition because this is prominent in vCJD, but that tissue was not available for analysis. Transmission of BSE isolates to transgenic mice expressing human PrP 129 valine results in clinical prion disease with undetectable PrP^{Sc}; however, transmission of vCJD isolates to the same mice produces PrP^{Sc} type 5 that shares the same predominance of diglycosylated PrP^{Sc} to that of PrP^{Sc} type 4, and these data suggest that the molecular signature of BSE may be preserved after BSE transmission to *PRNP* codon 129 VV humans.[3,12] Transmission studies of the current case in transgenic mice are now being undertaken to investigate transmission characteristics.

We have described a novel PrP^{Sc} type that would be designated type 7 by our classification. A firm connection between novel PrP^{Sc} types and BSE cannot be made on the basis of a single case, and it will be important to see whether other similar cases occur in the United Kingdom and other BSE-exposed countries but not elsewhere and to perform detailed transmission studies of prions from this patient into transgenic and conventional mice to compare with BSE-derived isolates from

cattle and other species. Two other cases of prion disease with valine homozygosity and atypical features have been reported in the United Kingdom and the Netherlands. One of these cases was atypical because of very young onset and a protracted psychiatric history[25]; the other was notable because certain clinical and molecular features of the case overlapped with those of vCJD, including Western blot analysis of autopsied brain showing a predominance of a diglycosylated PrP^{Sc} isoform.[26]

We recommend keeping an open mind about the etiology of such cases during the ensuing years. These cases emphasize the importance both of continued surveillance of prion disease and the further development and refinement of molecular classification of prion diseases of humans and animals. It will also be important to assess lymphoreticular involvement in subsequent cases either at diagnostic tonsil biopsy or at autopsy.

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Correspondence: John Collinge, FRS, MRC Prion Unit and Department of Neurodegenerative Disease, Institute of Neurology, University College London, National Hospital for Neurology and Neurosurgery, Queen Square, London WC1N 3BG, England (j.collinge@prion.ucl.ac.uk).
Author Contributions: *Study concept and design:* Mead and Collinge. *Acquisition of data:* Mead, Joiner, Desbruslais, O'Donoghue, Lantos, Wadsworth, and Collinge. *Analysis and interpretation of data:* Mead, Joiner, Desbruslais, Beck, Wadsworth, and Collinge. *Drafting of the manuscript:* Mead, Desbruslais, Beck, Lantos, Wadsworth, and Collinge. *Critical revision of the manuscript for important intellectual content:* Mead, Joiner, O'Donoghue, Wadsworth, and Collinge. *Statistical analysis:* Mead. *Obtained funding:* Collinge. *Administrative, technical, and material support:* Desbruslais, Beck, Lantos, and Collinge. *Study supervision:* Wadsworth and Collinge.
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REFERENCES

1. Will RG, Ironside JW, Zeidler M, et al. A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet.* 1996;347(9006):921-925.
2. Collinge J, Sidle KCL, Meads J, Ironside J, Hill AF. Molecular analysis of prion strain variation and the aetiology of "new variant" CJD. *Nature.* 1996;383(6602):685-690.
3. Hill AF, Desbruslais M, Joiner S, Sidle KCL, Gowland I, Collinge J. The same prion strain causes vCJD and BSE. *Nature.* 1997;389(6650):448-450.
4. Bruce ME, Will RG, Ironside JW, et al. Transmissions to mice indicate that "new variant" CJD is caused by the BSE agent. *Nature.* 1997;389(6650):498-501.
5. Hill AF, Zeidler M, Ironside J, Collinge J. Diagnosis of new variant Creutzfeldt-Jakob disease by tonsil biopsy. *Lancet.* 1997;349(9045):99-100.
6. Hill AF, Butterworth RJ, Joiner S, et al. Investigation of variant Creutzfeldt-Jakob disease and other human prion diseases with tonsil biopsy samples. *Lancet.* 1999;353(9148):183-189.
7. Wadsworth JDF, Joiner S, Hill AF, et al. Tissue distribution of protease resistant prion protein in variant CJD using a highly sensitive immuno-blotting assay. *Lancet.* 2001;358(9277):171-180.
8. Collinge J, Whitfield J, McKintosh E, et al. Kuru in the 21st century—an acquired human prion disease with very long incubation periods. *Lancet.* 2006;367(9528):2068-2074.
9. Collinge J, Beck J, Campbell T, Estibeiro K, Will RG. Prion protein gene analysis in new variant cases of Creutzfeldt-Jakob disease. *Lancet.* 1996;348(9019):56.
10. Collinge J. Variant Creutzfeldt-Jakob disease. *Lancet.* 1999;354(9175):317-323.
11. Mead S, Stumpf MP, Whitfield J, et al. Balancing selection at the prion protein gene consistent with prehistoric kurulike epidemics. *Science.* 2003;300(5619):640-643.
12. Wadsworth JD, Asante EA, Desbruslais M, et al. Human prion protein with valine 129 prevents expression of variant CJD phenotype. *Science.* 2004;306(5702):1793-1796.
13. Bishop MT, Hart P, Aitchison L, et al. Predicting susceptibility and incubation time of human-to-human transmission of vCJD. *Lancet Neurol.* 2006;5(5):393-398.
14. Asante EA, Linehan JM, Gowland I, et al. Dissociation of pathological and molecular phenotype of variant Creutzfeldt-Jakob disease in transgenic human prion protein 129 heterozygous mice. *Proc Natl Acad Sci U S A.* 2006;103(28):10759-10764.
15. Peden AH, Head MW, Ritchie DL, Bell JE, Ironside JW. Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. *Lancet.* 2004;364(9433):527-529.
16. Ironside JW, Bishop MT, Connolly K, et al. Variant Creutzfeldt-Jakob disease: prion protein genotype analysis of positive appendix tissue samples from a retrospective prevalence study [published correction appears in *BMJ.* 2006;333(7565):416]. *BMJ.* 2006;332(7551):1186-1188.
17. Kascsak RJ, Rubenstein R, Merz PA, et al. Mouse polyclonal and monoclonal antibody to scrapie-associated-fibril proteins. *J Virol.* 1987;61(12):3688-3693.
18. Hill AF, Joiner S, Beck JA, et al. Distinct glycoform ratios of protease resistant prion protein associated with *PRNP* point mutations. *Brain.* 2006;129(pt 3):676-685.
19. Wadsworth JDF, Hill AF, Joiner S, Jackson GS, Clarke AR, Collinge J. Strain-specific prion-protein conformation determined by metal ions. *Nat Cell Biol.* 1999;1(1):55-59.
20. Poulter M, Baker HF, Frith CD, et al. Inherited prion disease with 144 base pair gene insertion, I: genealogical and molecular studies. *Brain.* 1992;115:675-685.
21. Hill AF, Joiner S, Wadsworth JD, et al. Molecular classification of sporadic Creutzfeldt-Jakob disease. *Brain.* 2003;126(pt 6):1333-1346.
22. Parchi P, Giese A, Capellari S, et al. Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. *Ann Neurol.* 1999;46(2):224-233.
23. Parchi P, Castellani R, Capellari S, et al. Molecular basis of phenotypic variability in sporadic Creutzfeldt-Jakob disease. *Ann Neurol.* 1996;39(6):767-778.
24. Yull HM, Ritchie DL, Langeveld JP, et al. Detection of type 1 prion protein in variant Creutzfeldt-Jakob disease. *Am J Pathol.* 2006;168(1):151-157.
25. Hillier CE, Llewelyn JG, Neal JW, Ironside JW. Creutzfeldt-Jakob disease in a young person with valine homozygosity at codon 129: sporadic or variant? *J Neurol Neurosurg Psychiatry.* 2001;70(1):134-135.
26. Head MW, Tissingh G, Uitdehaag BMJ, et al. Sporadic Creutzfeldt-Jakob disease in a young Dutch valine homozygote: atypical molecular phenotype. *Ann Neurol.* 2001;50(2):258-261.

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別紙 3-1

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一般的名称			研究報告の公表状況	Species barrier for chronic wasting disease by in vitro conversion of prion protein. Li, L. et al, Biochem. Biophys. Res. Com. 364(4), 796-800 (2007)	公表国 カナダ	
販売名（企業名）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
本稿の著者らは，慢性消耗性疾患（北米シカに影響を及ぼす伝染性海綿状脳症）は，in vitro アッセイにおいてある特定の条件下で種の壁をすり抜けて感染することを明らかにした。本アッセイは，異種動物からの正常な脳ホモジネート（正常 PrP^{C}）を基質として，エルク（ヨーロッパヘラジカ）の異常プリオンタンパク質（PrP^{Sc}）とともにインキュベートするものである。標準の条件（pH 7.4）下では，エルク（ヨーロッパヘラジカ）PrP^{Sc} は同種系列〔トナカイ，ムース（アメリカヘラジカ），カリブー及びエルク（ヨーロッパヘラジカ）〕の PrP^{C} をタンパク質分解酵素耐性アイソフォームへと変換させたが，異種 PrP^{C}（ヒト，マウス，ヒツジ，ウシ，ハムスター）については，PrP^{C} のタンパク質配列が全ての種で 90%以上保持されているにもかかわらずタンパク質分解酵素耐性アイソフォームへ変換されたものは僅かであった。しかしながら，低 pH（3.5）による部分変性の条件下では，PrP^{Sc} によるタンパク質分解酵素耐性アイソフォームへの変換は全ての種で劇的に増大した。これより，基質の部分変性によって構造上の変化が起こり，遠隔種間の種の壁を越えることが示唆される。	BYL-2008-0303

報告企業の意見	今後の対応
異常プリオン PrP^{Sc} によるアイソフォーム変換への感度および耐性は，基質である PrP^{C} の立体構造が重要であるとしているが，生物学的な関連性については疑問が残る。	現時点で新たな安全対策上の措置を講じる必要はないと考える。

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Species barriers for chronic wasting disease by *in vitro* conversion of prion protein

Li Li [a], Michael B. Coulthart [b], Aru Balachandran [c], Avi Chakrabartty [d], Neil R. Cashman [a,*]

[a] *Brain Research Centre, Division of Neurology, Department of Medicine, University of British Columbia and Vancouver Coastal Health, UBC Hospital, 2211 Wesbrook Mall, Vancouver, BC, Canada V6T 2B5*
[b] *Prion Diseases Program, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Man., Canada R3E 3R2*
[c] *National Reference Laboratory for Scrapie and CWD, Animal Diseases Research Institute, Canadian Food Inspection Agency, 3851 Fallowfield Road, Nepean, Ont., Canada K2H 8P9*
[d] *University Health Network, Department of Medical Biophysics, University of Toronto, Toronto, Ont., Canada M5G 1L7*

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Abstract

Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy that can affect North American cervids (deer, elk, and moose). Using a novel *in vitro* conversion system based on incubation of prions with normal brain homogenates, we now report that PrP^{CWD} of elk can readily induce the conversion of normal cervid PrP (PrP^{C}) molecules to a protease-resistant form, but is less efficient in converting the PrP^{C} of other species, such as human, bovine, hamster, and mouse. However, when substrate brain homogenates are partially denatured by acidic conditions (pH 3.5), PrP^{CWD}-induced conversion can be greatly enhanced in all species. Our results demonstrate that PrP^{C} from cervids (including moose) can be efficiently converted to a protease-resistant form by incubation with elk CWD prions, presumably due to sequence and structural similarities between these species. Moreover, partial denaturation of substrate PrP^{C} can apparently overcome the structural barriers between more distant species.

Keywords: CWD; PrP^{C}; PrP^{Sc}; *In vitro* conversion; Species barrier

Chronic wasting disease (CWD) is a cervid form of transmissible spongiform encephalopathy (TSE) or prion disease. CWD's rapid spread from' Colorado to other states [1,2], to Canadian provinces (Alberta, Saskatchewan) [1] and to Korea [2,3] has raised concerns about its species tropism [4–6]. CWD has been transmitted to cattle via intracerebral inoculation [7], and to other animals, including ferrets, mink, and goats [8,9]. Reports documenting CWD prions in the muscle [10,11], blood, and saliva [12] of infected cervids, have heightened interest in the disease by public health agencies [13].

CWD and other TSEs are believed to be due to the template-directed accumulation of disease-associated prion protein, generically designated PrP^{Sc}. PrP^{C} in brain homogenates can be converted to a protease-resistant form by incubation with PrP^{Sc} "seeds" which are thought to recapitulate the template-directed misfolding of prion protein in disease [14,15], including protein misfolding cyclic amplification (PMCA) [15]. We have previously reported that partially denatured human brain PrP^{C} (which may mimic a PrP conversion intermediate [16]) is a superior substrate for templated *in vitro* conversion compared with untreated PrP^{C} in an incubation-shaking assay that does not utilize PMCA sonication [17].

Materials and methods

Reagents and antibodies. Proteinase K (PK) was purchased from Invitrogen. Mouse monoclonal antibody 6H4 was from Prionics Co. (Zürich, Switzerland). Horseradish peroxidase-conjugated sheep anti-

* Corresponding author. Fax: +1 604 822 7299.
E-mail address: Neil.Cashman@vch.ca (N.R. Cashman).

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mouse antibody was purchased from Amersham Biosciences. All other chemicals were purchased from Sigma unless specified otherwise.

Brain tissues and homogenate preparation. All brain samples were obtained from the disease control and surveillance programs of the Canadian Food Inspection Agency (CFIA) and were harvested within 24 h of death. The normal brain tissue was determined to be free of neurological disorders on the basis of neuropathological examination. The presence of PrP^{Sc} in brain tissue from an elk with clinical chronic wasting disease (CWD) was confirmed by immunohistochemistry and PK resistance on immunoblotting analysis. All tissues were frozen immediately after collection and stored at −80 °C. Ten percent (w/v) brain homogenates were prepared in lysis buffer (100 mM NaCl, 10 mM EDTA, 0.5% Nonidet P-40, 0.5% sodium deoxycholate, and 10 mM Tris–HCl, pH 7.5) as previously described [17].

Preparation of acid/GdnHCl-treated PrP^{C}. The preparation was followed as previously described [17], in brief, 100 µl of 10% brain homogenate was mixed with an equal volume of 3.0 M guanidine hydrochloride GdnHCl (final concentration of 1.5 M) in PBS at pH 7.4 or pH 3.5 adjusted with 1 M HCl, and incubated for 5 h at room temperature with shaking. After that, samples were precipitated with methanol and resuspended in 100 µl of PBS (pH 7.4) with 0.05% SDS, 0.5% Triton X-100.

In vitro conversion of acid/GdnHCl-treated PrP^{C}. In vitro conversion was performed in a 50 µl volume of the appropriate test substrate material (49 µl of normal brain homogenate + 1 µl CWD brain homogenate in a 1:50 dilution as the prion template). The sample was then incubated in a thermomixer at 37 °C for 12 h with shaking. After PK digestion and boiling in the loading buffer, the samples were subjected to SDS–PAGE and immunoblotting.

Proteinase K resistance and immunoblotting. To determine the PK-resistance of the PrP, 20 µl of the sample was incubated with PK at 100 µg/ml for 1 h at 37 °C, and the digestion reaction was terminated by addition of PMSF to 2 mM of final concentration. Proteins were separated by NuPAGE 4–12% pre-cast Bis–Tris gel (Invitrogen) and electrotransferred onto PVDF membranes. 6H4 was used as primary antibody (1:5000) and horseradish peroxidase-conjugated sheep anti-mouse IgG as secondary antibody. The proteins were visualized by enhanced chemiluminescence + Plus (ECL + Plus, Amersham Biosciences), the blots were scanned and were analyzed by Quantity One (Bio-Rad) software. At least eight experiments were performed on each species.

Results and discussion

Sequence alignment of prion protein

CWD appears to be freely transmitted among susceptible species of cervids by direct or environmentally medi-

A

```
    1                                                   50
Rangifer    MVKSHIGSWI LVLFVAMWSD VGLCKKRPKP GGGWNTGGSR YPGQGSPGGN
Elk         MVKSHIGSWI LVLFVAMWSD VGLCKKRPKP GGGWNTGGSR YPGQGSPGGN
Moose       MVKSHIGSWI LVLFVAMWSD VGLCKKRPKP GGGWNTGGSR YPGQGSPGGN

   51                                                  100
Rangifer    RYPPQGGGGW GQPHGGGWGQ PHGGGWGQPH GGGWGQPHGG GGWGQGGTHS
Elk         RYPPQGGGGW GQPHGGGWGQ PHGGGWGQPH GGGWGQPHGG GGWGQGGAHS
Moose       RYPPQGGGGW GQPHGGGWGQ PHGGGWGQPH GGGWGQPHGG GGWGQGGIHS

  101                                                  150
Rangifer    QWNKPSKPKT NMKHVAGAAA AGAVVGGLGG YMLGSAMSRP LIHFGNDYED
Elk         QWNKPSKPKT NMKHVAGAAA AGAVVGGLGG YMLGSAMSRP LIHFGNDYED
Moose       QWNKPSKPKT NMKHVAGAAA AGAVVGGLGG YMLGSAMSRP LIHFGNDYED

  151                                                  200
Rangifer    RYYRENMYRY PNQVYYRPVD QYNNQNTFVH DCVNITVKQH TVTTTTKGEN
Elk         RYYRENMYRY PNQVYYRPVD QYNNQNTFVH DCVNITVKQH TVTTTTKGEN
Moose       RYYRENMYRY PNQVYYRPVD QYNNQNTFVH DCVNITVKQH TVTTTTKGEN

  201                                                  250
Rangifer    FTETDIKMME RVVEQMCITQ YQRESQAYYQ RGASVILFSS PPVILLISFL
Elk         FTETDIKMME RVVEQMCITQ YQRESQAYYQ RGASVILFSS PPVILLISFL
Moose       FTETDIKMME RVVEQMCITQ YQRESQAYYQ RGASVILFSS PPVILLISFL

  251  256
Rangifer    IFLIVG
Elk         IFLIVG
Moose       IFLIVG
```

Fig. 1. Prion protein amino acid sequence alignment. (A) Prion protein sequence alignment of caribou/reindeer (rangifer), elk and moose. Protein sequences of PrP^{C} in cervid group are highly conserved, except for one amino acid polymorphism boxed in grey. (B) Prion protein sequence alignment of elk and other species (hamster, human, mouse, bovine, and sheep). PrP is >90% conserved.

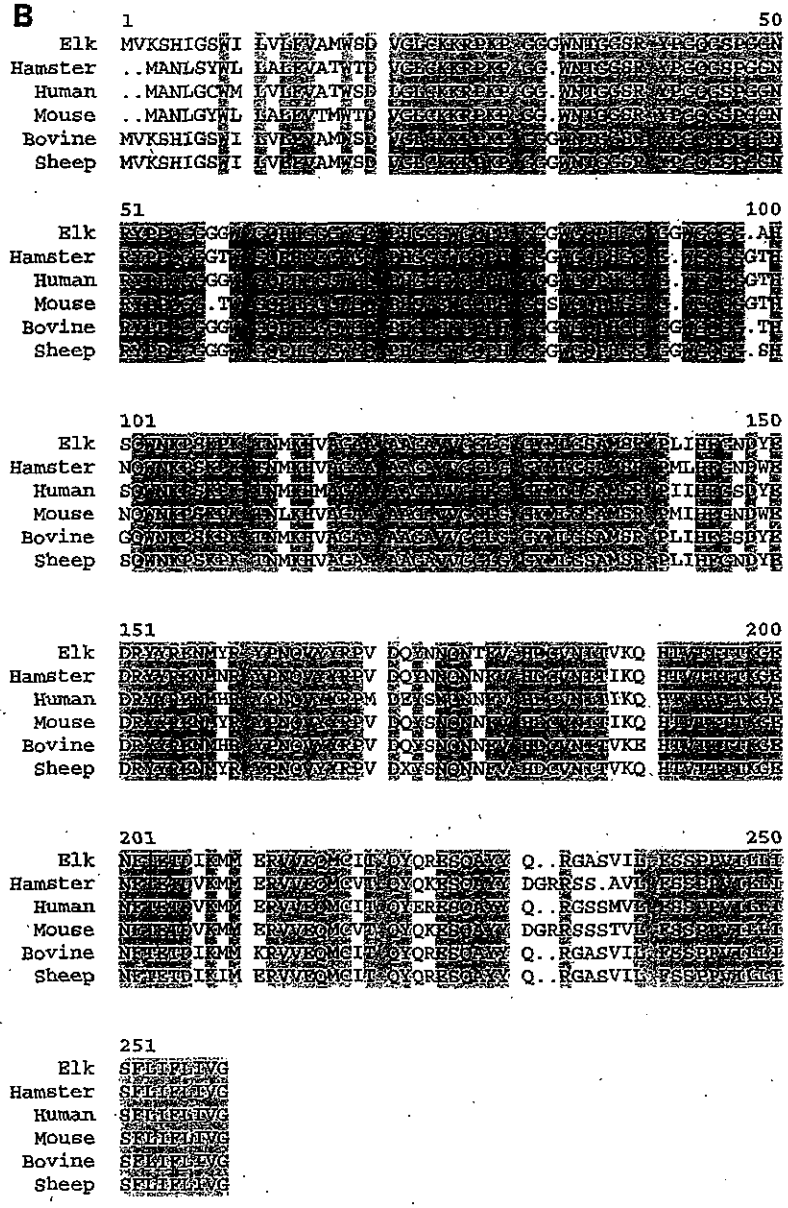

Fig. 1 (*continued*)

ated horizontal contact [5,9,18,19]. We aligned the amino acid sequences from species of cervid species which were used in the experiment: elk (*Cervus elaphus*; GenBank Accession No. CAA70902) reindeer/caribou, (*Rangifer tarandus*; GenBank Accession No. AAZ81477—reindeer is the European name for wild caribou), and moose (*Alces alces*; GenBank Accession No. AAZ81479) (Fig. 1A). The protein sequence of these three cervid species is highly conserved, with only one amino acid polymorphism reported in GenBank. We also aligned the amino acid sequences of elk with other species, such as hamster, human, mouse, bovine, and sheep, which reveals that the protein sequence of PrP^C is more than 90% conserved (Fig. 1B).

In vitro conversion of various species with CWD prion template

Normal brain homogenates from elk, reindeer, moose, caribou, human, hamster, mouse, bovine, and sheep, which were incubated with CWD-affected elk brain "seeds", were tested for conversion to a protease-resistant PrP isoform (Fig. 2) as previously described for human CJD *in vitro* conversion [17]. As a negative control, *Prnp* null mouse brain showed no signal corresponding to PK-resistant PrP^{Sc} (Fig. 2, K/O mouse bar). Partial denaturation of normal brain homogenates induced by exposure to low pH and guanidine enhanced *in vitro* conversion to PK-resistant PrP^{Sc} (Fig. 2) has been previously reported for the human

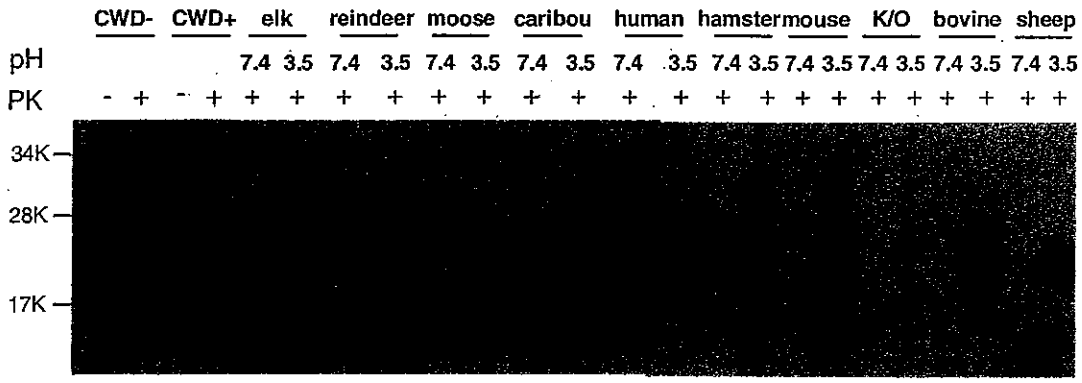

Fig. 2. *In vitro* conversion of treated PrP in the presence of PrP^{Sc} from CWD elk brain. Immunoblots of the PK-resistant PrP isoforms with 6H4 antibody. Samples were treated with GdnHCl and incubated in PBS (pH 7.4) with 0.05% SDS and 0.5% Triton X-100, at 37 °C for 12 h with shaking in the presence of trace amount of elk PrP^{Sc}. CWD−, normal elk brain homogenate as control; − and + indicates the PK treatment. CWD+, elk CWD brain homogenate as a control. The rests are the amplification of PrP^{Sc} in the different species, using elk CWD as seed, treated or untreated with acid (pH 7.4 or pH 3.5).

system [17]. All samples of normal brain contained PrP, which was sensitive to PK digestion (elk shown in Fig. 2, other species not shown). Five microliters of CWD brain homogenate was barely visible after PK digestion (Fig. 2), which was 25-fold greater than the dilution-adjusted CWD seed used in conversion system, excluding artifact from input PrP^{Sc}. Bands of the PK-resistant PrP^{Sc} form were present at ~21 kDa in all the species under acidic conditions (pH 3.5), except for the *Prnp* null mouse (Fig 2). However, PK-resistant PrP^{Sc} was poorly generated in some species in which the brain homogenates were treated under neutral conditions (pH 7.4), such as in human, hamster, mouse, bovine, and sheep. For homogenates treated at neutral pH (pH 7.4), the progression from most susceptible to least susceptible was: elk, reindeer > moose > caribou > hamster > human, bovine, sheep > mouse, with no detected conversion in *Prnp* null mouse brain.

PrP conversion efficiency enhancement by partial denaturation

Treatment of substrate brain with acidic pH (pH 3.5) enhanced PrP^{CWD}-induced conversion of all species, except *Prnp* null mice as expected (Fig. 3A). If the conversion of partially denatured PrP can be considered to be the maximum achievable conversion, the ratio of conversion of brain homogenates treated at pH 7.4 relative to pH 3.5 may provide a "conversion efficiency ratio" (CER) for that species. The comparative CER within different species is shown in Fig. 3B. Notably, some cervid species showed variability in crude conversion efficiency of native and denatured substrate, despite similar (or even identical) PrP amino acid sequences (e.g., caribou and reindeer). Although individual assays might vary for trivial reasons such as slightly differing concentration of brain homogenate, the adjusted CER seems to indicate all cervids display similar substrate conversion efficiency as expected from their evolutionary proximity. The CER analysis also

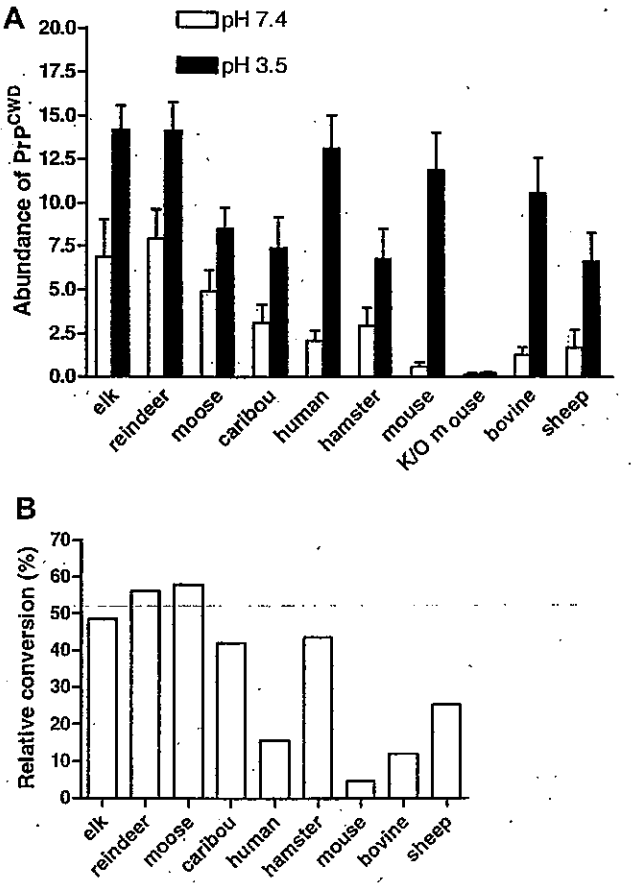

Fig. 3. (A) The immunoblots as in Fig. 2 were examined by densitometry to determine the ratio of neutral (pH 7.4) and acidic (pH 3.5) forms of PrP^{Sc} using Quantity One software (Bio-Rad). (B) Conservation efficiency ratio of native and denatured PrP substrate.

appears to show that hamster segregates with the cervids. Although Syrian hamsters were initially deemed resistant to CWD, a recent publication demonstrates that CWD can be transmitted and adapted to hamsters [20].

Measurement of species barriers by in vitro conversion assays

A number of studies have been published on the PrP^{Sc}-induced conversion of PrP^{C} [14,15,21–25]. However, in these assays require molecular cloning to obtain recombinant PrP of different species, derived from cells in culture that may not possess brain-specific PrP posttranslational modifications, and/or brain molecules which may facilitate PrP isoform conversion. Furthermore, it now appears that PMCA may trigger stochastic generation of PrP^{Sc} *de novo* [15], which may render this technique unsuitable for determining species barriers of prion infection.

Substrate denaturation and human health

We confirm with multiple species that acid/GdnHCl-treated brain PrP^{C} is a superior substrate for *in vitro* conversion than untreated PrP^{C}, possibly by overcoming conformational barriers in partial denaturation of substrate PrP^{C}. PrP conversion in scrapie-infected neuroblastoma cells is believed to occur in endosomes, a low-pH and reducing environment [26]. The non-ruminant stomach possesses a low pH lumen, and PrP^{C} is expressed in this organ [27]. Such acidic (denaturing) organ or cellular organellar environments might also promote CWD transmission to non-cervid species, including humans.

Acknowledgments

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References

[1] Alberta Government (Department of Agriculture, Food and Rural Development, Chronic wasting disease (CWD) of elk and deer. Available from: <http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex3594#occurred>, Ref Type: Internet Communication1-1-2006.

[2] Department of Agriculture – Animal and Plant Health Inspection Service. Chronic wasting disease herd certification program and interstate movement of farmed or captive deer, elk, and moose: final rule. Federal Register 71 (2006) 41681–41707.

[3] T.Y. Kim, H.J. Shon, Y.S. Joo, U.K. Mun, K.S. Kang, Y.S. Lee, Additional cases of chronic wasting disease in imported deer in Korea, J. Vet. Med. Sci. 67 (2005) 753–759.

[4] M.W. Miller, E.S. Williams, Prion disease: horizontal prion transmission in mule deer, Nature 425 (2003) 35–36.

[5] M.W. Miller, E.S. Williams, N.T. Hobbs, L.L. Wolfe, Environmental sources of prion transmission in mule deer, Emerg. Infect. Dis. 10 (2004) 1003–1006.

[6] E.S. Williams, Chronic wasting disease, Vet. Pathol. 42 (2005) 530–549.

[7] A.N. Hamir, R.A. Kunkle, J.M. Miller, J.C. Bartz, J.A. Richt, First and second cattle passage of transmissible mink encephalopathy by intracerebral inoculation, Vet. Pathol. 43 (2006) 118–126.

[8] J.C. Bartz, R.F. Marsh, D.I. McKenzie, J.M. Aiken, The host range of chronic wasting disease is altered on passage in ferrets, Virology 251 (1998) 297–301.

[9] E.S. Williams, Scrapie and chronic wasting disease, Clin. Lab. Med. 23 (2003) 139–159.

[10] R.C. Angers, S.R. Browning, T.S. Seward, C.J. Sigurdson, M.W. Miller, E.A. Hoover, G.C. Telling, Prions in skeletal muscle of deer with chronic wasting disease, Science (2006) 311:117.

[11] J.E. Jewell, M.M. Conner, L.L. Wolfe, M.W. Miller, E.S. Williams, Low frequency of PrP genotype 225SF among free-ranging mule deer (*Odocoileus hemionus*) with chronic wasting disease, J. Gen. Virol. 86 (2005) 8–34.

[12] C.K. Mathiason, J.G. Powers, S.J. Dahmes, D.A. Osborn, K.V. Miller, R.J. Warren, G.L. Mason, S.A. Hays, J. Hayes-Klug, D.M. Seelig, M.A. Wild, L.L. Wolfe, T.R. Spraker, M.W. Miller, C.J. Sigurdson, G.C. Telling, E.A. Hoover, Infectious prions in the saliva and blood of deer with chronic wasting disease, Science 314 (2006) 133–136.

[13] E.D. Belay, L.B. Schonberger, The public health impact of prion diseases, Ann. Rev. Public Health 26 (2005) 191–212.

[14] D.A. Kocisko, J.H. Come, S.A. Priola, B. Chesebro, G.J. Raymond, P.T. Lansbury, B. Caughey, Cell-free formation of protease-resistant prion protein, Nature 370 (1994) 471–474.

[15] G.P. Saborio, B. Permanne, C. Soto, Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding, Nature 411 (2001) 810–813.

[16] S. Hornemann, R. Glockshuber, A scrapie-like unfolding intermediate of the prion protein domain PrP(121–231) induced by acidic pH, Proc. Natl. Acad. Sci. USA 95 (1998) 6010–6014.

[17] W.Q. Zou, N.R. Cashman, Acidic pH and detergents enhance in vitro conversion of human brain PrPC to a PrPSc-like form, J. Biol. Chem. 277 (2002) 43942–43947.

[18] C.J. Sigurdson, T.R. Spraker, M.W. Miller, B. Oesch, E.A. Hoover, PrP(CWD) in the myenteric plexus, vagosympathetic trunk and endocrine glands of deer with chronic wasting disease, J. Gen. Virol. 82 (2001) 10–34.

[19] E.S. Williams, M.W. Miller, Chronic wasting disease in deer and elk in North America, Rev. Sci. et Tech. 21 (2002) 305–316.

[20] G.J. Raymond, L.D. Raymond, K.D. Meade-White, A.G. Hughson, C. Favara, D. Gardner, E.S. Williams, M.W. Miller, R.E. Race, B. Caughey, Transmission and adaptation of chronic wasting disease to hamsters and transgenic mice: evidence for strains, J. Virol. 81 (2007) 4305–4314.

[21] A. Bossers, P.B.G.M. Belt, G.J. Raymond, B. Caughey, V.R. de, M.A. Smits, Scrapie susceptibility-linked polymorphisms modulate the in vitro conversion of sheep prion protein to protease-resistant forms, Proc. Natl. Acad. Sci. USA 94 (1997) 4931–4936.

[22] D.A. Kocisko, S.A. Priola, G.J. Raymond, B. Chesebro, P.T. Lansbury Jr., B. Caughey, Species specificity in the cell-free conversion of prion protein to protease-resistant forms: a model for the scrapie species barrier, Proc. Natl. Acad. Sci. USA 92 (1995) 3923–3927.

[23] A. Bossers, V.R. de, M.A. Smits, Susceptibility of sheep for scrapie as assessed by in vitro conversion of nine naturally occurring variants of PrP, J. Virol. 74 (2000) 1407–1414.

[24] G.J. Raymond, A. Bossers, L.D. Raymond, K.I. O'Rourke, L.E. McHolland, P.K. Bryant III, M.W. Miller, E.S. Williams, M. Smits, B. Caughey, Evidence of a molecular barrier limiting susceptibility of humans, cattle and sheep to chronic wasting disease, EMBO J. 19 (2000) 4425–4430.

[25] G.J. Raymond, J. Hope, D.A. Kocisko, S.A. Priola, L.D. Raymond, A. Bossers, J. Ironside, R.G. Will, S.G. Chen, R.B. Petersen, P. Gambetti, R. Rubenstein, M.A. Smits, P.T. Lansbury Jr., B. Caughey, Molecular assessment of the potential transmissibilities of BSE and scrapie to humans, Nature 388 (1997) 285–288.

[26] L. Laszlo, J. Lowe, T. Self, N. Kenward, M. Landon, T. McBride, C. Farquhar, I. McConnell, J. Brown, J. Hope, Lysosomes as key organelles in the pathogenesis of prion encephalopathies, J. Pathol. 166 (1992) 333–341.

[27] Z. Marcos, K. Pfeifer, M.E. Bodegas, M.P. Sesma, L. Guembe, Cellular prion protein is expressed in a subset of neuroendocrine cells of the rat gastrointestinal tract, J. Histochem. Cytochem. 52 (2004) 1357–1365.

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研究報告の概要

ヒトおよび反芻動物における伝達性海綿状脳症(TSE)の診断は死後の脳組織中のプロテアーゼ抵抗性の宿主糖タンパク質 PrP の検出に依存している。この異常なアイソフォーム(PrP^{Sc})が組織中に存在することは TSE の感染性が存在することを示すものとされている。
本研究は、PrP^{Sc} のレベルが低いか、もしくは検出されない動物の TSE 疾患の臨床的および空胞化徴候を示す脳組織内に、高タイターの TSE 感染性が存在しうることを明確に示している。本研究は PrP^{Sc} のレベルと感染価との間の相関性に疑問を投げかけるものであり、プロテアーゼ K 抵抗性の PrP をほとんどもしくは全く含まない組織が感染源となりうること、および高タイターの TSE 感染性を有しうることを示すものである。
従って、プロテアーゼ抵抗性の PrP^{Sc} を感染性の唯一の尺度としてそれに依存することは、場合によっては診断しようとするサンプルの生物学的特性を著しく過小評価し、そのことによって TSE を防止し根絶しようとする努力を減弱させる可能性がある。

報告企業の意見	今後の対応
PrP^{Sc} のレベルが低いか、もしくは検出されない動物の TSE 疾患の臨床的および空胞化徴候を示す脳組織内に、高タイターの TSE 感染性が存在するとの報告である。 これまで血漿分画製剤によってvCJD、スクレイピー及びCWDを含むプリオン病が伝播したとの報告はない。しかしながら、万一vCJD感染者の血漿が本剤の原料に混入した場合には、製造工程においてプリオンを低減し得るとの報告があるものの、製剤から伝播する可能性を完全には否定し得ない。そのため、弊社の血漿分画製剤の製造工程におけるTSE感染性低減に関する検証実験を加速し、自社データを早期に取得し、工程評価を行い、必要に応じて工程改善を実施する予定である。	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。

使用上の注意記載状況・その他参考事項等

代表として献血ヴェノグロブリン-IH ヨシトミの記載を示す。
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。

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High Titers of Transmissible Spongiform Encephalopathy Infectivity Associated with Extremely Low Levels of PrP^{Sc} *in Vivo*[*] [S]

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Rona M. Barron[‡1,2], Susan L. Campbell[‡1,3], Declan King[‡], Anne Bellon[§], Karen E. Chapman[¶], R. Anthony Williamson[§], and Jean C. Manson[‡]

From the [‡]Neuropathogenesis Unit, Roslin Institute, Ogston Building, West Mains Road, Edinburgh EH9 3JF, Scotland, United Kingdom, the [§]Department of Immunology, Scripps Research Institute, La Jolla, California 92037, and the [¶]Centre for Cardiovascular Sciences, Queen's Medical Research Institute, University of Edinburgh, 47 Little France Crescent, Edinburgh EH16 4TJ, Scotland, United Kingdom

Diagnosis of transmissible spongiform encephalopathy (TSE) disease in humans and ruminants relies on the detection in post-mortem brain tissue of the protease-resistant form of the host glycoprotein PrP. The presence of this abnormal isoform (PrP^{Sc}) in tissues is taken as indicative of the presence of TSE infectivity. Here we demonstrate conclusively that high titers of TSE infectivity can be present in brain tissue of animals that show clinical and vacuolar signs of TSE disease but contain low or undetectable levels of PrP^{Sc}. This work questions the correlation between PrP^{Sc} level and the titer of infectivity and shows that tissues containing little or no proteinase K-resistant PrP can be infectious and harbor high titers of TSE infectivity. Reliance on protease-resistant PrP^{Sc} as a sole measure of infectivity may therefore in some instances significantly underestimate biological properties of diagnostic samples, thereby undermining efforts to contain and eradicate TSEs.

The transmissible spongiform encephalopathy (TSE)[4] diseases (also known as prion diseases) are infectious, fatal neurodegenerative diseases of animals, which include Creutzfeldt-Jacob disease (CJD) in humans and bovine spongiform encephalopathy (BSE) in cattle. The true identity of the infectious agent responsible for these diseases is not known. However, it has been proposed that TSE disease is caused by an abnormal form of the host glycoprotein, PrP (1). The abnormal, disease-associated form of the protein (PrP^{Sc}), is partially protease-resistant and detergent-insoluble unlike the normal cellular conformer (PrP^{C}), and is seen to accumulate in diseased tissues. The prion hypothesis predicts that PrP^{Sc} alone is the infectious agent of TSE and is able to induce the conversion of endogenous PrP^{C} into the abnormal form during disease (2).

Most human TSE diseases are familial or sporadic, but disease can also be acquired by surgical intervention (3) or blood transfusion from infected individuals (4–9), or possibly from the consumption of BSE-infected meat products, the presumed cause of variant CJD (vCJD) (10). The extent to which vCJD infection in particular is present in the United Kingdom population is unknown, but recent research has suggested there may be a higher rate of subclinical or preclinical vCJD than previously thought in different human PrP genotypes (7, 11–13). Although BSE is declining in the United Kingdom, cases have now been observed in cattle in countries that have not previously reported BSE. It is also unknown whether the agent responsible for BSE has re-entered the human food chain following transmission to sheep. For these reasons a high level of active and passive surveillance of ruminants is required at slaughter to monitor and prevent TSE-infected material from entering the human food chain. The introduction of ante-mortem surveillance in the human population is also critical to prevent the human-to-human transmission of vCJD by blood transfusion or surgical procedures. This will be of particular importance if subclinical disease proves to be a significant risk in vCJD transmission (12, 13).

Positive identification of TSE infectivity can only be demonstrated conclusively by transmission of disease to laboratory animals. Such assays are time-consuming, due to long incubation times, and expensive, and are therefore not suitable for the rapid diagnosis of all ante- or post-mortem samples. Current diagnostic tests instead rely on the detection of disease-associated PrP^{Sc} in samples taken from brain post-mortem. The development of ante-mortem diagnostic tests is also being based around more sensitive assays for PrP^{Sc}. Several diagnostic tests are available commercially, and most require proteinase K (PK) treatment of tissue homogenates to isolate disease-specific PK-resistant PrP^{Sc} (PrP-res). It has not yet been definitively proven that PrP^{Sc} is the TSE infectious agent, and whether it is present in all infected tissues. Studies using 263K hamster scrapie have shown a strong correlation between PrP-

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[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1–S3 and Table S1.

[1] Both authors contributed equally to this work.

[2] To whom correspondence should be addressed. Tel.: 44-131-667-5204; Fax: 44-131-668-3872; E-mail: rona.barron@bbsrc.ac.uk.

[3] Current address: Medical Research Council Clinical Sciences Centre, Imperial College, Hammersmith Campus, Du Cane Road, London W12 0NN, United Kingdom.

[4] The abbreviations used are: TSE, transmissible spongiform encephalopathy; CJD, Creutzfeldt-Jacob disease; vCJD, variant Creutzfeldt-Jacob disease; PK, proteinase K; GSS, Gerstmann Sträussler Scheinker; CDI, conformation-dependent immunoassay; IP, immunoprecipitation; IHC, immunohistochemistry; mAb, monoclonal antibody; BSE, bovine spongiform encephalopathy; PrP-res, PK-resistant PrP^{Sc}; $sPrP^{Sc}$, PK-sensitive form of PrP^{Sc}; ELISA, enzyme-linked immunosorbent assay; d/n, ratio of denatured to native signal; Wt, wild-type.

res and infectivity (2, 14, 15). However, other studies have demonstrated the transmission of disease from infected animals that appear to lack significant levels of PrP-res (16–19). In such cases it has been suggested that a PK-sensitive form of PrP^{Sc} ($sPrP^{Sc}$) may represent the infectious agent (20–22). Hence it is possible that infectivity may be associated with a specific isoform of abnormal PrP. The identification of this specific conformer is imperative for the future of TSE diagnosis. If present, large amounts of PrP^{Sc} may be a clear indication of the presence of infectivity in a tissue sample. However, if TSE infectivity does not always associate with high levels of PrP^{Sc}, current diagnostic methods may fail to identify all animals with TSE disease and may not provide a realistic estimate of the level of infectivity in an infected tissue. For the purposes of this study, PrP^{Sc} is used to define all abnormal forms of PrP, whereas PrP-res specifically defines PK-resistant PrP, and $sPrP^{Sc}$ defines PK-sensitive forms of PrP^{Sc}.

We have previously identified two mouse models of TSE disease (18, 19) that indicate that the association between PrP-res and infectivity is not as straightforward as predicted by the prion hypothesis. Unlike wild-type controls, transgenic mice homozygous for a targeted mutation at amino acid 101 (proline to leucine) in endogenous murine PrP (101LL) develop clinical TSE disease following inoculation with hamster 263K scrapie or human Gerstmann Sträussler Scheinker (GSS) P102L disease (patient shown to contain vacuolar pathology and PrP-res at post-mortem) (18, 19). Pathological analysis of brain tissue from these mice (101LL/GSS and 101LL/263K) showed TSE-associated vacuolization, and the disease could be further transmitted to 101LL mice with short incubation times of 100–160 days (18, 19). Such incubation times were indicative of a high titer of infectivity in the 101LL/GSS and 101LL/263K tissues, yet analysis by immunoblot revealed that most animals contained extremely low levels of PrP-res, and several contained no detectable PrP-res at all (18, 19). However, the presence of high titers of infectivity cannot be proven by a short disease incubation time. To establish the true relationship between PrP^{Sc} and infectivity we have now performed detailed and quantitative analyses of the disease in these mice. The ID_{50} (dilution at which 50% of the animals become infected) and titer of infectivity in several 101LL/GSS- and 101LL/263K-infected brains have been established by bioassay. Corresponding levels of PrP-res in the same tissues have also been established semi-quantitatively by immunoblot. These analyses have shown no relationship between infectivity titer and PrP-res level. Moreover no other disease-associated forms of PrP were detectable in these tissues. Thus within our model system there is a clear dissociation between titer of infectivity and level of PrP^{Sc}.

EXPERIMENTAL PROCEDURES

Transgenic Mouse Lines and Tissues—Inbred gene-targeted transgenic mouse line 101LL and the corresponding inbred 129/Ola wild-type control line have been described previously (18). 101LL/GSS tissues were produced by inoculation of 101LL transgenic mice with 1% brain homogenate prepared from the occipital cortex of a GSS P102L brain showing numerous multicentric plaques and abundant PrP-res by immunoblot. The individual was methionine 129 homozygous with a confirmed proline to leucine mutation at codon 102.[5] 101LL/263K tissues were produced by inoculation of 101LL transgenic mice with 1% brain homogenate from a 263K-infected hamster. Control tissues were produced by ME7 inoculation of 129/Ola wild-type mice and 101LL transgenic mice.

Preparation of Inocula—Separate inocula were prepared from the brains of two 101LL/GSS- and three 101LL/263K-infected mice with terminal TSE disease, which had been shown by immunohistochemical (IHC) analysis to contain extremely low levels of PrP deposition. Inocula were also prepared from brains of one wild-type and one 101LL mouse with terminal ME7 scrapie as controls. A 10% homogenate of each sample was prepared in sterile saline prior to use as an inoculum. This inoculum was then used to produce a series of 10-fold dilutions from 10^{-2} to 10^{-9} in sterile saline. Each dilution (20 μl) was inoculated intracerebrally under anesthesia into groups of 101LL mice for 101LL/ME7, 101LL/GSS, and 101LL/263K tissues, or wild-type 129/Ola mice for Wt/ME7 tissue. All experimental protocols were submitted to the Local Ethical Review Committee for approval before mice were inoculated. All experiments were performed under license and in accordance with the UK Home Office Regulations (Animals (Scientific Procedures) Act of 1986).

Scoring of Clinical TSE Disease—The presence of clinical TSE disease was assessed as described previously (23). Animals were scored for clinical disease without reference to the genotype of the mouse. Genotypes were confirmed for each animal by PCR analysis of tail DNA at the end of the experiment. Incubation times were calculated as the interval between inoculation and cull due to terminal TSE disease. Mice were killed by cervical dislocation at the terminal stage of disease, at termination of the experiment (between 500–700 days), or for welfare reasons due to intercurrent illness. The proportion of mice showing positive vacuolar pathology was calculated for each group, and the ID_{50} (dilution at which 50% of the mice became infected) was determined using the Karber method (24). This value was used to calculate the number of infectious units per gram wet weight of tissue (IU/g).

Genotyping of Mouse Tail DNA—A 2- to 3-cm portion of tail was removed post-mortem from each mouse. DNA was prepared, and the PrP genotype of each mouse was confirmed as described previously (18).

Immunoblot Analysis and Quantification of PrP-res—For immunoblot analysis, residual inocula (10% saline homogenate) were mixed with an equal volume of 2× Nonidet P-40 buffer (2% Nonidet P-40, 1% sodium deoxycholate, 300 mM NaCl, 100 mM Tris/HCl, pH 7.5) and further homogenized in a microcentrifuge tube using 20–30 strokes with a pre-cooled centrifuge tube pestle (Anachem). The homogenate was centrifuged at 11,000 × *g* for 10 min at 10 °C to remove cellular debris, and the supernatant stored in 50-μl aliquots at −70 °C. For quantification of PrP-res levels in each tissue, homogenates were digested with 20 μg/ml PK at 37 °C for 1 h. Digested homogenates were diluted to 1%, and 2-fold serial dilutions were prepared using PK-treated normal brain homogenate as

[5] J. W. Ironside and M. W. Head, personal communication.

the diluent to keep overall protein concentrations constant. Diluted samples were mixed with sample loading buffer and sample reducing agent (Invitrogen) and loaded across two 12% Tris/glycine polyacrylamide gels (Invitrogen) at concentrations ranging from 1 mg/ml to 3.9 μg/ml (200 μg to 0.8 μg of wet weight tissue equivalent). 50 ng of recombinant PrP was loaded onto each gel as an internal control. After separation, proteins were transferred onto a polyvinylidene difluoride membrane by electroblotting, and PrP was detected with mAb 8H4 (West Dura ECL substrate, Pierce). Monoclonal antibody 7A12 and polyclonal antibody 1B3 were also used to confirm the low PrP-res levels in 101LL/GSS and 101LL/263K tissues. Images were captured on both x-ray film and by a Kodak Digital Image Station 440. Experiments were repeated in duplicate or triplicate depending on sample availability.

Digital images of each gel were analyzed using Kodak ID software, and PrP-res levels were expressed as pixel intensities. Samples were normalized across the two blots and quantified using the recombinant PrP controls as standards. Each value was multiplied by the dilution factor, and an average was taken for all samples run per tissue to determine the level of PrP-res per gram wet weight brain tissue in each model. This value, combined with the titer of TSE infectivity measured in each tissue (IU/g) was used to calculate the number of molecules of PrP-res per infectious unit for each tissue as in Equations 1–3.

$$\text{Number of PrP-res molecules per g of tissue} = n \quad \text{(Eq. 1)}$$

$$n = [\text{PrP-res per g/Avagadro's number } (6.02 \times 10^{23})]/\text{molecular weight PrP } (30{,}000) \quad \text{(Eq. 2)}$$

$$\text{Number of molecules PrP-res per infectious unit} = n/\text{titer (IU/g)} \quad \text{(Eq. 3)}$$

Measurement of Alternative Forms of PrP—The PK resistance of PrP in all samples was analyzed by digestion with a range of PK concentrations. Individual 9-μl aliquots of each 5% Nonidet P-40 brain homogenate were incubated at 37 °C for 1 h with PK concentrations ranging from 1 to 20 μg/ml. The reaction was terminated by addition of phenylmethylsulfonyl fluoride to 1 mM, and samples were analyzed by SDS-PAGE and immunoblotting as described above.

For "cold PK" digestion, samples (10% homogenate) were incubated with 250 μg/ml PK on ice for 1 h. Digestion was stopped by the addition of phenylmethylsulfonyl fluoride to 1 mM. Samples were de-glycosylated with peptide *N*-glycosidase F (New England Biolabs) following the manufacturer's instructions and analyzed by SDS-PAGE and immunoblotting.

CDI Analysis—Samples were analyzed for the presence of PrP^{Sc} using conformation-dependent immunoassay (CDI) as described by Safar *et al.* (20). Briefly, abnormal PrP was precipitated from brain homogenates of 101LL/GSS, 101LL/263K, and 101LL/ME7 infected mice and uninfected 101LL mice using sodium phosphotungstate, and pellets were resuspended in either distilled water or 4 M guanidine hydrochloride to produce native and denatured samples. 4 M guanidine hydrochloride samples were further heat-denatured at 80 °C for 6 min. Samples were added to 96-well plates coated with mAb FH11, and PrP levels were detected using europium-labeled mAb 7A12 and a Victor 2 ELISA plate reader (PerkinElmer Life Sciences). The ratio of denatured to native signal (d/n) was calculated for each tissue to determine the presence of PrP^{Sc}.

Immunoprecipitation of PrP^{Sc}—Laterally bisected brain halves from 101LL transgenic mice were homogenized at 10% (w/v) in Tris-buffered saline and diluted to reach a concentration of 5% (w/v) in Tris-buffered saline containing 1% Triton. Homogenates were sonicated for three pulses of 4 s and clarified by centrifugation at 400 × *g* for 10 min at 4 °C. Phenylmethylsulfonyl fluoride was added to all samples to a concentration of 2 mM. Each sample was analyzed by dot blot to estimate the total PrP content. Briefly, brain homogenates were serially diluted (1:1) in Tris-buffered saline containing 1% Triton then denatured in Tris-SDS sample buffer at 100 °C for 5 min. Equivalent amounts of each sample were then deposited on a nitrocellulose membrane and left until dry. The membrane was probed with mAb 6H4 (Prionics) and a horseradish peroxidase-labeled anti-mouse secondary antibody (Pierce). The resulting signals were compared semi-quantitatively. These data were used to ensure equal PrP input into each individual immunoprecipitation (IP) reaction. For each IP reaction, the motif grafted antibodies or control antibodies were incubated at 10 μg/ml final concentration for 2 h at room temperature in a reaction mixture with 1% Triton. Rabbit anti-human antibodies (Jackson) coupled to magnetic Dynabeads (Dynal) were used to capture the PrP-specific antibodies as described (25, 26). Immunoblot membranes were probed with mAb 6H4 and developed using the ECL femtomolar kit (Pierce).

RESULTS

101LL Mice Infected with 263K and GSS P102L Show Little PrP Deposition in Brain—Brain tissue from 101LL transgenic mice, which showed TSE clinical signs and TSE-associated vacuolar pathology following inoculation with hamster 263K scrapie or human GSS P102L (18, 19), was screened for PrP deposition by IHC using anti-PrP mAb 6H4. As previously demonstrated, 101LL/GSS- and 101LL/263K-infected mice had low levels of PrP deposition in the brain, despite having confirmed TSE disease. Three 101LL/263K- and two 101LL/GSS-infected tissues, which showed extremely low PrP deposition in the brain, were selected for further analysis by bioassay (Fig. 1 and Table 1). In each case, PrP deposition was restricted to the thalamus and, in most cases, was only visible as small grainy deposits under high power microscopy (Fig. 1, *F–H*). Low or undetectable levels of PrP-res in each brain homogenate were confirmed by immunoblot following PK treatment of residual inoculum (Fig. 2).

High Levels of Infectivity Can Be Measured by Bioassay of 101LL/GSS and 101LL/263K Brain Tissue—Although short incubation times in mice can be indicative of high levels of TSE infectivity in an inoculum, the actual level can only be determined by establishing the ID_{50} (dilution at which 50% of the animals become infected) for the inoculum. Infectivity titers were therefore established for the five selected tissues: 101LL/263K(a), 101LL/263K(b), 101LL/263K(c), 101LL/GSS(d), and 101LL/GSS(e) (Table 1). It was considered extremely important in these experiments that, as far as possible, a single brain be

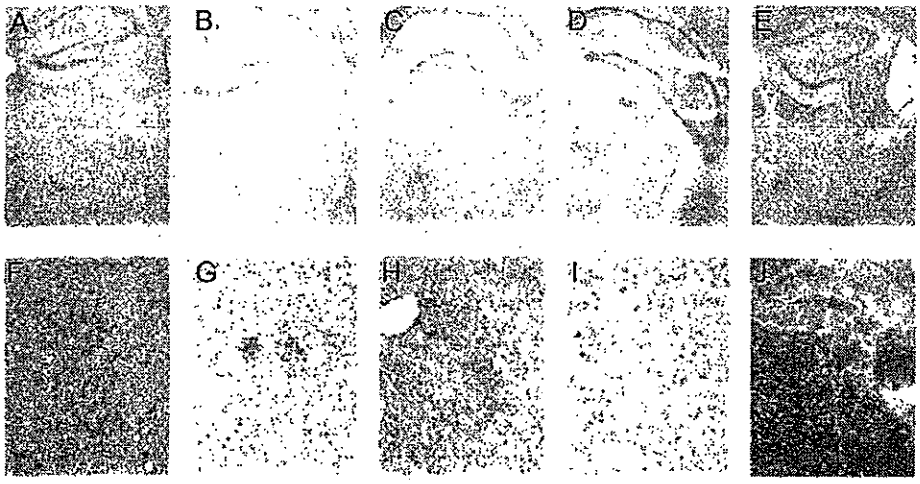

FIGURE 1. **Low levels of PrP deposition in 101LL/GSS- and 101LL/263K-infected brain.** Immunohistochemistry was performed on sections of brain from 101LL/263K- and 101LL/GSS-infected mice using mAb 6H4 to determine the levels of PrP deposition. ME7-infected control mouse brain was stained as control (*J*). Five brains shown in *A–E* (3× 101LL/263K and 2× 101LL/GSS) showing very low levels of deposition were selected for further analysis to quantify the levels of TSE infectivity and PrP^{Sc} in each tissue. Very low levels of PrP deposition were observed in brain tissue, which varied between each individual mouse brain. Deposition was mainly observed in the thalamus (*F–H*). Thalamus of an uninfected 101LL mouse is shown for background comparison (*I*). *A–E* and *J*, 4× magnification; *F–I*, 20× magnification. *A*, 101LL/263K(a); *B*, 101LL/263K(b); *C*, 101LL/263K(c); *D*, 101LL/GSS(d); *E*, 101LL/GSS(e); *F*, thalamus of 101LL/263K(a); *G*, thalamus of 101LL/263K(c); *H*, thalamus of 101LL/GSS(d); *I*, thalamus of 303-day-old uninfected 101LL mouse; and *J*, Wt/ME7 control.

TABLE 1

Tissues selected for analysis

Details of clinical disease and vacuolar pathology in the five tissues selected for analysis. All mice showed positive clinical and vacuolar signs of TSE disease and low levels of PrP deposition.

Tissue used for titration	Clinical TSE	Vacuolar pathology	PrP deposition[a]	Incubation period	
				Primary[b]	Secondary[c]
				days ± S.E.	
101LL/263K(a)	Positive	Positive	+	385	109 ± 2
101LL/263K(b)	Positive	Positive	+/−	464	129 ± 2
101LL/263K(c)	Positive	Positive	+/−	534	262 ± 4
101LL/GSS(d)	Positive	Positive	+	259	154 ± 3
101LL/GSS(e)	Positive	Positive	+/−	252	123 ± 1

[a] Scoring of PrP deposition: +++, high; ++, medium; +, low; +/−, very small grainy deposits.
[b] Incubation time of each individual mouse on primary transmission of either 263K or P102L GSS.
[c] Incubation time of 101LL mice inoculated with 1% brain homogenate from each specific 101LL/263K- or 101LL/GSS-infected tissue. Transmission of disease on subpass to 101LL mice was 100% in each case.

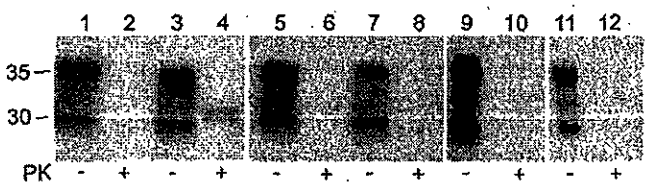

FIGURE 2. **Low or undetectable levels of PrP-res in 101LL/GSS- and 101LL/263K-infected brain.** Residual inoculum from the tissues selected for ID_{50} bioassay were analyzed by immunoblot following PK treatment to detect PrP-res. *Lanes 2, 4, 6, 8, 10,* and *12,* digested with PK at 20 μg/ml for 1 h at 37 °C; *lanes 1, 3, 5, 7, 9,* and *11,* no PK control; *lanes 1* and *2,* uninfected Wt 129/Ola mouse; *lanes 3* and *4,* 101LL/263K(a); *lanes 5* and *6,* 101LL/263K(b); *lanes 7* and *8,* 101LL/263K(c); *lanes 9* and *10,* 101LL/GSS(d); and *lanes 11* and *12,* 101LL/GSS(e). All samples were loaded at 10 mg/ml (w/v) wet weight tissue (200 μg per lane). Blots probed with mAb 8H4.

used for each series of procedures (ID_{50} determination, PK digestion, IHC, etc.). This allowed direct correlation to be made between the level of infectivity and PrP-res in each individual brain and avoided any variation that may occur between tissues, as is often observed on a primary transmission. Moreover this approach avoided the necessity of carrying out large numbers of titration experiments, which would have been both impractical and ethically unacceptable. Inocula were prepared from each individual tissue as 10% sterile saline homogenates and used to produce a series of 10-fold dilutions (10^{-2} to 10^{-9}) for inoculation. Wild-type control 129/Ola and transgenic 101LL mouse brains infected with the well characterized mouse scrapie strain ME7 (Wt/ME7 and 101LL/ME7, respectively) (18) were also assayed as controls. The seven samples were inoculated intracerebrally into groups of 129/Ola mice for Wt/ME7, and transgenic 101LL mice for all other samples. The percentage of mice that developed TSE pathology was calculated for each group in each dilution series, and the ID_{50} was determined using the Karber calculation (24). The numbers of infectious units per gram tissue (IU/g) for each individual mouse brain are shown in Table 2. Assuming a ±0.5 log error for each titer (24), all 101LL/GSS and 101LL/263K samples produced titers of infectivity ranging from ~10^7 to 10^9 IU/g. The highest titer ($10^{9.8}$) was identified in 101LL/GSS(d), however a titer of $10^{8.7}$ was also identified in 101LL/263K(a). Both of these brains showed low levels of PrP deposition by IHC, but titers were higher than that measured in control Wt/ME7 brain ($10^{8.5}$), which showed significantly more PrP deposition by IHC (Fig. 1). Titers in the other three tissues were similar ($10^{7.2}$ to $10^{7.5}$) and confirmed a high level of infectivity in the presence of extremely low or undetectable PrP deposition in the brain (Figs. 1 and 2). The results of the ID_{50} determination therefore prove the presence of high levels of infectivity in 101LL transgenic mice infected with P102L GSS or hamster 263K.

Little or No PrP-res Is Detected in Highly Infectious Tissue—IHC using anti-PrP monoclonal and polyclonal antibodies found little or no PrP deposition in brain tissue of 101LL/263K and 101LL/GSS infected mice (Fig. 1, and data not shown). However, IHC does not distinguish between different forms of PrP, therefore direct measurement of brain PrP-res levels was undertaken to determine the amount of PrP-res associated with titer of infectivity in each brain, listed in Table 1. Residual inoculum from each bioassay was mixed with detergent buffer and digested with PK (Fig. 2), and a 2-fold serial dilution from 1 mg/ml to 3.9 μg/ml (wet weight brain tissue) was analyzed by immunoblotting with mAb 8H4 (27). Recombinant PrP was loaded on each gel at 50 ng as an internal control. For the ME7-infected tissues, the limit of PrP-res detection was 15.6 μg/ml for Wt/ME7 homogenate and 31.3 μg/ml for 101LL/ME7 homogenate. Hence the same agent produced ~2-fold less PrP-

TABLE 2

Comparison of titer of infectivity and PrP-res level

PrP-res levels, quantified relative to recombinant PrP from digital immunoblot images, and infectivity titer, measured by ID_{50} bioassay. Detection limit of the immunoblot system was estimated to be equivalent to 25 μg of PrP-res/g wet weight brain.

Model	PrP-res	PrP-res	Titer[a]
	μg/g tissue[b]	*% of ME7*	*IU/g tissue*
Wt/ME7	1994	100	$10^{8.5}$
101LL/ME7	1040	52	$10^{7.8}$
101LL/263K(a)	498	25	$10^{8.7}$
101LL/263K(b)	<25	<1.3	$10^{7.3}$
101LL/263K(c)	<25	<1.3	$10^{7.5}$
101LL/GSS(d)	<25	<1.3	$10^{9.8}$
101LL/GSS(e)	<25	<1.3	$10^{7.2}$

[a] Titer of infectivity per gram of brain tissue as calculated from ID_{50} bioassay in mice using the Karber calculation.

[b] The actual amount of PrP-res quantified from the blots (0.5–2 mg/g) is higher than would be predicted for mouse tissue and may reflect the use of recombinant PrP for calibration, because this does not possess any post-translational modifications and may therefore display altered antibody affinity. However, this internal control acts to normalize each blot and, therefore, ensures that the relative proportions of PrP-res between each model are real, despite possible errors in the absolute quantification.

res in the 101LL transgenic mice compared with wild-type mice, although this was associated with a 0.7 log drop in titer (Table 2). In 101LL/263K(a) the limit of PrP-res detection was 62.5 μg/ml brain homogenate, which was approximately half the level in 101LL/ME7 and one quarter the level in Wt/ME7. For all other samples, no PrP-res was detectable in even the most concentrated (1 mg/ml) sample examined (Table 2, Fig. 2, and supplemental Fig. S1*A*). Digital imaging of immunoblots and quantitation of PrP-res relative to recombinant PrP control allowed the calculation of PrP^{Sc} concentration (mean PrP-res grams per gram wet weight of tissue) in each sample (Table 2). The level of sensitivity for the immunoblot, determined using recombinant PrP, was 5–10 ng, therefore the level of PrP-res in samples that showed no PK-resistant material must be below this threshold. Previous studies have shown that PrP-res from other well characterized rodent scrapie strains with titers ranging from $10^{5.5}$ to 10^{9} can be easily identified on immunoblot of 1% brain homogenate following PK treatment (supplemental Fig. S1*B*). These data would suggest that tissue containing titers of 10^{7} to 10^{9} IU/g should contain levels of PrP-res, which can be easily identified by immunoblot. However, for 101LL/GSS- and 101LL/263K-infected tissue this was clearly not the case. Although we cannot eliminate the possibility that PrP-res was indeed present below the threshold level of the immunoblot, a poor correlation between the level of infectivity and the amount of PrP-res in the brain is nevertheless clearly established. To confirm that the failure to detect PrP-res on these immunoblots was not simply a consequence of the loss of the monoclonal antibody epitope (8H4) duplicate blots were also probed with a second monoclonal antibody (7A12) and a polyclonal antibody (1B3), which detects multiple epitopes in PrP. These results confirmed the low PrP-res levels in 101LL/GSS and 101LL/263K tissues (data not shown). Although the combination of monoclonal and polyclonal antibodies used to examine these tissues makes it unlikely that a form of PrP-res exists that has not been detected in our immunoassays, this possibility has not been totally excluded and we continue to investigate these tissues with new antibodies.

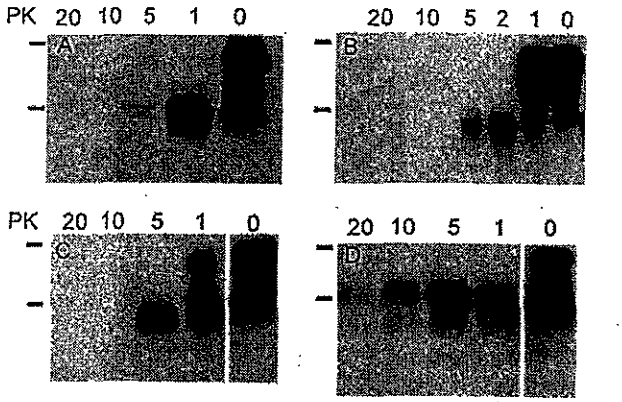

FIGURE 3. **PK resistance of PrP in 101LL/GSS and 101LL/263K brain tissue.** Brain homogenates in Nonidet P-40 lysis buffer were digested with varying concentrations of proteinase K at 37 °C for 1 h. Samples were subjected to SDS-PAGE and immunoblotting to determine the PK sensitivity of the PrP present in 101LL/GSS and 101LL/263K tissue. Representative images show: *A*, uninfected 101LL control mouse brain; *B*, uninfected Wt 129/Ola control mouse brain; *C*, 101LL/263K(b) mouse brain; and *D*, 101LL/263K(a) mouse brain. The PK concentration used for digestion is shown *above* each *lane* (micrograms/ml). Blots were probed with mAb 8H4. *Bars* indicate molecular mass markers of 36 and 30 kDa.

Are Alternative Forms of PrP Associated with Infectivity?—Although PrP-res was present at low or undetectable levels in tissues from 101LL/GSS- and 101LL/263K-infected mice, it is possible that forms of PrP other than PrP-res may be infectious (28). Alternative forms of PrP such as transmembrane PrP (29, 30), cytoplasmic PrP (31, 32), and PrP with amino acid insertions or deletions (33–36) have been linked with disease. In addition, a PK-sensitive variant of PrP^{Sc}, $sPrP^{Sc}$, has been recently described (20–22) that may represent an intermediate in the refolding of PrP^{C} to PrP^{Sc} during the disease process and could therefore be associated with infectivity. To test whether $sPrP^{Sc}$ may account for the dissociation between PrP-res and infectivity in 101LL/263K and 101LL/GSS tissues we examined the protease resistance of PrP in such brains by digesting with a range of PK concentrations from 1 μg/ml to 20 μg/ml. Homogenates from Wt/ME7, 101LL/ME7, and uninfected 101LL and 129/Ola mice were also treated with varying PK concentrations as controls. Digestion was stopped by the addition of phenylmethylsulfonyl fluoride to 1 mM, and samples were analyzed by immunoblot (Fig. 3). In the positive controls (Wt/ME7 and 101LL/ME7) PrP-res was evident in all dilutions, with the PK-resistant core still visible after treatment with 20 μg/ml PK (data not shown). PrP in the uninfected controls was found to be sensitive to PK concentrations >5 μg/ml, and produced mildly PK-resistant fragments at PK concentrations of 2–5 μg/ml under the digestion conditions used here (Fig. 3). PrP in the 263K-infected 101LL brains showed variable PK resistance, in agreement with the level of PrP-res detectable in each homogenate. Thus, 101LL/263K(a) showed PrP-res at 20 μg/ml, but 101LL/263K(b) and -(c) showed a similar pattern of PK resistance to uninfected mice (Fig. 3). In addition, samples from both 101LL/GSS(d) and 101LL/GSS(e) showed a PK-sensitivity pattern identical to that of uninfected 101LL mice (data not shown).

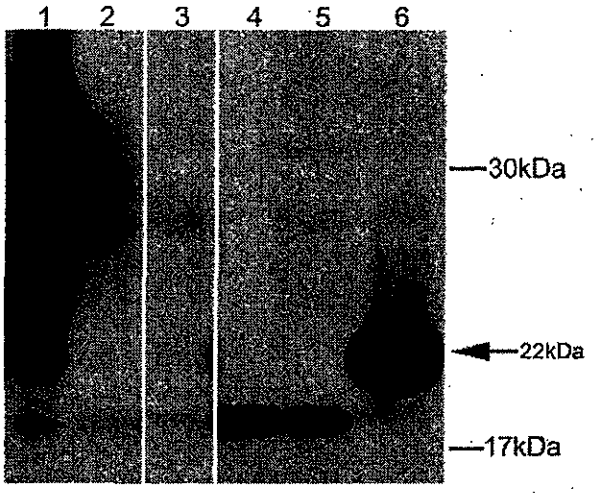

FIGURE 4. **Cold PK treatment of tissues from high titer/low PrP-res models.** 101LL/GSS and 101LL/263K tissues taken from the same transmission experiments as those shown in Table 1 were subjected to cold PK digestion on ice. Uninfected and Wt/ME7-infected brains were also digested as controls. *Lane 1*, undigested 101LL/GSS brain homogenate; *lane 2*, 101LL/263K(g); *lane 3*, 101LL/GSS(f); *lane 4*, 101LL uninfected control; *lane 5*, Wt129/Ola uninfected control; *lane 6*, Wt/ME7 infected control. *Lanes 2–6* were treated with 250 μg/ml PK on ice for 1 h and de-glycosylated with peptide *N*-glycosidase F. ME7 control was loaded at ~25% of the concentration of *lanes 2–5* to allow comparison. The blot was probed with mAb 7A12. The image has been cropped from a single blot to remove lanes with samples that are not relevant to this figure.

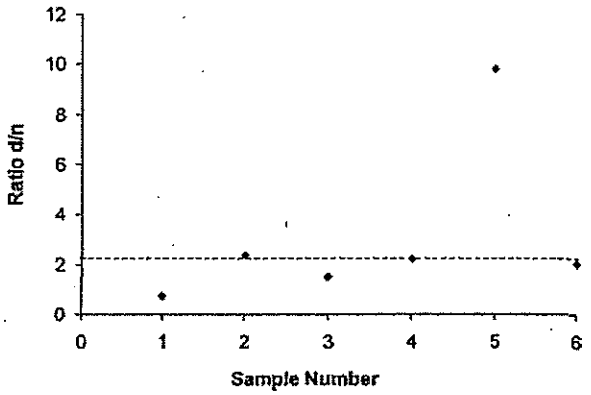

FIGURE 5. **CDI analysis of 101LL/GSS and 101LL/263K brain homogenate.** Samples of 101LL/GSS brain homogenate, 101LL/263K homogenate, and uninfected or ME7-infected controls were analyzed for the presence of PrP^{Sc} using a CDI. Samples were precipitated with sodium phosphotungstate, and pellets were resuspended in either distilled water or 4 M guanidinium-HCl to provide native and denatured samples. These were analyzed in a sandwich ELISA using mAb FH11 as capture and mAb 7A12 as detector. Ratio of denatured to native (*d/n*) signal plotted to show presence of PrP^{Sc}. *Sample 1*, 101LL/GSS(j); *sample 2*, 101LL/GSS(k); *sample 3*, 101LL/263K(m); *sample 4*, 101LL/263K(n); *sample 5*, 101LL/ME7; and *sample 6*, uninfected 101LL mouse. All samples were assayed in duplicate. *Dotted line* indicates cut-off value, which was calculated as the d/n ratio of the uninfected 101LL plus 10%.

The presence of $sPrP^{Sc}$ in brain tissue has also been demonstrated by performing cold PK digestion, *i.e.* PK digestion on ice (21, 22). $sPrP^{Sc}$ has been previously identified in samples that showed no PrP-res (using standard digestion conditions of 20 μg/ml for 1 h at 37 °C) by the presence of a 22-kDa band on immunoblot after digestion with PK on ice and subsequent de-glycosylation with peptide *N*-glycosidase F (21, 22). Although we aimed to perform all procedures on each individual mouse brain, the limited tissue size meant this was not possible for the cold PK analyses carried out here. However, cold PK digestion was performed on brain tissue taken from mice showing positive clinical and vacuolar signs of TSE, but low levels of PrP deposition in the same primary transmission experiments as those listed in Table 2 (details in supplemental Fig. S2 and Table S1). These tissues failed to demonstrate any marked increase in the 22-kDa PK-resistant PrP band after cold PK digestion (Fig. 4, *lanes 2* and *3*). When compared with the ME7 control (Fig. 4, *lane 6*, loaded at 25% concentration of *lanes 2–5*), the low levels of PrP apparent in *lanes 2* and *3* after digestion with PK on ice demonstrate that $sPrP^{Sc}$ cannot account for the high titer of infectivity in the 101LL/263K and 101LL/GSS models.

Although PrP^{Sc} is generally defined by its partial resistance to PK digestion, it can also be identified using immunoassays that exploit the differential binding of anti-PrP antibodies to PrP^{Sc} in the native and denatured state. Epitopes that are hidden in the native PrP^{Sc} conformation become exposed on denaturation in increasing concentrations of guanidine hydrochloride, leading to an increase in antibody binding. This observation is the basis of the CDI, where levels of PrP^{Sc} are calculated by measuring the ratio of the denatured to native signal (d/n ratio) in a sandwich ELISA (20, 37, 38). An increase in d/n ratio indicates the presence of PrP^{Sc}, which produces the increased signal obtained on denaturation of the sample. Because this assay does not use PK digestion to identify abnormal PrP, it can also be used to identify $sPrP^{Sc}$. To confirm the absence of large amounts of PrP-res or $sPrP^{Sc}$ in the models described here, CDI analysis was performed on tissues from 101LL/GSS- and 101LL/263K-infected mice. Tissue from animals detailed in Table 1 was not analyzed due to limited sample availability, but analysis was performed on tissues from 101LL/GSS- and 101LL/263K-infected mice with confirmed clinical and pathological TSE disease, but little or no PrP^{Sc} in the brain (supplemental Fig. S3 and Table S1). The d/n ratios obtained for all four infected animals ranged from 0.73 to 2.39, which were similar to or lower than the uninfected 101LL control (d/n ratio of 2.01). The 101LL/ME7 control gave a d/n ratio of 9.8 (Fig. 5). These data confirm the limited PK digestion studies, proving that no PrP^{Sc}-like conformers are present in 101LL/GSS- and 101LL/263K-infected tissues that could account for the observed titers of infectivity.

Immunoprecipitation Using PrP^{Sc}-specific Monoclonal Antibodies—Several mAbs have been generated that specifically bind PrP^{Sc} isoforms, but not PrP^{C}. These antibodies can therefore isolate PrP^{Sc} from non-PK-treated tissue homogenates by immunoprecipitation, ensuring that all abnormal PrP isoforms are identified. This technique has been used by others to demonstrate the presence of $sPrP^{Sc}$ in the brains of mice overexpressing 101L-PrP (22). Here, PrP^{Sc}-specific motif grafted mAbs 89–112 and 136–158 (25) were used to immunoprecipitate PrP from brain tissue homogenates of 101LL/GSS- and 101LL/263K-infected mice. Tissues analyzed were taken from mice showing positive clinical and vacuolar signs of TSE but low levels of PrP deposition in the same primary transmission experiments as those used to determine titer of infectivity in each model (details in supplemental Fig. S2 and Table S1). Positive control mAb D13 (which precipitates only the cellular form of PrP) and negative control mAb b12 were also

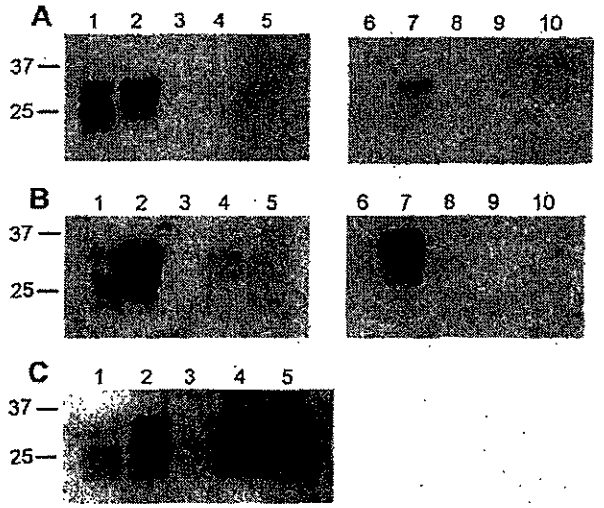

FIGURE 6. **Immunoprecipitation using PrP^{Sc}-specific monoclonal antibodies.** 101LL/GSS and 101LL/263K tissues taken from the same transmission experiments as those shown in Table 1 were subjected to IP using PrP^{Sc}-specific mAbs 89–112 and 136–158 to determine whether forms of PrP^{Sc}, which were sensitive to PK, were present in these tissues. mAb D13, which precipitates only cellular PrP, and mAb b12, which recognizes the HIV gp120 antigen, were used as IP controls. In *A*: *lanes 1–5*, 101LL/GSS(h); *lanes 6–10*, uninfected 101LL; in *B*: *lanes 1–5*, 101LL/263K(i); *lanes 6–10*, uninfected 101LL; in *C*: RML scrapie Wt control. *Lanes 1* and *6*, crude brain homogenate; *lanes 2* and *7*, IP with mAb D13 (positive control antibody); *lanes 3* and *8*, IP with mAb b12 (negative control antibody); *lanes 4* and *9*, IP with mAb 89–112; *lanes 5* and *10*, IP with mAb 136–158.

included in all experiments. For all 101LL/GSS and 101LL/263K tissues examined, extremely low levels of PrP^{Sc} were immunoprecipitated by both PrP^{Sc}-specific antibodies (Fig. 6). These levels were estimated by immunoblot to be 100- to 1000-fold less than those precipitated from control RML-infected mouse brain. Results from these immunoprecipitations therefore support our previous biochemical data, which show no evidence of PK-sensitive forms of PrP^{Sc} in brain tissue from 101LL/GSS- and 101LL/263K-infected mice.

DISCUSSION

PrP^{Sc} is thought to be the sole component of the prion, or TSE infectious agent. For this reason it has become the main target for TSE diagnostic assays, where identification of PrP^{Sc} in post-mortem brain tissue indicates a TSE-positive animal. However the relationship between PrP^{Sc} and TSE infectivity has not been definitively demonstrated, and concerns have been raised by earlier reports of disease transmission in the apparent absence of PrP-res (16, 18). In particular, 101LL gene-targeted transgenic mice inoculated with GSS P102L or 263K succumb to a disease, which is highly transmissible to both 101LL and wild-type mice but shows extremely low levels of PrP-res in the brain. Extended analyses of this model (described here) have now used quantitative assays to unequivocally demonstrate that titers of 10^7 to 10^9 IU/g can be present in brain tissue, which shows little or no abnormal PrP accumulation by standard immunoblot analysis, IHC, CDI, or immunoprecipitation. These titers are similar to or higher than those observed in our well characterized, high titer control strain ME7, but for 4 of 5 brains analyzed, PrP-res levels were below the limit of detection of our immunoblot assay (<1.3% of the amount of PrP-res in wild-type ME7 tissue). Previous studies have shown that PrP-res from other well characterized rodent scrapie strains with titers ranging from $10^{5.5}$ to 10^9 can be easily identified on immunoblot of 1% brain homogenate following PK treatment. Based on these previous data, it would be predicted that the tissues studied here should contain titers far below 10^5 IU/g tissue. However the transmission data clearly show that 101LL/GSS- and 101LL/263K-infected tissues contained high titers of infectivity, which exceed those measured in both 79V- and 22A-infected tissue (supplemental Fig. S1*B*). These data suggest that current diagnostic assay systems that rely on PrP^{Sc} detection might fail to identify some highly infectious tissues. To this end, tissues from 101LL/GSS- and 101LL/263K-infected mice are currently being assessed in several of these assay systems in our laboratory.

Several independent studies have previously shown that one TSE infectious unit is composed of ~10^5 PrP^{Sc} molecules (2, 14, 15). In contrast to these studies the data obtained from 101LL/GSS- and 101LL/263K-infected tissues indicate that the number of PrP^{Sc} molecules per unit of infectivity must display a wide range, with 101LL/GSS and 101LL/263K tissues showing between 10 to 1000 times fewer PrP-res molecules per unit infectivity than Wt/ME7. Alternatively, these data could indicate that only a very small proportion of PrP^{Sc} present in TSE-infected tissue is actually infectious. This lack of correlation between levels of PrP-res and infectivity does not support PrP-res as the infectious agent of TSE.

Because PrP-res does not appear to be a major component of infectivity in this study, it is possible that another form of PrP is responsible for disease in these mice. We have shown previously that 101LL mice can form PrP-res when inoculated with other rodent TSE strains (39); therefore, the lack of PrP^{Sc} in these models is not due to an inherent inability of 101L-PrP to convert to a protease-resistant isoform. In contrast to the gene-targeted transgenic 101LL mice described here, transgenic mice, which overexpress 101L-PrP at levels 8- to 16-fold higher than endogenous PrP, develop a spontaneous neurological disease that appears to be associated with a PK-sensitive form of PrP^{Sc} (21, 22). We have found no evidence of $sPrP^{Sc}$ in 101LL/GSS or 101LL/263K brain tissue by either limited PK digestion studies or CDI analysis. Additionally, motif-grafted mAbs 89–112 and 136–158, which specifically bind PrP^{Sc}, did not immunoprecipitate PK-sensitive forms of PrP^{Sc} from 101LL/GSS or 101LL/263K brain tissue, even though these mAbs have been shown to immunoprecipitate abnormal PK-sensitive PrP^{Sc} from 101L-overexpressing transgenic mice.[6] One possible reason for this discrepancy between models is that disease in 101LL/GSS and 101LL/263K mice is due to a TSE infection, which has been transmitted from a known infected source, and can be further passaged to both 101LL and wild-type 129/Ola mice (18, 19). In contrast the disease observed in transgenic mice overexpressing 101L PrP does not transmit to wild-type mice and only appears to accelerate the phenotype already present in mice expressing lower levels of the transgene (17, 22). This

[6] A. Bellon and R. A. Williamson, unpublished data.

suggests that $sPrP^{Sc}$ may instead be associated with overexpression or misfolding of 101L-PrP and not TSE. The species of abnormal PrP produced due to overexpression of 101L-PrP is therefore different from that produced by TSE infection. The nature of the infectious agent in the current study has yet to be established. We now aim to use this unique model to determine whether infectivity in these tissues is consistent with other abnormal conformations of PrP or with factors other than PrP.

The models of disease described herein demonstrate the potential for the existence of high levels of TSE infectivity with undetectable PrP-res in natural disease. Indeed, increased surveillance and sensitivity of testing methods has identified a new TSE of sheep, termed atypical scrapie. These animals were identified as TSE infected by one PrP^{Sc}-specific diagnostic ELISA, but could not be confirmed by other methods (40, 41). Such cases are now only identifiable using assays that require low concentrations of PK, or no PK, in the assay procedure. It is unknown whether this is truly a new TSE of sheep, or whether it has been present in sheep for some time (42) but was not detected due to the reduced PK resistance of PrP^{Sc}. However, the disease has been shown to be highly transmissible to transgenic mice expressing ovine PrP (43), indicating the presence of substantial levels of infectivity. The results of our study raise concern over the suitability of PrP^{Sc} as a sole diagnostic marker of TSE disease. It is vital that markers of TSE infectivity other than PrP^{Sc} are identified and validated in models such as those we have described and characterized here. We anticipate that such research will lead to the development of more robust diagnostic assays for TSE disease, which will have important implications for both animal and human health.

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REFERENCES

1. Prusiner, S. B. (1996) *Trends Biochem. Sci.* 21, 482–487
2. Prusiner, S. B. (1982) *Science* 216, 136–144
3. Brown, P., Preece, M., Brandel, J. P., Sato, T., McShane, L., Zerr, I., Fletcher, A., Will, R. G., Pocchiari, M., Cashman, N. R., D'Aignaux, J. H., Cervenakova, L., Fradkin, J., Schonberger, L. B., and Collins, S. J. (2000) *Neurology* 55, 1075–1081
4. Houston, F., Foster, J. D., Chong, A., Hunter, N., and Bostock, C. J. (2000) *Lancet* 356, 999–1000
5. Hunter, N., Foster, J., Chong, A., McCutcheon, S., Parnham, D., Eaton, S., MacKenzie, C., and Houston, F. (2002) *J. Gen. Virol.* 83, 2897–2905
6. Llewelyn, C. A., Hewitt, P. E., Knight, R. S. G., Amar, K., Cousens, S., Mackenzie, J., and Will, R. G. (2004) *Lancet* 363, 417–421
7. Peden, A. H., Head, M. W., Ritchie, D. L., Bell, J. E., and Ironside, J. W. (2004) *Lancet* 364, 527–529
8. Pincock, S. (2004) *Br. Med. J.* 329, 251
9. Wroe, S. J., Pal, S., Siddique, D., Hyare, H., Macfarlane, R., Joiner, S., Linehan, J. M., Brandner, S., Wadsworth, J. D. F., Hewitt, P., and Collinge, J. (2006) *The Lancet* 368, 2061–2067
10. Bruce, M. E., Will, R. G., Ironside, J. W., McConnell, I., Drummond, D., Suttie, A., McCardle, L., Chree, A., Hope, J., Birkett, C., Cousens, S., Fraser, H., and Bostock, C. J. (1997) *Nature* 389, 498–501
11. Hilton, D. A., Ghani, A. C., Conyers, L., Edwards, P., McCardle, L., Ritchie, D., Penney, M., Hegazy, D., and Ironside, J. W. (2004) *J. Pathol.* 203, 733–739
12. Ironside, J., Bishop, M. T., Connolly, K., Hegazy, D., Lowrie, S., Le Grice, M., Ritchie, D., McCardle, L., and Hilton, D. A. (2006) *Br. Med. J.* 332, 1164–1165
13. Bishop, M. T., Hart, P., Aitchison, L., Baybutt, H., Plinston, C., Thomson, V., Tuzi, N., Head, M., Ironside, J., Will, R., and Manson, J. (2006) *Lancet Neurol.* 5, 393–398
14. McKinley, M. P., Bolton, D. C., and Prusiner, S. B. (1983) *Cell* 35, 57–62
15. Beekes, M., Baldauf, E., and Diringer, H. (1996) *J. Gen. Virol.* 77, 1925–1934
16. Lasmezas, C. I., Deslys, J., Robain, O., Jaegly, A., Beringue, V., Peyrin, J., Fournier, J., Hauw, J., Rossier, J., and Dormont, D. (1997) *Science* 275, 402–405
17. Hsiao, K. K., Groth, D., Scott, M., Yang, S. L., Serban, H., Raff, D., Foster, D., Torchia, M., Dearmond, S. J., and Prusiner, S. B. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 9126–9130
18. Manson, J. C., Jamieson, E., Baybutt, H., Tuzi, N. L., Barron, R., McConnell, I., Somerville, R., Ironside, J., Will, R., Sy, M. S., Melton, D. W., Hope, J., and Bostock, C. (1999) *EMBO J.* 18, 6855–6864
19. Barron, R. M., Thomson, V., Jamieson, E., Melton, D. W., Ironside, J., Will, R., and Manson, J. C. (2001) *EMBO J.* 20, 5070–5078
20. Safar, J., Wille, H., Itri, V., Groth, D., Serban, H., Torchia, M., Cohen, F. E., and Prusiner, S. B. (1998) *Nat. Med.* 4, 1157–1165
21. Tremblay, P., Ball, H. L., Kaneko, K., Groth, D., Hegde, R. S., Cohen, F. E., DeArmond, S. J., Prusiner, S. B., and Safar, J. G. (2004) *J. Virol.* 78, 2088–2099
22. Nazor, K. E., Kuhn, F., Seward, T., Green, M., Zwald, D., Pürro, M., Schmid, J., Biffiger, K., Power, A. M., Oesch, B., Raeber, A., and Telling, G. (2005) *EMBO J.* 24, 2472–2480
23. Dickinson, A. G., Meikle, V. M., and Fraser, H. (1968) *J. Comp. Pathol.* 78, 293–299
24. Karber, G. (1931) *Arch. Exp. Pathol. Pharmacol.* 162, 480–483
25. Moroncini, G., Kanu, N., Solforosi, L., Abalos, G., Telling, G. C., Head, M., Ironside, J., Brockes, J. P., Burton, D. R., and Williamson, R. A. (2004) *Proc. Natl. Acad. Sci. U. S. A.* 101, 10404–10409
26. Solforosi, L., Bellon, A., Schaller, M., Cruite, J. T., Abalos, G. C., and Williamson, R. A. (2007) *J. Biol. Chem.* 282, 7465–7471
27. Zanusso, G., Liu, D., Ferrari, S., Hegyi, I., Yin, X., Aguzzi, A., Hornemann, S., Liemann, S., Glockshuber, R., Manson, J. C., Brown, P., Petersen, R. B., Gambetti, P., and Sy, M.-S. (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95, 8812–8816
28. Chiesa, R., and Harris, D. A. (2001) *Neurobiol. Dis.* 8, 743–763
29. Hegde, R. S., Tremblay, P., Gorth, D., DeArmond, S. J., Prusiner, S. B., and Lingappa, V. R. (1999) *Nature* 402, 822–826
30. Stewart, R. S., Piccardo, P., Ghetti, B., and Harris, D. A. (2005) *J. Neurosci.* 25, 3469–3477
31. Ma, J., Wollmann, R., and Lindquist, S. (2002) *Science* 298, 1781–1785
32. Ma, J., and Lindquist, S. (2002) *Science* 298, 1785–1788
33. Chiesa, R., Piccardo, P., Ghetti, B., and Harris, D. A. (1998) *Neuron* 21, 1339–1351
34. Chiesa, R., Drisaldi, B., Quaglio, E., Migheli, A., Piccardo, P., Ghetti, B., and Harris, D. A. (2000) *Proc. Natl. Acad. Sci. U. S. A.* 97, 5574–5579
35. Kitamoto, T., Iizuka, R., and Tateishi, J. (1993) *Biochem. Biophys. Res. Commun.* 192, 525–531
36. Zanusso, G., Petersen, R. B., Jin, T. C., Jing, Y., Kanoush, R., Ferrari, S., Gambetti, P., and Singh, N. (1999) *J. Biol. Chem.* 274, 23396–23404
37. Safar, J. G., Scott, M., Monaghan, J., Deering, C., Didorenko, S., Vergara, J., Ball, H., Legname, G., Leclerc, E., Solforosi, L., Serban, H., Groth, D., Burton, D. R., Prusiner, S. B., and Williamson, R. A. (2002) *Nat. Biotechnol.* 20, 1147–1150
38. Safar, J. G., Geschwind, M. D., Deering, C., Didorenko, S., Sattavat, M.,

Sanchez, H., Serban, A., Vey, M., Baron, H., Giles, K., Miller, B. L., DeArmond, S. J., and Prusiner, S. B. (2005) *Proc. Natl. Acad. Sci. U. S. A.* 102, 3501–3506
39. Barron, R. M., Thomson, V., King, D., Shaw, J., Melton, D. W., and Manson, J. C. (2003) *J. Gen. Virol.* 84, 3165–3172
40. Buschmann, A., Biacabe, A.-G., Ziegler, U., Bencsik, A., Madec, J.-Y., Erhardt, G., Luhken, G., Baron, T., and Groschup, M. H. (2004) *J. Virol. Methods* 117, 27–36
41. Buschmann, A., Luhken, G., Schultz, J., Erhardt, G., and Groschup, M. H. (2004) *J. Gen. Virol.* 85, 2727–2733
42. Bruce, M. E., Nonno, R., Foster, J., Goldmann, W., Di Bari, M., Esposito, E., Benestad, S. L., Hunter, N., and Agrimi, U. (2007) *Vet Rec.* 160, 665–666
43. Le Dur, A., Beringue, V., Andreoletti, O., Reine, F., Lai, T. L., Baron, T., Bratberg, B., Vilotte, J.-L., Sarradin, P., Benestad, S. L., and Laude, H. (2005) *Proc. Natl. Acad. Sci. U. S. A.* 102, 16031–16036

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No. 22

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研究報告の概要

○非定型ウシ海綿状脳症プリオン株のヒト伝播リスクの評価
ウシのプリオン疾患である、ウシ海綿状脳症(BSE)は、BSE-Cというたった1つの株から発生したと広く考えられている。BSE-Cは、ヒトにおいて変異性クロイツフェルト・ヤコブ病と称される致死性プリオン疾患を引き起こす。2004年以降、ウシアミロイド海綿状脳症(BASE、またはBSE-Lとも呼ばれる)およびBSE-Hという2つの非定型BSE株が複数の国で発見された。これらのヒトにおける伝播性と表現型は不明である。我々は、ヒトプリオンタンパク発現トランスジェニック(Tg)マウスに2つのBASE株感染ウシ由来ホモジネートを接種することにより、BASE株の感染性とヒトの表現型を検討した。接種20～22ヶ月後に接種実施Tgマウスの60%が感染し、これはBSE-Cで報告された伝播率よりも高かった。BASE株感染Tgマウスの4分の1が脾臓に病原性プリオンタンパクアイソフォームの存在を示し(孤発性ヒトプリオン疾患によるプリオン感染Tgマウスではゼロ)、BASEプリオンが本質的にリンパ向性であることを示した。BASE株に感染したヒト化Tgマウスの脳の病原性プリオンタンパクアイソフォームは、元のウシBASEまたは散発性ヒトプリオン疾患由来のアイソフォームとは異なった。BASE株感染Tgマウスでは脳の海綿化がごくわずかで、潜伏期間が長いことが観察された。以上の結果は、ヒトにおいて、BASE株は、BSE株よりも感染性が強く、リンパ向性が高いことを示している。

報告企業の意見	今後の対応
非定型ウシ海綿状脳症プリオン株は、ヒトにおいて通常のBSE株よりも感染性が強く、リンパ向性が高いことが示されたとの報告である。	今後も引き続き、プリオン病に関する新たな知見及び情報の収集に努める。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

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Evaluation of the Human Transmission Risk of an Atypical Bovine Spongiform Encephalopathy Prion Strain[▽]

Qingzhong Kong,[1]* Mengjie Zheng,[1] Cristina Casalone,[2] Liuting Qing,[1] Shenghai Huang,[1]† Bikram Chakraborty,[1] Ping Wang,[1] Fusong Chen,[1] Ignazio Cali,[1] Cristiano Corona,[2] Francesca Martucci,[2] Barbara Iulini,[2] Pierluigi Acutis,[2] Lan Wang,[1] Jingjing Liang,[1] Meiling Wang,[1] Xinyi Li,[1] Salvatore Monaco,[3] Gianluigi Zanusso,[3] Wen-Quan Zou,[1] Maria Caramelli,[2] and Pierluigi Gambetti[1]*

Department of Pathology, Case Western Reserve University, Cleveland, Ohio 44106[1]; CEA, Istituto Zooprofilattico Sperimentale, 10154 Torino, Italy[2]; and Department of Neurological and Visual Sciences, University of Verona, 37134 Verona, Italy[3]

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Bovine spongiform encephalopathy (BSE), the prion disease in cattle, was widely believed to be caused by only one strain, BSE-C. BSE-C causes the fatal prion disease named new variant Creutzfeldt-Jacob disease in humans. Two atypical BSE strains, bovine amyloidotic spongiform encephalopathy (BASE, also named BSE-L) and BSE-H, have been discovered in several countries since 2004; their transmissibility and phenotypes in humans are unknown. We investigated the infectivity and human phenotype of BASE strains by inoculating transgenic (Tg) mice expressing the human prion protein with brain homogenates from two BASE strain-infected cattle. Sixty percent of the inoculated Tg mice became infected after 20 to 22 months of incubation, a transmission rate higher than those reported for BSE-C. A quarter of BASE strain-infected Tg mice, but none of the Tg mice infected with prions causing a sporadic human prion disease, showed the presence of pathogenic prion protein isoforms in the spleen, indicating that the BASE prion is intrinsically lymphotropic. The pathological prion protein isoforms in BASE strain-infected humanized Tg mouse brains are different from those from the original cattle BASE or sporadic human prion disease. Minimal brain spongiosis and long incubation times are observed for the BASE strain-infected Tg mice. These results suggest that in humans, the BASE strain is a more virulent BSE strain and likely lymphotropic.

Overwhelming evidence indicates that bovine spongiform encephalopathy (BSE), a prion disease that has been detected in several hundred thousand cattle in the United Kingdom and many other countries since the 1980s, has been transmitted to humans through the consumption of prion-contaminated beef, causing a prion disease named variant Creutzfeldt-Jakob disease (vCJD) (5, 19, 24). Over 200 cases of vCJD have been reported around the world (19). In 2004, two types of bovine prion disease that differ from the original BSE, now named classical BSE (BSE-C), were reported (3, 8). The two atypical BSE types were associated with prion protein (PrP) scrapie isoforms (PrP^{Sc}) that after protease digestion, displayed distinct electrophoretic mobility or ratios of the PrP^{Sc} glycoforms different from those of BSE-C (3, 8). Currently, a total of at least 36 cases of these two atypical BSE types have been reported for cattle older than 8 years (5; M. Caramelli, unpublished data). The two atypical BSE types are identified as BSE-H and bovine amyloidotic spongiform encephalopathy (BASE, also named BSE-L); the "L" and "H" identify the higher and lower electrophoretic positions, respectively, of their protease-resistant PrP^{Sc} isoforms (7). The bovine phenotype and the PrP^{Sc} molecular features of BASE have previously been described in detail (8). The histopathology of BASE and the PrP immunostaining pattern of BASE strains are characterized by the presence of prion amyloid plaques and a more rostral distribution of the PrP^{Sc}, which at variance with BSE-C is present in the cerebral cortex, including the hippocampus, but is underrepresented in the brain stem (8). These phenotypic features and PrP^{Sc} characteristics resemble a subtype of sporadic Creutzfeldt-Jakob disease (sCJD) named sCJDMV2, which affects subjects who are methionine (M)/valine (V) heterozygous at codon 129 of the PrP gene, and it is associated with PrP^{Sc} identified as type 2 (15). This similarity has raised the question of whether sCJDMV2 is not sporadic but acquired from the consumption of BASE strain-contaminated meat (5, 8). To begin to investigate the transmissibility to humans and the "human" disease phenotype of BASE, including the involvement of the lymphoreticular system, we have inoculated brain homogenates from BASE-affected cattle to transgenic (Tg) mice expressing normal human PrP with Met at codon 129 (HuPrP-129M) in a mouse PrP-ablated background [Tg(HuPrP)] (13). The inoculated Tg mice were examined for attack rates and the disease phenotype, including the presence and characteristics of protease-resistant PrP^{Sc} in the brain and spleen and the histopathology, along with the PrP^{Sc} topography and pattern of deposition in the brain.

MATERIALS AND METHODS

Transgenic mice. Transgenic mice expressing human PrP-129M [Tg(HuPrP)] were reported previously (13). The Tg40 line that expresses human PrP-129M at

* Corresponding author. Mailing address: Department of Pathology, Case Western Reserve University, Cleveland, OH 44106. Phone for Pierluigi Gambetti: (216) 368-0586. Fax: (216) 368-2546. E-mail: pxg13@case.edu. Phone for Qingzhong Kong: (216) 368-1756. Fax: (216) 368-2546. E-mail: qxk2@case.edu.

† Present address: Department of Patient Education and Health Information, Cleveland Clinic Foundation, Cleveland, OH 44195.

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the wild-type level in the mouse PrP-ablated background was used in this study. Intracerebral (i.c.) inoculation of Tg mice and the monitoring of symptoms were performed as described previously (13). The mice were sacrificed 2 or 3 days after the appearance of symptoms or at death, and the brains and spleens were taken. The brains were sliced sagittally, with half frozen for immunochemical studies and the other half either fixed in formalin for histological and immunohistochemical staining or frozen for histoblot analysis (see below). Total PrP as well as proteinase K (PK)-resistant PrP^{Sc} was determined by immunoblotting in sodium dodecyl sulfate (SDS)-polyacrylamide gels as described below. This study was conducted with approvals from the Institutional Review Board and the Institutional Animal Care and Use Committee.

Immunoblotting, histology, histoblotting, and immunohistochemistry. Frozen brain or spleen tissues were homogenized in 2 volumes of cold phosphate-buffered saline to obtain 33% (wt/vol) crude homogenate for storage in aliquots at −80°C. The frozen 33% crude homogenate was thawed at 4°C for 2 h and diluted to 10% (wt/vol) with the lysis buffer (final concentration, 100 mM Tris, 10 mM EDTA, 100 mM NaCl, 0.5% sodium deoxycholate, 1.0% NP-40, pH 8.0). After incubation at room temperature for 15 min, the 10% homogenate was subjected to sonication with the Ultrasonic Dismembrator 100 (Fisher Scientific) for 3 min. The sonicated 10% homogenate was treated with 100 μg/ml PK (Roche Diagnostics GmbH, Mannheim, Germany) for 30 min at 37°C and denatured by being boiled at 100°C for 10 min after being mixed with an equal volume of 2× sample buffer (200 mM Tris-HCl, pH 6.8, 2% SDS, 40% glycerol, 0.04% Coomassie blue G-250, 2% β-mercaptoethanol). The enrichment of PrP^{Sc} by precipitation with sodium phosphotungstate (NaPTA) was performed virtually as previously reported (18), and special care and efforts were taken to ensure that the pellets were completely resuspended each time. Proteins were separated by precast 10 to 20% gradient Tris-Tricine gel (Bio-Rad), transferred to a polyvinylidene difluoride membrane, and subjected to Western blot analysis with monoclonal antibody (MAb) 8H4, 6H4, or 3F4 in conjunction with horseradish peroxidase-conjugated sheep anti-mouse immunoglobulin G Fc antibody (GE Healthcare, Buckinghamshire, United Kingdom) as described previously (13). The blots were developed with the ECL Western blotting detection reagent (GE Healthcare Amersham, Buckinghamshire, United Kingdom) and exposed to X-ray films. The blots were digitized by scanning the film. To determine the precise molecular weights of the bands, the digitized blots were analyzed by image acquisition and analysis software (UVP, Upland, CA) that automatically detects the midpoint of the band and calculates the molecular weight based on the sizes of the unglycosylated PK-resistant PrP fragments of sCJDMM1 and sCJDMM2; the values were statistically analyzed by Matlab 7.0 software (MathWorks, Natick, MA). To determine the glycoform ratios of PK-resistant PrP^{Sc} fragments, each PrP band on the digitized blots was quantified with UN-SCAN-IT software (Silk Scientific, Orem, UT); the values from duplicate blots were analyzed with Excel software to calculate the averages and standard deviations and to create the column chart.

Histological staining with hematoxylin and eosin (H&E) and immunohistochemical staining with 3F4 were performed as reported previously (13). Histoblot analysis was performed mostly as described previously (20), with the following modifications: the cryosections were 12 μm thick, and the sections were treated with 100 μg/ml of proteinase K for 4 h at 37°C, incubated with monoclonal antibody 3F4 (1:10,000 dilution) overnight at 4°C, followed by incubation with alkaline phosphatase-conjugated goat anti-mouse secondary antibody (1:500; DAKO), and developed with BCIP (5-bromo-4-chloro-3-indolylphosphate)-nitroblue tetrazolium solutions (Sigma).

RESULTS

To assess the transmissibility of BASE in humans, two BASE isolates (8) were used to intracerebrally inoculate 30 Tg40 mice that express normal levels of human PrP-129M. More than half of the inoculated mice (18/30) became infected, as determined by the presence of protease-resistant PrP^{Sc}, with average incubation times of 649 ± 34 days for BASE isolate 1 and 595 ± 28 days for BASE isolate 2, respectively (Table 1). Ten of the 18 infected mice that could be examined showed clear clinical signs of disease (Table 1), including hunched backs, ruffled fur, lethargy, occasional wobbling, and rigid tails. These signs were best detected in the younger mice, because in mice older than 24 months, the signs became difficult to distinguish from aging-related changes.

TABLE 1. BASE transmission in Tg(HuPrP) mice

Inoculum	Attack rate as determined by:			Incubation time (days)
	Clinical signs	Presence of PrP^{Sc}	Spongiform degeneration	
BASE-1	4/15	9/15	1 (focal)/8	649 ± 34
BASE-2	6/15	9/15	1 (focal)/11	595 ± 28
sCJDMM1	10/10	9/10	4/4	263 ± 13[a]
sCJDMM2	9/9	9/9	7/7	267 ± 17

[a] Reported previously (13).

All the Tg40 mice were examined for the presence of PK-resistant PrP^{Sc} in the brain by immunoblot analysis both directly and after enrichment with NaPTA precipitation. Such immunoblot analysis with three monoclonal antibodies (3F4, 6H4, and 8H4) to various PrP regions (12, 14, 25) showed that all 18 BASE strain-infected Tg40 mice accumulated comparable amounts of PK-resistant PrP^{Sc} in the brain (Fig. 1A, Table 1, and data not shown). The electrophoretic mobility of PK-resistant PrP^{Sc} fragments from all the BASE strain-infected Tg40 mice was indistinguishable from that of the PK-resistant PrP^{Sc} present in either the BASE strain inoculum or sCJDMM2, which contains type 2 PrP^{Sc} (Fig. 1A). The PK-resistant PrP^{Sc} fragments associated with both the BASE strain-infected Tg40 mice and the BASE isolates migrated slightly faster than those of BSE-C as originally reported (8). Measurements with software that automatically calculates the midpoint of the bands revealed a difference of 0.29 ± 0.12 kDa in gel mobility between the unglycosylated PK-resistant PrP^{Sc} bands of the BASE strain (native as well as from the Tg40 mice) and BSE-C.

The glycoform ratio of PrP^{Sc} in isolates from the BASE strain-infected Tg40 mice was slightly different from that of the BASE isolates (Fig. 1B), and both were quite different from that of BSE-C (Fig. 1B). The monoglycosylated form was the most prominent species in the BASE strain inocula, where the glycoform ratio (diglycosylated-to-monoglycosylated-to-unglycosylated) is 32:41:27, whereas the diglycosylated form was slightly more intense than the monoglycosylated form in BASE strain-infected Tg40 mice, where the glycoform ratio is 44:39:17 (Fig. 1B). In contrast, the diglycosylated form accounted for over 70% of the total PrP^{Sc} in BSE-C (glycoform ratio of 72:20:8).

PrP^{Sc} in the spleen was also examined after NaPTA enrichment for all 30 BASE strain-inoculated Tg40 mice. PK-resistant PrP^{Sc} was readily detected in the spleens of four mice (Fig. 1C), all of which also contained PK-resistant PrP^{Sc} in the brain. The electrophoretic mobility of the spleen PrP^{Sc} was similar to that of the brain PrP^{Sc}. The glycoform ratio of the spleen PrP^{Sc} was different from that of the brain and was characterized by the prominence of the monoglycosylated and unglycosylated forms (Fig. 1C), but the glycoform ratio may have been affected by the NaPTA enrichment. In contrast, none of the nine Tg40 mice inoculated with sCJDMM1 had detectable PK-resistant PrP^{Sc} in the spleen after NaPTA enrichment (data not shown).

None of the 12 BASE strain-infected Tg40 mice examined showed prominent and consistent histopathological changes related to prion diseases (Fig. 2A). Focal, ambiguous spongiform degeneration was observed for two mice. No PrP amyloid plaques were observed in BASE strain-infected Tg40 mice.

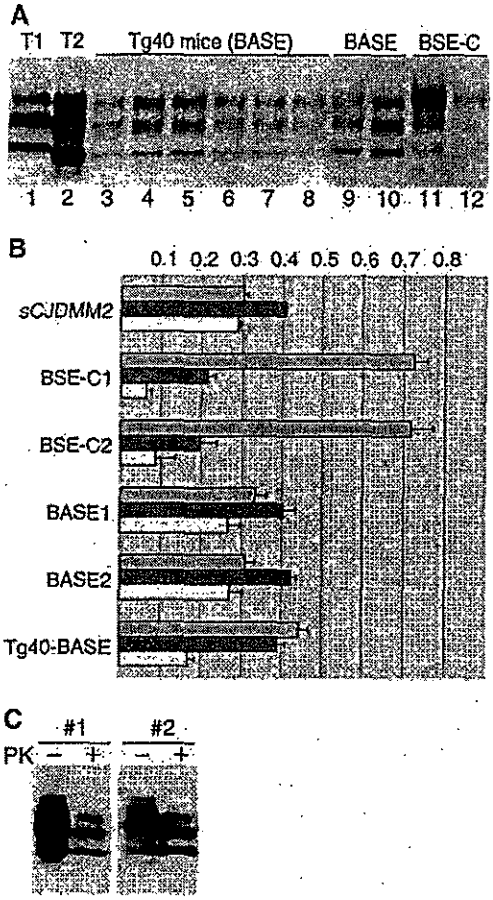

FIG. 1. Immunoblots and glycoform ratios of PK-resistant PrP^{Sc} from sCJD-affected, BASE strain-infected Tg(HuPrP) mice, the BASE strain inocula, and BSE-C and of PK-resistant PrP^{Sc} from the spleens of BASE strain-infected Tg(HuPrP) mice. (A) Immunoblot of PK-resistant PrP^{Sc} in the brain. Lanes 1 and 2, type 1 (sCJDMM1) (T1) and type 2 (sCJDMM2) (T2) sCJD, respectively; lanes 3 to 6, Tg(HuPrP) (Tg40) mice infected with BASE isolate 1 inoculum; lanes 7 to 8, Tg40 mice infected with BASE isolate 2 inoculum; lane 9, BASE isolate 1; lane 10, BASE isolate 2; lanes 11 and 12, two BSE-C isolates. All brain homogenates were treated with 100 μg/ml of PK for 30 min at 37°C and processed for immunoblot analysis with MAb 8H4. Five microliters of 10% brain homogenate was loaded for lanes 3 to 10. (B) Glycoform ratios of PK-resistant PrP^{Sc} in the brain. The upper (diglycosylated) (blue), middle (mostly monoglycosylated) (red), and lower (unglycosylated) (yellow) bands of PK-resistant PrP^{Sc} from BASE strain-infected Tg40 mice, the BASE strain, and BSE-C were quantified after optical scanning of duplicate immunoblots for panel A. Error bars indicate standard deviations. (C) PK-resistant PrP^{Sc} in the spleen. Ten milligrams of spleen tissue each from two of the BASE strain-infected Tg(HuPrP) (Tg40) mice (#1 and #2) was homogenized, PrP^{Sc} enriched by NaPTA precipitation, and either treated (+) or not treated (−) with 100 μg/ml of PK for 30 min at 37°C, followed by electrophoresis in a 10 to 20% Tris-Tricine SDS-polyacrylamide gradient gel and immunoblot analysis with MAb 8H4.

Histoblot analysis with MAb 3F4 showed a very distinct and selective distribution of PrP^{Sc} (Fig. 3A to D). Particular nuclei or groups of adjacent periventricular nuclei in the thalamus, hypothalamus, and brain stem were intensely immunostained for PrP^{Sc} (Fig. 3B to D). In contrast, PrP^{Sc} appeared to be overall less intense in the cerebral and cerebellar cortices (Fig. 3A to D). Immunohistochemical staining of paraffin-embedded brain tissue with 3F4 revealed PrP deposits in 5 of the 11 BASE strain-infected Tg40 mice examined. PrP^{Sc} deposits that stained intensely in the histoblots consisted of relatively large and well-circumscribed granules (Fig. 3E and G). Fine granular or small plaque-like aggregate patterns were occasionally seen in inferior regions of the cerebral cortex and in the thalamus (Fig. 3I and data not shown). In contrast, widespread, mostly fine-granular staining was detected in the cerebral cortex of symptomatic Tg40 mice inoculated with sCJDMM1 brain homogenate (Fig. 3J).

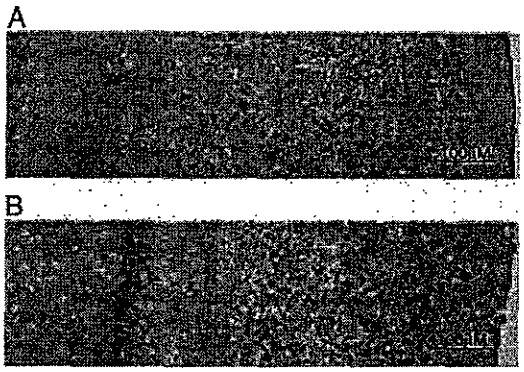

FIG. 2. Histopathology (with H&E) of BASE strain-infected and sCJDMM1-infected Tg(HuPrP) mice. (A) No consistent pathology was detected in the cerebral cortex as well as subcortical brain regions of symptomatic and immunoblot-positive BASE strain-infected Tg(HuPrP) (Tg40) mice. (B) In contrast, Tg40 mice inoculated with sCJDMM1 brain homogenate showed widespread spongiform degeneration.

The histopathological features of the BASE strain-inoculated Tg40 mice were quite different from those observed following inoculation with brain homogenates from the two forms of sCJD, sCJDMM1 and sCJDMM2. The sCJDMM1-inoculated Tg40 mice had widespread spongiform degeneration in the cerebrum (Fig. 2B) and moderate apoptosis of neuronal cells without spongiform degeneration in the cerebellum (13). Widespread spongiform degeneration was also seen in Tg40 mice inoculated with sCJDMM2 brain homogenate (data not shown).

DISCUSSION

We have shown that 60% of our Tg40 mice (in an inbred FVB background) that express normal levels of human PrP-129M became infected 20 to 22 months after i.c. inoculation with 0.3 mg of brain tissue from the two BASE isolates, suggesting a titer of approximately 3 50% infective dose units per milligram of brain tissue in the Tg40 line. An approximately 20% attack rate has been reported for the Tg650 line (in a mixed 129/Sv × C57BL/6 background) after i.c. inoculation with 2 mg brain tissues from BSE-C-infected cattle (2). It is noteworthy that the Tg650 mice express human PrP-129M at five to eight times the normal level, and high PrP levels are known to increase prion transmissibility (9, 17, 22). Inefficient BSE-C transmissions (0 to 30%) in Tg mouse lines of other genetic backgrounds expressing human PrP-129M at one or two times the normal level have also been reported by different groups (1, 4). Although it is difficult to compare results from different mouse lines, these findings suggest that the BASE strain has higher transmissibility than BSE-C does for human-

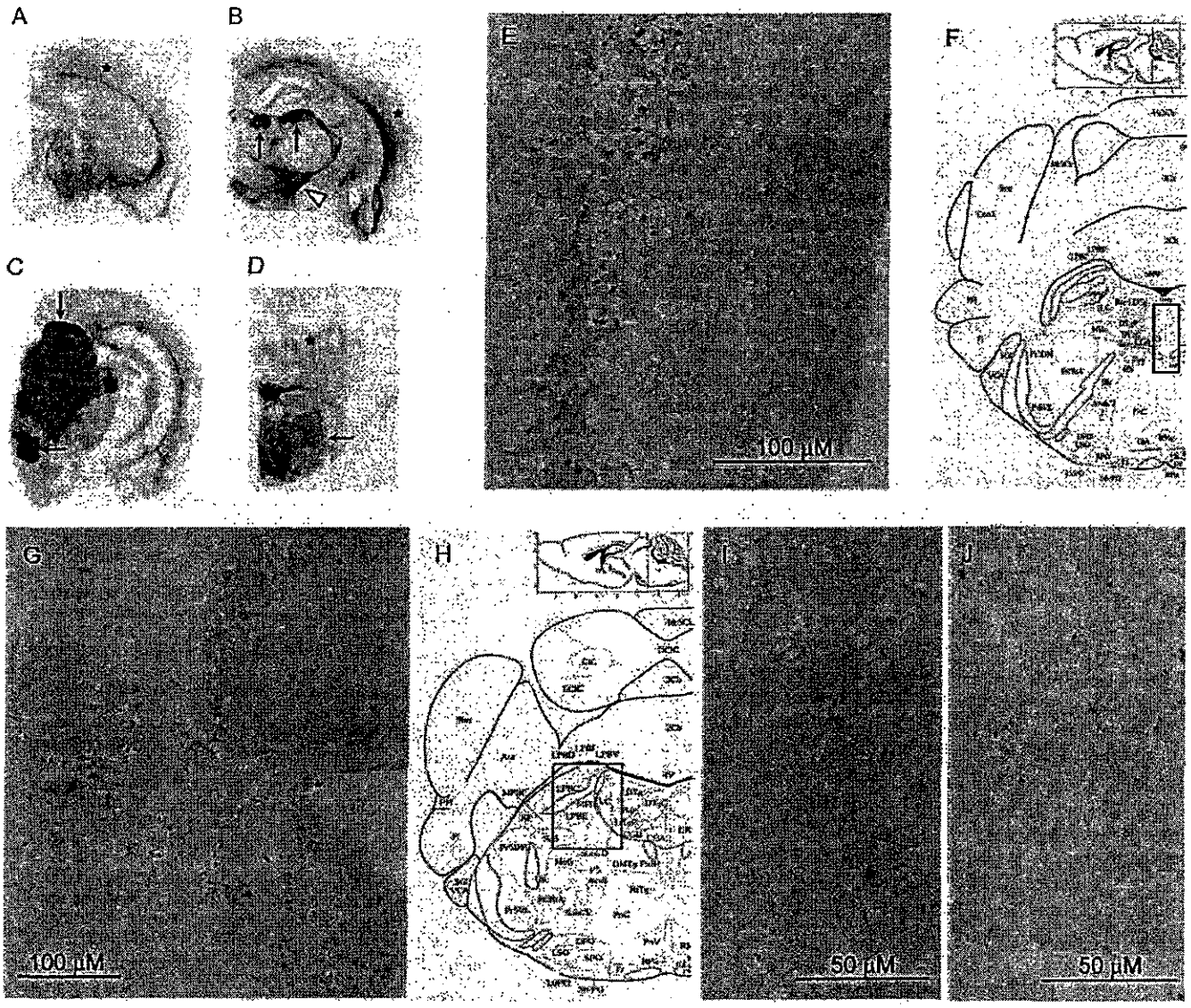

FIG. 3. Histoblot analysis and immunohistochemistry of BASE strain-infected and sCJDMM1-infected Tg(HuPrP) mice. (A to D) The histoblot analysis revealed preferential immunostaining of the PrP^{Sc} in the dorsal thalamic nuclei (arrows in panel B), along with hypothalamic nuclei (arrowhead) and brain stem nuclei (arrows in panels C and D), while PrP^{Sc} in the cerebral and cerebellar cortices (stars in panels A, B, and D) was mostly limited to the deep and inferior cortical regions. (E to J) The PrP immunostaining (E and G) of the intensely PrP-reactive brain stem nuclei in histoblot analysis (boxed regions in panels F and H) revealed coarse PrP granules, while the PrP immunostain in the cerebral cortex (I) was minimal and characterized mostly by a plaque-like pattern. In contrast, widespread fine-granular PrP immunostaining was observed in the cerebral cortex of symptomatic Tg40 mice following inoculation of sCJDMM1 brain homogenates (J). Monoclonal antibody 3F4 was used for all the staining.

ized Tg mice with PrP-129M and possibly for humans with PrP-129MM. The BASE strain also appears to be more virulent than BSE-C in bovinized Tg mice, since the incubation time for the BASE strain is 185 ± 12 days, whereas that for BSE-C is 230 ± 7 days (7). Nevertheless, compared with the 100% attack rate and incubation times of ~9 months for sCJDMM1 and sCJDMM2 in the Tg40 line (Table 1), the 60% attack rate and unusually long incubation times (20 to 22 months) for the BASE strain in the same Tg line suggest that the transmission barrier from the BASE strain to humans with PrP-129MM is still quite significant.

PK-resistant PrP^{Sc} was also detected in the spleen in 4 out of 18 BASE strain-infected Tg40 mice. In contrast, no spleen involvement could be demonstrated for the Tg40 mice following i.c. inoculation with human PrP^{Sc} from sCJDMM1. This is the first report of the presence of PrP^{Sc} in the spleens of humanized Tg mice after i.c. inoculation with a BSE strain, suggesting that the BASE strain, like BSE-C, where at least in vCJD-infected subjects PrP^{Sc} and prion infectivity have been detected in spleens and tonsils (6, 11), is intrinsically lymphotropic. Therefore, lymphoid tissues of BASE strain-infected individuals might also carry prion infectivity.

The gel mobility of the PK-resistant PrP^{Sc} recovered from the BASE strain-inoculated Tg40 mice was consistently slightly faster than the mobility of BSE-C, as originally reported for the BASE strain (8). The computed difference in gel mobilities

between BASE and BSE-C PrP^{Sc} is 0.29 ± 0.12 kDa, corresponding to 2 to 4 amino acid residues. In contrast, the gel mobilities of the PK-resistant PrP^{Sc} species from the BASE strain, BASE strain-infected Tg40 mice, and sCJDMM2, which was used as representative of human PrP^{Sc} of type 2, were indistinguishable. This finding suggests that the PK-resistant PrP^{Sc} electrophoretic heterogeneity between the BASE strain and BSE-C falls well within the 7-amino-acid variability of the N terminus (positions 92 to 99) that is consistently found in PK-resistant PrP^{Sc} of type 2 (16). Therefore, despite their minor but distinct variability in gel mobility, both the BASE strain and BSE-C PrP^{Sc} species appear to belong to the PrP^{Sc} of type 2. However, the PrP^{Sc} glycoform ratios of BASE strain-infected Tg40 mice and the BASE strain inocula display a small but statistically significant difference (Fig. 1). Therefore, PrP^{Sc} in BASE strain-infected human subjects may be expected to display a different glycoform ratio from that of the BASE strain. It is worth noting that the electrophoretic characteristics of the PK-resistant PrP^{Sc} of some human prion strains has been faithfully reproduced by our Tg40 line as well as by other humanized mouse lines (10, 13, 21).

Two distinct histopathological and PrP immunohistochemical phenotypes have been reported following BSE-C inoculation: one reproduced the distinctive features of vCJD with the "florid" plaques that intensely immunostained for PrP, and the other was reminiscent of sCJDMM1, with prominent spongiform degeneration and no plaque PrP immunostaining (1, 23). The brain histopathology, the PrP^{Sc} distribution, and the PrP immunostaining pattern of BASE strain-inoculated Tg40 mice were definitely distinct from such features described above (1, 23), further supporting the notion that BASE and classical BSE are associated with two distinct prion strains (8).

The relatively easy transmission of BASE to humanized Tg mice indicates that effective cattle prion surveillance should be maintained until the extent and origin of this and other atypical forms of BSE are fully understood.

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REFERENCES

1. **Asante, E. A., J. M. Linehan, M. Desbruslais, S. Joiner, I. Gowland, A. L. Wood, J. Welch, A. F. Hill, S. E. Lloyd, J. D. Wadsworth, and J. Collinge.** 2002. BSE prions propagate as either variant CJD-like or sporadic CJD-like prion strains in transgenic mice expressing human prion protein. EMBO J. 21:6358–6366.
2. **Béringue, V., O. Andreoletti, A. Le Dur, R. Essalmani, J. L. Vilotte, C. Lacroux, F. Reine, L. Herzog, A. G. Biacabe, T. Baron, M. Caramelli, C. Casalone, and H. Laude.** 2007. A bovine prion acquires an epidemic bovine spongiform encephalopathy strain-like phenotype on interspecies transmission. J. Neurosci. 27:6965–6971.
3. **Biacabe, A. G., J. L. Laplanche, S. Ryder, and T. Baron.** 2004. Distinct molecular phenotypes in bovine prion diseases. EMBO Rep. 5:110–115.
4. **Bishop, M. T., P. Hart, L. Aitchison, H. N. Baybutt, C. Plinston, V. Thomson, N. L. Tuzi, M. W. Head, J. W. Ironside, R. G. Will, and J. C. Manson.** 2006. Predicting susceptibility and incubation time of human-to-human transmission of vCJD. Lancet Neurol. 5:393–398.
5. **Brown, P., L. M. McShane, G. Zanusso, and L. Detwiler.** 2006. On the question of sporadic or atypical bovine spongiform encephalopathy and Creutzfeldt-Jakob disease. Emerg. Infect. Dis. 12:1816–1821.
6. **Bruce, M. E., I. McConnell, R. G. Will, and J. W. Ironside.** 2001. Detection of variant Creutzfeldt-Jakob disease infectivity in extraneural tissues. Lancet 358:208–209.
7. **Buschmann, A., A. Gretzschel, A. G. Biacabe, K. Schiebel, C. Corona, C. Hoffmann, M. Eiden, T. Baron, C. Casalone, and M. H. Groschup.** 2006. Atypical BSE in Germany—proof of transmissibility and biochemical characterization. Vet. Microbiol. 117:103–116.
8. **Casalone, C., G. Zanusso, P. Acutis, S. Ferrari, L. Capucci, F. Tagliavini, S. Monaco, and M. Caramelli.** 2004. Identification of a second bovine amyloidotic spongiform encephalopathy: molecular similarities with sporadic Creutzfeldt-Jakob disease. Proc. Natl. Acad. Sci. USA 101:3065–3070.
9. **Fischer, M., T. Rulicke, A. Raeber, A. Sailer, M. Moser, B. Oesch, S. Brandner, A. Aguzzi, and C. Weissmann.** 1996. Prion protein (PrP) with amino-proximal deletions restoring susceptibility of PrP knockout mice to scrapie. EMBO J. 15:1255–1264.
10. **Hill, A. F., M. Desbruslais, S. Joiner, K. C. Sidle, I. Gowland, J. Collinge, L. J. Doey, and P. Lantos.** 1997. The same prion strain causes vCJD and BSE. Nature 389:448–450.
11. **Hill, A. F., R. J. Butterworth, S. Joiner, G. Jackson, M. N. Rossor, D. J. Thomas, A. Frosh, N. Tolley, J. E. Bell, M. Spencer, A. King, S. Al-Sarraj, J. W. Ironside, P. L. Lantos, and J. Collinge.** 1999. Investigation of variant Creutzfeldt-Jakob disease and other human prion diseases with tonsil biopsy samples. Lancet 353:183–189.
12. **Kascsak, R. J., R. Rubenstein, P. A. Merz, M. Tonna-DeMasi, R. Fersko, R. I. Carp, H. M. Wisniewski, and H. Diringer.** 1987. Mouse polyclonal and monoclonal antibody to scrapie-associated fibril proteins. J. Virol. 61:3688–3693.
13. **Kong, Q., S. Huang, W. Zou, D. Vanegas, M. Wang, D. Wu, J. Yuan, H. Bai, M. Zheng, H. Deng, K. Chen, A. L. Jenny, K. O'Rourke, E. D. Belay, L. B. Schonberger, R. B. Petersen, M. S. Sy, S. G. Chen, and P. Gambetti.** 2005. Chronic wasting disease of elk: transmissibility to humans examined by transgenic mouse models. J. Neurosci. 25:7944–7949.
14. **Korth, C., B. Stierli, P. Streit, M. Moser, O. Schaller, R. Fischer, W. Schulz-Schaeffer, H. Kretzschmar, A. Raeber, U. Braun, F. Ehrensperger, S. Hornemann, R. Glockshuber, R. Riek, M. Billeter, K. Wüthrich, and B. Oesch.** 1997. Prion (PrPSc)-specific epitope defined by a monoclonal antibody. Nature 390:74–77.
15. **Parchi, P., A. Giese, S. Capellari, P. Brown, W. Schulz-Schaeffer, O. Windl, I. Zerr, H. Budka, N. Kopp, P. Piccardo, S. Poser, A. Rojiani, N. Streichemberger, J. Julien, C. Vital, B. Ghetti, P. Gambetti, and H. Kretzschmar.** 1999. Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. Ann. Neurol. 46:224–233.
16. **Parchi, P., W. Zou, W. Wang, P. Brown, S. Capellari, B. Ghetti, N. Kopp, W. J. Schulz-Schaeffer, H. A. Kretzschmar, M. W. Head, J. W. Ironside, P. Gambetti, and S. G. Chen.** 2000. Genetic influence on the structural variations of the abnormal prion protein. Proc. Natl. Acad. Sci. USA 97:10168–10172.
17. **Prusiner, S. B., M. Scott, D. Foster, K. M. Pan, D. Groth, C. Mirenda, M. Torchia, S. L. Yang, D. Serban, G. A. Carlson, P. C. Hoppe, D. Westaway, and S. J. DeArmond.** 1990. Transgenetic studies implicate interactions between homologous PrP isoforms in scrapie prion replication. Cell 63:673–686.
18. **Safar, J., H. Wille, V. Itri, D. Groth, H. Serban, M. Torchia, F. E. Cohen, and S. B. Prusiner.** 1998. Eight prion strains have PrP(Sc) molecules with different conformations. Nat. Med. 4:1157–1165.
19. **Seitz, R., F. von Auer, J. Blumel, R. Burger, A. Buschmann, K. Dietz, M. Heiden, W. E. Hitzler, H. Klamm, T. Kreil, H. Kretzschmar, M. Nübling, R. Offergeld, G. Pauli, V. Schottstedt, P. Volkers, and I. Zerr.** 2007. Impact of vCJD on blood supply. Biologicals 35:79–97.
20. **Taraboulos, A., K. Jendroska, D. Serban, S. L. Yang, S. J. DeArmond, and S. B. Prusiner.** 1992. Regional mapping of prion proteins in brain. Proc. Natl. Acad. Sci. USA 89:7620–7624.
21. **Telling, G. C., M. Scott, J. Mastrianni, R. Gabizon, M. Torchia, F. E. Cohen, S. J. DeArmond, and S. B. Prusiner.** 1995. Prion propagation in mice expressing human and chimeric PrP transgenes implicates the interaction of cellular PrP with another protein. Cell 83:79–90.
22. **Tremblay, P., Z. Meiner, M. Galou, C. Heinrich, C. Petromilli, T. Lisse, J. Cayetano, M. Torchia, W. Mobley, H. Bujard, S. J. DeArmond, and S. B. Prusiner.** *1998. Doxycyline control of prion protein transgene expression* modulates prion disease in mice. Proc. Natl. Acad. Sci. USA 95:12580–12585.
23. **Wadsworth, J. D., E. A. Asante, M. Desbruslais, J. M. Linehan, S. Joiner, I. Gowland, J. Welch, L. Stone, S. E. Lloyd, A. F. Hill, S. Brandner, and J. Collinge.** 2004. Human prion protein with valine 129 prevents expression of variant CJD phenotype. Science 306:1793–1796.
24. **Ward, H. J. T., D. Everington, S. N. Cousens, B. Smith-Bathgate, M. Leitch, S. Cooper, C. Heath, R. S. G. Knight, P. G. Smith, and R. G. Will.** 2006. Risk factors for variant Creutzfeldt-Jakob disease: a case-control study. Ann. Neurol. 59:111–120.
25. **Zanusso, G., D. Liu, S. Ferrari, L. Hegyi, X. Yin, A. Aguzzi, S. Hornemann, S. Liemann, R. Glockshuber, J. C. Manson, P. Brown, R. B. Petersen, P. Gambetti, and M. S. Sy.** *1998. Prion protein expression in different species:* analysis with a panel of new MAbs. Proc. Natl. Acad. Sci. USA 95:8812–8816.

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研究報告の概要

○スクレイピー感染ハムスター血漿中のPrP^{res}様タンパクの糖鎖依存性凝集検出による伝達性海綿状脳症血液検査の可能性

感染組織に多量のPrP^{res}を含有することが知られている感染動物モデルにおいても、血中のPrP^{res}は(白血球を除き)めったに検出されない。血中PrP^{res}の検出が困難なのは、血中感染価が低いことを反映していると見られる。ここでは、新規酸性SDS沈殿法と高感度化学発光法を組み合わせて、プロテイナーゼK耐性3F4反応性タンパクが、スクレイピー感染ハムスターの血漿中からは検出されるが、疑似感染ハムスターでは検出されないことを示す。高感度化学発光法では、$1.4x10^{-9}$gの脳ホモジネート、及び$1.5x10^{-12}$g ($6.5x10^{-17}$mol)のrPrPを従来型のウエスタンブロットで検出した。スクレイピー感染ハムスターの血漿中の3F4反応性タンパクは複数の分子量からなるタンパクバンドとなり、二糖鎖PrP分子のバンドよりも高い位置に検出された。スクレイピー感染ハムスター脳ホモジネートと疑似感染ハムスター血漿を混合することにより、3F4反応性タンパクと類似する分子量の位置にバンドが形成された。混合前に、血漿または脳ホモジネート中のタンパクから予め糖鎖を除去することにより、上記の複数の3F4反応性タンパクは検出できなくなった。これらの結果から、血漿中においてPrP^{res}は他の血漿タンパクと糖鎖を通じて凝集しており、スクレイピー感染ハムスター血漿において検出可能となったことが示唆される。スクレイピー感染ハムスターの血漿中でPrP^{res}様タンパクと凝集している相手の血漿タンパクが何であるかはまだ不明であるが、それはプロテイナーゼKに抵抗性を持っていると思われる。

報告企業の意見	今後の対応
新規酸性SDS沈殿法と高感度化学発光法を組み合わせて、スクレイピー感染ハムスターの血漿中からプロテイナーゼK耐性3F4反応性タンパクを検出したとの報告である。	今後も引き続き、検査法の研究を進めるとともに、プリオン病に関する新たな知見及び情報の収集に努める。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
新鮮凍結血漿-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

Microbiol. Immunol., 51(12), 1221–1231, 2007

Editor-Communicated Paper

A Potential Blood Test for Transmissible Spongiform Encephalopathies by Detecting Carbohydrate-Dependent Aggregates of PrPres-Like Proteins in Scrapie-Infected Hamster Plasma

Kazuo Tsukui*[,1], Masuhiro Takata[2], and Kenji Tadokoro[1]

[1]*Central Blood Institute, The Japanese Red Cross Society, Koto-ku, Tokyo 135–8521, Japan, and* [2]*Research Center for Prion Diseases, National Institute of Animal Health, Japan, Tsukuba-shi, Ibaraki 305–0856, Japan*

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Abstract: **PrPres has rarely been detected in blood (except in leukocytes) even in diseased animal models that are known to contain a large amount of PrPres in infected tissues. It seems likely that PrPres detection in blood is difficult because of the low titer of infectious material within the blood. Here, we demonstrate the detection of proteinase K-resistant 3F4-reactive protein in the plasma of scrapie-infected hamsters but not in the plasma of mock-infected hamsters by partial purification using a novel method termed "acidic SDS precipitation," in conjunction with a highly sensitive chemiluminescence detection system used to show the presence of PrP at a concentration equivalent to 1.4×10^{-9} g of brain homogenate or 1.5×10^{-12} g (6.5×10^{-17} mol) of rPrP by conventional Western blotting. The 3F4-reactive proteins in scrapie-infected hamster plasma often resulted in multiple Mw protein bands occurring at higher Mw positions than the position of the di-glycosyl PrP molecule. Mixing scrapie-infected hamster brain homogenate with mock-infected hamster plasma resulted in the formation of similar Mw positions for multiple 3F4-reactive proteins. Predigestion of carbohydrate side chains from the proteins in the plasma or brain homogenate before mixing resulted in failure to obtain these multiple 3F4-reactive proteins. These observations indicate that PrPres aggregated with other proteins in the plasma through carbohydrate side chains and was successfully detected in the plasma of scrapie-infected hamsters. Counterparts in these aggregates with PrPres-like proteins in scHaPl are not known but any that exist should resist the PK digestion.**

Key words: **PrPres-like protein, Carbohydrate, Scrapie infection, Discrimination**

Transmissible spongiform encephalopathy (TSE) is a fatal infectious neurodegenerative disease. It is characterized pathologically by spongy deterioration of the central nervous system (CNS) and by the deposition of amyloid plaques composed of an abnormal isoform of the prion protein (PrP^{sc}) in infected tissues (1, 2, 19). An important biochemical property of PrP^{sc} is its partial resistance to protease digestion, which results in the formation of a β-sheet-rich isoform. This molecule has therefore also been called PrPres, and it has been considered a disease-specific entity associated with TSE (1, 2, 9). Although the vCJD epidemic in the U.K. is declining, expansion of the disease throughout continental Europe and in many other countries has raised concern all over the world (9, 10, 28). After the appearance of three cases of transfusion-related vCJD infection

**Address correspondence to* Dr. Kazuo Tsukui, 2–1–67 Tatsumi, Koto-ku, Tokyo 135–8521, Japan. Fax: +81–3–5534–7588. E-mail: k-tsukui@bs.jrc.or.jp

Abbreviations: 2× acidic saline, 0.02 M acetic acid containing 0.15 M NaCl and 10 mM EDTA-2Na; Brh, brain homogenates; CNS, central nervous system; KLH, keyhole limpet hemocyanin; mAb, monoclonal antibody; mc, mock-infected; Pl, plasma; PMCA, protein misfolding cyclic amplification; PrP^{c}, normal prion protein; PrPres, proteinase K-resistant prion protein; PrP^{sc}, disease-associated prion protein; PTA, phosphotungstic acid; PVDF, polyvinylidene fluoride; RES, reticuloendothelial system; rPrP, recombinant hamster PrP(25-233); SB, super block; sc, scrapie-infected; TBST, Tris Buffered Saline containing 0.05% Tw20; TSE, transmissible spongiform encephalopathy.

in the U.K., transfusion-related iatrogenic expansion of vCJD between the asymptomatically infected donor and blood recipients has caused growing concern (13, 21, 26). In addition, the U.K. government has recently reported a fourth case of vCJD associated with a blood transfusion (HPA Press statement; 18 Jan. 2007, abbreviated in 13). For this reason, the aim of research into developing an antemortem test has changed from detecting infected persons in an endemic area to estimating the population size of infected persons within a more global area in order to prevent the iatrogenic infection by tainted blood (4, 8, 28). The most useful tissues for the diagnostic confirmation of vCJD in humans are CNS and reticulo-endothelial system (RES) tissue as well as the tonsils and appendix (14–16). The tonsils and appendix have been used successfully for the histopathological detection of PrPres in epidemiological studies of vCJD infection in the U.K.: an extremely high frequency of infection was detected compared to the frequency of so-called classical CJD (16, 17, 22). However, it is difficult to sample the tonsils and the appendix in living subjects. Therefore, the pathological diagnosis of TSE is currently made principally on the basis of postmortem preparations of CNS tissues, highlighting the need for the development of a more rapid diagnostic method using body fluids, especially blood (6, 27). For this purpose, several methods have been proposed and examined for prophylactic use (23, 30, 32). However, none of these methods has proved to be sufficient for the purposes (5, 7, 20, 29). To achieve this goal, several problems must be solved; solutions include using preparations with minimally invasive sampling techniques and establishing an effective and specific method for detecting the disease marker with sufficient sensitivity (5). The first problem can be solved by using body fluids such as blood or urine as test specimens. Therefore, the key is to develop a system with sufficient sensitivity to detect PrPres in blood or urine (5, 6, 20, 22, 29). The presence of PrPres in the urine of TSE-infected animals and humans has been reported previously (33). However, it has been suggested that this $uPrP^{sc}$ may be contaminated bacterial components in infected animal urine and not a marker of TSE (12). Blood has not been considered a highly infective source of classical CJD. The same was true for vCJD until the first victim of vCJD resulting from a blood transfusion was reported (21). This report was followed by reports of three more cases of possible transfusion-related transmission of vCJD (18, HPA Press statement; 18, Jan. 2007, 13). The development of a testing method using blood has therefore become a major goal of TSE research.

Here we show the successful detection (using a highly sensitive chemiluminescence immunoblotting system) of a PrPres-like protein molecule in plasma collected from scrapie-infected (sc) but not from mock-infected (mc) hamsters. Although the infectivity of this molecule has not been tested, and the immunoblot pattern of the anti-PrP reactive protein in plasma (scHaPl) was somewhat different from that of the brain homogenate (scHaBrh) in scrapie-infected hamsters, the specific reactivity of these proteins to anti-PrP mAb, the demonstration of carbohydrate side chain-mediated association between PrPres and plasma proteins, and the removal of the carbohydrate chain resulted in the appearance of similar Mw proteins in scBrh and scPl firmly support the conjecture that the extra Mw proteins observed in the trial were the aggregates of PrPres and some plasma proteins.

Materials and Methods

Enzymes, monoclonal antibodies (mAb) and recombinant hamster PrP peptide. Proteinase K (PK: 40.0 mAnson units/mg protein) was purchased from Merck Co. (Rahway, N.J., U.S.A.). Peptide *N*-glycosidase F (PNGaseF, 25,000 units/mg protein) was purchased from Roche Diagnostics Co., Ltd.

The anti-PrP mAbs 3F4 (Signet, Mass., U.S.A.) and 6H4 (Prionics AG, Zürich, Switzerland) were stored in aliquots at −80 C until use. mAb 5C8-113 was prepared by immunizing PrP knockout mice with bovine recombinant PrP (Prionics AG); screening was conducted using the same molecule. TA180 and TA181 were provided by Medical Biological Laboratory (MBL) and were prepared by immunizing conventional Balb/c mice with synthetic peptides of the hamster PrP sequence CERYYRE or CAVVGGLGGYML conjugated with keyhole limpet hemocyanin (KLH), respectively, then screened by the same peptides without KLH and conjugated with an ELISA plate. The epitope sites of the mAbs were 150–152 and 163–165 for TA180, and 129–131 for TA181. The epitope site of 5C8-113 has not yet been determined but is possibly an unknown conformation-dependent site. Anti-HIV P24 mAb (7A8.1; CHEMICON) was kindly donated by Dr. Iwakura of the Institute of Medical Sciences, Tokyo University and was used as a negative control for anti-PrP mAb reactions. Hamster recombinant PrP(25-233) (abbreviated rPrP hereafter) was purchased from Alicon AG (Switzerland).

Material from scrapie-infected and mock-infected hamsters. Twelve Syrian golden hamsters were inoculated with scrapie (Sc237)-infected hamster brain homogenate intra-cerebrally. Six hamsters were similarly inoculated with uninfected normal hamster brain

homogenate and were used as mock-infected hamsters. Hamsters from the two groups were anesthetized with ether at the terminal stage of disease among animals in the scrapie-infected group (approximately 50–70 days after inoculation) and after the same time interval among animals in the mock-infected group. Blood was collected from the animals with ACD containing 10 mM EDTA as an anticoagulant. Blood samples from scrapie-infected and mock-infected hamsters was centrifuged at low speed and the plasma fractions were collected (scPl and mcPl, respectively). Both scPl and mcPl were processed similarly thereafter. Brains were removed from the terminal-stage infected hamsters or the mock-infected hamsters and homogenized in TBS containing 0.5% NP40, 0.5% DOC and a protease inhibitor cocktail (Sigma) using a closed system homogenizer. These brain homogenates were then adjusted to a concentration of 10% with the above-mentioned buffer ($scBrh^{crude}$ or $mcBrh^{crude}$, respectively). $scBrh^{crude}$ or $mcBrh^{crude}$ were centrifuged at low speed to remove insoluble materials, and the supernatant fractions (scBrh or mcBrh) were processed as described below.

Enzyme treatment. The plasma or brain homogenates were diluted 4-fold with TBS containing 10 mM EDTA and digested with PK (50 μg/ml) at 37 C for 60 min. These reactions were stopped by adding 1 mM Pefablock. The digestion step was omitted in a set of controls. The samples were then treated with 3% SDS and 50 mM DTT in TBS before being inactivated at 100 C for 10 min and stored at −80 C in small aliquots.

Acidic SDS precipitation. Stored preparations were inoculated with equal volumes of 0.02 M acetic acid containing 0.15 M NaCl and 10 mM EDTA-2Na (2× acidic saline) at 10 C, followed by centrifugation at 15,000 rpm for 10 min. The resulting precipitates were dispersed in Tris Buffered Saline (TBS) with 5 mM EDTA and inoculated with equal amounts of 2× acidic saline again. After further centrifugation, the resulting precipitates were rinsed with a 5-fold volume of methanol, then dissolved in Laemmli's SDS sample buffer and analyzed thereafter.

Immunoblot detection of PrP-like proteins. SDS-PAGE was carried out on a 15% gel using Laemmli's conventional buffer system. The electrophoresed proteins were transferred onto polyvinylidene fluoride (PVDF) membranes using a semidry system. After Western blotting, the membranes were blocked with Super Block (SB; PIERCE, Rockford, Ill., U.S.A.) for 1 hr at room temperature then overnight at 4 C. The blocked membranes were first washed three times with TBS containing 0.05% Tween 20 (TBST), then incubated with an anti-PrP monoclonal antibody (mAb; 3F4, 6H4 or similar), in SB containing 10% Block Ace (Dainippon Pharmaceutical Co., Ltd.) and 0.01% BSA for 1 hr at room temperature then overnight at 4 C thereafter. For maximum detection of protein signals, the blotted membranes were incubated overnight at 4 C. After incubation, the membranes were washed five times with TBST, incubated with horseradish peroxidase-conjugated goat anti-mouse IgG (HRPGAM) in TBST cotaining 10% Block Ace and 0.1% BSA for 1 hr as a secondary antibody, washed five more times with TBST and incubated with a chemiluminescence substrate (Super Signal West Femto Maximum Sensitivity Substrates: SSWF; PIERCE). To obtain optimum chemiluminescence signals, HRPGAM was used at a concentration of 2 ng/ml according to the manufacturers instruction manual and chemiluminescence signals for antibody-reactive bands were detected using an LAS3000 image analyzer (Fuji Film, Tokyo).

Results

Sensitivity of Detection Systems (Fig. 1)

To determine the limits of the detection system, rPrP, 10% homogenates of sc- or mcBrh (crude or low-speed centrifugation supernatant) and PK predigested sc- or mcBrh were used. They were abbreviated as sc- or $mcBrh^{crude}$, sc- or $mcBrh^{sup}$ and sc- or mc $BrhPK_{50}$, respectively. In these experiments, the amount of PrP was indicated as brain equivalent (panel A) or brain protein (panel B) to enable convenient comparison between the equivalence to brain amount and brain protein. Protein amounts in the brain were determined before PK digestion. In panel A, 1.5×10^{-12} g (6.5×10^{-17} mol) for rPrP and PrPres in 1.4×10^{-9} g brain equivalent were detected. About 1/3 (equivalent to 2.25×10^{-6} g brain protein) of the PrP molecule in scBrh, and none of those in mcBrh looked like the PK-resistant molecule (PrPres; panel B). PrPres in scBrh was shifted from 30–32 kDa and 27–28 kDa before PK treatment to 25 and 20 kDa positions after PK treatment, respectively. As the total amount of PrPres plus PrPc in scBrh looked 3-fold larger than the amount of PrP in mcBrh, synthesis of the PrP was enhanced by scrapie infection in hamsters.

Discrimination of Scrapie Infection from Mock Infection by Plasma

When scrapie-infected or mock-infected Brh and plasma were pretreated with PK and subjected to immunoblot analysis, sc and mcBrh were easily discriminated by the PK treatment but sc and mcPl were not discriminated by the enzyme treatment (panel A). In mcPl, similar 3F4-reactive proteins were also

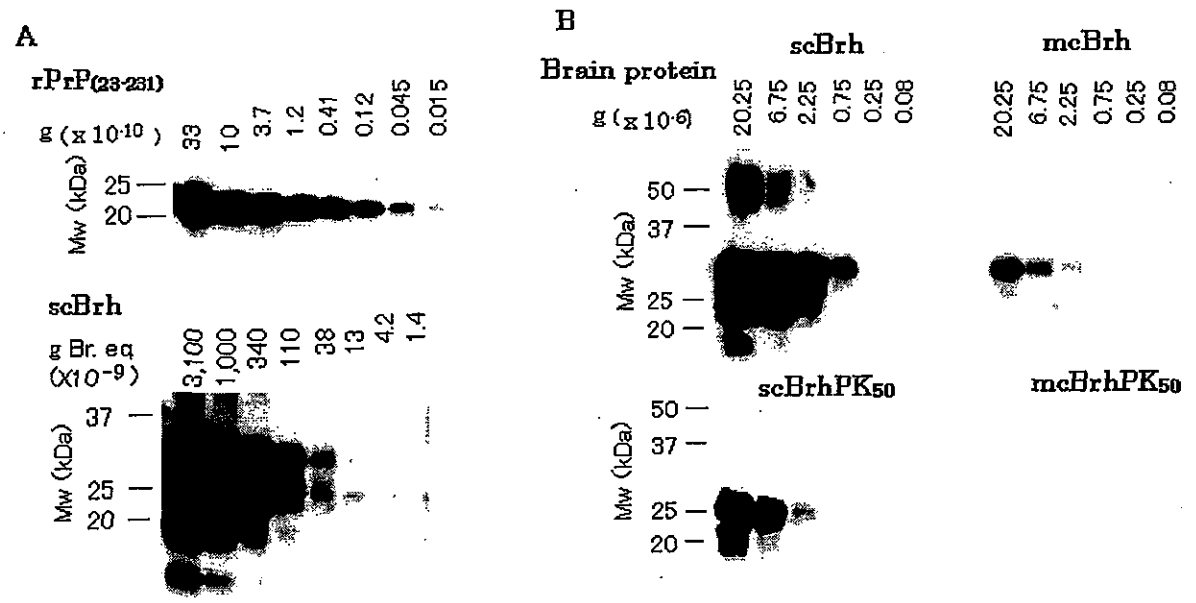

Fig. 1. Sensitivity and specificity determinations of immunoblot systems. Panel A: Sensitivity of used chemiluminescence immunoblots was determined using rPrP(25-233) (upper) or scBrh^{crude} (lower). These were diluted in the serial threefold manner as described in "Materials and Methods." Thereafter, each diluted preparation was subjected to chemiluminescence immunoblot detection. The amounts of each preparation used per lane are indicated in the figure as g ($\times 10^{-10}$) for rPrP(23-231) or g Br. eq. ($\times 10^{-9}$) for scBrh. Panel B: Specific detection of PrP in scBrh and mcBrh by chemiluminescence immunoblotting was indicated. scBrh or mcBrh (upper) or their PK-treated preparations (lower) were processed to serial threefold dilution series and subjected to chemiluminescence immunoblotting. PrP in each preparation was indicated per brain protein. Protein bands in scBrh showed the mixture of PrPres and PrPc. 3F4 and HRPGAM were used as the primary and secondary antibodies, respectively.

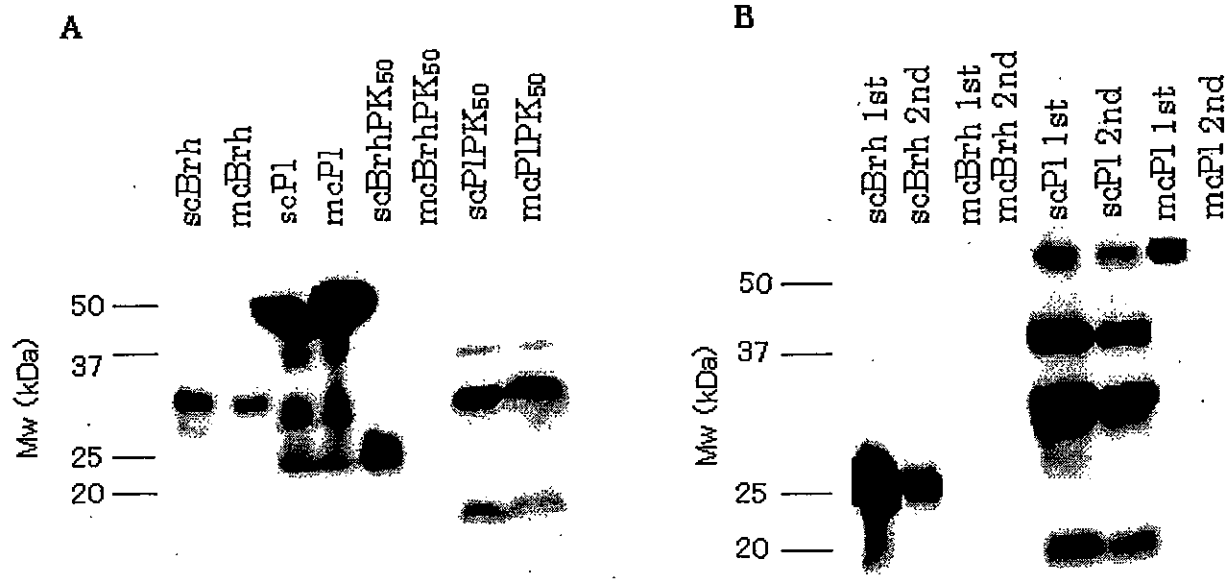

Fig. 2. Acidic SDS precipitation to discriminate scrapie infection and mock infection by their plasma. Panel A: scBrh, mcBrh, scPl and mcPl were processed and subjected to or not subjected to PK treatment. A chemiluminescence immunoblot analysis of PrP-like proteins was then performed using 3F4 primary and HRPGAM secondary antibodies. The PK treatment discriminated scBrh and mcBrh but did not discriminate between scPl and mcPl. Panel B: PK-pretreated scBrh, mcBRh, scPl and mcPl were subjected to acidic SDS precipitation condition, then analyzed by chemiluminescence immunoblotting. Acidic SDS precipitation condition was repeated twice (indicated as 1st and 2nd in the panel). This acidic SDS precipitation clearly discriminated scPl and mcPl as well as scBrh and mcBrh. The high MW protein band observed in the 1st precipitated fraction of mcPl was diminished in the 2nd precipitation. 3F4 and HRPGAM were used as the primary and secondary antibodies, respectively.

observed as in scPl. This observation was extremely different between the brain homogenate and plasma. From this observation, the presence of PK-resistant PrP molecules (PrPres-like molecules) in both sc- and mcPl was suspected (Fig. 2, panel A). These PrPres-like molecules in both plasma types have multiple inconstant Mw in experiments. Two patterns were often observed in 3F4-dependent immunoblot analysis; one was a 32 kDa major band with a 20 kDa minor band as well as 18, 25, 37 kDa faint bands (panel A; lane 7, 8), and the other was 20, 32, 40 and greater than 50 kDa dense bands as well as 27 kDa faint bands (Fig. 2, panel B; lane 5, 6).

The ability of the test to discriminate between scPl and mcPl was examined by acidic SDS precipitation (Fig. 2, panel B). PK-treated scBrh and mcBrh as well as scPl and mcPl preparations were subjected to acidic SDS precipitation condition (acidic SDS ppt) and analyzed with our immunoblotting system. In this experiment, precipitation was performed twice to ensure maximum removal of SDS soluble proteins. By this procedure, scPl and mcPl were clearly discriminated in the first precipitation and the higher Mw band that remained after the first precipitation was removed almost completely by the second precipitation. Discrimination between scPl and mcPl by acidic SDS precipitation was further confirmed by an experiment using 12 scPl and 6 mcPl samples (Fig. 3), but precipitation was only carried out once in this experiment. As shown in this figure, all 12 scPl samples showed the 3F4-reactive proteins but 4 mcPl samples did not. The mcPl of No. 1 and No. 6 showed weak 3F4-reactive bands. These observations confirm that scPl and mcPl can be successfully discriminated using the acidic SDS precipitation but that precipitation should be repeated twice. Weak bands observed in the mcPl No. 1 and No. 6 were expected to disappear by performing one more acidic SDS precipitation procedure.

Effect of Deglycosylation

It is known that three Mw species of PrP, di-, mono- and none-glycosylated molecules, exist in the brain and deglycosylation of the molecules causes the three protein species to accumulate into a single Mw. So, in order to determine whether deglycosylation affects the formation of multiple Mw protein bands in sc or mcPl, PK-treated sc and mcPl were deglycosylated or further processed using the acidic SDS precipitation procedure then compared to similarly processed scBrh. As shown in Fig. 4, 20–27 kDa proteins in scBrh and 19–50 kDa multiple Mw proteins in scPl and mcPl were detected following PK treatment (step 1). With deglycosylation of scBrh by PNGase F treatment, large amounts of 18 kDa protein appeared as was expected. Deglycosylation of scPl and mcPl resulted in 18 kDa proteins appearing but multiple higher Mw protein bands remained (step 2). After acidic SDS precipitation of these PK digested and deglycosylated materials, the multiple higher Mw protein bands in scPl disappeared, whereas a small amount of discrete 18 kDa protein bands remained in scBrh and scPl. These protein bands were not detected following similar treatment of mcPl (step 3). A long period of exposure (10 min) was necessary to obtain the protein signals described from step 3 of the experiment because the PrP-like proteins were difficult to detect after the deglycosylation step of the

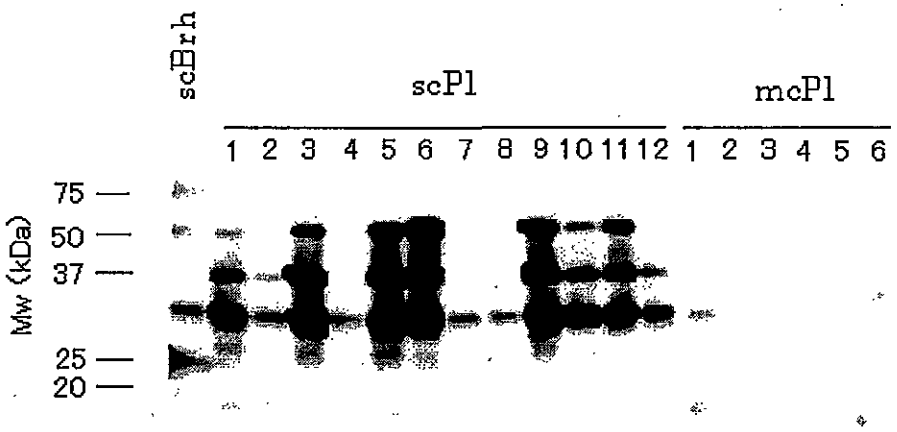

Fig. 3. Discrimination of PK-treated scPl and mcPl by acidic precipitation. Twelve preparations of scPl and 6 preparations of mcPl were pretreated with PK (50 μg/ml). scBrh was similarly treated before processing. After the pretreatment, the scPl and mcPl as well as scBrh were processed to the acidic SDS precipitation stage and analyzed by the normal immunoblot systems as described in "Materials and Methods." Anti-PrP mAb 3F4 and HRPGAM were used as the primary and secondary antibodies, respectively, for the immunoblot analysis.

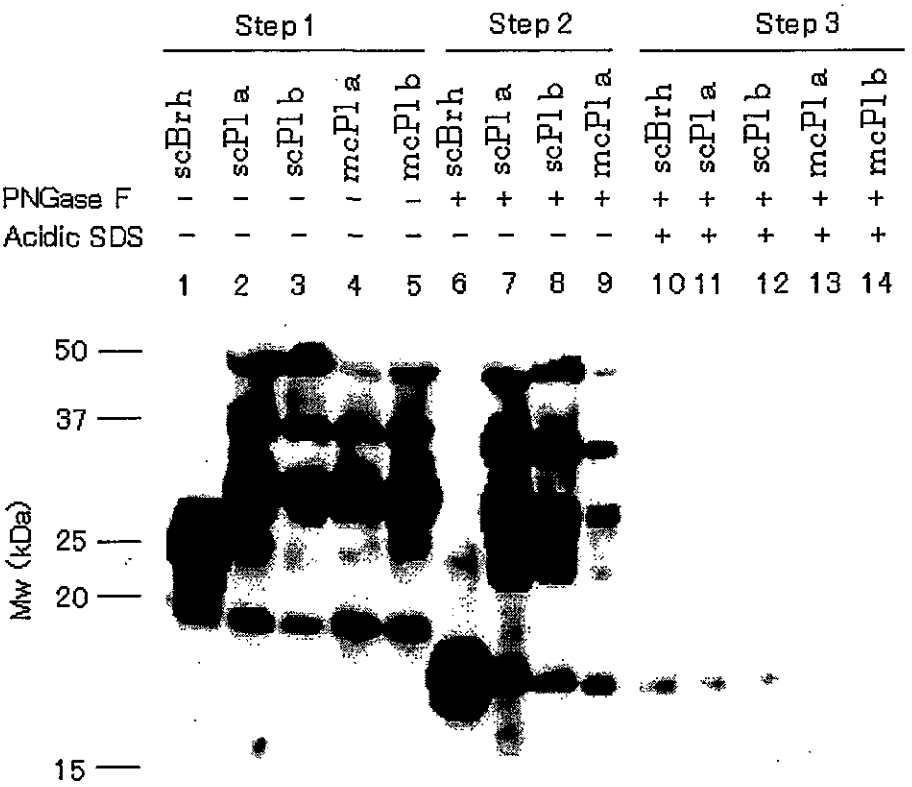

Fig. 4. Disappearance of extra Mw protein bands by digestion of carbohydrate side chains followed by acidic SDS precipitation. scBrh, two preparations of scPl and two preparations of mcPl were treated with PK (step 1, lanes 1–5). The PK-treated preparations were digested by PNGase F to remove the carbohydrate side chain on the protein molecules (step 2, lanes 6–9) then processed finally to the acidic SDS precipitation stage (step 3, lanes 10–14). Lanes were: Brain homogenate: 1, 6, 10; scPl: 2, 3, 7, 8, 11, 12; and mcPl: 4, 5, 9, 13, 14. The immunoblot pattern of each preparation during the three steps was determined. For the immunoblot analysis, 3F4 mAb and HRPGAM were used as the primary and secondary antibodies, respectively. Preparations treated with PNGase F or acidic SDS precipitation are indicated as (+) and untreated or unprocessed preparations are indicated as (−) in the figure. 3F4 and HRPGAM were used as the primary and secondary antibodies, respectively.

acidic SDS precipitation protocol. These observations suggested that carbohydrate side chains were involved in the formation of the multiple higher Mw protein bands (Fig. 4).

Appearance of Multiple Higher Mw Proteins by Mixing PK-Treated scBrh and PK-Treated or PK-Untreated mcPl

PK-treated Brh was mixed with PK-treated or PK-untreated mcPl then processed to the acidic SDS precipitation stage. These mixed preparations were compared with preparations of unmixed components in immunoblot analysis. Mixing of the PK-treated scBrh with PK-treated or PK-untreated mcPl resulted in the formation of higher Mw multiple protein bands as observed in scPl. Mixing with PK-treated mcPl seemed to show more discrete bands than mixing with PK-untreated mcPl. In PK-treated mcHaBrh, PK-treated or PK-untreated mcPl, these higher Mw protein bands were not observed. These immunoblot results suggested that the multiple Mw 3F4-reactive proteins were newly formed by the association between PrPres in scBrh and some PK-resistant plasma proteins in mcPl (Fig. 5).

Effect of Deglycosylation for the Association of PrPres in scBrh and PK-Resistant Protein in Plasma

As the deglycosylation of scPl resulted in failure to form the multiple higher Mw proteins but resulted in the appearance of a discrete 18 kDa band. As the Mw of which is similarly to the deglycosylated PrPres in scBrh, the possible involvement of saccharide chains was suspected for the formation of multiple extra Mw protein bands. To confirm this possibility, PK-pretreated scBrh and mcPl were deglycosylated by PNGase F or left untouched. After mixing the two preparations in the combination indicated in Fig. 6, acidic SDS precipitation was performed thereafter. As 3% SDS in the stored plasma or brain homogenates inhibits deglyco-

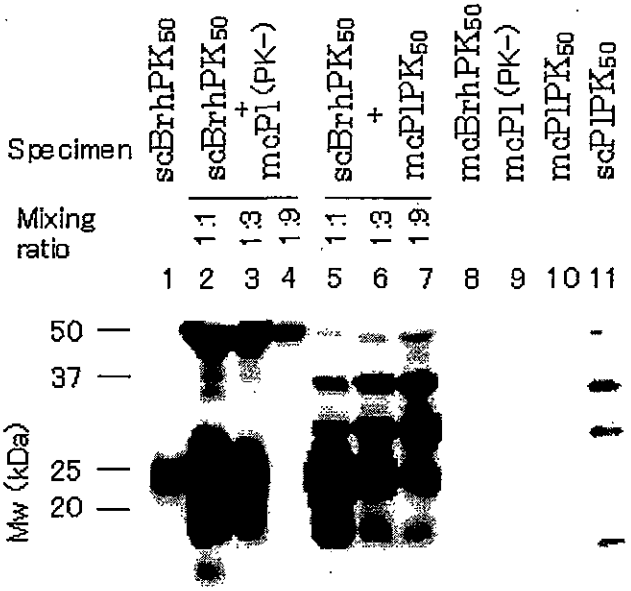

Fig. 5. Appearance of extra Mw proteins by mixing scBrh and mcPI after the acidic SDS precipitation. Proteinase K-pretreated scBrh was mixed with PK-treated or untreated mcPI and diluted to 1:3 or 1:9 in the presence of the PK-treated or untreated mcPI preparations. Then the mixed and unmixed preparations were processed to the acidic SDS precipitation stage. These processed preparations were compared by immunoblot analysis using 3F4 mAb and HRPGAM as the primary and secondary antibodies, respectively. Lanes: 1: PK treated scBrh; 2–4: PK-treated scBrh was mixed with an equal amount of PK-untreated mcPI (lane 2), diluted to 1:3 (lane 3), diluted to 1:9 (lane 4); 5–7: PK-treated scBrh was mixed with an equal amount of PK-treated mcPI (lane 5), diluted to 1:3 (lane 6), diluted to 1:9 (lane 7); 8: PK-treated mcBrh; 9: PK-untreated mcPI; 10: PK-treated mcPI; 11: PK-treated scPI. 3F4 and HRPGAM were used as the primary and secondary antibodies, respectively.

sylation reaction by PNGase F, the preparations for deglycosylation were diluted 30-fold before the reaction. After the deglycosylation, proteins in the reaction mixture of PNGase F treatment were precipitated by methanol and dissolved again to their original volumes with a primary buffer system that contained 3% SDS before mixing. Acidic SDS precipitation after the mixing of these deglycosylated preparations resulted in the appearance of an 18 kDa discrete band with a similar Mw to the deglycosylated PrPres in scBrh. Deglycosylation of brain proteins as well as of plasma proteins separately failed to form the higher Mw multiple protein bands. Mixing of PNGase F-treated scBrh and PNGase F-untreated mcPI formed a somewhat large amount of discrete 18 kDa proteins (lane 5).

Discussion

For antemortem diagnostic tests, body fluids such as blood or urine may be the most convenient specimens. The infectivity of blood in TSE-infected animals has already been determined to be 10–30 ID_{50}/ml (5). For this reason, the detection sensitivity of PrPres in blood is required to be in the order of ng/ml to test for TSE as has been mentioned elsewhere. Immunoblotting systems cannot detect such a low level of PrPres even in the blood of experimentally infected animals, so more sensitive methods to detect lower concentrations of PrP molecules need to be developed for antemortem diagnostic tests using blood or other body fluids. Various trials by several investigators have attempted to solve this extremely difficult problem (7, 19, 20, 30). In these studies, capillary electrophoresis analysis using a fluorescence-labeled synthetic PrP peptide, a combination of conformation-dependent PTA precipitation and ELISA, PCR of synthetic RNA conjugated with anti-PrP mAb and *in vitro* multiplication of abnormal PrP isoform (Protein Misfolding Cyclic Amplification; PMCA) have been suggested (3, 20, 29, 30). The PMCA method was shown to detect the presence of PrPres in scrapie-infected pre-mortem hamster blood using the buffy coat lysate (29). However, because these methods are complex and require a long time to obtain final results, their use in blood screening may be restricted. On the other hand, the common immunoblotting system used after PK treatment is excellent for detecting PrPres in the CNS or in other disease-affected tissues of infected animals. However, the usual immunoblot detection is less sensitive than the methods mentioned above. Therefore, a method that uses the common immunoblotting system would be the first choice for an antemortem test if its detection sensitivity could be enormously enhanced. It is suspected that the detection of PrPres molecules in blood is made more difficult by contamination from a large amount of protein, and so a method that will selectively concentrate the PrPres in blood to allow detection is therefore required. We tried to use the common immunoblotting systems in combination with a selective concentration method for PrPres-like protein aggregates and a highly sensitive chemiluminescence method. Using this combination, we successfully showed the presence of PrPres-like proteins in the scPI by means of reactivity to several anti-PrP mAbs, and by the similarity of Mw with the PrPres in infected hamster brains after deglycosylation. Moreover, carbohydrate may cause the PrPres-plasma protein aggregation and form the multiple Mw 3F4-reactive PrP-like proteins. PrP is a membrane protein and is known to aggregate frequently, especially after conversion to its disease-associated abnormal isoform. For this reason, detection of these aggregates is also the optimal way to develop an assay

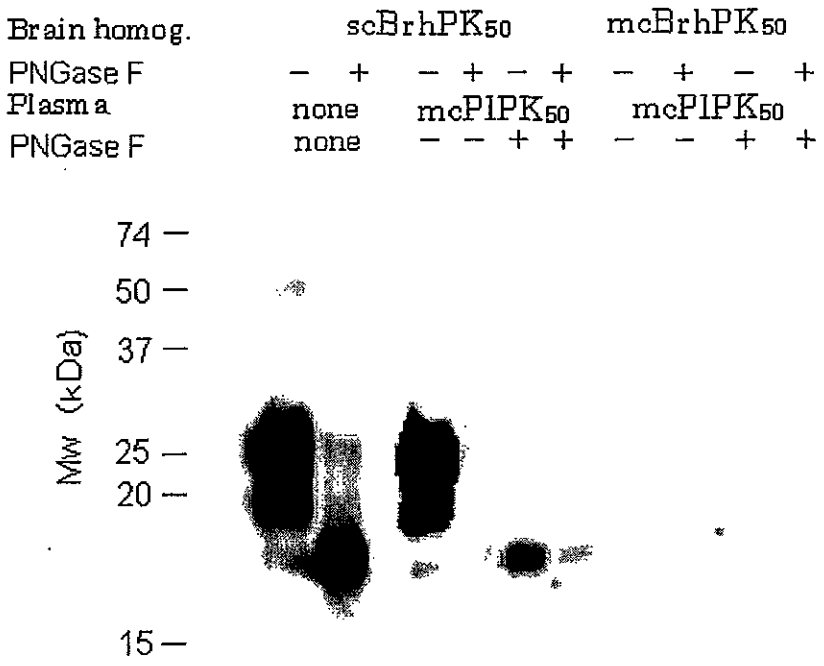

Fig. 6. Inability to form aggregate between scBrh and mcPl by digestion of carbohydrate before mixing. PK-treated scBrh, mcBrh and mcPl were further treated or not treated with PNGase F to digest the carbohydrate side chains on the proteins. These pretreated preparations were mixed with each other as indicated in the figure, and processed to the acidic SDS precipitation stage after mixing. Lanes: 1: scBrh (PNGase F−), 2: scBrh (PNGase F+); 3: scBrh (PNGase F−) mixed with mcPl (PNGase F−); 4: scBrh (PNGase F−) mixed with mcPl (PNGase F+); 5: scBrh (PNGase F+) mixed with mcPl (PNGase F−); 6: scBrh (PNGase F+) mixed with mcPl (PNGase F+); 7: mcBrh (PNGase F−) mixed with mcPl (PNGase F−); 8: mcBrh (PNGase F−) mixed with mcPl (PNGase F+); 9: mcBrh (PNGase F+) mixed with mcPl (PNGase F−); 10: mcBrh (PNGase F+) mixed with mcPl (PNGase F+), in which (PNGase F+) and (PNGase F−) mean digested or non-digested with PNGase F before mixing, respectively. 3F4 and HRPGAM were used as the primary and secondary antibodies, respectively.

method when using blood. However, previous tests for evaluating the sensitivity of detection systems using PrP molecules have frequently failed, presumably due to the tendency of the PrP molecule to form aggregates. We therefore evaluated the sensitivity of the detection system using SDS sample buffer which contained 0.1% BSA for the dilution buffer and by boiling the preparation throughout the serial dilution steps. This method allowed us to obtain a proper dispersion of the PrP aggregate in the test preparation and we successfully showed that the endpoint of the detection system was 1.5×10^{-12} g (6.05×10^{-17} mol) or more of rPrP and PrPres in 1.4×10^{-9} g brain equivalent of scHaBrh. As the scBrh has an infectivity titer of 10^{-7}–10^{-9} ID_{50}/ml, this chemiluminescence system can detect PrPres corresponding to 1 ID_{50}/ml or more, which is sufficiently greater than the value required to detect PrPres in blood (Fig. 1). We therefore decided to use this chemiluminescence system to detect PrPres in scPl. This system also allowed us to determine the detection limit of PrP protein in the brain (Fig. 1B).

Adding the acidic SDS precipitation stage to the protocol enabled successful discrimination of scPl and mcPl. The acidic SDS condition may selectively target aggregated PrP molecules, suggesting that PrP molecules in mcPl may not be aggregated. This observation is reasonable in that one of the main differences between PrPres and PrPc may be whether they exist in an aggregated form or not. Some investigators have tried to obtain PrPres in blood in an aggregated form (7). The aggregation of PrPres is thought to be a result of the more hydrophobic nature of the PrPres molecule than that of PrPc (24). However, the phenomenon observed here clearly suggests that an important factor for aggregation may be the presence of a carbohydrate side chain on both PrPres and plasma proteins rather than the hydrophobic nature of the PrPres. Carbohydrate has often been described as the outfitter for glycosylation and function (25).

The types of protein that aggregate with the PrPres-like molecules are not known. Some plasma proteins are known to associate with the PrP, but it is possible that the PrP molecules in hamster plasma may also be a candidate for these plasma proteins (11, 31, 34). PK-

resistant PrP molecules have recently been reported in uninfected human brains as well as in uninfected mouse and hamster brains and have been labeled a silent prion. PK-resistant protein in mcPl, which is able to aggregate with PrPres could be the silent prion in hamster plasma (34). Weakly observed 3F4-reactive protein bands in mcPl suggest the existence of the silent prion in plasma (Fig. 3).

In the lanes of scPl-2, -4, -7, -8, discrete bands in the Mw 32 kDa region were observed without other bands. As the band of this Mw region was weakly observed in mcPl-1 and scPl-6, it is somewhat difficult to decide the positive expression of 3F4-reactive protein for scPl-2, -4, -7 and -8. However, intensities of these signals in the scPl preparations were obviously strong compared to the signals in mcPl preparations. Thus it may be difficult to decide positive or not positive by performing acidic precipitation just one time. It is reasonable that an individual animal does not express the 3F4-reactive protein similarly in a time dependent manner and expression strength. Therefore, if blood testing is introduced, the plasma preparation should be processed twice with this acidic SDS precipitation, and the test should be conducted several times at different times.

Here we showed the successful discrimination of scrapie-infected and mock-infected hamsters by their plasma preparations using a novel combination method termed acidic SDS precipitation along with a highly sensitive chemiluminescence immunoblot system. In the immunoblots of PK-treated plasma preparations, multiple protein bands at Mw higher than the 25 kDa position were observed. These protein bands were observed in both scPl and mcPl after PK treatment. As these proteins were 3F4-reactive as well as PK-resistant, they were very likely to be PrPres molecules. However, observations showing multiple bands of higher than 25 kDa in Mw in mcPl as well as in scPl were very different from the electrophoresis pattern of scBrh. These differences between plasma and Brh have to be explained if the multiple PrPres-like proteins in plasma are aggregates of PrPres and some other plasma protein. This is similar with an observation in which the C-terminal domain of a recombinant mouse PrP peptide was aggregated spontaneously even in SDS sample buffer (24). Differences of electrophoresis patterns in Fig. 5, lanes 5–7 and Fig. 6, lane 3 or Fig. 2B, lane 5, 6 in spite of the same processing protocol may explain in which aggregation counterparts with PrPres in these plasma preparations may not be the same molecule, in preparation. After the PK treatment, an enormous amount of partial peptides was distributed in the broad Mw region if total protein was stained on WB membrane. This means that multiple partial peptides which possessing carbohydrate chains may have the potential to become the counterpart of these aggregates. We could not control the combination of the molecules. A deglycosylation experiment using both scPl and scBrh solved this question. After deglycosylation and acidic SDS precipitation, both scBrh and scPl showed a single discrete protein band at the 18 kDa Mw position. This observation strongly suggests that the carbohydrate side chain might be an important factor in the aggregation of the PrPres-like protein with some other proteins. From these observations, one of the components required to form aggregates must be the PrPres molecule but the other component need not be another PrPres molecule. That is, both self aggregation as well as aggregation of multiple hetero molecules could be resulted in the formation of the multiple Mw protein bands. Although dense bands at 25 kDa was observed in the scBrh and mcPl mixing (Fig. 5, lanes 5–7), the bands were obscure in scPl (Fig. 2, lanes 5, 6). This discrepancies between the preparations may conjectured by the differences of PrPres and plasma protein ratio. In Fig. 5, lanes 5–7, larger amount of scBrh compared to mcPl showed pattern more similar to that of scBrh, larger amount of mcPl showed more discrete band pattern after the PK treatment, in reverse. In this observation, 20 and 25 kDa protein bands were decreasing gradually along with mcPl was increasing. Therefore, it is conjectured that the 20 and 25 kDa proteins were not observed if less amounts of PrPres existed in scPl as observed in Fig. 2B, lane 5, 6.

PrPres was also found in uninfected human brains and labeled a silent prion (34). Similar molecules are likely to be present in non-infected hamsters and in mouse brains as well. If the silent prion in hamster and mouse brains is also exist in plasma, the PK-resistant 3F4-reactive proteins observed in mcPl in this experiment may be the candidate in hamster plasma. The silent prions in hamster plasma could aggregate with themselves or with other proteins to form the multiple higher Mw proteins in mcPl as well as scPl. But if the silent prion exists in mcPl, it must be discriminated through the blood tests. The acidic SDS precipitation process reported here may be useful for such trials.

So, as the PrPres molecules in hamster, 25 kDa, 20 kDa and 18 kDa proteins correspond to the di-, mono and no carbohydrate molecules, respectively. Multiple higher Mw protein bands were presumably aggregates with PrPres and other plasma proteins. The phenomenon that Mw of these aggregates were not found within a constant range indicated that counterparts of presumable PrPres might not be the specialized molecules in preparations; the silent prion may be included within these inconsistent molecules. Furthermore, we could

not control these combinations. The biological meaning of these aggregations is not known.

The observations from this experiment show that acidic SDS precipitation of plasma preparations enables discrimination between scrapie-infected and mock-infected hamsters and may be an extremely important finding for the developing of an antemortem blood test to diagnose TSE. The question as to why the silent prion is not precipitated by the acidic precipitation if it exists in mcPI remains to be answered.

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References

1) Agguzi, A., Montrasio, F., and Kaeser, P.S. 2001. Prions: health scares and biological challenge. Nat. Rev. Mol. Cell Biol. 2: 118–126.
2) Agguzi, A., and Polimenidou, M. 2004. Mammalian prion biology: one century of evolving concepts. Cell **116**: 313–327.
3) Bellon, A., Sayfert-Brandt, W., Lang, W., Baron, H., Gröner, A., and Vey, M. 2003. Improved conformation-dependent immunoassay: suitability for human prion detection with enhanced sensitivity. J. Gen. Virol. **84**: 1921–1925.
4) Beley, E.D., and Schonberger, L.B. 2005. The public health impact of prion diseases. Annu. Rev. Public Health **26**: 191–212.
5) Brown, P. 2005. Blood infectivity, processing and screening tests in transmissible spongiform encephalopathy. Vox Sang. **89**: 63–70.
6) Brown, P., Cerevenáková, L., and Diringer, H. 2001. Blood infectivity and the prospects for a diagnostic screening test in Creutzfeldt-Jakob disease. J. Lab. Clin. Med. **137**: 5–13.
7) Chang, B., Chen, X., Yin, S., Pan, T., Zhang, H., Wong, P., Kang, S.C., Yan, H., Li, C., Wolf, L., Miller, M.W., Wisniewski, T., Greene, M.I., and Sy, M.S. 2007. Test for detection of disease-associated prion aggregate in the blood of infected but asymptomatic animals. Clin. Vac. Immunol. **14**: 36–43.
8) Clerke, P., and Ghani, A.C. 2005. Projections of the future course of the primary vCJD epidemic in the UK: inclusion of subclinical infection and the possibility of wider genetic susceptibility. J. R. Soc. Interface **2**: 19–31.
9) Collins, S.J., Lawson, V.A., and Masters, C.L. 2004. Transmissible spongiform encephalopathies. Lancet **363**: 51–61.
10) Farrugia, A. 2006. Globalization and blood safety. ISBT Science Series **1**: 25–32.
11) Fischer, M.B., Roeckl, C., Patrizek, P., Schwarz, H.P., and Agguzi, A. 2000. Binding of disease-associated prion protein to plasminogen. Nature **408**: 479–483.
12) Furukawa, H., Doh-ura, K., Okuwaki, R., Shirabe, S., Yamamoto, K., Udono, H., Ito, T., Katamine, S., and Niwa, M. 2004. A pitfall in diagnosis of human prion diseases using detection of protease-resistant prion protein in urine. Contamination with bacterial outer membrane proteins. J. Biol. Chem. **279**: 23661–23667.
13) Hewitt, P.E., Llewlyn, C.A., MacKenzie, J., and Will, R.G. 2006. Creutzfeldt-Jakob disease and blood transfusion: results of the UK transfusion medicine epidemiological review study. Vox Sang. **91**: 221–230.
14) Hill, A.F., Butterworth, R.J., Joiner, S., Jackson, G., Rossor, M.N., Thomas, D.J., Frosh, A., Tolley, N., Bell, J.E., Spencer, M., King, A., Al-Sarraj, S., Ironside, J.W., Lantos, P.L., and Collinge, J. 1999. Investigation of variant Creutzfeldt-Jakob disease and other human prion diseases with tonsil biopsy samples. Lancet **353**: 183–189.
15) Hilton, D.A., Fathers, E., Edwards, P., Ironside, J.W., and Zajicek, J. 1998. Prion immunoreactivity in appendix before clinical onset of variant Creutzfeldt-Jakob disease. Lancet **352**: 703–704.
16) Hilton, D.A., Ghani, A.C., Conyers, L., Edwards, P., McCardle, L., Penny, M., Richie, D., and Ironside, J.W. 2002. Accumulation of prion protein in tonsil and appendix: review of tissue samples. Brit. Med. J. **325**: 633–634.
17) Hilton, D.A., Gahni, A.C., Conyers, L., Edwards, P., McCardle, L., Ritchie, D., Penny, M., Hegazy, D., and Ironside, J.W. 2004. Prevalence of lymphoreticular prion accumulation in UK tissue samples. J. Pathol. **203**: 732–739.
18) Ironside, J.W. 2006. Human prion diseases: biology and transmission by blood. ISBT Science Series **1**: 15–20.
19) Johnson, R.T., and Gibbs, C.J. 1994. Creutzfeldt-Jakob diseases and related transmissible spongiform encephalopathies. New Engl. J. Med. **339**: 1994–2004.
20) Laurenco, P.C., Scherr, M.J., MacGregor, I., Will, R.G., Ironside, J.W., and Head, M.W. 2006. Application of an immunocapillary electrophoresis assay to the detection of abnormal prion protein in brain, spleen and blood specimens from patients with variant Creutzfeldt-Jakob disease. J. Gen. Virol. **87**: 3119–3124.
21) Llewlyn, C.A., Hewitt, P.E., Knight, R.S.G., Amar, K., Cousens, S., MacKenzie, J., and Will, R.G. 2004. Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. Lancet **363**: 417–427.
22) MacGregor, I. 2001. Prion protein and developments in its detection. Transfusion Med. **11**: 3–14.
23) MacGregor, I., Hope, J., Barnard, G., Kirby, L., Drummond, O., Pepper, D., Hornsey, V., Barclay, R., Bessos, H., Turner, M., and Prowse, C. 1999. Application of a time-resolved fluoroimmunoassay for the analysis of normal prion protein in human blood and its components. Vox Sang. **77**: 88–96.

24) Martins, S.M., Frosoni, D.J., Martinez, A.M.B., DeFelice, F.G., and Ferreira, S.T. 2006. Formation of soluble oligomers and amyloid fibrils with physical properties of PrPsc from the C-terminal domain of recombinant murine prion protein, mPrP(121–231). J. Biol. Chem. **281**: 26121–26128.
25) Mitra, N., Siha, S., Ramya, T.N., and Surolia, A. 2006. N-linked oligosaccharides are outfitters for glycoprotein folding form and function. Trends Biochem. Sci. **31**: 156–163.
26) Peden, A.H., Head, M.W., Ritchie, D.L., Bell, J.E., and Ironside, J.W. 2004. Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. Lancet **363**: 417–427.
27) Prowse, C. 2006. Controlling the blood-born spread of human prion disease. ISBT Science Series **1**: 21–24.
28) Rickets, M.M. 2004. Public health and the BSE epidemic. Curr. Top. Microbiol. Immunol. **284**: 99–119.
29) Saa, P., Castilla, J., and Soto, C. 2006. Presymptomatic detection of prion proteins in blood. Science **313**: 92–94.
30) Saborio, G.P., Permanne, B., and Soto, C. 2001. Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. Nature **411**: 810–813.
31) Safar, J.G., Will, H., Geschwind, M.D., Deering, C., Latawiec, D., Serban, A., King, D.J., Legname, G., Weisgraber, K.H., Mahley, R.W., Miller, B., DeArmond, S.J., and Prusiner, S.B. 2006. Human prions and plasma lipoproteins. PNAS **103**: 11312–11317.
32) Schmerr, M.J., Jenny, A.L., Bulgin, M.S., Miller, J.M., Hamir, A.N., Cutlip, R.C., and Goodwin, K.R. 1999. Use of capillary electrophoresis and fluorescence labeled peptides to detect the abnormal prion protein in the blood of animals that are infected with a transmissible spongiform encephalopathy. J. Chromatogr. **A853**: 207–214.
33) Shaked, G.M., Shaked, Y., Kariv-Inbal, Z., Harimi, M., Avraham, I., and Gabizon, R. 2001. A protease resistant prion protein isoform is present in urine of animals and humans affected with prion diseases. J. Biol. Chem. **276**: 31479–31482.
34) Yuan, J., Xiano, X., McGeehan, J., Dong, Z., Cali, I., Fujioka, H., Kong, Q., Kneal, G., Gambetti, P., and Zou, W-Q. 2006. Insoluble aggregates and protease-resistant conformers of prion protein in uninfected human brains. J. Biol. Chem. **281**: 34846–34858.

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No. 24

医薬品 研究報告 調査報告書

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研究報告の概要

○プリオン病最新情報
[1]英国CJDサーベイランスユニット—月次統計と2007年の合計
月次CJD統計—2008年1月7日時点
以下の数字は英国CJDサーベイランスユニットに報告されたCJD疑い症例数及び確定・可能性例の死亡数である。
内訳は以下の通り: vCJD患者:vCJD確定例における死亡患者:114名。vCJD可能性例における死亡患者(神経病理学的に未確定):48名。vCJD可能性例における死亡患者(神経病理学的診断を保留):1名。死亡患者総数:163名。vCJD患者-存命中:3名。vCJD確定例または可能性例総数:166名。2007年12月の月例統計以来、新たにvCJDと診断された患者はないが、存命中の患者数は1名減少した。このデータは英国におけるvCJD流行は減少しつつあるとする見解に一致する。死亡患者数のピークは2000年の28名であり、その後2001年に20名、2002年に17名、2003年に18名、2004年に9名、2005年に5名、2006年に5名、2007年に5名と減少している。
2007年における全ての型のCJD症例の報告数は111名であった。死亡例は47名が孤発性CJD、2名が医原性CJD、4名が家族性CJD、1名がGSS、5名がvCJDだった。

報告企業の意見	今後の対応
2008年1月7日の時点で、英国CJDサーベイランスユニットに報告されたvCJD確定例または可能性例総数は166名、2007年中の死亡患者数は5名であり、英国におけるvCJD流行は減少しつつあるとする見解に一致するとの報告である。なお、2007年1月の同報告ではvCJD確定例または可能性例総数165名、死亡患者総数158名であったことから、2007年中のvCJD新規発症患者は1名、死亡患者は5名である。	日本赤十字社は、vCJDの血液を介する感染防止の目的から、献血時に過去の海外渡航歴(旅行及び居住)を確認し、欧州36ヶ国に一定期間滞在したドナーを無期限に献血延期としている。また、英国滞在歴を有するvCJD患者が国内で発生したことから、平成17年6月1日より1980～96年に1日以上の英国滞在歴のある方からの献血を制限している。今後もCJD等プリオン病に関する新たな知見及び情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-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

Back

Archive Number 20080107.0087
Published Date 07-JAN-2008
Subject PRO/AH/EDR> Prion disease update 2008 (02)

PRION DISEASE UPDATE 2008 (02)
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A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail is a program of the
International Society for Infectious Diseases
<http://www.isid.org>

[With the continuing decline of the number of cases of variant Creutzfeldt-Jacob disease (abbreviated previously as vCJD or CJD (new var.) in ProMED-mail) in the human population, it has been decided to broaden the scope of the occasional ProMED-mail reports to include other prion-related diseases. Data on vCJD cases from any part of the world are now included in these updates where appropriate, and other forms of CJD (sporadic, iatrogenic, familial, and GSS (Gerstmann-Straussler-Scheinker disease) are included also when they have some relevance to the incidence and etiology of vCJD. - Mod.CP]

In this update:
[1] UK: National CJD Surveillance Unit -- Monthly statistics & 2007 totals
[2] UK - New vCJD type
[3], [4], [5] vCJD in vitro assays

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[1] UK: National CJD Surveillance Unit -- Monthly statistics & 2007 totals
Date: Mon 7 Jan 2008
Source: UK National CJD Surveillance Unit, monthly statistics, 2007 [edited]
<http://www.cjd.ed.ac.uk/figures.htm>

Monthly Creutzfeldt-Jakob disease statistics -- as of 7 Jan 2008

These following figures show the number of suspect cases of CJD referred to the CJD surveillance unit in Edinburgh and the number of deaths of definite and probable variant Creutzfeldt-Jakob disease [abbreviated in ProMED-mail as CJD (new var.) or vCJD], the form of the disease thought to be linked to BSE (bovine spongiform encephalopathy).

Definite and probable vCJD cases in the UK as of 7 Jan 2008

Summary of vCJD cases -- deaths

Deaths from definite vCJD (confirmed): 114
Deaths from probable vCJD (without neuropathological confirmation): 48
Deaths from probable vCJD (neuropathological confirmation pending): 1
Number of deaths from definite or probable vCJD (as above): 163

Summary of vCJD cases -- alive

Number of probable vCJD cases still alive: 3

Total

Number of definite or probable vCJD (dead and alive): 166

These data indicate that there have been no new cases diagnosed during the past month, but the number of patients alive has decreased

by one.

These data are still consistent with the view that the vCJD outbreak in the UK is in decline (although the incidence curve may be developing a tail). 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, and 5 in 2007.

Totals for all types of CJD cases in the year 2007

As of 31 Dec 2007 in the UK in the year 2007, there were 111 referrals, 47 deaths from sporadic CJD, 2 deaths from iatrogenic CJD, 4 deaths from familial CJD, one from GSS, and 5 deaths from vCJD.

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ProMED-mail <promed@promedmail.org>

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[2] UK - New vCJD type
Date: Mon 7 Jan 2008
Source: Arch Neurol. 2007 Dec; 64(12):1780-4 [edited]
<http://archneur.ama-assn.org/cgi/content/abstract/64/12/1780>

[Prion disease update 2008 (01) contained brief press reports of the identification of a new form of vCJD in a young female patient, homozygote V/V at codon 129 of the PrPSc gene. The Abstract of the scientific paper describing this observation is reproduced below. - Mod.CP]

Creutzfeldt-Jakob disease, prion protein gene codon 129V/, and a novel PrPSc type in a young British woman
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By Mead S, Joiner S, Desbruslais M, Beck JA, O'Donoghue M, Lantos P, Wadsworth JD, Collinge J. MRC Prion Unit and Department of Neurodegenerative Disease, Institute of Neurology, University College London, National Hospital for Neurology and Neurosurgery, Queen Square, London, UK.

Background
Variant Creutzfeldt-Jakob disease (vCJD) is an acquired prion disease causally related to bovine spongiform encephalopathy that has occurred predominantly in young adults. All clinical cases studied have been methionine homozygotes at codon 129 of the prion protein gene (PRNP) with distinctive neuropathological findings and molecular strain type (PrPSc type 4). Modeling studies in transgenic mice suggest that other PRNP genotypes will also be susceptible to infection with bovine spongiform encephalopathy prions but may develop distinctive phenotypes.

Objective
To describe the histopathologic and molecular investigation in a young British woman with atypical sporadic CJD and valine homozygosity at PRNP codon 129.

Design
Case report, autopsy, and molecular analysis.

Setting
Specialist neurology referral center, together with the laboratory services of the MRC [Medical Research Council] Prion Unit.

Subject
Single hospitalized patient.

Main Outcome Measures
Autopsy findings and molecular investigation results.

Results
Autopsy findings were atypical of sporadic CJD, with marked gray and white matter degeneration and widespread prion protein (PrP) deposition. Lymphoreticular tissue was not available for analysis. Molecular analysis of PrPSc (the scrapie isoform of PrP) from cerebellar tissue demonstrated a novel PrPSc type similar to that

seen in vCJD (PrPSc type 4). However, this could be distinguished from the typical vCJD pattern by an altered protease cleavage site in the presence of the metal ion chelator EDTA.

Conclusions
Further studies will be required to characterize the prion strain seen in this patient and to investigate its etiologic relationship with bovine spongiform encephalopathy. This case illustrates the importance of molecular analysis of prion disease, including the use of EDTA to investigate the metal dependence of protease cleavage patterns of PrPSc.

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[The following 3 reports (3, 4, & 5), appearing during the past month (December 2007) describe new techniques for the in vitro assay of prions that promise to accelerate their characterization and epidemiology. - Mod.CP]

[3] vCJD in vitro assays
Date 11 Dec 2007
Source: PNAS, 26 Dec 2007, vol. 104, no. 52, 20908-20913 [edited]
<http://www.pnas.org/cgi/content/abstract/104/52/20908?etoc>

Prion strain discrimination in cell culture: The cell panel assay
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By Sukhvir P. Mahal*, Christopher A. Baker*, Cheryl A. Demczyk*, Emery W. Smith*, Christian Julius, and Charles Weissmann. At the Department of Infectology, Scripps Florida, 5353 Parkside Drive, Jupiter, FL 33458; and Institute of Neuropathology, University Hospital of Zurich, Schmelzbergstrasse 12, CH-8091 Zurich, Switzerland.

Abstract:
Prions are thought to consist mainly or entirely of misfolded PrP, a constitutively expressed host protein. Prions associated with the same PrP sequence may occur in the form of different strains; the strain phenotype is believed to be encoded by the conformation of the PrP. Some cell lines can be persistently infected by prions and, interestingly, show preference for certain strains. We report that a cloned murine neuroblastoma cell population, N2a-PK1, is highly heterogeneous in regard to its susceptibility to RML and 22L prions. Remarkably, sibling subclones may show very different relative susceptibilities to the 2 strains, indicating that the responses can vary independently. We have assembled 4 cell lines, N2a-PK1, N2a-R33, LD9 and CAD5, which show widely different responses to prion strains RML, 22L, 301C, and Me7, into a panel that allows their discrimination in vitro within 2 weeks using the standard scrapie cell assay (SSCA).

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[4] vCJD in vitro assays
Date: 20 Dec 2007
Source: Proc. Natl. Acad. Sci. USA, 10.1073/pnas.0710152105 [edited]
<http://www.pnas.org/cgi/content/abstract/0710152105v1?etoc>

Prion detection by an amyloid seeding assay

By David W. Colby, Qiang Zhang, Shuyi Wang, Darlene Groth, Giuseppe Legname, Detlev Riesner, and Stanley B. Prusiner. At the Institute for Neurodegenerative Diseases and Departments of Neurology and Biochemistry and Biophysics, University of California, San Francisco, CA 94143; and the Institut fur Physikalische Biologie, Heinrich-Heine Universitat, 40225 Dusseldorf, Germany.

Abstract:

Polymerization of recombinant prion protein (recPrP), which was produced in bacteria, into amyloid fibers was accompanied by the acquisition of prion infectivity. We report here that partially purified preparations of prions seed the polymerization of recPrP into amyloid as detected by a fluorescence shift in the dye Thioflavin T. Our amyloid seeding assay (ASA) detected PrPSc, the sole component of the prion, in brain samples from humans with sporadic Creutzfeldt-Jakob disease, as well as in rodents with experimental prion disease. The ASA detected a variety of prion strains passaged in both mice and hamsters. The sensitivity of the ASA varied with strain type; for hamster Sc237 prions, the limit of detection was approximately 1 fg. Some prion strains consist largely of protease-sensitive PrPSc (sPrPSc), and these strains were readily detected by ASA. Our studies show that the ASA provides an alternative methodology for detecting both sPrPSc and protease-resistant PrPSc that does not rely on protease digestion or immunodetection.

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[5] vCJD in vitro assays
Date: 9 Dec 2007
Source: Nature Neuroscience 11, 109 - 117 (2007) [edited]
<http://www.nature.com/neuro/journal/v11/n1/abs/nn2028.html>

A versatile prion replication assay in organotypic brain slices
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By Jeppe Falsig1, Christian Julius1, Ilan Margalith1, Petra Schwarz1, Frank L Heppner1,2 & Adriano Aguzzi. At the Institute of Neuropathology, University of Zurich, Schmelzbergstrasse 12, CH-8091 Zurich, Switzerland.

Abstract:
Methods enabling prion replication ex vivo are important for advancing prion studies. However, few such technologies exist, and many prion strains are not amenable to them. Here we describe a prion organotypic slice culture assay (POSCA) that allows prion amplification and titration ex vivo under conditions that closely resemble intracerebral infection. 35 days after contact with prions, mouse cerebellar slices had amplified the abnormal isoform of prion protein, PrPSc, >105-fold. This is quantitatively similar to amplification in vivo, but 5-fold faster. PrPSc accumulated predominantly in the molecular layer, as in infected mice. The POSCA detected replication of prion strains from disparate sources, including bovines and ovines, with variable detection efficiency. Pharmacogenetic ablation of microglia from POSCA slices led to a 15-fold increase in prion titers and PrPSc concentrations over those in microglia-containing slices, as well as an increase in susceptibility to infection. This suggests that the extensive microglial activation accompanying prion diseases represents an efficacious defensive reaction.

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[see also:
Prion disease update 2008 (01): correction 20080104.0046
Prion disease update 2008 (01) 20080102.0014
2007

Prion disease update 2007 (08) 20071205.3923
Prion disease update 2007 (07) 20071105.3602
Prion disease update 2007 (06) 20071003.3269
Prion disease update 2007 (05) 20070901.2879
Prion disease update 2007 (04) 20070806.2560
Prion disease update 2007 (03) 20070702.2112
Prion disease update 2007 (02) 20070604.1812
Prion disease update 2007 20070514.1542
CJD (new var.) update 2007 (05) 20070403.1130
CJD (new var.) update 2007 (04) 20070305.0780

CJD (new var.) update 2007 (03) 20070205.0455
CJD (new var.) update 2007 (02): South Korea, susp 20070115.0199
2006

CJD (new var.), blood transfusion risk 20061208.3468
CJD, transmission risk - Canada (ON) 20061207.3457
CJD (new var.) update 2006 (12) 20061205.3431
CJD (new var.) update 2006 (11) 20061106.3190
CJD (new var.) update 2006 (10) 20061002.2820
CJD (new var.) - Netherlands: 2nd case 20060623.1741
CJD (new var.) - UK: 3rd transfusion-related case 20060209.0432
CJD (new var.) update 2006 (02) 20060206.0386
CJD (new var.) update 2006 20060111.0101
2005

CJD (new var.) update 2005 (12) 20051209.3547
CJD (new var.) update 2005 (11) 20051108.3270
CJD (new var.) update 2005 (10) 20051006.2916
CJD (new var.) update 2005 (02) 20050211.0467
CJD (new var.) - UK: update 2005 (01) 20050111.0095
2004

CJD, genetic susceptibility 20041112.3064
CJD (new var.) - UK: update 2004 (14) 20041206.3242
CJD (new var.) - UK: update 2004 (10) 20040909.2518
CJD (new var.) - UK: update 2004 (02) 20040202.0400
CJD (new var.) - UK: update 2004 (01) 20040106.0064
CJD (new var.) - France: 8th case 20041022.2864
CJD (new var.) - France: 9th case 20041123.3138
CJD (new var.), blood supply - UK 20040318.0758
CJD (new var.), carrier frequency study - UK 20040521.1365
2003

CJD (new var.) - UK: update 2003 (13) 20031216.3072
CJD (new var.) - UK: update 2003 (01) 20030108.0057
2002

CJD (new var.) - UK: update Dec 2002 20021207.5997
CJD (new var.) - UK: update Jan 2002 20020111.3223
2001

CJD (new var.), incidence & trends - UK (02) 20011124.2875
CJD (new var.), incidence & trends - UK 20011115.2816
CJD (new var.) - UK: reassessment 20011029.2671
CJD (new var.) - UK: update Oct 2001 20011005.2419
CJD (new var.) - UK: regional variation (02) 20010907.2145
CJD (new var.) - UK: update Sep 2001 20010906.2134
CJD (new var.) - UK: update Aug 2001 20010808.1872
CJD (new var.) - UK: 9th Annual Report 20010628.1231
CJD (new var.) - UK: update June 2001 20010622.1188
CJD (new var.) - UK: update 3 Jan 2001 20010104.0025]
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識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	厚生労働省処理欄
				2008年3月24日	該当なし	

一般的名称	乾燥濃縮人アンチトロンビンⅢ	研究報告の公表状況	TRANSFUSION 2008; 48: 609-619	公表国 フランス
販売名（企業名）	①ノイアート（ベネシス） ②ノイアート静注用1500単位（ベネシス）			

研究報告の概要

＜背景＞輸血によるTSE感染のリスクはプリオンたん白の病理学的イソフォーム（PrP^{sc}）の血中濃度に依存するが、細胞PrP（PrP^{c}）の血中濃度によっても影響を受ける可能性がある。これらの濃度はPrPの血液クリアランスによって制御されるが、これについてはこれまで評価されたことはなかった。
＜研究デザイン及び方法＞ヒツジの精製された原核生物の組み換えPrP（rPrP）の血液（実際は血漿）クリアランスは、遺伝子型の異なるヒツジおよび腎摘出されたヒツジを用いて測定した。スクレイピー関連のフィブリルの静注後のプロテイナーゼK抵抗性PrP断片（PrP^{res}）への曝露についても、ヒツジで調査した。
＜結果＞rPrPのARR変異型は、VRQ変異型よりもより早く除去された。感受性の高いホモ接合体のVRQヒツジのPrP^{c}血漿濃度は、ホモ接合体のARR抵抗性ヒツジのそれよりも大きく、PrP^{c}のARR変異型のクリアランスがVRQ変異型のそれよりも大きいことを示唆している。rPrPの血漿クリアランスは、両方の腎臓摘出後は52%減少し、このことはrPrP除去において腎臓が重要な寄与をしていることを示している。PrP^{res}は、スクレイピー関連断片の静注後はゆっくりと除去されることが判明した。
＜結論＞PrP宿主の遺伝型及び生理病理学的要因は、血液のPrPクリアランスを調節することでTSE感染リスクに影響する。このリスクは、静注後にPrP^{res}へ曝露が続くことによって増大する。投与された物質は実際の種と異なるが、これらはPrPクリアランスのメカニズムを調査するためのプローブとして重要である。

使用上の注意記載状況・その他参考事項等

代表としてノイアート（献血）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。

報告企業の意見	今後の対応
ヒツジに静注したプリオンたん白の血液クリアランスは、宿主の遺伝子的及び生理病理学的要素に影響を受けるとの報告である。 これまで血漿分画製剤によってvCJD、スクレイピー及びCWDを含むプリオン病が伝播したとの報告はない。しかしながら、万一vCJD感染者の血漿が本剤の原料に混入した場合には、製造工程においてプリオンを低減し得るとの報告があるものの、製剤から伝播する可能性を完全には否定し得ない。そのため、弊社の血漿分画製剤の製造工程におけるTSE感染性低減に関する検証実験を加速し、自社データを早期に取得し、工程評価を行い、必要に応じて工程改善を実施する予定である。	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。

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Blood clearance of the prion protein introduced by intravenous route in sheep is influenced by host genetic and physiopathologic factors

Véronique Gayrard, Nicole Picard-Hagen, Catherine Viguié, Elisabeth Jeunesse, Guillaume Tabouret, Human Rezaei, and Pierre-Louis Toutain

BACKGROUND: The risk of transmissible spongiform encephalopathy (TSE) transmission by blood transfusion is dependent on the blood concentrations of the pathologic isoform of prion protein (PrPsc) but may also be influenced by blood concentrations of cellular PrP (PrPc). These concentrations are controlled by the blood clearance of PrP, which has never been evaluated.

STUDY DESIGN AND METHODS: The blood (actually plasma) clearance of ovine purified prokaryote recombinant PrP (rPrP) was measured in genotyped and in nephrectomized sheep. The exposure to proteinase K-resistant fragments of PrP (PrPres) after intravenous (IV) administration of scrapie-associated fibrils (SAFs) was also investigated in a sheep.

RESULTS: The ARR variant of rPrP was eliminated more rapidly than its VRQ counterpart. The PrPc plasma concentrations in homozygous highly susceptible VRQ sheep were greater than in homozygous ARR-resistant sheep, suggesting that clearance of the ARR variant of PrPc was higher than that of the VRQ variant. The plasma clearance of rPrP was decreased by 52 percent after a bilateral nephrectomy indicating the significant contribution of the kidneys in eliminating rPrP. PrPres was shown to be slowly eliminated after IV administration of scrapie-associated fibrils.

CONCLUSION: PrP host genotype and physiopathologic factors could influence the risk of TSE transmission by modulating blood PrP clearance. This risk was increased by the sustained exposure to PrPres after IV administration. It should be noted that although the materials that have been administered (rPrP and SAFs) were not the actual species of interest, they can be of value as probes for investigating PrP clearance mechanisms.

Transmissible spongiform encephalopathies (TSEs) are neurodegenerative diseases that occur in humans (e.g., Creutzfeldt-Jakob disease [CJD]) and animals including sheep (scrapie) and cattle (bovine spongiform encephalopathy [BSE]). These diseases are characterized by the cerebral deposition of the pathologic isoform (PrPsc) of a host-encoded cellular prion protein (PrPc) that is highly expressed in the brain.

Attempts to detect infectivity in the blood of animals naturally affected with TSE have often been inconclusive.[1,2] PrPsc or infectivity, however, has been evidenced in blood from intracerebrally inoculated rodents[3] and in

ABBREVIATIONS: MRT = mean residence time(s); Plgn = plasminogen; PrP = prion protein; PrPc = cellular PrP; PrPres = proteinase K-resistant fragment of PrP; PrPsc = pathologic isoform of PrP; rPrP = recombinant PrP; SAF(s) = scrapie-affected fibril(s); TSE(s) = transmissible spongiform encephalopathy(-ies).

From the Ecole Nationale Vétérinaire de Toulouse, UMR 181 Physiopathologie et Toxicologie Expérimentales, INRA, ENVT, UMR1225 Interactions Hôtes-Agents Pathogènes, INRA, ENVT, Toulouse; and Institut National de la Recherche Agronomique, UR892 Virologie et Immunologie Moléculaires, Jouy-en-Josas, France.

Address reprint requests to: Pierre-Louis Toutain, UMR 181 Physiopathologie et Toxicologie Expérimentales, INRA, ENVT, Ecole Nationale Vétérinaire de Toulouse, 23 chemin des Capelles, 31076 Toulouse, France; e-mail: pl.toutain@envt.fr.

This study was supported by grants from the French National Institute for Agronomical Research (INRA), from GIS prion, and DGER. The authors declare that there is no conflict of interest relative to the major sources of funding that would prejudice their impartiality relative to the results of the study.

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doi: 10.1111/j.1537-2995.2007.01628.x

TRANSFUSION 2008;48:609-619.

blood of sheep naturally[4] or experimentally infected with scrapie[5] or BSE.[4,6]

Evidence that variant CJD (vCJD) is transmitted by transfusion has accumulated, initially from animal models, including primates, in which the high efficiency of the intravenous (IV) route of infection for BSE transmission has been demonstrated.[7] There is no longer any doubt concerning the risk of blood products obtained from individuals during the presymptomatic phase of vCJD. A third case has been reported of autopsy-confirmed vCJD infection (and a second case of clinical vCJD) from a cohort of recipients who had received transfused blood products from individuals subsequently diagnosed with vCJD.[8] More recently, a fourth symptomatic case of vCJD infection has been identified in a patient who received a transfusion from the same donor as the third case.[9] This fourth incidence considerably strengthens the evidence for prion transmission by transfusion.[10]

The pathogenesis of TSE disease highlights the importance of the PrPc as a substrate for PrPsc replication in tissue. Hence, the risk of TSE transmission by blood transfusion could be affected by the level of blood PrPc. This is controlled by its own blood clearance and modified by factors affecting this variable. The transfusion risk will also depend on the ability of the body to clear the abnormal prion protein (PrPsc), that is, the plasma (blood) clearance. This clearance variable, essential to the estimation of systemic exposure, has never been evaluated.

The aim of this study was to document the exposure of sheep to the recombinant prion protein (rPrP) after IV administration and to identify genetic and pathophysiologic factors that might modulate such exposure. Sheep were chosen because of the similarity of the pathogenesis of scrapie with vCJD and because the impact of Prnp gene polymorphism on the susceptibility to scrapie infection is well documented. The $V_{136}R_{154}Q_{171}$ (VRQ) and ARR alleles have consistently been associated with high susceptibility and natural resistance to the clinical disease, respectively,[11-13] although atypical scrapie strain(s) can naturally infect sheep harboring the so-called resistant PrP genotype.[14] The ARR and VRQ genetic variants of the purified prokaryote rPrP were used as probes and only as probes (see Discussion) to test the hypothesis that the higher intrinsic stability of the VRQ variant relative to its ARR counterpart[15,16] could result in a lower in vivo clearance rate of the VRQ protein.

This approach also allowed us to examine the hypothesis that the subject's genetic background could directly influence PrP clearance and to evaluate the contribution of the kidneys to the overall clearance of plasma PrP, the kidney being a major organ for protein clearance.[17] The fate of ovine proteinase K-resistant fragment of PrP (PrPres) during the first hours after its direct entry in the blood was also evaluated in one sheep to assess the ability of the body to clear the scrapie agent.

MATERIALS AND METHODS

General

All experimental procedures were performed in accordance with French legal requirements regarding the protection of laboratory animals and under authorization number 31242 from the French Ministry of Agriculture.

Design

The objectives of Experiment 1 were 1) to compare the pharmacokinetics of the VRQ and ARR genetic variants of the purified prokaryote ovine rPrP; 2) to examine the influence of the genotype of the test animal on plasma pharmacokinetic variables of rPrP; and 3) to test the hypothesis of a first pass effect at the level of the brain, that is, direct trapping of the protein during its initial transit across the head.

The experiment was performed on eight healthy Romanov ewes: four homozygous VRQ at codons 136, 154, and 171 of the Prnp gene and four homozygous ARR. The VRQ ewes were 1 year old and weighed 42.1 ± 2.7 kg and the ARR ewes were 2 years old and weighed 51.2 ± 5.5 kg. The experiment involved two periods separated by 2 days. During the two periods, the ewes received an IV administration of rPrP (VRQ vs. ARR, 0.02 mg/kg) according to a crossover design. Ten days later, one ARR and two VRQ ewes received an intraarterial (external carotid) administration of the VRQ variant (0.02 mg/kg) to compare the pharmacokinetic variables of rPrP after arterial and IV administrations.

The ex vivo stability of the variants of rPrP in blood and plasma was compared by separately adding 145 ng of each of the ARR and VRQ variant of rPrP to 10-mL aliquots of fresh sheep blood and plasma that were incubated at 37°C under constant stirring. Samples were taken at 0.5, 1, 2, 3, 4, 5, 6, and 24 hours. The plasma was immediately separated from blood after centrifugation for 10 minutes at 1400 × *g* and all the plasma samples were stored at −20°C until PrP assay.

Experiment 2 was designed to evaluate the role of the kidneys in the clearance of rPrP and to examine the fate of PrPres after its direct entry into the blood. The first part of the experiment was performed with three Lacaune ewes aged from 5 to 8 years and weighing 38 to 55 kg. The pharmacokinetic variables of the VRQ variant of the rPrP intravenously administered (0.02 mg/kg) were determined before (control period) and immediately after a bilateral nephrectomy (experimental period). During the control period, one ewe was anesthetized according to the same protocol as that used for surgery. During the experimental period, which took place 1 to 14 days later, anesthesia was induced with sodium thiopental (Nesdonal, Merial, Lyon, France; 20 mg/kg) and maintained for 24 hours by repeated administrations of 0.2 to 0.3 mg per kg sodium

thiopental at 20- to 30-minute intervals. The ewes were bilaterally nephrectomized according to the method previously described.[18] Immediately after surgery, three control blood samples were obtained at 20-minute intervals and the VRQ variant was intravenously administered. The ewes were sacrificed in extremis. The second part of the experiment was performed with one young Lacaune ewe aged 6 months and weighing 30.5 kg that received an IV administration of 125 mL of scrapie-affected fibrils (SAFs) in 0.8 mol per L urea. The quantity of PrPres administered was evaluated at 75 µg equivalent of VRQ rPrP.

Ten-milliliter blood samples were collected at 20-minute intervals for 1 hour before administration; at 1, 2, 4, 8, 15, 30, 45, 60, 90, and 120 minutes after the variant administration; at 1-hour intervals for 12 hours; and finally at 24, 36, and 48 hours after administration, for Experiment 1 and the first part of Experiment 2, respectively. The same protocol was used for the second part of Experiment 2 except that 15-mL blood samples were also obtained every day until Day 7 after SAF administration.

For all experiments, in intact ewes, all the urine in the bladder was removed before the administrations and then at 1-hour intervals for 12 hours and at 3-hour intervals during the following 12 hours. The total volume of urine removed was measured in each case.

Brain extraction

Brain samples were obtained from three Romanov homozygous VRQ ewes naturally affected with scrapie. The frozen brain samples were homogenized to give a 20 percent (wt/vol) suspension in buffer (Bio-Rad, Marnes la Coquette, France) and PrPres was extracted with the purification protocol of the Bio-Rad TSE test (TeSeE sheep/goat purification kit, Bio-Rad) except that the final precipitate was solubilized with 4 mol per L urea (Sigma-Aldrich, Lisle d'Abeau Chesnes, France) and stored at –20°C until administration. The extract was diluted five-fold in 0.1 mol per L phosphate buffer containing 1 mg per mL bovine serum albumin (BSA) and 0.15 mol per L NaCl (Sigma-Aldrich), heated at 100°C for 5 minutes, and sonicated for 15 minutes at 560-W power setting (Transsonic 95HL, Prolabo, Fontenay sous Bois, France) in the hour preceding the administration. The PrPres content of the solution was measured by enzyme-linked immunosorbent assay (ELISA). Seventy-five micrograms of PrPres (equivalent to VRQ rPrP) was obtained from 100 g of brain tissue.

Administration and sampling

The IV administrations were performed in the right jugular vein via an indwelling catheter (Hemocath, Vygon, Ecouen, France). The intraarterial administrations were performed in the right external carotid artery via an intraarterial catheter (BD Careflow, Becton Dickinson, Le Pont-de-Claix, France) inserted in anesthetized ewes 2 days before the administrations. The VRQ and ARR variants of the ovine PrP were expressed in *Escherichia coli* and purified according to the method previously described.[15] Previous authors have shown that the recombinant proteins are monomeric in solution. The variants were kept at 4°C in solution in 20 mmol/L MOPS (Sigma-Aldrich), pH 7.25, at a concentration of approximately 1 mg per mL. The protein concentration was measured from the optical density at 280 nm with the extinction coefficient of 58718.0 mol per L per cm. ARR and VRQ solutions of rPrP were prepared in sheep plasma at a concentration of 0.2 mg per mL and kept at –20°C for all administrations.

Blood samples were collected from the left jugular vein via an indwelling catheter into ethylenediaminetetraacetate-containing tubes and centrifuged for 10 minutes at 1400 × g. The plasma was separated and stored at –20°C until assay. Urine was obtained via an indwelling closed urethral catheter (Rüsch, Teleflex Medical, Le Faget, France) that was kept in the bladder for 24 hours. Samples were stored at –20°C until analysis.

Quantification of PrP with a two-site enzyme immunoassay

Two immunometric assays were adapted from the method previously described.[19] The plasma and urine concentrations of purified prokaryote rPrP in all samples were measured by ELISA with BAR210, an anti-N terminal monoclonal antibody (MoAb)[20] recognizing residues 26 to 34 and the 12F10-AchE Spi Bio (Massy, France) antibody, an anti-C terminal MoAb recognizing residues between amino acids 154 and 171. Native plasma PrP, which lacks the BAR210 epitopes, was monitored by ELISA in plasma samples collected before administration of the rPrP, with the SAF34 and 12F10-AchE Spi Bio antibodies. Standard curves ranging from 0.5 to 20 ng per mL were established by diluting rPrP (VRQ and ARR) in ovine plasma, urine, or enzyme immunoassay (EIA) buffer (0.1 mol/L phosphate buffer, pH 7.4, 0.15 mol/L NaCl, 0.1% BSA, NaN_3 0.01%; Sigma-Aldrich).

Plasma (or urine) rPrP concentrations were calculated from the optical readings obtained by reference to the standard curve established with plasma (or urine) supplemented with the corresponding variant. Native PrP plasma concentrations were calculated from the optical readings obtained by reference to the standard curve established with EIA buffer solutions of the variants of rPrP. As an illustration, standard curves of ovine rPrP in EIA buffer or in plasma are shown in Fig. 1.

The limit of quantification of native and rPrP assays was 0.5 ng per mL. The accuracies of the PrP assay of plasma samples supplemented with the solution of VRQ

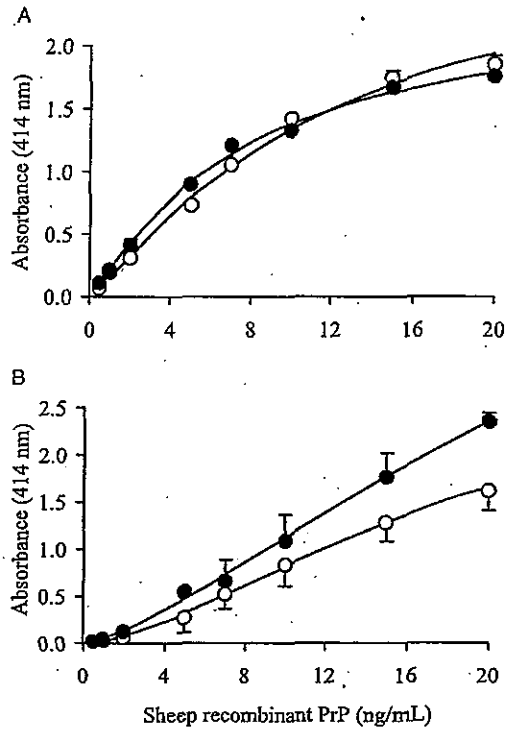

Fig. 1. Immunologic detection of ovine rPrP by two-site immunometric assays. ELISA plates were coated with capture antibodies SAF34 (A) or BAR210 (B) as described under Materials and Methods. Ovine recombinant ARR (○) or VRQ (●) variants in buffer (A) or in plasma (B) were detected by 12F10-AchE antibody. Data shown are mean ± SD of duplicate or triplicate wells. Lines represent the fitting with a non linear regression sigmoid model.

or ARR variants of rPrP at three concentrations (1.5, 6, and 12 ng/mL) were 91.1 and 94.3 percent, respectively. The mean intra- and interassay coefficients of variation for EIA buffer (plasma) solutions of the genetic variants of rPrP at these concentrations were 7.4 percent (6.5%) and 15.7 percent (13.5%), respectively.

Quantification of PrPres

Two methods were developed to measure the plasma PrPres concentrations after administration of the SAF preparation. The first method involved heating plasma at 60°C for 1 hour after diluting it by half in denaturing buffer from the TeSeE sheep/goat detection kit to denature the PrPres before assaying by ELISA. The second method was based on the natural affinity of plasminogen (Plgn) for PrPSc aggregates.[21,22] Superparamagnetic tosyl-activated M-280 microbeads (Invitrogen-Dynal, Cergy Pontoise, France) were coated with 100 µg of human Plgn (Sigma-Aldrich) in 0.1 mol per L carbonate-bicarbonate buffer, pH 9.4. The Plgn-coated microbeads were incubated at room temperature with plasma samples diluted (1:2, vol/vol) in phosphate buffer (10 mmol/L, pH 7.4) containing Sarkosyl (Sigma-Aldrich). After a 2-hour incubation with rotary shaking and three washes in 0.05 mol per L sodium phosphate buffer, pH 7.4, 1 percent Tween 20 (Sigma-Aldrich), the Plgn-coated microbeads were pelleted and heated at 105°C for 5 minutes in denaturing buffer from the TeSeE sheep/goat detection kit. The microbeads were then magnetized and discarded.

For the two methods, the supernatants and heated plasma samples were diluted fivefold in dilution buffer from the TeSeE sheep/goat detection kit before determination of plasma PrPres concentrations by ELISA (TeSeE sheep/goat detection kit). The plasma PrPres concentrations were calculated from the optical density readings obtained with reference to the standard curve established with solutions of the VRQ variant in dilution buffer and corrected for the mean extraction coefficient. The mean extraction coefficients of PrPres determined as the mean percentage of the PrPres recovered from plasma supplemented with an SAF solution (brain extract from a scrapie-affected homozygous VRQ sheep) at different levels (4, 8, and 16 mg of brain extract/mL) were 81.8 ± 21.1 and 97.6 ± 23.5 percent for the first (60°C heated plasma) and second method (Plgn-coated microbeads extraction), respectively.

Urine, half-diluted in denaturing buffer from the TeSeE sheep/goat detection kit, was heated at 105°C for 5 minutes and the PrPres concentrations were measured by ELISA with the SAF34 and 12F10-AchE Spi Bio antibodies. The extraction coefficient of PrPres, determined from urine spiked with the SAF in 0.8 mol per L urea to obtain the urinary PrPres concentration of 4 ng per mL, was 41 percent.

Kinetic analysis

Plasma PrP concentrations were analyzed with computer software (WinNonlin 5.0, Pharsight Corp., Mountain View, CA). Data were fitted to the biexponential equation

$$C(t) = A\exp(-\alpha t) + B\exp(-\beta t),$$

where A and B (ng/mL) are preexponential coefficients and α and β are exponents. The estimated variables (A, B, α, β) were used to solve the first-order rate constants of transfer from central to peripheral compartments (k_{10}, k_{12}, k_{21}) with classical equations.[23] The volume of the central compartment (mL/kg) was obtained from

$$V_C = \text{dose}/(A+B),$$

where dose is the administered dose. The steady-state volume of distribution (V_{ss}, mL/kg), which is the

appropriate volume to consider when determining the amount of PrP in the body at equilibrium, was obtained from

$$V_{SS} = V_C[1+(k_{12}/k_{21})],$$

where V_C is the volume of the central compartment, k_{12} is the first-order rate constant of transfer from the central compartment to the peripheral compartment, and k_{21} is the first-order rate constant of transfer from the peripheral compartment to the central compartment. The total plasma clearance, which expresses the capacity of the organism to eliminate proteins (Cl_{TOT}, mL/(kg·min)), was calculated with

$$Cl_{TOT} = \text{dose}/AUC,$$

where AUC is the area under the plasma PrP concentration–time curve obtained by integrating the equation

$$AUC = A/\alpha + B/\beta.$$

The clearance of distribution was calculated with

$$Cl_D = k_{12}V_C.$$

The appropriate volume to consider when calculating the amount of PrP remaining when the pseudodistribution equilibrium has been reached, V_{area} (mL/kg) was obtained from

$$V_{area} = Cl_{TOT}/\beta,$$

where β is the slope of the terminal phase. The terminal plasma half-life ($t_{1/2}$, min) was obtained from

$$t_{1/2} = \text{Log}2/\beta.$$

Different mean residence times (MRTs) were calculated.[24] The MRT (min), that is, the mean total time taken for each PrP molecule to transit through the body, was calculated with

$$MRT = (A/\alpha^2 + B/\beta^2)/(A/\alpha + B/\beta).$$

The MRT in the central compartment (MRT_C), that is, the mean time spent by the protein within the measured compartment was obtained from

$$MRT_C = 1/k_{10},$$

where k_{10} is the first-order rate constant of elimination from the central compartment. The MRT in the peripheral compartment (MRT_T), that is, the mean time spent by the protein outside the measured compartment, was obtained from

$$MRT_P = MRT - MRT_C.$$

Statistical analysis

Results are reported as mean ± standard deviation (SD). Statistical analyses were performed with computer software (SYSTAT 8.0, SPSS, Inc., Chicago, IL). PrP concentrations below the limit of quantification of the assay were arbitrarily fixed at 0.5 ng per mL. p Values lower than 0.05 were considered as significant. The pharmacokinetic variables of PrP genetic variants were compared between homozygous ARR and VRQ ewes with repeated-measures analysis of variance (ANOVA) with ewes as a random effect factor and the genetic variant of PrP, the ewe genotype, and their interactions as fixed effect factors. The genotype effect was tested with "ewe within-group variance" as the residual term. The mean pharmacokinetic variables of the VRQ variant of rPrP obtained after IV and intraarterial administration were compared by a paired t test. The effect of genotype on basal plasma PrPc concentrations was analyzed with repeated-measures ANOVA with ewes as a random effect factor, and genotype and time as fixed effect factors. The mean pharmacokinetic variables of the rPrP obtained before and after nephrectomy were compared by a paired t test. The effect of time on plasma PrP concentrations in vitro was analyzed by ANOVA with medium (blood vs. plasma), genetic variant, and time as fixed effect factors.

RESULTS

Pharmacokinetic variables of the prokaryote rPrP

Figure 2 shows, in a representative ewe, the semilogarithmic plots of the ARR and VRQ variants of the purified prokaryote rPrP after IV administration (0.02 mg/kg).

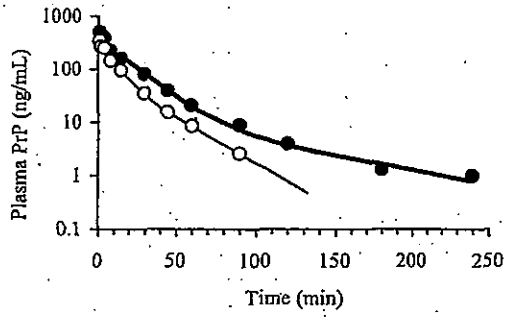

Fig. 2. Representative plasma concentrations of the genetic variants of PrP after IV administration. The observed ARR (○), VRQ (●), and corresponding fitted ARR (thin line) and VRQ (thick line) plasma concentrations were obtained in a representative homozygous VRQ sheep that received IV administrations of the ARR and VRQ genetic variants of the purified ovine prokaryote rPrP at a dose of 0.02 mg per kg.

Visual inspection of Fig. 2 shows that the ARR protein variant was eliminated faster than the VRQ protein. Table 1 gives the mean (±SD) pharmacokinetic variables of the VRQ and ARR variants of the rPrP intravenously administered to four healthy homozygous VRQ ewes and to four homozygous ARR ewes. Figure 3 gives a stochastic interpretation of the disposition of the ARR and VRQ variants of the rPrP. For a given genetic variant of the protein, the genotype of the recipient ewe did not influence any pharmacokinetic variables of the protein. The mean total plasma (blood) clearance (Cl_{TOT}) of the ARR variant of the rPrP was almost two times greater than that of the VRQ variant (ANOVA $p < 0.01$). The mean terminal half-life ($t_{1/2}$) of the ARR variant was nearly half that of the VRQ variant (ANOVA $p < 0.05$). The steady-state volume of distribution (V_{SS}) of the rPrP was low and was not affected by the genetic variant of the protein. The MRT of the protein was rather brief; the MRT in the central compartment (MRT_C) represented more than half the total MRT in the entire body for the two variants. The mean MRT and MRT_C of the ARR variant were lower than the values obtained for the VRQ variant (ANOVA $p < 0.01$).

TABLE 1. Mean (±SD) pharmacokinetic variables of the purified prokaryote rPrP*

	Variant		Genotype	
Variables	ARR	VRQ	ARR	VRQ
Cl_{TOT}, mL/(kg·min)	3.79 ± 0.94	2.00 ± 0.38†	2.69 ± 1.24	3.10 ± 1.11
Cl_D, mL/(kg·min)	2.99 ± 5.74	0.321 ± 0.215	2.41 ± 5.91	0.901 ± 0.917
$t_{1/2}$, min	29.9 ± 15.0	60.5 ± 27.8‡	48.9 ± 31.9	41.6 ± 22.0
V_C, min	46.4 ± 15.0	40.0 ± 9.1	42.9 ± 15.8	43.5 ± 9.12
V_{SS}, mL/kg	77.8 ± 34.2	60.9 ± 19.4	64.7 ± 28.6	74.1 ± 29.1
V_{area}, mL/kg	157.9 ± 83.6	167.3 ± 63.1	162.5 ± 91.4	162.7 ± 51.6
MRT, min	20.2 ± 6.00	30.9 ± 9.45†	25.3 ± 7.17	25.8 ± 11.8
MRT_C, min	12.5 ± 3.22	20.1 ± 3.25†	17.4 ± 5.49	15.2 ± 4.68
MRT_P, min	7.71 ± 4.77	10.77 ± 8.31	7.94 ± 4.25	10.54 ± 8.67

* The ARR and VRQ genetic variants of the purified prokaryote rPrP were intravenously administered at a dose of 0.02 mg per kg to four healthy homozygous VRQ sheep and four homozygous ARR sheep. The variant column indicates the mean (±SD) pharmacokinetic variables of the ARR and VRQ genetic variants of the rPrP while the genotype column gives the mean (±SD) pharmacokinetic variables of the two variants for the homozygous VRQ and ARR sheep, respectively.
† $p < 0.01$.
‡ $p < 0.05$.
Cl_{TOT} = total plasma (blood) clearance; Cl_D = clearance of distribution (equivalent to the clearance of redistribution, Cl_R); $t_{1/2}$ = terminal plasma half-life; V_C = volume of the central compartment; V_{SS} = steady-state volume of distribution; V_{area} = volume containing the amount of PrP remaining when the pseudodistribution equilibrium has been reached; MRT = mean residence time, that is, the mean total time taken for a PrP molecule to transit through the body; MRT_C = MRT in the central compartment, that is, the mean total time spent by the PrP molecule within the measured compartment; MRT_P = MRT in the peripheral compartment, that is, the mean time spent by the protein outside the measured compartment.

In the first experiment, PrP could not be detected in any of the urine samples collected. The mean basal plasma PrPc concentration of homozygous VRQ sheep was greater than that of homozygous ARR sheep (5.3 ± 1.2 ng/mL vs. 3.3 ± 0.7 ng/mL; ANOVA $p < 0.05$).

Figure 4 illustrates the plasma concentrations of the VRQ variant of the prokaryote rPrP after its administration at 0.02 mg per kg either by the intraarterial route or by the IV route to a representative homozygous ARR ewe. The time curve shows that both routes of administration gave very similar results. The mean pharmacokinetic variables of the VRQ variant of the rPrP obtained after intraarterial administration did not differ significantly ($p > 0.05$) from those obtained by the IV route (Table 2), thereby supporting the hypothesis of an absence of a brain first-pass effect to clear the protein.

When fresh blood or plasma samples were similarly spiked with either the ARR or the VRQ variant of the rPrP, the plasma PrP concentrations remained almost constant for incubation times ranging from 0 to 6 hours (no time effect, $p > 0.05$). After 24 hours of incubation, the mean decrease in the plasma PrP concentration ranged from 6 to 39 percent, suggesting a lack of (or very low) blood protease activity.

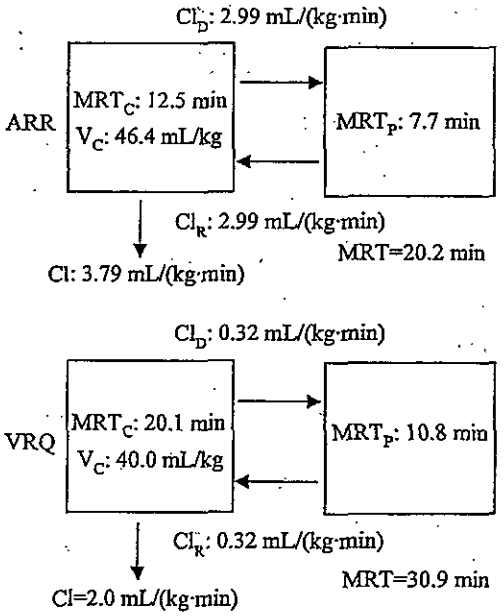

Fig. 3. Stochastic interpretation of the disposition of the ARR and VRQ variants of PrP. The values of the pharmacokinetic variables are the mean values obtained after administration of the variants of the purified ovine prokaryote rPrP by the IV route at 0.02 mg per kg to eight genotyped sheep. Abbreviations are explained in Table 1.

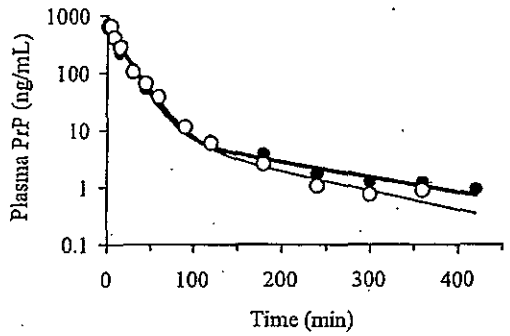

Fig. 4. Representative plasma concentrations of PrP according to its route of administration. The observed (●) and corresponding fitted (thick line) plasma concentrations of the VRQ variant of the purified ovine prokaryote rPrP were obtained after IV administration of the variant (jugular vein) at 0.02 mg per kg and the observed (○) and corresponding fitted (thin line) plasma concentrations of the VRQ variant were obtained after its administration at the same dose by the intraarterial route (external carotid) in a representative ARR sheep.

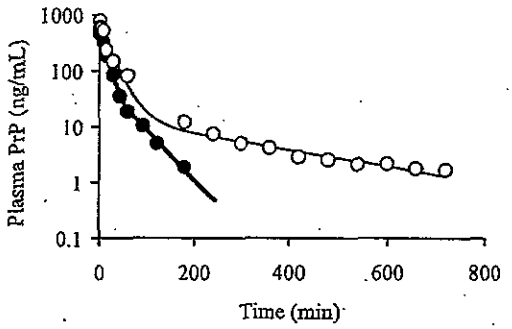

Fig. 5. Representative plasma concentrations of PrP after its administration before and after nephrectomy. The symbols represent the observed plasma concentrations of VRQ variant of the purified ovine rPrP obtained before (●) and after (○) a bilateral nephrectomy in a representative sheep intravenously administered with the VRQ variant at 0.02 mg per kg. The lines represent the corresponding fitted plasma concentrations of the VRQ variant obtained before (thick line) and after (thin line) nephrectomy.

TABLE 2. Pharmacokinetic variables (mean ± SD) of the VRQ variant of the purified prokaryote rPrP according to the route of administration*

Variables	Route of administration	
	IV	Intraarterial
Cl_{TOT}, mL/(kg·min)	1.98 ± 0.41	2.41 ± 0.90
$t_{1/2}$, min	85.8 ± 28.3	68.4 ± 18.8
V_C, min	40.6 ± 9.10	47.8 ± 12.2
V_{SS}, mL/kg	74.0 ± 26.7	72.4 ± 26.3
V_{area}, mL/kg	233.2 ± 30.1	227.6 ± 359.3
MRT, min	37.9 ± 12.9	30.8 ± 8.70
MRT_C, min	20.5 ± 1.48	19.5 ± 2.50
MRT_P, min	17.4 ± 11.5	11.3 ± 7.10

* The pharmacokinetic variables were obtained after an IV (jugular vein) or an intraarterial (external carotid) administration of the VRQ variant at a dose of 0.02 mg per kg to three sheep. Abbreviations are explained in Table 1.

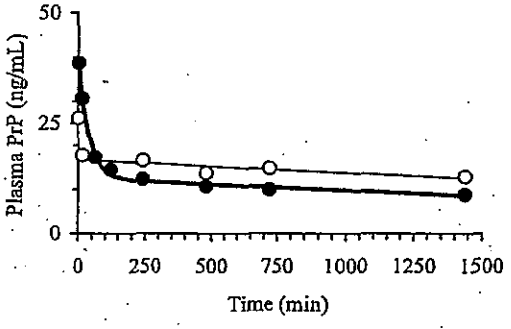

Fig. 6. Plasma PrPres concentrations after administration of SAF solution. The symbols represent the observed plasma concentrations of PrPres obtained with the PIgn-coated beads extraction method (○) or after a 60°C heat treatment (●) in a sheep intravenously administered an SAF solution containing a 75-µg equivalent of VRQ prokaryote rPrP. The lines represent the corresponding fitted plasma concentrations measured by the PIgn-coated beads method (thin line) and after the 60°C heat treatment (thick line).

Mechanisms of plasma PrP clearance

Figure 5 shows the temporal variations in plasma concentrations of the VRQ variant of rPrP after IV administration of 0.02 mg per kg to a representative sheep before and after a bilateral nephrectomy. Figure 5 shows the dramatic effect of nephrectomy, with a much slower elimination rate in the nephrectomized sheep. The mean plasma clearance of the VRQ variant was 2.1 times lower after bilateral nephrectomy (1.56 ± 0.66 mL/(kg·min) vs. 0.75 ± 0.36 mL/(kg·min); t test, $p < 0.05$). The renal PrP clearance derived from plasma clearance values obtained before and after nephrectomy (0.81 ± 0.30 mL/(kg·min)) represented 52 percent of the total clearance. The mean residence time of the protein was 2.6-fold higher (24.2 ± 7.6 min vs. 65.2 ± 2.6 min; $p < 0.05$, t test) after nephrectomy. The mean value of the volume of the central compartment obtained after nephrectomy was slightly lower than that obtained during the control period but this difference was not relevant (22.3 ± 9.82 mL/kg vs. 24.0 ± 9.69 mL/kg; t test, $p < 0.01$). No other pharmacokinetic variables were affected by nephrectomy.

Figure 6 shows the temporal decrease in plasma PrPres concentrations after the IV administration of an SAF solution containing 75 µg equivalent of VRQ rPrP. After a relatively fast distribution phase, the plasma PrPres concentrations decreased very slowly and reached the detection limit of the assay (7.5 ng/mL) 24 hours after administration. The estimated plasma clearance of PrPres

was 0.24 or 0.43 mL per (kg·min) based on the results obtained after Plgn-coated microbead capture or 60°C heating, respectively. The estimated mean terminal half-lives obtained after Plgn-coated microbead capture or by the 60°C heating method were 58 and 41 hours, respectively. The mean residence time and the steady-state distribution volumes were 5030 minutes (84 hr) versus 3435 minutes (57 hr) and 1187.0 versus 1487.4 mL per kg, respectively (Plgn-coated microbead capture method vs. 60°C plasma heating treatment). If the results obtained by Plgn-coated microbead capture are considered, then the MRT_C and MRT_P values are equivalent. PrPres could not be detected in any of the urine samples collected after administration of the SAF solution.

DISCUSSION

Before any discussion of the present experiments, we must acknowledge that we tested our different hypotheses with a rPrP of bacterial origin and not the native form of the PrP, which is not currently available for pharmacokinetic studies. The natural form is glycosylated and the glycosylation of a protein may considerably influence its fate in the body. We nevertheless believe that this pioneer work may have some value to explore the clearance pathways and the influence of the genotype and the role mediated by the conformation of the variant of PrP and/or by the clearance mechanisms. Similarly, the disease-associated isoform of PrP that was administered underwent a series of physicochemical processes before its administration (solubilization) that were able to partly denature the protein. Thus, the different test proteins used in the present experiment should be considered only as probes. We hypothesized they were able to explore, at least qualitatively, and possibly quantitatively (i.e., giving an order of magnitude), different disposition processes of physiologic interest, mainly concerning the overall plasma clearance that contributes to the systemic exposure.

Our results show that clearance of the rPrP is low and of the same order of magnitude as the glomerular filtration rate in sheep[25] (approx. 2 mL/(kg·min)). Clearance of the ARR variant of rPrP associated with resistance to scrapie was almost twice that of the VRQ variant associated with scrapie susceptibility. For a given variant, however, the genotype of the recipient had no effect on PrP plasma clearance, indicating that the protein conformation, not the animal's clearance mechanisms, was responsible for the difference between the two genotypes.

It is generally accepted that the kidney is the major organ of protein elimination[26] and this was confirmed in the present experiment where the kidney clearance of the VRQ variant of rPrP was about half the total clearance. Despite the high renal clearance, no PrP was found in urine. This is not really surprising because most of the low-molecular-weight proteins (here approx. 23 kDa for our variants) that are filtered through the glomerulus are metabolized by enzymes located in the brush border of the tubular lumen.[26] We did not investigate the renal clearance of the ARR variant. It is generally accepted, however, that the renal excretion of proteins is mainly governed by their size and that the sieving effect of glomerular filtration is independent, for small proteins, of molecular charge[27] or other conformational differences. Because ARR and VRQ are of the same molecular weight, it can be hypothesized that the observed difference in clearance of the ARR and VRQ recombinant proteins was of nonrenal origin.

Nonrenal clearance of rPrP was partly explored in our experiment and the direct metabolism of rPrP by plasma proteases can be excluded because we showed that the plasma concentrations obtained from blood or plasma supplemented with either the VRQ or the ARR variant and incubated at 37°C did not vary over a 6-hour period, that is, a time greater than that required to observe PrP elimination in vivo. Similarly, we can exclude a selective trapping of rPrP in the brain because there was no evidence of a first-pass effect when the recombinant protein was directly administered through the carotid artery. It is likely that, as for many other proteins, the main nonrenal clearance mechanism involves the reticuloendothelial system. The liver was shown to contribute significantly to protein metabolism, especially through receptor-mediated endocytosis followed by degradation in lysosomes. This mechanism, contrary to the bulk filtration of protein at the glomerular level, is likely to be a more specific process of protein elimination that could explain the difference in clearance that we observed between the two tested variants.

It is generally accepted that proteins are initially distributed into the plasma volume and then more slowly into the interstitial fluid space. This view is supported by the present experiment where the initial distribution volume of rPrP approximated to that of the plasma volume (overall mean $V_C = 43$ mL/kg); in contrast, the volume of distribution associated with the terminal disposition phase of rPrP was of the same order of magnitude as the volume of extracellular fluid ($V_{area} = 162$ mL/kg), indicating that at least a fraction of the administered rPrP gained access to the extracellular fluid. Owing to this restricted distribution, the overall mean residence time of the recombinant protein was rather short, ranging from 14.5 to 52 minutes, despite the low clearance rate. The greater mean residence time of the VRQ variant, when compared with that of the ARR variant, was mainly explained by the greater mean residence time of the VRQ variant in the central compartment as measured by the MRT_C (20 min vs. 12.5 min). By contrast the MRT_P (i.e., the overall MRT of the protein in the peripheral compart-

ment) was relatively similar for the two variants (8 min vs. 11 min).

These results obtained with the nonglycosylated purified prokaryote recombinant protein may provide useful information for plasma PrPc because some authors[28-33] have shown that the three-dimensional structure and the thermal stability of rPrP produced in *E. coli* are essentially identical to those of the natural glycoprotein. Thus, as expected, the glycosylation and the GPI anchor did not affect the folding of the PrP protein and transgenic mice expressing a nonglycosylated PrPc as well as transgenic mice harboring a PrP without a GPI anchor[34,35] are able to replicate the infectious agent. Furthermore, we have shown that the basal plasma PrPc concentrations of homozygous VRQ ewes were almost twice those of homozygous ARR ewes. This suggests that the plasma clearance of the ARR variant of PrPc is, like the same variant of rPrP, nearly twice that of its VRQ counterpart assuming that there is no difference in the synthesis of the protein and that rPrP may be a relevant probe for studying the fate of PrPc. Our results are in agreement with those of Halliday and coworkers[36] who showed that the level of PrPc expressed on the cell surface of peripheral blood mononuclear cells was influenced by the genotype, with the highest levels found in scrapie-susceptible homozygous VRQ sheep and the lowest in scrapie-resistant homozygous sheep. The level of PrP expression by blood cells was correlated with the level of ovine plasma PrPc by Thackray and colleagues[37] who showed genotypic differences in the level of ovine plasma PrPc, with the highest and lowest levels being observed in plasma from homozygous VRQ and ARR sheep, respectively. The higher plasma PrPc concentrations of dogs affected by renal insufficiency when compared to healthy ones (unpublished observations) together with previous observations in humans with extensive renal insufficiency[38,39] strongly suggest that the kidneys contribute highly to PrPc clearance.

Our results raise the question of the significance of host genetic and pathophysiologic (renal insufficiency)-caused variations of plasma PrPc levels with respect to TSE susceptibility. The implication of PrPc plasma levels for peripheral pathogenesis of scrapie is still debated because blood transmissions can occur in species such as hamsters where blood levels of normal PrP are exceedingly low. It cannot be ruled out, however, that some knowledge of the influence of genetic and pathophysiologic factors on plasma concentrations or clearance of the normal protein provides a ground for future investigations aimed at a better understanding of the role of plasma PrPc in the transmissibility of the infectious agent by the IV route.

For the single sheep that we investigated, the kinetics of the temporal decrease in plasma PrPres concentrations after the IV administration indicated that the disease-associated isoform of PrP (clearance of 0.24 mL/(kg·min)) is eliminated much more slowly than the recombinant genetic variants of PrP (2-3 mL/(kg·min)), resulting in a greater mean residence time (approx. 84 hr vs. 14-52 min). In addition, we observed a high steady-state volume of distribution for PrPres (1.2-1.5 L/kg) suggesting that PrPres is more widely distributed than rPrP. It should be stressed that our pharmacokinetic approach required the solubilization of PrPres recovered as pellets though we cannot exclude the persistence of insoluble aggregated forms of PrPres in the administered SAF preparation despite the ultrasonic and heating treatments. Despite such a limitation, the difference in clearance between the recombinant and pathologic isoforms of PrP is so great that we have no doubt that the disease associated isoform of PrP is eliminated much more slowly than the recombinant protein. The limit of our analytical method prevented us from evaluating PrPres concentrations below the level of quantification (7.5 ng/mL) and from ensuring that PrPres did not persist for a longer time at low concentrations.

Considerable uncertainty exists about the relevant spiking form of prion to document the risk of TSE transmission by blood transfusion.[40] Data from a rodent experimental model of TSE suggest that the infectious agent in plasma is very small, unsedimentable, and poorly aggregated,[41,42] but many attempts to solubilize PrPSc under nondenaturing conditions have been unsuccessful[43,44] until recently.[45] In the present clearance study, it must be assumed that the method used to prepare the prion material from sheep brain must be efficient and safe enough to obtain a PrPres dose that can be administered intravenously to a sheep. The purpose of this single PrPres infusion was no more than to obtain a first estimate of the order of magnitude of the clearance of the disease-associated form of PrP.

In conclusion, we have shown, by use of purified prokaryote rPrP, that the clearance of the ARR variant associated with resistance to scrapie is greater than the clearance of the VRQ variant associated with sensitivity to scrapie. This, together with the higher basal plasma PrPc concentrations observed in homozygous highly susceptible VRQ ewes compared with homozygous resistant ARR ewes, suggests that the ARR variant of PrPc is eliminated more rapidly than the VRQ variant. The 52 percent decrease in clearance of the prokaryote rPrP in nephrectomized ewes suggests that the kidneys contribute considerably to the elimination of the prion protein and that renal insufficiency could represent a risk factor for TSE disease transmission. The pathologic isoform of PrP was shown to be cleared very slowly from the blood, leading to sustained exposure after its direct IV administration.

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REFERENCES

1. Schmerr MJ, Jenny AL, Bulgin MS, Miller JM, Hamir AN, Cutlip RC, Goodwin KR. Use of capillary electrophoresis and fluorescent labeled peptides to detect the abnormal prion protein in the blood of animals that are infected with a transmissible spongiform encephalopathy. J Chromatogr A 1999;853:207-14.
2. Herrmann LM, Baszler TV, Knowles DP, Cheevers WP. PrP(Sc) is not detected in peripheral blood leukocytes of scrapie-infected sheep: determining the limit of sensitivity by immunohistochemistry. Clin Diagn Lab Immunol 2002; 9:499-502.
3. Brown P, Cervenakova L, McShane LM, Barber P, Rubenstein R, Drohan WN. Further studies of blood infectivity in an experimental model of transmissible spongiform encephalopathy, with an explanation of why blood components do not transmit Creutzfeldt-Jakob disease in humans. Transfusion 1999;39:1169-78.
4. Hunter N, Foster J, Chong A, McCutcheon S, Parnham D, Eaton S, MacKenzie C, Houston F. Transmission of prion diseases by blood transfusion. J Gen Virol 2002;83:2897-905.
5. Houston EF, Halliday SI, Jeffrey M, Goldmann W, Hunter N. New Zealand sheep with scrapie-susceptible PrP genotypes succumb to experimental challenge with a sheep-passaged scrapie isolate (SSBP/1). J Gen Virol 2002;83: 1247-50.
6. Houston F, Foster JD, Chong A, Hunter N, Bostock CJ. Transmission of BSE by blood transfusion in sheep. Lancet 2000;356:999-1000.
7. Herzog C, Sales N, Etchegaray N, Charbonnier A, Freire S, Dormont D, Deslys JP, Lasmezas I. Tissue distribution of bovine spongiform encephalopathy agent in primates after intravenous or oral infection. Lancet 2004;363:422-8.
8. Wroe SJ, Pal S, Siddique D, Hyare H, MacFarlane R, Joiner S, Linehan JM, Brandner S, Wadsworth JD, Hewitt P, Collinge J. Clinical presentation and pre-mortem diagnosis of variant Creutzfeldt-Jakob disease associated with blood transfusion: a case report. Lancet 2006;368:2061-7.
9. Editorial team. Fourth case of transfusion-associated vCJD infection in the United Kingdom. Euro Surveill 2007;12: E070118.4.
10. Wilson K, Ricketts MN. A third episode of transfusion-derived vCJD. Lancet 2006;368:2037-9.
11. Hunter N, Goldmann W, Foster JD, Cairns D, Smith G. Natural scrapie and PrP genotype: case-control studies in British sheep. Vet Rec 1997;141:137-40.
12. Elsen JM, Amigues Y, Schelcher F, Ducrocq V, Andreoletti O, Eychenne F, Khang JV, Poivey JP, Lantier F, Laplanche JL. Genetic susceptibility and transmission factors in scrapie: detailed analysis of an epidemic in a closed flock of Romanov. Arch Virol 1999;144:431-45.
13. Baylis M, Chihota C, Stevenson E, Goldmann W, Smith A, Sivam K, Tongue S, Gravenor MB. Risk of scrapie in British sheep of different prion protein genotype. J Gen Virol 2004; 85:2735-40.
14. Le Dur A, Beringue V, Andreoletti O, Reine F, Lai TL, Baron T, Bratberg B, Vilotte JL, Sarradin P, Benestad SL, Laude H. A newly identified type of scrapie agent can naturally infect sheep with resistant PrP genotypes. Proc Natl Acad Sci U S A 2005;102:16031-6.
15. Rezaei H, Marc D, Choiset Y., Takahashi M, Hui Bon Hoa G, Haertlé T, Grosclaude J, Debey P. High yield purification and physico-chemical properties of full-length recombinant allelic variants of sheep prion protein linked to scrapie susceptibility. Eur J Biochem 2000;267:2833-9.
16. Rezaei H, Choiset Y, Eghiaian F, Treguer E, Mentre P, Debey P, Haertle T, Grosclaude J. Amyloidogenic unfolding intermediates differentiate sheep prion protein variants. J Mol Biol 2002;322:799-814.
17. Maack T, Johnson V, Kau ST, Figueiredo J, Sigulem D. Renal filtration, transport, and metabolism of low-molecular weight proteins. A review. Kidney Int 1979;16: 251-70.
18. Horney D, Archibald J. Nephrectomy in cattle and sheep. J Am Vet Med Assoc 1961;138:551-2.
19. Grassi J, Creminon C, Frobert Y, Fretier P, Turbica I, Rezaei H, Hunsmann G, Comoy E, Deslys JP. Specific determination of the proteinase K-resistant form of the prion protein using two-site immunometric assays. Application to the post-mortem diagnosis of BSE. Arch Virol Suppl 2000;16: 197-205.
20. Feraudet C, Morel N, Simon S, Volland H, Frobert YF, Creminon C, Vilette D, Lehmann S, Grassi J. Screening of 145 anti-PrP monoclonal antibodies for their capacity to inhibit PrPSc replication in infected cells. J Biol Chem 2005;280:11247-58.
21. Fischer MB, Roeckl C, Parizek P, Schwarz HP, Aguzzi A. Binding of disease-associated prion protein to plasminogen. Nature 2000;408:479-83.
22. Maissen M, Roeckl C, Glatzel M, Goldmann W, Aguzzi A. Plasminogen binds to disease-associated prion protein of multiple species. Lancet 2001;357:2026-8.
23. Gibaldi M, Perrier D. Pharmacokinetics. 2nd ed. New York: Marcel Dekker; 1982.
24. Kong AN, Jusko WJ. Definitions and application of mean residence times in reference to the two-compartment mammillary plasma clearance model. J Pharm Sci 1988;77: 157-65.
25. Nesje M, Flaoyen A, Moe L. Estimation of glomerular filtration rate in normal sheep by the disappearance of iohexol from serum. Vet Res Commun 1997;21:29-35.
26. Rowland M, Tozer TN. Clinical pharmacokinetics. Concepts and applications. 3rd ed. New York: Lea & Febiger; 1995.
27. Maack T, Park CH, Camargo MJ. Renal filtration, transport and metabolism of proteins. In: Seldin DW, Giebish G, editors. The kidney physiology and pathophysiology. New York: Raven Press; 1992. p. 3005-38.

28. Calzolai L, Zahn R. Influence of pH on NMR structure and stability of the human prion protein globular domain. J Biol Chem 2003;278:35592-6.
29. De Simone A, Dodson GG, Verma CS, Zagari A, Fraternali F. Prion and water: tight and dynamical hydratation sites have a key role in structural stability. Proc Natl Acad Sci U S A 2005;102:7535-40.
30. Eghiaian F, Grosclaude J, Lesceu S, Debey P, Doublet B, Treguer E, Rezaei H, Knossow M. Insight into the $PrP^{C} \rightarrow PrP^{Sc}$ conversion from the structures of antibody-bound ovine prion scrapie-susceptibility variants. Proc Natl Acad Sci U S A 2004;101:10254-9.
31. Haire LF, Whyte SM, Vasisht N, Gill AC, Verma C, Dodson EJ, Dodson GG, Bayley PM. The crystal structure of the globular domain of sheep prion protein. J Mol Biol 2004; 336:1175-83.
32. Hornemann S, Schorn C, Wüthrich K. NMR structure of the bovine prion protein isolated from healthy calf brains. EMBO Rep 2004;5:1159-64.
33. Zahn R, Güntert P, Von Schroetter C, Wüthrich K. NMR structure of a variant human prion protein with two sulfide bridges. J Mol Biol 2003;326:225-34.
34. Chesebro B, Trifilo M, Race R, Meade-White K, Teng C, LaCasse R, Raymond L, Favara C, Baron G, Priola S, Caughey B, Masliah E, Oldstone M. Anchorless prion protein results in infectious amyloid disease without clinical scrapie. Science 2005;308:1435-9.
35. Legname G, Baskakov IV, Nguyen HO, Riesner D, Cohen FE, De Armond SJ, Prusiner SB. Synthetic mammalian prions. Science 2004;305:673-6.
36. Halliday S, Houston F, Hunter N. Expression of PrPC on cellular components of sheep blood. J Gen Virol 2005;86: 1571-9.
37. Thackray AM, Fitzmaurice TJ, Hopkins L, Bujdoso R. Ovine plasma prion protein levels show genotypic variation detected by C-terminal epitopes not exposed in cell-surface PrPc. Biochem J 2006;400:349-58.
38. Starke R, Drummond O, MacGregor I, Biggerstaff J, Camilleri R, Gale R, Lee CA, Nitu-Whalley S, Machin SJ, Harrison P. The expression of prion protein by endothelial cells: a source of the plasma form of prion protein? Br J Haematol 2002;119:863-73.
39. Starke R, Mackie I, Drummond O, MacGregor I, Harrison P, Machin S. Prion protein in patients with renal failure. Transfus Med 2006;16:165-8.
40. Foster PR. Selection of spiking materials for studies on the clearance of agents of transmissible spongiform encephalopathy during plasma fractionation. Biologicals 2007;Mar 30:1-2.
41. Brown P, Cervenakova L, McShane LM, Barber P, Rubenstein R, Drohan WN. Further studies of blood infectivity in an experimental model of transmissible spongiform encephalopathy, with an explanation of why blood components do not transmit Creutzfeldt-Jakob disease in humans. Transfusion 1999;39:1169-78.
42. Yakovleva O, Janiak A, McKenzie C, McShane L, Brown P, Cervenakova L. Effect of protease treatment on plasma infectivity in variant Creutzfeldt-Jakob disease mice. Transfusion 2004;44:1700-5.
43. Post K, Brown DR, Groschup M, Kretzschmar HA, Riesner D. Neurotoxicity but not infectivity of prion proteins can be induced reversibly in vitro. Arch Virol Suppl 2000;16: 265-73.
44. Wille H, Prusiner SB. Ultrastructural studies on scrapie prion protein crystals obtained from reversed micellar solutions. Biophys J 1999;76:1048-62.
45. Berardi VA, Cardone F, Valanzano A, Lu M, Pocchiari M. Preparation of soluble infectious samples from scrapie-infected brain: a new tool to study the clearance of transmissible spongiform encephalopathy agents during plasma fractionation. Transfusion 2006;46:652-8.

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研究報告の概要

本稿では，1990 年代にウシ海綿状脳症（BSE）が英国で流行したことを受けて，ヒト及び動物において伝染性海綿状脳症（TSE）を検出するために開発された様々な技法について焦点をあてている。脳組織中で典型的な病変及び異常プリオンタンパク PrP^{Sc} の蓄積を検出する従来の組織学的及び免疫組織学的方法に加えて，感受性の高いげっ歯類に疾患を実験的に伝播させることも確認手段としての機能を果たしてきた。さらに，ウェスタンブロット法も組織抽出物中の PrP^{Sc} 検出において高感度であることが実証されている。しかしながら，早急にハイスループットスクリーニングに適した手法が必要性であったことから，ウシ及び小型反芻動物（ヒツジ，ヤギ）の屠畜後に TSE を診断する，いわゆる「迅速な検査」が導入された。これらの多くは，感染動物又は非感染動物の脳組織を検討した大規模試験（対象は数百万検体）で妥当性が確認された後に，EU 保健・消費者保護総局（European Directorate General for Health and Consumer Protection）の承認を受けている。検査法の大半は ELISA 法であり，プロテイナーゼ K による PrPSc 消化とその後の変性及び特異的抗体による検出に依存している。現在，発症前での早期診断が可能となり得る屠畜前検査法が必要となっている。蛋白折りたたみ異常反復増幅法（Protein Misfolding Cyclic Amplification：PMCA）と呼ばれるアプローチ法は有望であると考えられていたが，最近では当初考えられていたほど特異的ではないことが明らかになっている。ヒトにおいて輸血により vCJD が伝播し得ることが示されていることから，プリオン研究分野では信頼性の高い血液検査法の開発が最優先事項となっている。様々な戦略が実施されているものの，いずれも未だ満足のいく結果にはつながっておらず，当該分野は依然として活発な研究領域である。

報告企業の意見	今後の対応
著者は，これまでに開発された動物及びヒトにおける TSE 検出法について詳しく調査している。本研究分野は，大規模な調査に基づいたスクリーニング方法の開発に成功したと考えられるが，依然として，血液における迅速な生前診断の必要性は高い。これが可能になれば，血液ドナーのスクリーニングにも使用でき，血液由来製剤の安全性も高まるであろう。	今後も引き続き，血漿分画製剤の製造に適用できるプリオンスクリーニング検査の有用性の情報収集に努める。

使用上の注意記載状況・その他参考事項等

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Review article

Progress and limits of TSE diagnostic tools

Jacques GRASSI*, Séverine MAILLET, Stéphanie SIMON, Nathalie MOREL

CEA, iBiTecS, Service de Pharmacologie et d'Immunoanalyse, bâtiment 136, CEA/Saclay, 91191 Gif-sur-Yvette, France

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Abstract – Following the two "mad cow" crises of 1996 and 2000, there was an urgent need for rapid and sensitive diagnostic methods to identify animals infected with the bovine spongiform encephalopathy (BSE) agent. This stimulated research in the field of prion diagnosis and led to the establishment of numerous so-called "rapid tests" which have been in use in Europe since 2001 for monitoring at-risk populations (rendering plants) and animals slaughtered for human consumption (slaughterhouse). These rapid tests have played a critical role in the management of the mad cow crisis by allowing the removal of prion infected carcasses from the human food chain, and by allowing a precise epidemiological monitoring of the BSE epizootic. They are all based on the detection of the abnormal form of the prion protein (PrP^{Sc} or PrP^{res}) in brain tissues and consequently are only suitable for post-mortem diagnosis. Since it is now very clear that variant Creutzfeldt-Jakob disease (vCJD) can be transmitted by blood transfusion, the development of a blood test for the diagnosis of vCJD is a top priority. Although significant progress has been made in this direction, including the development of the protein misfolding cyclic amplification (PMCA) technology, at the time this paper was written, this objective had not yet been achieved. This is the most important challenge for the years to come in this field of prion research.

TSE diagnosis / PrP / blood test / PMCA

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* Corresponding author: jacques.grassi@cea.fr

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1. INTRODUCTION

Humans have a long history of contact with animals affected by a transmissible spongiform encephalopathy (TSE), without apparent problems. Scrapie in sheep has been described since the 18th century and the available epidemiological data do not indicate a detectable risk for humans under natural conditions. The huge epizootic of bovine spongiform encephalopathy (BSE) in Great Britain that was first detected in 1986 (over 200 000 confirmed cases to date) and, above all, the announcement in 1996 of possible transmission of BSE to humans in the form of the variant Creutzfeldt-Jakob disease (vCJD), created enormous concern among European consumers and triggered the first European-wide mad cow crisis. In 1999, testing of at-risk populations was introduced in some European countries and subsequently identified cases of BSE in countries previously believed to be untouched by the epizootic (Germany, Italy, Spain). This realisation triggered a further mad cow crisis at the end of 2000 and prompted the European authorities to take a whole series of measures to stop the spread of the epizootic, and to protect consumers from possible contamination by BSE. In particular, there was a total ban on meat and bone meal (held to be the main reason for BSE propagation in cattle) in livestock feed. Consumer protection was essentially ensured by removal of organs most likely to contain prions (specified risk material), and the implementation of systematic testing of all cattle aged between 24 and 30 months, depending on the carcass category and country[1]. The BSE epizootic has clearly receded since 2001 in Western Europe, but the situation is less clear in Eastern Europe.

Although arguments have accumulated since 1996 to confirm a link between BSE and vCJD [10, 11], fewer people are affected by vCJD than might have been feared (201 as of August 2007, with 163 in the UK and 22 in France) all of them carrying the Met/Met genotype at codon 129 of the prion protein (PrP). It is possible, however, that affected carriers of other genotypes (Val/Val and Met/Val) may appear in the future. Furthermore, a risk of secondary transmission within the human species is now clearly identified, following the detection in Great Britain of four cases linked to blood transfusion [41, 56, 84].

In the late 1990s, there was a pressing need for rapid and sensitive diagnostic methods to identify animals infected by the BSE agent in order to define the extent of the epizootic and avoid transmission to humans. Surprisingly successful rapid tests have been developed since the mid-1990s and, as we shall see, have proven to be very useful.

The development of a blood test for the diagnosis of vCJD is now a priority, first to make blood transfusions safe, and secondly to identify affected individuals early so that treatment (which at present does not exist) can be initiated before neuroinvasion and onset of the first clinical signs. At the time this paper was written, this objective had not yet been achieved.

2. THE CURRENT STATUS OF TSE DIAGNOSIS

Before the mad cow epizootic, the diagnosis of prion diseases was not a public health or economic issue. For humans and live animals, it was essentially based on the analysis of clinical signs and post-mortem histological analysis.

2.1. Conventional methods

Historically, techniques used to diagnose TSE were designed to detect, in appropriate tissue samples, lesions characteristic of TSE, or disease associated forms of the prion protein (PrP^{Sc}), or the transmissible agent itself.

2.1.1. Histological and immunohistological techniques

Histological detection of lesions typical of TSE (spongiosis, astrogliosis, amyloid plaques), essentially in the tissue of the central nervous system, is the reference method

[1] This does not hold for the United Kingdom since, between 1996 and 2005, cattle over 30 months were not eligible for human consumption (certainly the more efficient protection), and consequently not systematically tested. The same active surveillance scheme as in other European countries has been in place since November 2005.

for confirming a clinical diagnosis [23]. It is very specific, since it allows direct observation of the signs of the disease, notably symmetrical spongiform lesions, but is less sensitive than other techniques [24, 83]. The sensitivity of microscopic observation can be increased by immunohistochemical techniques that use antibodies specific to PrP to detect accumulation of PrP^{Sc} in amyloid deposits [79, 80]. This technique's efficiency depends greatly on sample preparation and on the nature of the antibodies used. Although these methods are ill-suited to rapid, routine analysis, they are excellent for confirmation. They are also effective for the analysis of samples of lymphoid tissues (tonsils, Peyer's patches, lymph nodes) and can be utilised, for example, in preclinical diagnosis of scrapie in sheep [2, 79] and chronic wasting disease [73], i.e., diseases characterised by marked replication of the prion in the lymphoid organs during the presymptomatic phase. The same observation has been reported for the diagnosis of vCJD and retrospective examination of over 8 000 tonsil and appendix samples by immuno-histochemistry (IHC) identified one case of vCJD in Great Britain in a person presenting no clinical signs [32, 33].

2.1.2. Experimental infection tests

The most sensitive and specific method of diagnosing TSE is unquestionably experimental infection in laboratory animals. The animal is injected (usually by the intracranial route) with a homogenate prepared from the potentially infected tissue and is watched for the appearance of clinical signs. After the death of the experimentally infected animal, disease development is confirmed using classic techniques (histology, immunohistology, Western blot). For obvious practical reasons, these experiments are generally performed in rodents (mice, hamsters, bank voles). Recently, the availability of transgenic mice that overexpress the same PrP as that of the donor species has significantly increased the efficiency of experimental transmission and shortened incubation periods [71]. However, these methods are too labour-intensive and time-consuming for use in routine high-throughput screening.

2.1.3. Western blotting

Western blotting has been used to detect PrP^{Sc} in tissue extracts for 20 years now [6]. Since all samples also always contain PrP^{C}, the protease-sensitive prion protein, they are systematically treated with proteinase K. After denaturation of the tissue extract by heating with sodium dodecyl sulfate (SDS), it is analysed by polyacrylamide gel electrophoresis (PAGE) and the denatured protein is transferred to a solid support and detected with an enzyme-labelled antibody. The specificity of Western blotting stems, among other things, from the fact that proteolysis with proteinase K characteristically alters the molecular weight of the PrP^{res}, because of the partial degradation of the N-terminal part of the protein. As a consequence, in addition to the residual signal observed, the gel bands shift in a manner typical of PrP^{res}. This technique has enabled highly sensitive detection of PrP^{Sc} in various tissues from vCJD patients [81]. Western blotting is also commonly used to characterise prion strains. The characteristics of the molecular pattern (size and relative intensity of the bands) of the three glycoforms of PrP^{res}, and the reaction of certain antibodies directed against the N-terminal part of the PrP can, in some cases, be used to identify the molecular signature of the prion strain [75, 76].

2.2. Rapid tests for post-mortem diagnosis of TSE

2.2.1. General characteristics

None of the methods mentioned above are really suited to high-throughput screening, and cannot be automated. After the 1996 mad cow crisis, and the fear of possible transmission to humans, it became clear that there was a need to develop new simpler and faster diagnostic tests for large-scale epidemiological studies, and more accurate assessment of the characteristics of the epizootic, or for routine testing to warrant safety of animal meat, for instance, of all cattle before they enter the food chain or industrial circuits. A new generation of so-called "rapid" diagnostic tests emerged, all based on the immunological detection of PrP^{Sc}, the only identified reliable marker of TSE.

It has long been perfectly apparent that antibodies can distinguish between the different conformations of the same protein, and the abnormal form of the prion protein is known to differ from the normal cellular form by its conformation, which contains a much higher proportion of β sheets, and less α helices [57, 58]. Yet, despite several promising publications [17, 37, 54, 87], there are, as yet, no clearly identified antibodies that, under practical conditions, specifically recognise PrP^{Sc} with satisfactory affinity in its native form. This is not the least of the paradoxes encountered in this field of research, but is beyond the scope of the present review.

In the absence of antibodies that specifically recognise PrP^{Sc}, it was necessary to resort to indirect approaches to distinguish between PrP^{Sc} and PrP^{C} in tissue extracts, which is generally present at a higher concentration. In almost all the rapid tests developed hitherto, this distinction is based on the distinct biochemical properties of the two forms of the protein. Most tests utilise the relative resistance of PrP^{Sc} to degradation by proteolytic enzymes, particularly proteinase K. Other tests are based on the aggregation properties of PrP^{Sc} when extracted using detergents. Note that the extraction of PrP (PrP^{C} or PrP^{Sc}) is an indispensable step in all tests, because it is hard to envisage detecting PrP without extracting it from its neighbouring membrane structures. This is generally achieved by treating a tissue homogenate with one or more detergents. Lastly, all the rapid tests include a step in which PrP^{res} is denatured, to permit its detection by antibodies that recognise PrP^{C} or denatured PrP (whether from PrP^{C} or PrP^{Sc}).

2.2.2. *European validation campaigns*

In May 1999, the Directorate General XXIV (Consumer Policy and Consumer Health Protection) of the European Commission validated, under very strict conditions (blind testing in a limited time overseen by a European Commission representative), three tests (from Enfer Technology Ltd (Newbridge, Ireland), Prionics (Zurich, Switzerland), and CEA (Saclay, France)) that were suitable for rapid industrial development.

The Prionics test uses an industrialised format of Western blotting that enables large-scale analysis [49, 65], and was the first rapid test used in large-scale epidemiological studies, first in Switzerland and then in France [12].

A diagnostic test developed by the CEA since 1998 is another example of a rapid test based this time on a conventional immunoenzyme approach (enzyme-linked immunosorbent assay, ELISA). This test is now marketed by Bio-Rad (Hercules, CA, USA, TeSeE tests). In the first step of the test, PrP^{res} is selectively purified using proteinase K, centrifugation, and denaturation. In the second step, the solubilised and denatured PrP^{res} is measured by a two-site (so-called sandwich) immunoassay that uses two monoclonal antibodies [27].

Enfer Technology Ltd developed an ELISA [46] in which PrP^{Sc} is directly immobilised on a solid support in the presence of proteinase K, denatured, and then detected using a polyclonal antibody directed against a peptide sequence characteristic of PrP.

The evaluation of these three tests was performed on more than 1 600 brain stem samples from uninfected animals (1 000 animals from New Zealand) and from animals at the clinical stage of the disease (300 animals from the UK). Brain homogenates were also diluted to test the analytical sensitivity of the tests [46]. A fourth test (from Wallac, Bucks, UK) gave unsatisfactory results and was subsequently re-evaluated in a substantially different format in 2001. The Enfer Technology, Prionics, and CEA tests were found to have 100% sensitivity and specificity on the series studied. Later work demonstrated that the CEA test, and its industrial version developed by Bio-Rad, were as sensitive as intracerebral inoculation tests in conventional RIII mice [20, 27]. It was also shown that rapid tests can also detect the accumulation of PrP^{res} in nerve tissue before the appearance of clinical signs [3, 26].

In 2002 and 2004, 15 new tests were evaluated by the Directorate General for Health and Consumer Protection by a similar

procedure, albeit on fewer samples[2]. Nine of the tests were approved for the post-mortem diagnosis of BSE: Prionics-Check LIA and Prionics-Check Prio-Strip (both from Prionics), TSE Kit Version 2.0 (Enfer), CDI-5 (InPro, San Francisco, USA), Ceditect BSE (Cedi, Lelystad, The Netherlands), HerdChek BSE Test Kit (IDEXX, Westbrook, USA), Speed'it BSE (Institut Pourquier, Montpellier, France), Beta Prion BSE EIA (Roboscreen, Leipzig, Germany) and PrionScreen (Roche, Basel, Switzerland).

Most of these new validated tests work in an ELISA format except Prio-Strip (lateral flow technology). However, three are based on markedly different principles. InPro's CDI-5 and Cedi's Ceditect BSE detect PrP^{Sc} by taking advantage of the fact that its immunoreactivity increases upon denaturation due to the unmasking of cryptic epitopes [63, 64]. HerdChek BSE from IDEXX is unique in two aspects, in that it does not use proteinase K digestion, and uses an aggregate specific capture ligand on a dextran polymer (Seprion ligand technology, Microsens Biotechnologies, London, UK) of PrP^{Sc}, which after denaturation is detected using an anti-PrP antibody.

Today, virtually all testing of cattle is done with the tests from Bio-Rad, Prionics, IDEXX, and Enfer. Some of these tests have also proved effective in diagnosing chronic wasting disease in wild ruminants [31].

From 2002 to 2004, five tests validated for the post-mortem diagnosis of BSE in cattle were provisionally approved for the post-mortem diagnosis of TSE in small ruminants[3]: TeSeE (Bio-Rad), TSE Kit (Enfer), CDI-5 (InPro), Prionics-Check LIA and Prionics-Check Western (both Prionics). Between 2004 and 2005, the European Commission specifically assessed nine tests for application to small ruminants, and recommended eight of them: TeSeE and TeSeE sheep/goat (Bio-Rad), TSE post-mortem test (IDEXX), Prionics-Check Western SR and Prionics-Check LIA SR (both from Prionics), Enfer TSE test Version 2.0 (Enfer), CDI-5 (InPro), Institut Pourquier Scrapie ELISA test (Institut Pourquier). It should be noted, however, that only the first three of these effectively detect atypical scrapie (Nor98) in brain stem samples. In practice, almost all testing on small ruminants is now done with the Bio-Rad, Prionics, IDEXX, and Enfer tests.

Note too that all results recorded using the rapid tests are confirmed in national reference laboratories, essentially using histopathology, immunohistochemistry, and Western blotting.

2.2.3. *Large-scale use of rapid tests*

Between 1st of January 2001 and 31st of December 2006, nearly 60 million tests on cattle within the European Community (almost 90% at the slaughterhouse) detected over 4 800 cases of BSE, approximately 1 170 at the slaughterhouse and about 3 700 in at-risk animals collected in rendering plants[4]. Over the same period, passive surveillance detected only 2 361 cases of BSE.

Rapid tests have therefore contributed significantly to consumer protection, first by providing a basis for confidence in meat safety, and secondly, because they led to the withdrawal of over 1 000 infected carcasses from human consumption. In addition to the increased safety they provide, these large-scale analyses have detected BSE in

[2] The evaluation of five rapid tests for the diagnosis of spongiform encephalopathy in bovines (2nd study), 27 March 2002, http://ec.europa.eu/food/food/biosafety/bse/sci_advice_en.htm, and scientific report of the European Food Safety Authority on the evaluation of seven new rapid post mortem BSE tests, 16 November 2004, http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620780462.htm [consulted January 2008].

[3] Scientific report of the European Food Safety Authority on the evaluation of rapid post mortem TSE tests intended for small ruminants, adopted on 17 May 2005, http://efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620780483.htm [consulted on January 2008].

[4] Reports on the monitoring and testing for the presence of TSE in the EU, 2001–2006, http://ec.europa.eu/food/food/biosafety/bse/annual_reps_en.htm, and monthly report of Member States on BSE and TSE, http://ec.europa.eu/food/food/biosafety/bse/mthly_reps_en.htm [consulted on January 2008].

numerous European countries (Austria, Czech Republic, Finland, Germany, Greece, Holland, Italy, Luxembourg, Poland, Slovakia, Slovenia, Spain), as well as in Japan, Canada, and the USA. They have also shown that many countries with no recorded case up to 2000 (e.g. Germany, Italy, and Spain), and which denied the presence of BSE, had an incidence equivalent to or higher than that of France and Switzerland, which have been recording cases since the early 1990s. Lastly, rapid tests allow much more precise epidemiological follow-up, allowing the measurement of trends at low prevalence, and which has more clearly shown a spectacular decline in the BSE epizootic in Europe.

Active monitoring of TSE in small ruminants (sheep and goats) was set up in Europe in March 2002, essentially to gather epidemiological data, and obliges member states of the European Community to test a quota of animals slaughtered normally or from at-risk populations. Between 2002 and 2006, nearly three million tests were performed, which led to the detection of over 13 000 cases of scrapie[4]. Note that this active surveillance resulted in the detection of a great many cases of so-called atypical scrapie among European livestock. This form of scrapie, which very likely corresponds to strain Nor98 [7] identified in 1998 in Norway, now accounts for over 50% of TSE cases in small ruminants in many countries (France, Germany, Portugal, UK, etc.). The PrP^{Sc} associated with this strain is characterised by increased sensitivity to proteinase K, which makes its detection more difficult and explains why numerous rapid tests perform poorly in diagnosis. In practice, the vast majority of cases of atypical scrapie were identified using the tests from Bio-Rad (TeSeE since 2002) and IDEXX (post-mortem test, since 2005).

In view of the diversity of TSE strains present in small ruminants, the European Commission set up biochemical typing in 2005, mainly designed to identify the BSE strain in small ruminant populations[5]. Testing, performed exclusively in national reference laboratories, is based on Western blotting techniques [74, 76]. It is worth noting that, to date, only a single case of BSE, in a goat, was observed in France [21] before the implementation of this new regulation.

3. NEW APPROACHES TO ANTE-MORTEM TESTS

As we have seen, diagnosis of prion diseases depends principally on the detection of the abnormal form of PrP (PrP^{Sc} or PrP^{res}). This approach has been very useful in reacting to the BSE epizootic and in setting up active surveillance for TSE in ruminants, but to date has not met all the requirements of the diagnosis of prion diseases. These tests are only applicable to tissues collected after the death of the animal and so cannot be used for early preclinical diagnosis. So far, no test can give a reliable diagnosis using a readily available sample from a living animal or person, such as blood or urine. The problem is particularly acute for blood transfusion, insofar as it is now well established that vCJD can be transmitted by blood. Considerable effort has been devoted to the search for alternative markers enabling earlier diagnosis of TSE (for a review see Parveen et al. [55]).

3.1. The search for new markers

The search for alternative markers has grown greatly in recent years, boosted by the development of postgenomic approaches, which can be used for large-scale parallel analysis of the transcriptome, proteome, and metabolome of tissues. Attention naturally first turned to neuronal markers, which include protein 14-3-3 [28, 78], neurone-specific enolase [1], the protein S100B [5, 29], glial acidic fibrillar protein [44, 50], Tau protein [51], and prionins [59]. However, none of these markers has proved usable as a basis for a sufficiently sensitive and specific test allowing early preclinical diagnosis.

[5] Commission Regulation (EC) No 36/2005 of 12 January 2005 amending Annexes III and X to Regulation (EC) No 999/2001 of the European Parliament and of the Council as regards epidemio-surveillance for transmissible spongiform encephalopathies in bovine, ovine and caprine animals: http://ec.europa.eu/food/food/biosafety/bse/legisl_en.htm [consulted 11 January 2008].

Metabolic markers, such as fatty acid-binding proteins, interferon γ, prostaglandin E2, C-reactive protein, interleukin 6, cystatin C, and corticosteroids, have also been studied, but with no more success (for a review see Parveen et al. [55]).

Transcriptomic studies have revealed potential markers [70, 85], but to date none has proved of practical use in the diagnosis of prion diseases. Erythroid differentiation-related factor, for example, initially seemed highly promising (downregulation [43]), but its value was not confirmed in subsequent work [25].

Finally, some research groups have developed an approach based on serum analysis by Fourier transform infrared spectroscopy combined with data processing by the neural network method [13, 39, 40, 69, 77]. This approach has shown high (> 90%) sensitivity and specificity in cattle populations, but it remains to be seen whether it is usable under routine conditions, and can be used to make an early diagnosis of TSE.

3.2. Protein misfolding cyclic amplification

To facilitate preclinical detection of prions in peripheral tissues, notably blood, Claudio Soto's group developed an original approach in which the PrP^{Sc} in a sample is amplified by means of protein misfolding cyclic amplification (PMCA) [62]. In this approach, which seeks to mimic pathological processes and is akin to the polymerase chain reaction used to amplify DNA (but without addition of exogenous polymerase enzyme), PrP^{Sc} is incubated in the presence of excess PrP^{C} to allow expansion of aggregates of PrP^{Sc} which are then dispersed by sonication to generate smaller units and to encourage the formation of new aggregates. The quantity of PrP^{Sc} formed depends on the number of expansion/sonication cycles performed. In early articles [62, 72], amplification was modest (10- to 50-fold), but optimisation and automation subsequently enabled amplifications of several million fold [61]. In most studies, amplification is achieved by using as a source of PrP^{C}, a brain extract from the same species as that which produced the PrP^{Sc} to be amplified. Recent works [18, 19] have shown that PrP^{Sc} can be replicated in a more controlled "minimal" system in the presence of highly purified PrP^{C} (the only identified contaminant being lipids) and polyanions (polyA RNA in these studies).

Although most of the work by Soto's group concerns a hamster model infected by strain 263K, significant amplification has been achieved with the PrP^{Sc} produced by various mammalian species, including mice [47, 72], sheep, goats and cattle [72], cervids [38] and humans [36]. The PrP^{Sc} newly formed by PMCA has all the properties of the original PrP^{Sc}, notably its infectious character [14, 82]. Lastly, early detection of PrP^{Sc} in hamster blood fractions (buffy coats) was achieved at a sensitivity ranging between 0 and 89% and a specificity of 100% [15, 60].

PMCA has great potential and is certainly the most promising approach from the viewpoint of developing a blood test. It is, though, hampered by various fundamental and technical difficulties. Given the requirements imposed by a blood test (see paragraph below), notably in terms of practicability, sensitivity, and specificity, several technical improvements are needed. For adaptation to routine analysis, there is a need for simplification, reduction of the duration, and better control. Moreover, the obligatory requirement for a concentrated source of PrP^{C} (brain extract or purified PrP^{C}) of the same species as the target to be amplified constitutes an important practical handicap. This specific problem could be resolved by the use of recombinant PrP and accelerated procedures as recently shown [4], assuming the results obtained with the hamster model can be extended to other mammalian species. However, PMCA must also prove effective in terms of diagnosis (sensitivity and specificity close to 100%) using blood sample series more representative than those obtained with the hamster model. Finally, recent results from Supattapone's group show that infectious PrP^{Sc} can be generated de novo and stochastically by PMCA [18] in the absence of pre-existing prions, and this raises concerns about the specificity of this approach when used in routine conditions.

3.3. Blood tests: state of the art

As we have emphasised several times in this review, the development of a blood test is the top priority in prion disease diagnosis, notably to ensure the safety of blood transfusion in humans. Numerous difficulties, however, have to be overcome, which explains why no test is yet operational. Whereas blood from vCJD infected patients is clearly infectious [41, 56, 84], its concentration of infectious material is very likely much lower than that in the central nervous system, and its concentrations of PrP^{Sc} are estimated to be in the range of pg/mL [8, 9]. Given the efficacy of disease transmission by the intravenous route, and the large volume (commonly > 400 mL) of packed red blood cells transfused in humans, transmission can occur with very low levels of infectious material, and, as a consequence, candidate tests must have excellent analytical sensitivity. Also, blood is a complex tissue rich in cells and proteins, and little is known of the distribution of prions (and of PrP^{Sc}). Several studies indicate that the bulk of the infectious material is in the white blood cells, but the plasma is also clearly infectious [8, 9]. In a healthy individual, significant levels of PrP^{C} are present in white blood cells, red blood cells, platelets, and plasma, probably at much higher concentrations than PrP^{Sc}. A candidate test must therefore also be very selective. Also, we know very little about the biochemical properties of the PrP^{Sc} in the different blood fractions. Given its low concentration and its environment, it is not certain, for instance, that it can form aggregates resistant to proteinase K, the treatment on which most current rapid tests are based.

In terms of the risk of vCJD infection by blood transfusion, because the incidence of the disease is assumed to be very low, a highly specific test is needed, or it could lead to more false-positive results than detection of real cases. Such a situation would be very difficult to manage ethically, given that vCJD is a fatal disease for which at present there is no treatment. There is clearly a great need for at least one very specific confirmation test, which does not exist today.

Due to the above mentioned difficulties it is not surprising that very few publications report on blood tests for TSE.

The first promising results were obtained, as early as 1996, by the group of Mary-Jo Schmerr, which combined capillary electrophoresis with a competitive immunoassay to detect a PK resistant C-terminal sequence of PrP in the blood of sheep infected with scrapie [66–68]. The technique was subsequently improved and applied to more relevant series of scrapie infected sheep [34, 35, 42, 86], but despite achieved improvement, the method appeared insufficiently robust for routine use [22].

In recent years, many research groups or companies have developed original strategies to try to overcome the intrinsic difficulties associated with the blood test. These include:

- The use of ligands for a specific capture of PrP^{Sc} possibly present in blood fractions, which include the 15B3 antibody produced by Prionics [37, 48] and the Seprion resin of the Microsense company (already used in the IDEXX test for post-mortem diagnosis). In both cases, the idea is to concentrate abnormal PrP by immunoprecipitation, taking advantage of its polymerisation state (aggregate? polymers? oligomers?) to allow a more sensitive and more specific detection by ELISA or flow cytometry. Another approach developed by the bioMérieux company (Marcy l'Étoile, France) involves binding and aggregation of abnormal PrP in plasma by streptomycin [45], followed by a specific capture on calyx-Arenes "molecular basket" immobilised onto a solid phase, and final detection with an appropriate anti-PrP antibody.
- The development of immunoassays designed to detect polymerised PrP (AS-ELISA, for aggregate specific ELISA) and based on the use of the same monoclonal antibody for capture and detection [52]. The sensitivity of AS-ELISA was increased by combining signal amplification (fluorescence) and target amplification (prion amplification using a simplified PMCA like procedure). Using

this approach (named Am-A-FACTT) the group of Man-Sun Sy succeeded in detecting prion aggregates in plasma from mice or deer infected with scrapie or CWD respectively [16]. A similar approach has been developed by the Korean company PeopleBio (Seoul, Korea, Multimer Detection System (MDS)) without amplification of signal and target but details remain unpublished.

– The use of fluorescence labelled palindromic PrP peptides to detect misfolded PrP (MPD for misfolded protein diagnostic). In this approach, when the labelled peptide is in contact with PrP^{Sc}, it undergoes a large coil to a β-sheet conformational change which largely modifies the fluorescence properties of the pyrene label [30]. This method allowed discrimination between TSE infected and uninfected animals, albeit on a rather small series of blood samples [53].

However, even if some of these approaches seem promising, for the moment none of these tests has fulfilled the very strict analytical and diagnostic requirements described above. With the passage of time (some of these approaches were initially described a few years ago) it becomes apparent that they are facing real difficulties in establishing routine and robust assays, and that much more time and development is needed to achieve the goal of an operational blood test for TSE.

4. CONCLUSIONS

The successive "mad cow" crises of 1996 and 2000 have clearly boosted very significantly research in the field of prion diseases, and more data have been accumulated during the last ten years than during the previous century. This has considerably improved our knowledge on prion biology, but also provided much more relevant tools including: transgenic mice ($PrP^{0/0}$ or over-expressing various forms of wild-type or mutated PrP), cellular models of TSE infection, a large series of well characterised monoclonal antibodies and, of course, much more relevant analytical methods and diagnostic tests. As far as diagnosis is concerned, very significant progress has been made in the post-mortem detection of PrP^{Sc}, with the development of reliable and very sensitive methods suitable for routine analysis (results available within less than three hours, more than 20 000 tests performed every day throughout the world), having the capacity of diagnosing TSE before the onset of clinical signs. These tests have been used efficiently for managing the mad cow crisis, and are still very useful for monitoring the BSE epizootic as well as the various forms of TSE in small ruminants and cervids. The analytical sensitivity of these tests can now be considerably improved by coupling PMCA amplification with the appropriate detection techniques (ELISA, CDI, Western-blot), and this allows detection of minute amounts of PrP^{Sc} in the brain or in peripheral lymphoid tissues. However, so far, there is no test that delivers an early and specific diagnosis of TSE in live animals or patients, i.e. a test which can be easily applied to a body fluid like blood or urine. This is particularly critical for ensuring the safety of blood transfusion in countries that have experienced a large BSE epizootic (UK and Western Europe). We have seen that PMCA has shown a good potential, in terms of sensitivity, for achieving such an aim but its use in routine conditions and its actual specificity are questionable. There is thus a place for another approach, and the development of a blood test for TSE diagnosis remains the most important challenge for the years coming in this field of prion research.

REFERENCES

[1] Aksamit A.J. Jr., Preissner C.M., Homburger H.A., Quantitation of 14-3-3 and neuron-specific enolase proteins in CSF in Creutzfeldt-Jakob disease, Neurology (2001) 57:728–730.

[2] Andréoletti O., Berthon P., Marc D., Sarradin P., Grosclaude J., van Keulen L., et al., Early accumulation of PrP(Sc) in gut-associated lymphoid and nervous tissues of susceptible sheep from a Romanov flock with natural scrapie, J. Gen. Virol. (2000) 81:3115–3126.

[3] Arnold M.E., Ryan J.B., Konold T., Simmons M.M., Spencer Y.I., Wear A., et al., Estimating the temporal relationship between PrPSc detection and incubation period in experimental bovine spongiform encephalopathy of cattle, J. Gen. Virol. (2007) 88:3198–3208.

[4] Atarashi R., Moore R.A., Sim V.L., Hughson A.G., Dorward D.W., Onwubiko H.A., et al., Ultrasensitive

detection of scrapie prion protein using seeded conversion of recombinant prion protein, Nat. Methods. (2007) 4:645–650.

[5] Beekes M., Otto M., Wiltfang J., Bahn E., Poser S., Baier M., Late increase of serum S100 beta protein levels in hamsters after oral or intraperitoneal infection with scrapie, J. Infect. Dis. (1999) 180:518–520.

[6] Bendheim P.E., Bolton D.C., A 54-kDa normal cellular protein may be the precursor of the scrapie agent protease-resistant protein, Proc. Natl. Acad. Sci. USA (1986) 83:2214–2218.

[7] Benestad S.L., Sarradin P., Thu B., Schonheit J., Tranulis M.A., Bratberg B., Cases of scrapie with unusual features in Norway and designation of a new type, Nor98, Vet. Rec. (2003) 153:202–208.

[8] Brown P., Blood infectivity, processing and screening tests in transmissible spongiform encephalopathy, Vox Sang. (2005) 89:63–70.

[9] Brown P., Cervenakova L., Diringer H., Blood infectivity and the prospects for a diagnostic screening test in Creutzfeldt-Jakob disease, J. Lab. Clin. Med. (2001) 137:5–13.

[10] Brown P., Will R.G., Bradley R., Asher D.M., Detwiler L., Bovine spongiform encephalopathy and variant Creutzfeldt-Jakob disease: background, evolution, and current concerns, Emerging Infect. Dis. (2001) 7:6–16.

[11] Bruce M.E., Will R.G., Ironside J.W., McConnell I., Drummond D., Suttie A., *et al.*, Transmissions to mice indicate that "new variant" CJD is caused by the BSE agent, Nature (1997) 389:498–501.

[12] Calavas D., Ducrot C., Baron T., Morignat E., Vinard J.L., Biacabe A.G., et al., Prevalence of BSE in western France by screening cattle at risk: preliminary results of a pilot study, Vet. Rec. (2001) 149:55–56.

[13] Carmona P., Monleon E., Monzon M., Badiola J.J., Monreal J., Raman analysis of prion protein in blood cell membranes from naturally affected scrapie sheep, Chem. Biol. (2004) 11:759–764.

[14] Castilla J., Saa P., Hetz C., Soto C., In vitro generation of infectious scrapie prions, Cell (2005) 121:195–206.

[15] Castilla J., Saa P., Soto C., Detection of prions in blood, Nat. Med. (2005) 11:982–985.

[16] Chang B., Cheng X., Yin S., Pan T., Zhang H., Wong P., et al., Test for detection of disease-associated prion aggregate in the blood of infected but asymptomatic animals, Clin. Vaccine Immunol. (2007) 14:36–43.

[17] Curin S., V. Bresjanac M., Popovic M., Pretnar H.K., Galvani V., Rupreht R., et al., Monoclonal antibody against a peptide of human prion protein discriminates between Creutzfeldt-Jacob's disease-affected and normal brain tissue, J. Biol. Chem. (2004) 279:3694–3698.

[18] Deleault N.R., Harris B.T., Rees J.R., Supattapone S., From the cover: formation of native prions from minimal components in vitro, Proc. Natl. Acad. Sci. USA (2007) 104:9741–9746.

[19] Deleault N.R., Lucassen R.W., Supattapone S., RNA molecules stimulate prion protein conversion, Nature (2003) 425:717–720.

[20] Deslys J.P., Comoy E., Hawkins S., Simon S., Schimmel H., Wells G., et al., Screening slaughtered cattle for BSE, Nature (2001) 409:476–478.

[21] Eloit M., Adjou K., Coulpier M., Fontaine J.J., Hamel R., Lilin T., et al., BSE agent signatures in a goat, Vet. Rec. (2005) 156:523–524.

[22] Everest D.J., Waterhouse S., Kelly T., Velo-Rego E., Sauer M.J., Effectiveness of capillary electrophoresis fluoroimmunoassay of blood PrpSc for evaluation of scrapie pathogenesis in sheep, J. Vet. Diagn. Invest. (2007) 19:552–557.

[23] Fraser H., The pathology of a natural and experimental scrapie, Front. Biol. (1976) 44:267–305.

[24] Gavier-Widen D., Stack M.J., Baron T., Balachandran A., Simmons M., Diagnosis of transmissible spongiform encephalopathies in animals: a review, J. Vet. Diagn. Invest. (2005) 17:509–527.

[25] Glock B., Winter M., Rennhofer S.O., Brunholzl E., Troscher D., Reisacher R.B., Mayr W.R., Transcript level of erythroid differentiation-related factor, a candidate surrogate marker for transmissible spongiform encephalopathy diseases in blood, shows a broad range of variation in healthy individuals, Transfusion (2003) 43:1706–1710.

[26] Grassi J., Comoy E., Simon S., Creminon C., Frobert Y., Trapmann S., et al., Rapid test for the preclinical postmortem diagnosis of BSE in central nervous system tissue, Vet. Rec. (2001) 149:577–582.

[27] Grassi J., Creminon C., Frobert Y., Fretier P., Turbica I., Rezaei H., et al., Specific determination of the proteinase K-resistant form of the prion protein using two-site immunometric assays. Application to the post-mortem diagnosis of BSE, Arch. Virol. Suppl. (2000) 16:197–205.

[28] Green A.J., Cerebrospinal fluid brain-derived proteins in the diagnosis of Alzheimer's disease and Creutzfeldt-Jakob disease, Neuropathol. Appl. Neurobiol. (2002) 28:427–440.

[29] Green A.J., Jackman R., Marshall T.A., Thompson E.J., Increased S-100b in the cerebrospinal fluid of some cattle with bovine spongiform encephalopathy, Vet. Rec. (1999) 145:107–109.

[30] Grosset A., Moskowitz K., Nelsen C., Pan T., Davidson E., Orser C.S., Rapid presymptomatic detection of PrPSc via conformationally responsive palindromic PrP peptides, Peptides (2005) 26:2193–2200.

[31] Hibler C.P., Wilson K.L., Spraker T.R., Miller M.W., Zink R.R., DeBuse L.L., et al., Field validation and assessment of an enzyme-linked immunosorbent assay for detecting chronic wasting disease in mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), and Rocky Mountain elk (*Cervus elaphus nelsoni*), J. Vet. Diagn. Invest. (2003) 15:311–319.

[32] Hilton D.A., Ghani A.C., Conyers L., Edwards P., McCardle L., Penney M., et al., Accumulation of prion protein in tonsil and appendix: review of tissue samples, BMJ (2002) 325:633–634.

[33] Hilton D.A., Ghani A.C., Conyers L., Edwards P., McCardle L., Ritchie D., et al., Prevalence of lymphoreticular prion protein accumulation in UK tissue samples, J. Pathol. (2004) 203:733–739.

[34] Jackman R., Everest D.J., Schmerr M.J., Khawaja M., Keep P., Docherty J., Evaluation of a preclinical blood test for scrapie in sheep using immunocapillary electrophoresis, J. AOAC Int. (2006) 89:720–727.

[35] Jackman R., Schmerr M.J., Analysis of the performance of antibody capture methods using fluorescent peptides with capillary zone electrophoresis with laser-induced fluorescence, Electrophoresis (2003) 24:892–896.

[36] Jones M., Peden A., Prowse C., Groner A., Manson J., Turner M., et al., In vitro amplification and detection of variant Creutzfeldt-Jakob disease PrP(Sc), J. Pathol. (2007) 213:21–26.

[37] Korth C., Stierli B., Streit P., Moser M., Schaller O., Fischer R., et al., Prion (PrPSc)-specific epitope defined by a monoclonal antibody, Nature (1997) 390:74–77.

[38] Kurt T.D., Perrott M.R., Wilusz C.J., Wilusz J., Supattapone S., Telling G.C., et al., Efficient in vitro amplification of chronic wasting disease PrPres, J. Virol. (2007) 81:9605–9608.

[39] Lasch P., Beekes M., Schmitt J., Naumann D., Detection of preclinical scrapie from serum by infrared spectroscopy and chemometrics, Anal. Bioanal. Chem. (2007) 387:1791–1800.

[40] Lasch P., Schmitt J., Beekes M., Udelhoven T., Eiden M., Fabian H., et al., Antemortem identification of bovine spongiform encephalopathy from serum using infrared spectroscopy, Anal. Chem. (2003) 75:6673–6678.

[41] Llewelyn C.A., Hewitt P.E., Knight R.S., Amar K., Cousens S., Mackenzie J., Will R.G., Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion, Lancet (2004) 363:417–421.

[42] Lourenco P.C., Schmerr M.J., MacGregor I., Will R.G., Ironside J.W., Head M.W., Application of an immunocapillary electrophoresis assay to the detection of abnormal prion protein in brain, spleen and blood specimens from patients with variant Creutzfeldt-Jakob disease, J. Gen. Virol. (2006) 87:3119–3124.

[43] Miele G., Manson J., Clinton M., A novel erythroid-specific marker of transmissible spongiform encephalopathies, Nat. Med. (2001) 7:361–364.

[44] Missler U., Wiesmann M., Wittmann G., Magerkurth O., Hagenstrom H., Measurement of glial fibrillary acidic protein in human blood: analytical method and preliminary clinical results, Clin. Chem. (1999) 45:138–141.

[45] Moussa A., Coleman A.W., Bencsik A., Leclere E., Perret F., Martin A., Perron H., Use of streptomycin for precipitation and detection of proteinase K resistant prion protein (PrPsc) in biological samples, Chem. Commun. (Camb.) (2006) 9:973–975.

[46] Moynagh J., Schimmel H., Tests for BSE evaluated. Bovine spongiform encephalopathy, Nature (1999) 400:105.

[47] Murayama Y., Yoshioka M., Horii H., Takata M., Yokoyama T., Sudo T., et al., Protein misfolding cyclic amplification as a rapid test for assessment of prion inactivation, Biochem. Biophys. Res. Commun. (2006) 348:758–762.

[48] Nazor K.E., Kuhn F., Seward T., Green M., Zwald D., Purro M., et al., Immunodetection of disease-associated mutant PrP, which accelerates disease in GSS transgenic mice, EMBO J. (2005) 24:2472–2480.

[49] Oesch B., Doherr M., Heim D., Fischer K., Egli S., Bolliger S., et al., Application of prionics Western blotting procedure to screen for BSE in cattle regularly slaughtered at Swiss abattoirs, Arch. Virol. Suppl. (2000) 16:189–195.

[50] Otto M., Wiltfang J., Differential diagnosis of neurodegenerative diseases with special emphasis on Creutzfeldt-Jakob disease, Restor. Neurol. Neurosci. (2003) 21:191–209.

[51] Otto M., Wiltfang J., Cepek L., Neumann M., Mollenhauer B., Steinacker P., et al., Tau protein and 14-3-3 protein in the differential diagnosis of Creutzfeldt-Jakob disease, Neurology (2002) 58:192–197.

[52] Pan T., Chang B., Wong P., Li C., Li R., Kang S.C., et al., An aggregation-specific enzyme-linked immunosorbent assay: detection of conformational differences between recombinant PrP protein dimers and PrP(Sc) aggregates, J. Virol. (2005) 79:12355–12364.

[53] Pan T., Sethi J., Nelsen C., Rudolph A., Cervenakova L., Brown P., Orser C.S., Detection of misfolded prion protein in blood with conformationally sensitive peptides, Transfusion (2007) 47:1418–1425.

[54] Paramithiotis E., Pinard M., Lawton T., LaBoissiere S., Leathers V.L., Zou W.Q., et al., A prion protein epitope selective for the pathologically misfolded conformation, Nat. Med. (2003) 9:893–899.

[55] Parveen I., Moorby J., Allison G., Jackman R., The use of non-prion biomarkers for the diagnosis of transmissible spongiform encephalopathies in the live animal, Vet. Res. (2005) 36:665–683.

[56] Peden A.H., Head M.W., Ritchie D.L., Bell J.E., Ironside J.W., Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient, Lancet (2004) 364:527–529.

[57] Prusiner S.B., Prions, Proc. Natl. Acad. Sci. USA (1998) 95:13363–13383.

[58] Prusiner S.B., Scott M.R., DeArmond S.J., Cohen F.E., Prion protein biology, Cell (1998) 93:337–348.

[59] Ruth L., The quest for new prion tests, Anal. Chem. (2003) 75:32A–36A.

[60] Saa P., Castilla J., Soto C., Presymptomatic detection of prions in blood, Science (2006) 313:92–94.

[61] Saa P., Castilla J., Soto C., Ultra-efficient replication of infectious prions by automated protein misfolding cyclic amplification, J. Biol. Chem. (2006) 281:35245–35252.

[62] Saborio G.P., Permanne B., Soto C., Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding, Nature (2001) 411:810–813.

[63] Safar J., Wille H., Itri V., Groth D., Serban H., Torchia M., et al., Eight prion strains have PrP(Sc) molecules with different conformations, Nat. Med. (1998) 4:1157–1165.

[64] Safar J.G., Scott M., Monaghan J., Deering C., Didorenko S., Vergara J., et al., Measuring prions causing bovine spongiform encephalopathy or chronic wasting disease by immunoassays and transgenic mice, Nat. Biotechnol. (2002) 20:1147–1150.

[65] Schaller O., Fatzer R., Stack M., Clark J., Cooley W., Biffiger K., et al., Validation of a western immunoblotting procedure for bovine PrP(Sc) detection and its use as a rapid surveillance method for the diagnosis of bovine spongiform encephalopathy (BSE), Acta Neuropathol. (1999) 98:437–443.

[66] Schmerr M.J., Cutlip R.C., Jenny A., Capillary isoelectric focusing of the scrapie prion protein, J. Chromatogr. A (1998) 802:135–141.

[67] Schmerr M.J., Goodwin K.R., Cutlip R.C., Jenny A.L., Improvements in a competition assay to detect scrapie prion protein by capillary electrophoresis, J. Chromatogr. B, Biomed. Appl. (1996) 681:29–35.

[68] Schmerr M.J., Jenny A.L., Bulgin M.S., Miller J.M., Hamir A.N., Cutlip R.C., Goodwin K.R., Use of capillary electrophoresis and fluorescent labeled peptides to detect the abnormal prion protein in the blood of animals that are infected with a transmissible spongiform encephalopathy, J. Chromatogr. A (1999) 853:207–214.

[69] Schmitt J., Beekes M., Brauer A., Udelhoven T., Lasch P., Naumann D., Identification of scrapie infection from blood serum by Fourier transform infrared spectroscopy, Anal. Chem. (2002) 74:3865–3868.

[70] Schutz E., Urnovitz H.B., Iakoubov L., Schulz-Schaeffer W., Wemheuer W., Brenig B., Bov-tA short interspersed nucleotide element sequences in circulating nucleic acids from sera of cattle with bovine spongiform encephalopathy (BSE) and sera of cattle exposed to BSE, Clin. Diagn. Lab Immunol. (2005) 12:814–820.

[71] Simmons M.M., Spiropoulos J., Hawkins S.A.C., Bellworthy S.J., Tongue S.C., Approaches to investigating transmission of spongiform encephalopathies in domestic animals using BSE as an exemple, Vet. Res. (2008) 39:34.

[72] Soto C., Anderes L., Suardi S., Cardone F., Castilla J., Frossard M.J., et al., Pre-symptomatic detection of prions by cyclic amplification of protein misfolding, FEBS Lett. (2005) 579:638–642.

[73] Spraker T.R., O'Rourke K.I., Balachandran A., Zink R.R., Cummings B.A., Miller M.W., Powers B.E., Validation of monoclonal antibody F99/97.6.1 for immunohistochemical staining of brain and tonsil in mule deer (*Odocoileus hemionus*) with chronic wasting disease, J. Vet. Diagn. Invest. (2002) 14:3–7.

[74] Stack M., Jeffrey M., Gubbins S., Grimmer S., Gonzalez L., Martin S., et al., Monitoring for bovine spongiform encephalopathy in sheep in Great Britain, 1998–2004, J. Gen. Virol. (2006) 87:2099–2107.

[75] Stack M., Jeffrey M., Gubbins S., Grimmer S., Gonzalez L., Martin S., et al., Monitoring for bovine spongiform encephalopathy in sheep in Great Britain, 1998–2004, J. Gen. Virol. (2006) 87:2099–2107.

[76] Stack M.J., Chaplin M.J., Clark J., Differentiation of prion protein glycoforms from naturally occurring sheep scrapie, sheep-passaged scrapie strains (CH1641 and SSBP1), bovine spongiform encephalopathy (BSE) cases and Romney and Cheviot breed sheep experimentally inoculated with BSE using two monoclonal antibodies, Acta Neuropathol. (2002) 104:279–286.

[77] Thomzig A., Spassov S., Friedrich M., Naumann D., Beekes M., Discriminating scrapie and bovine spongiform encephalopathy isolates by infrared spectroscopy of pathological prion protein, J. Biol. Chem. (2004) 279:33847–33854.

[78] Tyler J.W., Lakritz J., Weaver D., Johnson G., VanMetre D., Smith K., et al., The 14-3-3 cerebrospinal fluid immunoassay lacks utility in the diagnosis of clinical scrapie, J. Vet. Diagn. Invest. (2001) 13:537–539.

[79] van Keulen L.J., Schreuder B.E., Meloen R.H., Mooij-Harkes G., Vromans M.E., Langeveld J.P., Immunohistochemical detection of prion protein in lymphoid tissues of sheep with natural scrapie, J. Clin. Microbiol. (1996) 34:1228–1231.

[80] van Keulen L.J., Schreuder B.E., Meloen R.H., Poelen-van den B.M., Mooij-Harkes G., Vromans M.E., Langeveld J.P., Immunohistochemical detection and localization of prion protein in brain tissue of sheep with natural scrapie, Vet. Pathol. (1995) 32:299–308.

[81] Wadsworth J.D., Joiner S., Hill A.F., Campbell T.A., Desbruslais M., Luthert P.J., Collinge J., Tissue distribution of protease resistant prion protein in variant Creutzfeldt-Jakob disease using a highly sensitive immunoblotting assay, Lancet (2001) 358:171–180.

[82] Weber P., Giese A., Piening N., Mitteregger G., Thomzig A., Beekes M., Kretzschmar H.A., Generation of genuine prion infectivity by serial PMCA, Vet. Microbiol. (2007) 123:346–357.

[83] Wells G.A., Wilesmith J.W., The neuropathology and epidemiology of bovine spongiform encephalopathy, Brain Pathol. (1995) 5:91–103.

[84] Wroe S.J., Pal S., Siddique D., Hyare H., Macfarlane R., Joiner S., et al., Clinical presentation and pre-mortem diagnosis of variant Creutzfeldt-Jakob disease associated with blood transfusion: a case report, Lancet (2006) 368:2061–2067.

[85] Xiang W., Windl O., Wunsch G., Dugas M., Kohlmann A., Dierkes N., et al., Identification of differentially expressed genes in scrapie-infected mouse brains by using global gene expression technology, J. Virol. (2004) 78:11051–11060.

[86] Yang W.C., Schmerr M.J., Jackman R., Bodemer W., Yeung E.S., Capillary electrophoresis-based noncompetitive immunoassay for the prion protein using fluorescein-labeled protein A as a fluorescent probe, Anal. Chem. (2005) 77:4489–4494.

[87] Zou W.Q., Zheng J., Gray D.M., Gambetti P., Chen S.G., Antibody to DNA detects scrapie but not normal prion protein, Proc. Natl. Acad. Sci. USA (2004) 101:1380–1385.

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研究報告の概要

本稿では，伝染性海綿状脳症（TSE）の伝播性を調べるための実験的アプローチ法を要約し，実験における所見と自然発生する TSE〔主にウシ海綿状脳症（BSE）及びスクレイピー〕との関連性を考察している。BSE はこれまで影響を受けなかった動物種における新規の海綿状脳症であり，点感染源の特徴を有する。本実験では，人工的感染経路（脳内接種）及び自然感染経路（経口）を用いて伝播の効率ならびに宿主の感受性を，特に食用動物種に焦点を当てて特定した。実験的伝播が認められても，曝露時の動物の年齢等，種々のパラメータの影響を受けることから結果の解釈は常に困難であった。しかしながら，ヒツジでは，BSE 陽性ウシの脳を経口投与した雌ヒツジから，その仔ヒツジへの BSE 伝播が示された。

これとは対照的に，スクレイピーは英国で数世紀にわたりヒツジ個体群において地域固有のものであった。それにもかかわらず，スクレイピーの真の垂直（子宮内）伝播は確認されておらず，一方で水平伝播が確認されている。すなわち，疾患を引き起こすには汚染された環境に曝露するだけで十分であると考えられる。特に胎盤はスクレイピーの自然伝播の原因とされており，感染性の PrP^{Sc} プリオンを含むことが立証されている。現時点では多くの疑問が依然として解明されておらず，結論として著者らは，様々な分野の研究者らに対して TSE の特性をより理解するため協力及び支援を強く呼び掛けている。

報告企業の意見	今後の対応
本概論は，TSE 研究の複雑さを明らかにしており，反芻動物でない，生物学上遠隔種のトランスジェニックマウスを用いた研究であっても，全ての研究結果は有益であり，疾患管理の向上及び公衆衛生を守る上で役立つであろう。	ヒトに影響するプリオン関連疾患伝播のメカニズムの更なる理解に関連した調査の情報を収集する以外，現時点で新たな安全対策上の措置を講じる必要はないと考える。

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Review article

Approaches to investigating transmission of spongiform encephalopathies in domestic animals using BSE as an example

Marion Mathieson SIMMONS[1]*, John SPIROPOULOS[1], Stephen Anthony Charles HAWKINS[1], Susan Jane BELLWORTHY[1], Susan Carol TONGUE[2]

[1] Pathology Department, Veterinary Laboratories Agency, Woodham Lane, New Haw, Addlestone, Surrey, KT15 3NB, UK
[2] Centre for Epidemiology and Risk Analysis, Veterinary Laboratories Agency, Woodham Lane, New Haw, Addlestone, Surrey, KT15 3NB, UK

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Abstract – Bovine spongiform encephalopathy was a novel spongiform encephalopathy, in an hitherto unaffected species, that had characteristics of a point source epidemic, with an agent that could have been incorporated into a wide variety of feedstuffs and iatrogenically administered to naïve populations, and there was early evidence that it was not restricted to bovines. It was vital to establish, albeit experimentally, which other species might be affected, and whether the epidemic could be maintained by natural transmission, if the source was removed. In contrast, scrapie has been endemic throughout Great Britain for centuries, is maintained naturally (even if we don't know exactly how) and has a known host range. The principles, process and integration of evidence from different types of studies, however, are similar for both of these transmissible spongiform encephalopathies (TSE) and can be applied to any emerging or suspected spongiform encephalopathy. This review discusses the experimental approaches used to determine TSE transmissibility and infectivity and how they relate to natural disease and control measures.

TSE / transmission / natural / experimental / domestic animals

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* Corresponding author:
m.m.simmons@vla.defra.gsi.gov.uk

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1. INTRODUCTION

1.1. Spongiform encephalopathies of animals

The spongiform encephalopathies of animals include scrapie, chronic wasting disease (CWD), transmissible mink encephalopathy (TME), bovine spongiform encephalopathy (BSE), feline spongiform encephalopathy (FSE), the spongiform encephalopathies seen in non-domestic captive ungulate species such as eland, oryx and greater kudu, and captive ostriches [85–87]. Spongiform change can also be seen in other diseases, such as rabies, other viral diseases [14, 29, 88], and hepatic encephalopathies. They may be encountered as a genetic or congenital problem [62, 63, 102], as an incidental finding in normal sheep [126], or even as an artefact [108].

However, the only observed natural animal-to-animal transmission of a spongiform encephalopathy occurs in ruminants: scrapie in small ruminant species, CWD in deer and elk, and possibly BSE in small ruminants (although this latter example has only been observed in an experimental flock [8]). Natural spongiform encephalopathies in other species, including humans, are either genetic in origin (e.g. Gerstmann-Sträussler-Scheinker disease, fatal familial insomnia) or have been linked predominantly with an idiopathic transmission mechanism i.e. exposure to contaminated feedstuffs (TME, BSE, FSE, and kuru in humans). There is no recorded occurrence of spongiform encephalopathies being able to transmit effectively within non-ruminant species.

The naturally occurring transmissible spongiform encephalopathies (TSE) are invariably fatal, have long incubation periods and provoke no overt immune response in the host. In some, such as scrapie, there are known genetic effects on whether exposure leads to the development of clinical disease [98, 100]. Additional factors that may affect host susceptibility have been proposed [25, 45, 93] and there could be other unconfirmed, or even as yet unidentified, factors that might affect host susceptibility.

1.2. Aim and objectives

An integral part of the classification of spongiform encephalopathies is whether they are transmissible or not. If it is possible to experimentally transmit "to pass or hand on" [4] i.e. transfer the disease, then it has the potential to be naturally infectious. An infectious disease is one that is due to the "transmission of a specific agent, or its toxic products from an infected person, animal, or reservoir to a susceptible host, either directly or indirectly through an intermediate plant or animal host, vector, or the inanimate environment" [71]. This has implications for disease control strategies; different approaches will be needed if there is an infectious component than if the disease was purely due to a nutritional or genetic cause. It should also be noted that an infectious disease may not be contagious – where contagious is defined as "the communication of disease by direct or indirect contact" i.e. it is communicable to other individuals [3].

Experimental approaches to the investigation of whether transmission occurs have become more sophisticated since the start of the 20th century when Cuillé and Chelle first achieved experimental transmission of sheep scrapie via the conjunctival route in France in 1936 [17, 18]. This experimental evidence of transmissibility was confirmed, somewhat unintentionally, by the iatrogenic transmission of scrapie from sheep to sheep via the medium of

a louping ill vaccine, which led to outbreaks in Great Britain during the 1930s [41].

In this review on the transmission of TSE in animals our first objective is to illustrate the route to the designation of a spongiform encephalopathy as "transmissible", through the example of BSE in the 20th century. The knowledge that a spongiform encephalopathy is transmissible then leads to the question of the relevance of experimental findings to the field situation, where the required outcomes are public health protection, disease control and, ultimately, disease eradication. This then is our second objective; to put transmissibility into a "real-world" context. Scrapie and BSE are our main examples, with other TSE of animals referred to where appropriate. We also aim to briefly highlight some of the challenges and unanswered (or unanswerable) questions that are inevitably raised when a novel spongiform encephalopathy is encountered, and its ability to transmit is investigated.

1.3. Definitions

- PrP^{Sc}: "Prion protein". An abnormal isoform of a naturally occurring host protein (PrP^{C}) which is resistant to proteolysis.
- End-stage/clinical disease: presence of clinical signs and PrP^{Sc} in brainstem and/or lymphoreticular system (LRS).
- Positive animal: PrP^{Sc} detectable, regardless of location (i.e. central nervous system (CNS), peripheral nervous system, lymphoreticular system) or clinical status.
- Exposed animal: known challenge with positive material, or contact with positive animals or a contaminated environment. May or may not also be in one of the categories above.
- Negative animal: no detectable PrP^{Sc} in any tissue tested (must include CNS (if animal dead) and/or LRS).
- Negative control: animal from a flock or farm with good records, no recorded TSE and a feeding history which does not include meat and bone meal supplements.
- Vertical transmission: transmission from one generation to the next via the germline or in utero [11].
- Horizontal transmission: lateral spread to others in the same group and at the same time; spread to contemporaries [11].
- Maternal transmission: there is some difficulty in separating possible horizontal and vertical components to transmission involved with the dam-offspring relationship, and so the term "maternal transmission" is often used in discussion of the transmission of scrapie, maternal transmission being defined as transmission before or immediately after birth.

2. CONFIRMATION OF DISEASE AND/OR INFECTION

The absolute nature of the infectious agent poses a unique challenge and is still a contentious issue. Accumulations of disease-specific prion protein (PrP^{Sc}) in the CNS can be demonstrated in all cases of clinical disease, so the detection of PrP^{Sc} is now used to confirm the disease status of a clinically suspect case at post-mortem [76]. PrP^{Sc} accumulations in a variety of tissues can also be seen in the absence of clinical signs and the demonstration of their presence is generally considered as evidence of exposure and infection. However, such PrP^{Sc} accumulation occurs relatively late in the incubation period of the disease [6, 117], so this reliance on the presence of PrP^{Sc} limits in vivo diagnosis of disease, and surveillance for evidence of exposure or infection, with current diagnostic tools [76]. The currently accepted paradigm is that accumulations of PrP^{Sc} are not only associated with disease, but are also associated with transmission and infectivity [92]. Whether it is the sole infectious component is still a subject of some dispute. Firstly, naturally occurring PrP^{Sc}, when used for transmission experiments, is inevitably contained in a suspension of the tissue in which it originated, and therefore the existence of another factor, or factors, coexisting with PrP^{Sc}, and responsible for infectivity cannot be unequivocally excluded. Secondly, disease has been experimentally produced by tissue suspensions from potentially infected animals in which no PrP^{Sc} was demonstrable with current diagnostic tools [69]. However, in order to investigate

transmission of spongiform encephalopathies, all studies currently use the presence of PrP^{Sc} as a confirmatory marker of disease or exposure/infection.

In experimental studies of TSE, the prolonged incubation periods and the availability of resources coupled with welfare considerations may not allow for individual animals to be followed up to the ultimate fatal endpoint. For this reason there is a lexicon of terms that are applied both in experimental studies and surveillance (see Section 1.3.).

3. THE SEARCH FOR EVIDENCE OF TRANSMISSION OF BOVINE SPONGIFORM ENCEPHALOPATHY

Experimental transmission studies in a wide range of recipient species have established that many species are susceptible to parenteral exposure with positive tissue from TSE cases under experimental circumstances (e.g. cattle, sheep, goats, cats, mink, deer, elk, exotic ungulates, primates, laboratory rodents). Detailed reviews of these transmissions have been published recently [52] and will not be repeated here.

3.1. Bovine spongiform encephalopathy – a TSE?

Following the identification of BSE in cattle [107] and its epidemiological link to contaminated feed [118, 119], the major transmission questions to be addressed, as in any other new disease, were:

- Can it be transmitted?
- Who or what can it be transmitted to in order to determine the potential host range, which food animal species are susceptible, and if there is a public health risk?
- How much is required to achieve transmission/infection, to define infectious dose and host susceptibility?

Then, if and when transmission is achieved:

- What is the pathogenesis of the resulting disease, what is the earliest time at which evidence of exposure can be detected and in which tissue(s)?
- What are the possible routes and mechanisms of transmission under natural as well as experimental conditions?
- What is the relative importance of identified routes and mechanisms in the transmission of the disease under natural conditions in the original host and other species?

Only then can fully effective steps be taken to intervene and minimise any risks to public or animal health that may arise.

3.2. Experimental transmission studies

3.2.1. Artificial exposure – artificial routes

Some of these questions were addressed for BSE initially by experimental transmission studies (see Tab. I for details [6, 8, 9, 20, 21, 26, 32, 35, 46, 49, 54, 58–60, 72, 83, 97, 109–114, 116, 117, 124]). In the case of BSE, a sense of urgency accompanied these investigations, partly as a consequence of the subsequent emergence of similar disease in a range of other species [57, 64, 125], and partly because infected animals would have entered the human food chain. Historically the most efficient transmission route to use to provide an indication of potential host range susceptibility was that of intracerebral inoculation (i.c.). Initial studies established that transmission of disease to food animal species using CNS tissues from natural cases of BSE in cattle was possible to cattle, sheep, goats and pigs but not chickens.

Table I summarises the experimental challenges that have been undertaken using cattle BSE as a source, and food animal species as recipients. A similar range of studies could be listed for other donor species/strains (in particular scrapie and CWD), and indeed for BSE challenges into non-food animal recipients, but exhaustively listing these is considered to be beyond the scope of this paper.

3.2.2. Artificial exposure – natural routes

The next stage was to establish if susceptibility could also be demonstrated by more natural routes of infection. The natural route(s) for the transmission of TSE in the field is still not known, but for most experimental purposes the oral route is considered appropriate.

Table I. Food animal species susceptibility to BSE – summary of experimental transmissions from bovine tissue.

Recipient species/ genotype (where relevant)	Route of inoculation	Donor tissue	Amount (g)	Titre in RIII mice* (if known)	No. of animals challenged	Age at dosing	Incubation period	Key policy outcomes/questions addressed
Cattle	i.c./i.v.	Brain	0.1/0.5	N/A	16	4–5 months	74–90 weeks	Proof of transmissibility within species[1] [20]. End stage disease looks the same as natural disease regardless of route [110]
Cattle	Oral	Brain	100	$10^{3.46}$	30	4 months	Timed kills	Pathogenesis of BSE in original host [111]. BSE infectivity identified in the CNS, ileum [109] and bone marrow [112] of pre-clinical cases. Endstage disease after experimental challenge is the same as natural disease [46, 110]
Cattle	Oral	Brain	3 × 100, 100, 10, 1, 0.1, 0.01, 0.001	$10^{3.5}$	10–15 per group (total n = 90)	4–6 months	34–98 months	Determination of LD_{50} and minimum infectious dose of BSE in cattle [117]. Establish attack rate for interpretation of pathogenesis study [6]. Establish minimum effective dose for epidemiological modelling. Confirm that experimental endstage disease looks the same regardless of dose and incubation period (Simmons, unpublished data)
Cattle	i.c.	Brain	Log dilutions	N/A	24 (4 per group)	4 months	20–39 months	Comparative titration BSE in cattle and mice showed that cattle approx. 500 times more sensitive than mice (Cattle 10^6, mice $10^{3.3}$)[2]
Cattle	i.c.	Range of tissues from initial pathogenesis study time kills	0.1	N/A	325 in groups of 5	4–6 months	23–45 months depending on tissue	In addition to CNS, palatine tonsil [114] and nictitating membrane (Wells, Hawkins, unpublished data) harbour BSE infectivity in cattle. The majority of peripheral tissues assayed were negative (Hawkins, unpublished data)
Cattle	Oral	Brain	100 – 3 × 100	$10^{2.86}$	24	6 months	Time kills	Early pathogenesis and the involvement of Peyer's patches in the distal ileum [97]

Table I. (continued).

Recipient species/genotype (where relevant)	Route of inoculation	Donor tissue	Amount (g)	Titre in RIII mice* (if known)	No. of animals challenged	Age at dosing	Incubation period	Key policy outcomes/questions addressed
Cattle	Oral	Brain		N/A	56	4–6 months	Time kills (ongoing)	Pathogenesis in original host [49]
Cattle	Oral	Brain	100 or 1	$10^{3.1}$	200	4 months	Timed kills up to a clinical end-point of 30–78 months	Pathogenesis of BSE in original host. Distribution of tissue infectivity in cattle using a range of statutory screening tests to ensure that SRM controls remain appropriate [6]. Provision of tissue bank (including milk) for future test evaluation. End-stage experimental disease looks like end-stage natural disease (Hawkins and Simmons, unpublished data). No PrP^{Sc} in milk from affected animals [26]
Cattle	Oronasal	Foetal membranes	90 mL oral, 5 mL nasal of a 50% homogenate	N/A	12	2–3 months	Animals survived to 7 years	No demonstrable infectivity in foetal membranes[2] [20]
Cattle	Embryo transfer	Live embryos from clinically affected donors	N/A	N/A	347	Young adult	N/A	BSE cannot be transmitted through embryo transfer [124]
Sheep (positive and negative line Cheviots)	i.c.	Brain	0.5 mL of 10% homogenate	N/A	11	6–18 months	440–994 days	Sheep are susceptible to BSE, including sheep not universally susceptible to scrapie [32]
Sheep (positive and negative line Cheviots)	Oral	Brain	50 mL of 1% homogenate	N/A	12	6–18 months	538–994 days	Sheep are susceptible to BSE by this route [32]
Sheep ARQ/ARQ	Oral	Brain	5 g	$10^{3.97}$	20	6 months	664-909 days	Distribution of infectivity in positive sheep [59]. Important for SRM and risk analysis. Verification that the BSE/scrapie discriminatory tests work in the ARQ/ARQ genotype [58]

Table I. (continued).

Recipient species/genotype (where relevant)	Route of inoculation	Donor tissue	Amount (g)	Titre in RIII mice* (if known)	No. of animals challenged	Age at dosing	Incubation period	Key policy outcomes/questions addressed
Sheep ARQ/ARQ	Oral	Brain	5, 0.5, 0.05, 0.005, 0.0005	$10^{3.97}$	120	3–6 months	Ongoing at VLA	Establishes minimum infectious dose of BSE in sheep by oral route, contributing to epidemiology and risk models. Endstage disease is the same regardless of dose (Bellworthy, Jeffrey, unpublished data)
Sheep ARQ/ARR ARR/ARR	Oral	Brain	5 g	$10^{3.97}$	20 each	6 months	Ongoing at VLA	Are these genotypes susceptible by the oral route? Relevant for genotype-based disease control strategies. What is distribution of infectivity if they are? Is there any evidence of carrier state?
Sheep ARQ/ARQ	Oral	Brain	5 g	$10^{3.97}$	30	6 months	569–1 058 days	Provision of material for statutory controls and other requests. Provision of milk from sheep with BSE. Create a BSE affected flock to establish if transmission could occur to in-contact animals and lambs [8]
Sheep ARQ/ARQ	Oral	Brain	5 g	$10^{3.97}$	8	2 weeks	535–824 days	Lower age at challenge reduces spread of incubation period, but does not shorten the minimum incubation period (Bellworthy, unpublished data)
Sheep AHQ/AHQ	Oral	Brain	5 g	$10^{3.97}$	5	6 months	568–665 days	Susceptibility and end-stage disease in particular genotype. Relevant for genotype-based disease control strategies. Verification that discriminatory tests work in the genotype. Contribution to BSE "flock" [8]
Sheep VRQ/VRQ	Oral	Brain	5 g	$10^{3.97}$	5	6 months	2 clinical supects at 1 570 days	Susceptibility of particular genotype. Relevant for genotype-based disease control strategies. Verification that discriminatory tests work in the genotype. Contribution to BSE "flock" [8]
Sheep ARQ/ARQ VRQ/VRQ VRQ/ARQ ARQ/ARR ARR/ARR	i.c.	Brain	0.05 g	N/A	19 (ARQ/ARQ) 10 (VRQ/VRQ) 10 (VRQ/ARQ) N/A (ARQ/ ARR) 19 (ARR/ARR)	N/A	495–671 days (ARQ/ARQ) 831–1 092 days (VRQ/ARQ and VRQ/VRQ) 1 008–1 127 days (ARR/ ARR)	The ARQ/ARQ, VRQ/VRQ, VRQ/ARQ and ARR/ARR genotypes of sheep are all susceptible to infection with BSE, with shorter incubation period (by this route) in ARQ/ARQ than other genotypes challenged [54]. There were survivors in all genotype groups at the time of publication. Sheep with resistant PrP genotypes are susceptible to BSE [54]. Potentially relevant for genotype-based disease control strategies

Table I. (continued).

Recipient species/genotype (where relevant)	Route of inoculation	Donor tissue	Amount (g)	Titre in RIII mice* (if known)	No. of animals challenged	Age at dosing	Incubation period	Key policy outcomes/questions addressed
Sheep ARQ/ARQ	Intraperitoneal/ intrasplenic	Brain	N/A	N/A	1 for each route	N/A	672 days and 1 444 days	Widespread peripheral tissue involvement, including muscle [9, 72]
Sheep ARR/ARR	Intraperitoneal/ intrasplenic	Brain	5 mL of 10% homogenate	N/A	1 for each route	N/A	No clinical disease	BSE-related PrP can accumulate in tissues of "scrapie resistant" sheep without any clinical signs. Evidence of potential carrier state? [9, 83]
Goats	i.c.	Brain	0.5 mL of 10% homogenate	N/A	3	4–6 years	506–570 days	Species susceptible [32]. Define end-stage disease [32, 35]
Goats	Oral	Brain	50 mL of 1% homogenate	N/A	3	2–5 years	941–1 501 days	Species susceptible by oral route (one survivor) [32]. Define end-stage disease [32, 35]. Discriminatory tests work in this species [60]
Pigs	i.c./i.v./i.p.	Brain	0.5 mL/1.2 mL/ 8–9 mL	N/A	10	1–2 weeks	69–150 weeks	Species susceptible [21]. Define end-stage disease [113]
Pigs	Oral	Brain	3 × MBM ration equivalent for an 8 week old pig	$10^{2.4}$	10	7–14 weeks	Time kills at 2 and 7 years	Species not susceptible to experimental challenge by this route [113]
Chicken	i.c./i.p.	Brain	50 µL/1 mL of 10% homogenate	N/A	12	1–14 days	N/A Survived up to 5years	Species not susceptible [116]
Chicken	Oral	Brain	5 g on 3 occasion	N/A	11	4–6 weeks	N/A Survived up to 5years	Species not susceptible to experimental challenge by this route [116]
Deer	i.c.	Brain	0.05 g	$10^{3.3}$	6	10–12 months	794–1 060 days (one still alive)	Species susceptible. Define endstage disease (Jeffrey M., personal communication)
Deer	Oral	Brain	25 g	$10^{3.1}$	18	4–6 weeks	Time kills	Is this species susceptible by this route? Ongoing. Negative to date – 4 years post challenge (Jeffrey M., personal communication)

* Mouse (i.c./i.p.) units LD_{50}/g.

[1] Dawson M., Wells G.A., Parker B.N.J., Scott A.C., Transmission studies of BSE in cattle, hamsters, pigs and domestic fowl, in: Bradley R., Savey M., Marchant B. (Eds.), Subacute spongiform encephalopathies, Proc. of a seminar in the CEC Agricultural Research Programme held in Brussels, 12–14 November 1990, Kluwer Academic Publishers, 1991, pp. 25–32.

[2] Hawkins S., Wells G., Austin A. et al., Comparative efficiencies of the bioassay of BSE infectivity in cattle and mice, in: Proc. of the Cambridge Healthtech Institute's 2nd Int. Transmissible Spongiform Encephalopathies Conference, 2–3 October 2000, Alexandria, VA, USA, 2000.

For BSE, epidemiological studies indicated that the oral ingestion of food contaminated with infected ruminant-derived protein, in the form of meat and bone meal (MBM), by cattle was likely to be a major route of transmission [118, 120]. Oral challenge studies showed that transmission of BSE was possible to sheep, goats and cattle by this route [32, 59, 111] and with very low challenge doses [117]. Transmission to pigs or chickens however was not achieved by this experimental route [113, 116].

One difficulty that arises with transmission experiments is the interpretation of a negative result; does it mean that transmission does not occur, or just that in this particular scenario it hasn't? The latter then raises questions as to why it may not have occurred. Is it the dose, route, or are there other factors involved such as species barriers or genetic influences? Given that BSE could be transmitted to pigs by the i.c. route, the absence of BSE transmission to pigs by the oral route indicated that there may be an effective species barrier, but a particular confounding factor in this type of study is that the infectious "dose" of any challenge inoculum is difficult to establish objectively. In most cases, inoculum titre (if known) is quoted as mouse LD50/g, using conventional inbred mice. However, we know that different hosts are differently susceptible [88] and that some TSE isolates do not transmit to particular species (including mice). Any experimentally-established titre is inevitably relative, and not necessarily informative for the recipient species in a particular experiment. Attempts to determine PrP^{Sc} concentration biochemically as a measure of titre are also limited by the assumption that PrP^{Sc} is an accurate and quantifiable marker for infectivity.

Conversely, a positive result in a transmission experiment only means that transmission can occur, not that it *does* in field conditions. It also leads to further questions. One is the relevance of such experiments to the field situation. There are a number of fundamental differences between natural exposure and experimental studies which should not be overlooked when comparing disease models with field cases. In natural disease, the incidence of TSE can be low but in experimental disease the aim is to achieve 100% morbidity, especially if the study contains a time-kill element. Very high doses can be given orally and such experimental exposures result in much higher attack rates than are observed naturally [117]. Time-kill approaches can then be used to study the disease pathogenesis in an experimental model, although it is not known what effect dose may have on pathogenesis. It is reassuring, therefore, that the end-stage disease resulting from such experimental exposure of cattle with BSE is virtually indistinguishable from natural cases in all but morbidity [46].

This experimental approach also assumes an oral route of transmission in the field, which is a reasonable assumption for BSE, given the clear epidemiological links with contaminated feed. However, the infectious material in feed has been subjected to a range of manufacturing processes and heat treatments in the course of MBM production; and experimental studies often use "neat" brain material (untreated) to achieve the best morbidity, since rendering has been shown to reduce titre [27, 91, 94–96]. The possible effects of rendering on the basic biological properties of any given TSE strain are very difficult to define, and almost impossible to control for in any experimental design.

It has also been suggested that age at exposure can affect susceptibility [5], but most experimental designs have focussed on a restricted range of ages at challenge from a logistical point of view.

None of these factors negates the data emerging from such experimental studies, as the studies provide a starting point. Once transmission has been achieved, further experimental protocols can be used to investigate aspects such as minimum effective doses [117], and inoculum can be treated to mimic more closely what is occurring in the field [95]. Data derived from transmission experiments can also be used in risk analyses and mathematical models, both of which may be used to inform the development of appropriate control strategies for TSE in animals, and thus to protect public and animal health. Further studies can also be implemented, as they were with BSE, to investigate hypotheses of the origin of

the disease (for example scrapie to cattle [66]), but countless variables prevent this approach from being comprehensive.

3.2.3. *Natural transmission*

With experimental confirmation that transmission is possible by a particular route, further investigation of the contribution of that route to natural transmission is vital.

For BSE it was clear that the principal driver of the epidemic was the feeding of contaminated MBM [120] – once relevant control measures were introduced the epidemic in Great Britain began to decline [50, 122] – but it was not initially known whether the disease could be sustained within an affected population by other natural or management means.

Evidence from a cohort study did not rule out the possibility of a maternal component to transmission [121]. The risk of developing BSE was slightly increased by being born to a dam approaching the clinical phase of the disease. Whether it represented genetic susceptibility, transmission or a combination of the two could not be determined. However, mathematical modelling indicated that if maternal transmission did occur, then it was highly unlikely to be at a rate that could sustain an epidemic [23]. The route through which such exposure might take place, whether it was due to true vertical transmission, or horizontal transmission through close contact also could not be established from the cohort study. A long-term large-scale experimental study to investigate the possibility of vertical transmission indicated that, "when appropriate sanitary protocols" were followed, "embryos derived from BSE-affected cattle did not transmit the disease" [124].

Ultimately for BSE in cattle the relative importance of the role of feed contaminated with infected MBM was confirmed, and the relative absence of evidence for maternal transmission [23] has enabled effective disease control interventions to be implemented.

4. BSE IN SMALL RUMINANTS

The positive results of oral transmission experiments to sheep and goats [32, 59], and the identification of a single natural case of BSE in a French goat [24] have, however, raised a new challenge: that of BSE in small ruminants. For Great Britain, this raised a concern about the national sheep population. With, hopefully, no naturally-occurring disease to study there remain only three alternatives.

Firstly, to set up small-scale animal experiments (as previously described) to investigate potential routes and mechanisms of transmission; secondly to set-up larger-scale natural transmission investigations, such as an experimental sheep flock; and thirdly, to find an alternative natural disease model that can be studied in the field.

4.1. Direct experimental exposure

Transmission of BSE in small ruminants by blood transfusion has been studied by the first method. Whilst experimental BSE can be transmitted by whole blood transfusion [53], this probably has more relevance in the establishment of a precedent for the protection of public health in the context of human-to-human transmission [1], rather than as a potential iatrogenic route in small ruminants.

4.2. Natural transmission experiments

The second method (the experimental sheep flock) has been used by both the Veterinary Laboratories Agency (VLA) and the Institute for Animal Health Neuropathogenesis Unit (NPU) in Edinburgh. The VLA has an experimental BSE-in-sheep flock in which lambs born to ewes that were orally dosed with 5 g of BSE-positive cattle brain have succumbed to clinical disease [8]. The age at onset for these lambs ranged from 654 to 968 days old. In all cases the birth of the lambs occurred within a few months prior to the onset of clinical disease in their dams. Thus we have evidence of natural transmission of BSE from sheep to sheep, albeit in experimental circumstances. Whether this represents true vertical or perinatal infection cannot be ascertained from this study. A similar but slightly different NPU study [36] did not result in transmission, however it could not be statistically ruled out.

4.3. Alternative disease models

The third method, to find an alternative natural disease model that can be studied in the field, is more problematic. Studies of the natural transmission of the only known naturally-occurring TSE of small ruminants, scrapie, might provide a model for BSE in sheep, should it occur under field conditions. Both scrapie and experimental BSE in sheep have similar clinical signs and they have similar diffuse tissue distributions of PrP^{Sc} [34, 35, 59, 115]. If natural ovine BSE is similar to experimental ovine BSE, then ovine BSE may potentially behave in a similar manner to scrapie as far as routes and mechanisms of transmission are concerned.

4.3.1. Scrapie

This is the most extensively studied TSE model. Several institutions have established, maintained and recorded naturally infected flocks of sheep in order to study various aspects of scrapie, including its transmission. These include the INRA Langlade flock of Romanov sheep, various Institute for Animal Health flocks and the VLA scrapie-affected flock.

Analyses of data collected over more than a decade from the first of these have provided epidemiological evidence for both a maternal and lateral component of transmission [22, 99]. Higher relative risks of clinical scrapie were observed associated with lambing periods. There was also a reduced risk of clinical scrapie in artificially-reared lambs from healthy dams, and an increased risk in maternally-reared lambs from scrapie-affected dams. They proposed that transmission may occur within the first 24 h of life with additional risk for those that then continue to share the maternal environment (all lambs remained on their dams for the first intake of colostrum and then for 24 h).

The Institute for Animal Health flocks have established that, despite earlier contradictory findings [30, 31, 33], true vertical transmission of ovine scrapie (via the germ-line or in utero) is improbable [36, 37]. A scrapie-free flock has been established by embryo-transfer (ET) from one with a long-standing scrapie problem. The ET-derived flock has remained scrapie-free since its establishment in 1996, even though it has a similar *PrP* genetic profile to the original flock. Of interest to mechanisms of horizontal/lateral transmission is the fact that the "clean" flock was established and maintained in a scrapie naïve environment; a parallel ET-derived flock that was maintained in close proximity to, but separate from, the original scrapie-affected flock did experience clinical scrapie cases [37]. Lateral transmission has also been shown to occur in the absence of lambing [38].

In the VLA flock it has been shown that lateral transmission occurs [84] and that exposure to a contaminated environment only is sufficient to produce disease (Dexter, Tongue, Bellworthy, unpublished data).

These flocks are managed in a way that maintains high frequencies of sheep with PrP genotypes at high risk of developing clinical disease. Thus with a high incidence of clinical disease and high infectious load, they also provide controlled environments in which to study the pathogenesis of naturally acquired disease. They effectively counter the difficulties of studying a disease that occurs at a low flock-level incidence, however it must be recognised that whilst they provide evidence for routes and mechanisms of natural transmission and estimates of transmission parameters, they are probably not representative of any but the most heavily affected (worst-case scenario) commercial flocks. They are also limited in the range of breeds present, and (potentially) in the number of different scrapie isolates/strains present. These flocks may mimic natural exposure, but at a level that no commercial flock-owner would be able to tolerate and remain as an economically viable unit. Because of this the relative importance of different components of transmission may vary in commercial field flocks and therefore intervention measures may have different outcomes. These institutionalised research flocks, therefore, act as an important bridge between the artificial exposure – natural route transmission experiments – and the true field situation.

A variety of experimental studies using the approaches outlined above have provided

evidence for possible routes of transmission of scrapie. PrP^{Sc} has been found in tissues that could be involved in the natural dissemination of the infectious agent i.e., routes that could lead to exit of the infectious agent from the animal, and result in either environmental contamination or direct transmission. These tissues include the lympho-reticular system of the gut [40, 103, 115], chronically inflamed mammary tissue associated with lymphocyctic mastitis [73], kidney tissue [90], salivary glands [104], nictitating membrane [77], and placentae [2, 81, 101].

For the majority of these tissues, evidence of infectivity or the presence of PrP^{Sc} in associated secretions and excretions is still elusive for scrapie in small ruminants. The exception is blood [55]. Although experimental blood transfusions have resulted in clinical scrapie [55], just as with BSE, it is unlikely to play a major role: blood transfusions are not regular occurrences in sheep veterinary practice.

On the other hand, not only has PrP^{Sc} and infectivity been demonstrated in placentae [3, 81, 101], but it has also been shown to produce clinical scrapie when administered orally to sheep [78, 79]. This was proposed by the authors as a mechanism for lateral transmission from ewe to ewe at lambing time. Placenta has also been cited as a possible explanation for some of the epidemiological findings thought to be associated with mechanisms of maternal transmission [74], although much of the epidemiological evidence may also be interpreted as a contribution to transmission via the lateral route, especially that of environmental contamination. For example, there are reduced odds of ever becoming a scrapie-affected flock if the flock sometimes lambs in different places, compared to those flocks that always lamb in the same place [74]; there is decreased risk of disease associated with lambing in individual pens [75], and there were increased odds for scrapie-positive status of a flock that was found to be associated with failure to remove placenta from bedding along with its disposal in compost.

Epidemiological cross-sectional [74, 75] and case-control studies [47, 51, 80] have provided supporting evidence for the role of various allied management practices in the transmission of scrapie in the field. So far they lack the consistency and specifics necessary for the development of appropriate intervention measures. The scrapie literature does however illustrate how the different types of investigations into aspects of transmission, and the different disciplines, are complementary. Experimental studies of transmission routes and epidemiological studies of risk factors are intrinsically linked in a positive feedback loop, each informing the other.

4.3.2. Chronic wasting disease

The other naturally occurring TSE, CWD of deer is probably less relevant as a model for BSE in small ruminants, has been recently reviewed elsewhere [123] and is covered by Sigurdson in this special issue [89].

4.3.3. Other disease models

Host-specific experimental studies in large animals are expensive and do take time to produce results. The former means that they are difficult to fund. The latter means that they may have to be run in parallel with other experiments, often with more start-up assumptions than desirable, rather than in a logical step-wise order following on from previous findings. They are, however, of paramount importance. They provide an opportunity to study the disease in the original host species; they can be comparable across studies, if standardised protocols are used, and they eliminate the noise of variability, the difficulties of loss to follow-up and the potential biases that are experienced with epidemiological studies. To counter the time and resource limitations, other models have been sought.

The role of hamsters, mice, the burgeoning range of murine transgenes and other models such as voles is a large subject in its own right, and is covered by Groschup and Buschmann in this special issue [44] and elsewhere [28, 43]. In the past such models have been useful [12, 13], but they also have limitations. For example, laboratory wild-type mice cannot replace the original donor species due to the species-transmission barrier and to their different biology and physiology compared

to ruminants. The former has been addressed with the advent of transgenic mice, the latter is insurmountable. Even these do not replicate reality, and the interpretation and extrapolation of any results back to the donor/host-species needs to be a considered, objective process. For example, data from different transgenic mouse lines are not directly comparable, even between lines which have a common transgene [16, 105].

5. PUBLIC HEALTH

The ultimate question of whether a TSE has implications for public health – i.e. is transmissible to man – is difficult to address in the absence of transmission experiments on people. The most appropriate alternative is to use non-human primates [48, 67, 68, 70] which have indicated that BSE transmits with a end-stage disease indistinguishable from variant Creutzfeldt-Jacob disease (vCJD). However these experiments are limited by ethical constraints. Here the development of transgenic mice has been of prospective value, but at the same time, can be misleading. For example, mice with a single copy of the human *PrP* gene were not susceptible experimentally to BSE [10] while at the same time, epidemiological and strain-typing studies were producing a very strong body of circumstantial evidence that vCJD was a consequence of BSE infection in man. The inevitable limitation of such transgenic mice is that only one human gene is present in the model, and disease susceptibility and incubation period are inevitably multi-factorial. Transgenic mouse models which overexpress human *PrP* are also available, and they are highly susceptible to BSE [7, 15, 65, 106] but these may not be a true indicator of susceptibility in humans. Detailed discussion of these models is outwith the scope of this paper and is covered in detail by Groschup and Buschmann in this special issue [44].

6. REMAINING CHALLENGES

Many challenges remain even when a spongiform encephalopathy has been identified as transmissible, and when routes and mechanisms have been proposed.

What are the effects of repeated low dose exposure? What happens when there is intercurrent disease? How do *PrP* genetics influence the transmission process? Is any apparent reduction in susceptibility actually an effect of incubation period prolongation to beyond the natural lifespan? What is the implication of carrier state/subclinical disease for disease control and health? How can we detect animals in the early stage of disease incubation – a phase "silent" to current investigative tools?

For BSE and scrapie some of these questions have been addressed partially [39, 42, 45, 49, 56, 61]. It is possible that for novel TSE many of these questions will remain unanswered or unpursued, except by the most determined of researchers after the funding, stimulated by the public health and political aspects of BSE and vCJD, has dwindled.

Perhaps the greatest conundrum for researchers faced with a new TSE in a species, or a TSE in a species in which it has not previously been described, is whether it is "new", or merely "newly observed". This is a particular issue for BSE, should it be found in the sheep population. With much speculation over the years that scrapie could be the origin of BSE, it might not be too surprising if a detailed study of scrapie isolates revealed one with BSE-like characteristics. A number of studies in the UK and elsewhere [19, 66, 82] have taken a direct approach to this question by looking at the experimental phenotype in cattle experimentally challenged with scrapie isolates, but the diversity of scrapie isolates precludes this approach being exhaustive.

Given that no one type of study can provide all the details or all the answers required, and because of the constraints implicit in each type of study, it is important that researchers respect and integrate the work from other areas, are rigorous, do not overestimate their findings despite various pressures to do so, and are honest: both in the presentation of their findings and in the value of the outcomes. Some of those interested in pure science may disparage studies that they deem to be of low scientific merit, but which are actually of high value to those involved in policy and decision-making: equally some work of high scientific merit may

be extremely interesting in its own right, but not actually necessary to advance disease control and protect public health.

7. CONCLUSION

The approaches to the investigation of the transmission of BSE and scrapie, outlined above, differ only slightly. Those differences are due to the nature of the two diseases. BSE was a novel spongiform encephalopathy, in a hitherto unaffected species, that had characteristics of a point source epidemic, with an agent that could have been incorporated into a wide variety of feedstuffs and iatrogenically administered to naïve populations, and there was early evidence that it was not restricted to bovines. It was vital to establish, albeit experimentally, which other species might be affected, and whether the epidemic could be maintained by natural transmission, if the source was removed. In contrast, scrapie has been endemic throughout Great Britain for centuries, is maintained naturally (even if we don't know exactly how) and has a known host range. The principles, process and integration of evidence from different types of studies, however, are similar for both of these TSE and can be applied to any emerging or suspected spongiform encephalopathy.

REFERENCES

[1] Aguzzi A., Glatzel M., vCJD tissue distribution and transmission by transfusion – a worst case scenario coming true?, Lancet (2004) 363:411–412.

[2] Andréoletti O., Lacroux C., Chabert A., Monnereau L., Tabouret G., Lantier F., et al., PrP^{Sc} accumulation in placentas of ewes exposed to natural scrapie: influence of foetal PrP genotype and effect on ewe-to-lamb transmission, J. Gen. Virol. (2002) 83:2607–2616.

[3] Anonymous, Encyclopaedic word dictionary, Hanks P. (Ed.), Paul Hamlyn, London, New York, Sydney, Toronto, 1971.

[4] Anonymous, The concise Oxford English dictionary of current English, 9th ed., Thomson D. (Ed.), Oxford university press, 1995.

[5] Arnold M., Wilesmith J.W., Estimation of the age-dependent risk of infection to BSE of dairy cattle in Great Britain, Prev. Vet. Med. (2004) 66:35–47.

[6] Arnold M.E., Ryan J.B.M., Konold T., Simmons M.M., Spencer Y.I., Wear A., et al., Estimating the temporal relationship between PrP^{Sc} detection and incubation period in experimental bovine spongiform encephalopathy (BSE) of cattle, J. Gen. Virol. (2007) 88:3198–3208.

[7] Asante E.A., Linehan J.M., Desbruslais M., Joiner S., Gowland I., Wood A.L., et al., BSE prions propagate as either variant CJD-like or sporadic CJD-like prion strains in transgenic mice expressing human prion protein, EMBO J. (2002) 21:6358–6366.

[8] Bellworthy S.J., Dexter G., Stack M., Chaplin M., Hawkins S.A.C., Simmons M.M., et al., Natural transmission of BSE between sheep within an experimental flock, Vet. Rec. (2005) 157:206.

[9] Bencsik A., Baron T., Bovine spongiform encephalopathy agent in a prion protein $(PrP)^{ARR/ARR}$ genotype sheep after peripheral challenge: complete immunohistochemical analysis of disease-associated PrP and transmission studies to ovine-transgenic mice, J. Infect. Dis. (2007) 195:989–996.

[10] Bishop M.T., Hart P., Aitchison L., Baybutt H.N., Plinston C., Thomson V., et al., Predicting susceptibility and incubation time of human-to-human transmission of vCJD, Lancet Neurol. (2006) 5:393–398.

[11] Blood D.C., Studdert V.P., Baillière's comprehensive veterinary dictionary, Baillière Tindall, London, UK, 1988.

[12] Bruce M.E., Strain typing studies of scrapie and BSE, in: Baker H.F., Ridley R.M. (Eds.), Methods in molecular medicine – Prion diseases, Humana Press, Totowa, USA, *1996*, pp. 223–236.

[13] Bruce M.E., Boyle A., Cousens S., McConnell I., Foster J., Goldmann W., Fraser H., Strain characterization of natural sheep scrapie and comparison with BSE, J. Gen. Virol. (2002) 83:695–704.

[14] Bundza A., Charlton K.M., Comparison of spongiform lesions in experimental scrapie and rabies in skunks, Acta Neuropathol. (1988) 76:275–280.

[15] Collinge J., Palmer M.S., Sidle K.C., Hill A.F., Gowland I., Meads J., et al., Unaltered susceptibility to BSE in transgenic mice expressing human prion protein, Nature (1995) 378: 779–783.

[16] Cordier C., Bencsik A., Philippe S., Betemps D., Ronzon F., Calavas D., Crozet C., Baron T., Transmission and characterization of bovine spongiform encephalopathy sources in two ovine transgenic mouse lines (TgOvPrP4 and TgOvPrP59), J. Gen. Virol. (2006) 87:3763–3771.

[17] Cuillé J., Chelle P.L., La maladie dite "tremblante" du mouton ; est-elle inoculable ?, C. R. Acad. Sci. Paris (1936) 203:1552–1554.

[18] Cuillé J., Chelle P.L., La maladie dite "tremblante" du mouton est bien inoculable, C. R. Acad. Sci. Paris (1938) 206:78–79.

[19] Cutlip R.C., Miller J.M., Race R.E., Jenny A.L., Katz J.B., Lehmkuhl H.D., et al., Intracerebral transmission of scrapie to cattle, J. Infect. Dis. (1994) 169:814–820.

[20] Dawson M., Wells G.A., Parker B.N., Preliminary evidence of the experimental transmissibility of bovine

spongiform encephalopathy to cattle, Vet. Rec. (1990) 126:112–113.

[21] Dawson M., Wells G.A., Parker B.N., Scott A.C., Primary parenteral transmission of bovine spongiform encephalopathy to the pig, Vet. Rec. (1990) 127:338.

[22] Diaz C., Vitezica Z.G., Rupp R., Andréoletti O., Elsen J.M., Polygenic variation and transmission factors involved in the resistance/susceptibility to scrapie in a Romanov flock, J. Gen. Virol. (2005) 86:849–857.

[23] Donnelly C.A., Ferguson N.M., Ghani A.C., Anderson R.M., Implications of BSE infection screening data for the scale of the British BSE epidemic and current European infection levels, Proc. Biol. Sci. (2002) 269:2179–2190.

[24] Eloit M., Adjou K., Coulpier M., Fontaine J.J., Hamel R., Lilin T. et al., BSE agent signatures in a goat, Vet. Rec. (2005) 156:523–524.

[25] Elsen J.M., Amigues Y., Schelcher F., Ducrocq V., Andréoletti A., Eychenne F., et al., Genetic susceptibility and transmission factors in scrapie: detailed analysis of an epidemic in a closed flock of Romanov, Arch. Virol. (1999) 144:431–445.

[26] Everest S.J., Thorne L.T., Hawthorn J.A., Jenkins R., Hammersley C., Ramsay A.M., et al., No abnormal prion protein detected in the milk of cattle infected with the bovine spongiform encephalopathy agent, J. Gen. Virol. (2006) 87:2433–2441.

[27] Fernie K., Steele P.J., Taylor D.M., Somerville R.A., Comparative studies on the thermostability of five strains of transmissible-spongiform-encephalopathy agent, Biotechnol. Appl. Biochem. (2007) 47:175–183.

[28] Flechsig E., Hegyi I., Raeber A.J., Cozzio A., Aguzzi A., Weissmann C., Knockouts and transgenic mice in prion research, in: Hörnlimann B., Riesner D., Kretzschmar H. (Eds.), Prions in humans and animals, de Gruyter, Berlin, Germany, 2007, pp. 134–150.

[29] Foley G.L., Zachary J.F., Rabies-induced spongiform change and encephalitis in a heifer, Vet. Pathol. (1995) 32:309–311.

[30] Foote W.C., Clark W., Maciulis A., Call J.W., Hourrigan J., Evans R.C. et al., Prevention of scrapie transmission in sheep using embryo transfer, Am. J. Vet. Res. (1993) 54:1863–1868.

[31] Foster J.D., McKelvey W.A., Mylne M.J., Williams A., Hunter N., Hope J., Fraser H., Studies on maternal transmission of scrapie in sheep by embryo transfer, Vet. Rec. (1992) 130:341–343.

[32] Foster J.D., Hope J., Fraser H., Transmission of bovine spongiform encephalopathy to sheep and goats, Vet. Rec. (1993) 133:339–341.

[33] Foster J.D., Hunter N., Williams A., Mylne M.J., McKelvey W.A., Hope J., Fraser H., Bostock C., Observations on the transmission of scrapie in experiments using embryo transfer, Vet. Rec. (1996) 138:559–562.

[34] Foster J.D., Parnham D.W., Hunter N., Bruce M., Distribution of the prion protein in sheep terminally affected with BSE following experimental oral transmission, J. Gen. Virol. (2001) 82:2319–2326.

[35] Foster J.D., Parnham D., Chong A., Goldmann W., Hunter N., Clinical signs, histopathology and genetics of experimental transmission of BSE and natural scrapie to sheep and goats, Vet. Rec. (2001) 148:165–171.

[36] Foster J.D., Goldmann W., McKenzie C., Smith A., Parnham D.W., Hunter N., Maternal transmission studies of BSE in sheep, J. Gen. Virol. (2004) 85:3159–3163.

[37] Foster J., McKenzie C., Parnham D., Drummond D., Goldmann W., Stevenson E., Hunter N., Derivation of a scrapie-free sheep flock from the progeny of a flock affected by scrapie, Vet. Rec. (2006) 159:42–45.

[38] Foster J., McKenzie C., Parnham D., Drummond D., Chong A., Goldmann W., Hunter N., Lateral transmission of natural scrapie to scrapie-free New Zealand sheep placed in an endemically infected UK flock, Vet. Rec. (2006) 159:633–634.

[39] Goldmann W., Houston F., Stewart P., Perucchini M., Foster J., Hunter N., Ovine prion protein variant A(136)R(154)L(168)Q(171) increases resistance to experimental challenge with bovine spongiform encephalopathy agent, J. Gen. Virol. (2006) 87:3741–3745.

[40] González L., Dagleish M.P., Bellworthy S.J., Sisó S., Stack M.J., Chaplin M.J., et al., Postmortem diagnosis of preclinical and clinical scrapie in sheep by the detection of disease-associated PrP in their rectal mucosa, Vet. Rec. (2006) 158:325–331.

[41] Gordon W.S., Advances in veterinary research. Louping-ill, tick borne fever and scrapie, Vet. Rec. (1946) 58:516–520.

[42] Gravenor M.B., Stallard N., Curnow R., McLean A.R., Repeated challenge with prion disease: the risk of infection and impact on incubation period, Proc. Natl. Acad. Sci. USA (2003) 100:10960–10965.

[43] Groschup M.H., Geissen M., Buschmann A., The experimental transmissibility of prions and infectivity distribution in the body, in: Hörnlimann B., Riesner D., Kretzschmar H. (Eds.), Prions in humans and animals, de Gruyter, Berlin, Germany, 2007, pp. 473–478.

[44] Groschup M.H., Buschmann A., Rodent models for prion diseases, Vet. Res. (2008) 39:32.

[45] Gruner L., Elsen J.M., Vu Tein Khang J., Eychenne F., Caritez J.C., Jacquiet P., et al., Nematode parasites and scrapie: experiments in sheep and mice, Parasitol. Res. (2004) 93:493–498.

[46] Hawkins S.A.C., Wells G.A.H., Simmons M.M., Blamire I.W.H., Meek S.C., Harris P., The topographic distribution pattern of vacuolation in the central nervous system of cattle infected orally with bovine spongiform encephalopathy, Bovine Practitioner (1997) 31.2:73–76.

[47] Healy A.M., Hannon D., Morgan K.L., Weavers E., Collins J.D., Doherty M.L., A paired case-control study of risk factors for scrapie in Irish sheep flocks, Prev. Vet. Med. (2004) 64:73–83.

[48] Herzog C., Salès N., Etchegaray N., Charbonnier A., Freire S., Dormont D., et al., Tissue distribution of bovine spongiform encephalopathy agent in primates after intravenous or oral infection, Lancet (2004) 363:422–428.

[49] Hoffmann C., Ziegler U., Buschmann A., Weber A., Kupfer L., Oelschlegel A., Hammerschmidt B., Groschup M.H., Prions spread via the autonomic nervous system from the gut to the central nervous system in cattle incubating bovine spongiform encephalopathy, J. Gen. Virol. (2007) 88:1048–1055.

[50] Hoinville L.J., Decline in the incidence of BSE in cattle born after the introduction of the "feed ban", Vet. Rec. (1994) 134:274–275.

[51] Hopp P., Ulvund M.J., Jarp J., A case-control study on scrapie in Norwegian sheep flocks, Prev. Vet. Med. (2001) 51:183–198.

[52] Hornlimann B., Riesner D., Kretzschmar H. (Eds.), Prions in humans and animals, de Gruyter Berlin, New York, 2007.

[53] Houston F., Foster J.D., Chong A., Hunter N., Bostock C.J., Transmission of BSE by blood transfusion in sheep, Lancet (2000) 356:999–1000.

[54] Houston F., Goldmann W., Chong A., Jeffrey M., González L., Foster J., et al., Prion diseases: BSE in sheep bred for resistance to infection, Nature (2003) 423:498.

[55] Hunter N., Foster J., Chong A., McCutcheon S., Parnham D., Eaton S., et al., Transmission of prion diseases by blood transfusion, J. Gen. Virol. (2002) 83:2897–2905.

[56] Jackman R., Everest D.J., Schmerr M.J., Khawaja M., Keep P., Docherty J., Evaluation of a preclinical blood test for scrapie in sheep using immunocapillary electrophoresis, J. AOAC Int. (2006) 89:720–727.

[57] Jeffrey M., Wells G.A.H., Spongiform encephalopathy in a nyala (*Tragelaphus angasi*), Vet. Pathol. (1988) 25:398–399.

[58] Jeffrey M., Martin S., Gonzalez L., Ryder S.J., Bellworthy S.J., Jackman R., Differential diagnosis of infections with the bovine spongiform encephalopathy (BSE) and scrapie agents in sheep, J. Comp. Pathol. (2001) 125:271–284.

[59] Jeffrey M., Ryder S., Martin S., Hawkins S.A., Terry L.M., Berthelin-Baker C., Bellworthy S.J., Oral inoculation of sheep with the agent of bovine spongiform encephalopathy (BSE). I. Onset and distribution of disease-specific PrP accumulation in brain and viscera, J. Comp. Pathol. (2001) 124:280–289.

[60] Jeffrey M., Martin S., Gonzalez L., Foster J., Langeveld J.P., van Zijderveld F.G., et al., Immunohistochemical features of PrP(d) accumulation in natural and experimental goat transmissible spongiform encephalopathies, J. Comp. Pathol. (2006) 134:171–181.

[61] Kao R.R., Houston F., Baylis M., Chihota C.M., Goldmann W., Gravenor M.B., et al., Epidemiological implications of the susceptibility to BSE of putatively resistant sheep, J. Gen. Virol. (2003) 84:3503–3512.

[62] Kelly D.F., Gaskell C.J., Spongy degeneration of the central nervous system in kittens, Acta Neuropathol. (1976) 35:151–158.

[63] Kinney H.C., Sidman R.L., Pathology of the spongiform encephalopathy in the gray tremor mutant mouse, J. Neuropathol. Exp. Neurol. (1986) 45:108–126.

[64] Kirkwood J.K., Cunningham A.A., Epidemiological observations on spongiform encephalopathies in captive wild animals in the British Isles, Vet. Rec. (1994) 135:296–303.

[65] Kong Q., Huang S., Zou W., Vanegas D., Wang M., Wu D., et al., Chronic wasting disease of elk: transmissibility to humans examined by transgenic mouse models, J. Neurosci. (2005) 25:7944–7949.

[66] Konold T., Ryder S.J., Lee Y.H., Stack M.J., Horrocks C., Green R., et al., Different prion disease phenotypes result from inoculation of cattle with two temporally separated sources of sheep scrapie from Great Britain, BMC Vet. Res. (2006) 2:31.

[67] Lasmézas C.I., Comoy E., Hawkins S., Herzog C., Mouthon F., Konold T., et al., Risk of oral infection with bovine spongiform encephalopathy agent in primates, Lancet (2005) 365:781–783.

[68] Lasmézas C.I., Deslys J.P., Demaimay R., Adjou K.T., Lamoury F., Dormont D., et al., BSE transmission to macaques, Nature (1996) 381:743–744.

[69] Lasmezas C.I., Deslys J.P., Robain O., Jaegly A., Béringue V., Peyrin J.M., et al., Transmission of the BSE agent to mice in the absence of detectable abnormal prion protein, Science (1997) 275:402–405.

[70] Lasmézas C.I., Fournier J.G., Nouvel V., Boe H., Marcé D., Lamoury F., et al., Adaptation of the bovine spongiform encephalopathy agent to primates and comparison with Creutzfeldt-Jakob disease: implications for human health, Proc. Natl. Acad. Sci. USA (2001) 98:4142–4147.

[71] Last J.M., A Dictionary of Epidemiology, 2nd ed., Oxford University Press, New York, USA, 1988.

[72] Lezmi S., Ronzon F., Bencsik A., Bedin A., Clavas D., Richard Y., et al., PrP^d accumulation in organs of ARQ/ARQ sheep experimentally infected with BSE by peripheral routes, Acta Biochim. Pol. (2006) 53:399–405.

[73] Ligios C., Sigurdson C.J., Santucciu C., Carcassola G., Manco G., Basagni M., et al., PrP^{Sc} in mammary glands of sheep affected by scrapie and mastitis, Nat. Med. (2005) 11:1137–1138.

[74] McIntyre M., Gubbins S., Sivam S.K., Baylis M., Flock-level risk factors for scrapie in Great Britain: analysis of a 2002 anonymous postal survey, BMC Vet. Res. (2006) 2:25.

[75] McLean A.R., Hoek A., Hoinville L.J., Gravenor M.B., Scrapie transmission in Britain: a recipe for a mathematical model, Proc. Biol. Sci. (1999) 266:2531–2538.

[76] Office Internationale Epizootie, Bovine spongiform encephalopathy, in: OIE manual of diagnostic

tests and vaccines for terrestrial animals, 5th ed., 2004. pp. 549–569.

[77] O'Rourke K.I., Baszler T.V., Besser T.E., Miller J.M., Cutlip R.C., Wells G.A., et al., Preclinical diagnosis of scrapie by immunohistochemistry of third eyelid lymphoid tissue, J. Clin. Microbiol. (2000) 38:3254–3259.

[78] Pattison I.H., Hoare M.N., Jebbett J.N., Watson W.A., Spread of scrapie to sheep and goats by oral dosing with foetal membranes from scrapie-affected sheep, Vet. Rec. (1972) 90:465–468.

[79] Pattison I.H., Hoare M.N., Jebbett J.N., Watson W.A., Further observations on the production of scrapie in sheep by oral dosing with foetal membranes from scrapie-affected sheep, Br. Vet. J. (1974) 130:65–67.

[80] Philippe S., Ducrot C., Roy P., Remontet L., Jarrige N., Calavas D., Sheep feed and scrapie, France, Emerging Infect. Dis. (2006) 11:1274–1279.

[81] Race R., Jenny A., Sutton D., Scrapie infectivity and proteinase K-resistant prion protein in sheep placenta, brain, spleen, and lymph node: implications for transmission and antemortem diagnosis, J. Infect. Dis. (1998) 178: 949–953.

[82] Robinson M.M., Hadlow W.J., Knowles D.P., Huff T.P., Lacy P.A., Marsh R.F., Gorham J.R., Experimental infection of cattle with the agents of transmissible mink encephalopathy and scrapie, J. Comp. Pathol. (1995) 113:241–251.

[83] Ronzon F., Benecsik A., Lezmi S., Vulin J., Kodjo A., Baron T., BSE inoculation to prion diseases-resistant sheep reveals tricky silent carriers, Biochem. Biophys. Res. Commun. (2006) 350:872–877.

[84] Ryder S., Dexter G., Bellworthy S., Tongue S., Demonstration of lateral transmission of scrapie between sheep kept under natural conditions using lymphoid tissue biopsy, Res. Vet. Sci. (2004) 76:211–217.

[85] Sharpe A.H., Hunter J.J., Chassler P., Jaenisch R., Role of abortive retroviral infection of neurons in spongiform CNS degeneration. Nature (1990) 346:181–183.

[86] Schoon H.A., Brunckhorst D., Pohlenz J., Spongiforme encephalopathie beim Rothalsstrauss (*Struthio camelus*) Eim Kasuistischer Beitrag, Teirartztl. Prax. (1991) 19:263–265.

[87] Schoon H.A., Brunckhorst D., Pohlenz J., Beitrag zur neuropathology beim Rothalsstrauss (*Struthio camelus*) – Spongiforme Encephalopathie, Verh. Ber. Erkrg. Zootiere (Akademischer Verlag) (1991) 33: 309.

[88] Schätzl H.M., The phylogeny of mammalian and nonmammalian prion proteins, in: Hörnlimann B., Riesner D., Kretzschmar H. (Eds.), Prions in humans and animals, de Gruyter, Berlin, Germany, 2007, pp. 119–121.

[89] Sigurdson C.J., A prion disease of cervids: chronic wasting disease, Vet. Res. (2008) 39:41.

[90] Sisó S., Gonzalez L., Jeffrey M., Martin S., Chianini F., Steele P., Prion protein in kidneys of scrapie-infected sheep. Vet. Rec. (2006) 159:327–328.

[91] Somerville R.A., Oberthur R.C., Havekost U., MacDonald F., Taylor D.M., Dickinson A.G., Characterization of thermodynamic diversity between transmissible spongiform encephalopathy agent strains and its theoretical implications, J. Biol. Chem. (2002) 277:11084–11089.

[92] Soto C., Prions: the new biology of proteins, CRC Press, Florida, USA, 2006.

[93] St Rose S.G., Hunter N., Matthews L., Foster J.D., Chase-Topping M.E., Kruuk L.E., et al., Comparative evidence for a link between Peyer's patch development and susceptibility to transmissible spongiform encephalopathies, BMC Infect. Dis. (2006) 6:5.

[94] Taylor D.M., Woodgate S.L., Atkinson M.J., Inactivation of the bovine spongiform encephalopathy agent by rendering procedures, Vet. Rec. (1995) 137:605–610.

[95] Taylor D.M., Woodgate S.L., Fleetwood A.J., Cawthorne R.J., The effect of rendering procedures on scrapie agent, Vet. Rec. (1997) 141:643–649.

[96] Taylor D.M., Woodgate S.L., Rendering practices and inactivation of transmissible spongiform encephalopathy agents. Rev. Sci. Tech. (2003) 22:297–310.

[97] Terry L.A., Marsh S., Ryder S.J., Hawkins S.A., Wells G.A., Spencer Y.I., Detection of disease-specific PrP in the distal ileum of cattle orally exposed to the agent of bovine spongiform encephalopathy, Vet. Rec. (2003) 152:387–392.

[98] Tongue S.C., Pfeiffer D.U., Warner R., Elliott H., del Rio Vilas V., Estimation of the relative risk of developing clinical scrapie: the role of prion protein (PrP) genotype and selection bias, Vet. Rec. (2006) 158:43–50.

[99] Touzeau S., Chase-Topping M.E., Matthews L., Lajous D., Eychenne F., Hunter N., et al., Modelling the spread of scrapie in a sheep flock: evidence for increased transmission during lambing seasons, Arch. Virol. (2006) 151:735–751.

[100] Tranulis M., Influence of the prion protein gene, *Prnp*, on scrapie susceptibility in sheep, APMIS (2002) 110:33–43.

[101] Tuo W., Zhuang D., Knowles D.P., Cheevers W.P., Sy M.S., O'Rourke K.I., PrP^{c} and PrP^{Sc} at the fetal-maternal interface, J. Biol. Chem. (2001) 276:18229–18234.

[102] Van den Ingh T.S., Mandigers P.J., van Nes J.J., A neuronal vacuolar disorder in young rottweiler dogs. Vet. Rec. (1998) 142:245–247.

[103] Van Keulen L.J., Schreuder B.E., Meloen R.H., Mooij-Harkes G., Vromans M.E., Langeveld J., Immunohistochemical detection of prion protein in lymphoid tissues of sheep with natural scrapie, J. Clin. Microbiol. (1996) 34:1228–1231.

[104] Vascellari M., Nonno R., Mutinelli F., Bigolaro M., Di Bari M.A., Melchiotti E., et al., PrP^{Sc} in salivary glands of scrapie-affected sheep. J. Virol. (2007) 81:4872–4876.

[105] Vilotte J.L., Soulier S., Essalmani R., Stinnakre M.G., Vaiman D., Lepourry L., et al., Markedly increased susceptibility to natural sheep scrapie of transgenic mice expressing ovine PrP. J. Virol. (2001) 75:5977–5984.

[106] Wadsworth J.D., Asante E.A., Desbruslais M., Linehan J.M., Joiner S., Gowland I., et al., Human prion protein with valine 129 prevents expression of variant CJD phenotype, Science (2004) 306:1793–1796.

[107] Wells G.A., Scott A.C., Johnson C.T., Gunning R.F., Hancock R.D., Jeffrey M., et al., A novel progressive spongiform encephalopathy in cattle, Vet. Rec. (1987) 121:419–420.

[108] Wells G.A., Wells M., Neuropil vacuolation in brain: a reproducible histological processing artefact, J. Comp. Pathol. (1989) 101:355–362.

[109] Wells G.A., Dawson M., Hawkins S.A., Green R.B., Dexter I., Francis M.E., et al., Infectivity in the ileum of cattle challenged orally with bovine spongiform encephalopathy, Vet. Rec. (1994) 135:40–41.

[110] Wells G.A., Simmons M.M., The essential lesion profile of bovine spongiform encephalopathy (BSE) in cattle is unaffected by breed or route of infection, Neuropathol. Appl. Neurobiol. (1996) 22:453.

[111] Wells G.A., Hawkins S.A., Green R.B., Austin A.R., Dexter I., Spencer Y.I., et al., Preliminary observations on the pathogenesis of experimental bovine spongiform encephalopathy (BSE): an update, Vet. Rec. (1998) 142:103–106.

[112] Wells G.A., Hawkins S.A., Green R.B., Spencer Y.I., Dexter I., Dawson M., Limited detection of sternal bone marrow infectivity in the clinical phase of experimental bovine spongiform encephalopathy (BSE), Vet. Rec. (1999) 144:292–294.

[113] Wells G.A., Spiropoulos J., Hawkins S.A., Ryder S.J., Pathogenesis of experimental bovine spongiform encephalopathy (BSE): preclinical infectivity in tonsil and observations on the distribution of lingual tonsil in slaughtered cattle, Vet. Rec. (2005) 156:401–407.

[114] Wells G.A., Hawkins S.A., Austin A.R., Ryder S.J., Done S.H., Green R.B., et al., Studies of the transmissibility of the agent of bovine spongiform encephalopathy to pigs. J. Gen. Virol. (2003) 84:1021–1031.

[115] Wells G.A., Ryder S.J., Hadlow W.J., The pathology of prion diseases in animals, in: Hörnlimann B., Riesner D., Kretzschmar H. (Eds.), Prions in humans and animals, de Gruyter, Berlin, Germany, 2007, pp. 315–323.

[116] Wells G.A., Pohlenz J., Hawkins S.A., Matthews D., Portrait of a spongiform encephalopathy in birds and the transmissibility of mammalian prion diseases to birds, in: Hörnlimann B., Riesner D., Kretzschmar H. (Eds.), Prions in humans and animals, de Gruyter, Berlin, Germany, 2007, pp. 279–282.

[117] Wells G.A., Konold T., Arnold M.E., Austin A.R., Hawkins S.A., Stack M., et al., Bovine spongiform encephalopathy: the effect of oral exposure dose on attack rate and incubation period in cattle, J. Gen. Virol. (2007) 88:1363–1373.

[118] Wilesmith J.W., Wells G.A., Cranwell M.P., Ryan J.B., Bovine spongiform encephalopathy: epidemiological studies, Vet. Rec. (1988) 123:638–644.

[119] Wilesmith J.W., Ryan J.B., Atkinson M.J., Bovine spongiform encephalopathy: epidemiological studies on the origin, Vet. Rec. (1991) 128:199–203.

[120] Wilesmith J.W., Ryan J.B., Hueston W.D., Bovine spongiform encephalopathy: case-control studies of calf feeding practices and meat and bonemeal inclusion in proprietary concentrates, Res. Vet. Sci. (1992) 52:325–331.

[121] Wilesmith J.W., Ryan J.B., Bovine spongiform encephalopathy: observations on the incidence during 1992, Vet. Rec. (1993) 132:300–301.

[122] Wilesmith J.W., Wells G.A., Ryan J.B., Gavier-Widen D., Simmons M.M., A cohort study to examine maternally-associated risk factors for bovine spongiform encephalopathy, Vet. Rec. (1997) 141:239–243.

[123] Williams E.S., Miller M.W., Portrait of chronic wasting disease in deer species, in: Hörnlimann B., Riesner D., Kretzschmar H. (Eds.), Prions in humans and animals, de Gruyter, Berlin, Germany, 2007, pp. 257–262.

[124] Wrathall A.E., Brown K.F., Sayers A.R., Wells G.A., Simmons M.M., Farrelly S., et al., Studies of embryo transfer from cattle clinically affected by bovine spongiform encephalopathy (BSE), Vet. Rec. (2002) 150:365–378.

[125] Wyatt J.M., Pearson G.R., Smerdon T.N., Gruffydd-Jones T.J., Wells G.A., Wilesmith J.W., Naturally occurring scrapie-like spongiform encephalopathy in five domestic cats, Vet. Rec. (1991) 129:233–236.

[126] Zlotnik I., Rennie J.C., A comparative study of the incidence of vacuolated neurons in the medulla from apparently healthy sheep of various breeds. J. Comp. Pathol. (1958) 68:411–415.

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研究報告の概要

ヒト顆粒球アナプラズマ症［Human Granulocytic Anaplasmosis (HGA)］の発生率は、1999年以来2倍になった。原因病原体のAnaplasma phagocytophilumは、ニューイングランドの風土病であり、主にマダニIxodes scapularisの流行によってヒトに感染する。A. phagocytophilumによって引き起こされる疾患は、無症候なものから重篤なものまであり、一様ではない。A. phagocytophilumの輸血感染が1例報告されているが、現在HGAのスクリーニングは実施されていない。
この病原体によって引き起こされる血液の安全リスクを調査するため、我々はコネチカット州及びマサチューセッツ州の血液ドナーの陽性率を測定した。血液サンプルを春の後半から冬の初め（2001-2005年）及び2006年の初めから1年間、採取した。参加ドナーからの血清について、間接蛍光分析（IFA）を使ってA. phagocytophilumのヒトIgG抗体の試験を実施した。IFA力価が≧1:64のときに陽性とした。IFAによって検査した15,828名のドナー中、432名（2.7%）がA. phagocytophilum抗体陽性であった。力価の分布は以下の通りであった。1:64が256名（59%）、1:128が115名（27%）、1:256が42名（9.7%）、1:512が14名（3.2%）、≧1:1024が5名（1.2%）であった。マサチューセッツ州ドナーの陽性率は2.2%（30/1,346）、コネチカット州ドナーの陽性率は2.8%（402/14,482）であった。血清陽性率ピークは、次の月に生じた：2月（4.7%）、12月（3.7%）と9月（3.4%）。全体的に、年間陽性率は1.7%（2004年）から4.1%（2001年）まで変化が見られた。年間血清陽性率で観察された変動は、おそらくA. phagocytophilumの複雑なライフサイクルに影響する気候および環境因子よるものであろう。
比較的高い陽性率が持続していることから、A. phagocytophilumの血液安全性に及ぼす影響を調査する必要がある。

報告企業の意見	今後の対応
米国ニューイングランド地方の供血者中のA. phagocytophilumの血清陽性率が比較的高い値を持続しているとの報告である。 アナプラズマ属菌は、ウシ科、シカ科、ラクダ科動物の赤血球内に寄生する直径0.2～1 μmのグラム陰性桿菌である。万一、原料血漿にアナプラズマ属菌が混入したとしても、除菌ろ過等の製造工程にて除去されるものと考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

代表としてノイアート（献血）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ろ過膜処理（ナノフィルトレーション）を施しているが、投与に際しては、次の点に十分注意すること。

study we assessed the field test version of the new WHO JE surveillance standards. We applied the clinical case definition of acute encephalitis syndrome (AES), laboratory diagnostic criteria and case classifications to patients with suspected central nervous system (CNS) infections in southern Vietnam. 380 patients (149 children) with suspected CNS infections were recruited and evaluable, of whom 296 (96 children) met the AES case definition: 54 children were infected with JE virus (JEV), of whom 35 (65%) had AES, giving a sensitivity of 65% (95%CI 56-73%), and specificity 39% (30-48%). 9 adults with JEV all presented with AES. The 19 JEV-infected children missed by the surveillance included 10 with acute flaccid paralysis, 2 with a flaccid hemiparesis, and 6 with meningism only. Altering the case definition to include limb paralysis and meningism improved the sensitivity to 89% (83-95), whilst reducing the specificity to 23% (15-30). An acute serum sample diagnosed 41(68%) of 60 JEV positive patients; an admission CSF diagnosed 33(72%) of 46 patients with this sample, including 7 that were serum negative. Examining a 2nd sample at day 10 diagnosed 61 of 62 patients. 5 patients with neurological manifestations of dengue infection had JEV antibodies in serum, and would have been misdiagnosed had we not tested for dengue antibodies in parallel. In conclusion, the case definitions detected about two thirds of the children infected with JEV, missing those presenting with acute flaccid paralysis. A modified case definition which included acute paralysis and meningism detected nearly 90% of children. An acute CSF sample is more sensitive and specific than an acute serum sample. This formal evaluation of surveillance standards during their development provides an evidence base to support their recommendation, and should be encouraged for future WHO standards.

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EPIDEMIC CHIKUNGUNYA FEVER, INDIA AND INDIAN OCEAN, 2006: LABORATORY-BASED SURVEILLANCE FOR IMPORTED CASES, UNITED STATES

Eileen C. Farnon, Amanda J. Panella, Roselyn Hochbein, Olga L. Kosoy, Janeen J. Laveen, Robert S. Lanciotti, Grant L. Campbell

Centers for Disease Control and Prevention, Fort Collins, CO, United States

Chikungunya virus (CHIKV) is a mosquito-borne alphavirus endemic to Africa and Asia. Chikungunya fever (CHIKF) is characterized by fever, rash, arthralgia, and sometimes arthritis; joint symptoms can be severe and prolonged. In 2005-2006, an unprecedented outbreak of CHIKF occurred on islands in the Indian Ocean and in India. Viremic travelers from epidemic areas could introduce CHIKV to the United States (U.S.) through infection of competent local mosquito species, including *Aedes aegypti* and *Aedes albopictus*, which are distributed throughout the southeastern U.S. and Hawaii. We investigated all cases of CHIKF among U.S.-bound travelers in 2006 that were confirmed at CDC. We searched the CDC Arboviral Diagnostic and Reference Laboratory's database for all patients with laboratory-confirmed CHIKF with onset in 2006, and abstracted demographic and travel information. Cases were confirmed using serology (IgM enzyme-linked immunosorbent assay and plaque reduction neutralization test), viral culture, and reverse transcriptase-PCR (RT-PCR). Thirty-eight people from 16 states and the District of Columbia had laboratory evidence of recent CHIKV infection. Their median age was 49 years (range, 22-78 years); 55% were female. India was the travel destination most frequently reported (87%), followed by Sri Lanka (11%), Réunion (3%) and Zimbabwe (3%). One person reported travel to both India and Sri Lanka. Evidence of recent infection was found by serology in 31 (82%), by viral culture and RT-PCR in 5 (13%), and by RT-PCR alone in 2 (5%). In contrast, only 3 cases of CHIKV infection among U.S.-bound travelers were diagnosed at CDC during the preceding 15-year period from 1991-2005. An unprecedented number of CHIKF cases were confirmed at CDC among travelers to the U.S. in 2006. The 5 culture-positive travelers, and others who might have had undetected viremia, posed a risk of introducing CHIKV into local mosquito populations. There was no evidence of local CHIKV transmission in the U.S. in 2006, but the potential for introducing CHIKV to the U.S. from areas with ongoing transmission still exists. Travelers to tropical areas of Asia and Africa should take precautions against mosquito bites. Travelers returning from epidemic or endemic areas with fever and joint symptoms should be tested for CHIKV infection, and positive cases reported promptly to local public health authorities.

O 1044

PERSISTENT SEROPREVALENCE OF *ANAPLASMA PHAGOCYTOPHILUM* IN NEW ENGLAND BLOOD DONORS

Melanie C. Proctor[1], David A. Leiby[1], Stephanie T. Johnson[2], Richard G. Cable[2]

[1]American Red Cross Holland Laboratory, Rockville, MD, United States, [2]American Red Cross, Farmington, CT, United States

The incidence of human granulocytic anaplasmosis (HGA) has doubled since 1999. The causative agent, *Anaplasma phagocytophilum*, is transmitted to humans primarily by the ixodid tick, *Ixodes scapularis*, endemic in New England. *A. phagocytophilum* causes an illness that ranges from asymptomatic to severe. There has been one reported case of transfusion-transmitted *A. phagocytophilum*, but blood donors are not currently screened for HGA. To determine the potential blood safety risk posed by this agent, we determined its seroprevalence in Connecticut (CT) and Massachusetts (MA) blood donors. Consenting CT and MA blood donors were enrolled in a comprehensive tick-borne disease study. Blood samples were collected during the late spring to early winter (2001-2005) and year round beginning in 2006. Serum collected from participating donors was tested for human IgG antibodies to *A. phagocytophilum* using an indirect immunofluorescent assay (IFA). A donor was considered positive if their IFA titer result was ≥ 1:64. Of 15, 828 donor sera tested by IFA, 432 (2.7%) were positive by IFA for *A. phagocytophilum* antibodies. The distribution of titers was as follows: 256 (59%) donors at 1:64, 115 (27%) at 1:128, 42 (9.7%) at 1:256, 14 (3.2%) at 1:512 and 5 (1.2%) at ≥1:1024. MA donors had a seroprevalence rate of 2.2% (30/1346), while the rate of CT donors was slightly higher, 2.8% (402/14,482). Seroprevalence peaks occurred in the following months: February (4.7%), December (3.7%) and September (3.4%). Overall, the seroprevalence data demonstrated variable yearly rates with a low of 1.7% in 2004 and a high of 4.1% in 2001. Observed fluctuations in yearly seroprevalence rates are likely the result of climactic and environmental factors that influence the complex lifecycle of *A. phagocytophilum*. The observed persistence of relatively high seroprevalence rates reinforces the need to examine the possible impact that *A. phagocytophilum* may have on blood safety. Limited transmission evidence to date may be attributable to the agent's short bacteremic phase, the effect of leukoreduction on this intra-granulocytic organism, or to transmission of primarily sub-clinical infection and resultant under-recognition.

(ACMCIP Abstract)

1045

FAILURE OF STANDARD BABESIOSIS THERAPY IN IMMUNOCOMPROMISED HOSTS

Peter J. Krause[1], Ben Gewurz[2], David Hill[3], Francisco Marty[2], Ivo Foppa[4], Edouard Vannier[5], Ellen Neuhaus[1], Gail Skowren[6], Shaili Gupta[7], Richard R. Furman[8], Carlo McCalla[9], Ed Pesanti[1], Mary Young[10], Donald F. Heiman[11], Jeffrey A. Gelfand[2], Gary Wormser[9], John Dickason[2], Samuel R. Telford[12], Barry Hartman[8], Frank Bia[7], Kenneth Dardick[1], Diane Christianson[1], Morton Coleman[13], Andrew Spielman[2]

[1]University of Connecticut School of Medicine, Farmington, CT, United States, [2]Harvard University School of Medicine, Boston, MA, United States, [3]Hospital for Tropical Diseases, London, United Kingdom, [4]University of South Carolina School of Medicine, Charleston, SC, United States, [5]Tufts University School of Medicine, Boston, MA, United States, [6]Brown University School of Medicine, Providence, RI, United States, [7]Yale University School of Medicine, New Haven, CT, United States, [8]Cornell University School of Medicine, New York, NY, United States, [9]New York

別紙様式第2-1

No. 3

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2008. 1. 15	該当なし	

一般的名称	（製造承認書に記載なし）	研究報告の公表状況	ABC Newsletter. 20008 Jan 11.	公表国
販売名（企業名）	合成血「日赤」（日本赤十字社） 照射合成血「日赤」（日本赤十字社） 合成血-LR「日赤」（日本赤十字社） 照射合成血-LR「日赤」（日本赤十字社）			米国

研究報告の概要

○血液安全パネルはHHSに病原体低減技術の開発を急ぐよう要請
血液安全・安定供給諮問委員会は、米国保健社会福祉省（HHS）事務局に対し、安全で効果的な輸血用血液製剤の病原体低減技術（不活化）の早急な開発を優先して進め、開発され次第実施するよう勧告した。
委員会はまた、HHSに低減技術の開発とバリデーションの障害を取り除くための手段を提供するよう要請した。現在、スクリーニング検査にかかる費用とその複雑さは、血液の安全性の革新を妨げている。これは、製造販売業者の血液安全技術への積極的な投資継続に見合うようなビジネスモデルがないためである。
2008年1月9日と10日の会合では、「病原体低減の効果と安全性を示すエビデンスの蓄積は、今後蔓延する可能性のある感染症に対し広く適応できるセーフガードとして、この技術の導入を保証する」という決議を採択した。こうした新しい技術の例は、血漿分画製剤では世界的に使用され、血液成分製剤ではヨーロッパで導入されている病原体低減システムである。
委員会はこの勧告の根拠として、受血者への既知の感染症の脅威をより低減する必要性を挙げた。また、感染性因子の特定後にドナーの検査を導入するという現行の方式では、新たな病原体が特定される前に感染が拡大する可能性があると指摘した。病原体低減技術の費用は高額になると予想されるが、ガンマ線照射、白血球除去、細菌培養、マラリア予防のための渡航歴による供血制限など、導入後に不要となる現行の血液安全対策の削減によって相殺されると委員会は考えている。また、病原体低減技術の導入で、検査の偽陽性や精度の低い渡航歴による供血制限のためのドナー喪失が減るため、血液の供給量が増加すると推測している。

報告企業の意見	今後の対応
血液安全・安定供給諮問委員会は、米国保健社会福祉省事務局に対し、安全で効果的な輸血用血液製剤の病原体低減技術（不活化）の早急な開発を優先して進め、開発され次第実施するよう勧告したとの報告である。	日本赤十字社では8項目の安全対策の一つとして、不活化技術の導入について、各不活化技術の効果、血液成分への影響、製造作業への影響などについて評価検討を行っている。外国での不活化実施状況や効果、新たな技術、副作用等の情報収集も含め総合的に評価し、導入について関係機関と協議する予定である。

使用上の注意記載状況・その他参考事項等

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2008 #2 January 11, 2008

INSIDE:

Blood Safety Panel Urges HHS to Speed Development of Pathogen Reduction Technologies

The Advisory Committee on Blood Safety and Availability recommended this week that the secretary of the Department of Health and Human Services (HHS) give priority to the urgent development of safe and effective pathogen reduction technologies for blood transfusion products and implement them as they become available.

The panel also urged HHS to provide resources to overcome current barriers to the development and validation of such technologies. Currently, the cost and complexity of individual screening tests is itself becoming a barrier to further blood safety innovations because business models do not appear to favor manufacturers' continued aggressive investments in blood safety technologies

Meeting in Washington, DC, on Wednesday and Thursday, the panel approved a resolution asserting that "accumulating evidence for the efficacy and safety of pathogen reduction warrants a commitment and concerted effort to add this technology as a broadly applicable safeguard against potential emerging infectious diseases." Examples of such emerging technologies are pathogen reduction systems used worldwide for plasma derivatives and being introduced for cellular blood components in Europe.

> **The committee based its recommendation on the need to further reduce known infectious threats to transfusion recipients from infectious agents. The Committee also indicated that the current strategy of implementing donor testing after the identification of new infectious agents may allow widespread transmission of disease before a new agent is recognized.**

Although the cost of pathogen reduction technologies are expected to be high, the committee felt that they likely will be offset by the elimination of current blood safety interventions that would be rendered redundant. These might include gamma irradiation, leukoreduction, bacterial cultures, and travel deferrals for malaria. The Committee also suggested that pathogen reduction could increase the availability of blood by reducing donor loss due to false positive test results and low specificity travel deferrals.

The tone of the meeting was set by Chairman Arthur Bracey, MD, from the St. Luke's Episcopal Hospital, Houston, Texas, who asked speakers to discuss

(continued on page 12)

Quick Test for Staph (continued from page 11)

Staph infections most frequently occur in hospitals and healthcare facilities among patient with weakened immune systems. Distinguishing between the two sources of infection is critical to successful treatment. The more common, less dangerous strain of staph results in infections that are generally mild and affect the skin with pimples or boils that can be swollen, painful and drain pus.

However, the MRSA staph bacterium is difficult to treat with ordinary antibiotics and can cause potentially life-threatening conditions such as blood stream infections, surgical site infections or pneumonia.

FDA cleared the BD GeneOhm StaphSR assay based on the results of a clinical trial at five locations. The new assay identified 100 percent of the MRSA-positive specimens and more than 98 percent of the more common, less dangerous staph specimens.

The FDA cautions that the test should be used only in patients suspected of a staph infection. The test should not be used to monitor treatment for staph infections because it cannot quantify a patient's response to treatment. Test results should not be used as the sole basis for diagnosis as they may reflect the bacteria's presence in patients who have been successfully treated for staph infections. Also, t he test will not rule out other complicating conditions or infections. (Source: FDA press release, 1/2/08)♦

Pathogen Reduction Technologies (continued from page 1)

"how safe is safe," what are the needs, what are the barriers to achieve an acceptable level of transfusion and transplantation safety and what are the pathways to be considered?

Roger Dodd, PhD, from the American Red Cross' Holland Laboratories, emphasized the current safety of the blood supply and the low risk of transfusion when compared to other medical procedures. Dr. Dodd challenged the committee to consider whether members could find a framework for appropriate decision-making instead of continuing to seek a zero-risk blood supply.

Dr. Dodd was followed by Marc J. Roberts, PhD, from the Harvard School of Public Health, who presented a review of the ethics of blood safety. According to Dr. Roberts, it would be unethical to adopt every possible increase in protection regardless of cost because that would put lower-income individuals at significantly higher risk than higher income individuals.

Celso Bianco, MD, executive vice president of America's Blood Centers, reviewed the current landscape of blood donor screening assays in the context of FDA's "five layers of safety" for the blood supply. These are: medical history, donor deferrals, product testing, quarantine of unsuitable products, and monitoring of collecting facilities. Dr. Bianco noted that the only layer that clearly contributes to safety is testing. He expressed his concern, however, that further development of donor screening tests is being threatened by a lack of investment on the part of assay manufacturers because they find investment in other diagnostic areas and pharmaceuticals much more profitable. Dr. Bianco's point of view was reinforced by Brian McDonough, vice president of World Wide Marketing for Ortho Clinical Diagnostics, who noted that "the market attractiveness" of assays for cardiovascular and metabolic diseases and for oncology is much higher than the "no growth" market of blood donor screening.

David Leiby, PhD, from the Holland Laboratories, and Mark Brecher, MD, from the University of North Carolina showed the need for assays and procedures that address infections like babesia, and malaria, for which blood centers do not test,

(continued on page 13)

Pathogen Reduction Technologies (continued from page 12)

and bacteria, for which screening is not completely effective. David Asher, MD from the Food and Drug Administration's Center for Biologics Evaluation and Research (CBER) reviewed the current epidemiology of variant Creutzfeldt Jakob disease (vCJD) and the status of assays being developed to detect vCJD and other prion diseases. He said that none of the tests under development produce satisfactory results.

"The Ultimate Precuationary Principle." The meeting then moved to the concept of pathogen reduction with Harvey Alter, MD from the NIH Blood Bank making an impassionate plea for examination of currently available processes for pathogen reduction and investment in further developments.

"Pathogen reduction is the ultimate precautionary principle by eradicating almost all potential for infectious disease transmission even before risk has been conclusively established, and possibly, even before the agent has been recognized" Dr. Alter said.

Dr. Alter was followed by John Chapman, PhD vice president of Research and Development for Thermogenesis Corp., who said that after many years in the area of pathogen reduction for cellular blood products he believes that various available procedures have acceptable toxicity. This was confirmed by Margarethe Heiden, PhD, from the Paul Erlich Institute in Germany, who spelled out the agency's reasoning in granting a CE mark to the process developed by the Cerus Corporation and the approval by the German regulatory authorities.

Harvey Klein, MD, from the National Institutes of Health's Blood Bank, summarized the conclusions of the panel of the Canadian Consensus Conference on Pathogen Inactivation that took place in March 2007 in Toronto, Canada. Dr. Klein was the chairman of the panel. The summary has been published in the journals *Transfusion* and the proceedings in *Transfusion Medicine Reviews*.

Dr. Klein's was followed by presentations by Larry Corash, MD, from Cerus Corporation, Ray Goodrich, PhD, from Navigant, and Marc Maltas, from Octapharma, about their respective pathogen inactivation processes and clinical trial results.

Finally, Jaroslav Vostal, MD, from CBER, reviewed the current requirements for FDA approval of a pathogen reduction process and provided the detailed reasoning for FDA's refusal to approve the Cerus pathogen reduction process for platelets without submission of additional clinical data. ♦

BRIEFLY NOTED

Hospitals in Vermont are joining those in two other states that have officially formed policies to stop billing patients and insurance companies for certain adverse events. Two more states are considering similar policies as well. The Vermont Association of Hospitals and Health Systems said its policy will cover eight serious events based on the list of 28 so-called "never events" identified by the National Quality Forum as preventable-care errors. Vermont's policy includes: air embolism-associated injury; artificial insemination/wrong donor; incompatible blood-associated injury; medication error injury; retention of foreign object; wrong-patient surgery; wrong-site surgery; and wrong surgical procedure. The hospital association said it expects to complete implementation by the fall. The Minnesota and Massachusetts hospital associations both announced similar policies last year. Minnesota will stop billing for all 28 events, but does not have an implementation schedule in place. Massachusetts, which will stop billing for nine of the 28 events while assessing the others, expects to initiate its policy by the end of January. The Colorado Hospital Association and Michigan Health & Hospital Association are considering non-billing policies as well. (Source: *Modern Healthcare*, 1/6/08)

(continued on page 14)

別紙様式第 2-1

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-7

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2007 年 12 月 5 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	Pathogen inactivation: a new paradigm for blood safety. Mc Cullough, J. Transfusion, 47, 2180-2184 (2007)	公表国 米国	
販売名（企業名）						

研究報告の概要

本論説は，血漿分画製剤業界における病原体不活化（PI）の新たな手法に焦点を当てた報告文献［BYL-2008-0306］と密接に関連している。本稿では，著者が病原体不活化に関するコンセンサス会議［BYL-2008-0306］で得られた結論を考察し，さらに展開している。過去 25 年間で血液の安全性については主要な改善が行われているものの，輸血伝播による感染を低減するための現在のアプローチ法にはいくつかの欠点がある。特に，新規病原体の脅威に対しては対応しきれていない。また，核酸標的薬剤を用いた特有の処理によって，多岐にわたる病原体が不活化されることが明らかになっているものの，この手法は現在ヨーロッパでは利用されているが北米では利用されていない。更に，PI は血液成分の絶対的な安全性を担保するものではないことも念頭に置いておく必要がある。結論として，利害関係者ら（規制当局，医師，血液バンク及び輸血医療業界）が長期的な展望に立って PI を検討し，北米での実施を著者は奨励している。

使用上の注意記載状況・その他参考事項等

BYL-2008-0305

報告企業の意見	今後の対応
著者らは，現在ヨーロッパで普及している PI を北米でも実施することを非常に推奨しており，それによる利点はリスクを上回ると確信している。 弊社のポリグロビン N の製造工程には，コーンの低温エタノール血漿分画法，限外ろ過，S/D 工程及び低 pH インキュベーション処理等の効果的なウイルスが除去・不活化工程が含まれている。	弊社の血漿分画製剤，及び遺伝子組換え製剤の製造工程で使用されている血漿分画製剤の原料が北米であることを考慮すると，本論説で推奨されている PI 法を導入することによって得られる血漿分画製剤の安全性について，検討する必要があると考えられる。

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Pathogen inactivation: a new paradigm for blood safety

In this issue of TRANSFUSION, Klein and colleagues[1] report the results of a consensus conference on pathogen inactivation (PI) sponsored by the Canadian Blood Service and Héma-Québec. The organizers of the conference have done an outstanding job of selecting the panel and posing questions that nicely frame the issues regarding PI. The panel has written an outstanding report that will be of interest to all of us in transfusion medicine and of great help in considering the future of PI. In this editorial, I will review and discuss some of the panel's findings and place them into context with my assessment of the present paradigm for minimizing transfusion-transmitted infections and the current status of PI. I will also provide some additional perspective to some of the issues that the panel identified in their extensive consideration of this evolving field and suggest that these issues will require extensive discussion with many stakeholders. Finally, I will offer my conclusions about where we need to move in the future.

SHORTCOMINGS OF THE PRESENT PARADIGM FOR MINIMIZING TRANSFUSION-TRANSMITTED INFECTIONS

Since the onset of the AIDS epidemic, the panel noted dramatic improvements that have been made in blood safety. These have come from new tests for transmissible diseases; seven have been introduced in the United States since 1985, along with many additional questions in the donor medical history. Current rates of posttransfusion infection from the most well-known agents are extremely low and range from 1 in 900,000 to 7.8 million (human immunodeficiency virus [HIV]) units of blood to 1 in 77,000 to 1.1 million (hepatitis B virus [HBV]).[1,2] On the basis of this background of data, the panel's position was that PI cannot be recommended for introduction "based on the relatively low rates of existing infectious transfusion-related complications *alone*" (italics are this author's). This conclusion illustrates that our present paradigm for the prevention of transfusion-transmissible infections has served us and patients extremely well over the past two decades. The issue then becomes whether this paradigm can be sustained in the future and can continue to be the best approach to maximize blood safety.

Our present paradigm for preventing transfusion-transmitted infections has several shortcomings including:

1. It applies only to known pathogens and transfusion-transmitted infections. Thus, the paradigm accepts that new agents will be allowed to enter the blood supply and our response will be reactive after the problem becomes apparent. West Nile virus (WNV) is the most recent example of the reactionary nature of our present paradigm. The blood banking and/or transfusion medicine community, industry, and regulators worked together to respond to the epidemic with unprecedented speed.[3] As many as several thousand patients may have been infected, however, and in one report 7 of 23 infected patients died.[4] Another example of a new infectious agent entering the blood supply is the Chikungunya virus epidemic that occurred in the island of Le Reunion,[5] a French department in the Indian Ocean. The outbreak was due to a new variant that may have enabled the virus to adapt to a new mosquito vector.[5] Because a large proportion of the population was infected, blood donation was halted on the island, red cells (RBCs) and plasma were shipped in, and PI procedures were put in place for island platelet (PLT) donations. At least 37 cases of infection by this virus are now known in the United States, although these cases occurred in travelers returning from epidemic areas.[6]
2. The current paradigm does not even prevent all known transfusion-transmitted infections. A test has recently become available for Chagas disease, but no practical steps are used to prevent babesiosis, Dengue, HHV-8, babesia, and others. Attempting to prevent transfusion-transmitted malaria by travel history is ineffective and defers many otherwise suitable donors. Cytomegalovirus (CMV) infection is another example of the shortcomings of our current paradigm. Even after leukodepletion or CMV antibody screening of donated blood, transfusion-transmitted CMV occurs.[7]
3. Because our present paradigm is reactive to the occurrence of new infectious agents, it accepts that some patients will be harmed before steps can be taken to minimize transmission of the agent. WNV and patients infected, some fatally, are the most recent examples of this shortcoming.

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4. Current methods to detect and/or prevent transfusion of bacterially contaminated products are inadequate. The AABB standard requiring methods to reduce bacterial contamination of PLTs led to the introduction of testing and has reduced the danger of transfusion-transmitted sepsis. The available test methods, however, are not really suitable for this purpose and even after introduction of screening, transfusion-related septic reactions continue to occur.[1,8]
5. Many donors whose blood does not pose a risk to patients are temporarily or permanently deferred because of the lack of precision of the present screening tests or deferral criteria. The best examples of this paradigm deficiency are donor history questions regarding travel to malaria areas and travel to the United Kingdom and France for new variant CJD.

The panel recognized these shortcomings, particularly the threat of emerging viruses, and recommended "that PI should be implemented when a feasible and safe method to inactivate a broad spectrum of infectious agents is available."[1] The panel based this recommendation in part on the precautionary principle. This principle recommends that when a threat to the public health can be reasonably predicted, a proactive approach should be taken and that the burden of proof is on those who advocate a restrictive approach.

CURRENT STATUS OF PI

Methods

Solvent/detergent (S/D) treatment has been used for years in the manufacture of plasma derivatives. S/D is also used to prepare individual units of frozen plasma from pools of approximately 2500 donors. Although this product is no longer available in the United States, it is used in some other countries primarily in Europe. S/D inactivates only lipid envelop viruses. Methylene blue can be added to plasma and, when exposed to visible light, inactivates most viruses and bacteria. Methylene blue treatment of plasma is used in some European countries.

Several other methods target and damage DNA or RNA thus preventing organisms from reproducing. The three that are most highly developed involve the use of riboflavin (vitamin B2) and UV light for PLTs, plasma, and RBCs (Navigant Corp.), the psoralen compound amotosalen and UV light for PLTs and plasma (Cerus Corp.), and a bifunctional alkylator for PI of RBCs (Cerus Corp.). Details of these methods can be found in recent reviews.[9,10]

Toxicity of compounds used for PI

The safety profiles of these compounds have been studied in ways consistent with general pharmacology[1,11] and are within safety limits. Although the alkylator compound used for RBC PI is similar to alkylators used in chemotherapy, it appears to have a satisfactory safety profile.[10]

Pathogens inactivated

Amotosalen, riboflavin, and the alkylator inactivate a wide variety of pathogens at up to 10^6 or more particles per milliliter.[9,10] The extent to which this level of PI reverses the threat from all pathogens that would be expected in an apparently healthy blood donor is difficult to conclude. Most commercial assays detect both full-length and incomplete noninfectious particles, making it difficult to determine the true level of infectivity in apparently healthy blood donors. For most transfusion-transmitted infections, the level of measurable particles in apparently healthy individuals is below the extent of inactivation obtained in vitro. PI with the amotosalen method effectively inactivated HBV and hepatitis C virus (HCV) in an animal model; and other studies suggest the efficacy of PI for other agents with other compounds.[12] It appears that these three compounds are very effective inactivating transfusion-transmitted pathogens including those for which no prevention strategy is currently in place.

Graft-versus-host disease

Because the PI process damages DNA and prevents the replication of nucleic acids, the process prevents replication of lymphocytes in treated blood components.[13,14] Thus, PI-treated blood components should not cause transfusion-related graft-versus-host disease (GVHD). This promise has been confirmed clinically in some centers in Europe that have discontinued irradiating PI PLTs produced with the amotosalen method without observed transfusion-related GVHD.[13,15]

Present use of PI worldwide

There is extensive literature that documents the in vitro and animal studies of cell and protein function that have occurred with PI compounds, a wide variety of in vivo Phase I studies, and a number of clinical trials of PI that have been widely discussed at international meetings and in excellent literature reviews.[9,10] As a result of this long and comprehensive developmental process, PI PLTs are being used in eight countries in Europe and work to gain experience using the technology is under way in four more. Approximately 80,000 units of PLTs PI using amotosalen have been transfused in Europe. Postmarketing studies of these PLTs as part of structured hemovigilance programs in Europe have not revealed unexpected problems or complications after approximately 20,000 units of amotosalen PI PLTs have been transfused to approximately 3,500 patients. The Phase III trials of amotosalen

fresh-frozen plasma (FFP) are completed; this product is approved in Europe and is now being used in two countries. Although PI of RBCs is technically more difficult and some methods were hampered by the development of antibodies in recipients, methods for PI of RBCs are under active study and may be available for implementation in coming years.

OTHER ISSUES CONSIDERED BY THE PANEL

Noninfectious hazards of transfusion

The panel recognized that the noninfectious hazards of transfusion such as TRALI and mistransfusion are more prevalent than currently recognized transmissible diseases and that PI does not address these problems. The panel did not believe that this issue should delay or inhibit the adoption of PI when the technology is ready. The panel urged that blood suppliers continue efforts to reduce these noninfectious complications but points out that the introduction of PI technology is not mutually exclusive of these efforts.

Rare risks

One concern with PI may be of a rare risk that would not be manifest until PI blood components have been transfused to a large number of patients. Although this problem may seem unique to PI, it really is not. Clinical trial data for licensure of any drug, biologic, or device will never be sufficiently extensive to identify very rare complications. The FDA must take rare risks into consideration with any drug, biologic, or device they license. Unfortunately, the United States does not have an effective system for postmarketing studies based on prelicensure data.[16] As the panel points out, this is the "weakest link in the regulatory process." They propose that licensure of PI mandate postmarketing studies as a condition of approval and that these studies might be somehow integrated with developing hemovigilance programs. An additional approach might include use of the RADAR project, which identifies previously unrecognized adverse drug and device reactions.[17] Follow-up of patients receiving amotosalen PI PLTs is linked with some hemovigilance programs in Europe.

Costs

The panel did not address the costs of implementing PI technology. They recommend that economic evaluations of PI should be carried out but emphasized that adoption of PI should be based on "considerations in addition to the results of an economic analysis."[1] Costs are "just one factor" in considering the use of PI. As the panel points out, many (most??) of the steps taken over the past two decades do not conform to the concepts of cost effectiveness used in other areas of medicine and health care. In the discussion of cost, the panel emphasized the importance of maintaining public confidence in the safety of the blood supply. This combined with the precautionary principle is consistent with other decisions regarding blood safety made over the past two decades and argues for the introduction of PI.

PI might not be as costly as some critics fear. In addition to elimination of the patient care costs of the diseases transmitted, transmission of agents not now tested should be prevented and those patients spared new infections. In the future, the countless hours spent in developing strategies to deal with new agents would be avoided and the costs of testing and loss of donors due to false-positive screening tests or medical history questions would be eliminated. In addition, irradiation of blood products, testing for bacterial contamination of PLTs, and testing for CMV and WNV could probably be eliminated; implementation of a test for trypanosomiasis could be avoided; and 7-day storage of PLTs could be reconsidered. Because plasma is replaced with a PLT additive solution during the amotosalen and potentially the riboflavin PI process, more plasma would become available for fractionation, thus providing some revenue. Because plasma is removed and because PI stops cytokine synthesis, transfusion reactions to PLTs should be decreased,[18] thus improving patient care and reducing the costs of managing these reactions.

Implications for developing countries

PI is discussed here in the context of developed countries. In many parts of the world, blood safety and transfusion-transmissible infections are a much greater problem than in developed countries. It is hoped that as PI becomes more widely used, the technology could be made available in some practical way in parts of the world where it is currently difficult to obtain an adequate supply of safe blood.

Implications of widespread adoption of PI

The panel also addresses several practical issues in the implementation of PI such as the problem of dual inventories. The amotosalen method for PI of plasma and PLTs widely used in Europe is different from that company's method under development for RBCs. Thus, that combination would not provide a single system for PI of all blood components. The riboflavin technology can be used for PLTs, plasma, and RBCs, making a single procedure effective for all components. Although currently there is no single licensed PI system for all blood components, the

panel felt that this should not delay adoption of PI for some components if overall considerations warrant its use.

If some, but not all, of the same blood component is subjected to PI, a dual inventory would arise. Both whole blood–derived (buffy coat) and apheresis PLTs are approved for use in Europe, so a single inventory of all PI PLTs is available there. It will be difficult to create a single inventory of PLTs in the United States, however, because whole blood–derived PLTs produced by the PLT-rich plasma method have not been studied in clinical trials. It seems unlikely that the United States would convert to buffy coat PLTs to adopt PI because only about 26 percent of PLTs in the United States are prepared from whole blood.[19] This problem could create pressure to speed the conversion to apheresis PLTs, motivate the manufacturers to develop a method for PI of PLTs produced with the PLT-rich plasma method, or provide incentive for the production of buffy coat–derived PLTs in the United States, (currently happening in Canada).

Patient selection issues

There is no evidence that components that have undergone PI pose a unique risk for any particular group of patients. The panel recommends that PI products be made available to all patients unless new data indicates an as yet unknown risk for specific patients. Thus, for instance, the panel concluded that there is no need to withhold PI components from neonates or pregnant women.

THE STAKEHOLDERS FOR OUR PI DELIBERATIONS

The panel recommends "broad public consultation" as part of the decision regarding adoption of PI. Stakeholders include industry, academia, the blood banking and/or transfusion medicine community including transfusion medicine physicians and leaders of blood supply organizations, physicians who use blood in their practice, regulators, and most of all patients.

Industry has done impressive work to develop PI technology and publicize their results. They have the responsibility to continue thorough, careful development of PI technology pursing appropriate safety and efficacy issues to produce a product that is helpful to patients and can be implemented into the blood supply system practically and realistically.

Academia also has a role. The companies developing PI technology do not have the breadth and depth of knowledge that exists in our universities. Thus, industry should avail themselves of this expertise and university scientists and physicians should collaborate when it is appropriate.

The blood banking and/or transfusion medicine community has the responsibility to consider PI with a view to the long-term future. Transfusion medicine physicians should have the patients' interest as their first priority. If PI improves transfusion therapy, which our European colleagues have concluded, then PI should be adopted more broadly. Leaders of blood supply organizations have the responsibility to consider PI with an open mind. The technology may be technically complex, but this issue should not deter us from being open to it. We have successfully implemented many complex technologies such as apheresis, radioimmunoassay, ELISA, and NAT. Thus, the consideration is whether it is time for a paradigm shift to further improve blood safety and, if so, whether PI is ready for adoption beyond Europe. PI may alter our current operations or be inconvenient, but these issues have been true of most improvements. Leaders of blood supply organizations have the responsibility to look beyond these short-term logistical issues.

Regulators play a key role in the evolution of PI. Their requirements must be consistent and based on scientifically sound and available data. It is essential that they speak with one voice and from a single point of view. It is reasonable to expect that they will look beyond the benefits of the elimination of existing transfusion-transmitted infections and take into account elimination of some current activities that may become redundant with PI introduction.

Physicians who use blood in their practice depend on those of us in the transfusion medicine and/or blood banking community to demonstrate leadership in providing high-quality transfusion therapy. Dialogue with and among these physician groups will be important to hear the concerns and questions of transfusing physicians, to educate them as to the benefits and unique aspects of PI products, and to determine the best ways to introduce PI blood components into clinical practice at the appropriate time.

Of course, the primary stakeholders are patients. They must be the focus of all of us in transfusion medicine and blood banking. It is our responsibility to provide adequate and safe transfusion therapy and to make available the appropriate blood products. To this end, we must ask the hard questions of the developers of PI, expect complete data and high-quality clinical trials, and be open to the introduction of technology that may be complex, challenging, or even disruptive to our present operations. If PI improves patient care, patients have a right to expect that we use our expertise and creativity to implement change.

CONCLUSIONS OF THE EDITORIALIST

The body of work to develop PI represents very substantial progress. PI is now widely used in Europe and has arrived at a point for realistic consideration in Canada and the

United States. I believe that the benefits of PI extend far beyond eliminating the small number of remaining infections from the traditional list of transfusion-transmitted infectious diseases such as hepatitis or HIV. The benefits include shortening the long list of other transfusion-transmitted infections that are not prevented by present technology or other methods of donor screening. The benefits will also be proven with emerging agents or changes in known agents such as SARS or Avian flu. In addition, irradiation of blood components could be eliminated, removing transfusion-associated GVHD as a lethal complication of transfusion. We are at the end of the usefulness of the present paradigm and must move to a new one. It is incumbent on all of us to consider PI in this broad context.

Jeffrey McCullough, MD
Department of Laboratory Medicine & Pathology
University of Minnesota
420 Delaware Street SE
Minneapolis, MN 55455
e-mail: mccul001@umn.edu

REFERENCES

1. Klein HG, Anderson D, Bernardi MJ, Cable R, Carey W, Hoch JS, Robitaille N, Sivilotti ML, Smaill F. Pathogen inactivation: making decisions about new technologies. Report of a consensus conference. Transfusion 2007;47: 2338-47.
2. Stramer SL. Current risks of transfusion-transmitted agents. Arch Pathol Lab Med 2007;131:702-7.
3. Peterson LR, Epstein JS. Problem solved? West Nile virus and transfusion safety. N Engl J Med 2005;353:516-7.
4. Pealer LN, Marfin AA, Petersen LR, Lanciotti RS, Page PL, Stramer SL, Stobierski MG, Signs K, Newman B, Kapoor H, Goodman JL, Chamberland ME. Transmission of West Nile virus through blood transfusion in the United States in 2002. N Engl J Med 2003;349:1236-45.
5. Charrel RN, Lamballerie X, Raoult D. Chikungunya outbreaks—the globalization of vectorborne diseases. N Engl J Med 2007;356:769-71.
6. Farnon E. Update: Chikungunya fever diagnosed among international travelers—United States, 2006. MMWR 2007; 56:276-7.
7. Nichols WG, Price TH, Gooley T, Corey L, Boeckh M. Transfusion-transmitted cytomegalovirus infection after receipt of leukoreduced blood products. Blood 2003;101: 4195-200.
8. Benjamin RJ, Wagner SJ. The residual risk of sepsis: modeling the effect of concentration on bacterial detection in two-bottle culture systems and an estimation of false-negative culture rates. Transfusion 2007;47:1381-9.
9. Bryant JB, Klein HG. Pathogen inactivation: the definitive safeguard for the blood supply. Arch Pathol Lab Med 2007; 131:719-33.
10. McCullough J. Pathogen inactivation: a new paradigm for preventing transfusion-transmitted infections. Am J Clin Pathol (in press).
11. Ciaravino V, McCullough T, Cimino G. The role of toxicology assessment in transfusion medicine. Transfusion 2003; 34;1481-92.
12. Corten L, Wieselhahn GP, Smyers JM, Murthy KK, McClure HM, Alter HJ. Photochemical inactivation of hepatitis B and hepatitis C viruses in human plasma as assessed in a chimpanzee infectivity model (abstract). Blood 2000;96: 60a.
13. Corash L, Lin L. Novel processes for inactivation of leukocytes to prevent transfusion-associated graft-versus-host disease. Bone Marrow Transplant 2004;33:1-7.
14. Fast LD, DiLeone G, Li J, Goodrich R. Functional inactivation of white blood cells by mirasol treatment. Transfusion 2006;46:642-8.
15. Osselaer JC, Doyen C. Routine use of platelet components prepared with photochemical treatment (INTERCEPT Platelets): impact on clinical outcomes and resources. Blood 2005;106:129a.
16. Psaty BM, Burke S. Institute of medicine on drug safety. N Engl J Med 2006;355:1753-9.
17. Bennett CL, Nebeker JR, Lyons E, Samore MH, Feldman MD, McKoy JM, Carson KR, Belknap SM, Trifilio SM, Schumock GT, Yarnold PR, Davidson CJ, Evens AM, Kuzel TM, Parada JP, Cournoyer D, West DP, Sartor O, Tallman MS, Raisch DW. The research on adverse drug events and reports (RADAR) project. JAMA 2005;293:2131-40.
18. McCullough J, Vesole DH, Benjamin RJ, Slichter SJ, Pineda A, Snyder E, Stadtmauer EA, Lopez-Plaza I, Coutre S, Strauss RG, Goodnough LT, Friday JL, Raife T, Cable R, Murphy S, Howard F, Davis K, Lin JS, Metzel P, Corash L, Koutsoukos A, Lin L, Buchholz DH, Conlan MG. Therapeutic efficacy and safety of platelets treated with a photochemical process for pathogen inactivation: the SPRINT Trial. Blood 2004;104:1534-41.
19. Sullivan MT, Cotton R, Read EJ, Wallace EL. Blood collection and transfusion in the United States in 2001. Transfusion 2007;47:385-94.

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研究報告の概要

○病原体不活化:新技術についての決断 コンセンサス会議報告
2007年3月29日～30日、カナダのトロントで、カナダ血液サービスとヘマ・ケベックが主催する病原体不活化(PI)に関するコンセンサス会議が開かれた。様々な分野の専門家9名で構成されたコンセンサス・パネルに対して提示された質問に回答する形で本報告はまとめられている。
近年の検査技術の発達により、現状の輸血感染症リスクは大変低く、PIを直ちに導入することは推奨しない。しかし、新興感染症のリスクは未知数であり、PIは予防手段として重要である。広範囲の病原体を不活化できる実現可能で安全な方法が確立されればPIを実施すべきである。
特に毒性の面では安全性と効果について厳格な基準を適用するべきである。各国の規制当局の間でデータを共有し、協力して取り組むことが望ましい。適切に計画された市販後調査も必要であり、副作用調査は全国的ヘモビジランスシステムと連携して行うべきである。
本格的な実施に先だって、安全性と効果に関するデータや採血・製造・保管など影響を受ける工程について、慎重に検討すべきである。患者や医師など関係者への十分な説明と、血液センターや病院などでの研修が必要である。最初は限定された地域でのパイロットプログラムとして導入すべきだろう。
不活化実施によって、現在行われている感染症検査など一部の安全対策を取りやめ、費用を削減できる可能性がある。
全ての血液製剤にPIを導入するためには、政府の支援と大規模な投資が必要である。

報告企業の意見	今後の対応
2007年3月29日～30日、カナダのトロントで行われた病原体不活化技術に関するコンセンサス会議の報告である。	日本赤十字社は8項目の安全対策の一環として不活化技術の導入について、各不活化技術の効果、血液成分への影響、製造作業への影響などの評価検討を行っている。細菌やウイルスを不活化する方策について今後も情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-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

CONFERENCE REPORT

Pathogen inactivation: making decisions about new technologies

Report of a consensus conference

Harvey G. Klein, David Anderson, Marie-Josée Bernardi, Ritchard Cable, William Carey, Jeffrey S. Hoch, Nancy Robitaille, Marco L.A. Sivilotti, and Fiona Smaill

Methods to remove and inactivate pathogens, used extensively in the manufacture of plasma protein fractions, have all but eliminated transmission of infectious agents by these products.[1] Technologies for reducing the risk of infection from single donor blood components have not been embraced as enthusiastically. Several methods have been introduced in Europe. Treatment with solvent/detergent (S/D) or methylene blue have both been applied to plasma components, and psoralen treatment of platelets (PLTs) has begun in several countries.[2-4] Although S/D-treated pooled plasma has been approved for use in the United States and Canada, none of these methods has been adopted for single-donor products in North America. Reasons for slow acceptance include 1) the current safety of the volunteer blood supply; 2) the success of surveillance and development of screening tests to deal with emerging pathogens; 3) the inability of current technologies to inactivate some agents such as spores, prions, and certain small nonencapsulated viruses; 4) concerns regarding remote risks from the residual chemical agents used during the pathogen inactivation (PI) process; 5) absence of any single method to treat whole blood or all components; and 6) the cost-effectiveness of these technologies especially compared to strategies to reduce noninfectious risks of transfusion.[5] The Canadian Blood Services and Héma-Québec, with support from the Biomedical Excellence for Safer Transfusion (BEST) Collaborative, organized a consensus conference entitled, "Pathogen Inactivation: Making Decisions About New Technologies," in Toronto, Ontario, Canada, March 29 through 30, 2007, to provide recommendations and guide decision-making in this area. The term "inactivation" was intended to include methods that reduce pathogen risk by any means, including physical removal.

The conference format was based on the model developed by the National Institutes of Health.[6] The steering committee was aware of the potential weaknesses of the consensus process and made every effort to minimize selection bias, particularly with respect to the choice of questions and panelists.[7] The Consensus Panel, selected by the steering committee, had been provided background materials regarding transfusion risk and PI technology as well as a series of six questions designed by the committee to focus debate on the major issues involving pathogen reduction of blood components. The Panel convened immediately before the conference to clarify objectives, principles, and roles. On the first conference day, invited experts made formal presentations on a variety of relevant topics including transfusion risks, inactivation technology, toxicology, regulatory approaches, risk analysis, and cost-benefit considerations. An open forum audience of approximately 270 international attendees participated. The audience and the nine-member independent Consensus Panel, which included a wide range of disciplines (transfusion medicine, hematology, epidemiology, microbiology, toxicology, critical care medicine, medical policy, and ethics) as well as a chronic transfusion

ABBREVIATIONS: PI = pathogen inactivation; WNV = West Nile virus.

From the National Institutes of Health, Bethesda, Maryland; QE II Health Sciences Center, Halifax, Nova Scotia, Canada; CHUM, Montreal, Quebec, Canada; American Red Cross Blood Services, Farmington, Connecticut; Owen Sound, Ontario, Canada; St. Michael's Hospital, Toronto, Ontario, Canada; CHU St. Justine, Montreal, Quebec, Canada; Queen's University, Kingston, Ontario, Canada; and McMaster University Faculty of Health Sciences, Hamilton, Ontario, Canada.

Address reprint requests to: Harvey G. Klein, MD, Department of Transfusion Medicine, Clinical Center, National Institutes of Health, Bethesda, MD 20892; e-mail: hklein@dtm.cc.nih.gov.

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TABLE 1. Risk per unit of selected transfusion-transmitted pathogens

Pathogen	Component	United States	Canada	Europe
HIV	All	1:2,000,000	1:7,800,000	1:900,000-5,500,000*
HCV	All	1:2,000,000	1:2,300,000	1:2,000,000-4,400,000*
HBV	All	1:277,000	1 in 153,000	1:77,000-1,100,000*
WNV	All	1:350,000	Rare	No reported cases
HTLV-I and/or -II	RBCs and/or PLTs	1:3,000,000	1:4,300,000	Not tested
Bacterial transmission	RBCs	1:40,000-1:5,000,000		
Bacterial sepsis	PLTs	1:59,000 single-donor	1:41,000 single-donor	1:11,000 (pooled)
Malaria	RBCs	1:1,000,000-1:5,000,000	Three cases in 10 years	11 cases in 10 years

* Variation between low and medium endemic areas. Modified from Bihl et al.[21]

recipient had an opportunity to question the presenters and add comment. The Consensus Panel reconvened in the evening to address the conference questions and prepare recommendations that could be applied both in Canada and internationally. On Conference Day 2, the Panel's draft statement was presented in its entirety to the experts and the audience for public comment. The Panel finalized the statement within a few weeks of the conference. A preliminary report has been published.[8]

This final Consensus Panel report is based on the information provided to the panelists before and during the conference, a review of background literature, and continued postconference discussion. The Panel by intent did not address advantages, disadvantages, current status, or cost of specific inactivation and/or reduction technologies or commercial products, although data regarding several technologies and trials were provided as background reading and presented at the conference. Several published summaries are available.[5,9-11] The conference questions and conclusions are summarized below.

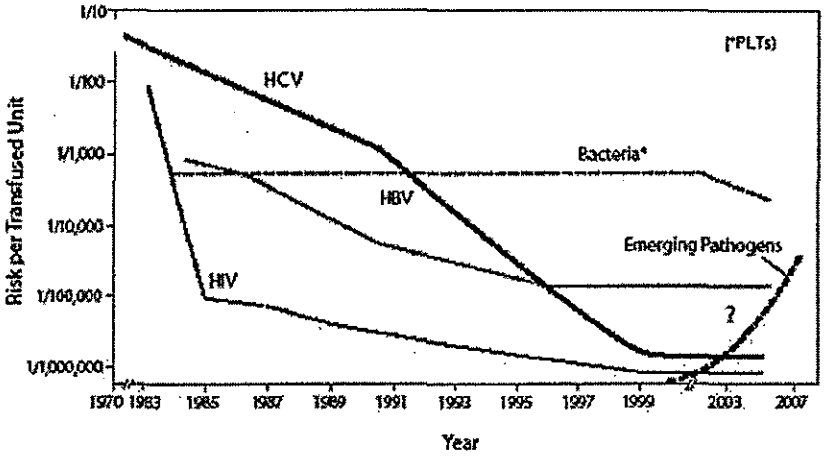

Fig. 1. Risks of transfusion-transmitted infections in the United States. Risk per unit transfused.

IS THE CURRENT RISK OF TRANSFUSION-TRANSMITTED DISEASES ACCEPTABLE IN RELATION TO OTHER RISKS OF TRANSFUSIONS?

Dramatic advances in the safety of allogeneic blood transfusion have been made during the past quarter of a century. At present, the estimated residual risk of transmission through transfusion of human immunodeficiency virus (HIV), hepatitis C virus (HCV), hepatitis B virus (HBV), and human T-lymphotropic virus (HTLV) in Canada is, respectively, 1 in 7.8 million donations, 1 in 2.3 million donations, 1 in 153,000 donations, and 1 in 4.3 million donations.[12] Risks still vary substantially even between low-endemic and high-endemic areas around the world (Table 1). For example, the residual risk of HBV per million blood donations is calculated to be 0.75 in Australia, 3.6 to 8.5 in the United States, 0.91 to 8.7 in Northern Europe, 7.5 to 13.9 in Southern Europe, and up to 200 in Hong Kong.[13-20] Nevertheless, the strategy of donor screening, testing, and deferral has proved remarkably successful in reducing the risk of transmission of the major viral pathogens (Fig. 1).[21]

Bacterial contamination of blood components was among the first recognized risks of transfusion.[22] The introduction of sterile interconnected plastic container systems and controlled refrigeration of blood components seemed to eliminate this risk by the 1960s; however, this conclusion proved illusory. Contamination of PLTs, the blood component stored at room temperature and therefore most susceptible to microbial growth, has been reported between 1 in 2000 and 1 in 5000 PLT collections (active surveillance in the United States) before the implementation of bacterial testing of PLTs, and bacterial sepsis has occurred on the order of 1 in 41,000 transfusions (voluntary reporting in Canada) after the introduction of screening cultures.[23-25] In the United States the frequency of septic reactions from single-donor (apheresis) PLTs before routine culture has been measured at 1 in 15,000 infusions.[26] Introduction of routine "in-process" culture of

PLTs has reduced the risk by about 50 percent. The American Red Cross now reports a residual risk of a septic transfusion reaction from a culture-negative single-donor unit at 1 in 50,200 (20 reported cases of sepsis including 3 fatalities associated with 1,004,000 single-donor PLT components tested).[27] These results are consistent with the Canadian experience. During the same period (2004-2006), septic transfusion reactions from whole blood–derived PLTs that were released without culture approached 1 in 33,000 (30 reported cases of sepsis in 1 million whole blood–derived PLT components released).[28]

Although Chagas disease, babesiosis, and West Nile virus (WNV) have been recent transfusion threats in the United States and Canada, published transmissions of other pathogens, such as hepatitis E and other viruses, other parasites, or prions that result in clinically important illness are very uncommon in the developed world.[21,29-31]

Hemovigilance data from developed countries suggest that the recognized noninfectious risks in aggregate are substantially higher than the current infectious risks of transfusion.[32] Transfusion-related acute lung injury (TRALI), which claims an estimated 50 to 100 lives in the United States each year, has been cited as the most frequent transfusion-related cause of death.[33,34] Acute transfusion reactions resulting from mistransfusion are fatal in about 1 in 1 million transfusions.[35] The frequency of acute and delayed hemolysis alone far exceeds that of clinically important pathogen transmission.[32] Based on the relatively low rates of existing infectious transfusion-related complications alone, the Panel does not recommend immediate introduction of PI with its attendant unknown risks. Even active surveillance, however, cannot estimate the risk of an emerging transfusion-transmitted pathogen. The Panel recognizes that such agents have been detected in blood donors at an increasing rate since the HIV epidemic.[36] The reactive strategy of surveillance, identification, test development, and screening permits a pathogen to disseminate widely even before clinical disease is recognized as was the case with HIV.[37] Furthermore, estimates presented at this conference by Dr Harvey J. Alter suggest that as many as 4.8 million cases of hepatitis, with an ensuing 768,000 cases of cirrhosis, resulted from transfusion in the 1970s and 1980s before a specific test for HCV was introduced. In addition to causing morbidity and mortality, the emergence of new pathogens also undermines public confidence in the blood supply. The Panel believes that such risks require a proactive approach in accordance with the precautionary principle (when facing public health threats for which the outcome can reasonably be predicted based, for example, on similar past issues, the precautionary principle dictates a risk assessment [which compares possible consequences of the action against the consequences of no action, according to available evidence and the rules of science], that favors a proactive approach, taking into account society's expectations that responsible actions be taken to circumscribe the threat. Under such circumstances, risks assessment that would favor inaction could be argued to be irresponsible and unethical, putting the public safety and the safety of future generations at greater risk. The active form of application of the principle places the burden of proof on those who propose a restrictive measure), which provides for a distinctive way of making decisions for managing serious threats to public health where there is scientific uncertainty to meet society's expectations that risks be addressed.[38,39]

If so, under what new circumstances should PI be implemented?

Given the recognition of transfusion-transmitted agents that are entering the blood supply and the risk of emerging infectious threats, the Panel believes that PI should be implemented when a feasible and safe method to inactivate a broad spectrum of infectious agents is available.

The Panel acknowledges that noninfectious hazards of transfusion can entail serious safety issues and deserve specific consideration. Blood services should direct attention to, and supply the necessary resources for, their resolution. For example, existing technology can provide a unified database for the patient's transfusion history, so that multiple collaborating hospitals could access patient blood type, antibody history, reactions to transfusion, and special transfusion needs in real time; one such system is operating in Quebec. Bedside bar-code systems and other technologic solutions have been introduced to improve positive patient identification and reduce transfusion errors.[40,41] The risk of TRALI can be reduced by excluding high-risk donors, limiting plasma use, and developing screening test technology.[34] All of these strategies are currently underfunded and underdeployed. A cost estimate by Dr Sunny Dzik presented to this conference, however, suggested that substantial risk reduction in TRALI and hemolytic transfusion reactions could be accomplished for $14 to $28 per unit, a sum that would raise the cost of blood in the United States by less than 10 percent (Table 2). Introduction of PI technology should not preclude vigorous efforts to reduce these noninfectious risks.

Should the criteria be the same for red cells, PLTs, and fresh-frozen plasma?

The same criteria of safety, feasibility, and efficacy should apply to all blood components. A single method for inactivating pathogens in all blood components would be ideal. No such system is likely to be introduced in the foreseeable future. The absence of an integrated system, however, does not imply that PI of any one component should be delayed until a method is proven satisfactory for all components.

TABLE 2. Costs to reduce noninfectious hazards*

Cost drivers	Patient bar code	Unified online database	TRALI: exclusion and/or HLA testing of high-risk donors	Total
Incremental cost/unit	$10-$20	$3-$6	$1-$2	$14-$28
× 27 million units†	$392 million	$90 million	$40 million	$432 million
Number of major events (hemovigilance data)†				295
Cost per event avoided				$1.5 million

* Adapted from S. Dzik as presented at Consensus Conference.
† Data from Stains by et al.[32]

Should different criteria be used for certain patient populations?

Once the decision has been made to move forward with a method for PI for a specific blood component, the treated product should be used universally. Traditionally, premature infants, children, and pregnant women have been considered "vulnerable populations." The same patients may be at particular risk for transfusion-transmitted pathogens, however, and might arguably derive special benefit from PI blood components. The Panel recognizes that there are few current data available on which to individualize risk-benefit assessment. For example, infection with HBV in infancy or early childhood may lead to a high rate of persistent infection (25%-90%) with significant morbidity.[42] Cytomegalovirus (CMV), in contrast, is readily transmitted by transfusion; however, infection does not necessarily result in increased morbidity and mortality, even for low-birth-weight and premature infants.[43] Similarly, blood component transmission of hepatitis C to neonates and children was common, but the epidemiologic data, histologic findings, and clinical outcomes are conflicting.[44,45] Even fewer data address the potential risk of trace amounts of residual additive, photoderivatives, or metabolites from the current inactivating agents. Until additional new information identifies groups of patients who should not receive the PI product, the Panel concluded that the product should be made universally available.

WHAT MINIMUM ACCEPTABLE SAFETY AND EFFICACY CRITERIA SHOULD BE PUT INTO PLACE FOR THE PREAPPROVAL ASSESSMENT OF PATHOGEN-INACTIVATED PRODUCTS? SPECIFICALLY:

What criteria should govern acceptable toxicology standards and how should they be assessed?

The Panel recognizes that the different regulatory authorities have established their own standard approaches to these assessments. Each agency has specific protocols and criteria for determining safety and efficacy. The Panel endorses the rigorous application of standards for safety and efficacy, particularly in the area of toxicology.[46,47] Established toxicology methods of systematically estimating hazards, anticipated exposure levels, and relevant dose-response relationships should be followed, to ensure a very high margin of safety for transfusion recipients. PI technologies that target nucleic acid should, for example, undergo careful scrutiny to assess the potential for genotoxicity, carcinogenicity, reproductive toxicity, and germline toxicity. These studies should be peer-reviewed and published.[48-50] The Panel strongly recommends that clinically relevant endpoints be selected when studying the direct toxicity of PI techniques on the blood product itself, rather than merely considering, for example, functional assays of oxygen delivery that have been proposed at this conference as one endpoint for evaluating PI of red cells (RBCs). The Panel recognizes that regulatory agencies may be constrained by issues of confidentiality in their ability to share proprietary information with the public.[48,49,51-53] The Panel encourages the harmonization of approaches and sharing of data among the various regulatory agencies internationally, however.[54]

What type of postmarketing surveillance should be required (if any) with the implementation of pathogen-inactivated blood components?

New drugs, biologics, and devices, such as modified blood components, blood containers, and anticoagulant-preservative solutions, undergo careful evaluations for efficacy and safety before approval. The premarketing randomized clinical trials are generally small, short-term studies that may fail to detect toxicities of low frequency (Table 3). New technologies are typically either approved or rejected based on these studies. In most countries, postapproval safety is monitored by a voluntary adverse event reporting system in which health-care professionals report adverse events thought to be related to the drug or biologic.[55] This collection of voluntarily submitted case reports represents the weakest link in the regulatory process. The Panel recognizes the difficulty of postmarketing surveillance studies.[56] Well-designed studies, however, should be mandated by the regulatory authorities and supported by the manufacturers and/or the blood

TABLE 3. Estimates of study size to rule out an adverse event frequency*

Study size to rule out an adverse event†	Adverse event frequency
100	1/33
300	1/100
1,000	1/333
3,000	1/1,000
10,000	1/3,333
225,000	1/75,000

* From Hanley and Lippman-Hand.[80]
† 95 percent upper confidence limit.

suppliers as a condition of approval. Postmarketing surveillance for adverse reactions to PI products should be linked to the national hemovigilance systems such as the Transfusion Transmitted Injuries Surveillance System (TTISS) in Canada. Depending on the new PI technologies implemented, specific additional surveillance outcomes may be identified. Annual reports on adverse reactions to specific products should be prepared, analyzed, and communicated to users.[56,57] In the case of PI, comparisons should be made to historical rates of adverse reactions with non-PI products. The Panel is uncertain as to what extent such information is proprietary or how quickly it is made available to regulatory agencies in different countries, but strongly recommends sharing of hemovigilance data across jurisdictions.

Research should be encouraged to identify rare and long-term consequences of transfusion of PI products. Chronically transfused patients might serve as an ideal surveillance population to identify long-term toxicities of PI products.

FOR PI TECHNOLOGIES THAT HAVE BEEN APPROVED BY THE REGULATORY AUTHORITIES, WHAT IMPLICATIONS SHOULD BE CONSIDERED BEFORE THEIR WIDESPREAD ADOPTION?

Regulatory agencies approve technologies based on their safety and efficacy. In Canada, and in many other countries, a distinction exists between regulatory authorization to market a drug and common practice.[58] Widespread implementation of novel technologies such as PI will have a number of implications for blood services (and beyond). Several technologies are already approved for fresh-frozen plasma treatment in some countries, and it is possible, even likely, that more than one technology will be approved for each of the labile blood components.[5] Suppliers will require a process to select the most appropriate PI technology. The Panel did not address the desirability of licensing or introducing any specific manufacturer's technology, but concentrated on the desirability of a PI technology and the process of implementation. The process should include the detailed review of the available safety and effectiveness data along with determination of how the adoption of a new technology will impact the processes of the organization. Collection methods, management of components, training of personnel, storage and transport, waste disposal, and methods of quality control may all be affected.

Treatment of a nation's blood supply requires societal informed consent. The Panel endorses the need for broad public consultation. Consultation with appropriate patient and physician stakeholder groups is essential. Consultation with hospital physician and transfusion groups is also a necessity. Inventory management is an important issue, particularly at the time of crossover from the current to the new technology. Once the final selection process has occurred, a detailed educational program should be put in place for blood centers, hospitals, healthcare providers, and patients before the introduction of the new product.

Initially, the new PI procedure should be introduced as a pilot project in one geographic area to work out logistical, environmental, and occupational health issues before the process is implemented more widely. For instance, a staged introduction of PI for PLTs is currently being conducted in France.

Should PI components differ in function from available non-PI products, this information should be disseminated to physicians and health-care providers and communicated to patients through an appropriate informed consent process. The manufacturer, the supplier, and provincial departments or ministries of health have the responsibility to ensure that this information is conveyed to physicians and health-care providers in a timely and effective manner. Finally, cost-effectiveness studies should be conducted by agencies such as the Canadian Agency for Drugs and Technologies in Health.[58]

IF PI WERE TO BE IMPLEMENTED FOR ALL COMPONENTS; IN PRINCIPLE:

What criteria would allow changes in donor deferral or testing?

After the implementation of PI for all components, it is possible that existing procedures could be modified to reduce costs or reduce donor deferrals. The rationale for PI implementation should be independent of these considerations, however. Specifically:

What criteria would allow the relaxation of any current donor deferral and/or exclusion policies?

The regulatory agencies and blood collectors should review the donor screening questionnaire to eliminate or modify questions that are believed to be of marginal value, such as tattooing and certain travel deferrals.[59,60]

What criteria would allow the cessation of any currently undertaken screening tests?

1. Screening tests for agents that are not readily transmissible by transfusion, for example, *Treponema pallidum* (syphilis).
2. Screening tests for agents of low infectious titer and high log kill by PI, for example, WNV.
3. Screening tests for agents that are sensitive to PI and for which redundant safety measures are in place, such as CMV, HTLV, and hepatitis B core antibody.
4. Screening tests for agents that are exquisitely sensitive to PI and for which the current tests have poor specificity and sensitivity, such as bacteria.
5. Although not a screening test, gamma irradiation of cellular blood components could be eliminated if nucleic acid–targeted PI technology were introduced. These technologies appear to inactivate contaminant lymphocytes and eliminate the risk of transfusion-associated graft-versus-host disease.[61-63]

What criteria would allow a decision not to implement new screening tests for agents susceptible to PI?
A candidate agent that is shown to be adequately inactivated by an implemented PI technology would not require screening tests, unless of unusually high infectious titer. Ideally PI treatment should reduce the pathogen load in a blood component by 6 to 10 log as measured with appropriate isolates in an in vitro assay of infectious units.[64] In certain cases virus-infected primate models may be desirable to define the efficacy of PI treatment in transfusion-mediated transmission.

Should multiple inventories be considered for each component and if yes how should allocation be decided?

The Panel recommends universal implementation of PI (or universal implementation for a particular component if PI methods for all components are not available). Consequently, unless special patient populations are identified which should not receive newly implemented PI components (see "Should different criteria be used for certain patient populations?" above), the Panel recommends against multiple inventories.

HOW SHOULD THE COSTS AND/OR BENEFITS OF PI BE ASSESSED?

The Panel appreciates that precaution must be tempered by the logic of cost-benefit analysis with its focus on scarcity and estimates of risk.[65] Country-specific studies of different PI technologies have been published, and the strengths and limitations of the existing studies were analyzed at this conference.[66-72] Economic evaluations of all PI procedures should be conducted. Implementation of PI, however, should be based on other considerations in addition to the results of an economic analysis; this practice is consistent with how economic evaluation results are used to assist with decisions in other areas of health care. For PI, the costs are currently unknown and the benefits are difficult to quantify. Even with perfect data, a decision should be made with the economic evidence as just one factor. Unlike many therapeutic interventions, PI is an intervention with "broad-spectrum" potential to reduce multiple infectious and noninfectious threats. Furthermore, blood safety interventions often do not conform to the traditional norms of cost effectiveness.[73,74] Economic evaluation is but one tool, albeit an important one, for assisting policy makers in arriving at a decision acceptable to their constituencies.[75]

Costs and benefits should be assessed with a societal perspective, examining both direct and indirect costs in accordance with published recommendations.[76] Analysts should strongly consider presenting the results in a disaggregate fashion with a cost consequence analysis in addition to a cost-effectiveness analysis.[75,77] Methods and models should be transparent with assumptions highlighted and tested for their effect on the results. Sensitivity analysis, at a bare minimum, should focus on variations in price and effectiveness. Uncertainty about these analyses should be considered, not only for the incremental cost-effectiveness ratio but also for the budget impact.

How should these be aligned with other blood safety interventions and/or other health-care interventions?

A judgment about whether the extra benefits outweigh the extra costs is context-specific. The Panel believes that it may be inappropriate to assign a single number as a cutoff threshold for the cost-effectiveness analysis.[75] Decision makers, however, should clearly state their reasoning for decisions with special emphasis on budget impact, the extra cost for improved patient outcome, and opportunity costs (i.e., what other safety improvements could be introduced for the cost of PI). Reasoning used for past decisions may not be applicable for current or future decisions involving new, expensive technologies. It is of utmost importance that decisions about scarce resources be made that are consistent with the values of the decision makers and the patients whom they represent.

WHAT OTHER INFORMATION, CONSIDERATIONS, AND RESEARCH-RELATED QUESTIONS WOULD NEED TO BE ANSWERED TO DECIDE WHETHER OR WHEN A PARTICULAR PI PROCEDURE SHOULD BE IMPLEMENTED?

The Panel recommends that consideration be given to robust governmental support for a large-scale investment

in developing an integrated PI technology for all blood components. Research initiatives should be directed toward a PI technology suitable for implementation in developing countries.[5,78]

Mathematical modeling should be used to develop credible scenarios for the unknown (emerging) pathogen risk; for example, what are the "break-even" threshold conditions and are they consistent with a worst-case scenario? Several different models might be constructed based on the extensive database developed during the HIV epidemic, which included a pathogen with an extended "silent period," high morbidity and mortality, secondary spread, surrogate testing, and clinical screening, contrasted with an agent such as WNV, which became clinically apparent quickly and involved limited morbidity and mortality and for which a screening test could be readily developed and introduced. These models could be used in economic analyses of candidate PI technologies to support decisions about PI implementation and investments for the research agenda.

Large, well-designed, randomized clinical trials should be performed to evaluate and/or confirm the effectiveness of any new PI technology. Postlicensure Phase IV studies should be integrated with hemovigilance systems to enhance the ability to detect adverse events.

Introduction of PI technologies might have unanticipated consequences for the health-care system. For example, the development and widespread availability of screening tests for new agents might be compromised.

Prion diseases have not been addressed by current PI technologies. New PI technologies should be investigated to address these and other resistant agents. Research should address the relative risks and benefits of PI pooled components versus PI single-donor components.[79]

CONCLUSION

PI or removal technologies hold considerable promise as a means of improving the safety of the blood supply, particularly against newly emergent or not-yet-discovered infectious threats. A number of PI technologies have already been adopted in different countries and some are expected to become available within a relatively short time in Canada. Implementation of PI will be complicated by considerations of efficacy, availability, logistics, cost-effectiveness, toxicity, and risk-benefit issues. Further, the extensive battery of screening assays for testing blood donations that has been developed since the mid-1980s greatly reduces the currently appreciated risk of blood transfusion. The success of this strategy has reduced the apparent benefit of PI. PI represents a prospective approach to blood safety that could add an important additional layer of safety to a nation's blood supply, however.

This consensus statement emerged from a consensus development process that involved experts and stakeholders in a variety of disciplines and a variety of roles in the process. The statement endeavors to answer six questions posed to the Consensus Panel by the conference organizers that address a number of the issues posed by the imminent availability of PI technologies. The Panel has prepared this statement in the anticipation that it will prove useful, not only to Canadian Blood Services and Héma-Québec, but also to the other stakeholders in Canada, and to planners and policy makers involved in blood services in other countries.

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REFERENCES

1. Burnouf T, Radosevich M. Reducing the risk of infection from plasma products: specific preventative strategies. Blood Rev 2000;14:94-110.
2. Solheim BG. Universal pathogen-reduced plasma in elective open-heart surgery and liver resection. Clin Med Res 2006;4:209-17.
3. Politis C, Kavallierou L, Hantziara S, Katsea P, Triantaphylou V, Richardson C, Tsoutsos D, Anagnostopoulos N, Gorgolidis G, Ziroyannis P. Quality and safety of fresh-frozen plasma inactivated and leucoreduced with the Theraflex methylene blue system including the Blueflex filter: 5 years' experience. Vox Sang 2007;92:319-26.
4. Janetzko K, Cazenave JP, Kluter H, Kientz D, Michel M, Beris P, Lioure B, Hastka J, Marblie S, Mayaudon V, Lin L, Lin JS, Conlan MG, Flament J. Therapeutic efficacy and safety of photochemically treated apheresis platelets processed with an optimized integrated set. Transfusion 2005; 45:1443-52.
5. Bryant BJ, Klein HG. Pathogen inactivation: the definitive safeguard for the blood supply. Arch Pathol Lab Med 2007; 131:719-33.
6. Ferguson JH; the NIH Consensus Development Program. The evolution of guidelines. Int J Technol Assess Health Care 1996;12:460-74.
7. Wortman PM, Vinokur A, Sechrest L. Do consensus conferences work? A process evaluation of the NIH Consensus

Development Program. J Health Polit Policy Law 1988;13: 469-98.
8. Klein HG, Anderson D, Bernardi MJ, Cable R, Carey W, Hoch JS, Robitaille N, Sivilotti ML, Smaill F. Pathogen inactivation: making decisions about new technologies—preliminary report of a consensus conference. Vox Sang 2007;93:179-82.
9. Allain JP, Bianco C, Blajchman MA, Brecher ME, Busch M, Leiby D, Lin L, Stramer S. Protecting the blood supply from emerging pathogens: the role of pathogen inactivation. Transfus Med Rev 2005;19:110-26.
10. Williamson LM, Cardigan R, Prowse CV. Methylene blue-treated fresh-frozen plasma: what is its contribution to blood safety? Transfusion 2003;43:1322-9.
11. Pelletier JP, Transue S, Snyder EL. Pathogen inactivation techniques. Best Pract Res Clin Haematol 2006;19:205-42.
12. O'Brien SF, Yi QL, Fan W, Scalia V, Kleinman SH, Vamvakas EC. Current incidence and estimated residual risk of transfusion-transmitted infections in donations made to Canadian Blood Services. Transfusion 2007;47:316-25.
13. Dodd RY, Notari EP, Stramer SL. Current prevalence and incidence of infectious disease markers and estimated window-period risk in the American Red Cross blood donor population. Transfusion 2002;42:975-9.
14. Soldan K, Barbara JA, Ramsay ME, Hall AJ. Estimation of the risk of hepatitis B virus, hepatitis C virus and human immunodeficiency virus infectious donations entering the blood supply in England, 1993-2001. Vox Sang 2003;84:274-86.
15. Pillonel J, Laperche S. Trends in risk of transfusion-transmitted viral infections (HIV, HCV, HBV) in France between 1992 and 2003 and impact of nucleic acid testing (NAT). Euro Surveill 2005;10:5-8.
16. Velati C, Fomiatti L, Baruffi L, Romano L, Zanetti A. Impact of nucleic acid amplification technology (NAT) in Italy in the three years following implementation (2001-2003). Euro Surveill 2005;10:12-4.
17. Niederhauser C, Schneider P, Fopp M, Ruefer A, Levy G. Incidence of viral markers and evaluation of the estimated risk in the Swiss blood donor population from 1996 to 2003. Euro Surveill 2005;10:14-6.
18. Alvarez do Barrio M, Gonzalez Diez R, Hernandez Sanchez JM, Oyonarte Gomez S. Residual risk of transfusion-transmitted viral infections in Spain, 1997-2002, and impact of nucleic acid testing. Euro Surveill 2005;10:20-2.
19. Offergeld R, Faensen D, Ritter S, Hamouda O. Human immunodeficiency virus, hepatitis C and hepatitis B infections among blood donors in Germany 2000-2002: risk of virus transmission and the impact of nucleic acid amplification testing. Euro Surveill 2005;10:8-11.
20. Coste J, Reesink HW, Engelfriet CP, Laperche S, Brown S, Busch MP, Cuijpers HT, Elgin R, Ekermo B, Epstein JS, Flesland O, Heier HE, Henn G, Hernandez JM, Hewlett IK, Hyland C, Keller AJ, Krusius T, Levicnik-Stezina S, Levy G, Lin CK, Margaritis AR, Muylle I, Neiderhauser C, Pastila S, Pillonel J, Pineau J, van der Poel CI, Politis C, Roth WK, Sauleda S, Seed CR, Sondag-Thull D, Stramer SI, Strong M, Vamvakas EC, Velati C, Vesga MA, Zanetti A. Implementation of donor screening for infectious agents transmitted by blood by nucleic acid technology: update to 2003. Vox Sang 2005;88:289-303.
21. Bihl F, Castelli D, Marincola F, Dodd RY, Brander C. Transfusion-transmitted infections. J Transl Med 2007; 5:25.
22. Pittman M. A study of bacteria implicated in transfusion reactions and of bacteria isolated from blood products. J Lab Clin Med 1953;42:273-88.
23. Yomtovian RA, Palavecino EL, Dyskra AH, Downes KA, Morrissey AM, Bajaksouzian S, Pokorny MA, Lazarus HM, Jacobs MR. Evolution of surveillance methods for detection of bacterial contamination of platelets in a university hospital, 1991 through 2004. Transfusion 2006;46:719-30.
24. Ramirez-Arcos S, Jenkins C, Dion J, Bernier F, Delage G, Goldman M. Canadian experience with detection of bacterial contamination in apheresis platelets. Transfusion 2007; 47:421-9.
25. Fang CT, Chambers LA, Kennedy J, Strupp A, Fucci MC, Janas JA, Tang Y, Hapip CA, Lawrence TB, Dodd RY. Detection of bacterial contamination in apheresis platelet products: American Red Cross experience, 2004. Transfusion 2005;45:1845-52.
26. Ness P, Braine H, King K, Barrasso C, Kickler T, Fuller A, Blades N. Single-donor platelets reduce the risk of septic platelet transfusion reactions. Transfusion 2001;41:857-61.
27. Eder AF, Kennedy JM, Dy BA, Notari EP, Weiss JW, Fang CT, Wagner S, Dodd RY, Benjamin RJ. Bacterial screening of apheresis platelets and the residual risk of septic transfusion reactions: the American Red Cross experience (2004-2006). Transfusion 2007;47:1134-42.
28. Stramer SL. Current risks of transfusion-transmitted agents: a review. Arch Pathol Lab Med 2007;131:702-7.
29. Cable RG, Leiby DA. Risk and prevention of transfusion-transmitted babesiosis and other tick-borne diseases. Curr Opin Hematol 2003;10:405-11.
30. Leiby DA. Threats to blood safety posed by emerging protozoan pathogens. Vox Sang 2004;87 Suppl 2:120-2.
31. Vamvakas EC, Kleinman S, Hume H, Sher GD: The development of West Nile virus safety policies by Canadian blood services: guiding principles and a comparison between Canada and the United States. Transfus Med Rev 2006;20:97-109.
32. Stainsby D, Jones H, Asher D, Atterbury C, Boncinelli A, Brant L, Chapman CE, Davison K, Gerrard R, Gray A, Knowles S, Love EM, Milkins C, McClelland DBL, Norfolk DR, Soldan K, Taylor C, John Revill J, Williamson LM, Cohen H; SHOT Steering Group. Serious hazards of transfusion: a decade of hemovigilance in the UK. Transfus Med Rev 2006;20:273-82.
33. Toy P, Popovsky MA, Abraham E, Ambruso DR, Holness LG, Kopko PM, Mcfarland JG, Nathens AB, Silliman CC,

Stroncek D. Transfusion-related acute lung injury: definition and review. Crit Care Med 2005;33:721-6.
34. Eder AF, Herron R, Strupp A, Dy B, Notari EP, Chambers LA, Dodd RY, Benjamin RJ. Transfusion-related acute lung injury surveillance (2003-2005) and the potential impact of the selective use of plasma from male donors in the American Red Cross. Transfusion 2007;47:599-607.
35. Linden JV, Wagner K, Voytovich AE, Sheehan J. Transfusion errors in New York State: an analysis of 10 years' experience. Transfusion 2000;40:1207-13.
36. Alter HJ, Stramer SL, Dodd RY. Emerging infectious diseases that threaten the blood supply. Semin Hematol 2007; 44:32-41.
37. Kroner BL, Rosenberg PS, Aledort LM, Alvord WG, Goedert JJ. HIV-1 infection incidence among persons with hemophilia in the United States and western Europe, 1978-1990. Multicenter Hemophilia Cohort Study. J Acquir Immune Defic Syndr 1994;7:279-86.
38. A Canadian perspective on the precautionary approach/ principle [monograph on the Internet]. Environment Canada; 2001 [cited 2007 Oct 30]. Available from: http://www.ec.gc.ca/econom/pamplet_e.htm
39. Stoto MA. The precautionary principle and emerging biologic risks: lessons from human immunodeficiency virus in blood products. Semin Hematol 2006;43(2 Suppl 3):S10-S12.
40. Davies A, Staves J, Kay J, Casbard A, Murphy MF. End-to-end electronic control of the hospital transfusion process to increase the safety of blood transfusion: strengths and weaknesses. Transfusion 2006;46:352-64.
41. Dzik WH. New technology for transfusion safety. Br J Haematol 2007;136:181-90.
42. Chang MH. Hepatitis B virus infection. Semin Fetal Neonatal Med 2007;12:160-7.
43. Galea G, Urbaniak SJ. The incidence and consequences of cytomegalovirus transmission via blood transfusion to low birth weight, premature infants in north east Scotland. Vox Sang 1992;62:200-7.
44. Aach RD, Yomtovian RA, Hack M. Neonatal and pediatric posttransfusion hepatitis C: a look back and a look forward. Pediatrics 2000;105(4 Pt 1):836-42.
45. Casiraghi MA, Romano L, Biffi R, Assi A, Binelli G, Zanetti AR. Long-term outcome (35 years) of hepatitis C after acquisition of infection through mini transfusions of blood given at birth. Hepatology 2004;39:90-6.
46. Pharmacology/Toxicology guidance documents [monograph on the Internet]. US Food and Drug Administration; 2006 [cited 2007 Oct 30]. Available from: http://www.fda.gov/cder/guidance/index.htm#Pharmacology/Toxicology
47. International Conference on Harmonization (ICH) safety guidelines [monograph on the Internet]. Health Canada; 2007 [cited 2007 Oct 30]. Available from: http://www.hc-sc.gc.ca/dhp-mps/prodpharma/applic-demande/guide-ld/ich/securit/index_e.html
48. Chapman JR, Moore K, Butterworth BE. Pathogen inactivation of RBCs: PEN110 reproductive toxicology studies. Transfusion 2003;43:1386-93.
49. Ciaravi V, Mccullough T, Dayan AD. Pharmacokinetic and toxicology assessment of INTERCEPT (S-59 and UVA treated) platelets. Hum Exp Toxicol 2001;20:533-50.
50. Goodrich RP. The use of riboflavin for the inactivation of pathogens in blood products. Vox Sang 2000;78 Suppl 2:211-5.
51. Orzack LH, Kaitin KI, Lasagna L. Pharmaceutical regulation in the European Community: barriers to single market integration. J Health Polit Policy Law 1992;17: 847-68.
52. Breckenridge A, Woods K. Medicines regulation and the pharmaceutical industry. BMJ 2005;331:834-6.
53. Kesselheim AS, Mello MM. Confidentiality laws and secrecy in medical research: improving public access to data on drug safety. Health Aff (Millwood) 2007;26: 483-91.
54. Farrugia A. The regulatory pendulum in transfusion medicine. Transfus Med Rev 2002;16:273-82.
55. Ravaud P, Tubach F. Methodology of therapeutic trials: lessons from the late evidence of the cardiovascular toxicity of some coxibs. Joint Bone Spine 2005;72:451-5.
56. Psaty BM, Burke SP. Protecting the health of the public—Institute of Medicine recommendations on drug safety. N Engl J Med 2006;355:1753-5.
57. McClellan M. Drug safety reform at the FDA—pendulum swing or systematic improvement? N Engl J Med 2007;356: 1700-2.
58. Kelly L, Lazzaro M, Petersen C. Canadian drug regulatory framework. Can J Neurol Sci 2007;34 Suppl 1:S3-10.
59. O'Brien SF, Ram SS, Vamvakas EC, Goldman M. The Canadian blood donor health assessment questionnaire: lessons from history, application of cognitive science principles, and recommendations for change. Transfus Med Rev 2007; 21:205-22.
60. Orton SL, Stramer SL, Dodd RY. Self-reported symptoms associated with West Nile virus infection in RNA-positive blood donors. Transfusion 2006;46:272-7.
61. Grass JA, Wafa T, Reames A, Wages D, Corash L, Ferrara JL, Lin L. Prevention of transfusion-associated graft-versus-host disease by photochemical treatment. Blood 1999;93: 3140-7.
62. Roback JD, Hossain MS, Lezhava L, Gorechlad JW, Alexander SA, Jaye DL, Mittelstaedt S, Talib S, Hearst JE, Hillyer CD, Waller EK. Allogeneic T cells treated with amotosalen prevent lethal cytomegalovirus disease without producing graft-versus-host disease following bone marrow transplantation. J Immunol 2003;171:6023-31.
63. Fast LD, Dileone G, Cardarelli G, Li J, Goodrich R. Mirasol PRT treatment of donor white blood cells prevents the development of xenogeneic graft-versus-host disease in Rag2-/-gamma c-/- double knockout mice. Transfusion 2006;46:1553-60.

64. Epstein JS, Vostal JG. FDA approach to evaluation of pathogen reduction technology. Transfusion 2003;43:1347-50.
65. Sunstein CR. Laws of fear: beyond the precautionary principle. Cambridge (UK): Cambridge University Press; 2005.
66. Jackson BR, Aubuchon JP, Birkmeyer JD. Update of cost-effectiveness analysis for solvent-detergent-treated plasma. JAMA 1999;282:329.
67. Pereira A. Cost-effectiveness of transfusing virus-inactivated plasma instead of standard plasma. Transfusion 1999;39:479-87.
68. Riedler GF, Haycox AR, Duggan AK, Dakin HA. Cost-effectiveness of solvent/detergent-treated fresh-frozen plasma. Vox Sang 2003;85:88-95.
69. Bell CE, Botteman MF, Gao X, Weissfeld JL, Postma MJ, Pashos CL, Triulzi D, Staginnus U. Cost-effectiveness of transfusion of platelet components prepared with pathogen inactivation treatment in the United States. Clin Ther 2003;25:2464-86.
70. Staginnus U, Corash L. Economics of pathogen inactivation technology for platelet concentrates in Japan. Int J Hematol 2004;80:317-24.
71. Postma MJ, van Hulst M, de Wolf JT, Botteman M, Staginnus U. Cost-effectiveness of pathogen inactivation for platelet transfusions in the Netherlands. Transfus Med 2005;15:379-87.
72. Moeremans K, Warie H, Annemans L. Assessment of the economic value of the INTERCEPT blood system in Belgium. Transfus Med 2006;16:17-30.
73. Custer B. Economic analyses of blood safety and transfusion medicine interventions: a systematic review. Transfus Med Rev 2004;18:127-43.
74. van Hulst M, de Wolf JT, Staginnus U, Ruitenberg EJ, Postma MJ. Pharmaco-economics of blood transfusion safety: review of the available evidence. Vox Sang 2002;83:146-55.
75. Detsky AS, Laupacis A. Relevance of cost-effectiveness analysis to clinicians and policy makers. JAMA 2007;298:221-4.
76. Gold M, Siegel J, Russell L, Weinstein M. Cost-effectiveness in health and medicine. New York: Oxford University Press; 1996.
77. Mauskopf JA, Paul JE, Grant DM, Stergachis A. The role of cost-consequence analysis in healthcare decision-making. Pharmacoeconomics 1998;13:277-88.
78. Barbara JA. The rationale for pathogen-inactivation treatment of blood components. Int J Hematol 2004;80:311-6.
79. Turner ML. Prion reduction filters. Lancet 2006;368:2190-1.
80. Hanley JA, Lippman-Hand A. If nothing goes wrong, is everything all right? Interpreting zero numerators. JAMA 1983;249:1743-5.

別紙様式第2-1

No. 37

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2007. 11. 22	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況		公表国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社) 合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)		Okazaki H. 18th Regional Congress of the ISBT, Asia; 2007 Nov 10-13; Hanoi.	日本

研究報告の概要

○よりよい患者治療のための日本のヘモビジランスの有用性

日本赤十字社(JRC)が全国的ヘモビジランス体制を導入してから14年が経過した。報告された輸血副作用症例数は年間約2000例で、過去3年間はほぼ一定数である。非溶血性輸血副作用は報告症例の約80%を占めており、これには輸血関連急性肺障害(TRALI)やアナフィラキシーが含まれる。過去3年間でTRALI症例92例、TRALI疑い症例44例を記録した。TRALIに関係した献血者の約40%に白血球抗体を認めた。非溶血性輸血副作用を起こした患者の血漿タンパク質の抗体と欠損のスクリーニングを継続し、2006年にハプトグロビン欠損者を新たに3例特定した。輸血感染症(TTI)の報告数は、2004年293例、2005年265例、2006年191例と年々減少しているが、献血者の保管検体のID-NATで感染が確認された症例数はこれよりかなり少ない。TTIリスクを低下させる新たな戦略として、2004年から、HBV/HCV/HIV NATのプールサイズ縮小と、受付時の本人確認が実施されている。近年、輸血伝播HEV感染が問題となっており、北海道では最近4症例を記録した。北海道地方ではブタの内臓を十分加熱せずに食べることがあるため、これが献血者に発現したHEVの原因と考えられる。現在北海道で研究的HEV NATを実施している。また、細菌感染も問題となっている。2006年には細菌感染症例を3例認めた。死亡例1例はStaphyloccocus aureusに汚染された濃厚血小板製剤、非死亡例2例はYersinia enterocoliticaに汚染された濃厚赤血球製剤に関連した。日本では、濃厚血小板の保存期間はわずか72時間であり、細菌検査は行っていない。2007年に全ての血液製剤について白血球除去と初流血除去を開始した。3つ目の問題はvCJDである。2005年には日本で最初のvCJD症例が診断された。厚生労働省は、輸血によるvCJD感染を防ぐために、特定の期間ヨーロッパに滞在した人を献血から除外することを決定した。JRCのヘモビジランスは病院の自発報告に基づいている。ヘモビジランスの向上には、病院と血液センターとの相互協力が不可欠である。

報告企業の意見	今後の対応
日本赤十字社の輸血副作用とヘモビジランスに関する報告である。	日本赤十字社では、薬事法に基づき輸血に関連する副作用・感染症症例を報告している。また、「血液製剤等に係る遡及調査ガイドライン」(平成17年3月10日付薬食発第0310009号)に基づき、輸血副作用・感染症の調査を行っている。輸血副作用・感染症に関する新たな知見等について今後も情報の収集に努める。次世代NATの導入に向けた準備を進めている。(2007年11月、血小板の有効期間を本文中の72時間から4日間に延長した。)

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-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

Simultaneous Session 13: Haemovigilance in Patients

3B-S13-2

TRANSFUSION-ASSOCIATED GRAFT-VERSUS-HOST DISEASE (T-A G-V-H D)

Holland PV
UC Davis Medical Center, Sacramento, USA

Transfusion-associated graft-versus-host disease may occur when viable lymphocytes in a blood component engraft in a susceptible recipient and reject the patient/host. It has the features of classical graft-versus-host disease, e.g. like that after an incompletely matched (allogeneic) bone marrow or stem cell transplant, with the added complication of bone marrow failure. The latter is responsible for the high mortality after T-A G-v-H D where death is usually due to sepsis and/or bleeding. Patients at risk of T-A G-v-H D may have cellular immunodeficiency states, either congenital or acquired, or may be immunocompetent when the right combination of HLA antigens occurs on the lymphocytes in the transfused blood component. Patients at risk include those with acute leukemia, lymphoma, stem cell transplants, and those on intense, immunosuppressive chemotherapy, especially those receiving drugs like fludarabine and 2CDA, or undergoing radiation therapy. Non-immunosuppressed patients may be at risk when the blood component comes from a donor homozygous for HLA locus antigens for which the patient is heterozygous. The relative risk of the latter is increased when components are from blood relatives or from the same ethnic group as the patient and have limited HLA diversity. HLA matched components for patients who have become refractory to random donor platelets may increase the risk of T-A G-v-H D. Prevention is the key to obviating T-A G-v-H D as treatment is limited and rarely effective in obviating death. While inactivation of lymphocytes in blood components is most often carried out using irradiation, pathogen inactivation (PI) processes similarly inactivate transfused white blood cells. Radiation may be carried out using cobalt 60 sources but is more conveniently performed with dedicated irradiators with a cesium 137 source or specialized X-ray irradiators. The latter instruments are expensive to purchase but easy to maintain while being convenient to use. Quality control of irradiation involves a method to map the absorbed dose periodically and a device (usually a radiosensitive label) to verify that the dose of irradiation has been delivered to the cellular blood component. Standard operating procedures (SOPs) are set up to ensure that patients at risk of T-A G-v-H D receive irradiated or PI blood components. Irradiated components are not radioactive and may be given to patients who do not require irradiated components. The main effect of the irradiation is to cause minimal ongoing hemolysis and increased potassium leakage of red blood cells, so RBCs have a dating period of 28 days after irradiation.

3B-S13-3

THE BENEFITS OF THE JAPANESE HAEMOVIGILANCE SYSTEM FOR BETTER PATIENT CARE

Okazaki H
Japanese Red Cross Society, Tokyo, Japan

The Japanese Red Cross (JRC) blood service headquarters is the one and only blood service institution in Japan. There are 69 blood centers and 116 blood donation rooms collecting almost 60% of all blood. Mobile units, on the other hand, collect 40% of all blood. There were about five million donations in 2006, which consisted of 400 mL of whole blood donations (50%), and 200 mL of whole blood (30%) and apheresis donations (20%). We issued 3.3 million bags of red cell concentrate, 0.7 million bags of apheresis platelet concentrate, and 1.3 million bags of fresh frozen plasma in 2006. Fourteen years has past since the JRC implemented the haemovigilance system nationwide. The number of reported cases is around 2000, which has been almost the same for the past three years. Non-hemolytic transfusion reactions account for 80% of reported cases, which include transfusion-related acute lung injury (TRALI) and anaphylaxis. In the last three years, we recorded 92 cases of TRALI and 44 cases of possible TRALI. We found leukocyte antibodies in around 40% of donors implicated in TRALI. We continued the screening of plasma protein antibody and deficiencies in patients showing non-hemolytic transfusion reactions and found three more cases of haptoglobin deficiency in 2006. The number of reported cases of transfusion-transmitted infections (TTI) gradually decreased yearly: 293 in 2004, 265 in 2005, and 191 in 2006, although the numbers of cases confirmed by ID-NAT of repository samples from implicated donors are much lower than these. New strategies to reduce the risk of TTI have been implemented since 2004, that is, the reduction of HBV/HCV/HIV NAT pool size from 50 to 20 and the implementation of the regulation regarding donor identification at the reception. Transfusion-transmitted HEV (TT-HEV) infection is our most recent concern. Recently, we have recorded four cases of TT-HEV infection in Hokkaido, which is the largest island north of Japan. The cause of the presence of HEV in donors is probably the local practice of eating rare pork innards in the Hokkaido area. We now implement investigative HEV NAT in the Hokkaido region. Bacterial contamination is another concern. In 2006, we encountered three cases of bacterial contamination. One fatal case was associated with a platelet concentrate contaminated with *Staphyloccocus aureus*. Two non-fatal cases were associated with red blood cell concentrate contaminated with *Yersinia enterocolitica*. In Japan, the storage period of platelet concentrate is only 72 hours without the need for bacterial examination. We started to implement universal leukoreduction and diversion of initial blood flow for all blood products from early 2007. The third concern is vCJD. The first vCJD case was diagnosed in Japan in 2005. The Ministry of health, labour and welfare decided to exclude donors who have traveled to Europe during a certain period to prevent vCJD infection via transfusion. Our haemovigilance system is based on voluntary reports from hospitals. Mutual cooperation between hospitals and blood centers is essential for improving the haemovigilance system.

3B-S13-4

DETECTION OF HPDEL AMONG THAIS, DELETED ALLELE OF HAPTOGLOBIN GENE THAT CAUSES CONGENITAL HAPTOGLOBIN DEFICIENCY

Shimada E[1], Odagiri M[1], Chaiwong K[2], Watanabe Y[1], Anazawa M[1], Mazda T[1], Okazaki K[1], Juji T[1], O'Charoen R[2], Tadokoro K[1]
[1]*The Japanese Red Cross, Tokyo, Japan* [2]*Thai Red Cross Society, Bangkok, Thailand*

Background: Congenital haptoglobin deficiency is a risk factor for anaphylactic non-hemolytic transfusion reactions in Japan. The deleted allele of the haptoglobin gene (Hp), Hpdel, in which there is a deletion larger than 20 kilobases in Hp and the tandemly arranged haptoglobin-related gene (Hpr), were identified from the Japanese patients with congenital haptoglobin deficiency who experienced anaphylactic transfusion reactions. The Hpdel allele has also been observed in other Northeast Asian populations, such as Koreans and Chinese. The same distribution in another part of Asia, specifically Southeast Asian countries, is thought to be worth investigating.
Aims: To investigate the distribution of congenital haptoglobin deficiency in Southeast Asian countries, we analyzed haptoglobin among the Thai population.
Methods: Blood samples collected from 200 randomly selected healthy Thai volunteers were analyzed for serum haptoglobin and the haptoglobin gene. 1) Plasma haptoglobin concentration was measured to identify haptoglobin deficiency. 2) Haptoglobin phenotyping was performed using SDS-PAGE followed by Western blotting. 3) The presence of the Hpdel allele was determined using genomic DNA by an Hpdel-specific PCR method.
Results: There were no haptoglobin-deficient subjects detected among the 200 Thais. Their haptoglobin phenotypes were as follows: Hp 1-1 in 10, Hp 2-1 in 81 and Hp 2-2 in 109. Six individuals heterozygous for Hpdel were

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2008. 3. 17	該当なし	

一般的名称	解凍人赤血球濃厚液	研究報告の公表状況		公表国
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)		Störmer M, Kleesiek K, Dreier J. Vox Sang. 2008 Apr;94(3):193-201. Epub 2007 Dec 11.	ドイツ

研究報告の概要

⊙Propionibacterium acnesは、濃厚血小板製剤中で増殖しない

背景および目的:Propionibacterium acnes(P. acnes)は、嫌気性培養による検出方法を用いた場合、血小板濃縮製剤(PC)でもっとも頻度の高い汚染菌のひとつと見なされている。しかし、プロピオン酸菌属は、すでに血液製剤が輸血された後で検出される場合が多い。また、P. acnes汚染PCを輸血された患者の転帰についての試験は現在もあまり行われていないことから、P. acnesと輸血の関連性の解明が望まれている。本試験では、輸血後に無菌試験で細菌が検出されたPCの受血者の臨床効果のモニタリングを行った。さらに、血小板細菌スクリーニングにおけるプロピオン酸菌属の重要性を明らかにするために、PCに接種したプロピオン酸菌属の細菌増殖を評価した。

材料および方法:ルックバック調査において、汚染が推定されるPCの保存から輸血までの経路を追跡した。In vitro試験ではPCにプロピオン酸菌属の臨床分離菌1～100 CFU/mLを接種した(n=10)。好気的に22℃で10日間保管している間にサンプルを摂取し、平板培養および自動BacT/Alert培養システムにより、細菌の有無を評価した。

結果:P. acnesは、PC保存条件下では、細菌の生育は緩慢であるか、または生育を認めなかった。汚染の可能性のあるPCを輸血した後の副作用は認めなかった。

結論:プロピオン酸菌属はPC保存条件下で増殖しないために、検出されないか、血液製剤がすでに輸血された後に検出されると考えられた。

報告企業の意見	今後の対応
Propionibacterium acnesをはじめとするプロピオン酸菌属は、濃厚血小板製剤の保存条件下では増殖せず、汚染の可能性のある製剤を輸血した後の副作用は認めなかったとの報告である。	日本赤十字社では、輸血による細菌感染予防対策として平成18年10月より血小板製剤について、また、平成19年3月より全血採血由来製剤について、初流血除去を導入した。また、全ての輸血用血液製剤について、平成19年1月より保存前白血球除去を実施している。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。今後も細菌やウイルスの検出や不活化する方策について情報の収集に努める。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

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Propionibacterium acnes lacks the capability to proliferate in platelet concentrates

M. Störmer, K. Kleesiek & J. Dreier

Institut für Laboratoriums- und Transfusionsmedizin, Herz- und Diabeteszentrum Nordrhein-Westfalen, Universitätsklinik der Ruhr-Universität Bochum, Bad Oeynhausen, Germany

Vox Sanguinis

Background and Objectives *Propionibacterium acnes* is considered to be one of the most frequent contaminants of platelet concentrates (PCs) when anaerobic culture-based detection methods are used. But *Propionibacteria* are often detected too late when blood products have already been transfused. Therefore, its transfusion relevance is still demanding clarification because studies of the outcome of patients transfused with *P. acnes*-contaminated PCs are still uncommon. In this study, we monitored clinical effects in patients after transfusion of PCs, which were detected too late in sterility testing. Furthermore, we assessed the bacterial proliferation of *Propionibacterium* species seeded into PCs to clarify their significance for platelet bacteria screening.

Materials and Methods In the look-back process, we followed the route of the putative contaminated PC units from storage to transfusion. In the *in vitro* study, PCs were inoculated with 1–100 colony-forming unit (CFU)/ml of clinical isolates of *Propionibacteria* ($n = 10$). Sampling was performed during 10-day aerobic storage at 22 °C. The presence of bacteria was assessed by plating culture and automated BacT/Alert culture system.

Results *Propionibacterium acnes* shows slow or no growth under PC storage conditions. Clinical signs of adverse events after transfusion of potentially contaminated PC units were not reported.

Conclusion *Propionibacteria* do not proliferate under PC storage conditions and therefore may be missed or detected too late when blood products have already been transfused.

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Key words: automated culture, bacterial detection, platelet contamination, *Propionibacterium acnes*, sterility testing.

Introduction

Bacterial contamination of platelet concentrates (PCs) is an ongoing problem associated with significant transfusion-related morbidity and mortality. Currently, PC transfusion-transmitted sepsis is recognized as the most frequent infectious complication in transfusion therapy, surpassing by up to two orders of magnitude the incidence of transfusion-associated viral transmission [1,2]. Most reports estimate that as many as 1 in 2000 to 3000 PCs, both apheresis-derived and buffy-coat-derived PCs are contaminated with bacteria [1,3]. Due to their storage at room temperature for up to 5 days, PCs are the most frequently affected blood product [1,4]. These conditions permit growth of bacteria with the potential for transmission to patients receiving platelet preparations [5]. Next to coagulase-negative *Staphylococci*, *Propionibacterium acnes* is implicated in most cases of bacterial contamination of PCs and is detected fairly frequently when anaerobic bottles are used [1,3,5,6]. Schmidt *et al.* [7] reported 20 of 37 initial

Correspondence: Jens Dreier, Institut für Laboratoriums- und Transfusionsmedizin, Herz- und Diabeteszentrum Nordrhein-Westfalen, Universitätsklinik der Ruhr-Universität Bochum, Georgstrasse 11, D-32545 Bad Oeynhausen, Germany
E-mail: jdreier@hdz-nrw.de

Abbreviations: DSM, Deutsche Stammsammlung für Mikroorganismen; IP, Propionibacterium isolate; OWL, Ostwestfalen-Lippe; PVX, PolyVitex; PCs, platelet concentrates; PBS, phosphate-buffered saline.

positive anaerobic cultures of which three were confirmed positive on reculture for *P. acnes* while Schrezenmeier *et al.* [8] reported 45 of 98 initial anaerobic positive samples with 20 confirmed positive on reculture for *P. acnes*. It accounts for approximately half of the total skin flora, with an estimated density of 10^2–10^6 organisms per cm^2 [9]. Accordingly, the bacterial entry from venepuncture during a conventional blood donation is expected to be 0·03 colony-forming unit (CFU)/ml [10]. Therefore, the donor phlebotomy site represents the major source of bacterial contamination of PCs [8]. In the UK, Serious Hazards of Transfusion (SHOT) reports that potentially 80% of bacterial transmissions, in which the source was defined, were derived from the donor's arm [11,12].

Propionibacterium acnes is a Gram-positive, slow-growing, non-sporeforming anaerobic bacterium that is commonly present as part of the normal skin flora and colonizes within the sebaceous glands, which are the likely sites of platelet contamination with a density of 10^2–10^3 organisms per cm^2. Even a careful disinfection of the donor phlebotomy site using a single-swab method with 70% isopropyl alcohol may result in incomplete disinfection of such organisms [13]. de Korte and colleagues [14] reported that surface disinfection will therefore be less adequate to remove diphtheroids like *P. acnes*, whereas diversion of the first 10 ml of a whole-blood donation will reduce all kind of skin flora. Limited reports have pointed out that *P. acnes* can be causative for a variety of infections, including endophthalmitis, neurosurgical wound infections, pulmonary infections and endocarditis. But, primarily it is considered as a contaminant of cultures obtained percutaneously, including blood cultures [15].

Since screening for bacterial contamination was recommended by the American Association of Blood Banks, several technologies including culture and rapid methods for bacterial detection have been developed [10,11,16]. Most facilities have adopted the semiautomated BacT/Alert 3D culture system (bioMérieux, Nürtingen, Germany), which is cleared for the quality control of PCs by the Food and Drug Administration (FDA), as the instrument to detect platelet contamination [17]. But despite the success of prevention of transfusion-transmitted infections, continued reports raise the possibility that this system has disadvantages and an appreciable failure rate [17–19]. On the one hand, slow-growing organisms may be detected after the product has already been transfused; on the other hand, two-bottle blood-culture systems allow for optimized growth of both aerobic and anaerobic organisms yet also enable detection of bacterial strains that are unable to proliferate in human PCs. Nevertheless, improvements from increasing the sensitivity and speed of this detection method are under development. Brecher and Hay [20] argue for the routine implementation of an anaerobic bottle together with an aerobic bottle for the detection of platelet bacteria contamination because of the great diversity of bacterial preferences for growth in either aerobic or anaerobic bottles. The addition of the anaerobic bottle slightly improves the time to first detection of some facultative anaerobes [20] and allows detection of obligate anaerobes, which have infrequently been implicated in transfusion-mediated bacterial sepsis [21]. Furthermore, doubling the platelet sample volume improves the detection of slow-growing organisms by approximately 25% [22].

In general, studies about bacterial contamination of PCs emphasize the incidence of *Propionibacteria* in platelet bacteria screening using automated culture but to date the significance of this organism in platelet bacteria screening is still not clear and badly needs clarification. Therefore, we monitored the clinical patients' outcome after transfusion of an initially culture-positive PC to clarify the clinical relevance of *P. acnes*. Moreover, we determined the bacterial growth kinetics of *Propionibacterium* species in PCs during storage. Subsequently, the significance of culture-positive detection at the end of PCs storage in platelet bacteria screening shall be discussed.

Materials and methods

Blood collection

Apheresis-derived single-donor platelets were obtained from the transfusion service UniBlutspendedienst Ostwestfalen-Lippe, Bad Oeynhausen, Germany, after standard processing with the Haemonetics MCS+ (Haemonetics GmbH, München, Germany) from healthy blood donors and stored at 20 to 24 °C with agitation. Predonation sampling was performed after donor arm disinfection using a single-swab method with 70% isopropyl alcohol.

Source of *Propionibacterium* isolates – routine sterility testing of PCs

This study was conducted with isolates of *Propionibacterium* (IP) species ($n = 6$; isolates IP540, IP240, IP016, IP551, IP095 and IP816), which were isolated from contaminated PCs during routine sterility testing of PCs at our transfusion service. All six cases of *P. acnes* were detected only in the anaerobic bottle in the automated culturing system. For routine screening of PCs, 15 ml of sample is taken under aseptic conditions after standard processing of PCs and storage of up to 24 h at 22 °C with agitation, and is used for microbial and molecular genetic sterility testing as described by Störmer *et al.* [23]. For this purpose, nucleic acids are extracted using magnetic separation technology (Chemagen, Baesweiler, Germany) and analysed by a one-step reverse transcriptase-polymerase chain reaction (RT-PCR) method using a primer and probe system for amplifying a 122-bp fragment of bacterial 23S ribosomal RNA. As an internal extraction and amplification control, human β_2-microglobulin (B2-MG) mRNA was coextracted and coamplified with each reaction to avoid

false-negative results due to PCR inhibition. The BacT/Alert (bioMérieux) automated culturing system served as reference method where 5 ml of PCs were inoculated into both the aerobic (BacT/Alert BPA; bioMérieux) and standard anaerobic culture bottle (BacT/Alert BPN) and were incubated for up to 7 days. Initial reactive [7] anaerobic culture bottles (BacT/Alert BPN; bioMérieux) were subcultured and the identification of bacterial isolates was performed by 16S rRNA analysis and biochemical tests.

In addition, *P. acnes* (IP3912), *Propionibacterium avidum* (IP4851) and *Propionibacterium granulosum* (IP5152) isolated from other clinical samples and reference strain *P. acnes* DSM (Deutsche Stammsammlung für Mikroorganismen) 1897, which was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Braunschweig, Heidelberg, Germany), were included in this study. The 10 *Propionibacterium* strains were cultured in Trypticase Soy Broth (TS; bioMérieux) at 37 °C under anaerobic conditions for 48 h. Serial 10-fold dilutions of grown cultures were made in phosphate-buffered saline (PBS) and plated on PolyVitex (PVX) blood agar plates (PVX; bioMérieux) to determine the bacterial titre (CFU/ml). Aliquots, taken from appropriate dilutions, were used for inoculation of the PCs.

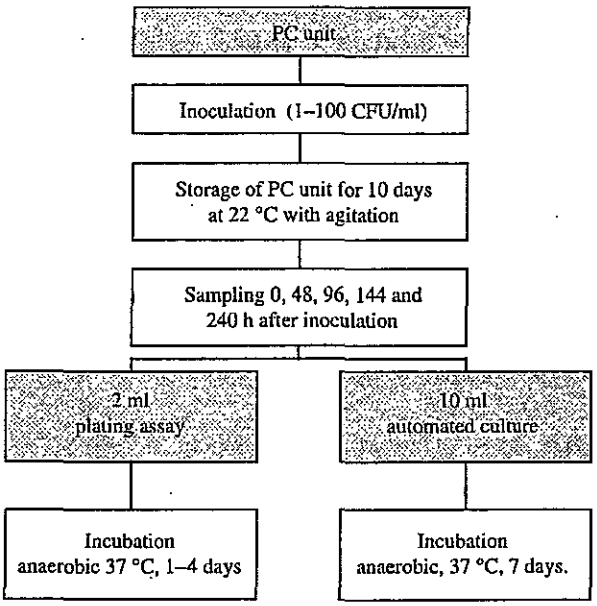

Fig. 1 Flow diagram representing the inoculation and sampling strategy. Inoculation of one single apheresis-derived platelet concentrate (PC) for one *Propionibacterium* species, and sampling for BacT/Alert are displayed.

Propionibacteria identification

Isolates of *Propionibacteria* were biochemically identified by using the API 20A multitest identification system (bioMérieux) in accordance with the manufacturer's instructions. For molecular genetic identification, PCR was performed using universal primers described by Ley *et al.* [24], which targets a conserved region of 16S ribosomal DNA. DNA sequencing and analysis was performed as described previously [25]. Sequence data have been submitted to GenBank and assigned accession numbers EF670439 to EF670442, EF670445, EF670450, EF680378 to EF680380, and EF680382.

Look-back process

In our PC-screening programme, we found six PCs tested positive for *P. acnes* [23]. In the look-back process, we followed the route of these putative contaminated PC units from storage to transfusion and monitored the clinical characteristics of the recipients. The donor directed look-back process summarized the detection time in the BacT/Alert system in relation to the time of transfusion of PCs. We reviewed the medical records of the six patients that received PCs tested positive for *P. acnes* in the BacT/Alert system. Medical records and laboratory information system searches were abstracted for primary diagnoses, kind of surgery, age at transfusion, microbiological findings, antibiosis at transfusion and markers of inflammatory events [C-reactive protein (CRP), leucocytes].

Inoculation and bacterial monitoring

To determine the growth kinetics of the isolated *P. acnes* strains during PC storage, we spiked PC units and monitored the presence of *P. acnes* during storage at 22 °C. All PCs used were sampled before bacterial inoculation to assure baseline sterility of the original apheresis bags. For this reason, 5 ml were inoculated into both the aerobic (BacT/Alert BPA; bioMérieux) and standard anaerobic culture bottles (BacT/Alert BPN) and incubated for up to 7 days.

For each bacterial strain, one PC was spiked with 1–100 CFU/ml of *Propionibacterium* species as shown in Fig. 1. To ensure the presence of *Propionibacteria* in the inoculated PC unit, a sample was taken immediately after inoculation (0 h) and analysed with the BacT/Alert 3D continuous monitoring system (bioMérieux).

To monitor the presence and proliferation of *Propionibacteria* in PCs by proliferation testing on blood agar plates and automated culture, sampling was performed during the 10-day storage at 22 °C with agitation at 48, 96, 144 and 240 h after inoculation. For this purpose, 5 ml aliquots of each PC unit were transferred in duplicate to the standard anaerobic culture bottle (BacT/Alert BPN). Incubation was performed using the BacT/Alert 3D continuous monitoring system at 37 °C until a reactive signal was detected, or for up to 7 days, if the signal remained negative. Samples that did not react after 7-day storage were considered sterile. Initially, reactive culture bottles were subcultured for confirmation and identification of *Propionibacteria*. Moreover, for visual inspection

and determination of the bacterial titre, 100 µl aliquots of serial dilutions of PC samples were plated in triplicate onto PVX blood agar and incubated at 37 °C for 48–168 h. To detect a bacterial level below 10 CFU/ml, 1 ml of sample was plated onto PVX blood agar, as well. After incubation, the number of colonies was counted and the concentration of *Propionibacteria* per ml of sample was calculated. Furthermore, to exclude donor-specific factors, like the presence of neutralizing antibodies, two further PC units from different donors were spiked with each *Propionibacterium* strain and bacterial proliferation was monitored by plate culture. All procedures were performed under sterile laminar air flow conditions.

Results

Study design

A total of 1533 apheresis-derived PC units were screened for bacterial contamination during a 20-month study period in our facility by automated culture and real-time RT-PCR as described previously [23]. In accordance with the definitions used by Schmidt *et al.* [7], we considered samples without a positive reaction in either test as negative. Samples with a reactive signal but no microbiological confirmation of the bacterial strain were labelled as initially reactive. Hence, a sample with both a reactive signal and microbiological confirmation was regarded as initially positive. Correspondingly, six anaerobic culture bottles were identified by the automated culture system as being initially positive (0·39%). An aliquot was removed from the initially positive culture bottle for Gram-staining and subculture to agar media. The six isolates were identified as *P. acnes* by biochemical and molecular genetic identification in all six cases. All strains were detected by the automated culture system between 5 and 6 days (5·19 ± 0·79) after sampling, or 6 and 7 days (6·19 ± 0·79) after donation, respectively. At that time, the platelet product had already been transfused and no sample or predonation bag was available for confirmation of the positive result, but no adverse reactions were noted after transfusion.

Look-back process

Because of the late detection of the automated culture system in our platelet bacteria screening study, all PCs had been transfused. Putative contaminated PCs were transfused within the first day ($n = 3$), second day ($n = 2$) and third day ($n = 1$) of storage whereas the BacT/Alert culture system detected these PC units between 5 and 6 days after donation. To exclude bacteraemia of the PC donors, the following PC donations were especially monitored for bacterial contamination using microbial and molecular genetic sterility testing, but without positive confirmation. In the look-back process, we reviewed the medical records of six patients that received PCs tested positive for *P. acnes* in the BacT/Alert system as shown in Table 1. All transfusion reports were returned to the blood bank and transfusion was documented without complications. Back-tracked PCs were transfused perioperatively or postoperatively to massively bleeding patients who underwent heart surgery. Because of bacterial infectious diseases prior to transfusion, most patients ($n = 5$) were under antibiotic therapy with drugs that should be effective against *P. acnes* as well. One patient was under immunosuppressant therapy due to heart transplantation. The progression of proinflammatory markers [procalcitonin (data not shown), CRP (reference range ≤ 5 mg/l) or leucocyte count] has to be regarded as crucial because of prior bacterial infectious diseases. Furthermore, the increase of these markers may be the result of a postoperative acute phase reaction. Blood cultures taken after transfusion of the PC unit were sterile.

Growth characteristics of *Propionibacteria* in platelet concentrates

In order to assess the bacterial proliferation of *Propionibacterium* species in PCs under storage conditions, the presence of bacteria was monitored by plate culture and enrichment culture as shown in Fig. 1. The results of the investigation are shown in Fig. 2. Sampling time, bacterial load (growth-curve of *Propionibacteria*) and detection time of the BacT/Alert culture system are presented for each *Propionibacterium* strain. Approximately 24 h after donation, PCs from different donors were spiked with one of the 10 *Propionibacterium* strains and bacteria contents were monitored by colony-forming assay and automated culture during a 10-day storage. The mean initial bacterial inoculum densities at the beginning of storage (day 0) for the PCs were determined by colony-forming assay and varied between 2 and 80 CFU/ml. Following inoculation, a slight increase to approximately 150 CFU/ml, a subsequent decrease or no change of the bacterial load were observed during storage at 22 °C depending on the *Propionibacterium* strain.

Propionibacterium isolates IP540, IP551, IP816 and IP095 showed a slightly increased bacterial load in the first 48 h of PC storage that decreased down to 10 CFU/ml in the following days. The bacterial load of the isolates IP016 and IP240 were already slightly reduced after 48 h and remained unchanged as well as for isolates IP3912, DSM1897, IP4851 and IP5152. Therefore, all *Propionibacteria* strains showed no proliferation in the PC within the 10 days. The influence of donor-specific factors was excluded, because all *Propionibacterium* strains showed similar growth kinetics in PC units from different donors (data not shown).

Automated culture monitoring of bacterial growth

As shown in Fig. 2, all day 0 inoculated samples cultured in the anaerobic bottles were signaled positive by the automated

Table 1 Outcome of recipients of putative contaminated platelet concentrate (PC) units

Donor							Recipient							
P. acnes isolate	Donor sex (age/years)	Time of donation	Time of TF[a]	Aerobic culture detection	Anaerobic culture detection[b]	Bacterial strain	Recipient sex (age/years)	Disease and surgical intervention	Microbiological diagnostic findings after TF	Antibiosis	CRP[c] pre-TF (mg/dl)	CRP post-TF (mg/dl)	Leucocytes pre-TF (10^9/l)	Leucocytes post-TF (10^9/l)
IP016	Female (32)	10 May 2006	12 May 2006	Negative[d]	Positive 107 h (5 days) (16 May 2006)	*P. acnes*	Male (77)	Aortic and mitral valve replacement, aortic plastic valvular prosthesis, ancurysma aorta acendes	Urinary tract infection with *P. aeruginosa* and *E. faecium*	No	0·38 (11 May 2006)	6·68 (13 May 2006)	6·4 (11 May 2006)	13·1 (13 May 2006)
IP540	Female (41)	23 October 2006	24 October 06	Negative	Positive 113 h (5 days) (29 October 2006)	*P. acnes*	Male (62)	Coronary heart disease, heart transplantation	Blood culture negative (2 November 2006)	Yes (vancomycin, imipenem)	0·74 (23 October 2006)	0·53 (24 October 2006)	7·7 (23 October 2006)	13·0 (24 October 2006)
IP551	Male (28)	26 October 2006	28 October 2006	Negative	Positive 159 h (6 days) (2 November 2006)	*P. acnes*	Female (87)	Aortic valve stenosis, aortic plastic valvular prosthesis aortic valve replacement aortocoronaric bypass	Blood culture negative (3 November 2006)	Yes (erythromycin, imipenem)	3·7 (27 October 2006)	12·6 (29 October 2006)	9·2 (27 October 2006)	10·1 (29 October 2006)
IP240	Male (57)	25 January 2007	26 January 2007	Negative	Positive 120 h (5 days) (31 January 2007)	*P. acnes*	Female (67)	Infectious endocarditis (*Enterococcus faecalis*), aortic and mitral valve replacement	Blood cultures negative (31 January 2007), tracheal secretion: *Klebsiella pneumoniae, Candida albicans*	Yes (vancomycin, imipenem)	3·93 (23 January 2006)	9·71 (27 January 2006)	18·6 (23 January 2006)	9·9 (27 January 2006)
IP816	Female (43)	20 March 2007	21 March 2007	Negative	Positive 132 h (6 days) (27 March 2007)	*P. acnes*	Male (47)	Pericardial lysis, aortic plastic valvular prosthesis aortocoronaric bypass	No microbiological Examination	Yes (cefazolin, clarithromycin)	0·49 (16 March 2007)	NT	8·0 (16 March 2007)	14·3 (22 March 2006)
IP095	Male (31)	29 June 2007	2 July 2007	Negative	Positive 116 h (5 days) (7 July 2007)	*P. acnes*	Male (74)	Ischemic cardiomyopathy, mitral valve replacement, aortocoronaric bypass	Blood culture (4 July 2007): *S. epidermidis*, tracheal secretion: *P. aeruginosa*	Yes (cefazolin, clarithromycin)	0·13 (29 June 2007)	4·88 (2 July 2006)	5·8 (28 June 2007)	13·2 (2 July 2007)

[a]TF, transfusion of platelet concentrate.

[b]Culture detection, detection time after sampling 24 h after donation.

[c]CRP, C-reactive protein (reference range ≤ 5 mg/l).

[d]Negative, negative after 7-day storage.

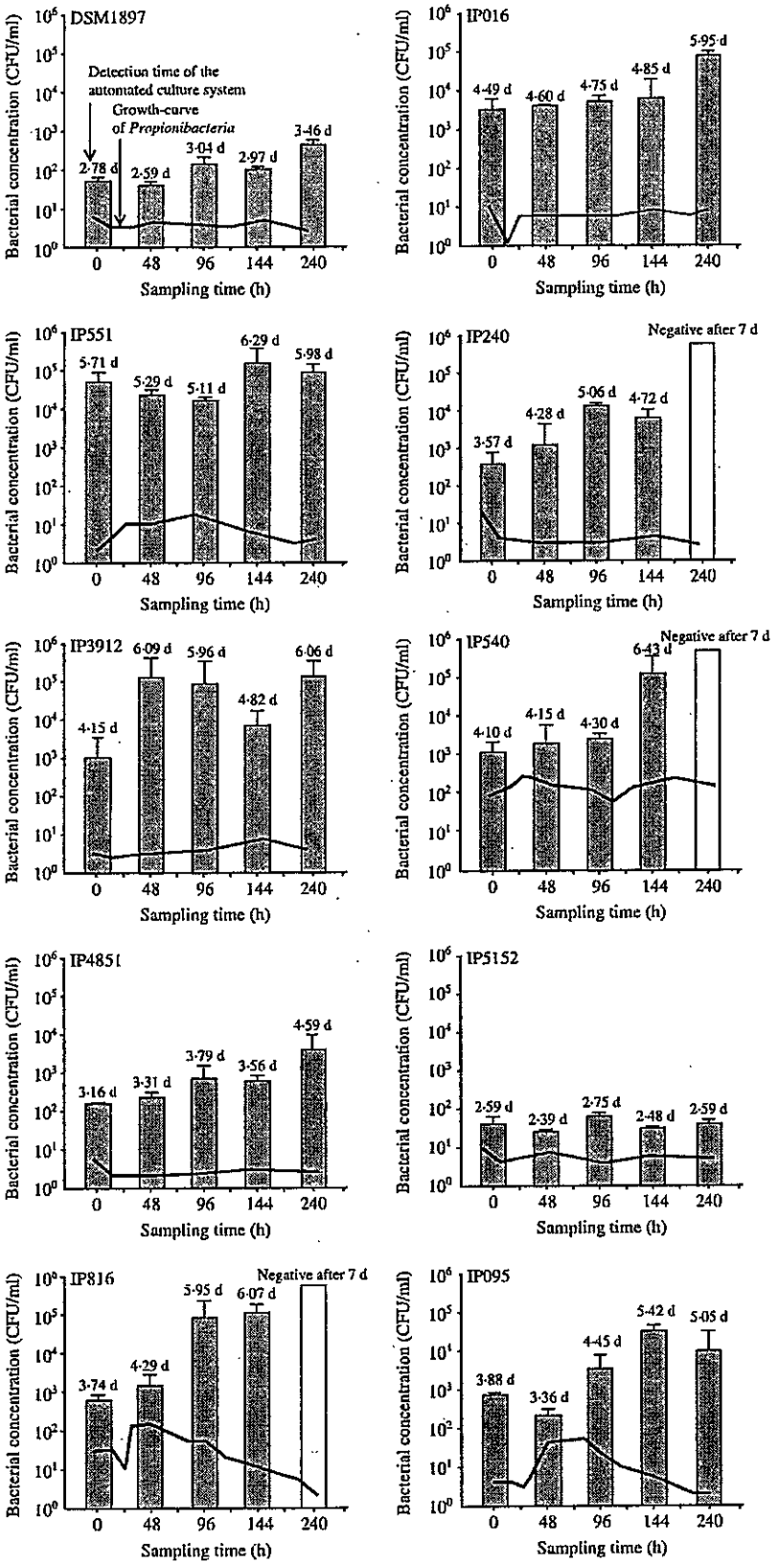

Fig. 2 Bacterial proliferation of *Propionibacterium* species in platelet concentrates (PCs) during storage and microbiological monitoring using an automated culture system. One single apheresis-derived PC unit was spiked with approximately 1–100 CFU/ml of *Propionibacterium* species and stored at 22 °C. Samples were taken in duplicate before inoculation (negative control) and at different times (0, 48, 96, 144 and 240 h after inoculation), enumerated by plating culture (line indicates the bacterial growth representing the bacterial load at the time of sampling) and inoculated into the anaerobic culture bottles for microbiological monitoring using an automated culture system (median times to first positive culture of the BacT/Alert detection is displayed in bars). d, days.

culture system, depending on the bacterial load in the PCs and growth characteristics of the strain. The BacT/Alert automated culture system detected all 10 *Propionibacterium* strains in the mean time of 2·59 to 5·71 days by sampling immediately after inoculation. During culture of inoculated PCs, all samples, with the exception of samples of IP240, IP540 and IP016, taken 240 h after inoculation were detected. Corresponding to the bacterial titres, the time to detection remained nearly constant (DSM1897, IP5152 and IP551) or slightly increased (IP540, IP016, IP240, IP3912, IP4851, IP816 and IP095) when samples were taken during the 10-day storage. Samples that did not react after 7-day incubation due to sampling error (samples of IP540, IP240 and IP816 after 240 h of storage) were subcultured for bacterial verification and considered sterile. Furthermore, no positive signal was recorded by the culture system for samples taken from unspiked PCs during incubation for up to 7 days.

Discussion

Contamination during blood donation or processing and subclinical infections in blood donors have all been implicated as sources of bacterial contamination in PCs [26]. Nevertheless, the predominant organisms implicated in platelet bacterial contamination are part of the human skin flora, including *Staphylococci*, *Corynebacterium* species and *Propionibacterium* species [3]. Coring of skin during the phlebotomy process may facilitate the entrance of bacteria into the collection bag [11]. In various studies, *P. acnes* was the most frequently implicated organism of bacterial contamination of PCs, but to date the clinical significance is debatable [8,14,27,28]. Thus, the principal objectives of this study were to discuss the meaning and appraisal of *Propionibacteria* detection at the end of storage using automated culture for platelet bacteria screening. Therefore, we simulated the bacterial contamination of PCs with 10 *Propionibacterium* species and monitored their growth characteristics in PCs during a 10-day storage at 22 °C. Although the bacterial contamination of apheresis products at collection may be as low as 1 to 10 CFUs per bag (0·003–0·03 CFU/ml) [17], it is common practice to perform *in vitro* experiments with an inoculum ensuring growth (1–100 CFU/ml) [29]. The results of our study agree to the findings of Mohr and colleagues [30] and show that *Propionibacterium* species do not proliferate under platelet storage conditions and therefore do not reach the level considered clinically significant (10^5 CFU/ml) [31]. These kinetics contribute to a very low bacterial concentration at the time of transfusion particularly considering that all implicated PCs were transfused within the first 3 days after donation, which is common practice in hospitals we serve. Hence, even the most sensitive assay based on the cultivation of bacteria misses *Propionibacteria* due to sampling error or detects *Propionibacteria* too late (5–7 days after PC preparation), when blood products have already been transfused. Therefore, sampling error and low rates of bacterial growth make it difficult to prevent transfusion of PCs contaminated with this organism [2].

Until today, different bacterial screening methods for the detection of bacterial contamination of PCs have been developed to reduce the risk of bacterial transmission by blood products [11]. But, to date none of these preventive methods is sufficient for the perfect preventive screening or detection of contaminated units. As shown in this study, *Propionibacterium* species may be missed or were detected most frequently in PCs with culture-based methods when blood products have already been transfused because of low bacterial numbers [6]. Inoculating anaerobic bottles in automated culture systems can detect these bacteria after 3- to 7-day incubation. Therefore, it must be pointed out that not all bacteria have the pathogenic capacity or growth characteristics to develop clinically significant inocula during the time period of platelet storage [32]. Nevertheless, automated bacterial screening methods based on carbon dioxide production or oxygen consumption as a function of bacterial growth have been regarded as the gold standard due to the high sensitivity with a stated detection limit of 1 CFU/ml [2,33–35]. Nonetheless, the use of the anaerobic culture bottle, in addition to the aerobic bottle, has a number of advantages. Most importantly, it enables detection of obligate anaerobes that have been implicated in transfusion-associated bacterial sepsis [21]. The need for detection of these organisms, however, requires clarification because of their slow growth and impaired survival [11]. In this study, we have shown that the growth of different bacterial species can vary widely in PCs. Similar data have been reported by others [25,30,36–39].

To approach this problem, we reviewed the medical records of six patients that received PCs tested positive for *P. acnes*. All patients neither showed symptoms of febrile transfusion complications, nor evidence of an inflammatory event associated with transfusion. Most patients transfused were under antibiotic therapy because of other infectious disease prior to transfusion. Therefore, our findings cannot be interpreted unequivocally. In moving forward, systematic studies of the outcome of patients transfused with *P. acnes*-contaminated PCs are needed. Although *P. acnes* is associated with serious infections like brain abscesses, osteomyelitis, endophthalmitis after intraocular surgey and lens implantation, subdural empyema, cerebal shunt infection and infective endocarditis [40], no correlation to transfusion transmission due to contaminated PCs has been reported and only a few cases have been described in transfusion-related sepsis [41–43]. As shown in our sterility testing study, in all cases of putative contaminated PC units, *P. acnes* was not isolated from the patients, and a cause-and-effect relation was not confirmed.

Moreover, Macauley *et al.* reported that eight units in which *P. acnes* was detected in the initial cultures were

transfused, but without adverse reactions associated to the unit [44]. In any event, transfusion-related clinical syndromes from PCs transfusion are often difficult, if not impossible to prove [43]. The lack of signs for transmissions of a bacterial infection is consistent with the assumption of either a low bacterial load or limited pathogenicity [10].

Therefore, further studies are needed to clarify the clinical significance of transfusion-transmitted bacterial infection in regard to *P. acnes*, taking into account that many recipients of PCs are immunosupressed or neutropenic. Studies of clinical syndromes including endocarditis, postcraniotomy infections, arthritis and spondylodiscitis, endophthalmitis and pansinusitis caused by *P. acnes* are currently being performed to confirm its pathogenic potential and clinical significance [15,45].

In conclusion, depending on the species and inoculums, differences in bacterial growth in PCs are often observed. Bacterial contamination of blood components may not always result in bacterial multiplication, because some organisms may not be able to survive the storage conditions due to autosterilization in the blood component. Other strains of bacteria may survive in the unit in low numbers but not multiply. In this study, we demonstrated that *P. acnes* is a frequent contaminant of blood components in platelet bacteria screening. But, due to its slow growth, the levels of bacteria in blood components may be too low to result in sepsis upon transfusion. However, optimized growth conditions using automated culture in platelet screening offers such species the opportunity to grow and be detected at the end of storage, but these conditions do not reflect the real storage and growth conditions of PCs.

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References

1 Yomtovian RA, Palavecino EL, Dysktra AH, Downes KA, Morrissey AM, Bajaksouzian S, Pokorny MA, Lazarus HM, Jacobs MR: Evolution of surveillance methods for detection of bacterial contamination of platelets in a university hospital, 1991 through 2004. *Transfusion* 2006; 46:719–730

2 Dreier J, Störmer M, Kleesiek K: Real-time polymerase chain reaction in transfusion medicine: applications for detection of bacterial contamination in blood products. *Transfus Med Rev* 2007; 21:237–254

3 Palavecino EL, Yomtovian RA, Jacobs MR: Detecting bacterial contamination in platelet products. *Clin Lab* 2006; 52:443–456

4 Riedel S, Siwek G, Beekmann SE, Richter SS, Raife T, Doern GV: Comparison of the BACTEC 9240 and BacT/Alert blood culture systems for detection of bacterial contamination in platelet concentrates. *J Clin Microbiol* 2006; 44:2262–2264

5 Blajchman MA, Beckers EA, Dickmeiss E, Lin L, Moore G, Muylle L: Bacterial detection of platelets: current problems and possible resolutions. *Transfus Med Rev* 2005; 19:259–272

6 Mohammadi T, Pietersz RNI, Vandenbroucke-Grauls CMJE, Savelkoul PHM, Reesink HW: Detection of bacteria in platelet concentrates: comparison of broad-range real-time 16S rDNA polymerase chain reaction and automated culturing. *Transfusion* 2005; 45:731–736

7 Schmidt M, Karakassopoulos A, Burkhart J, Deitenbeck R, Asmus J, Muller TH, Weinauer F, Seifried E, Walther-Wenke G: Comparison of three bacterial detection methods under routine conditions. *Vox Sang* 2007; 92:15–21

8 Schrezenmeier H, Walther-Wenke G, Muller TH, Weinauer F, Younis A, Holland-Letz T, Geis G, Asmus J, Bauerfeind U, Burkhart J, Deitenbeck R, Forstemann E, Gebauer W, Hochsmann B, Karakassopoulos A, Liebscher UM, Sanger W, Schmidt M, Schunter F, Sireis W, Seifried E: Bacterial contamination of platelet concentrates: results of a prospective multicenter study comparing pooled whole blood-derived platelets and apheresis platelets. *Transfusion* 2007; 47:644–652

9 Leyden JJ, McGinley KJ, Vowels B: *Propionibacterium acnes* colonization in acne and nonacne. *Dermatology* 1998; 196:55–58

10 Müller TH, Mohr H, Montag T: Methods for the detection of bacterial contamination in blood products. *J Lab Med* 2006; 30:74–90

11 McDonald CP: Bacterial risk reduction by improved donor arm disinfection, diversion and bacterial screening. *Transfus Med* 2006; 16:381–396

12 Shahar E, Wohl-Gottesman BS, Shenkman L: Contamination of blood cultures during venepuncture: fact or myth? *Postgrad Med J* 1990; 66:1053–1058

13 Kunishima S, Inoue C, Kamiya T, Ozawa K: Presence of *Propionibacterium acnes* in blood components. *Transfusion* 2001; 41:1126–1129

14 de Korte D, Curvers J, de Kort WLAM, Hoekstra T, van der Poel CL, Beckers EAM, Marcelis J: Effects of skin disinfection method, deviation bag and bacterial screening on clinical safety of platelet concentrates in The Netherlands. *Transfusion* 2006; 46:476–482

15 Pan SC, Wang JT, Hsueh PR, Chang SC: Endocarditis caused by *Propionibacterium acnes*: an easily ignored pathogen. *J Infect* 2005; 51:229–231

16 Hillyer CD, Josephson CD, Blajchman MA, Vostal JG, Epstein JS, Goodman JL: Bacterial contamination of blood components: risks, strategies, and regulation. Joint ASH and AABB Educational Session in Transfusion Medicine. *Hematology (Am Soc Hematol Educ Program)* 2003:575–589

17 Benjamin RJ, Wagner SJ: The residual risk of sepsis: modeling the effect of concentration on bacterial detection in two-bottle culture systems and an estimation of false-negative culture rates. *Transfusion* 2007; 47:1381–1389

18 Karahan ZC, Mumcuoglu I, Guriz H, Tamer D, Balaban N, Aysev D, Akar N: PCR evaluation of false-positive signals from two automated blood-culture sstems. *J Med Microbiol* 2006; 55:53–57

19 Kleinman SH, Kamel HAT, Harpool DR, Vanderpool SK, Custer B, Wiltbank TB, Nguyen KA, Tomasulo PA: Two-year eyperience

with aerobic culturing of apheresis and whole blood-derived platelets. *Transfusion* 2006; 46:1787–1794

20 Brecher ME, Hay SN: Investigation of an isolate of *Staphylococcus lugdunensis* implicated in a platelet fatality: a possible advantage of the use of an anaerobic bottle. *Transfusion* 2007; 47:1390–1394

21 McDonald CP, Hartley S, Orchard K, Hughes G, Brett MM, Hewitt PE, Barbara JAJ: Fatal *Clostridium perfringens* sepsis from a pooled platelet transfusion. *Transfus Med* 1998; 6:19–22

22 Wagner SJ, Eder AF: A model to predict the improvement of automated blood culture bacterial detection by doubling platelet sample volume. *Transfusion* 2007; 47:430–433

23 Störmer M, Kleesiek K, Dreier J: High-volume extraction of nucleic acids by magnetic bead technology for ultrasensitive detection of bacteria in blood components. *Clin Chem* 2007; 53:104–110

24 Ley BE, Linton CJ, Bennett DM, Jalal H, Foot AB, Millar MR: Detection of bacteraemia in patients with fever and neutropenia using 16S rRNA gene amplification by polymerase chain reaction. *Eur J Clin Microbiol Infect Dis* 1998; 17:247–353

25 Dreier J, Störmer M, Kleesiek K: Two novel real-time reverse transcriptase PCR assays for rapid detection of bacterial contamination in platelet concentrates. *J Clin Microbiol* 2004; 42:4759–4764

26 Nussbaumer W, Allersdörfer D, Grabmer C, Rheinschmidt M, Lin L, Schönitzer D, Lass-Flörl C: Prevention of transfusion of platelet components contaminated with low levels of bacteria: a comparison of bacteria culture and pathogen inactivation methods. *Transfusion* 2007; 47:1125–1133

27 Eady EA, Ingham E: *Propionibacterium acnes* – friend or foe? *Rev Med Microbiol* 1994; 5:163–173

28 Hinestrosa F, Djurkovic S, Bourbeau PP, Foltzer MA: *Propionibacterium acnes* as a cause of prosthetic valve aortic root abscess. *J Clin Microbiol* 2007; 45:259–261

29 Brecher ME, Holland PV, Pineda AA, Tegtmeier GE, Yomtovian R: Growth of bacteria in inoculated platelets: implications for bacteria detection an the extension of platelet storage. *Transfusion* 2000; 40:1308–1312

30 Mohr H, Bayer A, Gravemann U, Müller TH: Elimination and multiplication of bacteria during preparation and storage of buffy coat-derived platelet concentrates. *Transfusion* 2006; 46:949–955

31 Muder RR, Yee YC, Rihs J, Bunker M: *Staphylococcus epidermidis* bacteremia from transfusion of contaminated platelets: application of bacterial DNA analysis. *Transfusion* 1992; 32:771–774

32 AuBuchon JP: The reliability of bacterial detection in platelets. *ISBT Sci Series* 2006; 1:59–63

33 Brecher ME, Means E, Jere CS, Heath D, Rothenberg SJ, Stutzman LC: Evaluation of the BacT/ALERT 3D Microbial Detection System for platelet bacterial contamination: an analysis of 15 contaminating organisms. *Transfusion* 2001; 41:477–482

34 Brecher ME, Hay SN, Rothenberg SJ: Evaluation of a new plastic culture bottle using an automated microbial detection system for 9 common contaminating organisms found in platelet components. *Transfusion* 2004; 42:774–779

35 Eder AF, Kennedy JM, Dy BA, Notari EP, Weiss JW, Fang CT, Wagner S, Dodd RY, Benjamin RJ: Bacterial screening of apheresis platelets and the residual risk of septic transfusion reactions: the American Red Cross experience (2004–2006). *Transfusion* 2007; 47:1134–1142

36 Gong J, Högman CF, Hambraeus A, Johansson CS, Eriksson L: Transfusion-associated *Serratia marcescens* infection: studies of the mechanism of action. *Transfusion* 1993; 33:802–808

37 Mohammadi T, Pietersz R, Scholtalbers L, Vandenbroucke-Grauls CM, Savelkoul P, Reesink HW: Optimal sampling time after preparation of platelet concentrates for detection of bacterial contamination by quantitative real-time polymerase chain reaction. *Vox Sang* 2005; 89:208–214

38 Störmer M, Cassens U, Kleesiek K, Dreier J: Detection of bacteria in platelet concentrates prepared from spiked single donations using cultural and molecular genetic methods. *Transfus Med* 2007; 17:61–70

39 Heal JM, Singal S, Sardisco E, Mayer T: Bacterial proliferation in platelet concentrates. *Transfusion* 1986; 26:388–390

40 Günthard H, Hany A, Turina M, Wüst J: *Propionibacterium acnes* as a cause of aggressive aortic valve endocarditis and importance of tissue grinding: case report and review. *J Clin Microbiol* 1994; 32:3043–3045

41 Blajchmann MA, Goldman M, Baeza F: Improving the bacteriological safety of platelet transfusion. *Transfus Med Rev* 2004; 18:11–24

42 Pink JM, MacCallum S, Ribeiro A, Mylie BR: Platelet transfusion-related sepsis. *Aust N Z J Med* 1993; 23:717

43 Schneider T, Brevière D, Taillefer MF, Pujol-Rey A, Huart JJ: Bacterial contamination of platelet concentrates by *Propionibacterium acnes*. *Transfus Clin Biol* 2000; 7:540–546

44 Macauley A, Chandrasekar A, Geddis G, Morris KG, McChelland WM: Operational feasibility of routine bacterial monitoring of platelets. *Transfus Med* 2003; 13:189–195

45 Jakab E, Zbinden R, Gubler J, Ruef C, von Graevenitz A, Krause M: Severe infections caused by *Propionibacterium acnes*: an underestimated pathogen in late postoperative infections. *Yale J Biol Med* 1996; 69:477–482

別紙様式第2-1

No. 18

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2007. 12. 13	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	WHO, Epidemic and Pandemic Alert and Response (EPR). Available from: URL: http://www.who.int/csr/don/2007_12_09/en/index.html	公表国 中国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社) 合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)			

研究報告の概要

○鳥インフルエンザ―中国における状況―最新情報5
中国保健省は江蘇省におけるH5N1鳥インフルエンザの新たなヒト症例を報告した。この症例は12月6日に国立研究所にて感染が確認された。
患者は52歳の男性で、12月2日にH5N1感染のため死亡した24歳の男性の父親である。患者と密接な接触があった者であり、当局が医学的観察を行っていた。発症は12月3日で、直ちに治療のため病院に送られた。
12月9日までに中国では27例が確定され、17例が死亡例だった。

報告企業の意見	今後の対応
中国江蘇省において、H5N1鳥インフルエンザのため死亡した患者の父親がH5N1に感染、発症したとの報告である。	日本赤十字社では家禽に高病原性トリインフルエンザの流行が認められた場合、当該飼養農場の関係者や防疫作業従事者の献血制限を行っている。新型インフルエンザが流行した場合、献血者減少につながることも予想される。今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

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MedDRA/J Ver.11.0J

JRC2007T-090

World Health Organization

Avian influenza – situation in China - update 5

9 December 2007

The Ministry of Health in China has reported a new case of human infection with the H5N1 avian influenza virus in Jiangsu Province. The case was confirmed by the national laboratory on 6 December.

The 52-year old male is the father of the 24-year old man who died from H5N1 infection on 2 December 2007. He is one of the close contacts placed under medical observation by national authorities. He developed symptoms on 3 December and was sent immediately to hospital for treatment.

Of the 27 cases confirmed to date in China, 17 have been fatal.

感染症定期報告の報告状況(2008/3/1～2008/5/31)

血対ID	受理日	報告者名	一般名	生物由来成分名	原材料名	原産国	含有区分	文献	症例	適正使用措置
80030	2008/03/12	化学及血清療法研究所	乾燥濃縮人アンチトロンビンIII	アンチトロンビンIII	ヒト血液	日本	有効成分	有	有	無
80031	2008/03/12	化学及血清療法研究所	人免疫グロブリン ヒスタミン加人免疫グロブリン製剤	免疫グロブリン	ヒト血液	日本	有効成分	有	無	無
80032	2008/03/14	日本メジフィジックス	放射性医薬品基準テクネチウム大凝集人血清アルブミン(99mTc)	テクネチウム大凝集人血清アルブミン(99mTc)	生物学的製剤基準人血清アルブミン	日本	有効成分	無	無	無
80033	2008/03/18	ベネシス	ポリエチレングリコール処理人免疫グロブリン	人免疫グロブリンG	人血液	日本、米国	有効成分	有	無	無
80034	2008/03/18	ベネシス	乾燥濃縮人血液凝固第Ⅷ因子	血液凝固第Ⅷ因子	人血液	日本	有効成分	有	無	無
80035	2008/03/18	ベネシス	①人血清アルブミン ②乾燥濃縮人血液凝固第Ⅷ因子 ③乾燥濃縮人血液凝固第Ⅸ因子	人血清アルブミン	人血液	日本、米国	①有効成分 ②③添加物	有	有	無
80036	2008/03/24	化学及血清療法研究所	フィブリノゲン加第XIII因子	人血液凝固第XIII因子	ヒト血液	日本	有効成分	有	無	無
80037	2008/03/24	化学及血清療法研究所	フィブリノゲン加第XIII因子	人フィブリノゲン	ヒト血液	日本	有効成分	有	無	無
80038	2008/03/24	化学及血清療法研究所	①フィブリノゲン加第XIII因子 ②乾燥濃縮人活性化プロテインC ③乾燥濃縮人血液凝固第Ⅸ因子 ④乾燥スルホ化人免疫グロブリン ⑤人血清アルブミン ⑥乾燥濃縮人血液凝固第Ⅷ因子	人血清アルブミン	ヒト血液	日本	⑤有効成分 ①～④ ⑥添加物	有	有	無
80039	2008/03/24	化学及血清療法研究所	①フィブリノゲン加第XIII因子 ②乾燥濃縮人活性化プロテインC ③トロンビン	トロンビン	ヒト血液	日本	①、③有効成分、②製造工程	有	無	無
80040	2008/03/24	化学及血清療法研究所	フィブリノゲン加第XIII因子	アプロチニン	ウシ肺臓	ウルグアイ	有効成分	無	無	無
80041	2008/03/24	日本製薬	乾燥ポリエチレングリコール処理人免疫グロブリン	ポリエチレングリコール処理人免疫グロブリンG	人血液	日本	有効成分	有	有	無
80042	2008/03/24	日本製薬	トロンビン	トロンビン	人血液	日本	有効成分	有	無	無
80043	2008/03/24	日本製薬	乾燥濃縮人アンチトロンビンIII	人アンチトロンビンIII	人血液	日本	有効成分	有	無	無
80044	2008/03/24	日本製薬	人血清アルブミン(20%) 加熱人血漿たん白 人血清アルブミン(25%) 人血清アルブミン(5%)	人血清アルブミン	人血液	日本、(又は現在製造していない)	有効成分	有	無	無
80045	2008/03/25	日本赤十字社	人血清アルブミン	人血清アルブミン	人血液	日本	有効成分	有	無	無
80046	2008/03/25	日本赤十字社	(製造承認書に記載なし)	合成血	人血液	日本	有効成分	有	無	無
80047	2008/03/25	CSLベーリング	フィブリノゲン加第XIII因子	アプロチニン液	ウシ肺	ウルグアイ、ニュージーランド	有効成分	無	無	無
80048	2008/03/25	CSLベーリング	①人血清アルブミン ②人血液凝固第XIII因子 ③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(オンタリオ州ブラントフォード)	①有効成分 ②③添加物	有	有	無
80049	2008/03/28	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	無	有	無
80050	2008/03/28	バクスター	乾燥人血液凝固因子抗体迂回活性複合体	乾燥人血液凝固因子抗体迂回活性複合体	人血漿	米国	有効成分	無	有	無
80051	2008/03/28	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	無	有	無
80052	2008/04/02	化学及血清療法研究所	乾燥濃縮人活性化プロテインC	プロテインC	ヒト血液	日本	有効成分	有	無	無
80053	2008/04/02	化学及血清療法研究所	乾燥濃縮人活性化プロテインC 乾燥濃縮人血液凝固第Ⅸ因子	マウス由来モノクローナル抗体	マウス脾臓	日本	製造工程	無	無	無
80054	2008/04/11	ベネシス	乾燥抗HBs人免疫グロブリン ポリエチレングリコール処理抗HBs人免疫グロブリン	抗HBs抗体	人血液	米国	有効成分	有	無	無
80055	2008/04/11	ベネシス	乾燥濃縮人血液凝固第Ⅸ因子	ヤギIgG	ヤギ血液	オーストラリア	製造工程	無	無	無
80056	2008/04/11	ベネシス	乾燥濃縮人血液凝固第Ⅸ因子	ウサギIgG	ウサギ血液	日本	製造工程	無	無	無
80057	2008/04/11	ベネシス	乾燥濃縮人血液凝固第Ⅸ因子	マウスモノクローナル抗体	マウス脾臓細胞と骨髄腫細胞のハイブリドーマ	イギリス	製造工程	無	無	無
80058	2008/4/17	化学及血清療法研究所	抗HBs人免疫グロブリン	抗HBs人免疫グロブリン	ヒト血液	米国	有効成分	有	無	無
80059	2008/04/22	日本赤十字社	新鮮凍結人血漿	新鮮凍結人血漿	人血液	日本	有効成分	有	有	無
80060	2008/04/22	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	有	有	有
80061	2008/04/22	日本赤十字社	洗浄人赤血球浮遊液	洗浄人赤血球浮遊液	人血液	日本	有効成分	有	有	無
80062	2008/04/22	日本赤十字社	乾燥濃縮人血液凝固第Ⅷ因子	乾燥濃縮人血液凝固第Ⅷ因子	人血液	日本	有効成分	有	無	無
80063	2008/04/22	日本赤十字社	乾燥濃縮人血液凝固第Ⅷ因子	人血清アルブミン	人血液	日本	添加物	有	無	無
80064	2008/04/22	日本赤十字社	人免疫グロブリン	人免疫グロブリン	人血液	日本	有効成分	有	無	無
80065	2008/04/22	日本赤十字社	pH4処理酸性人免疫グロブリン	pH4処理酸性人免疫グロブリン	人血液	日本	有効成分	有	無	無
80066	2008/04/23	日本製薬	乾燥抗HBs人免疫グロブリン	抗HBs抗体	人血液	米国	有効成分	有	無	無
80067	2008/04/23	日本製薬	乾燥抗破傷風人免疫グロブリン	破傷風抗毒素	人血液	米国	有効成分	有	無	無
80068	2008/04/23	日本メジフィジックス	放射性医薬品基準人血清アルブミン五酢酸テクネチウム(99m Tc)注射液	人血清アルブミンジエチレントリアミン五酢酸テクネチウム(99m Tc)	生物学的製剤基準人血清アルブミン	日本	有効成分	無	無	無

80069	2008/04/24	CSLベーリング		ヒトアルブミン	ヒト血液	米国、ドイツ、オーストリア	添加物	有	無	無
80070	2008/04/24	CSLベーリング		アプロチニン	ウシ肺	ウルグアイ、ニュージーランド	有効成分	無	無	無
80071	2008/04/24	CSLベーリング		ウマコラーゲン	ウマアキレス腱	フランス、ドイツ、ベルギー、イタリア	支持体	無	無	無
80072	2008/04/24	CSLベーリング		トロンビン画分	ウシ血液	ニュージーランド	有効成分	無	無	無
80073	2008/04/24	CSLベーリング		トロンボプラスチン	ウサギ脳	ニュージーランド	製造工程	無	無	無
80074	2008/04/24	CSLベーリング		ヘパリン	ブタ腸粘膜	中国	製造工程	無	無	無
80075	2008/04/24	CSLベーリング		ヒトフィブリノゲン	ヒト血液	米国、ドイツ、オーストリア	有効成分	有	無	無
80076	2008/04/24	CSLベーリング		アンチトロンビンIII	ヒト血液	米国、ドイツ、オーストリア	製造工程	有	無	無
80077	2008/04/25	バイエル薬品	pH4処理酸性人免疫グロブリン	人免疫グロブリンG	ヒト血液	米国	有効成分	有	有	無
80078	2008/04/25	バイエル薬品	加熱人血漿たん白 オクトコグ アルファ(遺伝子組換え)	加熱人血漿たん白	ヒト血液	米国	有効成分 製造工程	有	無	無
80079	2008/04/25	バイエル薬品	①人血清アルブミン ②オクトコグ アルファ(遺伝子組換え)	人血清アルブミン	ヒト血液	米国	①有効成分 ②製造工程	有	有	無
80080	2008/04/25	バイエル薬品	オクトコグ アルファ(遺伝子組換え)	ウシインスリン	ウシ膵臓	米国	製造工程	有	無	無
80081	2008/04/25	バイエル薬品	オクトコグ アルファ(遺伝子組換え)	ヒトトランスフェリン	ヒト血液	米国	製造工程	有	無	無
80082	2008/04/25	CSLベーリング	フィブリノゲン加第XIII因子	アンチトロンビン	ヒト血液	米国、ドイツ、オーストリア	製造工程	有	有	無
80083	2008/04/25	CSLベーリング	フィブリノゲン加第XIII因子	トロンビン末	ヒト血液	米国、ドイツ、オーストリア	有効成分	有	有	無
80084	2008/04/25	CSLベーリング	乾燥pH4処理人免疫グロブリン	ペプシン	ブタ胃粘膜	米国	製造工程	無	無	有
80085	2008/04/25	CSLベーリング	乾燥pH4処理人免疫グロブリン	人免疫グロブリンG	ヒト血液	ドイツ	有効成分	有	無	無
80086	2008/04/25	CSLベーリング	フィブリノゲン加第XIII因子 人血液凝固第XIII因子	人血液凝固第XIII因子	ヒト血液	米国、ドイツ、オーストリア	有効成分	有	有	無
80087	2008/04/25	CSLベーリング	フィブリノゲン加第XIII因子	フィブリノゲン	ヒト血液	米国、ドイツ、オーストリア	有効成分	有	有	無
80088	2008/04/25	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	有	無	無
80089	2008/04/30	バクスター	人血清アルブミン	人血清アルブミン	人血漿	米国	有効成分	無	有	無
80090	2008/05/19	化学及血清療法研究所	乾燥ペプシン処理人免疫グロブリン	ペプシン処理人免疫グロブリンG分屑	ヒト血液	日本	有効成分	有	無	無
80091	2008/05/19	化学及血清療法研究所	乾燥ペプシン処理人免疫グロブリン	ペプシン	ブタ胃粘膜	米国、カナダ	製造工程	無	無	無
80092	2008/05/23	日本製薬	人免疫グロブリン	免疫グロブリンG	人血液	日本	有効成分	有	無	無
80093	2008/05/23	日本製薬	乾燥抗D(Rho)人免疫グロブリン	抗D(Rho)抗体	人血液	米国	有効成分	有	無	無
80094	2008/05/23	富士フィルムRIファーマ	テクネチウム人血清アルブミン(99mTc)	テクネチウム人血清アルブミン(99mTc)	ヒト血液	日本	有効成分	無	無	無
80095	2008/05/26	ベネシス	乾燥濃縮人アンチトロンビンIII	人アンチトロンビンIII	人血液	日本	有効成分	有	無	無
80096	2008/05/26	ベネシス	乾燥人フィブリノゲン	凝固性たん白質	人血液	日本	有効成分	有	無	無
80097	2008/05/26	ベネシス	乾燥抗D(Rho)人免疫グロブリン	抗D(Rho)抗体含有人免疫グロブリンG	人血液	米国	有効成分	有	無	無
80098	2008/05/26	ベネシス	乾燥濃縮人血液凝固第IX因子	血液凝固第IX因子	人血液	日本	有効成分	有	無	無
80099	2008/05/26	ベネシス	トロンビン	トロンビン	人血液	日本	有効成分	有	無	無
80100	2008/05/30	日本赤十字社	解凍人赤血球濃厚液	解凍人赤血球濃厚液	人血液	日本	有効成分	有	無	無

別紙様式第4

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回	10-1	感染症および寄生虫症	B型肝炎	ドイツ	不明	24	2008/01/10	不明	自発報告	外国製品	07000022、1回(完了) 平成20年2月7日 MedDRA ver.10.1
第8回	8-1	感染症および寄生虫症	C型肝炎	ドイツ	女	41	2006/11/21	不明	自発報告	外国製品	06000026、2回(完了) 平成18年12月27日 MedDRA ver.9.1
	8-1	感染症および寄生虫症	C型肝炎	ドイツ	女	41	2006/11/21	不明	自発報告	外国製品	06000026、1回(未完了) 平成18年12月8日 MedDRA ver.9.1
第6回	6-2	感染症および寄生虫症	C型肝炎	ドイツ	女	63	2005/11/10	不明	自発報告	外国製品	06000003、2回(追加) 平成18年5月15日 MedDRA ver.9.0
	6-2	感染症および寄生虫症	C型肝炎	ドイツ	女	63	2005/11/10	不明	自発報告	外国製品	06000003、1回(完了) 平成18年4月17日 MedDRA ver.9.0
	6-1	感染症および寄生虫症	B型肝炎	ドイツ	男	74	2005/10/21	未回復	自発報告	外国製品	05000491、1回(完了) 平成17年12月22日 MedDRA ver.8.1

80030	2008/03/12	化学及血清療法研究所	乾燥濃縮人アンチトロンビンIII	アンチトロンビンIII

別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	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 10 回	10-1	感染症および寄生虫症	Ｃ型肝炎	日本	男	71	2007 年 6 月 5 日	未回復	症例報告	当該製品	未完了報告日：2007 年 8 月 2 日 完了報告日　：2007 年 9 月 4 日 識別番号：A-07000069
第 7 回	7-1	感染症および寄生虫症	Ｂ型肝炎	日本	男	34	2006 年 1 月 5 日	未回復	症例報告	当該製品	未完了報告日：2006 年 2 月 22 日 取下げ報告日：2006 年 3 月 2 日 識別番号：A-05000255
	6-1	感染症および寄生虫症	Ｂ型肝炎	日本	不明	不明	不明	不明	症例報告	当該製品	取下げ報告日：2006 年 2 月 13 日 識別番号：A-05000183
第 6 回	6-1	感染症および寄生虫症	Ｂ型肝炎	日本	不明	不明	不明	不明	症例報告	当該製品	未完了報告日①：2005 年 10 月 26 日 未完了報告日②：2005 年 12 月 27 日 識別番号：A-05000183
第 3 回	3-1	感染症および寄生虫症	Ｃ型肝炎	日本	女	82	2003 年 8 月 5 日	軽快	症例報告	当該製品	完了報告日：2004 年 3 月 1 日 識別番号：A-03000155

80035	2008/03/18	ベネシス	①人血清アルブミン ②乾燥濃縮人血液凝固第Ⅷ因子	人血清アルブミン

別紙様式第4

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回	10-1	感染症および寄生虫症	B型肝炎	ドイツ	不明	24	2008/01/10	不明	自発報告	外国製品	07000022、2回(完了;第1回はアンスロビンPの番号10-1で報告。今回、本剤が同時期に投与されていたという情報を入手した。) 平成20年3月11日 MedDRA ver.10.1
第8回	7-2	感染症および寄生虫症	B型肝炎	日本	男	70	不明	死亡	自発報告	当該製品	06000076、2回(完了;因果関係が否定されたため、報告対象外として完了報告) 平成18年10月20日 (第7回の番号7-2の症例と同一である) MedDRA ver.9.0
第7回	7-2	感染症および寄生虫症	B型肝炎	日本	男	70	不明	死亡	自発報告	当該製品	06000076、1回(未完了) 平成18年7月21日 MedDRA ver.9.0
	7-1	感染症および寄生虫症	B型肝炎	日本	男	34	2005/12/21	回復	自発報告	当該製品	06000004、2回(完了) 平成18年5月15日 MedDRA ver.8.1
	7-1	感染症および寄生虫症	B型肝炎	日本	男	34	2005/12/21	回復	自発報告	当該製品	06000004、1回(未完了) 平成18年4月17日 MedDRA ver.8.1
第6回	5-1	臨床検査	C型肝炎抗体陽性	日本	女	87	2005/8/4	不明	自発報告	当該製品	05000116、2回(取下) 平成17年9月5日 (第5回の番号5-1の症例と同一である。副作用名が変更された。)MedDRA ver.8.0

	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5回	5-1	感染症および寄生虫症	C型肝炎	日本	女	87	2005/8/4	不明	自発報告	当該製品	05000116、1回(未完了) 平成17年8月9日 MedDRA ver.8.0

80038	2008/03/24	化学及血清療法研究所	①フィブリノゲン加第XIII因子 ②乾燥濃縮人活性化プロテインC ③乾燥濃縮人血液凝固第IX因子 ④乾燥スルホ化人免疫グロブリン	人血清アルブミン

別紙様式第４

感染症発生症例一覧

MedDRA/J：Ver10.1

	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10回	1	10022891/ 臨床検査 /Investigations	10057394/ Ｃ型肝炎陽性 /Hepatitis C positive	日本	女	20代	2005年 3月23日	未回復	症例報告	当該製品 (ｸﾞﾛﾍﾞﾆﾝ-I)	報告日： 2008年1月23日(第一報) 2008年2月21日(「因果関係なし」 のため、報告対象外報告) 識別番号：A-07000179

＊C型肝炎陽性患者の治療歴を調査したところ、約20年前に本剤が投与されていたとの情報に基づき、安全対策上、症例報告を行ったが、後の詳細調査において「因果関係なし」との報告を得たので「報告対象外症例」として追加報告(完了報告)を行った(グロベニンーＩは1999年2月に製造を中止している)。

80041	2008/03/24	日本製薬	乾燥ポリエチレングリコール処理人免疫グロブリン	ポリエチレングリコール処理人免疫グロブリンG

別紙様式第4

感染症発生症例一覧

別紙(5)

	番号	感染症の種類		発生国	性別	年齢	発現時期	転帰	出典	区分	備考
		器官別大分類	基本語								
第10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 追加報告提出日2007年8月6日 第9回報告症例番号1と同一症例
	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 追加報告提出日2007年8月6日 第9回報告症例番号1と同一症例
	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 追加報告提出日2007年8月6日 第9回報告症例番号1と同一症例
	2	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 提出日2007年9月21日
	2	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 完了報告提出日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 報告書提出日2007年7月24日
	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 追加報告提出日2007年7月26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 追加報告提出日2007年7月26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 追加報告提出日2007年7月26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 報告書提出日2007年2月23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 完了報告書提出日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 完了報告書提出日2007年3月30日

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80048	2008/03/25	CSLベーリング	①人血清アルブミン ②人血液凝固第XIII因子 ③フィブリノゲン加第XIII因子	人血清アルブミン

別紙様式第4

感染症発生症例一覧

報告回	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	備考			
		器官別大分類	基本語（PT）								識別番号	報告日	MedDRA（Ver.）	
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	* 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
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	

別紙様式第4

感染症発生症例一覧

報告回	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	備考			
		器官別大分類	基本語（PT）								識別番号	報告日	MedDRA（Ver.）	
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破棄
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	
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	

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報告回	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	備考			
		器官別大分類	基本語(PT)								識別番号	報告日	MedDRA(Ver.)	
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	
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	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	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	6-059	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	不明	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	

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報告回	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	備考			
		器官別大分類	基本語（PT）								識別番号	報告日	MedDRA（Ver.）	
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	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	6-045	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	
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	

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報告回	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	備考		
		器官別大分類	基本語（PT）								識別番号	報告日	MedDRA（Ver.）
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	6-013	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	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
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	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

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報告回	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	備考			
		器官別大分類	基本語（PT）								識別番号	報告日	MedDRA（Ver.）	
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	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	6-016	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	
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破棄
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	

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報告回	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	備考			
		器官別大分類	基本語（PT）								識別番号	報告日	MedDRA（Ver.）	
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	
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	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破棄
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	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	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破棄
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	

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		器官別大分類	基本語（PT）								識別番号	報告日	MedDRA（Ver.）
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
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

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報告回	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	備考		
		器官別大分類	基本語（PT）								識別番号	報告日	MedDRA（Ver.）
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
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

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報告回	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	備考		
		器官別大分類	基本語（PT）								識別番号	報告日	MedDRA（Ver.）
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
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

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報告回	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	備考		
		器官別大分類	基本語(PT)								識別番号	報告日	MedDRA(Ver.)
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
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	男性	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

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報告回	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	備考		
		器官別大分類	基本語(PT)								識別番号	報告日	MedDRA(Ver.)
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
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

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報告回	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	備考		
		器官別大分類	基本語(PT)								識別番号	報告日	MedDRA(Ver.)
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
第5回	5-137	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	05000102	2005/6/1	8.0
第5回	5-138	感染症および寄生虫症	HIV感染	香港	男性	不明	不明	死亡	症例報告	外国製品	05000103	2005/6/2	8.0
第5回	5-139	感染症および寄生虫症	HIV感染	香港	男性	不明	不明	死亡	症例報告	外国製品	05000104	2005/6/2	8.0
第5回	5-140	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	05000105	2005/6/2	8.0
第5回	5-141	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	05000085	2005/6/1	8.0
第5回	5-142	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	05000086	2005/6/1	8.0
第5回	5-142	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	05000086	2005/6/15	8.0
第5回	5-143	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	05000087	2005/6/1	8.0
第5回	5-143	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	05000087	2005/6/15	8.0
第5回	5-144	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	05000030	2005/5/30	8.0
第5回	5-144	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	05000030	2005/6/15	8.0
第5回	5-145	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	05000031	2005/5/30	8.0
第5回	5-145	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	05000031	2005/6/15	8.0
第5回	5-146	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	05000032	2005/5/30	8.0
第5回	5-146	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	05000032	2005/6/15	8.0
第5回	5-147	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	05000033	2005/5/30	8.0
第5回	5-148	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	05000034	2005/5/30	8.0
第5回	5-149	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	05000035	2005/5/30	8.0
第5回	5-150	感染症および寄生虫症	HIV感染	香港	男性	不明	不明	死亡	症例報告	外国製品	05000036	2005/5/30	8.0
第5回	5-151	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	05000111	2005/6/9	8.0
第5回	5-152	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	05000112	2005/6/9	8.0
第5回	5-153	感染症および寄生虫症	HIV感染	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	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	05000037	2005/5/30	8.0
第5回	5-155	感染症および寄生虫症	HIV感染	香港	男性	不明	不明	死亡	症例報告	外国製品	05000038	2005/5/30	8.0
第5回	5-156	感染症および寄生虫症	HIV感染	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	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	05000040	2005/5/30	8.0
第5回	5-158	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	05000116	2005/6/9	8.0
第5回	5-159	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	05000041	2005/5/30	8.0
第5回	5-160	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	05000042	2005/5/30	8.0

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報告回	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	備考		
		器官別大分類	基本語(PT)								識別番号	報告日	MedDRA(Ver.)
第5回	5-161	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	05000043	2005/5/30	8.0
第5回	5-162	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	05000044	2005/5/30	8.0
第5回	5-162	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	05000044	2005/6/27	8.0
第5回	5-163	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	05000045	2005/5/30	8.0
第5回	5-163	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	05000045	2005/6/15	8.0
第5回	5-164	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	05000046	2005/5/30	8.0
第5回	5-165	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	05000047	2005/5/30	8.0
第5回	5-166	感染症および寄生虫症	HIV感染	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	感染症および寄生虫症	HIV感染	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	感染症および寄生虫症	HIV感染	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	感染症および寄生虫症	HIV感染	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	感染症および寄生虫症	HIV感染	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	感染症および寄生虫症	HIV感染	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	感染症および寄生虫症	HIV感染	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	感染症および寄生虫症	HIV感染	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	感染症および寄生虫症	HIV感染	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	5-171	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	05000119	2005/6/13	8.0
第5回	5-172	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	05000120	2005/6/13	8.0
第5回	5-173	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	05000121	2005/6/13	8.0
第5回	5-174	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	05000122	2005/6/13	8.0
第5回	5-175	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	05000124	2005/6/13	8.0
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

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報告回	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	備考		
		器官別大分類	基本語(PT)								識別番号	報告日	MedDRA(Ver.)
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
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	男性	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

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報告回	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	備考		
		器官別大分類	基本語（PT）								識別番号	報告日	MedDRA（Ver.）
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	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	5-261	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
第5回	5-275	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
第5回	5-276	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
第5回	5-277	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
第5回	5-278	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
第5回	5-279	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
第5回	5-280	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
第5回	5-281	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
第5回	5-282	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
第5回	5-283	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
第5回	5-284	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
第5回	5-285	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
第5回	5-115	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
第5回	5-153	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
第5回	5-286	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
第5回	5-287	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
第5回	5-288	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
第5回	5-285	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
第5回	5-107	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
第5回	5-108	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
第5回	5-109	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
第5回	5-110	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
第5回	5-111	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
第5回	5-112	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
第5回	5-113	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
第5回	5-114	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
第5回	5-115	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

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報告回	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	備考		
		器官別大分類	基本語（PT）								識別番号	報告日	MedDRA（Ver.）
第5回	5-116	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
第5回	5-117	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
第5回	5-118	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
第5回	5-119	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
第5回	5-120	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
第5回	5-121	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
第5回	5-122	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
第5回	5-123	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
第5回	5-124	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
第5回	5-125	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
第5回	5-126	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
第5回	5-127	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
第5回	5-128	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
第5回	5-129	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
第5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	2005/5/30	8.0
第5回	5-131	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
第5回	5-132	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
第5回	5-133	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
第5回	5-134	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
第5回	5-135	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
第5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	2005/6/1	8.0
第5回	5-138	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
第5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	2005/6/2	8.0
第5回	5-140	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
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

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報告回	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	備考		
		器官別大分類	基本語（PT）								識別番号	報告日	MedDRA（Ver.）
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
第5回	5-196	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	05000187	2005/7/4	8.0
第5回	5-197	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	05000189	2005/7/4	8.0
第5回	5-198	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	05000190	2005/7/4	8.0
第5回	5-200	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	05000194	2005/7/4	8.0
第5回	5-201	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	05000202	2005/7/8	8.0
第5回	5-202	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	05000203	2005/7/8	8.0
第5回	5-203	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	05000204	2005/7/8	8.0
第5回	5-204	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	05000206	2005/7/8	8.0
第5回	5-205	臨床検査	C型肝炎ウイルス	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	5-207	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	05000213	2005/7/11	8.0
第5回	5-208	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	05000214	2005/7/11	8.0
第5回	5-209	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	05000219	2005/7/11	8.0
第5回	5-210	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	05000230	2005/7/11	8.0
第5回	5-211	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	05000232	2005/7/12	8.0
第5回	5-213	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	05000235	2005/7/12	8.0
第5回	5-215	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	05000240	2005/7/15	8.0
第5回	5-216	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	05000241	2005/7/15	8.0
第5回	5-217	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	05000242	2005/7/15	8.0
第5回	5-218	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	05000248	2005/7/15	8.0
第5回	5-219	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	05000249	2005/7/15	8.0
第5回	5-220	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	05000250	2005/7/15	8.0
第5回	5-221	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	05000251	2005/7/15	8.0
第5回	5-223	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	05000254	2005/7/19	8.0
第5回	5-224	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	05000256	2005/7/19	8.0

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報告回	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	備考		
		器官別大分類	基本語（PT）								識別番号	報告日	MedDRA（Ver.）
第5回	5-225	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	05000257	2005/7/22	8.0
第5回	5-226	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	05000258	2005/7/22	8.0
第5回	5-227	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	05000259	2005/7/22	8.0
第5回	5-228	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	05000261	2005/7/22	8.0
第5回	5-229	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	05000264	2005/7/22	8.0
第5回	5-230	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	05000267	2005/7/22	8.0
第5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	2005/7/26	8.0
第5回	5-232	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	05000276	2005/7/26	8.0
第5回	5-233	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	05000279	2005/7/26	8.0
第5回	5-234	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	05000297	2005/7/26	8.0
第5回	5-235	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	05000299	2005/7/26	8.0
第5回	5-236	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	05000302	2005/7/26	8.0
第5回	5-237	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	05000282	2005/7/26	8.0
第5回	5-239	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	05000284	2005/7/26	8.0
第5回	5-241	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	05000286	2005/7/26	8.0
第5回	5-242	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	05000287	2005/7/26	8.0
第5回	5-243	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	05000288	2005/7/26	8.0
第5回	5-245	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	05000293	2005/7/26	8.0
第5回	5-249	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	05000308	2005/7/26	8.0
第5回	5-250	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	05000314	2005/7/27	8.0
第5回	5-251	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	05000315	2005/7/27	8.0
第5回	5-252	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	05000316	2005/7/27	8.0
第5回	5-253	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	05000317	2005/7/27	8.0
第5回	5-254	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	05000320	2005/7/27	8.0
第5回	5-255	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	05000321	2005/7/27	8.0
第5回	5-256	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	05000334	2005/7/29	8.0
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

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		器官別大分類	基本語（PT）								識別番号	報告日	MedDRA (Ver.)
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
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	ブラジル	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

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報告回	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	備考		
		器官別大分類	基本語(PT)								識別番号	報告日	MedDRA(Ver.)
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
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	男性	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

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報告回	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	備考		
		器官別大分類	基本語(PT)								識別番号	報告日	MedDRA(Ver.)
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
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

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感染症発生症例一覧

報告回	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	備考		
		器官別大分類	基本語（PT）								識別番号	報告日	MedDRA（Ver.）
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

80049|2008/03/28|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

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	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	10-1	臨床検査	Ｃ型肝炎ウイルス	ブラジル	男	小児	2004/5/25	不明	症例報告	外国製品	登録番号：07000015 報告日：2007年10月29日 MedDRA: Version(10.1)
	10-1	臨床検査	Ｃ型肝炎ウイルス	ブラジル	男	小児	2004/5/25	不明	症例報告	外国製品	登録番号：07000015(追加報告) 報告日：2007年12月28日 MedDRA: Version(10.1)
第9回	0*	0	0	0	0	0	0	0	0	0	
第8回	0*	0	0	0	0	0	0	0	0	0	
第7回	0*	0	0	0	0	0	0	0	0	0	
第6回	0*	0	0	0	0	0	0	0	0	0	
第5回	5-1	感染症および寄生虫症	Ｃ型肝炎	フランス	男	不明	不明	不明	症例報告	外国製品	登録番号：05000319(追加報告) 報告日：2005年8月15日 MedDRA: Version(8.0)
	5-1	感染症および寄生虫症	Ｃ型肝炎	フランス	男	不明	不明	不明	症例報告	外国製品	登録番号：05000319(完了報告) 報告日：2005年7月27日 MedDRA: Version(8.0)

＊ 当該調査期間に対象となる感染症報告はなかった。

80050	2008/03/28	バクスター	乾燥人血液凝固因子抗体迂回活性複合体	乾燥人血液凝固因子抗体迂回活性複合体

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感染症発生症例一覧

報告回	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	備考			
		器官別大分類	基本語（PT）								識別番号	報告日	MedDRA（Ver.）	
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	* 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
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	

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報告回	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	備考			
		器官別大分類	基本語（PT）								識別番号	報告日	MedDRA（Ver.）	
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破棄
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	
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	

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報告回	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	備考			
		器官別大分類	基本語(PT)								識別番号	報告日	MedDRA(Ver.)	
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	
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	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	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	6-059	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	不明	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	

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報告回	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	備考			
		器官別大分類	基本語（PT）								識別番号	報告日	MedDRA（Ver.）	
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	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	6-045	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	
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	

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報告回	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	備考		
		器官別大分類	基本語（PT）								識別番号	報告日	MedDRA（Ver.）
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	6-013	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	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
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	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

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報告回	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	備考			
		器官別大分類	基本語（PT）								識別番号	報告日	MedDRA（Ver.）	
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	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	6-016	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	
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破棄
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	

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報告回	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	備考			
		器官別大分類	基本語（PT）								識別番号	報告日	MedDRA（Ver.）	
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	
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	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破棄
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	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	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破棄
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	

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		器官別大分類	基本語（PT）								識別番号	報告日	MedDRA（Ver.）
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
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

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感染症発生症例一覧

報告回	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	備考		
		器官別大分類	基本語(PT)								識別番号	報告日	MedDRA(Ver.)
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
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

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報告回	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	備考		
		器官別大分類	基本語（PT）								識別番号	報告日	MedDRA（Ver.）
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
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

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感染症発生症例一覧

報告回	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	備考		
		器官別大分類	基本語(PT)								識別番号	報告日	MedDRA(Ver.)
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
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	男性	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

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報告回	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	備考		
		器官別大分類	基本語(PT)								識別番号	報告日	MedDRA(Ver.)
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
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

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報告回	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	備考		
		器官別大分類	基本語（PT）								識別番号	報告日	MedDRA（Ver.）
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
第5回	5-137	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	05000102	2005/6/1	8.0
第5回	5-138	感染症および寄生虫症	HIV感染	香港	男性	不明	不明	死亡	症例報告	外国製品	05000103	2005/6/2	8.0
第5回	5-139	感染症および寄生虫症	HIV感染	香港	男性	不明	不明	死亡	症例報告	外国製品	05000104	2005/6/2	8.0
第5回	5-140	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	05000105	2005/6/2	8.0
第5回	5-141	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	05000085	2005/6/1	8.0
第5回	5-142	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	05000086	2005/6/1	8.0
第5回	5-142	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	05000086	2005/6/15	8.0
第5回	5-143	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	05000087	2005/6/1	8.0
第5回	5-143	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	05000087	2005/6/15	8.0
第5回	5-144	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	05000030	2005/5/30	8.0
第5回	5-144	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	05000030	2005/6/15	8.0
第5回	5-145	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	05000031	2005/5/30	8.0
第5回	5-145	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	05000031	2005/6/15	8.0
第5回	5-146	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	05000032	2005/5/30	8.0
第5回	5-146	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	05000032	2005/6/15	8.0
第5回	5-147	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	05000033	2005/5/30	8.0
第5回	5-148	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	05000034	2005/5/30	8.0
第5回	5-149	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	05000035	2005/5/30	8.0
第5回	5-150	感染症および寄生虫症	HIV感染	香港	男性	不明	不明	死亡	症例報告	外国製品	05000036	2005/5/30	8.0
第5回	5-151	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	05000111	2005/6/9	8.0
第5回	5-152	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	05000112	2005/6/9	8.0
第5回	5-153	感染症および寄生虫症	HIV感染	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	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	05000037	2005/5/30	8.0
第5回	5-155	感染症および寄生虫症	HIV感染	香港	男性	不明	不明	死亡	症例報告	外国製品	05000038	2005/5/30	8.0
第5回	5-156	感染症および寄生虫症	HIV感染	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	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	05000040	2005/5/30	8.0
第5回	5-158	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	05000116	2005/6/9	8.0
第5回	5-159	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	05000041	2005/5/30	8.0
第5回	5-160	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	05000042	2005/5/30	8.0

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報告回	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	備考			
		器官別大分類	基本語（PT）								識別番号	報告日	MedDRA（Ver.）	
第5回	5-161	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	05000043	2005/5/30	8.0	
第5回	5-162	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	05000044	2005/5/30	8.0	
第5回	5-162	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	05000044	2005/6/27	8.0	
第5回	5-163	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	05000045	2005/5/30	8.0	
第5回	5-163	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	05000045	2005/6/15	8.0	
第5回	5-164	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	05000046	2005/5/30	8.0	
第5回	5-165	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	05000047	2005/5/30	8.0	
第5回	5-166	感染症および寄生虫症	HIV感染	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	感染症および寄生虫症	HIV感染	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	感染症および寄生虫症	HIV感染	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	感染症および寄生虫症	HIV感染	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	感染症および寄生虫症	HIV感染	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	感染症および寄生虫症	HIV感染	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	感染症および寄生虫症	HIV感染	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	感染症および寄生虫症	HIV感染	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	感染症および寄生虫症	HIV感染	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	5-171	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	05000119	2005/6/13	8.0	
第5回	5-172	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	05000120	2005/6/13	8.0	
第5回	5-173	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	05000121	2005/6/13	8.0	
第5回	5-174	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	05000122	2005/6/13	8.0	
第5回	5-175	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	05000124	2005/6/13	8.0	
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	

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報告回	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	備考		
		器官別大分類	基本語(PT)								識別番号	報告日	MedDRA(Ver.)
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
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	男性	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

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報告回	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	備考		
		器官別大分類	基本語（PT）								識別番号	報告日	MedDRA（Ver.）
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	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	5-261	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
第5回	5-275	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
第5回	5-276	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
第5回	5-277	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
第5回	5-278	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
第5回	5-279	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
第5回	5-280	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
第5回	5-281	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
第5回	5-282	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
第5回	5-283	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
第5回	5-284	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
第5回	5-285	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
第5回	5-115	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
第5回	5-153	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
第5回	5-286	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
第5回	5-287	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
第5回	5-288	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
第5回	5-285	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
第5回	5-107	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
第5回	5-108	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
第5回	5-109	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
第5回	5-110	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
第5回	5-111	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
第5回	5-112	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
第5回	5-113	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
第5回	5-114	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
第5回	5-115	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

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報告回	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	備考		
		器官別大分類	基本語（PT）								識別番号	報告日	MedDRA（Ver.）
第5回	5-116	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
第5回	5-117	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
第5回	5-118	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
第5回	5-119	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
第5回	5-120	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
第5回	5-121	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
第5回	5-122	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
第5回	5-123	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
第5回	5-124	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
第5回	5-125	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
第5回	5-126	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
第5回	5-127	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
第5回	5-128	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
第5回	5-129	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
第5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	2005/5/30	8.0
第5回	5-131	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
第5回	5-132	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
第5回	5-133	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
第5回	5-134	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
第5回	5-135	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
第5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	2005/6/1	8.0
第5回	5-138	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
第5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	2005/6/2	8.0
第5回	5-140	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
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

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		器官別大分類	基本語（PT）								識別番号	報告日	MedDRA (Ver.)
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
第5回	5-196	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	05000187	2005/7/4	8.0
第5回	5-197	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	05000189	2005/7/4	8.0
第5回	5-198	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	05000190	2005/7/4	8.0
第5回	5-200	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	05000194	2005/7/4	8.0
第5回	5-201	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	05000202	2005/7/8	8.0
第5回	5-202	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	05000203	2005/7/8	8.0
第5回	5-203	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	05000204	2005/7/8	8.0
第5回	5-204	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	05000206	2005/7/8	8.0
第5回	5-205	臨床検査	C型肝炎ウイルス	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	5-207	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	05000213	2005/7/11	8.0
第5回	5-208	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	05000214	2005/7/11	8.0
第5回	5-209	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	05000219	2005/7/11	8.0
第5回	5-210	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	05000230	2005/7/11	8.0
第5回	5-211	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	05000232	2005/7/12	8.0
第5回	5-213	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	05000235	2005/7/12	8.0
第5回	5-215	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	05000240	2005/7/15	8.0
第5回	5-216	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	05000241	2005/7/15	8.0
第5回	5-217	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	05000242	2005/7/15	8.0
第5回	5-218	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	05000248	2005/7/15	8.0
第5回	5-219	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	05000249	2005/7/15	8.0
第5回	5-220	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	05000250	2005/7/15	8.0
第5回	5-221	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	05000251	2005/7/15	8.0
第5回	5-223	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	05000254	2005/7/19	8.0
第5回	5-224	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	05000256	2005/7/19	8.0

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		器官別大分類	基本語（PT）								識別番号	報告日	MedDRA（Ver.）
第5回	5-225	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	05000257	2005/7/22	8.0
第5回	5-226	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	05000258	2005/7/22	8.0
第5回	5-227	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	05000259	2005/7/22	8.0
第5回	5-228	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	05000261	2005/7/22	8.0
第5回	5-229	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	05000264	2005/7/22	8.0
第5回	5-230	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	05000267	2005/7/22	8.0
第5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	2005/7/26	8.0
第5回	5-232	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	05000276	2005/7/26	8.0
第5回	5-233	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	05000279	2005/7/26	8.0
第5回	5-234	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	05000297	2005/7/26	8.0
第5回	5-235	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	05000299	2005/7/26	8.0
第5回	5-236	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	05000302	2005/7/26	8.0
第5回	5-237	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	05000282	2005/7/26	8.0
第5回	5-239	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	05000284	2005/7/26	8.0
第5回	5-241	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	05000286	2005/7/26	8.0
第5回	5-242	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	05000287	2005/7/26	8.0
第5回	5-243	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	05000288	2005/7/26	8.0
第5回	5-245	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	05000293	2005/7/26	8.0
第5回	5-249	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	05000308	2005/7/26	8.0
第5回	5-250	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	05000314	2005/7/27	8.0
第5回	5-251	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	05000315	2005/7/27	8.0
第5回	5-252	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	05000316	2005/7/27	8.0
第5回	5-253	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	05000317	2005/7/27	8.0
第5回	5-254	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	05000320	2005/7/27	8.0
第5回	5-255	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	05000321	2005/7/27	8.0
第5回	5-256	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	05000334	2005/7/29	8.0
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

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		器官別大分類	基本語(PT)								識別番号	報告日	MedDRA(Ver.)
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
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	ブラジル	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

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		器官別大分類	基本語(PT)								識別番号	報告日	MedDRA(Ver.)
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
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	男性	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

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報告回	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	備考		
		器官別大分類	基本語(PT)								識別番号	報告日	MedDRA(Ver.)
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
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

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報告回	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	備考		
		器官別大分類	基本語（PT）								識別番号	報告日	MedDRA（Ver.）
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

80051	2008/03/28	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

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番号	感染症の種類		発現国	性別	年齢	発現時期	転帰	出典	区分	備考
	器官別大分類	基本語								
1	感染症および寄生虫症	B型肝炎	日本	男	66	2008/1/9	③未回復	症例報告	当該製品	2008/1/30提出、識別番号1-07000186　未完了報告
2	感染症および寄生虫症	サイトメガロウイルス感染	日本	女	2	2007/12/25	③未回復	症例報告	当該製品	2008/2/19提出、識別番号1-07000229　未完了報告
3	感染症および寄生虫症	B型肝炎	日本	女	53	2007/12/17	③未回復	症例報告	当該製品	2008/1/22提出、識別番号1-07000178　未完了報告
4	感染症および寄生虫症	C型肝炎	日本	男	62	2007/12/11	③未回復	症例報告	当該製品	2008/1/8提出、識別番号1-07000169　未完了報告
5	感染症および寄生虫症	C型肝炎	日本	男	53	2007/12/7	③未回復	症例報告	当該製品	2007/12/28提出、識別番号1-07000162　未完了報告
6	感染症および寄生虫症	細菌感染	日本	女	83	2007/11/19	①回復	症例報告	当該製品	2007/12/4提出、識別番号1-07000135　未完了報告
7	感染症および寄生虫症	細菌感染	日本	男	72	2007/11/17	①回復	症例報告	当該製品	2007/12/18提出、識別番号1-07000148　未完了報告
8	感染症および寄生虫症	伝染性紅斑	日本	女	55	2007/11/12	②軽快	症例報告	当該製品	2007/12/28提出、識別番号1-07000161　未完了報告
9	感染症および寄生虫症	B型肝炎	日本	女	61	2007/11/9	③未回復	症例報告	当該製品	2007/11/27提出、識別番号1-07000130　未完了報告
10	感染症および寄生虫症	B型肝炎	日本	女	26	2007/11/5	③未回復	症例報告	当該製品	2007/12/4提出、識別番号1-07000137　未完了報告
11	感染症および寄生虫症	C型肝炎	日本	男	58	2007/10/26	②軽快	症例報告	当該製品	2007/11/19提出、識別番号1-07000123　未完了報告
12	感染症および寄生虫症	B型肝炎	日本	女	34	2007/10/19	③未回復	症例報告	当該製品	2007/11/8提出、識別番号1-07000113　未完了報告
13	感染症および寄生虫症	B型肝炎	日本	男	56	2007/10/19	③未回復	症例報告	当該製品	2007/11/13提出、識別番号1-07000117　未完了報告
14	感染症および寄生虫症	B型肝炎	日本	女	30	2007/10/15	⑥不明	症例報告	当該製品	2007/12/4提出、識別番号1-07000136　未完了報告
15	感染症および寄生虫症	B型肝炎	日本	女	30	2007/10/15	⑥不明	症例報告	当該製品	2007/12/11提出、識別番号1-07000136　未完了報告（15番と同一症例）
16	感染症および寄生虫症	C型肝炎	日本	男	43	2007/9/10	③未回復	症例報告	当該製品	2007/10/11提出、識別番号1-07000098　未完了報告
17	感染症および寄生虫症	B型肝炎	日本	女	34	2007/8/6	②軽快	症例報告	当該製品	2007/12/28提出、識別番号1-07000160　未完了報告
18	感染症および寄生虫症	C型肝炎	日本	女	82	2007/6/21	③未回復	症例報告	当該製品	2007/9/13提出、識別番号1-07000089　未完了報告
19	感染症および寄生虫症	B型肝炎	日本	男	73	2007/5/16	⑥不明	症例報告	当該製品	2007/12/4提出、識別番号1-07000029　取り下げ 第10回症例番号19は前回報告における第9回症例番号2において報告したものの追加報告
20	感染症および寄生虫症	B型肝炎	日本	女	76	2006/8/25	①回復	症例報告	当該製品	2007/10/11提出、識別番号1-07000099　未完了報告
21	感染症および寄生虫症	B型肝炎	日本	女	53	2006/4/25	⑥不明	症例報告	当該製品	2007/12/17提出、識別番号1-06000025　完了報告 第10回症例番号21は前々々回報告における第7回症例番号17において報告したものの追加報告
22	感染症および寄生虫症	B型肝炎	日本	女	67	2006/4/14	⑥不明	症例報告	当該製品	2008/1/8提出、識別番号1-06000034　完了報告 第10回症例番号22は前々々回報告における第7回症例番号19において報告したものの追加報告
23	感染症および寄生虫症	B型肝炎	日本	男	85	2006/4/5	②軽快	症例報告	当該製品	2007/10/29提出、識別番号1-06000010　完了報告 第10回症例番号23は前々々回報告における第7回症例番号16において報告したものの追加報告
24	感染症および寄生虫症	B型肝炎	日本	女	4	2006/3/1	⑥不明	症例報告	当該製品	2007/10/29提出、識別番号1-05000274　完了報告 第10回症例番号24は前々々回報告における第7回症例番号14において報告したものの追加報告

別紙様式第4

感染症発生症例一覧

第10回	25	感染症および寄生虫症	C型肝炎	日本	男	68	2006/2/10	②軽快	症例報告	当該製品	2007/10/29提出、識別番号1-05000264　完了報告　第10回症例番号25は前々々々回報告における第6回症例番号1において報告したものの追加報告
	26	感染症および寄生虫症	A型肝炎	日本	男	3	2006/1/31	⑥不明	症例報告	当該製品	2007/12/14提出、識別番号1-05000266　完了報告　第10回症例番号26は前々々回報告における第7回症例番号11及び前々々々回報告における第6回症例番号2において報告したものの追加報告
	27	感染症および寄生虫症	B型肝炎	日本	女	74	2005/12/30	⑥不明	症例報告	当該製品	2007/10/29提出、識別番号1-05000265　完了報告　第10回症例番号27は前々々々回報告における第6回症例番号7において報告したものの追加報告
	28	感染症および寄生虫症	B型肝炎	日本	女	67	2005/12/26	②軽快	症例報告	当該製品	2007/12/17提出、識別番号1-06000029　完了報告　第10回症例番号28は前々々回報告における第7回症例番号18において報告したものの追加報告
	29	感染症および寄生虫症	B型肝炎	日本	男	37	2005/12/21	②軽快	症例報告	当該製品	2007/10/26提出、識別番号1-05000233　完了報告　第10回症例番号29は前々々々回報告における第6回症例番号9及び10において報告したものの追加報告
	30	感染症および寄生虫症	B型肝炎	日本	女	50	2005/12/2	①回復	症例報告	当該製品	2008/2/12提出、識別番号1-05000236　完了報告　第10回症例番号30は前々々回報告における第7回症例番号9及び前々々々回報告における第6回症例番号11において報告したものの追加報告
	31	感染症および寄生虫症	B型肝炎	日本	女	27	2005/9/27	⑥不明	症例報告	当該製品	2007/10/26提出、識別番号1-05000171　完了報告　第10回症例番号31は前々々々回報告における第6回症例番号15において報告したものの追加報告
	32	感染症および寄生虫症	B型肝炎	日本	女	94	2005/8/1	⑥不明	症例報告	当該製品	2007/10/29提出、識別番号1-05000131　完了報告　第10回症例番号32は前々々々々回報告における第5回症例番号2において報告したものの追加報告
	33	感染症および寄生虫症	B型肝炎	日本	男	88	2005/6/29	②軽快	症例報告	当該製品	2007/9/25提出、識別番号1-05000089　完了報告　第10回症例番号33は前々々々々回報告における第5回症例番号5において報告したものの追加報告
	34	感染症および寄生虫症	C型肝炎	日本	女	33	2005/6/22	⑥不明	症例報告	当該製品	2007/11/26提出、識別番号1-05000107　完了報告　第10回症例番号34は前々々々々回報告における第5回症例番号6において報告したものの追加報告
	35	感染症および寄生虫症	C型肝炎	日本	女	33	2005/6/22	⑥不明	症例報告	当該製品	2007/12/12提出、識別番号1-05000107　完了報告（34番と同一症例）
	36	感染症および寄生虫症	B型肝炎	日本	女	43	2005/5/30	②軽快	症例報告	当該製品	2008/2/12提出、識別番号1-05000059　完了報告　第10回症例番号36は前々々々回報告における第6回症例番号21及び前々々々々回報告における第5回症例番号7において報告したものの追加報告

別紙様式第4

感染症発生症例一覧

	37	感染症および寄生虫症	B型肝炎	日本	男	56	2005/5/16	⑥不明	症例報告	当該製品	2007/10/15提出、識別番号1-05000094　完了報告　第10回症例番号37は前々々々々回報告における第5回症例番号11において報告したものの追加報告
	38	感染症および寄生虫症	B型肝炎	日本	女	14	2005/4/19	①回復	症例報告	当該製品	2008/2/12提出、識別番号1-05000026　完了報告　第10回症例番号38は前々々々回報告における第6回症例番号23及び前々々々々回報告における第5回症例番号16において報告したものの追加報告
	39	感染症および寄生虫症	C型肝炎	日本	男	81	2005/3/30	⑥不明	症例報告	当該製品	2007/10/26提出、識別番号1-05000035　完了報告　第10回症例番号39は前々々々々回報告における第5回症例番号21において報告したものの追加報告
第9回	2	感染症および寄生虫症	B型肝炎	日本	男	73	2007/5/16	⑥不明	症例報告	当該製品	2007/5/30 提出、識別番号 1-07000029 未完了報告
第7回	9	感染症および寄生虫症	B型肝炎	日本	女	50	2005/12/2	①回復	症例報告	当該製品	2006/6/29 提出、識別番号 1-05000236 完了報告
	11	感染症および寄生虫症	A型肝炎	日本	男	3	2006/1/31	③未回復	症例報告	当該製品	2006/3/22 提出、識別番号 1-05000266 未完了報告
	14	感染症および寄生虫症	B型肝炎	日本	女	4	2006/3/1	③未回復	症例報告	当該製品	2006/3/24 提出、識別番号 1-05000274 未完了報告
	16	感染症および寄生虫症	B型肝炎	日本	男	85	2006/4/5	②軽快	症例報告	当該製品	2006/4/24 提出、識別番号 1-06000010 未完了報告
	17	感染症および寄生虫症	B型肝炎	日本	女	53	2006/4/25	⑥不明	症例報告	当該製品	2006/5/17 提出、識別番号 1-06000025 未完了報告
	18	感染症および寄生虫症	B型肝炎	日本	女	67	2005/12/26	②軽快	症例報告	当該製品	2006/5/23 提出、識別番号 1-06000029 未完了報告
	19	感染症および寄生虫症	B型肝炎	日本	女	67	2006/4/14	③未回復	症例報告	当該製品	2006/5/29 提出、識別番号 1-06000034 未完了報告
第6回	1	感染症および寄生虫症	C型肝炎	日本	男	68	2006/2/10	③未回復	症例報告	当該製品	2006/3/1 提出、識別番号 1-05000264 未完了報告
	2	感染症および寄生虫症	A型肝炎	日本	男	3	2006/1/31	③未回復	症例報告	当該製品	2006/3/3 提出、識別番号 1-05000266 未完了報告
	7	感染症および寄生虫症	B型肝炎	日本	女	74	2005/12/30	⑥不明	症例報告	当該製品	2006/3/3 提出、識別番号 1-05000265 未完了報告
	9	感染症および寄生虫症	B型肝炎	日本	男	37	2005/12/21	②軽快	症例報告	当該製品	2006/1/10 提出、識別番号 1-05000233 未完了報告
	10	感染症および寄生虫症	B型肝炎	日本	男	37	2005/12/21	②軽快	症例報告	当該製品	2006/1/24 提出、識別番号 1-05000233 未完了報告
	11	感染症および寄生虫症	B型肝炎	日本	女	50	2005/12/2	③未回復	症例報告	当該製品	2006/1/11 提出、識別番号 1-05000236 未完了報告
	15	感染症および寄生虫症	B型肝炎	日本	女	27	2005/9/27	③未回復	症例報告	当該製品	2005/10/12 提出、識別番号 1-05000171 未完了報告
	21	感染症および寄生虫症	B型肝炎	日本	女	43	2005/5/30	②軽快	症例報告	当該製品	2005/11/30 提出、識別番号 1-05000059 完了報告
	23	感染症および寄生虫症	B型肝炎	日本	女	14	2005/4/19	①回復	症例報告	当該製品	2005/11/28 提出、識別番号 1-05000026 完了報告
第5回	2	感染症および寄生虫症	B型肝炎	日本	女	94	2005/08/01	③未回復	症例報告	当該製品	2005/08/22 提出、識別番号 1-05000131 未完了報告
	5	感染症および寄生虫症	B型肝炎	日本	男	88	2005/06/29	①回復又は軽快	症例報告	当該製品	2005/07/26 提出、識別番号 1-05000089 未完了報告
	6	感染症および寄生虫症	C型肝炎	日本	女	33	2005/06/22	③未回復	症例報告	当該製品	2005/08/05 提出、識別番号 1-05000107 未完了報告
	7	感染症および寄生虫症	B型肝炎	日本	女	43	2005/05/30	⑧不明	症例報告	当該製品	2005/06/27 提出、識別番号 1-05000059 未完了報告
	11	感染症および寄生虫症	B型肝炎	日本	男	56	2005/05/16	⑧不明	症例報告	当該製品	2005/07/27 提出、識別番号 1-05000094 未完了報告
	16	感染症および寄生虫症	B型肝炎	日本	女	14	2005/04/19	③未回復	症例報告	当該製品	2005/05/06 提出、識別番号 1-05000026 未完了報告
	21	感染症および寄生虫症	C型肝炎	日本	男	81	2005/03/30	③未回復	症例報告	当該製品	2005/05/23 提出、識別番号 1-05000035 未完了報告

80059	2008/04/22	日本赤十字	新鮮凍結人血漿	新鮮凍結人血漿

別紙様式第4

感染症発生症例一覧

番号	感染症の種類		発現国	性別	年齢	発現時期	転帰	出典	区分	備考
	器官別大分類	基本語								
1	感染症および寄生虫症	細菌感染	日本	女	58	2008/2/10	②軽快	症例報告	当該製品	2008/2/28提出、識別番号1-07000239　未完了報告
2	感染症および寄生虫症	B型肝炎	日本	男	81	2008/2/4	③未回復	症例報告	当該製品	2008/2/21提出、識別番号1-07000231　未完了報告
3	感染症および寄生虫症	敗血症	日本	男	74	2008/1/9	②軽快	症例報告	当該製品	2008/1/30提出、識別番号1-07000185　未完了報告
4	感染症および寄生虫症	B型肝炎	日本	男	66	2008/1/9	③未回復	症例報告	当該製品	2008/1/30提出、識別番号1-07000186　未完了報告
5	感染症および寄生虫症	B型肝炎	日本	男	65	2008/1/8	⑥不明	症例報告	当該製品	2008/2/5提出、識別番号1-07000202　未完了報告
6	感染症および寄生虫症	サイトメガロウイルス感染	日本	女	2	2007/12/25	③未回復	症例報告	当該製品	2008/2/19提出、識別番号1-07000229　未完了報告
7	感染症および寄生虫症	C型肝炎	日本	女	42	2007/12/19	③未回復	症例報告	当該製品	2008/1/9提出、識別番号1-07000171　未完了報告
8	感染症および寄生虫症	B型肝炎	日本	女	53	2007/12/17	③未回復	症例報告	当該製品	2008/1/22提出、識別番号1-07000178　未完了報告
9	感染症および寄生虫症	C型肝炎	日本	男	74	2007/12/12	③未回復	症例報告	当該製品	2007/12/28提出、識別番号1-07000163　未完了報告
10	感染症および寄生虫症	細菌感染	日本	男	48	2007/11/24	②軽快	症例報告	当該製品	2007/12/12提出、識別番号1-07000141　未完了報告
11	感染症および寄生虫症	細菌感染	日本	男	52	2007/11/24	①回復	症例報告	当該製品	2007/12/18提出、識別番号1-07000147　未完了報告
12	感染症および寄生虫症	B型肝炎	日本	男	62	2007/11/15	③未回復	症例報告	当該製品	2007/12/14提出、識別番号1-07000145　未完了報告
13	感染症および寄生虫症	伝染性紅斑	日本	女	55	2007/11/12	③未回復	症例報告	当該製品	2007/12/28提出、識別番号1-07000161　未完了報告
14	感染症および寄生虫症	B型肝炎	日本	女	63	2007/11/6	⑥不明	症例報告	当該製品	2007/11/26提出、識別番号1-07000127　未完了報告
15	感染症および寄生虫症	B型肝炎	日本	女	34	2007/10/19	③未回復	症例報告	当該製品	2007/11/8提出、識別番号1-07000113　未完了報告
16	感染症および寄生虫症	B型肝炎	日本	男	56	2007/10/19	①回復	症例報告	当該製品	2007/11/13提出、識別番号1-07000117　未完了報告
17	感染症および寄生虫症	菌血症	日本	男	51	2007/9/30	①回復	症例報告	当該製品	2007/10/18提出、識別番号1-07000102　未完了報告
18	感染症および寄生虫症	細菌感染	日本	女	53	2007/9/27	①回復	症例報告	当該製品	2007/10/15提出、識別番号1-07000100　未完了報告
19	感染症および寄生虫症	B型肝炎	日本	女	81	2007/9/12	③未回復	症例報告	当該製品	2007/10/15提出、識別番号1-07000101　未完了報告
20	感染症および寄生虫症	細菌感染	日本	男	71	2007/8/29	③未回復	症例報告	当該製品	2007/9/20提出、識別番号1-07000091　未完了報告
21	感染症および寄生虫症	C型肝炎	日本	女	53	2007/8/28	②軽快	症例報告	当該製品	2007/10/19提出、識別番号1-07000104　未完了報告
22	感染症および寄生虫症	C型肝炎	日本	女	53	2007/8/28	②軽快	症例報告	当該製品	2007/11/20提出、識別番号1-07000104　未完了報告（21番と同一症例）
23	感染症および寄生虫症	B型肝炎	日本	男	73	2007/8/27	②軽快	症例報告	当該製品	2007/11/13提出、識別番号1-07000116　未完了報告
24	感染症および寄生虫症	B型肝炎	日本	男	73	2007/8/27	①回復	症例報告	当該製品	2008/2/1提出、識別番号1-07000116　取り下げ（23番と同一症例）
25	感染症および寄生虫症	B型肝炎	日本	男	16	2007/7/23	③未回復	症例報告	当該製品	2007/10/31提出、識別番号1-07000106　未完了報告
26	感染症および寄生虫症	B型肝炎	日本	男	16	2007/7/23	③未回復	症例報告	当該製品	2007/11/20提出、識別番号1-07000106　未完了報告（25番と同一症例）
27	感染症および寄生虫症	B型肝炎	日本	男	16	2007/7/23	③未回復	症例報告	当該製品	2008/2/19提出、識別番号1-07000106　完了報告（25番と同一症例）
28	感染症および寄生虫症	B型肝炎	日本	女	52	2007/7/9	②軽快	症例報告	当該製品	2007/11/6提出、識別番号1-07000078　取り下げ 第10回症例番号28は前回報告における第9回症例番号3において報告したものの追加報告
29	感染症および寄生虫症	B型肝炎	日本	男	58	2007/7/4	②軽快	症例報告	当該製品	2007/11/26提出、識別番号1-07000126　未完了報告
30	感染症および寄生虫症	B型肝炎	日本	男	58	2007/7/4	③未回復	症例報告	当該製品	2008/2/6提出、識別番号1-07000126　未完了報告（29番と同一症例）
31	感染症および寄生虫症	C型肝炎	日本	女	82	2007/6/21	③未回復	症例報告	当該製品	2007/9/13提出、識別番号1-07000089　未完了報告

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32	感染症および寄生虫症	B型肝炎	日本	女	64	2007/6/13	③未回復	症例報告	当該製品	2007/11/6提出、識別番号1-07000055　取り下げ 第10回症例番号32は前回報告における第9回症例番号6において報告したものの追加報告
33	感染症および寄生虫症	B型肝炎	日本	男	73	2007/5/16	⑥不明	症例報告	当該製品	2007/12/4提出、識別番号1-07000029　取り下げ 第10回症例番号33は前回報告における第9回症例番号15において報告したものの追加報告
34	感染症および寄生虫症	B型肝炎	日本	女	71	2007/4/26	③未回復	症例報告	当該製品	2008/2/14提出、識別番号1-07000223　未完了報告
35	感染症および寄生虫症	B型肝炎	日本	女	76	2006/8/25	①回復	症例報告	当該製品	2007/10/11提出、識別番号1-07000099　未完了報告
36	感染症および寄生虫症	B型肝炎	日本	男	71	2006/5/1	⑥不明	症例報告	当該製品	2008/1/11提出、識別番号1-06000032　完了報告 第10回症例番号36は前々々回報告における第7回症例番号19において報告したものの追加報告
37	感染症および寄生虫症	B型肝炎	日本	女	53	2006/4/25	⑥不明	症例報告	当該製品	2007/12/17提出、識別番号1-06000025　完了報告 第10回症例番号37は前々々回報告における第7回症例番号22において報告したものの追加報告
38	感染症および寄生虫症	B型肝炎	日本	男	58	2006/4/21	②軽快	症例報告	当該製品	2007/12/17提出、識別番号1-06000021　完了報告 第10回症例番号38は前々々回報告における第7回症例番号23において報告したものの追加報告
39	感染症および寄生虫症	B型肝炎	日本	男	70	2006/4/18	②軽快	症例報告	当該製品	2007/11/2提出、識別番号1-06000014　完了報告 第10回症例番号39は前々々回報告における第7回症例番号24において報告したものの追加報告
40	感染症および寄生虫症	B型肝炎	日本	男	85	2006/4/5	②軽快	症例報告	当該製品	2007/10/29提出、識別番号1-06000010　完了報告 第10回症例番号40は前々々回報告における第7回症例番号27において報告したものの追加報告
41	感染症および寄生虫症	B型肝炎	日本	男	54	2006/3/22	⑥不明	症例報告	当該製品	2008/1/8提出、識別番号1-06000037　完了報告 第10回症例番号41は前々々回報告における第7回症例番号28において報告したものの追加報告
42	感染症および寄生虫症	E型肝炎	日本	男	56	2006/3/20	②軽快	症例報告	当該製品	2007/12/14提出、識別番号1-06000020　完了報告 第10回症例番号42は前々々回報告における第7回症例番号29において報告したものの追加報告
43	感染症および寄生虫症	B型肝炎	日本	男	61	2006/3/17	⑥不明	症例報告	当該製品	2007/10/29提出、識別番号1-06000002　完了報告 第10回症例番号43は前々々回報告における第7回症例番号30において報告したものの追加報告
44	感染症および寄生虫症	B型肝炎	日本	女	36	2006/3/10	⑥不明	症例報告	当該製品	2007/10/29提出、識別番号1-05000276　完了報告 第10回症例番号44は前々々回報告における第7回症例番号31において報告したものの追加報告
45	感染症および寄生虫症	B型肝炎	日本	男	78	2006/2/3	⑥不明	症例報告	当該製品	2008/1/8提出、識別番号1-06000035　完了報告 第10回症例番号45は前々々回報告における第7回症例番号36において報告したものの追加報告

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46	感染症および寄生虫症	B型肝炎	日本	女	57	2006/1/30	⑥不明	症例報告	当該製品	2007/10/29提出、識別番号1-05000268　完了報告　第10回症例番号46は前々々々回報告における第7回症例番号38において報告したものの追加報告
47	感染症および寄生虫症	B型肝炎	日本	男	70	2006/1/12	⑥不明	症例報告	当該製品	2007/11/9提出、識別番号1-05000257　完了報告　第10回症例番号47は前々々々回報告における第6回症例番号3において報告したものの追加報告
48	感染症および寄生虫症	B型肝炎	日本	女	67	2005/12/26	②軽快	症例報告	当該製品	2007/12/17提出、識別番号1-06000029　完了報告　第10回症例番号48は前々々々回報告における第7回症例番号39において報告したものの追加報告
49	感染症および寄生虫症	B型肝炎	日本	男	37	2005/12/21	②軽快	症例報告	当該製品	2007/10/26提出、識別番号1-05000233　完了報告　第10回症例番号49は前々々々回報告における第6回症例番号7及び8において報告したものの追加報告
50	感染症および寄生虫症	B型肝炎	日本	男	57	2005/12/19	②軽快	症例報告	当該製品	2007/9/25提出、識別番号1-05000231　完了報告　第10回症例番号50は前々々々回報告における第6回症例番号9において報告したものの追加報告
51	感染症および寄生虫症	B型肝炎	日本	女	85	2005/12/6	②軽快	症例報告	当該製品	2007/9/12提出、識別番号1-05000230　完了報告　第10回症例番号51は前々々々回報告における第6回症例番号10において報告したものの追加報告
52	感染症および寄生虫症	B型肝炎	日本	男	56	2005/12/3	⑥不明	症例報告	当該製品	2007/9/26提出、識別番号1-05000258　完了報告　第10回症例番号52は前々々々回報告における第6回症例番号13において報告したものの追加報告
53	感染症および寄生虫症	B型肝炎	日本	女	56	2005/10/27	②軽快	症例報告	当該製品	2008/1/21提出、識別番号1-05000199　完了報告　第10回症例番号53は前々々々回報告における第6回症例番号21において報告したものの追加報告
54	感染症および寄生虫症	C型肝炎	日本	男	77	2005/10/22	⑥不明	症例報告	当該製品	2008/1/18提出、識別番号1-05000190　完了報告　第10回症例番号54は前々々々回報告における第6回症例番号22において報告したものの追加報告
55	感染症および寄生虫症	E型肝炎	日本	男	72	2005/10/1	⑥不明	症例報告	当該製品	2008/2/12提出、識別番号1-05000186　完了報告　第10回症例番号55は前々々回報告における第7回症例番号41、42及び前々々々回報告における第6回症例番号25において報告したものの追加報告
56	感染症および寄生虫症	B型肝炎	日本	男	60	2005/9/21	⑥不明	症例報告	当該製品	2007/10/29提出、識別番号1-05000166　完了報告　第10回症例番号56は前々々々回報告における第6回症例番号29において報告したものの追加報告
57	感染症および寄生虫症	細菌感染	日本	男	47	2005/9/20	①回復	症例報告	当該製品	2007/10/2提出、識別番号1-05000180　完了報告
58	感染症および寄生虫症	B型肝炎	日本	男	75	2005/8/24	②軽快	症例報告	当該製品	2007/11/9提出、識別番号1-05000150　完了報告　第10回症例番号58は前々々々回報告における第6回症例番号36において報告したものの追加報告

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	59	感染症および寄生虫症	C型肝炎	日本	女	71	2005/8/8	⑥不明	症例報告	当該製品	2007/10/29提出、識別番号1-05000133　完了報告第10回症例番号59は前々々々々回報告における第5回症例番号1において報告したものの追加報告
	60	感染症および寄生虫症	細菌感染	日本	男	75	2005/7/21	①回復	症例報告	当該製品	2007/10/2提出、識別番号1-05000111　完了報告第10回症例番号60は前々々々々回報告における第5回症例番号3において報告したものの追加報告
	61	感染症および寄生虫症	伝染性紅斑	日本	男	57	2005/7/8	①回復	症例報告	当該製品	2007/11/5提出、識別番号1-05000097　完了報告第10回症例番号61は前々々々々回報告における第5回症例番号5において報告したものの追加報告
	62	感染症および寄生虫症	B型肝炎	日本	男	61	2005/7/8	⑥不明	症例報告	当該製品	2008/2/12提出、識別番号1-05000141　完了報告第10回症例番号62は前々々々回報告における第6回症例番号43、44及び45において報告したものの追加報告
	63	感染症および寄生虫症	B型肝炎	日本	男	79	2005/7/7	②軽快	症例報告	当該製品	2008/1/18提出、識別番号1-05000187　完了報告第10回症例番号63は前々々々回報告における第6回症例番号46において報告したものの追加報告
	64	感染症および寄生虫症	伝染性紅斑	日本	男	41	2005/6/4	①回復	症例報告	当該製品	2007/11/5提出、識別番号1-05000063　完了報告第10回症例番号64は前々々々々回報告における第5回症例番号10において報告したものの追加報告
	65	感染症および寄生虫症	B型肝炎	日本	女	43	2005/5/30	②軽快	症例報告	当該製品	2008/2/12提出、識別番号1-05000059　完了報告第10回症例番号65は前々々々回報告における第6回症例番号47及び前々々々々回報告における第5回症例番号11において報告したものの追加報告
	66	感染症および寄生虫症	B型肝炎	日本	女	75	2005/4/22	⑥不明	症例報告	当該製品	2008/1/18提出、識別番号1-05000181　完了報告第10回症例番号66は前々々々回報告における第6回症例番号49において報告したものの追加報告
	67	感染症および寄生虫症	B型肝炎	日本	女	14	2005/4/19	①回復	症例報告	当該製品	2008/2/12提出、識別番号1-05000026　完了報告第10回症例番号67は前々々々回報告における第6回症例番号50及び前々々々々回報告における第5回症例番号22において報告したものの追加報告
	68	感染症および寄生虫症	B型肝炎	日本	女	60	2005/2/25	⑥不明	症例報告	当該製品	2008/1/21提出、識別番号1-05000113　完了報告第10回症例番号68は前々々々々回報告における第5回症例番号34において報告したものの追加報告
	69	感染症および寄生虫症	E型肝炎	日本	男	64	2004/10/1	②軽快	症例報告	当該製品	2008/2/12提出、識別番号1-04000223　完了報告第10回症例番号69は前々々々回報告における第6回症例番号52及び前々々々々々回報告における第4回症例番号29において報告したものの追加報告
第9回	3	感染症および寄生虫症	B型肝炎	日本	女	52	2007/7/9	③未回復	症例報告	当該製品	2007/8/16 提出、識別番号 1-07000078 未完了報告
	6	感染症および寄生虫症	B型肝炎	日本	女	64	2007/6/13	③未回復	症例報告	当該製品	2007/7/5 提出、識別番号 1-07000055 未完了報告

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	15	感染症および寄生虫症	B型肝炎	日本	男	73	2007/5/16	⑥不明	症例報告	当該製品	2007/5/30 提出、識別番号 1-07000029 未完了報告
第7回	19	感染症および寄生虫症	B型肝炎	日本	男	71	2006/5/1	⑥不明	症例報告	当該製品	2006/5/25 提出、識別番号 1-06000032 未完了報告
	22	感染症および寄生虫症	B型肝炎	日本	女	53	2006/4/25	⑥不明	症例報告	当該製品	2006/5/17 提出、識別番号 1-06000025 未完了報告
	23	感染症および寄生虫症	B型肝炎	日本	男	58	2006/4/21	②軽快	症例報告	当該製品	2006/5/17 提出、識別番号 1-06000021 未完了報告
	24	感染症および寄生虫症	B型肝炎	日本	男	70	2006/4/18	②軽快	症例報告	当該製品	2006/5/2 提出、識別番号 1-06000014 未完了報告
	27	感染症および寄生虫症	B型肝炎	日本	男	85	2006/4/5	②軽快	症例報告	当該製品	2006/4/24 提出、識別番号 1-06000010 未完了報告
	28	感染症および寄生虫症	B型肝炎	日本	男	54	2006/3/22	⑥不明	症例報告	当該製品	2006/6/1 提出、識別番号 1-06000037 未完了報告
	29	感染症および寄生虫症	E型肝炎	日本	男	56	2006/3/20	②軽快	症例報告	当該製品	2006/5/12 提出、識別番号 1-06000020 未完了報告
	30	感染症および寄生虫症	B型肝炎	日本	男	61	2006/3/17	③未回復	症例報告	当該製品	2006/4/6 提出、識別番号 1-06000002 未完了報告
	31	感染症および寄生虫症	B型肝炎	日本	女	36	2006/3/10	③未回復	症例報告	当該製品	2006/3/29 提出、識別番号 1-05000276 未完了報告
	36	感染症および寄生虫症	B型肝炎	日本	男	78	2006/2/3	③未回復	症例報告	当該製品	2006/5/29 提出、識別番号 1-06000035 未完了報告
	38	感染症および寄生虫症	B型肝炎	日本	女	57	2006/1/30	③未回復	症例報告	当該製品	2006/3/6 提出、識別番号 1-05000268 未完了報告
	39	感染症および寄生虫症	B型肝炎	日本	女	67	2005/12/26	②軽快	症例報告	当該製品	2006/5/23 提出、識別番号 1-06000029 未完了報告
	41	感染症および寄生虫症	E型肝炎	日本	男	72	2005/10/1	⑥不明	症例報告	当該製品	2006/4/24 提出、識別番号 1-05000186 完了報告
	42	感染症および寄生虫症	E型肝炎	日本	男	72	2005/10/1	⑥不明	症例報告	当該製品	2006/6/6 提出、識別番号 1-05000186 追加報告
第6回	3	感染症および寄生虫症	B型肝炎	日本	男	70	2006/1/12	③未回復	症例報告	当該製品	2006/2/24 提出、識別番号 1-05000257 未完了報告
	7	感染症および寄生虫症	B型肝炎	日本	男	37	2005/12/21	②軽快	症例報告	当該製品	2006/1/10 提出、識別番号 1-05000233 未完了報告
	8	感染症および寄生虫症	B型肝炎	日本	男	37	2005/12/21	②軽快	症例報告	当該製品	2006/1/24 提出、識別番号 1-05000233 未完了報告
	9	感染症および寄生虫症	B型肝炎	日本	男	57	2005/12/19	⑥不明	症例報告	当該製品	2006/1/5 提出、識別番号 1-05000231 未完了報告
	10	感染症および寄生虫症	B型肝炎	日本	女	85	2005/12/6	②軽快	症例報告	当該製品	2005/12/28 提出、識別番号 1-05000230 未完了報告
	13	感染症および寄生虫症	B型肝炎	日本	男	56	2005/12/3	③未回復	症例報告	当該製品	2006/2/24 提出、識別番号 1-05000258 未完了報告
	21	感染症および寄生虫症	B型肝炎	日本	女	56	2005/10/27	③未回復	症例報告	当該製品	2005/11/22 提出、識別番号 1-05000199 未完了報告
	22	感染症および寄生虫症	C型肝炎	日本	男	77	2005/10/22	③未回復	症例報告	当該製品	2005/11/14 提出、識別番号 1-05000190 未完了報告
	25	感染症および寄生虫症	E型肝炎	日本	男	72	2005/10/1	③未回復	症例報告	当該製品	2005/11/9 提出、識別番号 1-05000186 未完了報告
	29	感染症および寄生虫症	B型肝炎	日本	男	60	2005/9/21	⑥不明	症例報告	当該製品	2005/10/11 提出、識別番号 1-05000166 未完了報告
	36	感染症および寄生虫症	B型肝炎	日本	男	75	2005/8/24	③未回復	症例報告	当該製品	2005/9/16 提出、識別番号 1-05000150 未完了報告
	43	感染症および寄生虫症	B型肝炎	日本	男	61	2005/7/8	③未回復	症例報告	当該製品	2005/9/5 提出、識別番号 1-05000141 未完了報告
	44	感染症および寄生虫症	B型肝炎	日本	男	61	2005/7/8	⑥不明	症例報告	当該製品	2005/11/30 提出、識別番号 1-05000141 完了報告
	45	感染症および寄生虫症	B型肝炎	日本	男	61	2005/7/8	⑥不明	症例報告	当該製品	2005/12/9 提出、識別番号 1-05000141 完了報告
	46	感染症および寄生虫症	B型肝炎	日本	男	79	2005/7/7	②軽快	症例報告	当該製品	2005/11/9 提出、識別番号 1-05000187 未完了報告
	47	感染症および寄生虫症	B型肝炎	日本	女	43	2005/5/30	②軽快	症例報告	当該製品	2005/11/30 提出、識別番号 1-05000059 完了報告
	49	感染症および寄生虫症	B型肝炎	日本	女	75	2005/4/22	③未回復	症例報告	当該製品	2005/10/26 提出、識別番号 1-05000181 未完了報告
	50	感染症および寄生虫症	B型肝炎	日本	女	14	2005/4/19	①回復	症例報告	当該製品	2005/11/28 提出、識別番号 1-05000026 完了報告
	52	感染症および寄生虫症	E型肝炎	日本	男	64	2004/10/1	②軽快	症例報告	当該製品	2005/10/6 提出、識別番号 1-04000223 完了報告
	1	感染症および寄生虫症	C型肝炎	日本	女	71	2005/08/08	③未回復	症例報告	当該製品	2005/08/24 提出、識別番号 1-05000133 完了報告
	3	感染症および寄生虫症	細菌感染	日本	男	75	2005/07/21	⑧不明	症例報告	当該製品	2005/08/08 提出、識別番号 1-05000111 未完了報告

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感染症発生症例一覧

第5回	5	感染症および寄生虫症	伝染性紅斑	日本	男	57	2005/07/08	③未回復	症例報告	当該製品	2005/07/29 提出、識別番号 1-05000097 未完了報告
	10	感染症および寄生虫症	伝染性紅斑	日本	男	41	2005/06/04	⑧不明	症例報告	当該製品	2005/06/29 提出、識別番号 1-05000063 未完了報告
	11	感染症および寄生虫症	B型肝炎	日本	女	43	2005/05/30	⑧不明	症例報告	当該製品	2005/06/27 提出、識別番号 1-05000059 未完了報告
	22	感染症および寄生虫症	B型肝炎	日本	女	14	2005/04/19	③未回復	症例報告	当該製品	2005/05/06 提出、識別番号 1-05000026 未完了報告
	34	感染症および寄生虫症	B型肝炎	日本	女	60	2005/02/25	⑧不明	症例報告	当該製品	2005/08/09 提出、識別番号 1-05000113 未完了報告
第4回	29	感染症および寄生虫症	E型肝炎	日本	男	64	2004/10/1	⑧不明	症例報告	当該製品	2004/10/20 提出、識別番号 1-04000223 未完了報告

80060|2008/04/22|日本赤十字|人血小板濃厚液|人血小板濃厚液

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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10回	1	感染症および寄生虫症	B型肝炎	日本	男	73	2007/8/27	②軽快	症例報告	当該製品	2007/11/13提出、識別番号1-07000116　未完了報告
	2	感染症および寄生虫症	B型肝炎	日本	男	73	2007/8/27	①回復	症例報告	当該製品	2008/2/1提出、識別番号1-07000116　取り下げ（1番と同一症例）
	3	感染症および寄生虫症	B型肝炎	日本	男	16	2007/7/23	③未回復	症例報告	当該製品	2007/10/31提出、識別番号1-07000106　未完了報告
	4	感染症および寄生虫症	B型肝炎	日本	男	16	2007/7/23	③未回復	症例報告	当該製品	2007/11/20提出、識別番号1-07000106　未完了報告（3番と同一症例）
	5	感染症および寄生虫症	B型肝炎	日本	男	16	2007/7/23	③未回復	症例報告	当該製品	2008/2/19提出、識別番号1-07000106　完了報告（3番と同一症例）
	6	感染症および寄生虫症	B型肝炎	日本	男	56	2005/12/3	⑥不明	症例報告	当該製品	2007/9/26提出、識別番号1-05000258　完了報告 第9回症例番号17は前々々々回報告における第6回症例番号1において報告したものの追加報告
	7	感染症および寄生虫症	B型肝炎	日本	女	60	2005/2/25	⑥不明	症例報告	当該製品	2008/1/21提出、識別番号1-05000113　完了報告 第9回症例番号17は前々々々々回報告における第5回症例番号2において報告したものの追加報告
第6回	1	感染症および寄生虫症	B型肝炎	日本	男	56	2005/12/3	③未回復	症例報告	当該製品	2006/2/24 提出、識別番号 1-05000258 未完了報告
第5回	2	感染症および寄生虫症	B型肝炎	日本	女	60	2005/02/25	⑧不明	症例報告	当該製品	2005/8/9 提出、識別番号 1-05000113 未完了報告

80061	2008/04/22	日本赤十字社	洗浄人赤血球浮遊液	洗浄人赤血球浮遊液

別紙4

別紙様式第4

別紙5

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10回	10-1	臨床検査	Ｂ型肝炎抗体陽性	アメリカ	男	21歳	2007/12/18	不明	症例報告	外国製品	2008/02/01、3-07000019
	10-2	臨床検査	Ｂ型肝炎抗体	アメリカ	女	35歳	2007/10/12	不明	症例報告	外国製品	2007/10/31、3-07000014
	10-3	臨床検査	Ａ型肝炎抗体陽性	アメリカ	女	10歳	2007/10/11	不明	症例報告	外国製品	2007/11/08、3-07000016
	10-2	臨床検査	Ｂ型肝炎陽性	アメリカ	女	35歳	2007/10	不明	症例報告	外国製品	2007/10/31、3-07000014
	10-2	臨床検査	抗ＨＢｃＩｇＭ抗体陽性	アメリカ	女	35歳	2007/9/20	不明	症例報告	外国製品	2007/10/31、3-07000014
	10-4	臨床検査	単純ヘルペス血清学的検査陽性	アメリカ	男	29歳	2007/6	回復	症例報告	外国製品	2008/01/10、3-07000013
	9-2	臨床検査	Ｃ型肝炎陽性	カナダ	男	55歳	2007/1/3	未回復	症例報告	外国製品	2007/12/19、3-07000007
	9-1	感染症および寄生虫症	進行性多巣性白質脳症	カナダ	男	18歳	2006/12	死亡	症例報告	外国製品	2008/01/23、3-07000006
	9-5	臨床検査	Ｃ型肝炎ウイルス	アメリカ	男	47歳	不明	未回復	症例報告	外国製品	2007/10/04、3-07000008
第9回	9-1	感染症および寄生虫症	ＪＣウイルス感染	カナダ	男	18歳	2007/2	死亡	症例報告	外国製品	2007/07/11、3-07000006
	9-2	臨床検査	Ｃ型肝炎陽性	カナダ	男	55歳	2007/1/3	未回復	症例報告	外国製品	2007/07/25、3-07000007
	8-1	感染症および寄生虫症	ブドウ球菌感染	カナダ	女	50歳	2006/10/20	不明	症例報告	外国製品	2007/03/02、3-06000024
	8-2	感染症および寄生虫症	Ｂ型肝炎	アメリカ	男	46歳	2006/7/17	不明	症例報告	外国製品	2007/03/02、3-06000030
	9-5	臨床検査	Ｃ型肝炎ウイルス	アメリカ	男	47歳	不明	未回復	症例報告	外国製品	2007/07/17、3-07000008
第8回	8-1	感染症および寄生虫症	ブドウ球菌感染	カナダ	女	50歳	2006/10/20	不明	症例報告	外国製品	2006/11/14、3-06000024
	8-2	感染症および寄生虫症	Ｂ型肝炎	アメリカ	男	46歳	2006/7/17	不明	症例報告	外国製品	2007/02/20、3-06000030
第7回	7-1	臨床検査	抗ＨＢｓ抗体陽性	アメリカ	男	17歳	2006/2/18	不明	症例報告	外国製品	2006/04/13、3-05000685
	7-1	感染症および寄生虫症	Ｂ型肝炎	アメリカ	男	17歳	2006/2	不明	症例報告	外国製品	2006/04/13、3-05000685
	7-2	感染症および寄生虫症	Ｂ型肝炎	カナダ	女	13歳	2005/12/1	不明	症例報告	外国製品	2006/06/28、3-06000002
	7-2	臨床検査	Ｂ型肝炎陽性	カナダ	女	13歳	2005/7/22	不明	症例報告	外国製品	2006/06/28、3-06000002
第6回	6-1	感染症および寄生虫症	ウエストナイルウイルス感染	アメリカ	男	43歳	不明	不明	症例報告	外国製品	2006/02/15、3-05000574

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別紙5

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5回	4-8	臨床検査	Ｂ型肝炎陽性	アメリカ	男	66歳	2005/1/13	不明	症例報告	外国製品	2005/03/30、3-04000093
	3-10	感染症および寄生虫症	Ｃ型肝炎	アメリカ	男	26歳	2002/11/1	不明	症例報告	外国製品	2005/06/28、3-04000022
	2-4	感染症および寄生虫症	Ｃ型肝炎	アメリカ	男	26歳	2002/8/30	不明	症例報告	外国製品	2005/03/09、3-03000017
	4-9	臨床検査	Ｂ型肝炎抗体陽性	アメリカ	女	32歳	不明	不明	症例報告	外国製品	2005/03/28、3-04000097
	3-10	感染症および寄生虫症	Ｂ型肝炎	アメリカ	男	26歳	不明	不明	症例報告	外国製品	2005/06/28、3-04000022
第4回	3-1	臨床検査	Ｂ型肝炎抗体陽性	カナダ	女	56歳	2004/3/10	不明	症例報告	外国製品	2004/09/17、3-04000007
	3-3	臨床検査	ＨＴＬＶ－１検査陽性	カナダ	女	不明	2003/10/10	不明	症例報告	外国製品	2004/09/03、3-04000025
	3-5	臨床検査	ＨＴＬＶ－１検査陽性	カナダ	女	不明	2003/8/25	不明	症例報告	外国製品	2004/08/31、3-04000026
	3-6	臨床検査	ＨＴＬＶ－１検査陽性	カナダ	女	不明	2003/6/20	不明	症例報告	外国製品	2004/09/03、3-04000027
	3-8	臨床検査	ＨＴＬＶ－１検査陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2004/10/01、3-04000028
	3-10	感染症および寄生虫症	Ｂ型肝炎	アメリカ	男	不明	不明	不明	症例報告	外国製品	2004/09/17、3-04000022
	3-10	感染症および寄生虫症	Ｃ型肝炎	アメリカ	男	不明	不明	不明	症例報告	外国製品	2004/09/17、3-04000022
	4-7	感染症および寄生虫症	単純ヘルペス	アメリカ	女	不明	不明	不明	症例報告	外国製品	2005/01/12、3-04000089
	4-8	臨床検査	Ｂ型肝炎陽性	アメリカ	不明	不明	不明	不明	症例報告	外国製品	2005/02/02、3-04000093
	4-9	臨床検査	Ｂ型肝炎抗体陽性	アメリカ	女	不明	不明	不明	症例報告	外国製品	2005/02/23、3-04000097
第3回	3-1	肝胆道系障害	肝炎	カナダ	女	56歳	2004/3/12	不明	症例報告	外国製品	2004/05/07、3-04000007
	3-2	臨床検査	Ｂ型肝炎陽性	アメリカ	女	48歳	2004/3	未回復	症例報告	外国製品	2004/06/21、3-04000017
	3-3	臨床検査	Ｃ型肝炎陽性	アメリカ	女	48歳	2004/3	未回復	症例報告	外国製品	2004/06/21、3-04000017
	3-4	臨床検査	ＨＴＬＶ－１検査陽性	カナダ	女	不明	2003/10/10	不明	症例報告	外国製品	2004/07/20、3-04000025
	2-5	臨床検査	ＨＴＬＶ－１検査陽性	カナダ	女	25歳	2003/9/26	回復	症例報告	外国製品	2004/07/06、3-03000010
	3-5	臨床検査	ＨＴＬＶ－１検査陽性	カナダ	女	不明	2003/8/25	不明	症例報告	外国製品	2004/08/31、3-04000026
	3-6	臨床検査	ＨＴＬＶ－１検査陽性	カナダ	女	不明	2003/6/20	不明	症例報告	外国製品	2004/07/20、3-04000027
	2-3	臨床検査	ＨＴＬＶ－１検査陽性	カナダ	女	不明	2003/5/26	回復	症例報告	外国製品	2004/08/09、3-03000008
	3-8	臨床検査	ＨＴＬＶ－１検査陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2004/08/31、3-04000028
	3-9	感染症および寄生虫症	Ｃ型肝炎	カナダ	女	不明	不明	不明	症例報告	外国製品	2004/05/31、3-04000011
	3-10	感染症および寄生虫症	Ｂ型肝炎	アメリカ	男	不明	不明	不明	症例報告	外国製品	2004/06/30、3-04000022
	3-10	感染症および寄生虫症	Ｃ型肝炎	アメリカ	男	不明	不明	不明	症例報告	外国製品	2004/06/30、3-04000022

別紙5

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2回	2-1	臨床検査	ＨＴＬＶ－１検査陽性	日本	男	26歳	2003/12	不明	症例報告	当該製品	2004/02/26、1-03000112
	2-2	感染症および寄生虫症	マイコプラズマ感染	日本	女	不明	2003/7	不明	症例報告	当該製品	2004/01/14、1-03000016
	2-3	臨床検査	ＨＴＬＶ－１検査陽性	カナダ	女	不明	2003/5	不明	症例報告	外国製品	2004/02/23、3-03000008
	2-4	感染症および寄生虫症	Ｃ型肝炎	アメリカ	男	26歳	2002/8/30	不明	症例報告	外国製品	2004/01/13、3-03000017
	2-5	臨床検査	ＨＴＬＶ－１検査陽性	カナダ	女	25歳	不明	不明	症例報告	外国製品	2004/01/05、3-03000010

MedDRAバージョン10.1により集計した。

第10回症例番号9-1, 9-2, 9-5は、それぞれ第9回症例番号9-1, 9-2, 9-5において報告したものの追加報告。

第10回症例番号9-1の有害事象名は，第9回報告時では「ＪＣウイルス感染」であったが追加情報により，「進行性多巣性白質脳症」に変更された。

第9回症例番号8-1, 8-2は、それぞれ第8回症例番号8-1, 8-2において報告したものの追加報告。

第5回症例番号4-8, 3-10, 2-4, 4-9, 3-10は、それぞれ第4回、第3回および第2回症例番号4-8, 3-10, 2-4, 4-9, 3-10において報告したものの追加報告。

第4回症例番号3-1, 3-3, 3-5, 3-6, 3-8, 3-10は，それぞれ第3回症例番号3-1, 3-3, 3-5, 3-6, 3-8, 3-10において報告したものの追加報告。

第4回症例番号3-1の有害事象名は，第3回報告時では「肝炎」であったが追加情報により，「Ｂ型肝炎抗体陽性」に変更された。

第3回症例番号2-5, 2-3は，それぞれ第2回症例番号2-5, 2-3において報告したものの追加報告。

第1回調査期間中に当該生物由来製品によると疑われる感染症の報告はなかった(初回および追加報告ともに)。

80077	2008/04/25	バイエル薬品	pH4処理酸性人免疫グロブリン	人免疫グロブリンG

別紙様式第4

別紙5

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10回	9-1	臨床検査	C型肝炎陽性	カナダ	男	55歳	2007/1/3	未回復	症例報告	外国製品	2007/12/19、3-07000007
第9回	9-1	臨床検査	C型肝炎陽性	カナダ	男	55歳	2007/1/3	未回復	症例報告	外国製品	2007/07/25、3-07000007
第7回	7-1	感染症および寄生虫症	C型肝炎	アメリカ	女	不明	不明	不明	症例報告	外国製品	2006/06/19、3-06000020
第6回	5-2	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2005/10/25、3-05000341
	6-2	感染症および寄生虫症	肝炎ウイルスキャリアー	フランス	男	不明	不明	不明	症例報告	外国製品	2005/11/15、3-05000462
第5回	5-1	臨床検査	C型肝炎陽性	カナダ	男	31歳	1998/6/4	不明	症例報告	外国製品	2005/03/25、3-04000123
	5-2	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2005/08/01、3-05000341
第4回	4-1	臨床検査	C型肝炎陽性	カナダ	女	31歳	2004/5/25	不明	症例報告	外国製品	2004/11/12、3-04000085
	4-2	感染症および寄生虫症	細菌性関節炎	フランス	男	不明	2004/3	不明	症例報告	外国製品	2004/09/28、3-04000067
	4-3	臨床検査	C型肝炎陽性	カナダ	男	63歳	2001/5/17	不明	症例報告	外国製品	2004/09/10、3-04000064
	3-35	臨床検査	C型肝炎陽性	カナダ	女	68歳	2001/3/27	不明	症例報告	外国製品	2004/09/14、3-04000057
	4-5	臨床検査	C型肝炎陽性	カナダ	女	47歳	2000/2/25	不明	症例報告	外国製品	2004/10/05、3-04000071
	4-6	臨床検査	C型肝炎陽性	カナダ	女	65歳	1999/3/11	不明	症例報告	外国製品	2004/10/06、3-04000070
	3-6	臨床検査	C型肝炎陽性	カナダ	男	72歳	1998/7/13	不明	症例報告	外国製品	2004/08/31、3-04000056
	4-8	臨床検査	C型肝炎陽性	カナダ	男	58歳	1998/6/2	不明	症例報告	外国製品	2004/09/17、3-04000063
	3-8	臨床検査	C型肝炎陽性	カナダ	女	34歳	1996/10/16	不明	症例報告	外国製品	2004/08/31、3-04000042
	3-34	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2004/08/31、3-04000054
	3-36	臨床検査	C型肝炎陽性	カナダ	男	不明	不明	不明	症例報告	外国製品	2004/08/31、3-04000058
	4-12	臨床検査	C型肝炎陽性	カナダ	男	不明	不明	不明	症例報告	外国製品	2004/09/17、3-04000061
	4-13	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2004/09/10、3-04000062
第3回	3-1	臨床検査	C型肝炎陽性	カナダ	男	69歳	2004/1/13	不明	症例報告	外国製品	2004/04/27、3-04000005
	3-2	臨床検査	C型肝炎陽性	カナダ	男	43歳	2003/8/20	不明	症例報告	外国製品	2004/04/20、3-04000004
	2-2	臨床検査	C型肝炎陽性	カナダ	男	29歳	2000/2/14	不明	症例報告	外国製品	2004/08/05、3-03000002
	3-4	臨床検査	C型肝炎陽性	カナダ	男	57歳	1999/11/9	不明	症例報告	外国製品	2004/05/10、3-04000008
	3-5	臨床検査	C型肝炎陽性	カナダ	男	84歳	1999/3/16	不明	症例報告	外国製品	2004/07/29、3-04000046

別紙様式第4

別紙5

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3回	3-6	臨床検査	C型肝炎陽性	カナダ	男	72歳	1998/7/13	不明	症例報告	外国製品	2004/08/31、3-04000056
	3-7	臨床検査	C型肝炎陽性	カナダ	男	52歳	1997/1/9	不明	症例報告	外国製品	2004/07/23、3-04000037
	3-8	臨床検査	C型肝炎陽性	カナダ	女	34歳	1996/10/16	不明	症例報告	外国製品	2004/08/31、3-04000042
	3-9	臨床検査	C型肝炎陽性	カナダ	男	80歳	1996/2/26	不明	症例報告	外国製品	2004/08/05、3-04000031
	3-10	臨床検査	C型肝炎陽性	カナダ	男	72歳	1996/1/31	不明	症例報告	外国製品	2004/08/24、3-04000045
	3-11	臨床検査	C型肝炎陽性	カナダ	女	76歳	1996/1/11	不明	症例報告	外国製品	2004/07/13、3-04000033
	2-3	臨床検査	C型肝炎陽性	カナダ	男	75歳	1995/12/13	不明	症例報告	外国製品	2004/08/03、3-03000001
	3-13	臨床検査	C型肝炎陽性	カナダ	女	21歳	1992/2/11	不明	症例報告	外国製品	2004/07/13、3-04000040
	3-14	臨床検査	C型肝炎陽性	カナダ	男	49歳	1992/2/10	不明	症例報告	外国製品	2004/05/19、3-04000010
	2-8	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2004/08/04、3-03000003
	2-10	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2004/03/25、3-03000023
	2-11	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2004/03/25、3-03000024
	2-12	臨床検査	C型肝炎陽性	カナダ	男	不明	不明	不明	症例報告	外国製品	2004/03/25、3-03000025
	2-13	臨床検査	C型肝炎陽性	カナダ	不明	不明	不明	不明	症例報告	外国製品	2004/03/25、3-03000027
	3-20	臨床検査	C型肝炎陽性	カナダ	不明	不明	不明	不明	症例報告	外国製品	2004/05/19、3-04000009
	3-21	感染症および寄生虫症	C型肝炎	ドイツ	女	不明	不明	不明	症例報告	外国製品	2004/06/01、3-04000012
	3-22	臨床検査	C型肝炎陽性	カナダ	不明	不明	不明	不明	症例報告	外国製品	2004/07/01、3-04000024
	3-23	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2004/06/28、3-04000019
	3-24	臨床検査	C型肝炎陽性	カナダ	女	46歳	不明	不明	症例報告	外国製品	2004/07/13、3-04000036
	3-25	臨床検査	C型肝炎陽性	カナダ	女	70歳	不明	不明	症例報告	外国製品	2004/07/23、3-04000035
	3-26	臨床検査	C型肝炎陽性	カナダ	男	95歳	不明	不明	症例報告	外国製品	2004/07/13、3-04000034
	3-27	臨床検査	C型肝炎陽性	カナダ	男	68歳	不明	不明	症例報告	外国製品	2004/07/13、3-04000041
	3-28	臨床検査	C型肝炎陽性	カナダ	不明	不明	不明	不明	症例報告	外国製品	2004/07/23、3-04000039
	3-29	臨床検査	C型肝炎陽性	カナダ	不明	74歳	不明	不明	症例報告	外国製品	2004/07/13、3-04000038

別紙様式第4

別紙5

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3回	3-30	臨床検査	C型肝炎陽性	カナダ	不明	不明	不明	不明	症例報告	外国製品	2004/07/28、3-04000044
	3-31	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2004/08/24、3-04000050
	3-32	臨床検査	C型肝炎陽性	カナダ	男	不明	不明	不明	症例報告	外国製品	2004/08/24、3-04000051
	3-33	臨床検査	C型肝炎陽性	カナダ	男	不明	不明	不明	症例報告	外国製品	2004/08/17、3-04000053
	3-34	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2004/08/31、3-04000054
	3-35	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2004/08/31、3-04000057
	3-36	臨床検査	C型肝炎陽性	カナダ	男	不明	不明	不明	症例報告	外国製品	2004/08/31、3-04000058
第2回	2-1	臨床検査	C型肝炎陽性	カナダ	男	88歳	2001/4/20	不明	症例報告	外国製品	2003/12/26、3-03000016
	2-2	臨床検査	C型肝炎陽性	カナダ	男	29歳	2000/2/14	不明	症例報告	外国製品	2004/01/13、3-03000002
	2-3	臨床検査	C型肝炎陽性	カナダ	男	不明	1995/12/13	不明	症例報告	外国製品	2004/01/14、3-03000001
	2-4	臨床検査	C型肝炎陽性	カナダ	女	不明	1991/9	不明	症例報告	外国製品	2003/12/26、3-03000015
	2-5	臨床検査	C型肝炎陽性	カナダ	男	不明	不明	不明	症例報告	外国製品	2003/10/07、D03-00050
	2-6	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2003/10/07、D03-00049
	2-7	臨床検査	C型肝炎陽性	カナダ	男	不明	不明	不明	症例報告	外国製品	2003/10/07、D03-00048
	2-8	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2003/11/13、3-03000003
	2-9	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2003/12/24、3-03000009
	2-10	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2004/02/26、3-03000023
	2-11	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2004/02/26、3-03000024
	2-12	臨床検査	C型肝炎陽性	カナダ	男	不明	不明	不明	症例報告	外国製品	2004/02/26、3-03000025
	2-13	臨床検査	C型肝炎陽性	カナダ	不明	不明	不明	不明	症例報告	外国製品	2004/02/25、3-03000027
	2-14	臨床検査	C型肝炎陽性	カナダ	男	不明	2003/5	不明	症例報告	外国製品	2003/09/17、D03-00045
	2-15	臨床検査	C型肝炎陽性	カナダ	男	不明	不明	不明	症例報告	外国製品	2003/09/17、D03-00046

MedDRAバージョン10.1により集計した。

第10回症例番号9-1は，第9回症例番号9-1において報告したものの追加報告。

第8回調査期間中に当該生物由来製品によると疑われる感染症の報告はなかった(初回および追加報告ともに)。

第6回症例番号5-2は，第5回症例番号5-2において報告したものの追加報告。

第4回症例番号3-35, 3-6, 3-8, 3-34, 3-36は，それぞれ第3回症例番号3-35, 3-6, 3-8, 3-34, 3-36において報告したものの追加報告。

第3回症例番号2-2, 2-3, 2-8, 2-10, 2-11, 2-12, 2-13は，それぞれ第2回症例番号2-2, 2-3, 2-8, 2-10, 2-11, 2-12, 2-13において報告したものの追加報告。

第1回調査期間中に当該生物由来製品によると疑われる感染症の報告はなかった(初回および追加報告ともに)。

80079	2008/04/25	バイエル薬品	①人血清アルブミン ②オクトコグ　アルファ(遺伝子組換え)	人血清アルブミン

別紙様式第4

別紙(5)

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10回	1	感染症および寄生虫症	B型肝炎	ドイツ	男	24	2007/12/21	不明	症例報告	外国製品	識別番号3-07000026 完了報告提出日2008年2月19日
	2	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 未完了報告提出日2007年9月21日
	2	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 完了報告提出日2007年10月11日
第9回		報告なし									

80082	2008/04/25	CSLベーリング	フィブリノゲン加第XIII因子	アンチトロンビン

別紙様式第4

別紙(5)

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 未完了報告提出日2007年9月21日
	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 完了報告提出日2007年10月11日
第9回		報告なし									

80083	2008/04/25	CSLベーリング	フィブリノゲン加第XIII因子	トロンビン末

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 未完了報告提出日2007年9月21日
	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 完了報告提出日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 提出日2007年7月24日
	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 追加報告提出日2007年7月26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 追加報告提出日2007年7月26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 追加報告提出日2007年7月26日
	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 追加報告提出日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 追加報告提出日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 追加報告提出日2007年8月6日

80086	2008/04/25	CSLベーリング	フィブリノゲン加第XIII因子人血液凝固第XIII因子	人血液凝固第XIII因子

別紙様式第4

別紙(5)

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 未完了報告提出日2007年9月21日
	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 完了報告提出日2007年10月11日
第9回		報告なし									

80087	2008/04/25	CSLベーリング	フィブリノゲン加第XIII因子	フィブリノゲン	

別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回	10-1	感染症および寄生虫症	Ｂ型肝炎	ドイツ	男性	24歳	2008/1/10	不明	症例報告	外国製品	識別番号：07000029 報告日：2008年2月28日 MedDRA: Version(10.1)
	10-2	臨床検査	Ｃ型肝炎陽性	日本	女性	78歳	2008/2	不明	症例報告	当該製品	識別番号：07000245 報告日：2008年3月5日 MedDRA: Version(10.1)
	10-2	臨床検査	Ｃ型肝炎陽性	日本	女性	78歳	2008/12	不明	症例報告	当該製品	識別番号：07000245 報告日：2008年3月21日 2008年3月5日に報告したもの追加報告 MedDRA: Version(10.1)
	10-3	臨床検査	Ｃ型肝炎陽性	日本	女性	64歳	1992/7	未回復	症例報告	当該製品	識別番号：07000252 報告日：2008年3月12日 MedDRA: Version(10.1)
	10-4	感染症および寄生虫症	Ｃ型肝炎	ドイツ	男性	44歳	2007/10/29	未回復	症例報告	外国製品	識別番号：07000032 報告日：2008年3月21日 MedDRA: Version(11.0)
第9回	9-1	臨床検査	Ｃ型肝炎陽性	日本	男性	58歳	2007/5/31	未回復	症例報告	当該製品	識別番号：07000048 報告日：2007年6月29日 MedDRA: Version(10.0)
	9-1	臨床検査	Ｃ型肝炎陽性	日本	男性	58歳	2007/5/31	未回復	症例報告	当該製品	識別番号：07000048 報告日：2007年8月29日 2007年6月29日に報告したもの追加報告 MedDRA: Version(10.0)
	9-1	臨床検査	Ｃ型肝炎陽性	日本	男性	58歳	2007/5/31	未回復	症例報告	当該製品	識別番号：07000048 報告日：2007年9月4日 2007年6月29日に報告したもの完了報告 MedDRA: Version(10.0)

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	転帰	出典	区分	備考
		器官別大分類	基本語				(年/月/日)				
第８回	該当なし										
第７回	7-1	臨床検査	Ｃ型肝炎ウイルス	米国	女性	不明	不明	不明	症例報告	当該製品	識別番号：06000021 報告日：2006年８月１日 MedDRA: Version(9.1)
第６回	該当なし										
第５回	該当なし										
第４回	4-1	感染症および寄生虫症	Ｃ型肝炎	日本	男性	68歳	2004/7/6	未回復	症例報告	当該製品	識別番号：04000186 報告日：2004年12月16日 MedDRA: Version(7.1)
第３回	1-1	臨床検査	Ｃ型肝炎抗体陽性	日本	女性	78歳	2003/4/2	未回復	症例報告	当該製品	識別番号：04000023 報告日：2004年４月27日 第１回症例番号1-1において報告したもの（未完了報告）の完了報告 MedDRA : Version(7.0)
第２回	該当なし										
第１回	1-1	臨床検査	Ｃ型肝炎抗体陽性	日本	女性	78歳	2003/4/2	不明	症例報告	当該製品	登録番号：A03-51 報告日：2003年８月８日 （未完了報告） MedDRA: Version(6.1)