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。

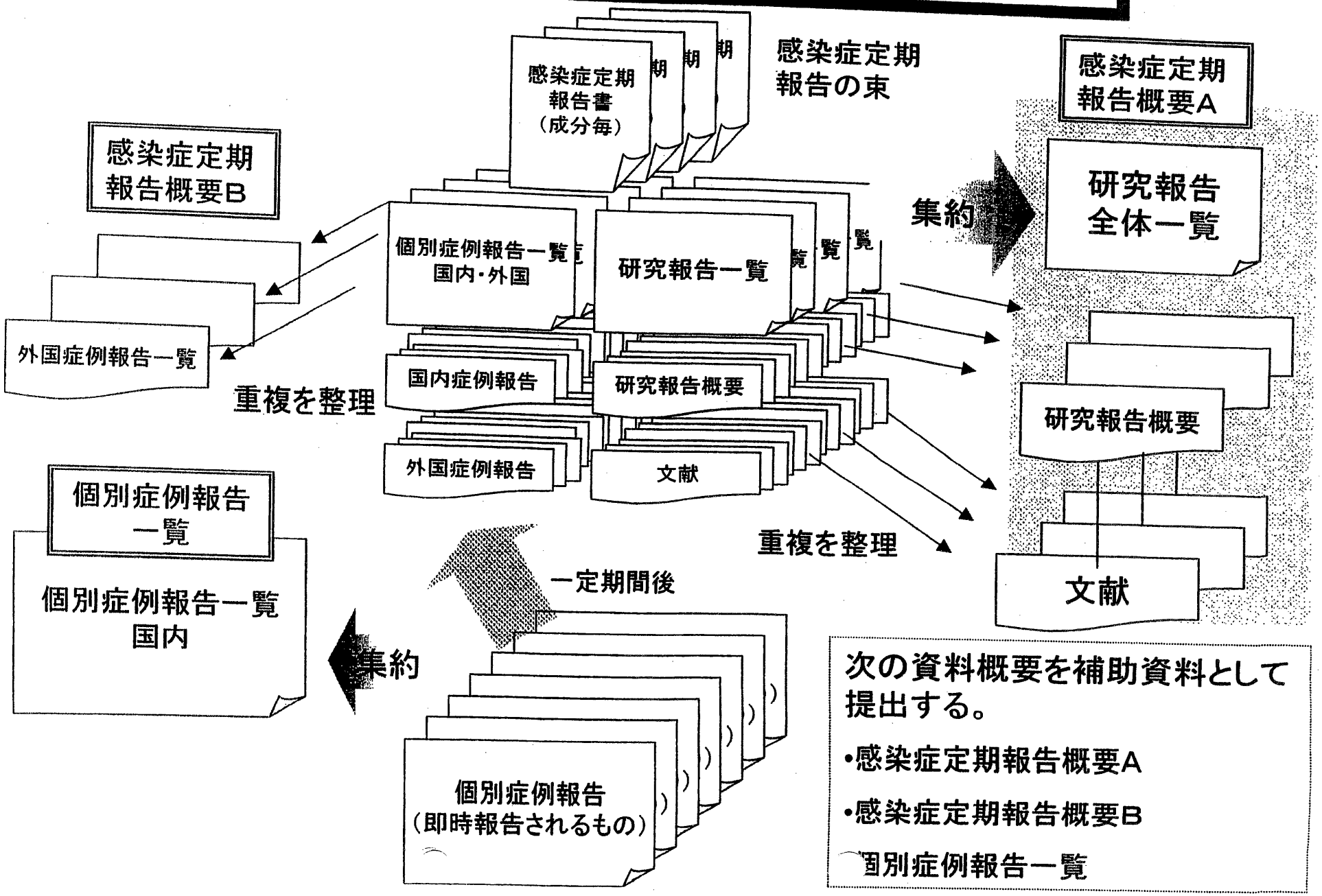
② 感染症発生症例報告のうち、発現国が「外国」の血漿分画製剤の使用による症例は、同一製品毎に報告期間を代表する感染症発生症例一覧（別紙様式第4）をまとめた**「資料概要B」**を事務局が作成し、送付する。

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２１年２月１０日）

平成２０年９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９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（2008/9/1～2008/11/30）

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
90013	2008/09/29	80516	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。	
90013	2008/09/29	80516	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症例数である。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。	
90030	2008/10/23	80632	B型肝炎	Clin Infect Dis 2008; 47: e52-56	2000年1月から2004年12月に日本で新たにB型肝炎表面抗原陽性となった患者を調査したところ、552名中23名(4%)がHBV再活性化で、529名が急性B型肝炎であった。再活性化群は急性B型肝炎群に比べ、年齢およびHBV DNA値が有意に高く、ALTおよびアルブミンピーク値は低かった。また再活性化群の4分の1の患者が劇症肝不全となり、死亡した。肝臓関連死亡率は再活性化群の方が有意に高かった。	1
90013	2008/09/29	80516	B型肝炎	FDA/CBER 2008年5月 業界向けガイダンス（案）	FDAはB型肝炎コア抗原に対する抗体(抗HBc抗体）が陽性となったために供血延期となった供血者のリエントリー・アルゴリズムを提案するガイダンス案を発表した。これまで、抗HBc抗体が2回以上陽性となった供血者は無期限に供血延期とされていたが、本ガイダンスでは2回目に陽性となった後、8週間以上経ってからHBs抗原、抗HBc抗体および高感度HBV NATによってHBV感染が否定された場合は供血可能となる。	2
90060	2008/11/25	80719	B型肝炎	J Hepatol 2008; 48: 1022-1025	スロヴェニアで、HBs抗原陰性で抗HBc抗体陽性、抗HBs抗体低力価陽性、HBV DNA陽性の濃厚赤血球と新鮮凍結血漿を輸血された59歳の患者が4ヶ月後に急性B型肝炎を発症した。また同じ供血血液由来のRCCの輸血を受けた71歳の患者も7ヶ月後にHBV感染を認めた。2例ともドナーと同じ配列を有するジェノタイプDが感染していた。潜在性B型肝炎ウイルス感染者の血液は抗HBs抗体が陽性にかかわらず、感染性を有した。	3
90007	2008/09/26	80498	B型肝炎	Transfusion 2008; 48: 286-294	最小感染量を求めるために、遺伝子型Aまたは遺伝子型CのHBVを含む急性期前の接種株をチンパンジーに接種したところ、最小50%チンパンジー感染量(CID50）は各々約10コピーと推定された。最低感染量を接種したチンパンジーにおけるHBV DNA ウィンドウ期は遺伝子型Aでは55-76日、遺伝子型Cでは35-50日、HBs Agウィンドウ期は遺伝子型Aでは69-97日、遺伝子型Cでは50-64日であった。またHBV DNAダブリングタイムは遺伝子型Cの方が遺伝子型Aに比べ有意に短かった。	

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
90013	2008/09/29	80516	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。	
90006	2008/09/26	80497	B型肝炎、E型肝炎	Veterinary Science in China 2007; 37: 921-925	中国の畜殺場から集めたブタの肝臓と血清からDNAを抽出し、PCRを使ってs遺伝子を増幅し配列決定を行った結果、ブタとヒトのHBVのS遺伝子の配列は98-100%の相同性を示した。また、RT-PCRによるHEV RNA検出を行った結果、HEV RNAがブタの肝臓に存在することが示された。	4
90013	2008/09/29	80516	B型肝炎C型肝炎	第56回日本輸血・細胞治療学会総会 2008年4月25-27日 P-033	2007年に医療機関から日本赤十字社に報告された輸血関連感染症の報告数は124例(10月末現在)であり、一昨年及び昨年の同期間に比べ減少傾向にある。内訳はHBVが61例、HCV32例、細菌24例、その他のウイルスが7例であった。ウイルス感染(疑)症例の調査結果により病原体を確認した症例は、HBVの12例とHCVの1例であった。HCVの1例は20プールNAT開始後(2004年8月開始)初めての検出限界以下の献血血液による感染症例であった。	
90030	2008/10/23	80632	C型肝炎	Clin Infect Dis 2008; 47: 627-633	フランスの大学病院の血液透析ユニットでのHCV伝播リスクにおける環境汚染および標準的注意の非遵守の役割を評価した。試験期間中にHCV陽性となった2名のうち1名は、同ユニットで治療中の慢性感染患者と同じウイルス株に感染していることが系統遺伝学的解析により明らかとなった。環境表面検体740例中82例がヘモグロビンを含み、その内6例がHCV RNAを含んでいた。手の衛生に関する遵守率は37%、患者ケアの直後に手袋をはずしていたのは33%であった。	5
90013	2008/09/29	80516	E型肝炎	Am J Trop Med Hyg 2008; 78: 1012-1015	スペインでブタに曝露しているヒト101名と曝露していないヒト97名におけるHEV感染の有無を調べた。抗HEV IgG保有率は曝露群では18.8%、非曝露群では4.1%であった。ブタに接するヒトの抗HEV IgG保有リスクは5.4倍(P=0.03)であった。HEV感染は養豚作業員の職業病として扱うべきである。	6
90007	2008/09/26	80498	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。	

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
90060	2008/11/25	80719	E型肝炎	Transfusion 2008; 48: 1368-1375	2004年9月20日に39歳日本人男性から献血された血液はALT高値のため不適当とされ、HEV陽性であった。当該ドナーの遡及調査の結果、9月6日にも献血を行い、HEV RNAを含有する血小板が輸血されていた。当該ドナーと親戚は8月14日にブタの焼肉を食べており、父親は9月14日に急性肝炎を発症し、E型劇症肝炎で死亡した。他に7名がHEV陽性であった。レシピエントは輸血22日目にALTが上昇し、HEVが検出された。	7
90040	2008/10/24	80645	E型肝炎	Vox Sanguinis 2008; 95(Suppl.1): 282-283	2005年の中国の4都市(Beijing、Urmuchi、KunmingおよびGuangzhou)における供血検体のHEV感染率を調べた。その結果、ルーチン検査(抗HCV、抗HIV1/2、HBsAg、梅毒およびALT)陰性供血者の約1%は抗HEV IgMまたはHEV Ag陽性で、HEV感染の可能性があった。また、ALTスクリーニングは中国のHEV感染血排除に役立つ可能性があった。	8
90013	2008/09/29	80516	E型肝炎	第56回日本輸血・細胞治療学会総会 2008年4月25-27日 O-026	北海道地区において現行プールNATスクリーニングの残量を用いてTaqMan RT-PCR法によるHEV NATスクリーニングを行った。陽性献血者85例について追跡調査および遡及調査などを行なった。陽性献血者の多くは動物内臓肉を食してHEVに感染したと考えられる新規感染者で、GenotypeはG3が多かった。多くは症状が現れないまま抗体が陽転化し、典型的な無症候性一過性感染の経過をたどった。	
90014	2008/09/29	80517	HIV	ABC Newsletter 2008; No.26 2008年7月4日	米国医師会(AMA)は、男性同性愛行為を行った男性(MSM)の供血延期期間を生涯としている連邦の方針を5年間に変更することを支持するという声明を採択した。AMAはこの新方針をFDAに通告し、この方針を推し進めるグループと協力していく。FDAは1977年以降、MSMの供血を生涯延期することを血液事業者に要求しているが、アメリカ血液センターなどからは反対意見が出されている。	9
90013	2008/09/29	80516	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。	
90014	2008/09/29	80517	アメリカ・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。	

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
90014	2008/09/29	80517	アメリカ・トリパノソーマ症	Vox Sanguinis 2008; 95(Suppl.1): 39	米国で全供血者を対象にしたTripanosoma Cruzi検査が導入された2007年1月30日以降、最初の10ヶ月間、供血者の調査を行った。適合供血のうちELISA法で反復陽性(RR)となったのは0.013%(90/651471)で、そのうちRIPA陽性は34%(28/82)で、陽性確認率は0.0043%であった。全供血のスクリーニングは費用対効果が低く、出生地と初回供血者に絞った対策の検討が示唆された。	10
90013	2008/09/29	80516	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。	
90013	2008/09/29	80516	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。	
90013	2008/09/29	80516	インフルエンザ	Vox Sanguinis 2008; 95(Suppl. 1): 40	米国におけるパンデミックインフルエンザの血液供給に対する影響をシミュレーションした。3ヶ月間の血液供血量が50%減少した場合、血液需要に制限がない場合は在庫のほとんどを使い尽くしたが、血液の使用を必要最低限に制限した場合は在庫がなくなることはなかった。	11
90018	2008/09/29	80523	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。	
90007	2008/09/26	80498	ウイルス感染	Emerg Infect Dis 2008; 14: 834-836	カナダにおいて、Saffoldウイルスに関連するカルジオウイルス分離株が呼吸器症状を有する3名の小児の鼻咽頭吸引物から検出された。Can112051-06分離株のポリプロテイン配列は、Saffoldウイルスと91.2%のアミノ酸同一性を有した。しかし、ウイルス表面のEF及びCDのループは、かなり異なっていた。	
90045	2008/10/29	80668	ウイルス感染	Lancet Infect Dis 2008; 8: 355	ボリビア、ペルーおよび米国CDCのチームはボリビアの出血熱の致死症例から新規のアレナウイルスを発見し、Chapare virusと名付けられた。また、ウガンダでは赤オナガザルで新型ポックスウイルスの可能性があるウイルスが発見された。これら野生動物のウイルスがヒトへの感染能を獲得し、重篤な疾患を引き起こす可能性がある。	12

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
90003	2008/09/26	80493	ウイルス感染	PLoS Pathogens 2008; 4: e1000047	出血熱症例の小さな流行が、2003年12月と2004年1月にボリビアのCochabamba付近で発生した。1死亡例から検体を入手し、患者血清検体から非細胞障害性ウイルスを単離し、アレナウイルスと同定した。RT-PCR分析、並びにS及びL RNAセグメント配列の解析の結果、このウイルスはサビアウイルスに最も近縁であるが、新規のウイルスであることが示された。我々はこのウイルスをChapareウイルスと命名することを提案する。	
90052	2008/11/14	80708	ウイルス感染	Proc Natl Acad Sci USA 2008; 105: 14124-14129	インフルエンザ様疾患の小児の呼吸分泌物中から、汎ウイルスマイクロアレイ法を用いて、初めてヒトカルディオウイルスを同定した。系統遺伝学的分析から、このウイルスはTheilerのネズミ脳脊髄炎ウイルス亜型に属し、Saffoldウイルスと最も近縁であった。また、胃腸疾患患者群498名から得た751例の糞便検体中6検体からカルディオウイルスが検出された。	13
90013	2008/09/29	80516	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。	
90013	2008/09/29	80516	ウイルス感染	ProMED-mail20080720.2201	オーストラリアBrisbaneの動物病院のスタッフが致死性のヘンドラウイルスに感染した。看護師1名と獣医1名が、感染したウマ数頭を治療後、感染した。前回のアウトブレイクは1994年で調教師1名とウマ14頭が死亡した。同ウイルスがヒト-ヒト感染するとのエビデンスはなく、拡大する危険性はない。	14
90007	2008/09/26	80498	ウイルス感染	Transfusion 2008; 48: 1180-1187	米国テキサス南東部の健康な成人ドナー100名の血液中のヒトヘルペスウイルス(HHV)陽性率とウイルスDNA量をRT-PCRにより調べた。その結果、HSV-1、HSV-2、VZV及びHHV-8 DNAはどの検体からも検出されなかった。一方、EBVは72%、HHV-7は65%、HHV-6は30%、CMVは1%に検出された。また、1名の血液から6.1×10^7geq/mlを超えるHHV-6 Type Bが検出されたが、健常者における異常な高値は活動性感染や免疫不全とは関連が無いと思われる。	15

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
90054	2008/11/14	80710	ウイルス感染	WHO/EPR 2008年10月13日	南アフリカとザンビア出身者の最近の死亡例3例はアレナウイルス科のウイルスが原因あることが、NICDおよびCDCで行われた検査の結果明らかとなった。このウイルスに関する詳細な分析が継続されている。一方、南アフリカでは患者と密接に接触した看護師が感染し、入院中である。	16
90036	2008/10/24	80638	ウイルス性脳炎	ProMED-mail20080828.2697	インド東部のウッタルプラデシ州で小児を死亡させている原因不明のウイルスは、インド保健省の専門家らにより急性脳炎症候群と診断された。同州の13の地区では、数週間におよそ800人の患者が発生し150人が死亡したと報告され、その数は増加すると見られている。血液検査で日本脳炎陽性となった患者は5%以下であった。日本脳炎とエンテロウイルスとの混合感染の可能性について調査中である。	17
90018	2008/09/29	80523	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。	
90013	2008/09/29	80516	ウエストナイルウイルス	Rev Panam Salud Publica 2006; 19: 112-117	文献および未発表データから、ラテンアメリカやカリブ海地域のウエストナイルウイルス(WNV)感染の現状をまとめた。WNV感染は2001年にCayman諸島とFlorida Keysの住民で見られ、2002～2004年にジャマイカ、メキシコなど周辺地域で動物や鳥類での感染が確認されている。しかし、疾患報告数は少ない。この不可解な熱帯生態系でのウイルス減弱または他の可能性を検討するためには分離株が必要である。	18
90013	2008/09/29	80516	コンゴ・クリミア出血熱	ProMED-mail20080709.2092	2008年7月7日、トルコのBursa、CanakkaleおよびSamsunの病院でダニ媒介性疾患であるクリミア・コンゴ出血熱により3名が死亡し、この2ヶ月での死者数は37名となった。保健省はダニに注意するよう呼びかけ、咬まれた場合は決して手でつぶさずに、医師にピンセットで注意深く取り除いてもらい、ヨードで消毒することを推奨している。	19
90014	2008/09/29	80517	サルモネラ	CDC 2008年7月8日	CDCは関係機関と協力して複数の州で発生したサルモネラ血清型セントポールのアウトブレイクを調査している。生のトマトの摂食が原因と考えられている。2008年4月以降2008年7月7日までに、米国の41の州、ワシントンD.C.およびカナダで991名の患者が同じ遺伝子パターンのサルモネラ血清型セントポールに感染したことが確認された。	20

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
90040	2008/10/24	80645	チクングニヤウイルス感染	Transfusion 2008; 48: 1333-1341	2005年から2007年に、チクングニヤウィルス(CHIKV)はレユニオン島で大流行し、供血は2006年1月に中断された。大流行中のウイルス血症血供血の平均リスクは、10万供血あたり132と推定された。2006年2月の最流行時におけるリスクは、10万供血あたり1500と最高であった。この期間中、757000人の住民のうち推定312500人が感染した。2006年1月から5月の平均推定リスク(0.7%)は、CHIKV NAT検査による血小板供血のリスク(0.4%)と同じオーダーであった。	21
90007	2008/09/26	80498	手足口病	WHO Represetative Office in China 2008年5月19日	2008年3月下旬、中国Anhui省Fuyang市で未就学児3名が重症の肺炎と急激な悪化により死亡し、4月中旬までに15名の小児が同様の疾患で死亡した。調査の結果、エンテロウイルス71による手足口病と確定された。同市では、3月1日から5月9日の間に、6,049例報告され、353例が重篤で、22例が死亡した(致死率0.4%)。患者数は、4月の初めに増加し始めて、4月28日にピークに達し、5月5日以後減少した。	
90013	2008/09/29	80516	デング熱	Hong Kong Med J 2008; 14: 170-177	1998～2005年に香港の公立病院に入院したデング確定患者全員の医療記録をレトロスペクティブに検討した。126例中123例(98%)がデング熱、3例(2%)がデング出血熱であった。1例が輸血により感染したデング熱であった。116例が輸入症例、10例が地域症例であった。デングウイルス1型が最も多く、次に2型、3型、4型の順であった。死亡例はなかった。発熱、皮疹を呈し、血小板減少などを示す渡航歴のある患者には鑑別診断にデング熱を含めるべきである。	22
90020	2008/09/29	80525	デング熱	Transfusion 2008; 48: 1342-1347	高力価の培養デングウイルス　セロタイプ2をアルブミンおよび免疫グロブリンの各種製造工程(低温エタノール分画、陽イオン交換クロマトグラフィー、低温殺菌、S/D処理およびウイルスろ過)前の検体に加え、各工程での同ウイルスのクリアランスをVero E6細胞培養におけるTCID50アッセイおよびRT-PCRで測定した。その結果、全ての工程が不活化・除去に有効であることが示された。	23
90040	2008/10/24	80645	デング熱	Transfusion 2008; 48: 1348-1354	2005年9月20日～12月4日のプエルトルコの米国赤十字におけるすべての供血16521検体中のデングウイルス(DENV) RNAをTMA(transcription-mediated amplification)法で測定したところ、12検体(0.07%)がTMA陽性であった。4検体は、RT-PCR(DENVセロタイプ2および3)陽性であった。RT-PCR陽性4検体中3検体でウイルスを培養することができた。TMA陽性12検体中1検体がIgM陽性であった。1:16に希釈した場合は5検体のみTMA陽性であった	24

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
90045	2008/10/29	80668	鳥インフルエンザ	N Engl J Med 2008; 358: 2573-2584	細胞培養で製造したH5N1鳥インフルエンザワクチンのPhase 1および2試験を行った。その結果、大多数の被験者においてアジュバントなしの7.5μgまたは15μgのヘマグルチニン抗原を含有するワクチンの2回接種が、様々なH5N1ウイルス株に対するする中和抗体の合成を誘発することが示された。注射部位での軽度の痛みと頭痛が主な有害反応であった。このワクチンが有用であることが示唆された。	25
90018	2008/09/29	80523	鳥インフルエンザ	Proc Natl Acad Sci USA 2008; 105: 7558-7563	ユーラシアおよび北米系統のH7型トリインフルエンザウイルスの受容体結合能およびフェレットモデルにおける感染性を調べた。その結果、2004年にカナダで分離されたH7N3型、2002-2003年に米国北東部で分離されたH7N2型はα2-6結合シアル酸に対する親和性を高めたHAを保有していた。また2003年にニューヨークの男性から分離された低病原性H7N2型はフェレットの上気道で効率的に増殖し、直接接触で感染できることが確認された。	
90014	2008/09/29	80517	バベシア症	American Society for Microbiology 108th General Meeting 2008年6月1-5日、Boston	米国中南部では稀な輸血によると考えられるBabesia microti感染症例の報告である。61歳の女性患者で、赤血球輸血後、吐き気と発熱を訴え、敗血症の症状を呈し、死亡した。血液塗抹標本で赤血球の5～15%にトロフォゾイト(栄養体)があった。患者血液検体中でBabesiaは形態学的に確認され、PCRでB. microti陽性であった。輸血された製剤の供血者のうち1名がB. microti陽性であった。	
90018	2008/09/29	80523	バベシア症	Blood 2007; 110(11, Part 1): 853	米国コネチカット州での輸血によるBabesia microti感染の危険性を評価するため、2004-2007年に収集されたドナーとレシピエントの保存検体を検査した。その結果、45回の赤血球輸血を受けていた患者1例でBabesia microti感染が確認されたが、血清検体陽性の ドナーを特定することはできなかった。危険性は1920回の赤血球輸血で0例または1例と計算された。	
90045	2008/10/29	80668	パルボウィルス	Biotechnol Prog 2008; 24: 554-560	レジン上に3量体ペプチドを結合し、PBSまたは血漿溶液に添加したブタパルボウイルス(PPV)への選択的な結合能を有するペプチドをスクリーニングした。その結果、WRW(トリプトファン-アルギニン-トリプトファン)結合レジンは7.5%ヒト血漿中のPPVを検出限界以下に除去することができた。	26
90003	2008/09/26	80493	パルボウィルス	FDA/CBER 2008年7月 業界向けガイダンス(案)	血漿由来製品によるパルボウイルスB19伝播リスクを低減するための核酸増幅検査(NAT)についてのガイダンス案が示された。全ての血漿由来製剤について、製造プール中のパルボウイルスB19 DNAのウイルス負荷を確実に10000 IU/ml未満とするため、製造過程の品質管理検査としてNATを実施すべきである。ミニプール中でのNATの感度は少なくとも1000000 IU/mlとするべきである。これらの基準を超えるものは使用してはならない。	27

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
90013	2008/09/29	80516	パルボウィルス	Transfusion 2008; 48: 1036-1037	大阪における1997-1999年の献血者979052名中102名がヒトパルボウイルスB19感染者であった。B19感染者のうち20名のB19 DNA、IgGおよびIgMを長期間フォローアップしたところ、B19持続感染が観察されたが、B19感染の症状を報告した者はいなかった。B19急性感染後の血漿ウイルス力価は約1年で10^4IU/mL未満、約2年で10^3IU/mL未満まで下がることが示された。	
90045	2008/10/29	80668	パルボウィルス	Transfusion in press	3つの血液凝固因子製剤(第VIII因子インヒビター-バイパス活性、第IX因子複合体および第VII因子)の製造工程においてSTIM-4蒸気加熱処理を用いた不活性化処理を行い、ヒトパルボウイルスB19(B19V)とマウス微小ウイルス(MMV)間で不活性化効果の比較を行った。その結果、血液凝固因子製剤の中間体の種類に関わらず、試験に用いたB19V(遺伝子型1型、2型)はいずれもMMVと比較して効果的に不活性化された。	
90013	2008/09/29	80516	ハンタウイルス	Emerg Infect Dis 2008; 14: 808-810	スウェーデンにおけるPuumalaウイルスの予期せぬ大規模アウトブレイクにより、2007年のVästerbotten地方の流行性腎症患者の数は100,000人当り313人に至った。齧歯類の増加の他、気候温暖化および地表を覆う積雪の減少により、ウイルスを媒介するハタネズミの活動が活発だったことが、当該アウトブレイクの一因であろうと考えられる。	
90007	2008/09/26	80498	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。	
90014	2008/09/29	80517	ブルセラ症	Clin Infect Dis 2008; 46: e131-136	急性ブルセラ症患者39名の血液検体中のBrucella DNAの存在をRT PCR法により調べた。その結果、治療終了時では87%、治療完了後6ヶ月では77%、治療後2年を過ぎても70%の患者で、無症候性であるにもかかわらず、Brucella DNAが検出された。適切な治療を行い、回復したように見えても、Brucella DNAは存続する。ブルセラ菌は除去不可能な持続性の病原体である。	28
90014	2008/09/29	80517	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。	

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
90014	2008/09/29	80517	リケッチア症	Emerg Infect Dis 2008; 14: 1019-1023	ネコノミが媒介するRickettsia felis感染症のヒト症例は世界中で報告されている。症状は発疹熱やデング熱などに類似しており、実際よりも少なく推定されている可能性が高い。ヒトの健康を脅かす感染症として今後調査が必要である。	29
90010	2008/09/26	80501	リケッチア症	ProMED-mail20080728.2306	オランダ・ブラバント州の公衆衛生局が行った調査でQ熱の症例報告数が急激に増加し、2008年7月21日付けで491症例が報告されている。感染症管理センター長によると、実際の感染者数は報告された症例数の10倍であると思われる。2007年まではQ熱はオランダではほとんど存在しなかった。	30
90003	2008/09/26	80493	リンパ性脈絡髄膜炎	boston.com 2008年5月13日	2008年5月12日の保健当局発表によると、ボストンの病院で検出が難しいウイルスに感染したドナーから腎臓を移植された70歳女性が死亡し、57歳男性が危篤である。ドナーと患者2名の検体をCDCが検査したところ、全員、リンパ球性脈絡髄膜炎ウイルス(LCMV)陽性であり、ドナーからの伝播であったことが確認された。移植前にはエイズウイルス、肝炎ウイルスなどの検査は行ったが、LCMVの検査は行っていなかった。	
90003	2008/09/26	80493	リンパ性脈絡髄膜炎	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、リンパ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。	
90007	2008/09/26	80498	リンパ性脈絡髄膜炎	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、リンパ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。	
90010	2008/09/26	80501	レプトスピラ症	Infect Genet Evol 2008, doi:10.1016	コスタリカにおいて、レプトスピラ症の入院患者から分離されたレプトスピラは、Javanica血清群型に分類される新しい血清型で、Arenalと命名された。同じ地区の重症患者から分離された株も同じ血清型であったことから、この株は、この地域に流行する新規の高病原性の血清型であると考えられた。	

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
90036	2008/10/24	80638	レプトスピラ症	Infect Genet Evol 2008; 8: 529-533	コスタリカにおいて、レプトスピラ症の入院患者から分離されたレプトスピラは、Javanica血清群型に分類される新しい血清型で、Arenalと命名された。同じ地区の重症患者から分離された株も同じ血清型であったことから、この株は、この地域に流行する新規の高病原性の血清型であると考えられた。	
90029	2008/10/21	80614	異型クロイツフェルト・ヤコブ病	American Society of Hematology/Press Releases 2008年8月28日	Blood誌のprepublished onlineに掲載されたヒツジにおける研究によると、輸血によるBSE伝播のリスクは驚くほど高い。エジンバラ大学で行われた9年間の研究は、BSEまたはスクレイピーに感染したヒツジからの輸血による疾病伝播率を比較した。その結果、BSEおよびスクレイピーとも輸血によりヒツジに効率よく伝播された。症状を呈する前のドナーから採取された血液によっても伝播することが示された。	31
90007	2008/09/26	80498	異型クロイツフェルト・ヤコブ病	Ann Neurol 2008; 63: 697-708	米国の国立プリオン病病因調査センターに2002年5月から2006年1月に紹介された患者11名(平均発症年齢62歳)を調べたところ、海綿状変性の型、PrP免疫染色パターンおよびマイクロプラークの存在が、既知のプリオン病とは異なり、通常の方法では典型的なプロテアーゼ抵抗性PrPは検出されなかった。我々はこれらをプロテアーゼ感受性プリオン病(PSPr)と名付けた。PSPrは、プリオン病の中では稀ではなく、我々のデータが示すよりもさらに多い可能性がある。	32
90055	2008/11/19	80714	異型クロイツフェルト・ヤコブ病	Cell 2008; 134: 757-768	マウスPrPScと混合させることによって折り畳み異常が起こったハムスターPrPCは、野生型ハムスターに対して感染性を起こす新規なプリオンを生成した。同様の結果は、反対方向でも得られた。PMCA増幅を繰り返すとin vitro産生プリオンの順応が起こる。このプロセスは、in vivoでの連続継代に観察される株の安定化を暗示させる。種の壁と株の生成がPrP折り畳み異常の伝播によって決定されることが示唆される。	33
90045	2008/10/29	80668	異型クロイツフェルト・ヤコブ病	dailypress.com 2008年4月11日	米国Portsmouthで、脳変性疾患を呈し死亡した女性の死因を、vCJD疑いのため調査中である。MRIまたは脳スキャンの結果がアトランタの疾病対策センターに送付され、バージニア大学および国立プリオン病病因サーベイランスセンターで更に検査される。結果が出るまでには数ヶ月を要すると思われる。	
90030	2008/10/23	80632	異型クロイツフェルト・ヤコブ病	Emerg Infect Dis 2008; 14: 1406-1412	263Kスクレイピーの臨床症状を呈するハムスター22匹の尿にTSE感染性があることが示された。これらの動物の腎臓と膀胱のホモジネートは20000倍以上希釈してもTSE感染性があった。組織学的、免疫組織化学的分析では、腎臓における疾患関連PrPの散発的な沈着以外、炎症や病変は見られなかった。尿中のTSE感染性が、自然のTSEの水平感染に何らかの役割を果たす可能性がある。	34

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
90013	2008/09/29	80516	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。	
90045	2008/10/29	80668	異型クロイツフェルト・ヤコブ病	Medgadget.com 2008年4月9日	カナダQuebecのProMetic Life Science社は血液中のvCJDプリオンを除去する使い捨てフィルターを開発した。何百万ものペプチドをスクリーニングし、プリオンに最も親和性のあるものを探し、市販の樹脂に固定し、膜状にし、何層にも重ねた。本フィルターは汚染血液からのプリオン除去が可能であった。また、フィルターで処理したプリオン感染ハムスターの血液をプリオン非感染ハムスターに投与しても疾患は発現しなかった。	
90013	2008/09/29	80516	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。	
90030	2008/10/23	80632	異型クロイツフェルト・ヤコブ病	PLoS ONE 2008; 3: e2878	野生型マウスおよびヒトPrPを発現しているトランスジェニックマウスに、輸血関連vCJD感染第1号症例由来の脳材料を接種し、輸血によるヒト-ヒト間の2次感染後のvCJD病原体の性質について調べた。その結果、潜伏期間、臨床症状、神経病理学的特徴およびPrP型について、vCJD(輸血)接種群はvCJD(BSE)接種群と類似していた。vCJD病原体は、ヒトにおける2次感染により、有意な変化が起こらないことが明らかとなった。	35
90055	2008/11/19	80714	異型クロイツフェルト・ヤコブ病	PLoS ONE 2008; 3: e3017	非定型BSE(BASE)に感染した無症候のイタリアの乳牛の脳ホモジネートをカニクイザルに脳内接種した。BASE接種サルは生存期間が短く、古典的BSEまたはvCJD接種サルとは異なる臨床的展開、組織変化、PrPresパターンを示した。感染牛と同じ国の孤発性CJD患者でPrPが異常なウエスタンブロットを示す4例のうち3例のPrPresに同じ生化学的特徴を認めた。BASEの霊長類における高い病原性および見かけ上孤発性CJDである症例との関連の可能性が示唆された。	36

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
90007	2008/09/26	80498	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。	
90007	2008/09/26	80498	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、更なる調査が必要である。	
90045	2008/10/29	80668	感染	Transfusion 2008; 48: 1739-1753	最近米国で承認された2つのフィブリノゲンおよびトロンビンについて、HIV、HCV、HBV、HAV、パルボウイルスB19およびvCJDに関する病原体感染リスクを評価した。これら血液製剤の製造過程では2つの異なった不活化および除去工程が使われている。全製剤とも1バイアル当たりの残存する病原体感染リスクは極めて低いことが示された。	37
90014	2008/09/29	80517	感染	Transfusion 2008; 48: 304-313	血小板濃厚液におけるUVC照射の病原体不活化能を検討した。UVC照射は、血小板の品質に影響を及ぼさずに、細菌(表皮ブドウ球菌、黄色ブドウ球菌および大腸菌)ならびに伝播性胃腸炎ウイルスなど広範なウイルス(HIVおよびシミアンウイルス40を除く)を不活化することができた。しかし、HIVのような血液感染性ウイルスに対応するには、UVC法をさらに最適化することが必要である。	
90014	2008/09/29	80517	感染	Transfusion 2008; 48: 697-705	欧州の3つの血液センターにおけるアモトサレンおよびUVAによるフォトケミカル処理(PCT)過程のプロセスバリデーション試験を行った。フィブリノーゲンおよび第VIII因子はPCTにより平均26%減少したが、治療用血漿として十分なレベルを保持していた。他の凝固因子は対照FFPのレベルの81-97%であった。PCT処理済FFP中の凝固因子が治療用血漿に関する欧州規制および国内基準の範囲内に保持されることが示された。	
90014	2008/09/29	80517	感染	Vox Sanguinis 2008; 94: 315-323	アモトサレンと紫外線A波で光化学処理した血小板(PCT-PLT)の輸血に関連する有害事象を調べるために能動的血液安全監視プログラムを実施した。患者1400名に7437件のPCT-PLTが輸血され、その内、68件が有害事象と関連付けられた。PCT-PLT輸血に関連した急性輸血反応は発現頻度が低く、ほとんどが軽度であった。	

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
90014	2008/09/29	80517	感染	Vox Sanguinis 2008; 95(Suppl. 1), 2A-S01-02	化学的または光化学的遺伝子修飾に基づいた血液製剤中の病原体不活化(PI)は広範囲のスペクトルの予防的アプローチである。溶媒界面活性剤(SD)およびメチレンブルー法は欧州の多くの国で使われている。アモトサレン(Intercept)、リボフラビンを用いた新しい方法が導入されている。リボフラビン、UVおよび可視光線を用いる血小板(PC)、血漿および赤血球のためのPI法が開発中である。	38
90040	2008/10/24	80645	狂犬病	ProMED-mail20080826.2660	1990年から2007年の中国における狂犬病発生傾向を調べた研究によると、最近8年間でヒト狂犬病症例数が急激に増加したことが明らかとなった。ヒト狂犬病は1990年から1996年の間は全国的な狂犬病ワクチン接種プログラムにより抑制され、わずか159症例が報告されただけであるが、2006年は3279症例と激増した。	39
90014	2008/09/29	80517	原虫感染	Emerg Infect Dis 2008; 14: 1013-1018	リーシュマニア症は生物媒介性疾患で、南ヨーロッパに定着しており、毎年700例近く、トルコを含めると3950例のヒトでの感染が報告されている。無症候症例は臨床症例の30～100倍とみられ、また飼い犬の血清陽性率は25%と推定される。薬剤耐性Leishmania infantumがイヌを介して拡大するおそれもある。全ヨーロッパレベルでの研究が必要である。	40
90013	2008/09/29	80516	細菌感染	American Society for Microbiology 108th General Meeting 2008年6月1-5日	マサチューセッツの医療センターで品質管理のため使用された廃棄製剤、使用期限切れロット、アフェレーシスの残りの人血清アルブミン製剤を入手し、クラミジアの有無を調べた。その結果、PCR及びウエスタンブロットにより、4社の20製剤全てにおいてクラミジアの存在が確認された。また、in vitro培養を行ったところ11検体(55%)でクラミジア生菌が生育した。	41
90030	2008/10/23	80632	細菌感染	Transfusion 2008; 48: 1520-1521	骨髄異形成症候群と汎血球減少症の79歳男性が、血小板輸血と続いて赤血球1単位の輸血を受けた。 40分後に39.6℃の発熱、硬直、背部痛、低血圧および低酸素症を呈し、輸血は中止された。患者は抗菌剤による治療で回復した。患者の血液および赤血球バッグの残存物からStreptococcus pneumoniae血清型4が検出された。赤血球輸血によるS pneumoniae感染の初めての症例である。	42
90014	2008/09/29	80517	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。	

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
90014	2008/09/29	80517	細菌感染	第56回 日本輸血・細胞治療学会総会 2008年4月25-27日 WS-3-3	血小板濃厚液の輸血後に、TRALI様の急性呼吸不全と髄膜炎を併発し、血小板残液からBacillus cereusが検出された症例の報告である。TRALI様の急性呼吸不全を呈した際は、輸血後感染症も視野に入れた対応が必要である。髄膜炎併発例の報告はこれまでに無いが、輸血後感染症治療では髄液移行性も考慮した抗生剤選択が求められる。培養検査だけでなく、遺伝子検査まで施行することが、診断及び同一菌株の証明に重要である。	
90040	2008/10/24	80645	手足口病	WHO Represetative Office in China 2008年5月19日	2008年3月下旬、中国Anhui省Fuyang市で未就学児3名が重症の肺炎と急激な悪化により死亡し、4月中旬までに15名の小児が同様の疾患で死亡した。調査の結果、エンテロウイルス71による手足口病と確定された。同市では、3月1日から5月9日の間に、6,049例報告され、353例が重篤で、22例が死亡した(致死率0.4%)。患者数は、4月の初めに増加し始めて、4月28日にピークに達し、5月5日以後減少した。	
90014	2008/09/29	80517	梅毒	SignOnSanDiego.com 2008年3月26日	カリフォルニア州サンディエゴ郡の年間梅毒症例数は、最低となった2000年の28例から昨年(2007年)は340例まで急増した。州の他の大都市の郡と比べて非常に急激な増加である。増加率は州全体の2倍以上、全国の3倍以上になる。州から派遣された5名の専門家チームは、梅毒と診断された人々と連絡をとって、性的パートナーを探し、検査を受けるよう勧めている。	

別紙様式第2-1

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. 8. 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		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)		Umemura T, Tanaka E, Kiyosawa K, Kumada H; Japan de novo Hepatitis B Research Group. Clin Infect Dis. 2008 Sep 1;47(5):e52-6.	日本

研究報告の概要

○日本においてB型肝炎の治癒後再燃した患者の劇症肝不全による死亡率
本邦において、新たにB型肝炎表面抗原が陽性化した552名のうち、B型肝炎の治癒後に再燃した患者は23名(4%)であることが判明した。このうち5名が劇症肝不全を発症し、死亡率は100%だった。B型肝炎再燃患者の転帰は、6名(26%)が肝臓関連死、11名(48%)が感染消失、6名(26%)が慢性化した。急性感染の患者(529名)では490名(93%)が自然治癒、16名(3%)が慢性化、21名(4%)が死亡となり、B型肝炎再燃患者の死亡率が急性感染患者と比較して有意に高いことが示された。
B型肝炎再燃で劇症肝不全を発症した患者(5名)は、発症しなかった患者(18名)と比べて悪性リンパ腫の罹患率が高く、全員がリツキシマブを含む化学療法を受けていた。劇症肝不全を発症しなかった患者18名中16名がラミブジンの投与を受けていた。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
日本において、新たにB型肝炎表面抗原が陽性化した552名のうち、B型肝炎の治癒後に再燃した患者は23名(4%)で、急性感染患者と比較して劇症肝不全、慢性化、肝臓関連死に至る割合が高いことが判明したとの報告である。輸血後HBV感染症の調査では、化学療法などに伴うB型肝炎の再燃について考慮する必要がある。	日本赤十字社では、HBs抗原検査及びHBc抗体検査を実施することに加えて、HBVについて20プールでスクリーニングNATを行い、陽性血液を排除している。また、これまでの凝集法と比べて、より感度の高い化学発光酵素免疫測定法(CLEIA)及び精度を向上させた新NATシステムを導入した。HBV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。

BRIEF REPORT

Mortality Secondary to Fulminant Hepatic Failure in Patients with Prior Resolution of Hepatitis B Virus Infection in Japan

Takeji Umemura,[1] Eiji Tanaka,[1] Kendo Kiyosawa,[1,a] Hiromitsu Kumada,[2] and the Japan de novo Hepatitis B Research Group[b]

[1]Department of Internal Medicine, Hepatology, and Gastroenterology, Shinshu University School of Medicine, Matsumoto, and [2]Department of Hepatology, Toranomon Hospital, Tokyo, Japan

Hepatitis B virus (HBV) reactivation in patients with resolved HBV infection was found in 23 (4%) of 552 newly hepatitis B surface antigen–positive patients in Japan. Because one-fourth of cases develop into fulminant hepatic failure and mortality is 100%, management of HBV reactivation in patients with resolved HBV infection should be discussed.

Reactivation of hepatitis B virus (HBV) is becoming a well-recognized complication in patients with chronic HBV infection who are undergoing cytotoxic chemotherapy or immunosuppressive therapy [1–5]. HBV reactivation has a variety of manifestations, ranging from subclinical increases in transaminase activity to severe and potentially fatal fulminant hepatic failure (FHF). Because clinical studies have demonstrated that lamivudine therapy reduces the rate of HBV reactivation and mortality [6–9], prophylactic antiviral therapy is advised for HBV carriers at the onset of chemotherapy [10].

The clearance of hepatitis B surface antigen (HBsAg) and the appearance of antibody to HBsAg, with normalization of liver function, is generally accepted as evidence of clinical and serologic recovery from acute hepatitis B. However, HBV replication has been shown to persist at low levels in the liver for decades [11–13], which may explain the recent increase in the rate of HBV reactivation in patients with resolved infection during or after chemotherapy and transplantation [1, 5, 14–16]. Although reactivation led to FHF and even death in some cases [17–22], the incidence of and mortality associated with HBV reactivation have not been fully clarified in this context. Recently, a prospective study [23] from Hong Kong revealed that 3.3% of HBsAg-negative patients developed HBV reactivation after chemotherapy. In Japan, because ~20% of individuals are positive for at least 1 HBV marker [24], HBV reactivation during or after immunosuppressive treatment may become a critical issue in the near future. Thus, we investigated the mortality associated with and prevalence and clinical significance of HBV reactivation in Japanese patients with resolved HBV infection in a multicenter, cross-sectional study.

Methods. In 2005, we sent a questionnaire to 230 hospitals certified by the Japan Society of Hepatology; this included questions about patients who had become newly positive for serum HBsAg from January 2000 through December 2004 [25]. A total of 1239 patients were registered by 93 hospitals (40%). Of those patients, 55 were recorded as having experienced HBV reactivation after having resolved HBV infection, and the remaining 1184 patients were classified as having acute hepatitis B. Sixty-three (68%) of 93 hospitals responded to a second survey and provided information on 552 patients enrolled in this study; 23 of these patients developed HBV reactivation, and 529 had acute hepatitis B.

HBV reactivation was defined (according to a slight modification of the report by Hui et al. [23]) as a decrease in the level of antibody to HBsAg that was associated with the reappearance of HBsAg, a 3-fold elevation of serum alanine aminotransferase (ALT) level above normal, and detection of HBV DNA in serum during or after chemotherapy. The diagnoses of acute hepatitis B and FHF were defined as reported elsewhere [26]. Patients with other liver diseases were excluded. Serum HBV markers were determined as reported elsewhere [26]. Serum levels of HBV DNA were determined with use of Amplicor HBV Monitor kits (Roche Diagnostics) at each hospital when the patients were admitted. HBV genotypes were determined with use of the PCR-invader method, with genotype-specific probes [27]. This study was approved by the ethics committees of appropriate institutional review boards. Informed consent was obtained from each patient in accordance with the Helsinki Declaration.

The Mann-Whitney U test was used to analyze continuous variables. The χ^2 test with Yate's correction was used for analysis of categorical data. In cases in which the number of patients was <5, Fisher's exact test was used. $P \leq .05$ was considered to

Received 25 March 2008; accepted 16 May 2008; electronically published 21 July 2008.

[a] Present affiliation: Nagano Red Cross Hospital, Nagano, Japan.

[b] Members of the study group are listed at the end of the text.

Reprints or correspondence: Dr. Takeji Umemura, Dept. of Internal Medicine, Gastroenterology, and Hepatology, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621, Japan (tumemura@shinshu-u.ac.jp).

Clinical Infectious Diseases 2008; 47:e52–6

© 2008 by the Infectious Diseases Society of America. All rights reserved.

1058-4838/2008/4705-00E2$15.00
DOI: 10.1086/590968

be statistically significant. Statistical analyses were performed using SPSS, version 15.0J (SPSS).

Results. We first compared the demographic, clinical, and virologic features of the 23 patients who experienced HBV reactivation with those of the 529 patients with acute hepatitis B (table 1). The reactivation group had a significantly higher median age and median serum HBV DNA level ($P < .001$) and significantly lower peak ALT and albumin levels ($P < .001$). Although HBV genotype was not determined for one-half of the patients with acute hepatitis B, marked differences in the distribution of genotypes were seen; HBV type A occurred less frequently ($P = .003$) among patients with HBV reactivation than among those with acute hepatitis. However, HBV type B occurred more frequently among patients with HBV reactivation ($P < .001$).

FHF was more common among patients with HBV reactivation than among those with acute hepatitis ($P = .048$). Of the 23 cases of HBV reactivation, 6 (26%) resulted in liver-related death, 11 (48%) resolved, and 6 (26%) led to chronic hepatitis B. In contrast, of the 529 cases of acute hepatitis B, 490 (93%) were self-limited, 16 (3%) became chronic, and 21 (4%) resulted in death. These results revealed that liver-related mortality was significantly higher in the group with HBV reactivation than in the group with acute hepatitis ($P < .001$).

We then compared the clinical features of FHF between the groups (table 2). Patients with HBV reactivation had a higher median age, significantly lower peak ALT levels ($P = .006$), higher HBV DNA levels ($P = .035$), and higher mortality ($P = .031$) than did patients with acute hepatitis B.

Malignant lymphoma–associated morbidity was significantly higher among patients with HBV reactivation who developed FHF than among those who did not develop FHF (table 3). A rituximab-containing treatment regimen was administered to all patients who experienced FHF, compared with only 4 (22%) of 18 patients who did not experience FHF ($P = .004$). Lamivudine was administered to 16 (89%) of 18 patients who did not experience FHF and to all patients who experienced FHF at 7 and 20 days after hospital admission, respectively; this suggests that lamivudine treatment could not prevent FHF after HBV reactivation. Eventually, liver-related mortality occurred exclusively in patients who experienced FHF. There were no statistically significant differences between the 2 subgroups regarding HBV markers.

Discussion. Although a prospective study by Hui et al. [23] revealed that the incidence of HBV reactivation among HBsAg-negative patients after chemotherapy was 3.3%, there are no data available on HBV reactivation in Japan. In our nationwide cross-sectional study, a total of 552 newly HBsAg-positive patients were registered from 63 tertiary care hospitals. Overall, HBV reactivation was found in 4% of patients with resolved infection after chemotherapy. Serum and liver samples were not available before chemotherapy for most of these patients; therefore, we were unable to prove specifically whether reactivation was a result of occult or acute HBV infection. However,

Table 1. Demographic and clinical characteristics of patients with hepatitis B virus (HBV) reactivation, compared with those of patients with acute hepatitis B.

Characteristic	Patients with HBV reactivation	Patients with acute hepatitis B	*P*
Age, median years (95% CI)	63 (39–83)	33 (19–64)	<.001
Male sex	14/23 (61)	374/529 (71)	NS
Peak ALT level, median IU/L (95% CI)	929 (137–2441)	2300 (299–6626)	<.001
Peak bilirubin level, median mg/dL (95% CI)	10.3 (0.3–58.6)	6.4 (1.0–23.7)	NS
Lowest albumin level, median g/dL (95% CI)	3.2 (2.1–3.7)	3.6 (2.7–4.2)	<.001
Most prolonged PT%, median % (95% CI)	65.0 (10.2–121.4)	75.0 (11.0–103.1)	NS
HBV DNA level, median log copies/mL (95% CI)	7.5 (4.0 to >7.6)	5.5 (2.6 to >7.6)	<.001
Genotype			
A	0/19 (0)	57/232 (25)	.003
B	8/19 (42)	27/232 (12)	<.001
C	11/19 (58)	141/232 (61)	NS
Other	0/19 (0)	7/232 (3)	
Treatment			
Lamivudine	20/23 (87)	118/529 (22)	<.001
IFN	5/23 (22)	12/529 (2)	<.001
Fulminant hepatic failure	5/23 (22)	45/529 (9)	.048
Liver-related death	6/23 (26)	21/529 (4)	<.001

NOTE. Data no. (%) of patients, unless otherwise indicated. ALT, alanine aminotransferase; NS, not statistically significant; PT, prothrombin time.

Table 2. Demographic and clinical characteristics of patients with hepatitis B virus (HBV) reactivation who experienced fulminant hepatic failure (FHF), compared with those of patients with acute hepatitis B who experienced FHF.

Characteristic	Patients with FHF		P
	With HBV reactivation	With acute hepatitis B	
Age, median years (95% CI)	63 (47–64)	48 (18–72)	.029
Male sex	3/5 (60)	26/45 (58)	NS
Peak ALT level, median IU/L (95% CI)	907 (359–1823)	5995 (589–11,858)	.006
Peak bilirubin level, median mg/dL (95% CI)	20.8 (10.2–45.7)	9.9 (4.9–30.5)	.099
Lowest albumin level, median g/dL (95% CI)	2.6 (2.1–3.0)	2.9 (1.9–3.9)	NS
Most prolonged PT%, median % (95% CI)	22.0 (8.7–32.3)	16.0 (0.2–37.0)	NS
HBV DNA level, median log copies/mL (95% CI)	7.6 (5.6 to >7.6)	5.7 (2.6 to >7.6)	.035
Genotype			
A	0/5 (0)	2/16 (13)	NS
B	1/5 (20)	3/16 (19)	NS
C	4/5 (80)	11/16 (69)	NS
Received lamivudine treatment	5/5 (100)	29/45 (81)	NS
Liver-related death	5/5 (100)	21/45 (47)	.031

NOTE. Data are no. (%) of patients, unless otherwise indicated. ALT, alanine aminotransferase; NS, not statistically significant; PT, prothrombin time.

because all patients were negative for HBsAg and positive for antibody to hepatitis B core antigen before treatment, we presumed that reactivation was occult in nature.

In our study, patients who experienced HBV reactivation were significantly older and had lower serum albumin levels, compared with patients with acute hepatitis B. The immune status of many patients may have been further decreased by cytotoxic chemotherapy. Approximately 20% of the patients who experienced HBV reactivation developed FHF. Surprisingly, mortality was 100%, implying that FHF in these cases is severe. Both the prevalence of and mortality associated with FHF were significantly higher among patients who experienced HBV reactivation than among those with acute HBV infection. Although the group with HBV reactivation also had lower albumin levels at the onset of lamivudine therapy, the development of FHF could not be predicted from this study. Thus, it is crucial to prevent FHF in patients with HBV reactivation with use of agents other than—or complimentary to—lamivudine. Unfortunately, preemptive therapy is not recommended because of the difficulties in detecting reactivation. Hui et al. [23] recommended monthly testing of HBV DNA levels and immediate antiviral therapy when levels are 100-fold the levels before chemotherapy. However, this strategy is still controversial [28, 29] and needs testing in a randomized study.

A recent study revealed that HBV type Bj and G1896A mutations were independently associated with a fulminant outcome in patients with acute HBV infection [30]. However, HBV genotype, serum HBV DNA level, or mutations in G1896A or A1762T/G1764A did not influence the development of FHF in patients who experienced HBV reactivation in this study. HBV reactivation in patients infected with HBV genotype A was also not found in this study, which may be explained by the fact that this genotype occurs in only 1.7% of patients with chronic hepatitis B in Japan [31].

Because our study and other studies [23] have confirmed that HBV reactivation can be fatal, we need to emphasize greater testing of HBV markers, including antibody to hepatitis B core antigen, antibody to HBsAg, and HBV DNA levels before administration of chemotherapy, especially therapy containing rituximab. Patients with resolved HBV infection should be routinely monitored for liver function and HBV DNA levels, and antiviral therapy should be administered immediately when evidence of HBV reactivation is found.

In conclusion, HBV reactivation is found in 4% of newly HBsAg-positive patients with resolved HBV infection in Japan. One-fourth of cases of HBV reactivation develop into FHF, and mortality is extremely high. Because our study was unable to distinguish HBV reactivation from occult HBV infection and could not clarify whether antiviral therapy was effective, a prospective study is being planned to clarify the mechanism of HBV reactivation and the benefits of antiviral therapy.

Japan de novo Hepatitis B Research Group. Tetsuya Ishikawa (Aichi Medical University), Takaaki Otake (Asahikawa Medical University), Kunihide Ishii (Asakura Hospital), Kenichi Fukai (Chiba University), Yoichi Hiasa and Morikazu Onji (Ehime University), Yuichi Tanabe (Fukuoka City Hospital), Kozou Fujio (Fukuyama City Hospital), Tatsuro Sakata (Fukuyama Medical Center), Eiichi Tomita (Gifu Municipal Hospital), Hideki Hagiwara (Higashiosaka City General Hospital), Mikiya Kitamoto (Hiroshima Prefectural Hospital), Shoichi

Table 3. Demographic and clinical characteristics of patients with hepatitis B virus (HBV) reactivation who did and did not experience fulminant hepatic failure (FHF).

Characteristic	Patients with HBV reactivation		
	Experienced FHF (n = 5)	Did not experience FHF (n = 18)	P
Age, median years (95% CI)	63 (47–64)	63 (39–78)	NS
Male sex	3 (60)	11 (61)	NS
Peak ALT level, median IU/L (95% CI)	907 (359–1823)	1016 (124–2524)	NS
Peak bilirubin level, median mg/dL (95% CI)	20.8 (10.2–45.7)	7.6 (0.3–24.9)	.094
Lowest albumin level, median g/dL (95% CI)	2.6 (2.1–3.0)	3.3 (2.2–3.6)	.015
Most prolonged PT%, median % (95% CI)	22.0 (8.7–32.3)	77.5 (18.0–101.8)	<.001
ALT level,[a] median IU/L (95% CI)	176 (83–1035)	266 (58–1690)	NS
Bilirubin level,[a] median mg/dL (95% CI)	0.7 (0.4–7.2)	0.7 (0.3–13.6)	NS
Albumin level,[a] median g/dL (95% CI)	3.4 (2.5–3.5)	3.9 (2.8–4.5)	.035
PT%,[a] median % (95% CI)	42.2 (16.4–46.4)	83.7 (38.7–123.5)	NS
HBV DNA level, median log copies/mL (95% CI)	7.6 (5.6 to >7.6)	7.5 (4.0 to >7.6)	NS
Genotype			
B	1 (20)	7/14 (50)	NS
C	4 (80)	7/14 (50)	NS
Mutation			
G1896A	4 (80)	5/12 (42)	NS
A1762T/G1764A	2 (40)	2/12 (17)	NS
Non-Hodgkin lymphoma	5 (100)	8 (44)	.046
Received a rituximab-containing treatment regimen	5 (100)	4 (22)	.004
Treatment			
Lamivudine	5 (100)	16 (89)	NS
IFN	1 (20)	4 (22)	NS
Liver-related death	5 (100)	1 (6)	<.001

NOTE. Data are no. (%) of patients, unless otherwise indicated. ALT, alanine aminotransferase; NS, not statistically significant; PT, prothrombin time.

[a] Laboratory data are from the start of lamivudine therapy.

Takahashi (Hiroshima University), Yuji Oka (Hitachi General Hospital), Koichi Abe (Iwate Medical University), Makoto Oketani and Hirohito Tsubouchi (Kagoshima University), Akito Sakai (Kanazawa University), Mutsumi Tsuchishima (Kanazawa Medical University), Gotaro Yamada (Kawasaki Medical University), Yoshihiro Akahane (Kofu Municipal Hospital), Akihide Masumoto (Kokura National Hospital), Hiroyuki Shimomura (Kurashiki Central Hospital), Tatsuya Ide (Kurume University), Masahito Minami (Kyoto Prefectural University), Sawako Inoue (Kyushu University), Michimori Kono (Matsue City Hospital), Motoo Iwasa (Mie University), Naoya Murashima (Mishuku Hospital), Shigehiko Sainokami (Mizusawa Hospital), Masayuki Kurosaki Musashino Red Cross Hospital), Toru Ishikawa (Nagaoka Red Cross Hospital), Hiroshi Yatsuhashi (Nagasaki Medical Center), Shogo Okoshi (Niigata University), Kenji Soga (Nippon Dental University Niigata), Shigetoshi Fujiyama (NTT West Japan Kyushu Hospital), Yuji Kato (Oita Prefectural Hospital), Tetsuo Takehara and Morikazu Seki (Osaka University), Hiromasa Yoshihara (Osaka Rosai Hospital), Tadakazu Sekine (Saiseikai Kawaguchi General Hospital), Shuichi Miyase (Saiseikai Kumamoto Hospital), Tomoteru Kamimura (Saiseikai Niigata Daini Hospital), Shuichi Kubo (Saiseikai Yokohama Nanbu Hospital), Yoshiyasu Karino (Sapporo-Kosei General Hospital), Masamichi Nagasawa (Seirei Hamamatsu General Hospital), Takayoshi Ito (Showa University), Chiaki Okuse (St. Marianna University), Akira Sato (St. Marianna University Yokohama City Seibu Hospital), Atsushi Tanaka (Teikyo University), Jong-Hon Kang (Teine Keijinkai Hospital), Yoshiyuki Ueno (Tohoku University), Yusei Ikeda (Tokyo Kosei Nenkin Hospital), Naoaki Hashimoto (Tokyo Teishin Hospital), Takuji Yamada (Tomei Atsugi Hospital), Kenji Ikeda (Toranomon Hospital), Mari Kawakami (Tottori University), Terumi Takahara (Toyama University), Sumio Kawata (Yamagata University), Takahiro Kodama (Yamaguchi Grand Center), and Takaaki Ikeda (Yokosuka Kyousai Hospital).

Acknowledgments

Financial support. Ministry of Health, Labour, and Welfare of Japan (to K.K.) and Viral Hepatitis Research Foundation of Japan (to T.U.).

Potential conflicts of interest. All authors: no conflicts.

References

1. Wands JR, Chura CM, Roll FJ, Maddrey WC. Serial studies of hepatitis-associated antigen and antibody in patients receiving antitumor chemotherapy for myeloproliferative and lymphoproliferative disorders. Gastroenterology 1975; 68:105–12.
2. Galbraith RM, Eddleston AL, Williams R, Zuckerman AJ. Fulminant hepatic failure in leukaemia and choriocarcinoma related to withdrawal of cytotoxic drug therapy. Lancet 1975; 2:528–30.
3. Hoofnagle JH, Dusheiko GM, Seeff LB, Jones EA, Waggoner JG, Bales ZB. Seroconversion from hepatitis B e antigen to antibody in chronic type B hepatitis. Ann Intern Med 1981; 94:744–8.
4. Hoofnagle JH, Dusheiko GM, Schafer DF, et al. Reactivation of chronic hepatitis B virus infection by cancer chemotherapy. Ann Intern Med 1982; 96:447–9.
5. Lok AS, Liang RH, Chiu EK, Wong KL, Chan TK, Todd D. Reactivation of hepatitis B virus replication in patients receiving cytotoxic therapy: report of a prospective study. Gastroenterology 1991; 100:182–8.
6. Lau GK, Yiu HH, Fong DY, et al. Early is superior to deferred preemptive lamivudine therapy for hepatitis B patients undergoing chemotherapy. Gastroenterology 2003; 125:1742–9.
7. Leaw SJ, Yen CJ, Huang WT, Chen TY, Su WC, Tsao CJ. Preemptive use of interferon or lamivudine for hepatitis B reactivation in patients with aggressive lymphoma receiving chemotherapy. Ann Hematol 2004; 83:270–5.
8. Yeo W, Chan PK, Ho WM, et al. Lamivudine for the prevention of hepatitis B virus reactivation in hepatitis B s-antigen seropositive cancer patients undergoing cytotoxic chemotherapy. J Clin Oncol 2004; 22: 927–34.
9. Yeo W, Ho WM, Hui P, et al. Use of lamivudine to prevent hepatitis B virus reactivation during chemotherapy in breast cancer patients. Breast Cancer Res Treat 2004; 88:209–15.
10. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology 2007; 45: 507–39.
11. Kuhns M, McNamara A, Mason A, Campbell C, Perrillo R. Serum and liver hepatitis B virus DNA in chronic hepatitis B after sustained loss of surface antigen. Gastroenterology 1992; 103:1649–56.
12. Fong TL, Di Bisceglie AM, Gerber MA, Waggoner JG, Hoofnagle JH. Persistence of hepatitis B virus DNA in the liver after loss of HBsAg in chronic hepatitis B. Hepatology 1993; 18:1313–8.
13. Michalak TI, Pasquinelli C, Guilhot S, Chisari FV. Hepatitis B virus persistence after recovery from acute viral hepatitis. J Clin Invest 1994; 93:230–9.
14. Dhedin N, Douvin C, Kuentz M, et al. Reverse seroconversion of hepatitis B after allogeneic bone marrow transplantation: a retrospective study of 37 patients with pretransplant anti-HBs and anti-HBc. Transplantation 1998; 66:616–9.
15. Seth P, Alrajhi AA, Kagevi I, et al. Hepatitis B virus reactivation with clinical flare in allogeneic stem cell transplants with chronic graft-versus-host disease. Bone Marrow Transplant 2002; 30:189–94.
16. Kempinska A, Kwak EJ, Angel JB. Reactivation of hepatitis B infection following allogeneic bone marrow transplantation in a hepatitis B–immune patient: case report and review of the literature. Clin Infect Dis 2005; 41:1277–82.
17. Dervite I, Hober D, Morel P. Acute hepatitis B in a patient with antibodies to hepatitis B surface antigen who was receiving rituximab. N Engl J Med 2001; 344:68–9.
18. Westhoff TH, Jochimsen F, Schmittel A, et al. Fatal hepatitis B virus reactivation by an escape mutant following rituximab therapy. Blood 2003; 102:1930.
19. Sarrecchia C, Cappelli A, Aiello P. HBV reactivation with fatal fulminating hepatitis during rituximab treatment in a subject negative for HBsAg and positive for HBsAb and HBcAb. J Infect Chemother 2005; 11:189–91.
20. Law JK, Ho JK, Hoskins PJ, Erb SR, Steinbrecher UP, Yoshida EM. Fatal reactivation of hepatitis B post-chemotherapy for lymphoma in a hepatitis B surface antigen–negative, hepatitis B core antibody–positive patient: potential implications for future prophylaxis recommendations. Leuk Lymphoma 2005; 46:1085–9.
21. Sera T, Hiasa Y, Michitaka K, et al. Anti-HBs–positive liver failure due to hepatitis B virus reactivation induced by rituximab. Intern Med 2006; 45:721–4.
22. Kitano K, Kobayashi H, Hanamura M, et al. Fulminant hepatitis after allogenic bone marrow transplantation caused by reactivation of hepatitis B virus with gene mutations in the core promotor region. Eur J Haematol 2006; 77:255–8.
23. Hui CK, Cheung WW, Zhang HY, et al. Kinetics and risk of de novo hepatitis B infection in HBsAg-negative patients undergoing cytotoxic chemotherapy. Gastroenterology 2006; 131:59–68.
24. Kiyosawa K, Tanaka E, Sodeyama T, et al. Transmission of hepatitis C in an isolated area in Japan: community-acquired infection. The South Kiso Hepatitis Study Group. Gastroenterology 1994; 106:1596–602.
25. Umemura T, Kiyosawa K. Fatal HBV reactivation in a subject with anti-HBs and anti-HBc. Intern Med 2006; 45:747–8.
26. Umemura T, Tanaka E, Ostapowicz G, et al. Investigation of SEN virus infection in patients with cryptogenic acute liver failure, hepatitis-associated aplastic anemia, or acute and chronic non–A-E hepatitis. J Infect Dis 2003; 188:1545–52.
27. Tadokoro K, Kobayashi M, Yamaguchi T, et al. Classification of hepatitis B virus genotypes by the PCR-invader method with genotype-specific probes. J Virol Methods 2006; 138:30–9.
28. Keeffe EB, Dieterich DT, Han SH, et al. A treatment algorithm for the management of chronic hepatitis B virus infection in the United States: an update. Clin Gastroenterol Hepatol 2006; 4:936–62.
29. Liu CJ, Kao JH, Chen DS. Kinetics of hepatitis B virus reactivation after chemotherapy: more questions than answers. Gastroenterology 2006; 131:1656 (author reply: Gastroenterology 2006; 131:1657).
30. Ozasa A, Tanaka Y, Orito E, et al. Influence of genotypes and precore mutations on fulminant or chronic outcome of acute hepatitis B virus infection. Hepatology 2006; 44:326–34.
31. Orito E, Mizokami M, Sakugawa H, et al. A case-control study for clinical and molecular biological differences between hepatitis B viruses of genotypes B and C. Japan HBV Genotype Research Group. Hepatology 2001; 33:218–23.

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
				2008. 6. 23	該当なし	

一般的名称	(製造販売承認書に記載なし)	研究報告の公表状況	FDA, CBER. 2008 May 20; Available from: URL: http://www.fda.gov/cber/gdlns/reentrybld.htm	公表国 米国
販売名(企業名)	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

○業界向けガイダンス案:B型肝炎コア抗体(抗HBc抗体)検査陽性により供血延期となった供血者の供血再開(リエントリー)についての適格性確認方法

米国食品医薬品局(FDA)は、B型肝炎コア抗体が陽性となったために供血延期となった供血者のリエントリー・アルゴリズムを提案するガイダンス案を発表した。これまで、HBc抗体が2回以上陽性となった供血者は無期限に供血延期とされていた。FDAの試算では、1980年代後半から90年代にかけて、HBc抗体が偽陽性だったために供血延期となった人は毎年約21,500名にのぼり、これまでに20万人以上の供血適格者が供血できなくなっている。

本ガイダンスでは、HBc抗体検査が2回目に陽性となった後、8週間以上経ってから高感度のHBV NATによってHBV感染が否定された場合は供血可能となる。フォローアップの際に、HBV NAT陽性、HBs抗原繰り返し陽性、HBc抗体繰り返し陽性のいずれかに該当する場合は無期限供血延期となる。

報告企業の意見	今後の対応
米国食品医薬品局(FDA)は、B型肝炎コア抗体が陽性となったために供血延期となった供血者のリエントリー・アルゴリズムを提案するガイダンス案を発表したとの報告である。米国ではもともとHBV感染者が少なくワクチンも普及していることから、日本と状況は異なるが、偽陽性者のリエントリーの方法としては参考になると考えられる。	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)及び精度を向上させた次世代NATを導入した。

使用上の注意記載状況・その他参考事項等

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

(2)

Guidance for Industry

Requalification Method for Reentry of Blood Donors Deferred Because of Reactive Test Results for Antibody to Hepatitis B Core Antigen (Anti-HBc)

DRAFT GUIDANCE

This guidance document is for comment purposes only.

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

Additional copies of this draft guidance are available from the Office of Communication, Training and Manufacturers Assistance (HFM-40), 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448, or by calling 1-800-835-4709 or 301-827-1800, or from the Internet at http://www.fda.gov/cber/guidelines.htm.

For questions on the content of this guidance, contact Robin Biswas, M.D., at 301-827-3011.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
May 2008

Contains Nonbinding Recommendations

Draft –Not For Implementation

Table of Contents

I. INTRODUCTION 1
II. BACKGROUND 2
III. RECOMMENDATIONS 4
IV. IMPLEMENTATION 5
V. REFERENCES 6
APPENDIX 8

Contains Nonbinding Recommendations

Draft –Not For Implementation

Guidance for Industry

Requalification Method for Reentry of Blood Donors Deferred Because of Reactive Test Results for Antibody to Hepatitis B Core Antigen (Anti-HBc)

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

I. INTRODUCTION

We, FDA, are providing recommendations to you, establishments that collect human blood or blood components, for a requalification method or process for the reentry of deferred donors into the donor pool based on a determination that previous tests that were repeatedly reactive for antibodies to hepatitis B core antigen (anti-HBc) were falsely positive and that there is no evidence of infection with hepatitis B virus (HBV). Currently, donors who are repeatedly reactive on more than one occasion for anti-HBc (samples from more than one collection from the donor are repeatedly reactive for anti-HBc), must be indefinitely deferred, in accordance with Title 21 Code of Federal Regulations, section 610.41(a) (21 CFR 610.41(a)). Although it may seem unlikely that two anti-HBc tests would be false positives, such situations have occurred with some frequency because of the relative non-specificity of these tests. The result is that many otherwise suitable donors are indefinitely deferred because of their anti-HBc test results even though medical follow-up of such donors indicates that they are not infected with HBV.

The availability of an FDA-licensed hepatitis B virus nucleic acid test (HBV NAT), which is particularly sensitive when single samples are tested, provides an additional, powerful method of determining whether a donor who has been deferred because of anti-HBc reactivity is truly infected by HBV. Due to the availability of this licensed HBV NAT and the improved specificity of anti-HBc assays, we are recommending in this guidance a reentry algorithm for donors deferred due to falsely positive repeatedly reactive tests for anti-HBc.

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

II. BACKGROUND

A. Clinical Significance of Donor Screening for Hepatitis B Virus Infection

HBV is a major human pathogen that causes acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma (Ref. 1). HBV is an enveloped virus with a partially duplex circular deoxyribonucleic acid (DNA) genome of approximately 3,200 bases. Most primary infections in adults are self-limited; the virus is cleared from the blood and liver, and individuals develop a lasting immunity. Fewer than 5% of infected adults develop chronic infections that can be asymptomatic (i.e., a carrier state). About 20% of chronically infected individuals develop cirrhosis. Chronically infected subjects have 100 times higher risk of developing hepatocellular carcinoma than non-carriers. The mortality of acute HBV infection is about 1%. In the United States, deaths from chronic HBV infection are estimated to range from 3,000 to 5,000 individuals per year (Ref. 2).

Currently, HBV is transmitted by blood transfusions more frequently than hepatitis C virus or human immunodeficiency virus (HIV). The residual risk of post-transfusion HBV infection from donations screened for hepatitis B surface antigen (HBsAg) and anti-HBc have been estimated as 1 in 63,000 donations (Ref. 3) to 1 in 180,000 donations (Ref. 4). The major cause of HBV transmission by blood is attributable to donations from asymptomatic donors with acute HBV infections who have not yet developed HBsAg (i.e., donors in the seronegative window period), and from donors with chronic infections in which serological markers are not detected (occult hepatitis B). Seronegative blood donations from infected individuals can transmit hepatitis B. In such cases, lookback studies using polymerase chain reaction have shown that HBV DNA can be detected at low levels in the donor's blood (Ref. 5).

HBsAg becomes detectable in blood 30 to 60 days after infection followed by emergence of anti-HBc. Viremia develops by the time HBsAg is detected, and can reach 10^9-10^{10} virions/ml in acute infections (Ref. 1). Upon clearance of the HBV infection by the immune response, the HBsAg antigen disappears from the circulation and detectable anti-HBc and antibody to hepatitis B surface antigen (anti-HBs) usually persists indefinitely. There is evidence that anti-HBc can decrease and even disappear over a period of decades in resolved infections (Ref. 6). In chronically infected individuals, tests for HBsAg and anti-HBc usually remain positive for life and lower viral titers can be detected in blood for a long period but tend to decline over time.

HBV NAT assays for detection of HBV DNA have been developed. So far, one test has been licensed for screening blood donations using a minipool sample format. This assay is also indicated for testing samples from individual donations, thus increasing test sensitivity. In a BPAC meeting on October 21, 2004 (Ref. 15), we proposed a revised reentry algorithm in which subsequent testing of the donor for HBsAg and anti-HBc is

retained, but sensitive HBV DNA testing using a licensed NAT would replace anti-HBs testing. While the Committee did not take a formal vote on FDA's proposed algorithm, the Committee did not raise any objections to FDA's proposal. We are not proposing additional testing for anti-HBs as part of donor reentry because extensive hepatitis B vaccination programs have been in place for a number of years, resulting in many individuals having anti-HBs from vaccination. As a result, anti-HBs now has questionable value as a marker of hepatitis B infection.

Since October 21, 2004, we have licensed a qualitative test for the direct detection of HBV DNA in human plasma from donations of Whole Blood and blood components for transfusion, and Source Plasma. The availability of a sensitive, FDA-licensed HBV NAT assay, particularly when single samples are tested, provides an additional, powerful method of determining whether a donor who has been deferred because of anti-HBc reactivity is truly infected by HBV. Due to the availability of a licensed HBV NAT and the improved specificity of anti-HBc assays, we are proposing a reentry algorithm for anti-HBc in this guidance.

B. Rationale for the Requalification Method for Reentry

Under 21 CFR 610.40(a), you must test each donation of human blood or blood component intended for use in preparing a product, including donations intended as a component of, or used to prepare, a medical device, for evidence of infection due to HBV. Testing for evidence of infection includes testing for the presence of HBsAg and anti-HBc. In addition, some blood establishments also test blood donations for HBV DNA by NAT.

Under 21 CFR 610.41(a), blood establishments must defer donors who test reactive[1] by a screening test for evidence of infection due to a communicable disease agent(s) listed in 21 CFR 610.40(a). However, donors who test repeatedly reactive for anti-HBc on only one occasion need not be deferred (21 CFR 610.41(a)(1)), although the donation collected would be unsuitable (Ref. 11). Donors who test reactive on more than one occasion do not fall within this provision and must be deferred under 21 CFR 610.41(a).

Under 21 CFR 610.41(b), we provided for reentry of a deferred donor who is subsequently "found to be suitable as a donor of blood or blood components by a requalification method or process found acceptable for such purposes by FDA."

Until now, we have not recommended a requalification method for reentry of donors deferred due to repeatedly reactive test results for anti-HBc because there was no

[1] In 21 CFR 610.41(a), FDA requires that blood establishments defer donors who test reactive by a screening test for evidence of infection due to a communicable disease agent listed in section 610.40(a). In section 610.41(a)(1), however, a donor who tests reactive for anti-HBc on only one occasion is not required to be deferred. In this guidance, we refer to reactive test results for HBsAg and anti-HBc as "repeatedly reactive" to accurately describe the testing algorithm for anti-HBc.

supplemental (additional, more specific) test available. Although donor screening for anti-HBc has contributed to blood safety, a large proportion of donors with anti-HBc reactivity who fulfill all other donor suitability criteria have been indefinitely deferred on the basis of potentially false positive anti-HBc test results (Refs. 7, 16). It is estimated that as many as 21,500 potentially eligible donors were deferred annually in the late 1980s and 1990s because of false positive anti-HBc results, and that over 200,000 donors could be eligible for reentry (Ref. 7).

III. RECOMMENDATIONS

For purposes of reentering into the donor pool a donor who has been indefinitely deferred because of having tested repeatedly reactive for anti-HBc on more than one occasion, we recommend that, after a minimum of 8 weeks following the last repeatedly reactive anti-HBc test, you obtain a pre-donation blood sample (i.e., a blood sample which is obtained prior to any next donation) from the donor for follow-up testing, using licensed tests for HBsAg, anti-HBc and HBV DNA by NAT. Provided that the blood sample test results are negative for HBsAg, anti-HBc and HBV NAT, the donor may, at a later date, return to donate blood. When the donor returns to donate, subsequent to the negative tests for HBsAg, anti-HBc, and HBV NAT on the pre-donation sample, we recommend that you reenter the donor as eligible to donate Whole Blood and blood components, provided that all other suitability criteria are met.

For donor retesting, we recommend that a minimum 8-week (56 days) period elapse following the last repeatedly reactive anti-HBc test, because this time period provides sufficient confidence that at least one of the three HBV markers (HBsAg, anti-HBc, and HBV DNA) will be detectable if the donor had been truly infected with HBV at the time of that last anti-HBc reactive donation (Ref. 1). In addition, 56 days is the minimum time period permitted between donations of Whole Blood (21 CFR 640.3(b)).

For purposes of reentry, we recommend that you use a licensed HBV NAT labeled as having a sensitivity of $\leq$ 10 copies /mL (at 95 % detection rate). This sensitivity reflects the current technological capabilities regarding sensitivity of HBV NAT assays. Depending upon the assay and the platform used, this sensitivity may only be achieved when testing individual donor samples.

Donor reentry following deferral for repeatedly reactive tests for anti-HBc on more than one occasion:

A. You may reenter into the donor pool a donor who has been indefinitely deferred solely because of repeatedly reactive tests for anti-HBc on more than one occasion if (see flow chart in the Appendix):

   1. After a minimum of 8 weeks following the last repeatedly reactive anti-HBc test, you collect a follow up sample from the donor, and this sample tests

negative on licensed tests for HBsAg, anti-HBc, and HBV DNA by NAT (sensitivity at 95% detection rate of ≤ 10 copies/mL)

and

2. When the donor presents to donate, subsequent to the negative tests for HBsAg, anti-HBc, and HBV NAT, you determine that the donor meets all eligibility criteria for donors of Whole Blood and blood components

B. You should continue to indefinitely defer a donor who was deferred for anti-HBc reactivity on more than one occasion and whose sample or donation tests repeatedly reactive on the: 1) HBsAg test (whether or not the neutralization test is positive), 2) anti-HBc test, or 3) HBV NAT. Positive results on tests for HBsAg, anti-HBc or HBV NAT may be useful in donor counseling.

C. If you wish to perform follow-up testing on a donor who is deferred because of anti-HBc test results, you may do so before the end of the 8-week waiting period for donor notification purposes or for medical reasons. Negative test results on follow-up for HBsAg, anti-HBc, and HBV NAT (sensitivity at 95% detection rate of ≤ 10 copies/mL), may be useful in donor counseling. However, only negative results for all three tests, obtained at least 8 weeks after the last repeatedly reactive anti-HBc result, would qualify the donor for reentry. If you obtain a reactive HBV NAT, or repeatedly reactive HBsAg or anti-HBc, or positive HBsAg result on any of these tests during this 8-week waiting period, the donor would not be eligible for reentry, and we recommend that you defer the donor indefinitely.

IV. IMPLEMENTATION

This guidance is being distributed for comment purposes only.

V. REFERENCES

1. Ganem, D., Prince, A.M. (2004). "Hepatitis B Virus Infection – Natural History and Clinical Consequences." *N. Engl. J. Med., 350,* 1118-1129.

2. Alter, M.J. Centers for Disease Control and Prevention. Epidemiology of HBV Infection and Prevention Programs. Presentation to the Advisory Committee on Blood Safety and Availability, August 27, 2004.

3. Schreiber, G.B., Busch, M.P., Kleinman, S.H., Korelitz, J.J. (1996). "The Risk of Transfusion-Transmitted Viral Infections." *N. Engl. J. Med., 334(26),* 1685-1690.

4. Busch MP. Overview of NAT and Reduction of Residual Risk in Infectious Disease Transmission. Presentation at the FDA Workshop on Application of Nucleic Acid Testing to Blood Pathogens and Emerging Technologies. December 4 – 5, 2001. http://www.fda.gov//cber/minutes/workshop-min.htm#01

5. Roth, W.K., Weber, M., Petersen, D., Drosten, C., Buhr, S., Sireis, W., Weichert, W., Hedges, D., Seifried, E. (2002). "NAT for HBV and anti-HBc testing increase blood safety." *Transfusion, 42,* 869-875.

6. Seeff, L.B., Beebe, G.W., Hoofnagle, J.H., et al. (1987). "A Serologic Follow-Up of The 1942 Epidemic of Post-Vaccination Hepatitis in the United States Army." *N. Eng. J. Med., 316(16),* 965-970.

7. Kleinman, S.H., Kuhns, M.C., Todd, D.S., Glynn, S.A. McNamara, A., DiMarco, A., Busch, M.P. for the Retrovirus Epidemiology Donor Study. (2003). "Frequency of HBV DNA detection in US blood donors testing positive for the presence of anti-HBc: implications for transfusion transmission and donor screening." *Transfusion, 43,* 696-704.

8. Allain, J-P. (2004). "Occult hepatitis B virus infection: implications in transfusion." *Vox Sang, 86,* 83-91.

9. Busch, M.P. (2004). "Should HBV DNA NAT replace HBsAg and/or anti-HBc screening of blood donors?" *Transfusion Clinique et Biologique, 11,* 26-32.

10. Hoofnagle, J.H. (1990). "Posttransfusion Hepatitis B" (Editorial). *Transfusion, 30,* 384-386.

11. FDA Memorandum to All Registered Blood Establishments: "FDA Recommendations Concerning Testing for Antibody to Hepatitis B Core Antigen (Anti-HBc)," September 10, 1991. http://www.fda.gov/cber/memo.htm.

12. NIH Consensus Statement: "Infectious Disease Testing for Blood Transfusions." Volume 13, Number 1, January 9-11, 1995.

13. FDA Memorandum to All Registered Blood and Plasma Establishments: "Recommendations for the Quarantine and Disposition of Units from Prior Collections from Donors with Repeatedly Reactive Screening Tests for Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) and Human T-Lymphotropic Virus Type I (HTLV-I)," July 19, 1996. http://www.fda.gov/cber/memo.htm.

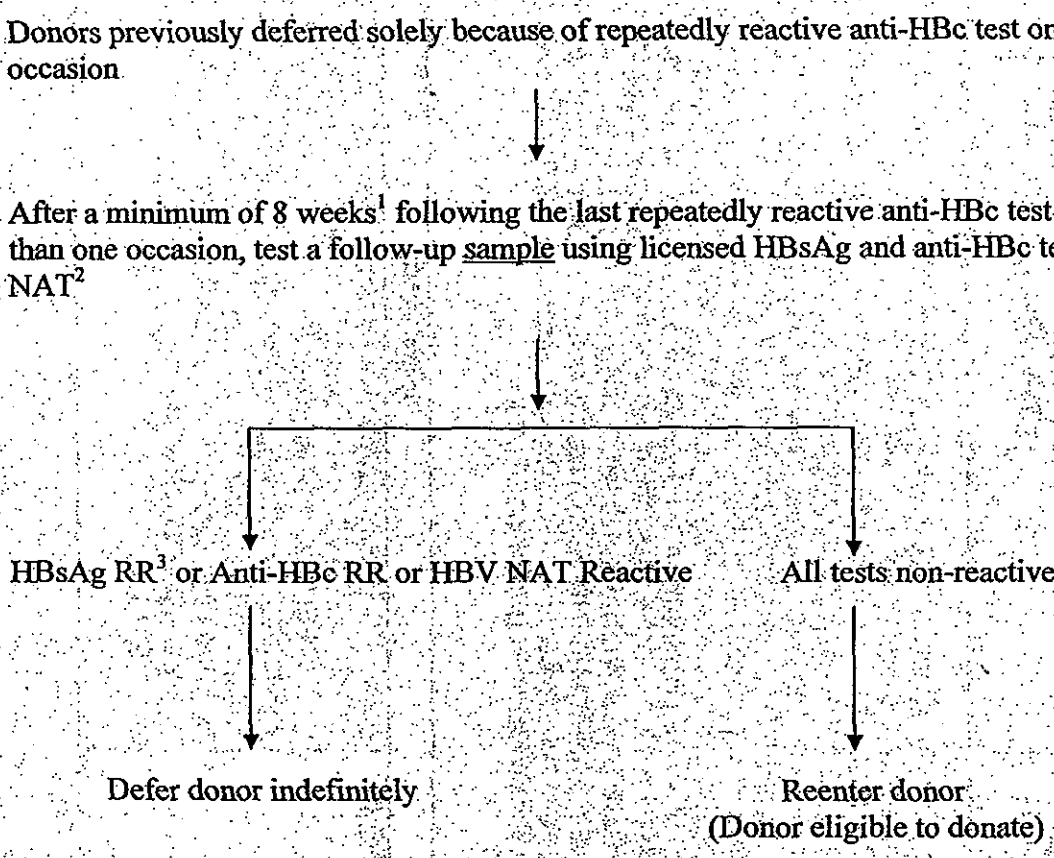
14. Hepatitis B Anti-Core Reentry (Anti-HBc). Blood Products Advisory Committee 61st Meeting; Transcript, December 10, 1998. www.fda.gov/ohrms/dockets/ac/98/transcpt/3479t1.pdf

15. FDA's Current Thinking on Reentry of Donors Deferred for Repeated Detection of Antibody to Hepatitis B Core Antigen: Blood Products Advisory Committee 81st Meeting; Transcript, October 21, 2004. www.fda.gov/ohrms/dockets/ac/cber04.html#BloodProducts

16. Tegtmeier, G, Henderson, S, McNamara, A, Kuhns, M. (1997). Contribution of Anti-HBc Screening to Blood Safety at a Regional Blood Center in the United States *Transfusion, 37*, 110S (Abstr. S439).

APPENDIX

REENTRY FOR DONORS DEFERRED BECAUSE OF REPEATEDLY REACTIVE TEST RESULTS FOR ANTI-HBc



[1] If the donor sample is tested before 8 weeks following the last repeatedly reactive anti-HBc test results on more than one occasion, a) if the sample tests HBsAg RR or anti-HBc RR or HBV NAT reactive, the donor is indefinitely deferred, and b) if the sample tests negative on all three of these tests, the donor should be retested after a minimum of 8 weeks following the last repeatedly reactive anti-HBc test result on more than one occasion using licensed HBsAg and anti-HBc tests, and HBV NAT.

[2] The sensitivity of the HBV NAT used should be ≤ 10 copies/mL, at 95% detection rate.

[3] Regardless of the neutralization test result.

別紙様式第２－１

医薬品　研究報告・調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
一般的名称	新鮮凍結血漿、濃厚赤血球		研究報告の公表状況	Journal of hepatology (England) Jun2008, 48 (6) p1022-5.	公表国 英国	
販売名（企業名）	－					

研究報告の概要

潜在性 B 型肝炎ウイルス感染者 (OBI) の血液は抗 HBs 抗体が陽性であれば感染性がないと考えられているが、スロヴェニアにおいて、冠動脈バイパス術で濃厚赤血球と新鮮凍結血漿 (HBs 抗原陰性で抗 HBc 抗体陽性、抗 HBs 抗体低力価陽性、HBV DNA 陽性) が輸血された 59 歳の患者が、その 4 ヵ月後に急性 B 型肝炎を発症した。
また、もう一例、先の例の感染源と同じ供血血液から得られた濃厚赤血球 (RCC) の輸血を受けた 71 歳の患者が、受血の 7 ヵ月後に HBV 感染を認めた (HBV に感染した 2 例はドナーと同じ配列を有するジェノタイプ D 型が感染していた)。
原因となった供血血液は、抗 HBc 抗体及び抗 HBs 抗体 (12IU/L) が陽性であったが、HBV DNA も陽性であり、この供血者のこれまで及びそれ以後のサンプルには低量のウイルスと抗 HBs 抗体が含まれていたが、過去 2 回分の供血血液では HBV 感染は起きていなかった。
今回の 2 例の受血者は手術の外傷に加え、加齢により免疫が低下していたことがウイルスに対する感受性を増大させたとも考えられる。
OBI は感染性を持つが HBV DNA スクリーニングで検出可能であるので、抗 HBc 抗体も HBV NAT も実施されていない国の保健当局は慎重に考慮すべきである。

使用上の注意記載状況・その他参考事項等

重要な基本的注意

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

報告企業の意見	今後の対応
HBs 抗原陰性で抗 HBs 抗体低力価陽性、HBV DNA 陽性の血液による B 型肝炎感染の報告である。 血漿分画製剤の原料血漿はミニプール血漿における NAT 検査で HBV DNA 陰性を確認しており、最終製品においても HBV DNA 陰性を確認している。	今後ともに潜在性 B 型肝炎ウイルス感染に関する安全性情報に留意していく。

③

NIHONSEIYAKI
2008-005

ELSEVIER

Journal of Hepatology 48 (2008) 1022–1025

Journal of Hepatology

www.elsevier.com/locate/jhep

Case Report

Anti-HBs positive occult hepatitis B virus carrier blood infectious in two transfusion recipients☆

Snezna Levicnik-Stezinar[1,*], Urska Rahne-Potokar[1], Daniel Candotti[2], Nico Lelie[3], Jean-Pierre Allain[4]

[1]*Blood Transfusion Center of Slovenia, Ljubljana, Slovenia*
[2]*National Health Service Blood and Transplant, Cambridge, UK*
[3]*Chiron/Novartis Europe, Suresnes, France*
[4]*Department of Haematology, University of Cambridge, Cambridge, UK*

***Background/Aims*: Occult hepatitis B infection (OBI) in blood donations is not considered infectious when anti-HBs is present.**

***Methods*: Four months after transfusion of eight blood components during coronary arterial bypass surgery, a 59-year-old patient developed acute hepatitis B. A second 71-year-old patient transfused with a red cell concentrate (RCC) from one of these donations had early HBV infection 7 months post-transfusion. Samples were tested for HBV serological markers and HBV DNA was quantified and sequenced.**

***Results*: One implicated donation contained anti-HBc, anti-HBs (12 IU/L) and 180 IU/ml of HBV DNA. Previous and subsequent samples contained 3–10 times lower viral load and slightly variable anti-HBs. Two previous donations did not cause HBV infection. Recipients of the FFP and RCC from the index donation were both HBV infected and carried genotype D strains with sequences identical to the donor strain.**

***Conclusions*: Despite anti-HBs, an OBI carrier transmitted HBV to two immunocompetent transfusion recipients.**

© 2008 European Association for the Study of the Liver. Published by Elsevier B.V. All rights reserved.

***Keywords*: HBV; Occult HBV; Infectivity; Blood transfusion**

1. Introduction

In Slovenia, approximately 100,000 donations per year are collected. However, in 2005–2007, six cases of HBV transmission by transfusion were reported. Incidence was probably underestimated due to a high frequency of subclinical infection. Since HBsAg serological screening with a sensitive assay is systematically performed, transfusion transmission of HBV can originate from either recent infections in the pre-HBsAg seroconversion window period or occult HBV infection (OBI). OBI is defined as an atypical carrier state characterized by the presence of HBV DNA in plasma without detectable hepatitis B surface antigen (HBsAg) with or without antibodies to hepatitis B core antigen (anti-HBc) and hepatitis B surface antigen (anti-HBs) [1].

Received 14 January 2008; received in revised form 14 February 2008; accepted 19 February 2008
Associate Editor: M. Colombo

☆ The work reported has in part been presented in abstract form at the European congress of the International Society of Blood Transfusion in Madrid, Spain, June 2007. Dr. Nico Lelie is an employee of Chiron/Novartis but was not involved in the writing of the drafts of the manuscript except for specific comments. Prof J.P. Allain has been an occasional speaker at meetings organised by Chiron/Novartis but does not otherwise have a conflict of interest. The other co-authors do not have any declared conflict of interest.

* Corresponding author. Tel.: +386 1 5438 150; fax: +386 1 430 27 84.
E-mail address: snezna.levicnik@ztm.si (S. Levicnik-Stezinar).

Abbreviations: OBI, occult HBV infection; HBV, hepatitis B virus; RCC, red cell concentrate; FFP, fresh frozen plasma; anti-HBc, antibody to hepatitis B virus core antigen; anti-HBs, antibody to hepatitis B virus surface antigen; QPCR, real-time PCR; BCP/PC, basic core promoter/pre-core.

0168-8278/$34.00 © 2008 European Association for the Study of the Liver. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.jhep.2008.02.016

It is generally accepted that HBV DNA in blood may carry the risk of transmission, particularly in the pre-HBsAg window phase [2]. However, the transmission risk of OBIs is not well defined, although some cases of OBIs with anti-HBc only infectious by transfusion were described [2,3].

HBV transmission by blood components from a single anti-HBs positive OBI donation to two recipients is presented.

2. Case report

A patient who had been transfused 4 months previously with five units of fresh frozen plasma (FFP) and three units of RCC was suspected of acute hepatitis B. Stored samples from each implicated donation were tested for HBV markers. Seven samples were HBV marker negative. One sample was anti-HBc reactive and contained HBV DNA. The implicated donor was identified and stored samples from eight previous donations and one donation subsequent to the index donation as well as three follow-up samples were tested for HBV markers.

The first recipient of an FFP unit from the index donation was a 59-year-old male who was screened negative for HBV markers 3 days prior to cardiac arterial bypass. He was transfused on 23rd June, 2005. Four months later, clinical and laboratory evidence of acute Hepatitis B was obtained. ALT level was 1821 IU/L, HBsAg and anti-HBc IgM became reactive. No sample was available for HBV DNA testing. In a sample collected 4 months later, HBsAg was undetectable, IgM anti-HBc remained present and HBV DNA was at low level (Table 1).

The second recipient of the index donation was a 71-year-old female who received two units of RCC following orthopedic surgery. No pre-surgical HBV screening was performed and no post-surgical evidence of HBV infection was noted. A blood sample obtained 7 months after transfusion was anti-HBc negative but HBsAg positive and contained a high level of HBV DNA (Table 1). Nine months post-transfusion, ALT level was 566 IU/L. At 14 months post-transfusion the patient had recovered.

2.1. Methods

Routine blood donation screening for HBsAg was performed using Abbott PRISM (Abbott laboratories, Delkenheim, Germany). HBsAg repeat testing, anti-HBc and anti-HBs assays were performed with Abbott AxSYM. Cobas Amplicor HBV Monitor (Roche, Basel, Switzerland) and in-house real-time PCR (QPCR) as previously described were used to detect and quantify HBV DNA [4]. Basic core promoter/pre-core region (BCP/PC), Pre-S/S regions and full HBV genome were amplified, sequenced and phylogenetically analyzed as described [5].

3. Results and discussion

The index donation met the criteria defining 'occult' hepatitis B virus carriage since the plasma contained no detectable HBsAg but HBV DNA, anti-HBc and low titer of anti-HBs. This pattern was consistent 7 and 16 months after the index donation. Seven prior donations carried anti-HBc and anti-HBs although HBV DNA ranged between 7 and 63 IU/ml when tested

Table 1
Hepatitis B virus markers in the OBI donor and two HBV infected recipients

	Time from Index donation (m)	HBsAg	Anti-HBc	Anti-HBc IgM	Anti-HBs (IU/L)	HBV DNA (IU/ml)		HBV genotype
						Amplicor	QPCR	
Donor	−45	−	+	ND	29	Neg	63	
	−37	−	+	ND	15	Neg	Neg	
	−31	−	+	ND	16	Neg	35	
	−23	−	+	ND	16	Neg	45	
	−19	−	+	ND	19	Neg	7	
	−13	−	+	ND	15	Neg	ND	
	−6	−	+	ND	15	Neg	ND	
	Index	−	+	ND	12	<60	180	D
	+3	−	+	ND	53	Neg	ND	
	+7	−	+	ND	31	Neg	16	
	+12	−	ND	ND	ND	ND	Neg	
	+16	−	+	ND	25	Neg	40	
Recipient 1	−3 days	−	−	ND	−	ND	ND	
	+4	+	+	+	−	ND	ND	
	+8	−	+	+	−	12	185	D
Recipient 2	+7	+	−	−	−	1.1×10^6	1.7×10^6	D
	+14	−	+	+	−	Neg	ND	

−, non-reactive; ND, not done; Neg, negative.

with a sensitive in-house assay but was consistently undetectable by a commercial assay except in the Index sample. This pattern indicates recovery from >5 years past HBV infection (Table 1). Despite being tested with the high sensitivity assay, two of the nine donor samples tested remained HBV DNA negative, suggesting fluctuations of viremia. Prior to the index donation, anti-HBs levels were essentially stable (15–29 IU/L) but increased from 12 to 53 IU/L 3 months later suggesting minimal immune response. There was no clinical evidence that 14 previous donations and one subsequent donation were infectious to recipients. Pre- and post-transfusion samples from recipients of −71 and −13 month-donations showed no serological evidence of HBV infection. The −71 recipient was negative for HBsAg, anti-HBc and anti-HBs pre-transfusion, and 4 months post-transfusion, HBsAg was negative but anti-HBc was not tested. The −13 month recipient did not carry HBsAg, anti-HBc or anti-HBs 42 months after transfusion.

In contrast, there is strong evidence that both recipients of the index donation were HBV infected since acute hepatitis B occurred in recipient 1, 4 months after transfusion. In recipient 2, the 7-month post-transfusion sample containing HBsAg and high HBV DNA load without anti-HBc strongly suggested recent acute HBV infection and was followed by serological evidence of recovery (Table 1). A high ALT level 9 months post-transfusion that normalized after 14 months further supported this conclusion. The 4-month and probably 7-month long incubation time observed in recipients 1 and 2, respectively, could be explained by a relatively low infectious dose further decreased by partial anti-HBs neutralization (calculated on the basis of 180 IU/ml of HBV DNA and 200 ml of FFP for recipient 1 at 200,000 copies and 20,000 copies in 20 ml of RCC plasma for recipient 2). Published data indicated that lower infectious dose prolonged HBV incubation time and milder symptoms [6]. Transfusion transmission was further demonstrated by the Pre-S/S sequence identity between the index donation, recipient 1 and recipient 2 strains from follow-up samples. The whole genome sequences of recipient 2 and index donation were identical. Strains were of genotype D. Of note, the deduced amino acid sequence of the S protein was wild-type when compared to the genotype D consensus sequence except for A117T and S133Y, neither of these substitutions being recognized as escape mutants. An escape mutant mechanism explaining the infectivity of the index donation but not of the other donations from the donor was thus excluded. Similar cases of breakthrough HBV infection with wild-type strains have been described [7]. Although suppression of the HBV replication and gene expression is a reported cause of occult HBV [8], no mutation in the parts of the genome implicated in replication was found. Imperfect containment of viral replication by the donor immune system is the most likely cause of low levels of HBV DNA.

The stability of HBV DNA load and anti-HBs in multiple samples preceding the index donation and tested simultaneously contained 6–10 times less viral DNA than the index donation (Table 1). It is therefore speculated that the main factor singling out the index donation was a temporarily higher viral load sufficient to overcome the relatively weak neutralizing capacity of a low anti-HBs level (Table 1). This interpretation is supported by the subsequent increase in anti-HBs level suggesting a weak immune response.

Published data reporting the infectivity of OBIs by transfusion are rare. One case of transmission by a donation carrying anti-HBc without anti-HBs was reported in Japan [2]. Another study reported five donors (4 genotype D, one genotype A2) with OBI also carrying only anti-HBc transmitting to recipients. Of 51 traced recipients, 28 (54.9%) either developed fulminant, fatal, hepatitis B (3 cases) or carried anti-HBc post-transfusion although no pre-transfusion testing was performed [3]. In the Japanese study, 16 donations contained both anti-HBc and anti-HBs and no evidence of HBV transmission was found [2] confirming previous results [9]. The two cases reported here appear to be the first related to an OBI donor with anti-HBs. Data collected in Poland indicated that approximately 50% of OBIs in asymptomatic, apparently healthy, blood donors carry anti-HBs [10] and that levels of DNA and anti-HBs are variable as reported here.

Considering that the recipients at age 59 and 71, respectively, might have presented a mild, age-related, immunodeficiency added to the trauma of major surgery might have played a role in increasing susceptibility to viral infection [11]. The fact that approximately 50% of recipients of blood components in Western Europe present some degree of immunodeficiency related to age, chemotherapy or therapeutic immunosuppression suggests an increased susceptibility to HBV infection [12]. Limited but convincing evidence that OBIs can be infectious and can be detected by HBV DNA screening should be carefully considered by the health authorities of countries where neither anti-HBc nor HBV NAT are implemented.

Despite their apparent uniqueness, our two cases of HBV transmission need to be factored in discussions regarding HBV blood safety policy. They clearly illustrate that the neutralizing capacity of low-level anti-HBs is limited and reinforce the validity of considering anti-HBs below 100 IU/L to be poorly protective from infectivity when HBV DNA is present. However, even in the presence of higher levels of anti-HBs, in a severely immunodeficient recipient, HBV DNA-containing blood might be infectious and the clinical expression severe.

Acknowledgements

This work was supported in part by grants from the International Society of Blood Transfusion (2007-01) and Chiron/Novartis Corporation.

We gratefully acknowledge Drs. Irena Kramar and Slavica Maver for their contribution in tracing the recipients of previous donations.

References

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

[2] Satake M, Taira R, Yugi H, Hino S, Kanemitsu K, Ikeda H, et al. Infectivity of blood components with lower hepatitis B virus DNA levels identified in a lookback program. Transfusion 2007;47:1197–1205.

[3] Gerlich WH, Wagner FF, Chudy M, Harrishoj LH, Lattermenn A, Wienzek S, et al. HBsAg non-reactive HBV infection in blood donors: transmission and pathogenicity. J Med Virol 2007;79:S32–S36.

[4] Allain JP, Candotti D, Soldan K, Sarkodie F, Phelps B, Giachetti C, et al. The risk of hepatitis B virus infection by transfusion in Kumasi, Ghana. Blood 2003;101:2419–2425.

[5] Candotti D, Opare-Sem O, Rezvan H, Sarkodie F, Allain JP. Molecular and serological characterization of hepatitis B virus in deferred Ghanaian blood donors with and without elevated alanine amino transferase. J Viral Hepat 2006;13:715–724.

[6] 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.

[7] Gerlich WH. Breakthrough of hepatitis B virus escape mutants after vaccination and virus reactivation. J Clin Virol 2006;36:S18–S22.

[8] Zahn A, Li C, Danso K, Candotti D, Owusu-Ofori S, Temple J, et al. Molecular characterization of occult hepatitis B in genotype E-infected subjects. J Gen Virol 2008;89:409–418.

[9] Mosley JW, Stevens CE, Aach RD, Hollinger FB, Mimms LT, Solomon LR, et al. Donor screening for antibody to hepatitis B core antigen and hepatitis B virus infection in transfusion recipients. Transfusion 1995;35:5–12.

[10] Brojer E, Grabarczyk P, Liszewski G, Mikulska M, Allain J-P, Letowska M. Characterization of HBV DNA positive/HBsAg negative blood donors identified in the Polish NAT screening program. Hepatology 2006;44:1666–1674.

[11] Reed W, Lee TH, Norris PJ, Utter GH, Busch MP. Transfusion-associated microchimerism: a new complication of blood transfusions in severely injured patients. Semin Hematol 2007;44:24–31.

[12] Llewelyn C, Williamson L. Immunodeficiency and recipients of blood transfusion. EuroSat 2004; Paris, France [Abstract].

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 3 月 13 日	新医薬品等の区分	厚生労働省処理欄

一般的名称	①献血アルブミン-Wf ②献血アルブミン(5%)-Wf ③ノイアート ④ノイアート静注用 1500 単位 ⑤ハプトグロビン注-ヨシトミ ⑥コンコエイト-HT	研究報告の公表状況	Veterinary Science in China 2007; 37(11): 921-925	公表国 中国
販売名 （企業名）	①②人血清アルブミン ③④乾燥濃縮人アンチトロンビンⅢ ⑤人ハプトグロビン ⑥乾燥濃縮人血液凝固第Ⅷ因子			

研究報告の概要

文献中のヒト HBV の S 遺伝子の配列に従って、ブタ HBV の S 遺伝子のための 2 つのプライマーを設計し合成した。ブタの肝臓と血清のサンプルを中国の畜殺場から集めた。次いで、RT-PCR を使って S 遺伝子を増幅し配列決定を行った。その結果、ブタとヒトの HBV の S 遺伝子の配列は 98-100%の相同性を示した。HBV 陽性血漿の発光透過型電子顕微鏡による測定の結果、ウイルス粒子は直径 20 および 40nm であることが分かった。それら粒子は、ヒトの HBV 粒子と直径と形状が類似していた。陽性血清は、ELISA 法による HBV の表面抗原の存在によって確認した。ORF2/ORF3 のオーバーラップ領域から設計された 1 対の degenerated primers に対する nested RT-PCR アッセイから、HEV の遺伝子配列はブタの肝臓には存在するが、血清には存在しないことが示唆された。

報告企業の意見	今後の対応
中国の畜殺場から集めたブタの肝臓および血清からブタ B 型肝炎ウイルス、ブタ肝臓から E 型肝炎ウイルスを検出したとの報告である。 万一、ヘパリンの原料であるブタ小腸粘膜にHBVまたはHEVが混入したとしてもそれぞれPRVおよびPPVをモデルウイルスとしたウイルスバリデーション試験成績から、ヘパリンの製造工程において十分に不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

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ウイルス除去膜によるろ過処理を施しているが、投与に際しては、次の点に十分注意すること。

中国兽医科学 2007,37(11):921-925
Veterinary Science in China

BENE2008-010
管理部

中图分类号:S 852.659.1 文献标识码:A 文章编号:1673-4696(2007)11-0921-05

屠宰猪肝和血清中乙型肝炎病毒及戊型肝炎病毒的检测

李文贵[1,3],佘锐萍[1],韦海涛[2],赵景义[2],刘利强[1],李秀敏[2],王英华[1],王德成[1]
(1.中国农业大学 动物医学院,北京 100094;2.北京市畜牧兽医总站,北京 100107;
3.云南农业大学 动物科学学院,云南 昆明 650201)

摘要:应用1对乙型肝炎病毒(HBV)S基因保守区的引物,采用PCR方法从屠宰猪肝、血清中检测到了HBV,序列分析表明,扩增片段与已发表的HBV S基因的同源性高达98%~100%。电镜负染色样品观察结果表明,在HBV表面抗原ELISA检测强阳性反应的血清样品中存在有形态、大小与人HBV Dane颗粒和小球状颗粒相似的病毒粒子。针对戊型肝炎病毒(HEV) ORF2/ORF3重叠区设计了简并引物,采用巢式RT-PCR对屠宰猪肝和血清样品进行了检测。结果表明,部分屠宰猪肝中存在HEV。

关键词:猪;乙型肝炎病毒;戊型肝炎病毒;电镜观察

Detection of swine hepatitis B virus and E viruses in the liver and serum in pigs in China

LI Wen-gui[1,3], SHE Rui-ping[1], WEI Hai-tao[2], ZHAO Jing-yi[2], LIU Li-qiang[1],
LI Xiu-min[2], WANG Ying-hua[1], WANG De-cheng[1]
(1. *College of Veterinary Medicine, China Agricultural University, Beijing* 100094, *China*; 2. *Institute of Beijing Animal Husbandry and Veterinary, Beijing* 100107, *China*; 3. *College of Animal Science and Technology, Yunnan Agricultural University, Kunming* 650201, *China*)

Abstract: The study reported the presence of swine hepatitis B(HBV) and E(HEV) viruses in the liver and serum in pigs in China. Two primers for S gene of swine HBV were designed and synthesized according to sequences of the S genes of human HBV in the literature. Swine liver and serum samples were collected from a slaughter house. Swine S genes were amplified with RT-PCR and sequenced. Results showed that sequences of S genes of swine and human HBV viruses shared 98%—100% homology. Emission transmission electron microscopy examination of HBV-positive serum revealed presence of virus partides 40—20 nm in diameter. These particles were similar to human HBV particles in terms of both diameters and shapes. A positive serum was defined by the presence of the HBV surface antigen using the ELISA method. Responses in a nested RT-PCR assay to a pair of degenerated primers designed from ORF2/ORF3 overlapping region indicated presence of gene sequence of HEV in the liver, but not serum, in some pigs.

Key words: swine; hepatitis B virus; hepatitis E virus; electron microscopy

嗜肝DNA病毒科(Hepadnaviridae)有正嗜肝DNA病毒属(Orthohepadnavirus)及禽类嗜肝DNA病毒属(Avihepadnavirus)2个属。正嗜肝DNA病毒属包括人乙型肝炎病毒(HBV)、灵长类嗜肝病毒和啮齿动物嗜肝病毒,禽类嗜肝DNA病毒属包括鸭乙型肝炎病毒、苍鹭乙型肝炎病毒、雪雁乙型肝炎病毒等。这些病毒的共同特点为:基因组长3.0~3.3 kb,具有部分双链的环状DNA,外有包膜,核心内有基因组及病毒所编码的特异DNA聚合酶。除病毒颗粒外,产生大量的病毒包膜脂蛋白

收稿日期:2007-06-11;修回日期:2007-10-28
基金项目:国家自然科学基金项目(30771588)
作者简介:李文贵(1973-),男,云南昆明人,讲师,博士生。佘锐萍为通讯作者,E-mail:sheruiping@126.com

颗粒仅有很窄的宿主谱，一般可致持续病毒感染，而且有较明确的嗜肝性[7]。资料表明，畜禽中也存在一个嗜肝病毒群。自20世纪80年代以来，国内有关猪、鸡、牛、羊、犬等动物检出乙肝病毒表面抗原(HBsAg)的报道屡见不鲜，并有分离到类乙肝病毒的报道[2-6]。2006年余锐萍等[10-11]应用免疫组织化学方法对北京、河南等地肉联厂屠宰猪肝的人乙型肝炎病毒抗原进行检测，结果发现人乙型肝炎病毒抗原总检出率高达73%～100%。

戊型肝炎(Hepatitis E，HE)是由戊型肝炎病毒(Hepatitis E virus，HEV)感染引起的经肠道传播的急性病毒性肝炎，主要通过粪便经口传播。我国是HE高发区，被列为戊肝发病和死亡所致经济负担最严重的国家之一。有学者认为，猪是HEV主要自然宿主，日本已有因食用HEV污染的猪肝导致人感染的报道。对戊肝非流行区从事与猪相关职业人群和对照人群的研究表明，从事与猪相关职业者可增加HEV感染的风险。最近又发现了一些猪HEV感染人的证据[12-13]。总之，HB和HE严重地威胁着人类的健康，而人类病毒性肝炎的发病率还在不断上升。动物，尤其是与人类关系十分密切的猪体内的HBV和HEV带毒情况如何，它们与人的HBV和HEV的同源性怎样，都还不十分清楚。本试验应用PCR技术，在原有研究的基础上，进一步对屠宰猪血清和肝的HBV S基因、HEV RNA进行了检测，并应用免疫电镜负染色技术观察了屠宰猪血清中的HBV。

1 材料与方法

1.1 样品

屠宰猪肝、血液采自北京市某肉联厂。

1.2 酶与试剂

Trizol购自北京普博欣生物科技公司；*Taq*酶、dNTP购自北京博大泰克公司；Oligo(dT)、引物购自上海生工生物工程技术服务有限公司；MMLV Reverta Ace和RNA酶抑制剂为TOYOBO公司产品；NCR quick柱式DNA凝胶回收试剂盒购自新长江生物科技有限公司；各种限制性内切酶、pMD18-T载体为大连宝生物工程有限公司产品。

1.3 SHBV的PCR检测

1.3.1 DNA提取 血清中DNA的提取：取猪血清100 μL加入300 μL TES裂解液(10 mmol/L Tris-HCl，pH8.0，5 mmol/L EDTA，5 g/L SDS，200 mg/L蛋白酶K)，55 ℃消化5 h，用酚-氯仿-异戊醇抽提2～3次，取上清加1/10体积3 mol/L NaAc，再加入2.5倍体积无水乙醇进行沉淀，用700 mL/L乙醇洗沉淀1次，干燥后溶于20 μL灭菌水中备用。

肝中DNA的提取：取新鲜肝组织标本称重约1～2 g，加液氮研成粉末，取约20 mg加入DNA提取液(0.5 mmol/L Tris-HCl，0.02 mol/L EDTA，10 g/L SDS，0.01 mol/L NaCl，500 μg/mL蛋白酶K)，42～48 ℃过夜，用酚-氯仿-异戊醇法提取DNA。

1.3.2 PCR扩增 采用针对HBV S基因保守区设计的引物(见表1)进行PCR扩增。PCR体系：10×PCR缓冲液2.5 μL，引物HBV-FP、HBV-RP各0.5 μL，200 μmol/L dNTPs 0.5 μL，*Taq*酶0.5 μL(2.5 U)，加灭菌水补足至25 μL。扩增条件：94 ℃预变性4 min；94 ℃ 30 s，58 ℃ 30 s，72 ℃ 40 s，30个循环；72 ℃延伸5 min。于10 g/L琼脂糖凝胶中电泳，用凝胶成像仪观察、照相。

表1 用于检测猪体内HBV和HEV的引物

Table 1 Primers used in detection of swine hepatitis B virus and hepatitis E virus

编号 No.	核苷酸序列 Nucleotide sequence	产物大小/bp Amplicon size
HBV-FP	5′-GATGTGTCTGCGGCGTTTTA-3′	281
HBV-RP	5′-CTGAGGCCCACTCCCATAGG-3′	
HE164F1	5′-GCRGTGGTTTCTGGGGTGAC-3′	164
HE164R1	5′-CTGGGMYTGGTCDCGCCAAG-3′	
HE137F2	5′-GYTGATTCTCAGCCCTTCGC-3′	137
HE137R2	5′-GMYTGGTCDCGCCAAGHGGA-3′	

1.3.3 PCR产物的克隆和测序 采用NCR quick柱式DNA凝胶回收试剂盒回收扩增片段，将其克隆至pMD18-T载体，鉴定后送北京奥科生物公司测序，用DNAman(version5.2.2，Lynnon biosoft)分析测序结果。2份送测片段编号分别为SHBV_bj1、SHBV_bj2。

1.4 免疫电镜负染色样品的制备

取HBV表面抗原ELISA检测强阳性和HBV PCR检测阳性反应的血清样品，4 000 r/min离心30 min，取上清液，加入适度稀释的抗HBV单克隆抗体，4 ℃过夜；低温15 000 r/min离心1 h，弃上清液；用少量PBS稀释沉淀；吸取少量悬液于铜网上，用10 g/L醋酸铀负染后，电镜观察。

1.5 HEV RNA的巢式PCR检测

按Trizol法提取总RNA。用Inoue等[11]设计的巢式PCR引物(见表1)进行RT-PCR。逆转录体系：5×逆转录缓冲液6 μL，20 mmol/L Oligo(dT) 0.5 μL，10 mmol/L dNTP 2 μL，MMLV(100

U/μL) 1 μL，DEPC 水 6 μL，RNA 酶抑制剂 1 μL；RNA 经 70 ℃变性 5 min，迅速置于冰上 10 min，取 14 μL 作为模板。混匀后 42 ℃ 1 h，95 ℃ 5 min，取 4 μL 产物作为模板进行 PCR。PCR 体系：10×PCR 缓冲液 2.5 μL，20 mmol/L 引物 HE164F1、HE164R1 各 0.5 μL，200 μmol/L dNTP 0.5 μL，*Taq* 酶 0.5 μL(2.5 U)，加灭菌水补足至 25 μL。按文献[14]进行扩增，第 1 轮扩增结束后，取 2 μL 作为模板进行第 2 轮扩增。最后于 20 g/L 琼脂糖凝胶中电泳，用凝胶成像仪观察、照相。

2　结果

2.1　SHBV 检测

2.1.1　PCR 检测　从屠宰猪肝、血清中提取 DNA，应用 HBV S 基因区引物进行 PCR 扩增，获得的产物大小约为 300 bp，与预期的片段大小相符(见图 1)。

2.1.2　PCR 产物的测序结果　对 SHBV_bj1、SHBV_bj2 片段进行测序，结果发现，所扩增区域与 GenBank 中 HBV 毒株的同源性达 100%。两片段序列仅在第 519、520 位碱基存在差异(见图 2)。

2.1.3　电镜观察　在血清负染色样品中，见到大量的密集排列的病毒样粒子，病毒粒子表面可见明显的表面蛋白颗粒，但未观察到管状颗粒。根据大小不同，这些病毒样粒子主要可分为 2 种类型，一种直径为 40 nm 左右(图 3 细箭头所示)，与人类 HBV 的 Dane 颗粒相类似；另一种直径为 20 nm 左右(图 3 粗箭头所示)，类似于人类 HBV 的小球状粒子。

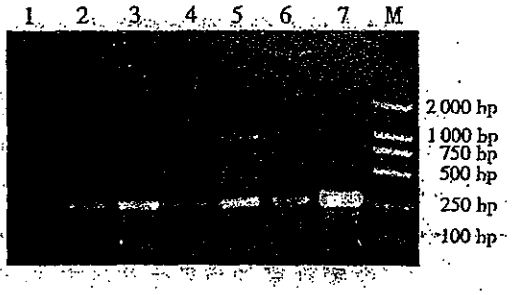


图 1　猪血清和肝中 HBV S 基因 PCR 产物的电泳结果

Fig. 1　Electrophoresis of the PCR-amplified S gene of HBV from porcine livers and sera

M：DNA 分子质量标准；1：阴性对照；2～4：血清样品；5～7：肝样品

M：DL2000 DNA Marker；1：Negative control；2—4：Serum samples；5—7：Liver samples

```
HBV(x04615)   GATGTGTCTGCGGCGTTTTATCATATTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGTTCTTCTGGACTACCAAGGTATGTTGCCCGTTTGT
SHBV_bj1      ....................................................................................................
SHBV_bj2      ....................................................................................................
HBV(x04615)   CCTCTACTTCCAGGAACATCAACTACCAGCACGGGACCATGCAGAACCTGCACGATTCCTGCTCAAGGAACCTCTATGTTTCCCTCTTGTTGCTGTACAA
SHBV_bj1      ..........................................AG..........................................................
SHBV_bj2      ..........................................GA..........................................................
HBV(x04615)   AACCTTCGGACGGAAACTGCACTTGTATTCCCATCCCATCATCCTGGGCTTTCGCAAGATTCCTATGGGAGTGGGCCTCAG
SHBV_bj1      ..................................................................................
SHBV_bj2      ..................................................................................
```

图 2　扩增片段与 HBV X04615 株序列的同源性比较

Fig. 2　Homologous comparison of the amplified S gene of HBV with the published S gene of HBV X04615 strain

2.2　HEV 的 RT-PCR 扩增

经 2 轮扩增后，从部分猪肝组织中扩增到 1 条约 137 bp 的产物带，与预期片段大小相符(见图 4)。

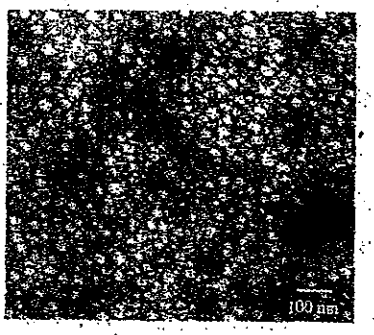


图 3　电镜下观察到的猪乙肝病毒颗粒

Fig. 3　Porcine HBV particles observed by TEM

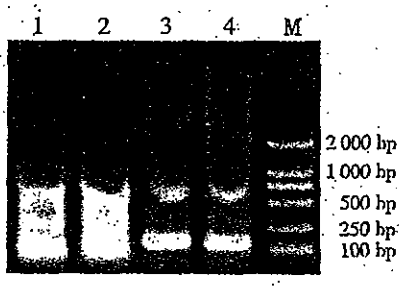


图 4　屠宰猪肝 HEV 的巢式 RT-PCR 电泳结果

Fig. 4　Electrophoresis of the nested RT-PCR-amplified fragment of HEV from porcine livers

M：DNA 分子质量标准；1、2：第 1 轮扩增结果；3、4：第 2 轮扩增结果

M：DL2000 DNA Marker；1，2：Products of the first PCR；3，4：Products of the second PCR

3 讨论

3.1 关于猪乙肝病毒

关于动物乙肝病毒的检测，国内已有一些报道，但有关屠宰猪体内乙肝病毒的检测鲜有报道[15]。本试验在过去观察研究的基础上，应用PCR技术和透射电镜技术对屠宰猪血清和肝中HBV抗原进行了检测。电镜负染色样品观察结果表明，在HBV表面抗原ELISA检测强阳性反应的血清样品中存在有形态、大小与人HBV Dnae颗粒和小球状颗粒相似的病毒粒子。在人HBV携带者血清中一般以小球状颗粒为主，Dnae颗粒较少，从本试验观察结果来看，在电镜观察的负染色样品中Dnae颗粒并不少(见图3)。

目前，国外尚未见畜禽HBV的相关报道，国内现有的研究多采用HBV检测试剂进行血清标志物和相关抗原的检测，也有人对其形态和S区基因等进行了研究，但对于动物的HBV分子病毒学及其致病性、与人HBV之间的关系等的研究还很少。本试验应用HBV S区引物从屠宰猪血清、肝中检测出了预期片段，序列分析结果表明，与HBV同源性高达98%～100%。虽然本试验所测片段仅占HBV全基因组的9%左右，但这至少在一定程度上说明了猪HBV与人HBV有较高的同源性。

一般认为，畜禽乙肝病毒对人没有致病性，但对动物是否有致病性，经肉类食品进入人体后是否可以引起相应的免疫应答，现在尚未可知。我国约有1.2亿人为乙肝病毒携带者，这么高的感染率是否与畜禽乙肝病毒有某种联系，这个问题值得进一步研究。

由于HBV至今无法体外培养，且宿主范围非常狭窄，尚没有合适的小型动物作为动物模型供病原、发病机理、疫苗和治疗性药物的研究，加之道德等原因，非人灵长类动物模型的使用受到了限制[1]。畜禽乙肝病毒的发现不仅将为嗜肝病毒科增加新的成员，也必将为嗜肝病毒的起源、进化、持续性感染、发病机理、慢性病毒性肝细胞癌起源等方面的研究提供研究对象。

3.2 关于戊肝病毒

很多研究结果表明，HE是一种人畜共患病，经口感染，猪是重要的储存宿主。日本、印度等国家已发生多起因食用未煮熟的猪肝和猪肉而引起人感染HEV的报道，日本、美国的研究表明，与猪接触的职业人群的血清抗HEV抗体高于非职业人群，在猪场周围的污水中能检测到HEV的存在[16-18]。曹海俊等[19]对浙江地区从事生猪屠宰和销售的职业人群的HEV感染情况进行了调查，结果显示，浙江省从事生猪屠宰和销售人群的阳性率为77.25%，远远高于1992年全国13个省市HE血清流行病学调查的1～59岁人口HEV阳性率(17.2%)。还有报道表明，我国4月龄以上的猪血清抗体阳性率平均为40%，而猪饲养员的血清抗体阳性率高达100%；泰国3月龄以上的猪阳性率为9%～20%；其饲养员的阳性率为71%。上述研究结果说明，人的HE阳性检出率与从事和猪接触的相关职业有一定的关系，也说明HE是一种人兽互传病。

Meng[20]曾在不同月龄的猪血清中检测出了HEV RNA，国内外还未见有屠宰猪肝HEV RNA的检出报道。本试验应用RT-PCR方法从屠宰猪肝中扩增出了HEV RNA，说明屠宰猪肝中也存在HEV RNA。本实验室过去的研究结果表明，在屠宰猪肝中HEV相关抗原的阳性检出率高达95%～100%，这是很值得注意的问题，因为从公共卫生的角度来看，屠宰猪已经进入到了猪肉品生产链的末端。虽然还没有从猪胴体肉中检测到HEV的报道，但肝中HEV相关抗原的阳性检出率如此之高，无疑会对人类健康构成潜在的威胁。因此，笔者建议在屠宰猪检疫中将HEV列入检测项目。

参考文献(References)

[1] 陈欣如，燕顺生，张勇. 野生动物嗜肝病毒的研究进展[J]. 地方病通报，2006，21(1)：89-94.
CHEN Xin-ru, YAN Shun-sheng, ZHANG Yong. Advance in hepadnaviruses in wild life[J]. *Endemic Diseases Bulletin*, 2006, 21(1): 89-94. (in Chinese)

[2] 徐宜为，初秀. 动物的类乙型肝炎研究进展[J]. 中国兽医科技，1993，23(7)：16-20.
XU Yi-wei, CHU Xiu. Study progress on HBV-like virus in animals[J]. *Chinese Journal of Veterinary Science and Technology*, 1993, 23(7): 16-20. (in Chinese)

[3] 沈柏青，任政华. 猪源乙型肝炎病毒样病毒的提纯与鉴定[J]. 黑龙江畜牧兽医，1993(7)：7-9.
SHEN Bai-qing, REN Zheng-hua. Purification and identification of pig HBV-like virus[J]. *Heilongjiang Animal Science and Veterinary Medicine*, 1993(7): 7-9. (in Chinese)

[4] 律祥君，侯安祖. 黄牛感染人乙型肝炎病毒的研究[J]. 中国兽医科技，1992，22(4)：3-4.
LÜ Xiang-jun, HOU An-zu. Studies on human hepatitis B virus infection in farm cattle[J]. *Chinese Journal of Veterinary Science and Technology*, 1992, 22(4): 3-4. (in Chinese)

[5] 李块，李红，张鹏举，等. PCR和ELISA法检测畜禽类乙型肝炎病毒的研究[J]. 郑州牧专学报，1997，17(1)：21-23.

LI Jue, LI Hong, ZHANG Peng-ju, *et al*. Detection of hepatitis B like virus in chicken by PCR and ELISA[J]. *Journal of Zhengzhou College of Animal Husbandry Engineering*, 1997, 17(1): 21-23. (in Chinese)

[6] 杜念兴,黄吉凤.从猪和牛检出类乙肝病毒抗原和抗体[J].畜牧与兽医,2002,34(1):3-5.
DU Nian-xing, HUANG Ji-feng. Detection of hepatitis type B virus-like antigen and antibody from pigs and cattle[J]. *Animal Husbandry and Veterinary Medicine*, 2002, 34(1): 3-5. (in Chinese)

[7] 丁壮,金宁一,陈创夫,等.羊源乙肝病毒与人乙肝病毒 S 基因序列同源性研究[J].动物医学进展,2001,22(4):54-58.
DING Zhuang, JIN Ning-yi, CHEN Chuang-fu, *et al*. Study on S gene sequence homologous analysis between the hepatitis B virus from sheep and human[J]. *Progress in Veterinary Medicine*, 2001, 22(4): 54-58. (in Chinese)

[8] 丁壮,金宁一,陈振文,等.鸡源类人乙肝病毒与人乙肝病毒 S 基因序列分析[J].中国兽医学报,1999,19(1):18-21.
DING Zhuang, JIN Ning-yi, CHEN Zhen-wen, *et al*. S gene sequence comparison between the HBV like virus from chicken and human HBV[J]. *Chinese Journal of Veterinary Science*, 1999, 19(1): 18-21. (in Chinese)

[9] 丁壮,王承宇,金宁一,等.犬乙肝病毒 S 基因遗传变异研究[J].中国预防兽医学报,2003,25(1):24-28.
DING Zhuang, WANG Cheng-yu, JIN Ning-yi, *et al*. Hereditary variation in S gene sequence of hepatitis B virus from canine[J]. *Chinese Journal of Preventive Veterinary Medicine*, 2003, 25(1): 24-28. (in Chinese)

[10] 许江城,余锐萍,林剑波,等.应用免疫组织化学方法检测屠宰猪肝脏中人乙型肝炎病毒抗原[J].中国兽医杂志,2004,40(4):46-47.
XU Jiang-cheng, SHE Rui-ping, LIN Jian-bo, *et al*. Immunohistochemistry detection of human hepatitis B virus antigen in slaughtered swine livers[J]. *Chinese Journal of Veterinary Medicine*, 2004, 40(4): 46-47. (in Chinese)

[11] 余锐萍,李文贵,王英华,等.病毒性肝炎:值得警惕的重要人兽互传病[J].科技导报,2007,25(4):44-52.
SHE Rui-ping, LI Wen-gui, WANG Ying-hua, *et al*. Viral hepatitis: a dangerous zoonosis[J]. *Science and Technology Review*, 2007, 25(4): 44-52. (in Chinese)

[12] RENOU C J, CADRANEL J F, BOURLIERE M, *et al*. Possible zoonotic transmission of hepatitis E from pet pig to its owner[J]. *Emerg Infect Dis*, 2007, 13(7): 1094-1096.

[13] COLSON P, KABA M, BERNIT E, *et al*. Hepatitis E associated with surgical training on pigs[J]. *The Lancet*, 2007, 370(9591): 935.

[14] INOUE J, TAKAHASHI M, YAZAKI Y, *et al*. Development and validation of an improved RT-PCR assay with nested universal primers for detection of hepatitis E virus strains with significant sequence divergence[J]. *J Virol Methods*, 2006, 137(2): 325-333.

[15] 余锐萍,郑志伟,江维文,等.屠宰猪血清及肝脏中人乙型肝炎表面抗原的检测[J].中国畜禽传染病,1998,20(增刊):160-161.
SHE Rui-ping, ZHENG Zhi-wei, JIANG Wei-wen, *et al*. Detection of human hepatitis B surface antigen in serum and liver of slaughtered pigs[J]. *Chinese Journal of Animal and Poultry Infectious Diseases*, 1998, 20(Suppl): 160-161. (in Chinese)

[16] DROBENIUC J, FAVOROV M O, SHAPIRO C N, *et al*. Hepatitis E virus antibody prevalence among persons who work with swine[J]. *J Infect Dis*, 2001, 184(12): 1594-1597.

[17] MENG X J, 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]. *J Clin Microbiol*, 2002, 40(1): 117-122.

[18] WU J, CHEN C, CHIANG T, *et al*. Potential subclinical spread of hepatitis E virus among swine, swine handlers and different countries: a longitudinal study[J]. *J Hepatol*, 2001, 34(1): 190.

[19] 曹海俊,王法弟,高眉扬,等.生猪屠宰销售职业人群戊型肝炎病毒感染的危险因素研究[J].中国人兽共患病杂志,2004,20(7):607-609.
CAO Hai-jun, WANG Fa-di, GAO Mei-yang, *et al*. Risk of contract with hepatitis E virus in occupational populations [J]. *Chinese Journal of Zoonoses*, 2004, 20(7): 607-609. (in Chinese)

[20] MENG X J. Hepatitis E virus: cross-species infection and zoonotic risk[J]. *Clin Microbiol New*, 2005, 27(6): 43-48.

(责任编辑　胡弘博)

食肉処理したブタの肝臓および血清中における B 型肝炎ウイルスおよび E 型肝炎ウイルス検査

ヘパドナウイルス科 (Hepadnaviridae) はオルトヘパドナウイルス属 (Orthohepadnavirus)とトリヘパドナウイルス属(Avihepadnavirus)の 2 属に分類される。オルトヘパドナウイルス属にはヒト B 型肝炎ウイルス(HBV)、霊長類ヘパドナウイルス、げっ歯類ヘパドナウイルスがあり、トリヘパドナウイルスにはアヒル B 型肝炎ウイルス、アオサギ B 型肝炎ウイルス、ハクガン B 型肝炎ウイルスなどがある。これらのウイルスは、ゲノム全長が 3.0~3.3kb であること、一部に環状二重鎖 DNA 構造をとること、外側にエンベロープを有すること、またコア内部にゲノムおよびウイルスにコードされた特殊な DNA ポリメラーゼを有することを共通の特徴とする。ウイルス粒子以外にも大量のウイルス外皮タンパク粒子を生成し、宿主域は極めて狭く、通常、ウイルスの持続感染を引き起こし明らかな肝向性を有する[1]。データによれば禽獣においても肝向性ウイルス群の存在が確認されている。1980 年代以降、中国においてブタ、ニワトリ、ウシ、ヒツジ、イヌなどの動物の B 型肝炎ウイルス表面抗原 (HBsAg)検出に関する報告が多くなり、各種 B 型肝炎ウイルスの分離が報告されるようになった[2~9]。2006 年、余鋭萍[10~11]らは免疫組織化学法を応用し、北京、河南などの食肉生産連合において豚の食肉処理およびその肝臓の解体に従事する人員の B 型肝炎ウイルス抗原検査を実施した結果、B 型肝炎ウイルス抗原の検出率は実に 73~100%という高率に達することが明らかになった。

E 型肝炎(Hepatitis E, HE)は E 型肝炎ウイルス(Hepatitis E virus, HEV)感染により引き起こされ、腸を経由して伝播される急性ウイルス性肝炎であり、主要な伝播経路は排泄物を介した経口感染である。中国は HE 高発症地域であり、E 型肝炎の発症および E 型肝炎での死亡により引き起こされる経済的負担が最も深刻な国の 1 つに数えられている。ブタが HEV の主要な宿主であると提唱する学者も存在し、日本においては HEV に汚染された食用ブタの肝臓からヒトへの感染が報告されている。E 型肝炎非流行地域におけるブタに関連する職業の従事者群と対照群の研究では、ブタに関連する職業の従事者に HEV 感染リスクの上昇が認められた。さらに、近年ではブタ HEV のヒトへの感染を示す複数の証拠が発見されている[12、13]。つまり、HB および HE はヒトの健康に深刻な脅威を与える疾患であり、ヒトのウイルス性肝炎の発症率は今なお上昇し続けている。動物、とりわけヒトとの関係が緊密なブタ体内の HBV および HEV 保有状況、ブタ HBV、HEV とヒト HBV、HEV の相同性などについては未だ明らかにされていない部分が多い。本試験では既存の研究を基礎とし、PCR 技術を応用し、食肉処理したブタの血清および肝臓の HBV S 遺伝子、HEV RNA に対しさらなる検査を行うとともに、免疫電子顕微鏡を用いたネガティブ染色法にて食肉処理したブタ血清中の HBV の観察を行った。

1 材料および方法

1.1 サンプル

食肉処理したブタの肝臓および血液は、北京の某食肉生産連合にて採集を行った。

1.2 酵素および試薬

Trizol は北京普博欣生物科学技術公司より購入した。*Taq* 酵素、dNTP は北京博大泰克公司より購入した。Oligo(dT)、プライマーは上海生工生物工程科学技術サービス有限公司より購入した。MMLV Revertra Ace および RNA 酵素阻害剤は TOYOBO 社(東洋紡)の製品とした。NCR quick カラム式 DNA ゲル回収試薬キットは新長江生物科技有限公司より購入した。各種制限酵素、pMD18-T キャリヤーは大連宝生物工程有限公司の製品とした。

1.3 SHBV の PCR 検査

1.3.1 DNA 抽出　血清中の DNA 抽出：ブタの血液 100μL を採取し、TES 分解液 300μL(10mmol/L Tris-HCl、pH8.0、5mmol/L EDTA、SDS 5g/L、プロテイナーゼ K 200mg/L)中に加え、55℃下にて 5 時間消化を行い、フェノール-クロロフォルム-イソアミルアルコールを用いて 2～3 回抽出を行った。その後、上澄みを採取し 1/10 体積の 3mol/L NaAc および 2.5 倍体積の無水エチルアルコールを順次加えて沈殿を行い、エチルアルコール 700mL/L を用いて沈殿を 1 回洗浄し、乾燥後に滅菌水 20μL 中に溶解し保存した。

肝臓中の DNA 抽出：新鮮な肝臓組織標本約 1～2g を秤量し、液体窒素を加えて粉末状にしたものを、約 20mg 採取して DNA 抽出液(0.5mmol/L Tris-HCl、0.02mol/L EDTA、SDS 10g/L、0.01mol/L NaCl、プロテイナーゼ K 500μg/mL)中に加え、42～48℃下にて一晩静置した。その後、フェノール-クロロフォルム-イソアミルアルコールを用いて DNA の抽出を行った。

1.3.2 PCR による増幅　HBV S 遺伝子保存領域をターゲットとして設計されたプライマー(表 1 を参照のこと)を用いて PCR による増幅を行った。PCR システム：10×PCR 緩衝液 2.5μL、プライマーHBV-FP、HBV-RP 各 0.5μL、200μmol/L dNTPs 0.5μL、*Taq* 酵素 0.5μL(2.5U)に滅菌水を加え 25μL とした。増幅条件：94℃下にて 4 分間予備変性を行った後、94℃下にて 30 秒、58℃下にて 30 秒、72℃下にて 40 秒の変性を 30 サイクル行った。さらに 72℃下にて 5 分間伸張を行い、アガロースゲル 10g/L 中にて電気泳動を行い、ゲルイメージングシステムを用いて観察および画像の記録を行った。

1.3.3 PCR 生成物のクローンおよびシークエンシング　NCR quick カラム式 DNA ゲル回収試薬キットを用いて回収した増幅断片のクローンを pMD 18-T キャリヤーに吸着させ、評定を行った後、北京奥科生物公司に送付してシークエンシングを行い、DNAman(version5.2.2、Lynnon biosoft)を用いてシークエンシング結果に対する分析を行った。増幅断片 2 サンプルの番号はそれぞれ SHBV_bj1、SHBV_bj2 とした。

1.4 免疫電子顕微鏡を用いたネガティブ染色サンプルの調製

ELISA 法にて HBV の表現抗原に強陽性を示す血清サンプルおよび HBV PCR 検査にて陽性反応を示す血清サンプルを採取し、4000r/分にて 30 分間遠心分離を行い、上

澄みを採取した後、適切に希釈を行った抗 HBV 単クローン抗体中に加え、4℃下にて一晩静置した。その後、低温下において 15000r/min にて 1 時間遠心分離を行い、上澄みを廃棄し、少量の PBS を用いて沈殿の希釈を行った。少量の懸濁液を銅メッシュに吸着させ、ウラニルアセテート 10g/L を用いてネガティブ染色を行った後、電子顕微鏡による観察を行った。

1.5 ネステッド PCR 法を用いた HEV RNA の検出

Trizol 法に照らして総 RNA を抽出した。Inoue ら[11]の設計によるネステッド PCR プライマー(表 1 を参照のこと)を用いて RT-PCR を行った。逆転写システム：5×逆転写緩衝液 6μL、20mmol/L Oligo(dT)0.5μL、10mmol/L dNTP 2μL、MMLV(100U/μL)1μL、DEPC 水 6μL、RNA 酵素阻害剤 1μL とした。70℃下にて 5 分間変性を行った RNA を採取し、速やかに氷上に 10 分間静置した後、採取した生成物 14μL をテンプレートとした。さらに 42℃下にて 1 時間、95℃下にて 5 分間混合し、得られた生成物 4μL をテンプレートとして PCR を行った。 PCR システム：10×PCR 緩衝液 2.5μL、20mmol/L プライマー HE164F1、HE164R1 各 0.5μL、200μmol/L dNTP 0.5μL、*Taq* 酵素 0.5μL(2.5U) に滅菌水を加え、25μL とした。文献[14]に照らして増幅を行い、第 1 サイクルの増幅終了後に 2μL を採取し、これを第 2 サイクルの増幅におけるテンプレートとした。最後にアガロースゲル 20g/L 中にて電気泳動を行い、ゲルイメージングシステムを用いて観察および画像の記録を行った。

2 結果

2.1. SHBV 検査

2.1.1 PCR 検査 食肉処理したブタの肝臓、血清中より DNA を抽出し、HBV S 遺伝子領域のプライマーを用いて PCR による増幅を行った結果、獲得された生成物のサイズは 300bp であり、予測断片のサイズと近似するものであった(図 1 を参照のこと)。

2.1.2 PCR 生成物のシークエンシング結果 SHBV_bj1、SHBV_bj2 の各断片に対するシークエンシングを行った結果、すべての増幅領域および GenBank 中における HBV ウイルス株の相同性は 100%に達することが明らかになった。両断片の序列はわずかに 519、520 位の塩基に差異が認められるのみであった(図 2 を参照のこと)。

2.1.3 電子顕微鏡による観察 血清のネガティブ染色サンプルに大量かつ密集した配列のウイルス様粒子が観察され、ウイルス粒子表面には鮮明な表面タンパク粒子が認められたものの、管状粒子は認められなかった。これらウイルスはそのサイズに基づき 2 種類に分けることができ、うち一方は、直径が約 40nm(図 3 中、細矢印にて表示)であり、ヒト HBV の Dane 粒子と類似する。もう一方は直径約 20nm(図 3 中、太矢印にて表示)であり、ヒト HBV の小球状粒子と類似する。

2.2 HEV の RT-PCR による増幅

2 サイクルの増幅を行い、ブタの一部肝臓組織中より増幅された約 137bp の帯状生成物のサイズは予測断片サイズと一致するものであった(図 4 を参照のこと)。

3 考察

3.1 ブタのB型肝炎ウイルスについて

動物の B 型肝炎ウイルスの検出については中国においても複数の報告がされているが、食肉処理したブタ体内の B 型肝炎ウイルスの検出に関する報告は稀有なケースである[15]。本試験では既存の研究を基礎としつつ、PCR 技術および透過電子顕微鏡技術を応用し、食肉処理したブタの血清および肝臓中の HBV 抗原に対する検査を行った。電子顕微鏡を用いてネガティブ染色サンプルを観察した結果、ELISA 法にて HBV の表現抗原に強陽性を示す血清サンプルに、ヒト HBV の Dane 粒子および小球状粒子に形態およびサイズの類似するウイルス粒子の存在が認められた。ヒト HBV キャリアの血清中においては、通常、小球状粒子が多数を占め、Dane 粒子は少数であるが、本試験では電子顕微鏡による観察を行ったネガティブ染色サンプル中に数多くの Dane 粒子が存在する結果となった(図 3 を参照のこと)。

現在、海外において禽獣の HBV に関する報告はなく、中国においては HBV 検査試薬を用いた血清マーカーおよび関連抗原の検査、ならびにウイルスの形態および遺伝子の S 領域などに対する研究に従事する研究者は存在するものの、動物の HBV に対する分子ウイルス学およびその病原性に関する研究、ヒトの HBV との関連性に関する研究は非常に少ない。本試験において HBV S 領域のプライマーを用いてブタの血清および肝臓中より予測断片を検出し、シークエンシング分析を行った結果、HBV の相同性は実に 98～100%に達することが明らかになった。本試験において検出を行った断片の占める割合は HBV 全遺伝子の約 9%に過ぎないが、少なくとも一定レベルにおいてブタ HBV とヒト HBV が高い相同性を有することを証明した。

一般的には、禽獣の B 型肝炎ウイルスはヒトに対する病原性を持たないと認識されているが、動物に対する病原性の有無、ならびに食肉および食肉加工製品を介して人体に摂取された後にこれに対する免疫反応を引き起こす可能性の有無については、現在もなお不明である。中国には 1.2 億人の B 型肝炎キャリアが存在すると見られ、この高い感染率に禽獣の B 型肝炎ウイルスが何らかの関連を持つか否かについては、今後さらに研究を進める価値がある。

HBV は現在においても体外での培養が不可能であり、また宿主領域が極めて狭いことから、適切な小型動物を動物モデルとした病原、発症機序、ワクチンおよび治療薬に対する研究はなく、さらに倫理的理由からヒトを除く霊長類動物モデルの使用は制限を受ける[1]。禽獣の B 型肝炎の発見は、肝向性ウイルス学に新たな研究対象を追加するのみならず、肝向性ウイルスの起源、進化、持続感染、発症機序、慢性ウイルス性肝細胞ガンの起源など各方面の研究に新たな研究対象を提供する。

3.2 E型肝炎について

多数の研究を通じ、HE は人畜共通感染症であること、経口感染すること、またブタが重要なウイルスキャリアであることが明らかになった。日本、インドなどでは加

熱不十分な食用ブタレバーおよびブタ肉の摂取による HEV 感染が報告されている。日本、米国の研究においてはブタと接触する職業に従事する人員群の血清抗 HEV 抗体はその他職業に従事する人員群よりも高く、また養豚場周辺の汚水中から HEV が検出されたことも明らかになった[16~18]。曹海俊[19]らが、浙江地域においてブタの食肉処理および販売に従事する人員群の HEV 感染状況について調査を行った結果、浙江省にてブタの食肉処理および販売に従事する人員群の 77.25%が HEV 陽性であり、1992 年に全国 13 省および市において実施された HE 血清流行病調査中の 1～59 歳人口に占める陽性率(17.2%)をはるかに上回ることが明らかになった。さらに別の報告では、中国の月齢 4 ヶ月以上のブタにおける血清抗体陽性率の平均が 40%にのぼり、ブタの飼育者の血清抗体陽性率に至っては 100%に達することも明らかになっている。その他タイでは、月齢 3 ヶ月以上のブタにおける陽性率が 9～20%に達し、ブタの飼育者の陽性率は 71%にのぼる。上記の研究結果は、ヒトの HE 陽性検出率とブタに接触する職業への従事者の間に一定の関連性があること、ならびに HE は人畜相互間の感染症であることを証明するものである。

かつて Meng[20]は異なる月齢のブタ血清から HEV RNA の検出を行ったが、中国内外において食肉処理したブタの肝臓から HEV RNA の検出を行ったという報告はない。本試験では RT-PCR 法を用いて食肉処理したブタ肝臓中の HEV RNA の増幅を行った結果、食肉処理したブタの肝臓中にも HEV RNA の存在が確認された。本試験室における過去の研究において食肉処理したブタ肝臓中の HEV に関連を有する抗原の陽性検出率が 95～100%と高率にのぼることが明らかになり、また食肉処理したブタが精肉製品生産網の末端に組み込まれていることを考慮すると、公衆衛生の見地からも、この問題は決して放置できない問題である。現在のところ、ブタの生肉中より HEV が検出されたという報告はなされていないものの、肝臓中の HEV に関連する抗原の陽性検出率が上記のように高いことが、人体の健康に対する潜在的な脅威となることは確実である。以上より、筆者は食肉処理したブタの検疫において HEV を検査項目として採用することを提案する。

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. 8. 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	Girou E, Chevaliez S, Challine D, Thiessart M, Morice Y, Lesprit P, Tkoub-Scheirlinck L, Soing-Altrach S, Cizeau F, Cavin C, André M, Dahmanne D, Lang P, Pawlotsky JM. Clin Infect Dis. 2008 Sep 1;47(5):627-33.	公表国 フランス
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

○環境汚染および標準的予防法の非遵守が血液透析装置のC型肝炎感染リスクに及ぼす影響

背景:C型肝炎ウイルス(HCV)感染症の原因の第2位は院内感染である。環境汚染および血液透析装置のHCV交差感染を予防する標準的予防法の非遵守が院内感染に及ぼす影響を検討するため、前向き観察試験を実施した。

方法:フランスの大学病院において長期血液透析を受けている患者を系統的にスクリーニングし、HCV散発感染症例2例が認められた。試験を行い、当該患者の感染が血液透析装置によるものかどうかを判定し、環境汚染と予防のための標準法非遵守が院内感染に影響する可能性を調べた。新規HCV感染症例と、血液およびHCV RNAによる環境汚染、手の衛生と手袋使用ガイドラインの非遵守との関連性を検討した。

結果:患者2例が試験期間中にHCV抗体陽性となった。系統発生解析では、これらの患者の1例が、同一ユニット内で治療を受けている慢性感染患者と相同なウイルス株に感染していることが示された。環境表面検体740検体中82検体(11%)にヘモグロビンが含まれ、6検体(7%)にHCV RNAが含まれていた。手の衛生に関する遵守率は37%(95%信頼区間、35%～39%)であり、患者ケアの直後に手袋をはずしていたのは33%(95%信頼区間、29%～37%)だった。環境表面のヘモグロビンの存在を予測する独立因子は、患者に対する看護師数の少なさ、手の衛生の不良であった。

結論:血液に汚染された表面は、血液透析装置がHCV交差感染の原因である可能性がある。血液透析患者間のHCV交差感染リスクを低減させるためには、手の衛生および手袋使用の厳重遵守と治療手順の徹底が必要である。

報告企業の意見	今後の対応
フランスの大学病院において長期血液透析を受けている患者2名がHCVに感染し、患者に対する看護師数の少なさと手の衛生の不良が、病院内の環境汚染の原因であることが示された。院内感染リスク低減のためには、手の衛生および手袋使用の厳重遵守と治療手順の徹底が必要であるとの報告である。輸血後HCV感染の調査では、院内感染など輸血以外の伝播ルートについて考慮する必要がある。	日本赤十字社では、HCV抗体検査を実施することに加えて、HCVについて20プールでスクリーニングNATを行い、陽性血液を排除している。また、これまでの凝集法と比べて、より感度の高い化学発光酵素免疫測定法(CLEIA)及び精度を向上させた新NATシステムを導入した。HCV感染に関する新たな知見等について今後も情報の収集に努める。

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

MAJOR ARTICLE

Determinant Roles of Environmental Contamination and Noncompliance with Standard Precautions in the Risk of Hepatitis C Virus Transmission in a Hemodialysis Unit

Emmanuelle Girou,[1,2] Stéphane Chevaliez,[2] Dominique Challine,[2] Michaël Thiessart,[1] Yoann Morice,[2] Philippe Lesprit,[1] Latifa Tkoub-Scheirlinck,[1] Sophan Soing-Altrach,[1] Florence Cizeau,[1] Celine Cavin,[1] Martine André,[3] Djamel Dahmanne,[3] Philippe Lang,[3] and Jean-Michel Pawlotsky[2]

[1]Infection Control Unit, [2]French National Reference Center for Viral Hepatitis B, C, and delta, Department of Virology & INSERM U635, and [3]Nephrology Ward, Henri Mondor Hospital, Assistance Publique–Hôpitaux de Paris, Université Paris 12, Créteil, France

Background. Nosocomial transmission is the second most frequent cause of hepatitis C virus (HCV) infection. A prospective observational study was conducted to assess the roles of environmental contamination and noncompliance with standard precautions in HCV cross-transmission in a hemodialysis unit.

Methods. Patients undergoing chronic hemodialysis in a French university hospital unit were systematically screened, revealing 2 sporadic cases of HCV transmission. An investigation was launched to determine whether the patients were infected in the hemodialysis unit and the possible roles of environmental contamination and noncompliance with standard precautions. We examined possible relationships among new cases of HCV infection, environmental contamination by blood and HCV RNA, and compliance with guidelines on hand hygiene and glove use.

Results. Two patients experienced seroconversion to HCV during the study period. Phylogenetic analyses showed that 1 of these patients was infected with the same strain as that affecting a chronically infected patient also treated in the unit. Of 740 environmental surface samples, 82 (11%) contained hemoglobin; 6 (7%) of those contained HCV RNA. The rate of compliance with hand hygiene was 37% (95% confidence interval, 35%-39%), and gloves were immediately removed after patient care in 33% (95% confidence interval, 29%-37%) of cases. A low ratio of nurses to patients and poor hand hygiene were independent predictors of the presence of hemoglobin on environmental surfaces.

Conclusion. Blood-contaminated surfaces may be a source of HCV cross-transmission in a hemodialysis unit. Strict compliance with hand hygiene and glove use and strict organization of care procedures are needed to reduce the risk of HCV cross-transmission among patients undergoing hemodialysis.

Hepatitis C virus (HCV) infection is a major health problem. Worldwide, >170 million individuals carry the virus, and the infection becomes chronic in ~80% of adult cases. Approximately 20% of patients with chronic HCV infection develop cirrhosis, and the incidence of hepatocellular carcinoma is 4%–5% per year in cirrhotic patients [1].

HCV is principally, if not exclusively, transmitted by blood. Historically, the 2 main routes of transmission have been blood transfusion and injection drug use. Since the implementation, in the United States and Europe, of blood-donor screening with highly sensitive EIAs for anti-HCV antibodies and minipool testing for HCV RNA, the incidence of transfusion-transmitted hepatitis C has decreased to ~1 case per 2 million transfused blood units [2, 3]. In France, 3000–4000 new cases of HCV infection still occur every year [4]. Approximately two-thirds of these cases are related to injection drug use, but nosocomial transmission is the

Received 14 November 2007; accepted 7 May 2008; electronically published 28 July 2008.

Presented in part: 45th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, D.C., 2005 (abstract K-1113), and at the 2nd Society of Hospital Epidemiology of America Annual Meeting, Chicago, 2006.

Reprints or correspondence: Dr. Emmanuelle Girou, Unité de Contrôle, Epidémiologie et Prévention de l'Infection, Hôpital Henri Mondor, 51 Ave. du Maréchal de Lattre de Tassigny, 94010 Créteil, France (emmanuelle.girou@hmn.aphp.fr).

Clinical Infectious Diseases 2008; 47:627–33
© 2008 by the Infectious Diseases Society of America. All rights reserved.
1058-4838/2008/4705-0007$15.00
DOI: 10.1086/590564

second most common source of HCV infection. Most cases of HCV transmission in the hospital setting are attributable to patient-to-patient transmission through invasive procedures, such as insertion of an intravascular catheter, colonoscopy, sharing of dialysis equipment, surgery, and sharing of multidose vials [5–11].

The prevalence of HCV infection is high among patients who undergo hemodialysis, because of both contaminated transfusions before the early 1990s and nosocomial transmission. Several outbreaks and sporadic cases of nosocomial HCV or hepatitis B virus transmission in dialysis units have been linked to poor disinfection of dialysis equipment and to poor compliance with standard infection-control measures [9, 12–18]. However, the exact route and mechanism of transmission were unknown in most cases. Here, we examined the intricate roles of noncompliance with standard precautions, environmental contamination, and low nurse-to-patient ratio in cross-transmission of HCV within a dialysis unit.

PATIENTS AND METHODS

Setting and patients. Henri Mondor University Hospital has a 9-bed hemodialysis unit that mainly treats patients with chronic renal failure. A case of HCV seroconversion was detected by systematic screening during the study period. The study period was defined as the interval between the probable date of infection and the discovery of the index case—that is, January–September 2004. Patients' medical files were exhaustively reviewed to eliminate a potential external source of HCV transmission. None of the health care personnel was known to be infected with HCV. No systematic screening of personnel was undertaken. No isolation policy was implemented in the unit. Multidose vials were not in use in the unit.

All patients who undergo regular hemodialysis are screened for anti-HCV antibodies every 3 months, in an effort to detect seroconversion. On 27 July 2004, a case of HCV seroconversion was detected through this screening. To determine whether this case was sporadic or part of an outbreak, all 52 patients with chronic renal failure who were undergoing regular hemodialysis in the unit were tested for anti-HCV antibodies and HCV RNA, as were all patients treated for acute renal failure in the unit during the at-risk period. Six (12%) of the 52 patients (patients 3–8) were known to be chronically infected with HCV, with HCV RNA levels ranging from 4.4 to >6.9 $\log_{10}$ IU/mL at the time of the study. All but 1 of these patients were to known to have been infected for several years (e.g., patient 3 has been infected since 2001). A second patient undergoing hemodialysis was found to be HCV RNA positive through culture of a blood sample obtained in July 2004 (tests for anti-HCV antibodies were negative), and an investigation was then launched.

Virological studies and phylogenetic analyses. Anti-HCV antibodies were detected with a third-generation EIA (Vitros ECi; Ortho-Clinical Diagnostics). We tested for HCV RNA in all patients' blood and in hemoglobin-positive surface swab eluates through use of a sensitive RT-PCR assay (Amplicor HCV, version 2.0; Roche Molecular Systems), with a detection limit of 50 IU/mL.

To estimate the genetic relatedness of HCV strains, 2 HCV genomic regions were PCR amplified and sequenced, including a 328–base pair portion of the nonstructural 5B (NS5B) coding region (nucleotide positions 8271–8597) and the 81–base pair region coding for hypervariable region 1 (HVR1) of the E2 envelope glycoprotein.[19]. HCV genotyping was based on phylogenetic analysis of NS5B sequences, which included prototype sequences of various subtypes of HCV genotypes 1–6. The genetic relatedness of HCV strains was studied by phylogenetic analysis of both the NS5B and HVR1 regions. Sequences were aligned with ClustalW software [20]. Phylogenetic relationships were deduced with the DNADIST-NEIGHBOR module of the Phylogeny Interference Package, version 3.5 [21]. For neighbor-joining analysis, a distance matrix was calculated using a Kimura 2-parameter distance matrix with a transition/transversion ratio of 4.0. Trees were drawn with TREVIEW or NJ-Plotprograms [22]. Their robustness was assessed by bootstrap analysis of 1000 replicates with the SEQBOOT module of the Phylogeny Interference Package program.

The index patient (patient 1) experienced HCV seroconversion in July 2004. The second case of HCV seroconversion during the study period (patient 2) was identified by systematic screening for HCV RNA. To determine whether chronically infected patients were the source of the new cases, the sequences of 2 HCV genomic regions, including a portion of the NS5B coding sequence and the sequence coding for HVR1, were compared among the 8 infected patients, relative to reference sequences. Phylogenetic analyses of the NS5B region (figure 1) and the HVR1 (figure 2) showed that newly infected patient 2 was infected with the same HCV genotype 1 strain as was chronically infected patient 3. In contrast, patient 1 was infected with an HCV genotype 3a strain that was unrelated to the strains infecting the other 6 chronically infected patients (all infected with genotype 1). Despite the proximity of the HCV strains from patients 4–8 in the NS5B phylogenetic tree (figure 1), HVR1 analysis showed that those patients were infected with unrelated strains (figure 2).

Thus, 2 patients were infected during the at-risk period, 1 of whom (patient 2) was infected with the same strain as was a chronically infected patient (patient 3). The other newly infected patient (patient 1) was infected with a genotype 3a strain, which could have been acquired either from a patient occasionally treated in the dialysis unit or from an external source.

Risk factors of HCV transmission. Potential risk factors of HCV transmission were hypothesized—namely, contamination of dialysis equipment (through machine sharing and inadequate

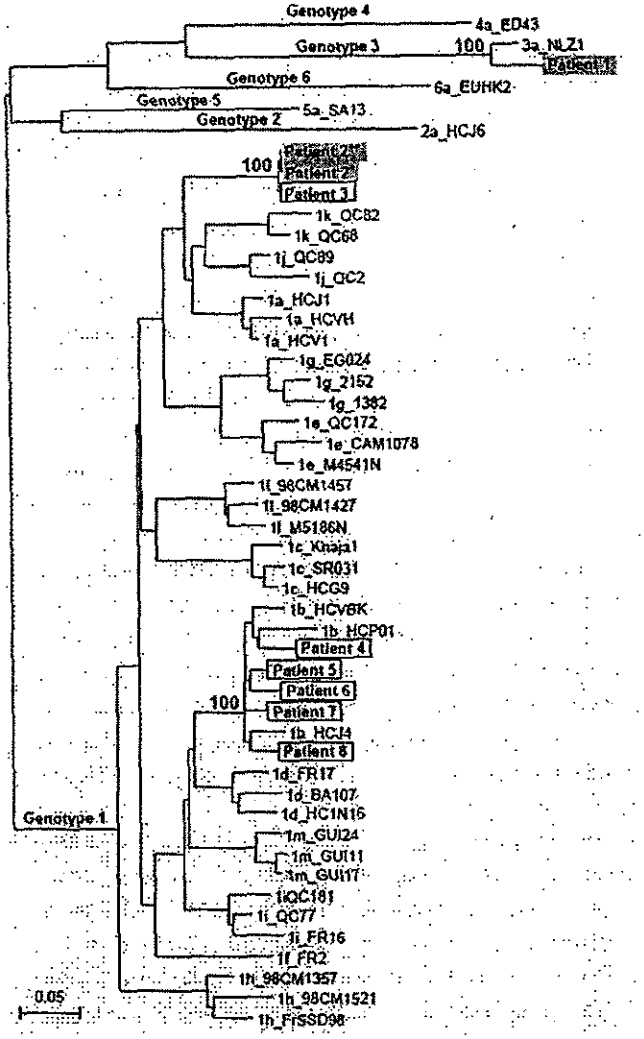


Figure 1. Phylogenetic tree plotted with nonstructural 5B sequences (nucleotide positions 8271–8597) from the 2 patients recently infected in our hemodialysis unit (patients 1 and 2; *shaded boxes*), the 6 patients known to be chronically infected with hepatitis C virus (HCV) and regularly treated in our hemodialysis unit (patients 3–8; *unshaded boxes*), and reference HCV strains of different genotypes (the type and subtype are indicated just before the strain identification letters and/or numbers). Two samples were available and were included in the analysis for patient 2, *July 2004; **September 2004. Nucleotide sequence of the nonstructural 5B gene of HCV-ED43 was used as an outgroup root.

environmental disinfection), noncompliance with standard precautions, and variation of the nurse-to-patient ratio in the hemodialysis unit.

The use and maintenance of dialysis equipment was reviewed by the local infection control team according to the written local procedures that are based on published data and recommendations. Dialyzers were not being reused, and dialysis machines (AK100; Gambro) were disinfected after each session, according to a written protocol combining chemical (peroxyacetic acid [Dialox]) and sodium hypochlorite) and heat disinfection.

Surfaces at risk of contamination with infected blood were defined as the most frequently manipulated surfaces—including dialysis machines, shared waste carts, patients' removable tables, and work benches. At-risk surfaces were swabbed during dialysis sessions (30 swabs per day on 25 consecutive days) on a surface area of ~100 cm^2, by using a cotton swab moistened with sterile distilled water that was then eluted in 1 mL of sterile distilled water. Hemoglobin was detected with reagent strips (Hemastix; Bayer HealthCare) with a detection limit of 150 μg Hb/L—that is, the equivalent of 5 erythrocytes per microliter. All hemoglobin-positive samples were tested for HCV RNA [23, 24].

Compliance with standard precautions (hand hygiene and glove use) was studied in the dialysis unit each day for three 30-min periods—during the morning, afternoon, and night shifts—for 7 weeks (2 weeks during September 2004 immediately after the first case alert and 5 weeks during June–July 2005). All staff categories were studied, in an open, unobtrusive manner, by 5 specially trained members of the infection control team, with use of a standardized questionnaire [25]. Hand hygiene opportunities tailored to the care activities in the hemodialysis unit were listed in the questionnaire (i.e., before and

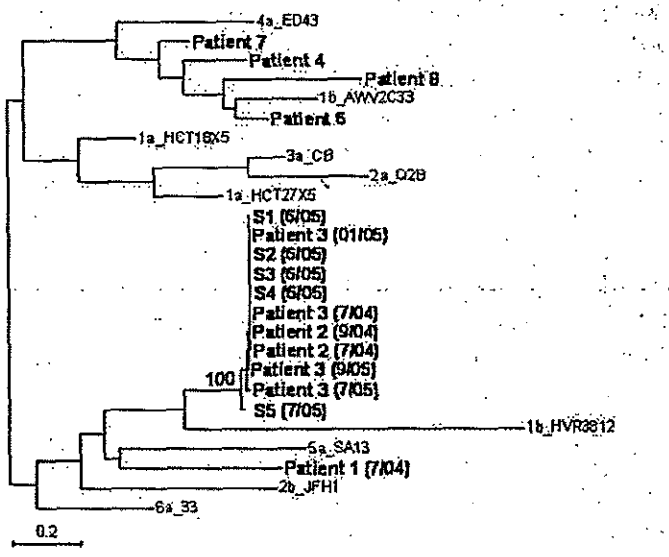


Figure 2. Phylogenetic tree plotted with hypervariable region (HVR) 1 sequences from the 6 patients known to be chronically infected with hepatitis C virus (HCV) and regularly treated in our hemodialysis unit (patients 3–8), including the patient who transmitted HCV to patient 2 (i.e., patient 3), the 2 patients infected in our hemodialysis unit (patients 1 and 2), the 5 environment surfaces that tested positive for HCV RNA and that could be PCR amplified in that region (S1–S5), and reference HCV strains of different genotypes (the type and subtype are indicated just before the strain identification letters and/or numbers). Dates of sampling are shown in parentheses.

after central venous catheter or fistula handling; preparation of material, connection, disconnection, dressing, and manipulation of lines and before and after direct contact with a patient; handling of other invasive devices, if present; measurement of temperature; measurement of arterial pressure; etc.). The handling of catheter and fistula were considered to be activities with high risk of HCV transmission. Overall, 2382 opportunities were observed during 197 shifts, with a total of 98 h of observation.

Glove use was observed during the same periods as was hand hygiene. For each care activity, the following variables were collected on the same standardized questionnaire as that used for hand hygiene: type of contact, wearing gloves during contact, and glove removal immediately after contact. Wearing gloves is recommended in the unit when exposure to body fluids is anticipated.

With consideration that the nurse-to-patient ratio (including nurses and nurse assistants) may influence the risk of HCV transmission, the ratio was recorded during each observation period, and the average nurse-to-patient ratio per shift (morning, afternoon, and night) was determined by calculating the median ratio for all the relevant observation periods. Hand hygiene compliance was also calculated for each of the 3 shifts.

Statistical analysis. Percentages and 95% CIs were calculated. The χ^2 test or Fisher's exact test was used, as appropriate, to compare proportions. The Mann-Whitney nonparametric test was used to compare continuous variables. Each potential risk factor for environmental hemoglobin contamination (i.e., nurse-to-patient ratio and hand hygiene compliance) was tested in a univariate model, and results were then entered in a logistic regression model. Variables were not dichotomized. To take into account the interdependence of observations made during the same shift, we used robust estimates of variance (generalized estimating equations) in which each shift observation was included as a cluster. Goodness of fit was assessed using the Hosmer-Lemeshow χ^2 test, and discrimination was determined from the area under the receiver operating characteristics curve. Accuracy was considered to be good when the area under the receiver operating characteristics curve had a range of 0.70–0.80 and was considered to be excellent when it was >0.80. The adjusted OR and 95% CI were calculated for each factor that was statistically significant in the logistic regression model. *P* values <.05 were considered to be statistically significant. All tests were 2 tailed. Statistical tests were performed using Intercooled Stata software, version 8.2 (Stata).

RESULTS

Virological study of environmental surfaces. A total of 740 surface samples were collected in the dialysis unit during June–August 2005, comprising 663 (90%) from dialysis machines and 77 (10%) from other surfaces (table 1). Hemoglobin was found in 82 samples (11%), including 71 (10%) from surfaces where blood was not evident. Among the 25 hemoglobin-positive samples collected from dialysis machines, 5 had been obtained after external disinfection of the machine. Six (7%) of the 82 hemoglobin-positive samples contained detectable levels of HCV RNA, comprising 4 samples taken from a dialysis machine and 2 from a shared waste cart (table 1). The HVR1-coding region could be PCR-amplified and sequenced in 5 of these 6 samples, designated S1–S5. These sequences were compared with HVR1 sequences recovered from patients 1–8 during the at-risk period (except for patient 5, in whom HVR1 could not be amplified) and also from patient 3 at the time of surface sampling (figure 2). As shown in figure 2, phylogenetic analysis revealed that all sequences found in environmental samples were closely related to those isolated from patient 2 when he was infected in 2004 and to those from patient 3, from whom samples were obtained both in 2004 and in 2005. Note also in figure 2 the very slow genetic evolution of the HVR1 in patient 3 (only 4 nucleotide substitutions accumulated in 14 months; data not shown), probably because of hemodialysis-associated immune suppression. Interestingly, the same HCV strain was isolated from 2 environmental samples taken at a 6-h interval from the same machine that had been used to treat 2 different patients.

Assessment of practices. Compliance with local precautions for machine use and internal disinfection was adequate. Multidose vials were never shared between patients. The finding that patients 2 and 3, who were infected with closely related HCV strains (figures 1 and 2), had always undergone dialysis during the same sessions but had never shared the same machine strongly suggested that patient 2 had been infected by patient 3 via the hands of a health care worker.

Compliance with standard precautions during the investigation is shown in figure 3. Overall, 2382 opportunities for hand hygiene were observed (2358 [99%] for nurses; 24 [1%]

Table 1. Environmental samples containing hemoglobin and/or hepatitis C virus (HCV) RNA.

Sample site	No. of samples	Positive samples, no. (%)	
		Hemoglobin	HCV RNA
Dialysis machine	663	36 (5)	4 (11)
Shared waste cart	27	24 (89)	2 (8)
Patients' removable table	9	6 (67)	0 (0)
Miscellaneous[a]	41	16 (39)	0 (0)
Total	740	82 (11)	6 (7)

NOTE. HCV RNA–positive findings are percentages of the number of hemoglobin-positive samples.

[a] Including nursing preparation area, wheelchairs, and patient file cart.

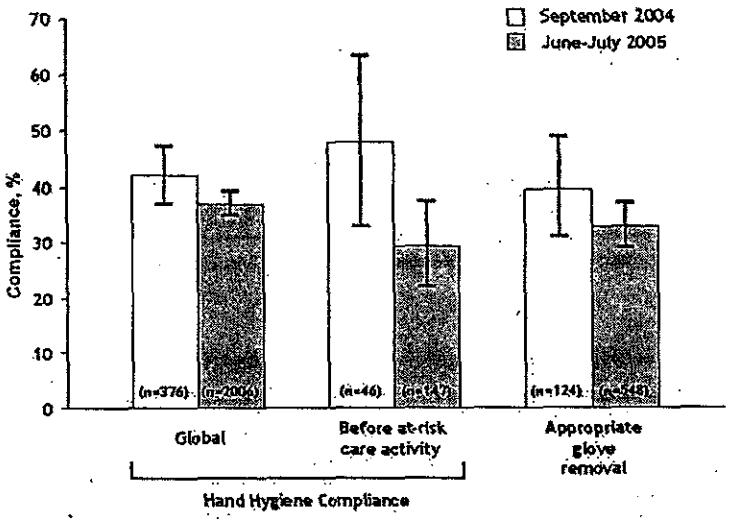


Figure 3. Compliance with guidelines for health care worker hand hygiene and appropriate glove use during dialysis. At-risk care activities consisted of handling dialysis catheters or fistulas. Whiskers, 95% CIs.

for nurse assistants). Immediately after the infection alert (September 2004), compliance with hand hygiene immediately before handling a dialysis catheter or fistula was significantly higher ($P < .001$) than it was several months later (figure 3). Globally, gloves were worn in 857 (36%) of observed contacts with a patient or the environment. When worn, gloves were removed immediately after a contact in only 672 (34.1%) of cases (95% CI, 30.5%–37.8%). There was no statistically significant difference between the findings of the 2 periods of observation. As shown in table 2, a low nurse-to-patient ratio and a poor rate of hand hygiene compliance were independently associated with the detection of hemoglobin on environmental surfaces.

DISCUSSION

Several reports of nosocomial HCV transmission in the hemodialysis setting have been published, but the investigations were incomplete and the routes of transmission remained unclear [13, 17, 18, 26]. Allander et al. [26] reported nosocomial HCV transmission in a series of patients who underwent dialysis at the same time but who did not share dialysis equipment. Those authors postulated, but did not show, that the environment was contaminated. Compliance with standard precautions was not studied.

To our knowledge, ours is the first study to demonstrate that a low nurse-to-patient ratio and poor compliance with guidelines for hand hygiene and glove use are independent predictors of environmental contamination by blood and HCV. By combining genetic and phylogenetic analyses of HCV recovered from patients' blood and the environment with measurements of compliance with standard precautions, we showed that: (1) 2 sporadic cases of HCV transmission occurred in the dialysis unit during the study period, 1 of which was unequivocally due to patient-to-patient transmission within the unit; (2) the dialysis environment was frequently contaminated by blood, including HCV-infected patients' blood, as shown by the detection of hemoglobin, sometimes associated with detectable levels of HCV RNA in a substantial proportion of swabs; and (3) compliance with guidelines for hand hygiene and glove use during patient care was poor, raising the possibility of HCV transmission via the hands of health care workers. Interestingly, all HCV-infected blood found in environmental samples belonged to the patient who indirectly infected another patient undergoing dialysis.

In our study, hemoglobin was found in 11% of environmental samples, and 7% of those positive samples contained detectable HCV RNA. Hepatitis B virus transmission has been linked to the presence of the virus on environmental surfaces—in the absence of visible blood [27]. Hepatitis B virus has been reported to remain viable on environmental surfaces for at least 7 days at room temperature [28, 29]. HCV RNA has been shown to be resistant for at least 48 h on inert surfaces at room temperature [24, 30, 31]. A robust cell culture system for HCV was recently developed, but it cannot be infected with viruses other than those produced after cell culture transfection of a specific HCV clone [32–34]. Cell culture systems that can be directly infected by HCV-infected patients' blood will be needed to determine how long HCV remains infective in the environment. Even in the absence of such data, our results strongly suggest that infectious HCV is present in the dialysis environment and that HCV can be transmitted by the hands of health care workers. We did not, however, sample health care workers'

Table 2. Factors independently associated with environmental blood contamination during nursing shifts.

Variable	Univariate analysis of environmental hemoglobin, by daily shifts		Multivariate analysis	
	Hemoglobin found (n = 28)	Hemoglobin not found (n = 14)	OR (95% CI)	P
Nurse-to-patient ratio, mean ± SD	0.55 ± 0.23	0.78 ± 0.50	0.03 (0.002–0.39)	.008
Hand hygiene compliance, mean % ± SD	39 ± 15	44 ± 17	0.93 (0.88–0.99)	.036

NOTE. Performance of the model, Hosmer-Lemeshow goodness-of-fit; P = .386; area under receiver operating characteristics curve, 0.768.

gloved or ungloved hands during care activities, because this would have hindered the assessment of compliance with standard precautions by increasing the Hawthorne effect.

The rate of compliance with standard precautions in our study was similar to that reported elsewhere about a similar setting [35, 36]. A recent survey of hand hygiene practices in 9 Spanish hemodialysis units showed poor compliance, both before and after contact with patients (14% and 36%, respectively) [36].

Permanent glove use can impair compliance with hand hygiene [37] and may thus lead to cross-transmission of infectious agents. This is the first time that glove use and removal have been studied in relation to the risk of environmental contamination. Gloves are worn mainly for health care worker self-protection, rather than to prevent patient cross-infection. The recommendation that gloves always be worn in the hemodialysis setting, whatever the type of contact (environment or patient) [38], therefore, may be confusing and may expose patients to HCV transmission if not followed properly, with systematic glove removal and hand hygiene between care procedures.

We found that a nurse-to-patient ratio <0.60 was independently associated with hemoglobin contamination of environmental surfaces. Understaffing is a recognized major risk factor for nosocomial infection [39–41]. Recently, a Brazilian study of 22 dialysis centers showed that the number of patients per health care worker was independently related to the risk of hepatitis B virus infection [16]. Petrosillo et al. [42] showed, in a prospective multicenter study in Italian hemodialysis units, that a low staff-to-patient ratio is an independent predictor of the risk of HCV nosocomial transmission. Therefore, to limit the spread of blood in the dialysis environment, we recommend that at-risk care procedures, such as connection and disconnection of equipment to the patient, be performed by a pair of nurses: one working with the patient and the other working with the machine.

In conclusion, blood-contaminated surfaces may represent a source of HCV transmission, via health care workers' hands or gloves. Environmental contamination is mainly a consequence of poor adherence to standard precautions in the hemodialysis setting. Strict adherence to guidelines for hand hygiene and glove use and strict organization of care procedures, with an adequate nurse-to-patient ratio, should help to reduce the risk of environmental contamination and, thus, HCV transmission in patients undergoing dialysis.

Acknowledgments

We are very grateful to the medical and paramedical personnel of the hemodialysis unit and nephrology ward who agreed to participate in this study.

Potential conflicts of interest. All authors: no conflicts.

References

1. McHutchison JG, Bacon BR. Chronic hepatitis C: an age wave of disease burden. Am J Manag Care 2005; 11:S286–95.
2. Busch MP, Glynn SA, Stramer SL, et al. 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.
3. 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.
4. Delarocque-Astagneau E, Pioche C, Desenclos JC. National surveillance of hepatitis C by voluntary hepatology reference centres, 2001–2004. Bull Epidemiol Hebdomadaire 2006; 51–52:414–8.
5. Allander T, Gruber A, Naghavi M, et al. Frequent patient-to-patient transmission of hepatitis C virus in a haematology ward. Lancet 1995; 345:603–6.
6. Bronowicki JP, Venard V, Bott, C, et al. Patient-to-patient transmission of hepatitis C virus during colonoscopy. N Engl J Med 1997; 337: 237–40.
7. Sartor C, Brunet P, Simon S, Tamalet C, Berland Y, Drancourt M. Transmission of hepatitis C virus between hemodialysis patients sharing the same machine. Infect Control Hosp Epidemiol 2004; 25:609–11.
8. Esteban JI, Gomez J, Martell M, et al. Transmission of hepatitis C virus by a cardiac surgeon. N Engl J Med 1996; 334:555–60.
9. Furusyo N, Kubo N, Nakashima H, Kashiwagi K, Etoh Y, Hayashi J. Confirmation of nosocomial hepatitis C virus infection in a hemodialysis unit. Infect Control Hosp Epidemiol 2004; 25:584–90.
10. Ross RS, Viazov S, Gross T. Transmission of hepatitis C virus from a patient to an anesthesiology assistant to five patients. N Engl J Med 2000; 343:1851–4.
11. Massari M, Petrosillo N, Ippolito G, et al. Transmission of hepatitis C virus in a gynecological surgery setting. J Clin Microbiol 2001; 39: 2860–3.
12. Schneeberger PM, Keur I, van Loon AM, et al. The prevalence and incidence of hepatitis C virus infections among dialysis patients in The Netherlands: a nationwide prospective study. J Infect Dis 2000; 182: 1291–9.
13. Halfon P, Roubicek C, Gerolami V, et al. Use of phylogenetic analysis of hepatitis C virus (HCV) hypervariable region 1 sequences to trace

an outbreak of HCV in an autodialysis unit. J Clin Microbiol 2002; 40:1541–5.
14. Kokubo S, Horii T, Yonekawa O, Ozawa N, Mukaide M. A phylogenetic-tree analysis elucidating nosocomial transmission of hepatitis C virus in a haemodialysis unit. J Viral Hepat 2002; 9:450–4.
15. Jadoul M, Poignet JL, Geddes C, et al. The changing epidemiology of hepatitis C virus (HCV) infection in haemodialysis: European multicentre study. Nephrol Dial Transplant 2004; 19:904–9.
16. Carrilho FJ, Moraes CR, Pinho JR, et al. Hepatitis B virus infection in haemodialysis centres from Santa Catarina State, Southern Brazil: predictive risk factors for infection and molecular epidemiology. BMC Pub Health 2004; 4:13.
17. Izopet J, Sandres-Saune K, Kamar N, et al. Incidence of HCV infection in French hemodialysis units: a prospective study. J Med Virol 2005; 77:70–6.
18. Lavillette D, Morice Y, Germanidis G, et al. Human serum facilitates hepatitis C virus infection, and neutralizing responses inversely correlate with viral replication kinetics at the acute phase of hepatitis C virus infection. J Virol 2005; 79:6023–34.
19. Pawlotsky JM, Pellerin M, Bouvier M, et al. Genetic complexity of the hypervariable region 1 (HVR1) of hepatitis C virus (HCV): influence on the characteristics of the infection and responses to interferon alfa therapy in patients with chronic hepatitis C. J Med Virol 1998; 54: 256–64.
20. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 1994; 22:4673–80.
21. Felsenstein J. PHYLIP: phylogeny inference package, version 3.5c (distributed by the author). Department of Genetics, University of Washington, Seattle, 1993.
22. Page RD. TreeView: an application to display phylogenetic trees on personal computers. Comput Appl Biosci 1996; 12:357–8.
23. Carducci A, Verani M, Casini B, et al. Detection and potential indicators of the presence of hepatitis C virus on surfaces in hospital settings. Lett Appl Microbiol 2002; 34:189–93.
24. Piazza M, Borgia G, Picciotto L, Nappa S, Cicciarello S, Orlando R. Detection of hepatitis C virus-RNA by polymerase chain reaction in dental surgeries. J Med Virol 1995; 45:40–2.
25. Girou E, Oppein F. Handwashing compliance in a French university hospital: new perspective with the introduction of hand-rubbing with a waterless alcohol-based solution. J Hosp Infect 2001; 48:S55-7.
26. Allander T, Medin C, Jacobson JA, Grillner L, Persson MAA. Hepatitis C transmission in a hemodialysis unit: molecular evidence for spread of virus among patients not sharing equipment. J Med Virol 1994; 43: 415–9.
27. Bond WW, Petersen NJ, Favero MS. Viral hepatitis B: aspects of environmental control. Health Lab Sci 1977; 14:235–52.
28. Favero MS, Maynard JE, Petersen NJ, et al. Letter: hepatitis-B antigen on environmental surfaces. Lancet 1973; 2:1455.
29. Bond WW, Favero MS, Petersen NJ, Gravelle CR, Ebert JW, Maynard JE. Survival of hepatitis B virus after drying and storage for one week. Lancet 1981; 7:550–1.
30. Piazza M, Borgia G, Picciotto L, Cicciarello S, Nappa S. HCV-RNA survival as detected by PCR in the environment. Boll Soc Ital Biol Sper 1994; 70:167–70.
31. Froio N, Nicastri E, Comandini UV, et al. Contamination by hepatitis B and C viruses in the dialysis setting. Am J Kidney Dis 2003; 42: 546–50.
32. Lindenbach BD, Evans MJ, Syder AJ, et al. Complete replication of hepatitis C virus in cell culture. Science 2005; 309:623–6.
33. Wakita T, Pietschmann T, Kato T, et al. Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. Nat Med 2005; 11:791–6.
34. Zhong J, Gastaminza P, Cheng G, et al. Robust hepatitis C virus infection in vitro. Proc Natl Acad Sci U S A 2005; 102:9294–9.
35. Arenas JMD, Sanchez-Paya J, Gonzales C, Rivera F, Antolin A, Arenas JD. Audit on the degree of application of universal precautions in a haemodialysis unit. Nephrol Dial Transplant 1999; 14:1001–3.
36. Arenas MD, Sanchez-Paya J, Barril G, et al. A multicentric survey of the practice of hand hygiene in haemodialysis units: factors affecting compliance. Nephrol Dial Transplant 2005; 20:1164–71.
37. Girou E, Chai SH, Oppein F, et al. Misuse of gloves: the foundation for poor compliance with hand hygiene and potential for microbial transmission? J Hosp Infect 2004; 57:162–9.
38. Centers for Disease Control and Prevention. Recommendations for preventing transmission of infections among chronic hemodialysis patients. MMWR Recomm Rep 2001; 50(RR-5):1–43.
39. Harbarth S, Sudre P, Dharan S, Cadenas M, Pittet D. Outbreak of *Enterobacter cloacae* related to understaffing, overcrowding, and poor hygiene practices. Infect Control Hosp Epidemiol 1999; 20:598–603.
40. Andersen BM, Lindemann R, Bergh K, et al. Spread of methicillin-resistant *Staphylococcus aureus* in a neonatal intensive unit associated with understaffing, overcrowding and mixing of patients. J Hosp Infect 2002; 50:18–24.
41. Needleman J, Buerhaus P, Mattke S, Stewart M, Zelevinsky K. Nurse-staffing levels and the quality of care in hospitals. N Engl J Med 2002; 346:1715–22.
42. Petrosillo N, Gilli P, Serraino D, et al. Prevalence of infected patients and understaffing have a role in hepatitis C virus transmission in dialysis. Am J Kidney Dis 2001; 37:1004–10.

別紙様式第2-1

No. 41

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. 6. 23	該当なし	

一般的名称	(製造販売承認書に記載なし)	研究報告の公表状況	Galiana C, Fernández-Barredo S, García A, Gómez MT, Pérez-Gracia MT. Am J Trop Med Hyg. 2008 Jun;78(6):1012-5.	公表国 スペイン
販売名(企業名)	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

○養豚業従事者のE型肝炎ウイルス(HEV)への職業的曝露
本研究の目的は、ブタ接触群(養豚業従事者)と非接触群のHEV陽性率とウイルス感染リスク因子を調べることであった。合計198名[非接触者97名(49%)、接触者101名(51%)]を対象にHEV感染の有無を調べた。抗HEV IgG抗体陽性率はブタ接触群が18.8%、非接触群が4.1%であった。ブタ接触者の抗HEV IgG抗体陽性リスクは5.4倍(P = 0.03)であった。IgG抗体陽性者10名(52.6%)は、未処理水の摂取およびブタへの接触という2つの汚染リスク因子を示した。以上のデータは、HEV感染を養豚従事者の職業病として扱うべきことを裏付けるものである。したがって、当該ウイルスへの曝露を予防するために、当該集団における包括的な衛生措置の適用が強く推奨される。

報告企業の意見	今後の対応
職業上のブタ接触群と非接触群のHEVの陽性率とウイルス感染リスク因子を調べたところ、抗HEV IgG抗体陽性率はブタ接触群が有意に高く、陽性者は未処理水の摂取およびブタへの接触という2つのリスクファクターを示したとの報告である。HEV感染については血液の安全対策上だけではなく、公衆衛生及び食品衛生上の問題でもある。	日本赤十字社では、輸血による肝炎ウイルス感染防止のため、血液中のALT値61IU/L以上の血液を排除している。また、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。加えて、北海道における輸血後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。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-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

Am. J. Trop. Med. Hyg., 78(6), 2008, pp. 1012–1015
Copyright © 2008 by The American Society of Tropical Medicine and Hygiene

Short Report: Occupational Exposure to Hepatitis E Virus (HEV) in Swine Workers

Carolina Galiana, Salceda Fernández-Barredo, Angel García, María Teresa Gómez, and Maria Teresa Pérez-Gracia*

Departamento de Atención Sanitaria, Salud Pública y Sanidad Animal, Facultad de Ciencias Experimentales y de la Salud, Universidad CEU Cardenal Herrera, Moncada, Valencia, Spain

Abstract. The aim of this work was to study the prevalence of hepatitis E virus (HEV) and the risk factors for the acquisition of the virus in a population in contact with swine and unexposed to swine. A total of 198 individuals, 97 unexposed (49%) and 101 exposed (51%) to swine, were tested for the presence of HEV infection. The prevalence of anti-HEV IgG in the exposed group was 18.8% versus 4.1% in the unexposed to swine group. People exposed to swine were observed to be 5.4 times ($P = 0.03$) at risk of having anti-HEV IgG. Ten (52.6%) of the IgG-positive individuals showed two concomitant risk factors: untreated water consumption and exposure to swine. These data support that HEV infection should be treated as a vocational illness in swine workers. Therefore, systematic application of hygiene measures in this collective is highly recommended to avoid the exposition to this virus.

Hepatitis E virus (HEV) is the main causative agent of enterically transmitted non-A non-B hepatitis and self-limiting clinical presentation in humans.[1] It is a non-enveloped virus with a positive-sense, single-stranded RNA genome of ~7,200 nucleotides in length and contains three open reading frames (ORFs). Nowadays, HEV is classified into the family *Hepeviridae*, genus *Hepevirus*. Regarding the phylogeny, HEV has been divided into four genotypes,[2] although only one serotype of HEV is recognized.[3] Transmission of HEV infection primarily occurs through contaminated water, although person to person transmission and sexual transmission occur infrequently.

Hepatitis E has been considered an infectious endemic in developing areas such as India, Africa, and Southeast Asia, because of poor sanitary conditions in drinking water.[4] The mortality rate of hepatitis E in the normal population is generally < 1%, but it can be as high as 20–25% among pregnant women.

In industrialized countries, HEV has been found mainly in individuals who had traveled to endemic zones. Actually, the increasing number of autochthonous cases of hepatitis E[5] and the recent findings of HEV in domestic animals such as swine give rise to the suspicion that HEV is underdetected in idiopathic non-A non-B hepatitis. Therefore, the transmission pathways from animals to humans remain obscure. However, in developed countries, seroprevalence ranges varying from 1–18% have been reported. In the last years, several studies have been published describing differences in the prevalence of anti-HEV antibodies between people exposed and not exposed to swine,[6–12] but the risk factors for the acquisition of the virus have not been studied.

Accordingly, the aim of this work was to study the prevalence of HEV and the risk factors for the acquisition of the virus in healthy Spanish people distributed in exposed and unexposed to swine groups.

A retrospective study was carried out to determine the prevalence of HEV during the period from October 2004 to July 2007 in Spain.

A total number of 198 healthy individuals, 101 (51%) men and 97 (49%) women, were included in this study to detect the prevalence of HEV. Participants filled out an epidemiologic questionnaire including name, age, area of residence, travel abroad, exposure to swine, and consumption of raw vegetables, raw shellfish, and untreated water. Informed approval was obtained from all participants. Individuals were divided into two separate groups taking into consideration exposition to swine: 97 unexposed (NE; 27 men and 70 women) and 101 exposed (E; 74 men and 27 women). Individuals included in the E group were made up of swine farmers, pig handlers, and swine veterinarians, whereas the NE group was made up of volunteers with no contact with swine.

Blood samples were obtained from all the participants by venipuncture, and sera were obtained and frozen at −20°C until used. RNA was extracted from 140 μL of each serum using a commercial kit following the manufacturer's instructions (QIampViral RNA Kit; Qiagen, Valencia, CA). Two pairs of degenerate oligonucleotide primers[13] were used to amplify a 348-bp fragment of ORF-2 of HEV using a reverse transcriptase–nested polymerase chain reaction (PCR).[14] These primers were based on 18 human HEV sequences and the swine HEV prototype strain from the United States. A positive control from a naturally infected pig (GenBank accession number AY323506) was included in each procedure. Different stages of assay were performed in different places to avoid the possibility of cross-contamination. The PCR products were separated by electrophoresis in 2% agarose and were detected by staining with ethidium bromide.

Sera from all individuals were tested for the presence of HEV antibodies (anti-HEV IgG and IgM) using a commercial ELISA (Fortress Diagnostics, Antrim, UK) according to the manufacturer's instructions. This kit used polystyrene microwell strips precoated with recombinant HEV antigens (HEV-Ag) corresponding to structural proteins ORF2, derived from genotype 1. The sensitivity and specificity of the ELISA assay use in this study were determined by the manufacturer as 92% and 88%, respectively. Positive results obtained using this assay were confirmed by means of an HEV immunoblot test (Recomblot HEV-IgG/IgM; Mikrogen, Martinsried, Germany). Antigens used in this kit were the N-terminal part of the capsid antigen (GST fusion protein O2N, 50 kd), the C-terminal part of the capsid antigen (triple band; O2C 38–41 kd), the middle part of the capsid antigen (O2M; 28 kd), and the ORF3 protein (O3; 15 kd) of genotypes 1 and 2.

* Address correspondence to M. T. Pérez-Gracia, Departamento de Atención Sanitaria, Salud Pública y Sanidad Animal, Facultad de Ciencias Experimentales y de la Salud, Universidad CEU Cardenal Herrera, Avenida Seminario s/n 46113, Moncada, Valencia, Spain. E-mail: teresa@uch.ceu.es

Liver function tests, including transaminase levels (aspartate aminotransferase [ALT] and alanine aminotransferase [AST]) in serum were determined using a Thermo Spectronic spectrophotometer (Helios, Barcelona, Spain).

To determine the correlation between the data obtained from the questionnaire and the laboratory results, odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were calculated using binary logistic regression analysis by means of SPSS version 15.0 statistical software. For the statistical comparison of the seroprevalence obtained in the E and NE groups, the Pearson χ^2 test and Student t test were applied.

All individuals tested negative for the presence of HEV RNA in serum. The overall prevalence of anti-HEV IgG confirmed by immunoblotting was 11.6% (23/198). The seroprevalence of anti-HEV IgG in the E group and in NE group was 18.8% (19/101) and 4.1% (4/97), respectively (Table 1). Values of transaminase enzymes were located within the normal range (ALT: men < 45 IU/L, women < 36 IU/L; AST: < 34 IU/L for men and women) in all individuals. No significant differences in the levels of transaminases were observed between the anti-HEV IgG-positive group (ALT: 22 ± 14; AST: 12 ± 7.5) and the anti-HEV IgG-negative group (ALT: 15 ± 12.2; AST: 11 ± 6.8). The statistical analysis showed a significant association ($P < 0.05$) between the presence of anti-HEV IgG and the consumption of untreated water with an OR value of 5.6 ($P = 0.01$). Additionally, people exposed to swine were observed to be 5.4 times ($P = 0.03$) at risk of having anti-HEV IgG antibodies. Ten (52.6%) of the IgG-positive individuals showed two concomitant risk factors: untreated water consumption and exposure to swine. The χ^2 goodness-of-fit test showed a good fit with the observed and expected frequencies in the E and NE groups ($\chi^2 = 10.4$, $P = 0.01$) and consumption of untreated water ($\chi^2 = 12.9$, $P = 0.01$). No significant differences were observed between the rest of the study parameters.

This is the first study in Spain reporting the prevalence of IgG anti-HEV antibodies in swine workers (18.8%) and in people unexposed to swine (4.1%). The increased risk (5.4 times at risk) of having IgG anti-HEV observed in swine workers in this work is not surprising, taking into account the high number of farms (76%) and pigs (23%) testing positive for HEV RNA in the same area.[14] This datum is higher than the OR (1.46) reported by Meng and others[6] in 2002 in the only study that calculated the risk for a veterinarian to be positive for IgG anti-HEV. The fact that the values of transaminases were similar between positive and negative individuals suggests that HEV might be responsible for subclinical infections, because none of the participants reported any past clinical signs of acute hepatitis. The factors triggering the development of an acute or a subclinical hepatitis E infection remain obscure in industrialized countries. Some authors point to several contributing factors such as age,[15] preexisting hepatopathy,[16] and the genotype of the strain.[17]

It has been reported for autochthonous hepatitis E in developed regions that swine isolates from genotype 3 are more related to human strains from the same geographic region than to swine strains from different areas. Moreover, HEV strains circulating in Spanish swine farms are highly homologous with Spanish human strains, which raises the possibility of HEV transmission from swine to humans.[18] HEV has been suggested to be a zoonotic infection where pigs play an important role in the spreading of the disease. HEV is capable of crossing the species barrier, as has been shown by means of experimental infections in pigs with a human HEV strain and in non-human primates with a swine HEV strain.[19]

The results obtained in this study support the link between the presence of anti-HEV antibodies and direct contact with swine, as reported by several authors. Thus, in the United States,[6,7] significant prevalences between veterinarians working with swine (26% and 10.9%, respectively) and unexposed people (18% and 2.4%, respectively) were reported. Similar results were described in The Netherlands, Moldova, and Taiwan,[8–10] with values for those exposed to swine of 11%, 51%, and 27% versus 2%, 24.7%, and 2.4%, respectively. In contrast, studies in Sweden[11] found no significant differences be-

TABLE 1
Characteristics and risk factors of the studied population according to the presence or absence of anti-HEV IgG

	Anti-HEV IgG positive	Anti-HEV IgG negative	P	OR	95% CI
Sex					
Male	21 (20.8%)	80 (79.2%)	0.01	0.08	0–0.3
Female	2 (2%)	95 (97.9%)			
Age (years)	38.2 ± 10.4	26 ± 9.0			
ALT	22 ± 14	15 ± 12.2			
AST	12 ± 7.5	11 ± 6.8			
RNA-HEV	0 (0%)	0 (0%)			
Consume raw vegetables					
No	2 (7.4%)	25 (92.6%)	0.46	1.75	0.3–7.9
Yes	21 (12.3%)	150 (87.7%)			
Consume raw shellfish					
No	23 (11.6%)	175 (88.4%)	0	0	0
Yes	0 (0%)	0 (0%)			
Consume untreated water					
No	13 (7.8%)	154 (92.2%)	0.01	5.6	12.2–14.5
Yes	10 (32.2%)	21 (67.8%)			
Travel abroad					
No	18 (13.2%)	118 (86.8%)	0.29	0.6	0.2–1.6
Yes	5 (8%)	57 (92%)			
Exposure to swine					
No	4 (4.1%)	93 (95.9%)	0.03	5.4	1.7–16.5
Yes	19 (18.8%)	82 (81.2%)			

OR = odds ratio; CI = confidence interval; ALT = alanine aminotransferase; AST = aspartate aminotransferase.

tween those exposed (13%) and unexposed to swine (9.3%), and in Italy,[12] prevalences of 3.3% in swine farmers and 2.9% in people without occupational exposure to swine were reported. The high variation among the prevalences described above might be caused by differences in sample size, country of origin, and the diagnostic assay used. In this context, it has been described that there are significant sensitivity variations in developed countries depending on the type of ELISA kit used, as well as immunoblotting confirmation of the ELISA-positive samples. The data obtained by Herremans and others[20] in 2007 suggest that there are few differences in the sensitivity of ELISAs based in genotype 1 or 3 antigens. Therefore, the number of false negatives in the healthy population is expected to be low. In our study, to minimize the possibility of false positives and yield more accurate prevalence results, positive samples were confirmed by means of an immunoblot assay (Recomblot HEV; Mikrogen).

Regarding other risk factors studied in this work, an elevated prevalence (32.2%) and risk (OR = 5.6) in people who reported consumption of untreated water from water fountains in the countryside was recorded. The relationship between untreated water consumption and exposure to swine in swine workers is not surprising because the farms are located in the countryside where untreated water fountains are numerous. Additionally, it is very common among farmers to fertilize cultivated fields with manure from swine farms, which could infiltrate down through the ground, contaminating subterranean water and reaching to the water fountains. However, this hypothesis needs to be confirmed by further studies detecting HEV in water fountains.

The seroprevalence observed in other industrialized countries such as the United Kingdom,[19] Italy,[21] France,[22] New Zealand,[23] and Brazil,[24] with 6.3%, 2.6%, 3.2%, 4%, and 2.3%, respectively, was lower than the value reported in our study. The overall percentage found in this study (11.6%) is also higher than the one observed by Mateos and others[25] (2.8%) and the rate obtained by Buti and others (7.3%)[26] in a normal Spanish population. These cannot be properly compared with the data obtained in this study because of the high number of exposed people (50%). These high prevalences suggest that autochthonous HEV is circulating in Spain, and the infection is underdiagnosed. Although transfusion-transmitted HEV is probably much too rare to sustain HEV transmission, it should be taken into account that HEV is spread through uncertain routes, and the potential risk of transfusion-transmitted HEV infection should be considered.[27]

In conclusion, this is the first study in Spain reporting a high prevalence of IgG anti-HEV antibodies in swine workers. These data support that HEV infection should be treated as a vocational illness in swine workers. Therefore, systematic application of hygiene measures in this group is highly recommended to avoid the exposition to this virus.

Received October 16, 2007. Accepted for publication January 3, 2008.

Financial support: This project was supported by UCH-CEU (PRUCH 06/21), EVES (053/2005), and Generalitat Valenciana (GV05/132).

Authors' addresses: Carolina Galiana, Salceda Fernández-Barredo, Angel García, María Teresa Gómez, and María Teresa Pérez-Gracia, Area Microbiología, Facultad de Ciencias Experimentales y de la Salud, Universidad CEU Cardenal Herrera, Avenida Seminario s/n 46113, Moncada, Valencia, Spain.

REFERENCES

1. Perez-Gracia MT, Rodriguez-Iglesias M, 2003. [Hepatitis E virus: current status]. *Med Clin (Barc) 121:* 787–792.
2. Schlauder GG, Dawson GJ, Erker JC, Kwo PY, Knigge MF, Smalley DL, Rosenblatt JE, Desai SM, Mushahwar IK, 1998. The sequence and phylogenetic analysis of a novel hepatitis E virus isolated from a patient with acute hepatitis reported in the United States. *J Gen Virol 79:* 447–456.
3. Meng XJ, Halbur PG, Shapiro MS, Govindarajan S, Bruna JD, Mushahwar IK, Purcell RH, Emerson SU, 1998. Genetic and experimental evidence for cross-species infection by swine hepatitis E virus. *J Virol 72:* 9714–9721.
4. Aggarwal R, Krawczynski K, 2000. Hepatitis E: an overview and recent advances in clinical and laboratory research. *J Gastroenterol Hepatol 15:* 9–20.
5. Perez-Gracia MT, Garcia-Valdivia MS, Galan F, Rodriguez-Iglesias MA, 2004. Detection of hepatitis E virus in patients sera in southern Spain. *Acta Virol 48:* 197–200.
6. Meng XJ, Wiseman B, Elvinger F, Guenette DK, Toth TE, Engle RE, Emerson SU, Purcell RH, 2002. 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 40:* 117–122.
7. Withers MR, Correa MT, Morrow M, Stebbins ME, Seriwatana J, Webster WD, Boak MB, Vaughn DW, 2002. Antibody levels to hepatitis E virus in North Carolina swine workers, non-swine workers, swine, and murids. *Am J Trop Med Hyg 66:* 384–388.
8. Bouwknegt M, Engel B, Herremans MM, Widdowson MA, Worm HC, Koopmans MP, Frankena K, Husman AM, De Jong MC, Der Poel WH, 2007. Bayesian estimation of hepatitis E virus seroprevalence for populations with different exposure levels to swine in The Netherlands. *Epidemiol Infect 136:* 1–10.
9. Drobeniuc J, Favorov MO, Shapiro CN, Bell BP, Mast EE, Dadu A, Culver D, Iarovoi P, Robertson BH, Margolis HS, 2001. Hepatitis E virus antibody prevalence among persons who work with swine. *J Infect Dis 184:* 1594–1597.
10. Hsieh SY, Meng XJ, Wu YH, Liu ST, Tam AW, Lin DY, Liaw YF, 1999. Identity of a novel swine hepatitis E virus in Taiwan forming a monophyletic group with Taiwan isolates of human hepatitis E virus. *J Clin Microbiol 37:* 3828–3834.
11. Olsen B, Axelsson-Olsson D, Thelin A, Weiland O, 2006. Unexpected high prevalence of IgG-antibodies to hepatitis E virus in Swedish pig farmers and controls. *Scand J Infect Dis 38:* 55–58.
12. Vulcano A, Angelucci M, Candelori E, Martini V, Patti AM, 2007. HEV prevalence in the general population and among workers at zoonotic risk in Latium Region. *Ann Ig 19:* 181–186.
13. Huang FF, Haqshenas G, Guenette DK, Halbur PG, Schommer SK, Pierson FW, Toth TE, Meng XJ, 2002. Detection by reverse transcription-PCR and genetic characterization of field isolates of swine hepatitis E virus from pigs in different geographic regions of the United States. *J Clin Microbiol 40:* 1326–1332.
14. Fernandez-Barredo S, Galiana C, Garcia A, Vega S, Gomez MT, Perez-Gracia MT, 2006. Detection of hepatitis E virus shedding in feces of pigs at different stages of production using reverse transcription-polymerase chain reaction. *J Vet Diagn Invest 18:* 462–465.
15. 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.
16. Perez-Gracia MT, Mateos ML, Galiana C, Fernández-Barredo S, García A, Goméz MT, Moreira V, 2007. Autochthonous hepatitis E infection in a slaughterhouse worker. *Am J Trop Med Hyg 77:* 893–896.
17. Mizuo H, Yazaki Y, Sugawara K, Tsuda F, Takahashi M, Nishizawa T, Okamoto H, 2005. Possible risk factors for the transmission of hepatitis E virus and for the severe form of hepatitis E acquired locally in Hokkaido, Japan. *J Med Virol 76:* 341–349.

18. Fernandez-Barredo S, Galiana C, Garcia A, Gomez-Munoz MT, Vega S, Rodriguez-Iglesias MA, Perez-Gracia MT, 2007. Prevalence and genetic characterization of hepatitis E virus in paired samples of feces and serum from naturally infected pigs. *Can J Vet Res 71:* 236–240.
19. 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.
20. Herremans M, Bakker J, Duizer E, Vennema H, Koopmans MP, 2007. Use of serological assays for diagnosis of hepatitis E virus genotype 1 and 3 infections in a setting of low endemicity. *Clin Vaccine Immunol 14:* 562–568.
21. Gessoni G, Manoni F, 1996. Hepatitis E virus infection in northeast Italy: serological study in the open population and groups at risk. *J Viral Hepat 3:* 197–202.
22. Boutrouille A, Bakkali-Kassimi L, Cruciere C, Pavio N, 2007. Prevalence of anti-hepatitis E virus antibodies in French blood donors. *J Clin Microbiol 45:* 2009–2010.
23. Dalton HR, Fellows HJ, Gane EJ, Wong P, Gerred S, Schroeder B, Croxson MC, Garkavenko O, 2007. Hepatitis E in New Zealand. *J Gastroenterol Hepatol 22:* 1236–1240.
24. Bortoliero AL, Bonametti AM, Morimoto HK, Matsuo T, Reiche EM, 2006. Seroprevalence for hepatitis E virus (HEV) infection among volunteer blood donors of the Regional Blood Bank of Londrina, State of Parana, Brazil. *Rev Inst Med Trop Sao Paulo 48:* 87–92.
25. Mateos ML, Camarero C, Lasa E, Teruel JL, Mir N, Baquero F, 1998. Hepatitis E virus: relevance in blood donors and other risk groups. *Vox Sang 75:* 267–269.
26. Buti M, Dominguez A, Plans P, Jardi R, Schaper M, Espunes J, Cardenosa N, Rodriguez-Frias F, Esteban R, Plasencia A, Salleras L, 2006. Community-based seroepidemiological survey of hepatitis E virus infection in Catalonia, Spain. *Clin Vaccine Immunol 13:* 1328–1332.
27. Boxall E, Herborn A, Kochethu G, Pratt G, Adams D, Ijaz S, Teo CG, 2006. Transfusion-transmitted hepatitis E in a 'nonhyperendemic' country. *Transfusion Med 16:* 79–83.

医薬品　研究報告・調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
一般的名称	血小板		研究報告の公表状況	Transfusion(United States)Jul2008,48(7)p1368-75.	公表国 米国	
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
HEV の輸血伝染のリスクは低いと思われるが、これまでに非流行国において 5 例の輸血伝染の HEV 感染が報告されており、原因供血者の HEV 感染経路が知られている例はない。 今回、原因供血者の感染経路が確認された最初の症例である、動物原性食品伝染経路を介して HEV に感染した供血者からの輸血伝染によって引き起こされた急性 E 型肝炎の症例を報告する。 HEV に汚染されていた血小板が、血清中 HEV マーカー陰性の 64 歳の日本人男性非ホジキンリンパ腫患者に輸血された。輸血後 3 週間の肝機能検査は正常であったが、約 22 日後に ALT 値が一過性に 67 IU/L まで上昇し、血清中に HEV が検出され、急性 E 型肝炎と診断された。原因となった供血者は供血の約 1 ヵ月前に親族 12 名と焼肉レストランでブタの肝臓や腸などを食べており、血液サンプル中の HEV マーカーを検査したところ、13 例中 7 例に抗 HEV 抗体が検出された。 これまでの日本における数例の E 型肝炎症例は動物原性食品伝染経路を介して生じたことを示唆しており、最近の研究では HEV は加熱不活性化に対して中等度の耐性を有することが示されている。 抗 HCV 抗体検査開始後は HCV 感染に対して ALT 検査はほとんど貢献しないことから、米国などでは ALT スクリーニングは中止されているが、今回の症例は HEV が存在する血液を排除する上で ALT 検査が貢献することを示唆している(日本赤十字血液センターでは ALT 検査を実施している)。 血液原性の HEV 伝染のリスクを抑制する最も効果的な予防策は、供血の HEV をスクリーニングすること、または病原体の不活性化を実施することである。日本赤十字社は日本人血液供血者における HEV 感染の疫学的研究、および北海道における HEV の NAT スクリーニングの実行可能性試験を計画している。	2004 年の感染例に関する報告であり、北海道赤十字血液センター管内における献血者の HEV 保有状況の調査結果などについては、薬事・食品衛生審議会運営委員会(2006 年 1 月 26 日開催)で報告されている。

報告企業の意見	今後の対応
輸血による HEV 感染に関する情報である。現在まで、血漿分画製剤による伝播の報告はなく、製造工程中には複数のウイルス不活化除去工程を設けているが、今後とも関連情報の収集に努める。	今後とも同様な情報に留意し、関連情報の収集に努めていく。

NIHONSEIYAKI
2008-006

A case of transfusion-transmitted hepatitis E caused by blood from a donor infected with hepatitis E virus via zoonotic food-borne route

Keiji Matsubayashi, Jong-Hon Kang, Hidekatsu Sakata, Kazuaki Takahashi, Motohiro Shindo, Masaru Kato, Shinichiro Sato, Toshiaki Kato, Hiroyuki Nishimori, Kunihiko Tsuji, Hiroyuki Maguchi, Jun-ichi Yoshida, Hiroshi Maekubo, Shunji Mishiro, and Hisami Ikeda

BACKGROUND: Five cases of transfusion transmission of hepatitis E virus (HEV) have been reported so far. The infection routes of the causative donors remain unclear, however. Also, the progress of virus markers in the entire course of HEV infection has not been well documented.

STUDY DESIGN AND METHODS: Nucleic acid testing was performed by real-time reverse transcription–polymerase chain reaction targeting the open reading frame 2 region of HEV. Full-length nucleotide sequences of HEV RNA were detected by direct sequencing.

RESULTS: Lookback study of a HEV-positive donor revealed that the platelets (PLTs) donated from him 2 weeks previously contained HEV RNA and were transfused to a patient. Thirteen relatives including the donor were ascertained to enjoy grilled pork meats together in a barbecue restaurant 23 days before the donation. Thereafter, his father died of fulminant hepatitis E and the other 6 members showed serum markers of HEV infection. In the recipient, HEV was detected in serum on Day 22 and reached the peak of 7.2 log copies per mL on Day 44 followed by the steep increase of alanine aminotransferase. Immunoglobulin G anti-HEV emerged on Day 67; subsequently, hepatitis was resolved. HEV RNA sequences from the donor and recipient were an identical, Japan-indigenous strain of genotype 4. HEV RNA was detectable up to Day 97 in serum, Day 85 in feces, and Day 71 in saliva.

CONCLUSION: A transfusion-transmitted hepatitis E case by blood from a donor infected via the zoonotic food-borne route and the progress of HEV markers in the entire course are demonstrated. Further studies are needed to clarify the epidemiology and the transfusion-related risks for HEV even in industrialized countries.

Hepatitis E virus (HEV) infection has been considered to occur mainly via fecal-oral transmission and is an important public health concern in developing countries.[1] In industrialized countries including Japan, cases have been rarely reported and hepatitis E has been regarded as an imported infectious disease from its endemic areas. Recently, however, increasing numbers of sporadic cases have been reported,[2-11] some of which resulted from infection via a zoonotic food-borne route by consumption of raw or undercooked meats of wild boar, wild deer, or farmed pig that was contaminated with HEV.[8-11]

In 2004, we reported the first molecularly confirmed case of transfusion transmission of HEV.[12] The infection route in the causative donor was not very clear, however. Thereafter, at least four cases of transfusion transmission of HEV have been reported in Japan, the United Kingdom,

ABBREVIATIONS: FAM = 6-carboxyfluorescein; HEV = hepatitis E virus; ORF = open reading frame; PSL = predonisolone; TAMRA = 6-carboxy-tetramethylrhodamine.

From the Hokkaido Red Cross Blood Center, Sapporo; the Center for Gastroenterology, Teine Keijinkai Hospital, Sapporo; the Department of Medical Sciences, Toshiba General Hospital, Tokyo; the Third Department of Internal Medicine, Asahikawa Medical College, Asahikawa; the Department of Internal Medicine, Kitami Red Cross Hospital, Kitami; and the Department of Internal Medicine, Teine Keijinkai Hospital, Sapporo, Japan.

Address reprint requests to: Keiji Matsubayashi, Hokkaido Red Cross Blood Center, 2-2 Yamanote, Nishi-ku, Sapporo 063-0002, Japan; e-mail: kmatsu@hokkaido.bc.jrc.or.jp.

Received for publication September 5, 2007; revision received January 20, 2008, and accepted January 20, 2008.

doi: 10.1111/j.1537-2995.2008.01722.x

TRANSFUSION 2008;48:1368-1375.

and France,[13-16] where hepatitis E is nonendemic and HEV infection routes remained to be obscure.

Here, we report a case of acute hepatitis E caused by transfusion transmission from the donor who was infected with HEV via a zoonotic food-borne manner. To our knowledge, this is the first case in which the infection route of the causative donor has been confirmed. Also, in this report, we describe, for the first time, the virus kinetics and changes of anti-HEV in serum, prospectively monitored from latent period of infection until convalescence, accompanied by disease progression in the patient.

MATERIALS AND METHODS

Detection and quantitation of HEV RNA

For reverse transcription–polymerase chain reaction (RT-PCR) to detect HEV RNA in the samples, the following oligonucleotides were designed to detect 75 nucleotides of highly conserved sequence in the open reading frame (ORF) 2 region of all HEV genotypes: forward primer 5′-CGGCGGTGGTTTCTGG-3′, reverse primer 5′-AAGG GGTTGGTTGGATGAATA-3′, and mixed probes with a 5′-reporter dye (6-carboxyfluorescein, FAM) and a 3′-quencher dye (6-carboxy-tetramethylrhodamine, TAMRA) and FAM-5′-TGACAGGGTTGATTCTCAGCCCTTCG-3′-TAMRA, FAM-5′-TGACCGGGTTGATTCTCAGCCCTTC-3′-TAMRA, and FAM-5′-TGACCGGGCTGATTCTCAGCCC TT-3′-TAMRA (Sigma-Aldrich Japan, Tokyo, Japan). Nucleic acid was extracted from 200 µL of serum and saliva and from 100 µL of 10 percent (wt/vol) fecal suspension in saline with kits (QIAamp MinElute virus spin kit, Qiagen K.K., Tokyo, Japan; and SMITEST R&D-EX, Medical & Biological Laboratories, Nagoya, Japan). Before extraction, the samples were centrifuged at 6000 × *g* at 4°C for 10 minutes; thereafter the clear supernatant was subjected to nucleic acid extraction. Before RT-PCR, RNA preparation of feces was diluted at 10 times with nuclease-free water to reduce the effect of inhibitors. Twenty microliters of nucleic acid sample was used for each reaction. Each 50 µL of reaction mixture contained 25 µL of 2× RT-PCR kit master mix (QuantiTect Probe RT-PCR kit, Qiagen), 0.5 µL of RT mix (QuantiTect Probe RT-PCR kit, Qiagen), 400 nmol per L each of forward and reverse primer, and 67 nmol per L each of three probes. RT-PCR mixture was incubated at 50°C for 30 minutes and at 95°C for 15 minutes, followed by 50 cycles of 94°C for 15 seconds, and 60°C for 1 minute utilizing a thermocycler (Applied Biosystems 7500, real time PCR system, Applied Biosystems, Tokyo, Japan). HEV nucleic acid testing (NAT) was performed individually. The analytical sensitivity of the HEV NAT was determined to be 25 (13-166) copies per mL (with 95% confidence interval) by logistic analysis. HEV viral load was determined from standard curves generated by using 10^1 to 10^7 copies of HEV RNA per reaction. The HEV quantitation standard was generated by transcribing HEV cDNA of HEV ORF2 region that was cloned into a plasmid (pCRII-TOPO, Invitrogen, Carlsbad, CA), using the in vitro transcription kit (MAXIscript T7 high-yield transcription kit, Ambion, Austin, TX). Purified plasmid DNA was linearized with *Hin*dIII restriction endonuclease and transcribed to yield 717-nucleotide-long RNA transcripts containing 75-nucleotide target sequence.

Phylogenetic analysis of HEV isolates

Entire or nearly entire sequences of HEV isolates were determined as previously described by Takahashi and coworkers.[4] The sequences were aligned together with reported HEV strains with a computer program (CLUSTAL W, Version 1.8).[17] A phylogenetic tree based on the nearly entire HEV RNA sequence was constructed by the neighbor-joining method,[18] and the final tree was obtained by a computer program (TreeView, Version 1.6.6).[19] Bootstrap values were determined by resampling 1000 times of the data sets. The nucleotide sequences isolates HRC-HE14C, JST-KitAsa04C, and JTC-Kit-FH04L reported in this study have been assigned DDBJ/EMBL/GenBank nucleotide sequence databases with the accession numbers AB291965, AB291966, and AB291959, respectively.

Detection of serum anti-HEV

Samples were tested for immunoglobulin M (IgM)- and immunoglobulin G (IgG)-class antibodies against HEV using a commercial enzyme-linked immunosorbent assay kit (Viragent HEV-Ab, Cosmic Corp., Tokyo, Japan).[5,20]

Alanine aminotransferase testing

Alanine aminotransferase (ALT) testing was carried out using transaminase-HRII Nisseki/GPT (Wako Pure Chemical Industries Ltd, Osaka, Japan) on an automatic analyzer (ACA5400, Olympus Corp., Tokyo, Japan).

RESULTS

A lookback study of a causative blood donor

Blood from a 39-year-old Japanese male on September 20, 2004, was disqualified because of the elevated ALT level at 236 IU per L and tested for hepatitis viruses because of the abnormal ALT result. His blood sample turned out to be positive for the presence of HEV RNA at 4.8 log copies per mL as well as anti-HEV IgM and IgG and negative for the presence of any marker of hepatitis B virus (HBV) or hepatitis C virus (HCV). A lookback study revealed that his donated blood on September 6, 2004, 2 weeks before the last donation, was positive for the presence of HEV RNA at 3.1 log copies per mL and negative for the presence of IgM- or IgG-class anti-HEV. The HEV isolate, HRC-HE14C, was classified as genotype 4 of a Japan-indigenous strain (Fig. 1). The blood (platelet [PLT] concentrate) donated on

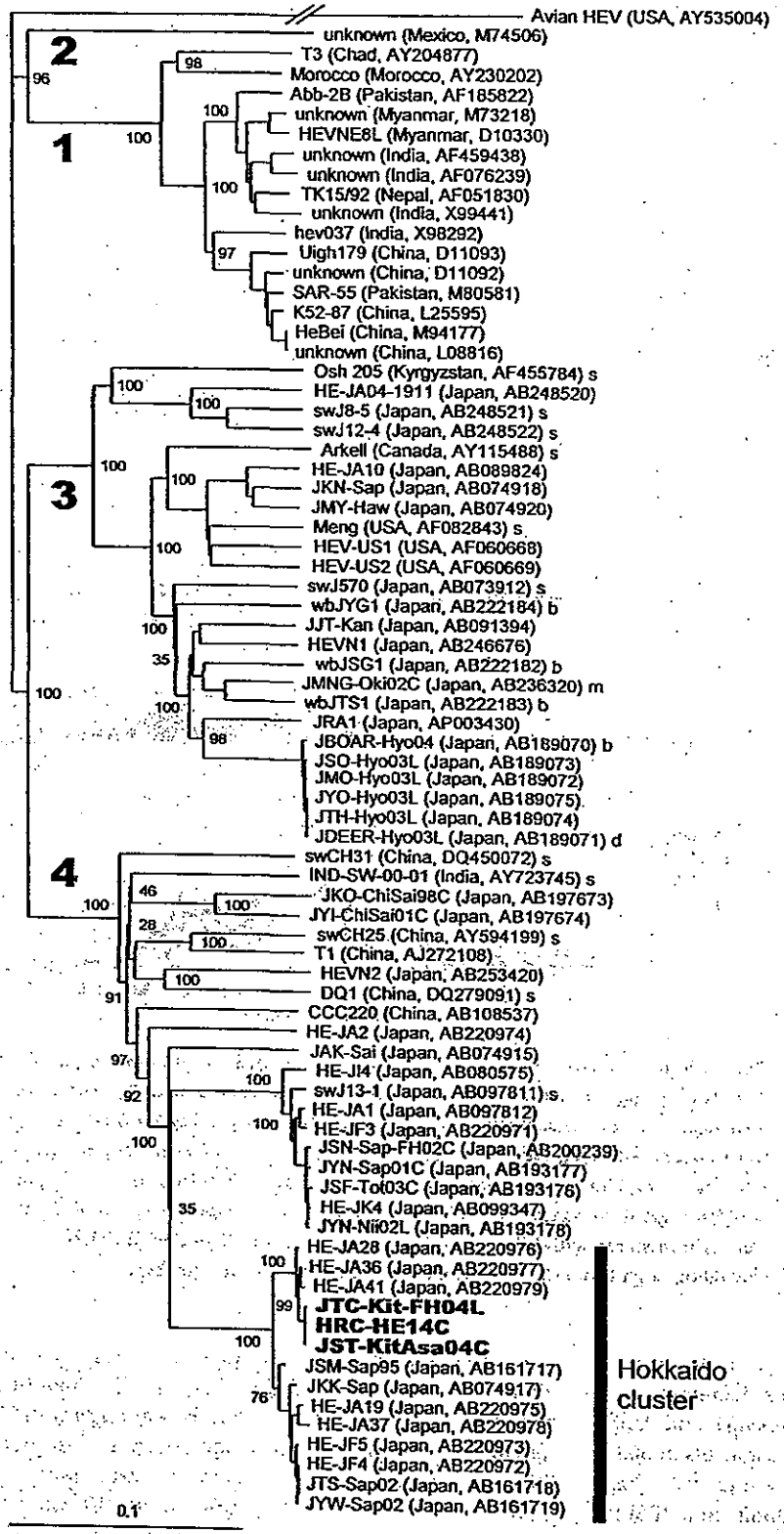


Fig. 1. Phylogenetic tree constructed by the neighbor-joining method based on the entire or nearly entire sequences of HEV genome of 77 isolates using an avian HEV (AY535004) as an outgroup. After the isolate name, the name of the country where the strain was isolated and accession numbers are shown in parentheses. The numbers 1, 2, 3, and 4 in bold indicated HEV genotypes. The 3 isolates HRC-HE14C from the causative donor, JST-KitAsa04C from the patient, and JTC-Kit-FH04L from the donor's father are indicated in bold. The letters "s," "b," "d," and "m" after parentheses denote HEV isolates from farmed pig, wild bore, wild deer, and mongoose, respectively. A vertical bar represents a cluster consisting of strains indigenous to Hokkaido, Japan. Bootstrap values are indicated for the major nodes as a percentage of the data set obtained from 1000 resamplings.

September 6 was released because it showed normal ALT and passed all the current blood screening tests. Transfusion was carried out 3 days after the blood donation, and the total amount of HEV in the PLT concentrate was estimated to be approximately 5.4 log copies. He was asymptomatic and did not feel tired or febrile in the periods near the two occasions of blood donation.

A minioutbreak of HEV infection in family members of the causative donor

Besides the causative donor, HEV RNA was detected in the blood of his 69-year-old father, who developed acute hepatitis on September 14, 2004, and finally died of fulminant hepatitis on October 14. Retrospective analysis of the father's blood sample taken on September 24, 41 days after the dining, revealed that the HEV strain, JTC-Kit-FH04L, was genotype 4. HEV RNA sequence analysis of the HEV isolates from the causative donor and his father showed only 9-nucleotide differences of 6588 nucleotides, suggesting that the two strains were extremely close but not identical (Fig. 1).

By retroactive interviewing, it was revealed that the causative donor and his 12 relatives gathered to enjoy grilled meats

including pig liver and intestines at a barbecue restaurant on August 14, 2004.[21] Blood samples from the relatives were tested for HEV markers with informed consent. Seven of the family members who ate grilled pig liver and/or intestines had IgM- and/or IgG-class anti-HEV in the blood samples taken 37 to 92 days after the barbecue party. Retrospectively, in the previous 6 months or more, dining out at that restaurant was the only occasion all the 13 relatives had eaten together.

Clinical course of the patient

It was confirmed that the PLT concentrate (approx. 200 mL) contaminated with HEV was transfused to a 64-year-old Japanese male patient with non-Hodgkin's lymphoma on September 9, 2004, as shown Day 0 in Fig. 2. The patient had been treated with autologous peripheral blood stem cell transplantation accompanied with heavy chemotherapy since July 30, 2004. In the first 3 weeks after the transfusion, liver function tests sustained to be normal. On Day 22, the ALT level increased transiently at 67 IU per L, and HEV was detected in serum. While the ALT level returned to normal, the viral load in serum showed an exponential increase. Levels of aspartase aminotransferase (AST) and ALT took an upward turn on Day 41. There was no evidence for acute infection of hepatitis A virus, HBV, HCV, cytomegalovirus, or Epstein-Barr virus. He was diagnosed as acute hepatitis E. On Day 45, he was referred to the liver unit of Teine Keijinkai Hospital to treat presumed developing acute hepatitis E. Despite antiviral therapy with interferon (IFN) from Day 45, 2′,5′-oligoisoadenylate synthetase in serum never showed apparent increase and no obvious decrement of viral load had obtained (Fig. 2A). Levels of AST and ALT indicated creeping increase to reach highest levels of 903 and 673 IU per L on Day 59, respectively (Fig. 2C). The treatment was switched from IFN to predonisolone (PSL) in expectation of its anti-inflammatory effect. From Day 59 after induction of PSL treatment, AST and ALT showed rapid decrease and improvement of prothrombin time was observed (data not shown). Dosage of PSL was tapered gradually and discontinued on Day 113. Soon after anti-HEV IgG emerged on Day 67, HEV load in the serum sample had declined rapidly, although anti-HEV IgM in the serum sample remained negative (Figs. 2A and 2B). The levels in aminotransferases were normalized after Day 95 (Fig. 2C). The HEV strain JST-KitAsa04C detected in the patient was genotype 4 and its entire sequence analysis showed only a 1-nucleotide difference of 7255 nucleotides, suggesting the two isolates were identical (Fig. 1).

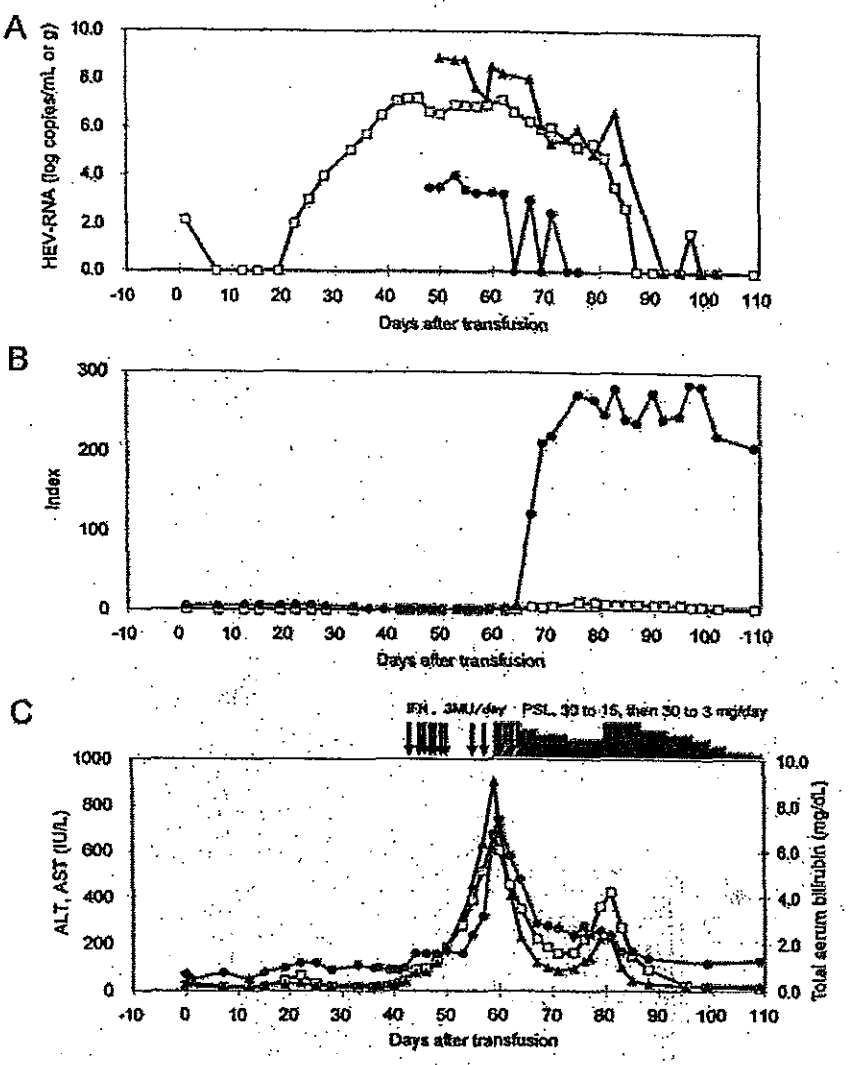


Fig. 2. Clinical course of transfusion-transmitted hepatitis E with kinetics of (A) HEV RNA, (B) serologic, and (C) biochemical markers after transfusion. The patient had transfusion of PLT concentrates contaminated with HEV on Day 0. (A) HEV RNA load was represented as log copies per mL of serum (□) or saliva (●) or per g of feces (▲). There were no data between Day 0 and Day 44 in feces and saliva. (B) Cutoff values of anti-HEV IgM (□) and IgG (●) antibodies are 30 and 13, respectively. (C) Medications were administered with IFN-α from Day 43 through Day 62 and with PSL from Day 59 through Day 112. (□) ALT; (▲) AST; (●) total serum bilirubin.

Serial quantitative changes of HEV load in serum, saliva, and feces of the patient

HEV RNA and anti-HEV were measured for every serum sample before and after the transfusion. In addition, HEV loads were also assessed prospectively for feces and saliva after his transference to the liver unit on Day 45. Any marker for HEV was not detected in serum sampled 37 days before the transfusion. A small amount of HEV RNA was transiently detected in his serum on Day 1, the next day of the transfusion. After the reappearance on Day 22, HEV RNA showed exponential increment with doubling every 29 hours and reached the peak level of 7.2 log copies per mL on Day 44. Beyond its plateau phase lasting 3 weeks, viral load revealed gradual decline over 2 weeks and thereafter decreased promptly. HEV viremia had been finally sustained for 63 days. HEV RNA remained detectable up to Day 97 in serum, Day 71 in saliva, and Day 85 in feces. Peak levels of HEV RNA were found on Day 53 in saliva at 4.0 log copies per mL and on Day 50 in feces at 8.9 log copies per g, respectively. HEV RNA was no longer detectable after Day 99 (Fig. 2A).

DISCUSSION

In Japan, a nonendemic country for hepatitis E, HEV infection is occurring more frequently than previously recognized. The prevalence of anti-HEV IgG in healthy Japanese persons ranged from 1.9 to 14.1 percent, depending on the geographic area,[20] and the prevalence of HEV RNA among Japanese blood donors with ALT level of at least 201 IU per L was 2.8 percent.[21] The risks of transfusion transmission of HEV might be low; however, five molecularly confirmed cases of transfusion-transmitted HEV infection have been reported in nonendemic countries so far.[12-16] In none of them, HEV infection routes of the causative donors are known. In this report, we have described the first case that the infection route of donor is clarified as zoonotic food-borne. The conclusion is based mainly on two observations.

First, by the epidemiologic study, the donor was determined to be infected in a minioutbreak of HEV infection in the context of food-borne transmission. Six of the 13 relatives who dined out together were positive for the presence of HEV RNA and/or IgM anti-HEV in their serum samples obtained 37 to 92 days after dining at the restaurant (Appendix 1). As for 4 relatives who were positive for the presence of IgM anti-HEV, HEV viremia might have transiently occurred without any symptom and had subsided by the time when blood samples were taken. Since IgM anti-HEV are regarded as the markers of acute HEV infection besides HEV RNA,[10] these facts strongly suggest that family members had recently become infected with HEV probably at the same time and remained asymptomatic. The party at the barbecue restaurant was the only opportunity all the 13 members had eaten together in the estimated period of HEV infection, 2 to 10 weeks.[22,23] Although it was difficult to identify the source of infection because no meat was left, they ingested various kinds of pig meats including liver and intestines, according to the replies to the questionnaire from the family members.[24] From this retrospective research, it is strongly suspected that the family members shared the motive of infection with HEV by ingestion of pig liver and intestines. In Japan, HEV has been isolated from farmed pigs,[9,25] wild deer,[8,26,27] and wild boar[10,11,26,27] as well as humans and recent studies also indicated that HEV is moderately resistant to heat inactivation.[28,29] Some reports suggest that a number of hepatitis E cases in Japan may be via a zoonotic food-borne route.[8-11,25-27,30]

Second, a single transmission route of HEV in this minioutbreak is corroborated by molecularly confirmed facts. From full-length sequence analysis, HEV RNAs detected in the donor and recipient were identical and closely related to that in his father. Among the strains of genotype 4 indigenous to Hokkaido, Japan, these three strains were segregated into a distinct cluster with a bootstrap value of 99 percent in a phylogenetic tree based on the entire or nearly entire sequences of HEV genome. Moreover, when comparing 412-nucleotide sequences (nucleotides 5985-6396 of HRC-HE14C) of ORF2 region, where many sequences of Japanese swine HEV are retrievable in DDBJ/EMBL/GenBank nucleotide sequence databases, high similarity (409/412 nucleotides, 99.3%) was observed between the HEV sequences derived from the causative donor and his father and strain swJL145 (AB105902),[9] which was detected in pig liver sold at a drug store in Hokkaido, Japan.

To date, in acute hepatitis E including transfusion transmission cases, dynamic relationships between infection markers for HEV and disease progression throughout the course from HEV transmission to convalescence of disease have not been demonstrated. This is the first case of acute hepatitis E, in which HEV kinetics in serum as well as in feces and saliva were described by using quantitative RT-PCR for HEV RNA from transfusion up to the end of viremia accompanied by disease progression, and the emergence and increase of anti-HEVs. In the current case, HEV viremia had lasted for 9 weeks or more and viral load reached its peak 15 days before the peak of aminotransferase level and died out promptly right after the appearance of anti-HEV IgG on Day 67. The results led us to understand the chronologic relationship between preceding viremia and after emergence and increase of anti-HEV.

Besides serum, the kinetics of HEV load in feces and saliva were concomitantly observed for the first time in hepatitis E in humans. After the transmission, HEV RNA remained detectable until Day 71 in saliva and Day 85 in feces. Among sera, saliva, and feces, every time point at peak viral loads resembled each other, 50 to 60 days after transmission. These facts may indicate that viral loads in

saliva and feces would also reflect viremia state. In addition, the results for saliva suggest that besides fecal-oral route, oral-oral transmission manner can be another route of human-to-human infection of HEV.

Soon after the transference to liver unit in the hospital, IFN-α therapy was started against HEV infection, indicating the exponential increase of viral load in sera. The levels in 2′,5′-oligoadenylate synthetase, however, induced by IFN and regarded as a predictive marker for favorable IFN efficacy,[31] did not show sufficient increase in serum (data not shown), and HEV load monitored concomitantly indicated no actual decrement during treatment. Thereafter, single-nucleotide polymorphisms in markers predicting the therapeutic efficacy of IFN, such as mannose-binding lectin,[32] MxA,[33] LMP7,[34] and osteopontin,[35] were examined, and all of them did not show the phenotype associated with favorable efficacy of IFN (data not shown).

Throughout his clinical course, no distinct positive result for IgM anti-HEV was observed. It is possible that the concentration of IgM anti-HEV was too low to be detected by the method we used. In fact, some of his samples showed equivocal reaction. Furthermore, underlying disease and the preceding treatment including autologous peripheral blood stem cell transplantation and large dosage chemotherapy might have led the patient to an immunocompromised state that responds inadequately for HEV infection. In fact, both serum levels in IgG and IgM had been indicated consistently less than lower limitation of normal ranges in the entire course (data not shown).

We should note that the present case was not revealed if the two practices had not been introduced, which are not widespread outside Japan. They are ALT screening and donor blood sample repository system. As a safety measure, the Japanese Red Cross Blood Center introduced ALT testing for a surrogate marker for non-A, non-B hepatitis virus infection. Because ALT testing contributes little for HCV infection after HCV antibody testing started, ALT screening has been discontinued in the United States and some other countries. Although the cutoff value may need to be reevaluated, the current case suggests that ALT testing may contribute to excluding blood with the presence of HEV. On the other hand, the Japanese Red Cross has established storing repository samples of all donations since 1996. Blood samples are collected from each donation and stored for 10 years at −30°C to investigate for lookback study such as the suspected cases of transfusion-transmitted infection and alloantibodies for TRALI. This system plays a very important role in the hemovigilance system in Japan.[36,37]

In the present case of transfusion-transmitted acute hepatitis E, the infection route in the blood donor was, for the first time, clarified to be zoonotic food-borne manner. In addition, the entire course including incubation period and disease progression in acute HEV infection was followed by serologic and virologic markers, and the patient was treated by monitoring them. To our knowledge, this is the first report for acute HEV infection in humans, in which various infection markers were prospectively monitored simultaneously with disease progression, excepting experimental hepatitis E in a volunteer.[38]

Our data suggest that hepatitis E is likely caused by consumption of contaminated pig meat, and there is a risk of transfusion transmission of HEV in Japan. The most effective preventive measure to reduce the risk of blood-borne transmission is to screen the blood supply for HEV or to implement pathogen inactivation. The epidemiology and the transfusion-related risks for HEV infection have not been fully understood in industrialized countries including Japan. We are undertaking epidemiologic studies of HEV infection in Japanese blood donors and a feasibility study of NAT screening for HEV in Hokkaido, Japan.

ACKNOWLEDGMENTS

We are grateful to the patient, the donor, and his relatives who were the subjects of the family study. This study was supported in part by grants from the Ministry of Health, Labor, and Welfare of Japan.

REFERENCES

1. Purcell RH, Emerson SU. Hepatitis E virus. In: Knipe GM, Howley PM, editors. Fields virology. 4th ed. Philadelphia (PA): Lippincott, Williams & Wilkins; 2001. p. 3051-61.
2. Schlauder GG, Dawson GJ, Erker JC, Kwo PY, Knigge MF, Smalley DL, Rosenblatt JE, Desai SM, Mushahwar IK. The sequence and phylogenetic analysis of a novel hepatitis E virus isolated from a patient with acute hepatitis reported in the United States. J Gen Virol 1998;79:447-56.
3. Worm HC, Wurzer H, Frosner G. Sporadic hepatitis E in Austria. N Engl J Med 1998;339:1554-5.
4. Takahashi K, Iwata K, Watanabe N, Hatahara T, Ohta Y, Baba K, Mishiro S. Full-genome nucleotide sequence of a hepatitis E virus strain that may be indigenous to Japan. Virology 2001;287:9-12.
5. Mizuo H, Suzuki K, Takikawa Y, Sugai Y, Tokita H, Akahane Y, Itoh K, Gotanda Y, Takahashi M, Nishizawa T, Okamoto H. Polyphyletic strains of hepatitis E virus are responsible for sporadic cases of acute hepatitis in Japan. J Clin Microbiol 2002;40:3209-18.
6. Mansuy JM, Peron JM, Abravanel F, Poirson H, Dubois M, Miedouge M, Vischi F, Alric L, Vinel JP, Izopet J. Hepatitis E in the south west of France in individuals who have never visited an endemic area. J Med Virol 2004;74:419-24.
7. 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. Non-travel-associated hepatitis E in England and Wales: demographic, clinical, and molecular epidemiological characteristics. J Infect Dis 2005;192:1166-72.
8. Tei S, Kitajima N, Takahashi K, Mishiro S. Zoonotic transmission of hepatitis E virus from deer to human beings. Lancet 2003;362:371-3.
9. Yazaki Y, Mizuo H, Takahashi M, Nishizawa T, Sasaki N, Gotanda Y, Okamoto H. Sporadic acute or fulminant hepatitis E in Hokkaido, Japan, may be food-borne, as suggested by the presence of hepatitis E virus in pig liver as food. J Gen Virol 2003;84:2351-7.
10. Tamada Y, Yano K, Yatsuhashi H, Inoue O, Mawatari F, Ishibashi H. Consumption of wild boar linked to cases of hepatitis E. J Hepatol 2004;40:869-70.
11. Li TC, Chijiwa K, Sera N, Ishibashi T, Etoh Y, Shinohara Y, Ishida M, Sakamoto S, Takeda. N Miyamura T. Hepatitis E virus transmission from wild boar meat. Emerg Infect Dis 2005;11:1958-60.
12. Matsubayashi K, Nagaoka Y, Sakata H, Sato S, Fukai K, Kato T, Takahashi K, Mishiro S, Imai M, Takeda N, Ikeda H. Transfusion-transmitted hepatitis E caused by apparently indigenous hepatitis E virus strain in Hokkaido, Japan. Transfusion 2004;44:934-40.
13. Mitsui T, Tsukamoto Y, Yamazaki C, Masuko K, Tsuda F, Takahashi M, Nishizawa T, Okamoto H. Prevalence of hepatitis E virus infection among hemodialysis patients in Japan: evidence for infection with a genotype 3 HEV by blood transfusion. J Med Virol 2004;74:563-72.
14. Boxall E, Herborn A, Kochethu G, Pratt G, Adams D, Ijaz S, Teo CG. Transfusion-transmitted hepatitis E in a "nonhyperendemic" country. Transfus Med 2006;16:79-83.
15. Tamura A, Shimizu YK, Tanaka T, Kuroda K, Arakawa Y, Takahashi K, Mishiro M, Shimizu K, Moriyama M. Persistent infection of hepatitis E virus transmitted by blood transfusion in a patient with T-cell lymphoma. Hepatol Res 2007;37:113-20.
16. Colson P, Coze C, Gallian P, Henry M, De Micco P, Tamalet C. Transfusion-associated hepatitis E, France. Emerg Infect Dis 2007;13:648-9.
17. Thompson JD, Higgins DG, Gibson TJ, CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 1994;22:4673-80.
18. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 1987;4:406-25.
19. Page RD. TreeView: an application to display phylogenetic trees on personal computers. Comput Appl Biosci 1996;12: 357-8.
20. Li TC, Zhang J, Shinzawa H, Ishibashi M, Sata M, Mast EE, Kim K, Miyamura T, Takeda N. Empty virus-like particle-based enzyme-linked immunosorbent assay for antibodies to hepatitis E virus. J Med Virol 2000;62:327-33.
21. Gotanda Y, Iwata A, Ohnuma H, Yoshikawa A, Mizoguchi H, Endo K, Takahahsi M, Okamoto H. Ongoing subclinical infection of hepatitis E virus among blood donors with an elevated alanine aminotransferase level in Japan. J Med Virol 2007;79:734-42.
22. Khuroo MS. Study of an epidemic of non-A, non-B hepatitis. Possibility of another human hepatitis virus distinct from post-transfusion non-A, non-B type. Am J Med 1980; 68:818-24.
23. Balayan MS, Andjapardze AG, Savinskaya SS, Ketiladze ES, Braginsky DM, Savinov AP, Poleschuk VF. Evidence for a virus in non-A, non-B hepatitis transmitted via the fecal-oral route. Intervirology 1983;20:23-31.
24. Kato M, Taneichi K, Matsubayashi K. A mini-outbreak of HEV infection in those who enjoyed *Yakiniku* party: one died of fulminant hepatitis. Kanzo 2004;45:688.
25. Takahashi M, Nishizawa T, Miyajima H, Gotanda Y, Iita T, Tsuda F, Okamoto H. Swine hepatitis E virus strains in Japan form four phylogenetic clusters comparable with those of Japanese isolates of human hepatitis E virus. J Gen Virol 2003;84:851-62.
26. Takahashi K, Kitajima N, Abe N, Mishiro S. Complete or near-complete nucleotide sequences of hepatitis E virus genome recovered from a wild boar, a deer, and four patients who ate the deer. Virology 2004;330:501-5.
27. Sonoda H, Abe M, Sugimoto T, Sato Y, Bando M, Fukui E, Mizuo H, Takahashi M, Nishizawa T, Okamoto H. Prevalence of hepatitis E virus (HEV) infection in wild boars and deer and genetic identification of a genotype 3 HEV from a boar in Japan. J Clin Microbiol 2004;42:5371-4.
28. Emerson SU, Arankalle VA, Purcell RH. Thermal stability of hepatitis E virus. J Infect Dis 2005;192:930-3.
29. Tanaka T, Takahashi M, Kusano E, Okamoto H. Development and evaluation of an efficient cell-culture system for hepatitis E virus. J Gen Virol 2007;88:903-11.
30. Abe T, Aikawa T, Akahane Y, Arai M, Asahina Y, Atarashi Y, Chayama K, Harada H, Hashimoto N, Hori A. Demographic, epidemiological, and virological characteristics of hepatitis E virus infections in Japan based on 254 human cases collected nationwide. Kanzo 2006;47:384-91.
31. Schattner A, Merlin G, Wallach D, Rosenberg H, Bino T, Hahn T, Levin S, Revel M. Monitoring of interferon therapy by assay of 2'-5' oligo-isoadenylate synthetase in human peripheral white blood cells. J Interferon Res 1981;1:587-94.
32. Matsushita M, Hijikata M, Ohta Y, Iwata K, Matsumoto M, Nakao K, Kanai K, Yoshida N, Baba K, Mishiro S. Hepatitis C virus infection and mutations of mannose-binding lectin gene MBL. Arch Virol 1998;143:645-51.
33. Mochida S, Hashimoto M, Matsui A, Naito M, Inao M, Nagoshi S, Nagano M, Egashira T, Mishiro S, Fujiwara K. Genetic polymorphisms in promoter region of osteopontin gene may be a marker reflecting hepatitis activity in chronic hepatitis C patients. Biochem Biophys Res Commun 2004;23:1079-85.

34. Hijikata M, Ohta Y, Mishiro S. Identification of a single nucleotide polymorphism in the MxA gene promoter (G/T at nt -88) correlated with the response of hepatitis C patients to interferon. Intervirology 2000;43:124-7.
35. Sugimoto Y, Kuzushita N, Takehara T, Kanto T, Tatsumi T, Miyagi T, Jinushi M, Ohkawa K, Horimoto M, Kasahara A, Hori M, Sasaki Y, Hayashi N. A single nucleotide polymorphism of the low molecular mass polypeptide 7 gene influences the interferon response in patients with chronic hepatitis C. J Viral Hepat 2002;9:377-84.
36. Satake M. Japanese repositories. Transfusion 2007;47:1105.
37. Okazaki H. The benefits of the Japanese haemovigilance system for better patient care. ISBT Sci Ser 2007;2:104-9.
38. Chauhan A, Jameel S, Dilawari JB, Chawla YK, Kaur U, Ganguly NK. Hepatitis E virus transmission to a volunteer. Lancet 1993;341:149-50.

APPENDIX 1

HEV infection markers in the 13 family members who participated in the dinner on August 14, 2004

Number*	Age (years)	Sex	Days after Aug 14, 2004	ALT (IU/L)	HEV markers		
					RNA (10^6/mL)	IgM† (index)	IgG‡ (index)
1	39	Male	23	27	+(3.1)	–(3.4)	–(2.0)
			37	236	+(4.8)	+(60.4)	+(14.2)
			49	70	+(2.1)	+(269.5)	+(154.7)
			53	44	–	+(257.8)	+(150.5)
			77	20	–	+(174.6)	+(163.0)
2	69	Male	41	1511	+(2.6)	+(187.2)	+(271.4)
3	43	Male	92	34	–	+(174.7)	+(297.7)
4	68	Male	79	15	–	+(51.7)	+(283.3)
5	37	Female	79	13	–	+(110.9)	+(90.3)
6	15	Male	90	17	–	+(63.3)	+(250.6)
7	58	Female	79	25	–	–(4.0)	+(25.9)
8	67	Female	79	15	–	–(1.4)	–(12.9)
9	38	Female	89	12	–	–(6.1)	–(1.1)
10	15	Male	77	19	–	–(0.3)	–(0.5)
11	14	Male	77	19	–	–(7.5)	–(0.3)
12	46	Male	90	15	–	–(2.2)	–(0.4)
13	6	Female	90	15	–	–(26.6)	–(1.1)

Data shown were originally reported by Kato et al.[24] without describing quantitative test results of antibodies and viral RNA and follow-up data of the causative donor.
* Number 1 is the causative donor; Number 2 is the donor's father and died of hepatitis E; others are their relatives.
† Positive ≥30 index.
‡ Positive ≥13 index.

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8月21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	Vox Sanguinis 2008; 95(SUPPL. 1): 282-283	公表国 中国	
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

＜目的＞
中国の4つの都市における血液ドナー中のHEV陽性率を評価し、HEV感染を排除するためのALT測定の価値を評価すること。
＜方法＞
ルーチンのスクリーニング検査（HCV抗体、HIV 1/2抗体、HBsAg、梅毒およびALT）で陰性と判定されたドナー検体とALT値が高いだけの検体を、中国の4つの都市（北京、ウルムチ、昆明、広州）の4つの血液センターから2005年に収集し、-40℃で冷凍した。全部で6,665の血液ドナーの検体について、2007年にHEV IgG抗体、HEV IgM抗体、HEV Agの測定を行った。
＜結果＞
検査を実施した6,665の血液ドナーのうち、HEV IgG抗体、HEV IgM抗体、HEV Agの各々の陽性率は、24.23%(1,615/6,665)、1.08%(72/6,665)、0.03%（2/6,665）であった。ALTのみが高かった487のドナーのHEV IgG抗体、HEV IgM抗体、HEV Agの陽性率（30.80%、2.05%、0.21%）はすべて、ルーチンスクリーニングで陰性であった6,178のドナーの陽性率（23.71%、1.00%、0.02%）よりも高かった（P<0.05）。2名のHEV Ag陽性ドナーのうち、1名はルーチンのスクリーニングで陰性で、HEV Ag ELISA S/COの平均値が3.4、HEV IgG抗体が陰性、HEV IgM抗体が陰性であった。他方の1名はALTのみが高く、HEV Ag ELISA S/COの平均値が18.0、HEV IgG抗体が陽性でS/COの平均値が10.8、HEV IgM抗体が陰性であった。
＜結論＞
HEVは中国における風土病である。中国におけるルーチンのスクリーニングで陰性と判定された血液ドナーの中で、1%がHEV IgM抗体陽性またはHEV Ag陽性であり、HEVに感染性がある可能性がある。ALTスクリーニングは、中国ではHEV感染血液の排除に一定の役割を有している可能性がある。

報告企業の意見	今後の対応
中国における血液ドナーの約1%は、抗HEV IgM陽性又はHEV抗原陽性であり、HEV感染の可能性があるとの報告である。 静注用ヘブスブリン-IHについては、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。 ヘブスブリンについては、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では本剤の製造工程において十分なLRVが得られないため、製造工程における不活化・除去が十分であるとは説明困難である。そのため、ヘブスブリン用の原料血漿については、弊社にてHEVについてのミニプールNATを試行的に導入した。	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。

easy to use, FDA approved test to confirm repeat reactives or to resolve discrepant results is lacking.
Aims: To develop a supplemental test for confirming the presence of antibodies to *T. cruzi* in repeatedly reactive blood or plasma units identified by a screening assay.
Methods: The immunoblot assay is based on four different recombinant antigens (rAgs) FP3, FP6, FP10, and TcF, for the detection of antibodies to *T. cruzi*. Each rAg was constructed with multiple antigenic domains of *T. cruzi* including repetitive sequences and non-repetitive sequences. The rAgs are fitted as discrete lines onto the strip. Antibody responses were visually assessed against two internal calibrators (low and high) also applied to the immunostrip as discrete lines. The immunoblot assay sensitivity was evaluated with 688 RIPA confirmed chagasic specimens. The specificity was evaluated with 821 unscreened specimens from random U.S. blood donors and 531 specimens of 30 different unrelated medical conditions, including leishmaniasis, malaria, and autoimmune diseases, or potentially interfering substances. The interpretation of results was as follows: (a) no bands or a single test band = NEGATIVE; (b) two or more test bands with a least on band having intensity of 1+ or higher = POSITIVE; and (c) multiple faint test bands (±) = INDETERMINATE. All samples were initially tested in the PRISM Chagas screening assay; and reactive samples were also tested in two different ELISA and in a radio-immunoprecipitation assay (RIPA).
Results: All 688 chagasic samples showed two to four rAg test bands and were interpreted as positive in the immunoblot assay; sensitivity of 100% (688/688). Among 821 unscreened specimens of random donors, 819 showed none or a single test band, and one gave two faint test bands. One specimen was repeatedly reactive in PRISM Chagas assay, two reference ELISAs, and confirmed in RIPA as positive; while another specimen was non-reactive in these reference tests. Of the 531 specimens with disease states or potentially interfering substances, 525 tested negative, two confirmed positive, 1 false-positive, and three indeterminate.
Conclusions: The sensitivity of the immunoblot assay in the geographically-diverse group of chagasic specimens was 100% (688/688). The resolved specificity of random donor specimens was also 99.88% (819/820). The recombinant antigen based immunoblot assay, in multiple lots and run by multiple technicians, has demonstrated great potential as a supplemental test to confirm the presence of antibodies to *T. cruzi* in blood specimens. Design verification and validation of this assay are ongoing.

P-615

HEPATITIS B VIRUS DETECTION AMONG VOLUNTARY BLOOD DONORS IN THE MUNICIPALITY OF STRUMICA

Timova TT[1], Kocovska EK[1], Maninska LM[1], Momirovska TM[2], Stamholieva DS[1], Gorgevska VG[1], Kostovska SK[1]
[1]*General Hospital, Strumica, Macedonia* [2]*General Hospital -Prilep, Prilep, Macedonia* [3]*National Institute for Transfusion Medicine, Skopje, Macedonia*

In spite of the progress in the development of diagnostic, therapeutic and prophylactic methods, virus hepatitis still present a serious global health problem. The possibility of transmission of these infections through transfusion of blood and blood derivates implies obligatory control of the donated blood.
Aim: To show the prevalence of Hepatitis B (HBsAg) in volunteer blood donors for the period from 2001 till 2006.
Materials: The presence of virus markers was analyzed in the serum of 9166 blood donors who donated blood at the Department of transfuziology, General Hospital-Strumica, in the period from 2001 till 2006.
Methods: The samples were tested for the presence of viral markers (HBsAg), using tests for HBsAg (Abbott Auxyme Monoclonal EIA).
Results: The presence of markers for Hepatitis B (HBsAg) were found in 89 (0.97%) blood donors. In 2001 the presence of HBsAg was found in 12 blood donors, 2002 – in 20 blood donors, 2003 in 14 blood donors, 2004 in 17 blood donors, 2005 in 14 blood donors, 2006 in 12 blood donors. With A blood group were 42 (47.2%) blood donors, with O blood group were 28 (31.4%) blood donors, with B blood group were 10 (11.2%) blood donors and with AB blood group were nine (10.2%) blood donors.
Conclusion: The obligatory testing of the donors blood is of exceptional importance to prevent the transmission of diseases. Moreover, a significant ring in the chain for ensuring safe blood is the selection of a qualitative donor, that is a donor who donates blood volontarily, freely, anonymously and periodically.

P-616

OCCULT HEPATITIS B VIRUS INFECTION IN BLOOD DONORS FROM CENTRAL PORTUGAL

Teixeira MJ, Henggeler F, Diniz C, Caldeira M, Mateus F, Freitas C, Gonçalves H
Instituto Português di Sangue, Coimbra, Portugal

Background: The detection of HBV DNA in serum without HBsAg and with/without the presence of antibodies (anti-HBc/anti-HBs), defines the state of the occult hepatitis B virus infection. The prevalence in endemic areas varies from 7% to 19%, while in the west countries varies from 0% to 9%, being greater in people with anti-HBc and/or anti-HBs. Low serum HBV DNA titers, in the range of 100–1000 copies/mL, are typical in occult HBV infection. A high prevalence of occult HBV has been reported in hepatocellular carcinoma (HCC).
Aims: The appearance of the nucleic acid testing (NAT) with great sensibility allows us to identify a population with HBsAg negative but with low levels of HBV DNA in serum. In our Centre all donors are screened for HBV DNA, HIV RNA and HCV RNA.
Methods: In the screening of the hepatitis B serologic markers we have used ELISA and quimioluminiscence tests. In the screening of the HBV DNA we have used the Transcription Mediated Amplification (TMA) technology, in single testing, with predicted HBV detection rate of 50% and 95% of 3.1 and 7.4 IU/mL, respectively.In the screening of HBV viral load we have used PCR technology, with detection limit of 60 IU/mL.
Results: The Regional Blood Centre (Coimbra) started the screening of the HBV DNA to all donors in October 2006. Until November 2007, we have studied 20.881 donors. We found three cases of occult hepatitis B virus infection.
Conclusions: Some aspects need to be investigated, especially the relationship between the occult hepatitis B virus infection and the infectivity of the different blood components. The sensibility of the NAT is very important in the precocious detection of the HBV DNA in blood donors.

P-617

PREVALENCE OF HEPATITIS E VIRUS INFECTION IN BLOOD DONORS IN DIFFERENT CITIES OF CHINA

Ren FR[1], Wang ZY[1], Gong XY[1], Song ML[1], Lv QS[1], Tiemuer MHL[2], Yao FZ[3], Zheng YR[4], Wang YC[5], Zhuang H[6]
[1]*Beijing Red Cross Blood Center, Beijing, China* [2]*Urumchi Blood Center of Xinjiang Uygur Autonomous Region, Urumchi, China* [3]*Yunnan Kunming Blood Center, Kunming, China* [4]*Guangzhou Blood Center, Guangzhou, China* [5]*National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China* [6]*Peking University Health Science Center, Beijing, China*

Background: Hepatitis E virus (HEV) is a single strand and non-enveloped RNA virus. HEV infection is normally transmitted via the faeco-oral route. However HEV recently emerged as a transfusion-transmitted pathogen. Several transfusion-transmitted HEV infections have been reported in

© 2008 The Authors
Journal compilation © 2008 Blackwell Publishing Ltd.

HEV-hyperendemic or nonhyperendemic countries. In China, neither HEV antibodies nor HEV RNA are systematically tested in blood donors. Alanine aminotransferase (ALT) in serum/plasma has been tested in all blood donors since 1960s in China, before hepatitis B surface antigen screening. With the introduction of specific anti-HCV and viral nucleic acid testing (NAT), ALT test is no longer used in routine donor screening in many countries. However, ALT measurement is still retained as a screening tool for blood donors in China, in consideration that viral hepatitis is endemic in China, although ALT has low specificity for detecting individuals with transfusion-transmitted virus infection risk and its value is controversial.

Aims: To evaluate the prevalence of HEV infection among blood donors in four cities of China and to evaluate the value of ALT measurement for eliminating HEV infectious blood in blood donors.

Methods: Donor samples with negative results in routine screening (anti-HCV, anti-HIV1/2, HBsAg, syphilis and ALT) and samples with ALT elevated alone were collected from four blood centers in four Chinese cities, Beijing (North), Urumchi (Northwest), Kunming (Southwest), and Guangzhou (South) in 2005 and were frozen at -40?. A total of 6665 blood donor samples were tested for anti-HEV IgG, anti-HEV IgM and HEV Antigen (Ag) by enzyme-linked immunoassays (WANTAI Biological Enterprise Co. Ltd. Beijing, China) in 2007. Repeated positive results defined as a positive result. The Person Chi-Squared test or Fisher's exact test were used for the statistical analysis.

Results: Of the 6665 blood donors tested, the prevalence of anti-HEV IgG, anti-HEV IgM and HEV Ag were 24.23% (1615/6665), 1.08% (72/6665) and 0.03% (2/6665) respectively. The prevalence of anti-HEV IgG, anti-HEV IgM and HEV Ag were all higher in 487 donors with elevated ALT alone (30.80%, 2.05% and 0.21%, respectively) than in 6178 donors with negative results in routine screening (23.71%, 1.00% and 0.02%)

Table HEV Seroprevalence in blood donors

Samples	Cities	Numbers Tested	Anti-HEV IgG (%)	Anti-HEV IgM (%)	HEV Ag (%)
Samples with negative results in routine screening	Beijing	2378	458 (19.26%)	30 (1.26%)	0 (0.00%)
	Urumchi	1910	341 (17.85%)	14 (0.73%)	1 (0.05%)
	Kunming	1170	431 (36.84%)	11 (0.94%)	0 (0.00%)
	Guangzhou	720	235 (32.64%)	7 (0.97%)	0 (0.00%)
	Total	6178	1465 (23.71%)	62 (1.00%)	1 (0.02%)
Samples with elevated ALT alone	Beijing	72	16 (22.22%)	2 (2.78%)	0 (0.00%)
	Urumchi	247	45 (18.22%)	1 (0.40%)	0 (0.00%)
	Kunming	152	84 (55.26%)	6 (3.95%)	0 (0.00%)
	Guangzhou	16	5 (31.25%)	1 (6.25%)	1 (6.25%)
	Total	487	150 (30.80%)	10 (2.05%)	1 (0.21%)
Total		6665	1615 (24.23%)	72 (1.08%)	2 (0.03%)

Data were shown as "numbers of positive samples (positive rate)"

($P < 0.05$). Of the two HEV Ag positive donors, one had negative results in routine screening and had average HEV Ag ELISA S/CO ratio of 3.4, anti-HEV IgG (-), anti-IgM (-); the other had elevated ALT alone and had average HEV Ag ELISA S/CO ratio of 18.0, anti-HEV IgG (+) with average S/CO ratio of 10.8, anti-HEV IgM (-). The following table shows the more detailed results.

Conclusions: Hepatitis E virus is endemic in China. Among blood donors with negative results in routine screening in China, about 1% are anti-HEV IgM (+) or HEV Ag (+) and may be HEV infectious. ALT screening may have some role in eliminating HEV infectious blood in China.

Acknowledgements: This work was supported by the '863' project (grant No. 2006AA02Z453) from Chinese Ministry of Science and Technology in 2006.

P-618

Abstract withdrawn.

P-619

POLYMORPHISM OF HLA-DRB1 OF THE UYGHURS IN CHRONIC HEPATISIS B IN KHOTAN AREA XINJIANG CHINA

Kurexijiang KT[1], Wupuer H[2], Yunusi K[2], Zhang Z[3], Shawuer R[1]
[1]*Uyghur Traditional Medicine Hospital of Khotan Area, Xinjiang, Khotan, China* [2]*Xinjiang Medical University, Urumqi, China* [3]*HLA Laboratory, Beijing Red Cross Blood Center, Beijing, China*

This abstract is read by title only.

P-620

IMPACT OF PHOTOCHEMICAL TREATMENT OF PLATELET COMPONENTS (INTERCEPT™) ON PLATELET AND RBC COMPONENT USE BY HEMATOLOGY PATIENTS DURING 3 YEARS OF ROUTINE PRACTICE

Osselaer JC[1], Doyen C[1], Defoin L[1], Debry C[1], Goffaux M[1], Messe N[1], Van Hooydonk M[2], Bosly A[2], Lin JS[3], Corash LM[3]
[1]*Cliniques Universitaires de Mont Godinne, Yvoir, Belgium* [2]*Hematology Svc, Cliniques Universitaire de Mont Godinne, Yvoir, Belgium* [3]*Cerus Corporation, Concord, CA, USA*

Background: In 2003 the Blood Transfusion Center (BTC), Cliniques Universitaires Mont Godinne (CUMG) initiated universal use of pathogen inactivated INTERCEPT Platelets (I-P, Cerus Europe BV, Amersfoort, Netherlands) for transfusion (txn) support of thrombocytopenia. Hematology patients require intensive txn support.

Aims: To examine the impact of I-P adoption on platelet (PLT) and red blood cell concentrate (RBC) use by hematology patients, the duration of support, the number of PLT txn per patient, total PLT dose per patient, and total RBC units per patient were compared for 3 years before I-P adoption, when only conventional PLT (C-P) were used, and for 3 years after adoption of I-P. RBC use served as a surrogate for hemostasis efficacy of PLT txn and was evaluated during periods of PLT support and periods without PLT txn support.

Methods: In both periods, PLT were collected by apheresis in reduced plasma concentration with process leukocyte reduction. For C-P, T-Sol (Fenwal, La Chatre, France) with a ratio to plasma of 70:30% was used. For I-P, Intersol (Cerus) with a ratio to plasma of 65:35% was used. I-P components (2.5–6.0-E11 PLT) were treated with amotosalen (150 μmol/L) plus UVA (3 J/cm sq) to inactivate pathogens and leukocytes. I-P replaced gamma irradiation, bacteria detection, and CMV serology. I-P and C-P were available for issue the day after collection. Days of txn support were calculated from the first PLT txn until 5 days after the last PLT txn. An

Effect of I-P Adoption on Platelet and RBC Use

Parameter	CP	IP	P
Platelet Use (mean/median)			
Patients supported	272	276	
Days of PLT support	31.6/15	33.1/15	0.70
PLT txn/pt	20.8/10	24.2/11	0.17
Total PLT dose (10^{11})/pt	87.3/41	100.8/43	0.19
RBC Use During Platelet Support (mean/median)			
Patients transfused	222	244	
Total RBC units/pt	16.4/8.0	17.6/7.0	0.64
RBC Use Outside of Platelet Support (mean/median)			
Patients transfused	237	235	
Total RBC units/pt	12.7/8.0	12.7/8.0	0.99

© 2008 The Authors

別紙様式第2-1

No. 30

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2008. 7. 22	該当なし	

一般的名称	(製造販売承認書に記載なし)	研究報告の公表状況		公表国
販売名(企業名)	合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)		ABC Newsletter, No. 26. 2008 Jul 4.	米国

研究報告の概要

○米国医師会がゲイ男性の供血5年延期を「容認できる」との考え

米国医師会(AMA)は、男性同性愛行為を行った供血者の供血延期期間を生涯から5年間に変更するとした連邦の方針を支持するという声明を採択した。この声明は2008年のAMA年次総会で採択され、「AMAは、現在の科学的エビデンスとリスク分析モデルに基づき、MSMに対する5年間の供血延期は容認できる(supportable)と認める」と述べている。AMAによると、「容認できる」という言葉は、基本的に、FDAに対して新しい方針を通知し「実施に協力する」ことを意味している。また、AMAは今回の変更に対して反対を主張しない。

FDAは1977年以降、採血事業者に対し、MSMの供血を生涯延期とすることを求めてきた。AMAの声明は、血液事業者団体が主張する1年間の供血延期により近いものとなっている。血液事業者は、供血延期は金銭や薬物と引き替えのセックスなどハイリスク行為に対して実施すべきであると主張してきた。また、最近ではゲイ・グループによる反対運動、政府機関や大学での議論も行われ、一部の大学では構内での移動採血を中止しようとする動きが出ていた。

報告企業の意見	今後の対応
米国医師会は、男性同性愛行為を行った供血者の供血延期期間を生涯から5年間に変更するとした連邦の方針を支持するという声明を採択したとの報告である。MSMのHIV等ウイルス感染率は高く、日本においても1年間の献血延期の他、検査目的の献血禁止などの対策を引き続き行っていく必要がある。	日本赤十字社は、輸血感染症対策として、男性と性的接触を持った男性は1年間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-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

America's Blood Centers®
It's About Life.

ABC NEWSLETTER

CURRENT EVENTS AND TRENDS IN BLOOD SERVICES

Visit ABC's Web site at: www.americasblood.org

2008 #26 July 4, 2008

INSIDE:

Finnish Parliament Finds Red Cross MSM Policy Justified 3
Umbilical Cord Blood Cell Transplants Help Mice With ALS 4
House Leader Seeks FDA Documents on Preemption Policy Change 5
Labor-HHS Bill Markup Hearing Fiasco May Mean NCBI Spending Set by CR 5

AMA Deems Five-Year Blood Donor Deferral for Gay Men "Supportable"

The American Medical Association (AMA) has adopted a statement indicating it may support changing the federal policy imposing a lifetime deferral for potential blood donors who have had sex with men to a five-year deferral.

The statement, adopted by the AMA House of Delegates at the 2008 AMA Annual Meeting June 14-18 in Chicago, reads: "*The AMA recognizes that based on existing scientific evidence and risk assessment models, a shift to a five-year deferral policy for blood donation from men who have sex with men (MSM) is supportable.*"

According to the AMA, the word "supportable" basically means the organization will notify the Food and Drug Administration of its new policy and "will be open to work with groups to advance the policy." In addition, the AMA will not speak up against efforts to examine changing the federal deferral requirement.

The FDA requires blood collectors to permanently defer men who have had sex with men (MSM) since 1977 from blood donation. The AMA statement, recommended by its Council on Science and Public Health, hews closer to the one-year deferral for MSM called for in a joint recommendation by America's Blood Centers, AABB, and the American Red Cross. The organizations said such a policy is more consistent with deferrals for other high-risk activities, such as receiving money or drugs for sex. They have argued that public education and the development of sensitive nucleic acid amplification tests have significantly reduced the residual risk of sexually transmitted diseases entering the blood supply.

In recent years, the controversial federal policy has sparked a number of protests by gay groups, who say it was inspired by and promotes unfair stereotypes, and arguments among government officials and academics, who say it is violates non-discrimination policies. This year alone, California's San Jose State University decided to ban blood drives on its 30,000-student campus over discrimination concerns. At Sonoma State University in Santa Rosa, a professor suggested ending blood drives there because the lifetime deferral violates the university's non-discrimination policy, though after a protracted debate involving faculty and students the university decided to allow blood collection to continue. The Santa Clara County Board of Supervisors in February voted unanimously to oppose the federal policy and encourage federal lobbyists to work to overturn the ban.

(continued on page 2)

AMA Statement (continued from page 1)

The AMA statement is expected help in those efforts because it underlines the problems of the mathematical models being used to assess risk.

"Any policy decision on blood donation deferral of the MSM population must be governed by the best available scientific evidence, but there are inherent weaknesses in mathematical models used in the risk assessments on this issue that continue to generate some uncertainty. With respect to the MSM population, it appears that a policy change from a permanent lifetime deferral to a five-year deferral following the last MSM contact may be supportable, but societal and ethical consequences must be analyzed should this decision be advanced," according to the statement.

The AMA considers current risk models weak because they rely on an insufficient number of studies and study groups that aren't large enough to provide predictive results, the organization said. AMA also found that, depending on the inputs, modeling studies reflect different risk assessments, creating uncertainty in the data.

The residual risk that an HIV-infected unit of blood will enter the blood supply is estimated at about 1 infected donation for every 2.1 million donations. Given that there are about 14.5 million blood donations annually, the residual risk is about 7 infected units every year. However, the AMA said, it is clear that 7 HIV-infected units do not enter the US blood supply annually undetected. Since the implementation of NAT in 1999, there have been four incidences where HIV has been transmitted via a blood transfusion, the last in 2002. In all four cases, the donors denied engaging in risky behavior at screening. So, out of more than 112 million whole blood units transfused, only 4 resulted in HIV transmission – far lower than predicted by the risk models.

In suggesting that a five-year deferral might be warranted, the AMA pointed to a study that found, compared to blood donors who did not report MSM contact, blood donors who reported the behavior within five years had five times the number of reactive test results. However, those who had not practiced male-to-male sex in at least five years had no significant difference in reactive tests than those who did not report MSM contact at all. The organization reasoned then that data suggest men who practice five-year abstinence from homosexual sex "essentially present no greater risk than the general population."

(continued on page 3)

The *ABC Newsletter* (ISSN: #1092-0412) is published 46 times a year by America's Blood Centers® and distributed by e-mail.
Contents and views expressed are not official statements of ABC or its Board of Directors. Copyright 2008 by America's Blood Centers. Reproduction of the *ABC Newsletter* is forbidden unless permission is granted by the publisher. (ABC members need not obtain prior permission if proper credit is given.)

ABC is an association of not-for-profit, independent community blood centers that helps its members provide excellence in transfusion medicine and related health services. ABC accomplishes its mission by providing leadership in donor advocacy, education, national policy, quality, safety, funding efficiencies for the benefit of donors, patients and healthcare facilities, by encouraging collaboration among blood organizations, and by acting as a forum for its members to share information and best practices.

America's Blood Centers
President: Don Doddridge
Chief Executive Officer: Jim MacPherson
ABC Newsletter Co-Editors:
Robert Kapler and Jane Starkey
Classified Advertising Manager: Deanna Du Lac
Annual Subscription Rate: $348
(Residents, Fellows and SBB Students: $120)
Publication Office
America's Blood Centers
725 15th St NW, Suite 700, Washington, DC 20005
Tel: (202) 393-5725
E-mail: newsletter@americasblood.org

Finnish Parliament Finds Red Cross MSM Policy Justified

The Finnish Red Cross Blood Service policy imposing a lifetime ban on blood donation on men who have had sex with men cannot be considered unlawful, Finland's parliamentary ombudsman said in a statement Monday (6/30/08).

The ombudsman, Riitta-Leena Paunio, said in the statement that the decision was based on "appropriately reasoned epidemiological information to the effect that sex between men clearly increases the risk of contracting serious blood-transmitted diseases, such as HIV and hepatitis B and C, and thereby increases the safety risk in blood transfusion.... The ombudsman emphasizes that the ban is not due to sexual orientation, which enjoys constitutional protection against discrimination, but rather to sexual behavior."

The ombudsman pointed out that in addition to gay men, the Finnish Red Cross does not accept blood from anyone over 65 years of age or people who had visited Britain during the bovine spongiform encephalopathy outbreak. The ombudsman was responding to two complaints that alleged the Blood Service was violating the constitutional prohibition of discrimination in considering sex between men to be a permanent obstacle to blood donation.

According to the ombudsman's opinion, the measures undertaken by the Blood Service are not discriminatory and, hence, not in contravention of the Constitution. "The ombudsman considers that there is appropriate justification for regarding sex between men as a permanent obstacle to blood donation. ...At present, sex between men still carries an elevated risk of HIV infection. Statistics from the National Public Health Institute of Finland indicate that 330 men contracted HIV through sex between men and 247 men through heterosexual intercourse in Finland during the period 2000-2007.

"It is estimated that some 5 percent of all men have had sexual contacts with other men, which makes the risk of recent HIV infection through sex between men about 25-fold compared with that in heterosexual relationships. The selection of blood donors is largely based on assessment of risks in various donor groups and less so on individual risk behaviour." (Sources: NewsRoom Finland, 6/30/08; Ombudsman Statement, 6/30/08; Finnish Red Cross release, 6/30/08)

AMA Statement (continued from page 2)

As for a one-year deferral, the AMA said "while the increased risk with a one-year abstinence from blood donation from the last MSM contact would be very small, it is not zero. This small but scientifically real increase in risk represents a clear violation of ethical principles and therefore is not tolerable. If a 5- or 10-year deferral policy is considered, risk management calculations would yield risks at a level that many might consider acceptable."

The AMA had considered other language pointing out the weaknesses of current risk assessment models and a recommendation to ask the AMA Ethical and Judicial Council to examine the societal and ethical impacts of moving to a five-year deferral.

But the organization concluded that the data and explanations offered in the report itself, combined with the discussion at the hearing, supported a decision to remove the wording relating to the weakness of the models. The House of Delegates also removed the second recommendation of the report because the issue at hand was a risk- and science-based decision and further ethical scrutiny by the Council was deemed unnecessary. The Council's examination of any issue is always science-based, while any consideration of the ethical impact of a change in policy for MSM would be based, at least in part, on societal values, the AMA said. The AMA statement can be found at www.ama-assn.org/ama/pub/category/18644.html ♦

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. 6. 23	該当なし	

一般的名称	(製造販売承認書に記載なし)	研究報告の公表状況	Custer S, Kamel HT, Tomasulo PA, Murphy EL, Busch MP. XXIIXth Congress of the International Society of Blood Transfusion; 2008 Jun 7-12; Macao.	公表国 米国
販売名(企業名)	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

○米国における供血者の*Tripanosoma Cruzi*(*T. cruzi*)スクリーニング10ヶ月間の経験:検出頻度、リスク要因、費用対効果

背景:供血者の*T. Cruzi*スクリーニングは血液の安全性を高めるが、財政的な負担と潜在的な供血者損失の原因ともなり得る。ここでは、米国の全供血者を対象に*T. cruzi*検査が導入された2007年1月30日以降、10ヶ月間の経験を報告する。

方法:供血者は、供血前の問診の際に、出生国と*T. cruzi*流行地の中南米で過ごした期間についての質問に回答した。ELISA法で*T. cruzi*繰り返し陽性(RR)となった供血者は通知を受け、シャーガス病のリスク要因と症状についてのインタビューに回答した。ELISA RRの供血はRIPAで確認試験を行った。また、費用対効果分析によって全供血者対象の*T. cruzi*スクリーニングの医療経済的な面を検討した。

結果:約652,000名の供血適格者のうち、リピートドナーの2.1%、初回ドナーの4.8%が、問診で中南米に3ヵ月以上の滞在歴があると回答した。期間中に93名(うち3名は自己血ドナー)が*T. cruzi* RRとなった。適合血供血のRR発生率は0.0138% (90/651,471; 1:7,239)だった。RRの供血のうちRIPA陽性は34% (28/82)、陽性確認率は特異度99.99%で0.0043% (1:23,267)だった。リスク要因としては、中南米の農村部居住歴、わらぶき屋根や泥の壁の家の居住歴、母方の家族が中南米出身、などが報告された。シャーガス病関連の症状を報告した人の割合は、RIPA陽性及び陰性供血者で同程度だったが、無症候のドナーはそれよりも多く、ELISA RRの供血者でも20%では症状が報告されなかった。予備的費用対効果分析では、スクリーニングはスクリーニング未実施と比較して$10,000,000/QALYを超える費用効果であることが示された。

結論:*T. cruzi*感染のリスク要因発現率は、検査前の予想と同程度だった。RR供血の大半はRIPAで陰性だったが、ELISAの特異度は、供血者損失と比較して良好だった。RIPA陽性の供血者は地理的な暴露リスクを報告したが、シャーガス病関連の症状を報告した人は少数だった。症状に関連した質問は、別の疾患で同じ症状を発症する場合があるため、地理的なリスク要因の質問よりも有益ではないと考えられた。全供血のスクリーニングは費用対効果が低く、出生地と初回供血者に対象を絞った対策の検討が示唆された。

報告企業の意見	今後の対応
米国の供血者を対象にT. cruzi検査が導入された後10ヶ月間で、陽性確認率は0.0043%だった。症状に関連した質問は地理的なリスク要因の質問よりも有益ではないと考えられること、全供血のスクリーニングは費用対効果が低く、出生地と初回供血者に対象を絞ったスクリーニング戦略の検討が示唆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献血時に海外渡航歴の有無を確認し、シャーガス病の既往がある場合には献血不適としている。日本在住の中南米出身献血者については、厚生労働科学研究「献血血の安全性確保と安定供給のための新興感染症等に対する検査スクリーニング法等の開発と献血制限に関する研究」班と共同して検討する予定である。今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

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

JRC2008T-038

S19 - Emerging Infections

3C-S19-01

10-MONTH EXPERIENCE SCREENING USA BLOOD DONORS FOR *TRYPANOSOMA CRUZI*: YIELD, RISK FACTORS, AND COST EFFECTIVENESS

Custer S[1], Kamel HT[2], Tomasulo PA[2], Murphy EL[3], Busch MP[1]
[1]*Blood Systems Research Institute, San Francisco, USA* [2]*Blood Systems Inc., Scottsdale, AZ, USA* [3]*University of California, San Franscisco, CA, USA*

Background: Screening blood donors for the parasite *Trypanosoma cruzi*, the cause of Chagas disease, can improve transfusion safety but may come at a high price financially and potentially in donors lost. Since January 30, 2007 all donors have been tested for *T. cruzi* by an USA FDA-approved ELISA. Here we report our experience during the first 10 months of testing and interviewing donors.

Methods: Donors complete a pre-donation health questionnaire that includes questions on country of birth and time spent in Mexico, Central and South America, areas endemic for *T. cruzi*. Donors who test ELISA repeat reactive (RR) for *T. cruzi* are informed by telephone and asked to complete an interview to assess risk factors for and symptoms of Chagas disease. ELISA RR donations are tested by radioimmunoprecipitation assay (RIPA) to discriminate confirmed- from false-positive results. We also conducted a cost-effectiveness analysis to assess the health economics of universal donor screening for *T. cruzi* in the USA using an updated version of a published model [].

Results: Of nearly 652;000 eligible allogeneic donors, 2.1% of repeat donors and 4.8% of first-time donors report having spent 3 months or more in Latin America based on pre-donation questions. 93 donors (including 3 autologous donors) tested T cruzi RR in the first 10 months of testing. The RR rate for allogeneic donations was 0.0138% (90/651;471; 1:7239). Only 34% (28 of 82 tested to date) RR donations tested RIPA-positive, for a confirmed yield of 0.0043% (1:23,267) with a specificity of 99.99%. The yield of RIPA-positive donations according to region of birth is provided in the table.

Reported risk factors include previously living in rural areas of Latin America, living in housing with thatched roofs and/or mud walls, and maternal family history in Latin America. RIPA-positive and negative donors reported similar frequencies of symptoms that could indicate Chagas disease, yet no symptom was reported by more that 20% of ELISA RR donors. Preliminary cost effectiveness analysis comparing no screening to screening using ELISA and supplemental RIPA indicated a cost-effectiveness of >$10,000,000/QALY.

Birth country or region	RIPA positive prevalence
USA	1:108,207
Mexico	1:1800
Central or South America	1:154
All other countries	1:13,410
Missing/Unknown	1:82,485

Conclusion: The prevalence of and risk factors for *T. cruzi* infection are consistent with pre-testing expectations. Although the majority of RR donations did not test RIPA-positive, the specificity of the ELISA was good with substantial donor loss not evident. RIPA-reactive donors have reported geographical exposure risks and a small number have indicated symptoms consistent with Chagas disease. Symptom-related questions appear less valuable for targeting screening than geographic risk factor questions due to the potential for other health conditions to cause the same symptoms. The cost-effectiveness of screening all donations is poor and may represent an extremely inefficient use of resources, indicating that targeted screening strategies focused on country of birth and first-time donor-status should be considered.

Reference: Wilson LS, Strosberg AM, Barrio K. Cost-effectiveness of Chagas disease interventions in latin America and the Caribbean: Markov models. Am J Trop Med Hyg 2005; 73: 901–910.

3C-S19-02

EVALUATING THE EFFECTIVENESS OF MALARIA DEFERRALS THROUGH ANTIBODY TESTING

Leiby D, Nguyen L, Goff T, Gibble J
American Red Cross, Rockville, MD, USA

Background: For decades US blood collection organizations have used risk-factor questions to defer donors deemed to be at-risk for infection with *Plasmodium* spp., the etiologic agents of malaria. Risk factors are broadly classified as travel to or residence in a Plasmodium-endemic country, or past history of malaria. Affirmative responses to any one these risk-factor questions results in deferral from donating blood for 1–3 years. In recent years it has become clear that this approach has a negative impact on blood availability. Despite < 5 cases of transfusion-transmitted malaria in the US since 1998, over 100,000 potential donors are lost to malaria related deferrals each year. Thus, malaria can now viewed primarily as a blood availability issue, as opposed to a blood safety issue.

Aim: Assess the effectiveness of current malaria risk-factor questions by testing groups of deferred and non-deferred donors.

Methods: Blood donors previously deferred for malaria risk, defined as travel to or residence in *Plasmodium* spp. endemic areas or a prior history of malaria, were recruited and enrolled in the present study following administration of consent. Each study subject provided 10 ml of blood (EDTA) and completed a detailed questionnaire regarding risk factors for exposure to *Plasmodium* spp. Blood samples were tested by EIA (NewMarket Laboratories, UK) for *Plasmodium* spp. antibodies as per the manufacturers' instructions. Those samples found to be repeat reactive by EIA were considered positive and tested by real-time PCR for the presence of parasite DNA, and subsequent speciation. In addition, a group of randomly selected, non-deferred donors was selected and tested to determine assay specificity.

Results: A total of 1473 deferred donors enrolled in the study and provided a blood sample for EIA testing. Among those tested, 21 (1.43%) were initially reactive and 20 (1.36%) were repeat reactive. All samples tested by real-time PCR were negative for parasite DNA. The distribution of the 20 repeat reactive donors among the deferral categories was as follows: 14 for travel, 5 for residency and 1 for malaria history. The results of the risk factor questionnaire revealed that most seropositive donors had multiple risk factors including 17 (85%) with either residence in an endemic country or a past history of malaria. A group of non-deferred donors (n = 3229) was also tested by EIA and 21 (0.65%) were initially reactive and 11 (0.34%) were repeat reactive. Four of these 11 had a past history of malaria and three others had spent extensive time in *Plasmodium*-endemic countries.

Conclusions: Blood donors seropositive for *Plasmodium* spp. were detected among non-deferred and deferred donors. The relationship between long-term antibody titers and the risk for transmitting infection remains unclear, but semi-immune donors have been implicated in transfusion cases previously. The current approach to donor deferral is inconsistent, failing to defer donors with residence in endemic areas and/or a past history of malaria, two factors shown to be associated with transfusion transmission. In contrast, excessive donor deferral for travel to Latin America produces unnecessary donor loss, despite minimal risk for transmitting infection.

3C-S19-03

GENETIC VARIABILITY OF WEST NILE VIRUS (WNV) IN CLINICAL ISOLATES FROM US

Rios M, Grinev A, Chancey C, Daniel S, Rios M
Food and Drug Administration, Bethesda, MD, USA

Background: WNV is endemic in the US and has caused 1.5–3.5 million human infections since 1999, with >1000 cases of neurological diseases and ≥100 deaths yearly since 2002. WNV is transmissible by transfusion

© 2008 The Authors
Journal compilation © 2008 Blackwell Publishing Ltd.

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. 6. 23	該当なし	

一般的名称	(製造販売承認書に記載なし)	研究報告の公表状況		公表国
販売名(企業名)	合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)		Walderhaug M, Menis M. XXIIXth Congress of the International Society of Blood Transfusion; 2008 Jun 7-12; Macao.	米国

研究報告の概要

○パンデミックインフルエンザが米国の血液供給に与える影響のシミュレーション
米国におけるパンデミックインフルエンザ発生に備えて、パンデミックによる供血の減少と製造担当職員の不足により、供血数と職員数が通常程度に回復する前に在庫がなくなる可能性を分析した。米国では、年間約1450万製剤分の供血が行われ、約530万件の輸血が行われている。パンデミック中に起こりうるシナリオを検証するために、米国の血液供給量、1日当たりの供血数、1日当たりの需要について、個々にコンピュータシミュレーションを行った。シミュレーションは、製剤に関しては「先入れ先出し」法で行い、各製剤の供血後の日数の経過を追った。1日のシミュレーションで保存期間が42日を超えた製剤は供給から排除された。1日当たりの供血数については、供血記録から得られた通常の供給量と標準的逸脱数に基づく確率的シミュレーションを行った。1日当たりの需要のデータは、米国メディケア&メディケイドサービス由来の、65歳以上の入院患者の1日当たりの輸血実施数に関するデータと同様の方法で算定した。1日当たりの供血数と血液需要に関する分析は、1週間のうち日曜日の供血と需要が最も少なく、週半ばが最も多いというパターンを示した。1日の血液供給のシミュレーションを複数年分続けた場合では、血液供給量の見積もりは夏に減少し冬に回復するパターンを示した。パンデミックインフルエンザの影響を検証するため、3ヶ月間の供血量が50%減少したとしてシミュレーションを行ったところ、血液需要に何も制限がない場合は、血液供給量のほとんどを使い尽くした。しかし、血液の使用を必要最低限に制限した場合は、3ヶ月間供血が減少した場合でも血液在庫がなくなることはなかった。このシミュレーションモデルは、実際の血液供給量に関して適切であり、パンデミックインフルエンザ中に考えられるシナリオの範囲を策定する際に有用と考えられる結果を導き出した。

報告企業の意見	今後の対応
米国におけるパンデミックインフルエンザのシミュレーションで、3ヶ月間の血液供血量が50%減少した場合、血液需要に制限がない場合は血液在庫のほとんどを使い尽くしたが、血液の使用を必要最低限に制限した場合は血液在庫がなくなることはなかったとの報告である。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。	日本においてもパンデミックインフルエンザの発生が予期されることから、安全な血液の安定供給を確保し血液事業を継続するための対応計画を検討する必要がある。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

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

JRC2008T-039

and since 2003 blood donations are screened for WNV RNA. Investigation of WNV genetic variation is important since persistent reoccurrence suggests viral adaptation through mutations that can potentially interfere with diagnostic and screening assays, pathogenesis and therapeutic approaches. This study reports the genomic variation of WNV observed in 67 clinical isolates obtained in the continental US during 6 consecutive years (2002–2007).

Methods: RNA extracts were prepared from WNV and subjected to RT-PCR and sequencing. Sequences were compared to the prototype WN-NY99 and other isolates previously studied using NTI Vector. We also developed and validated a multiplex RT-PCR assay to investigate if the newly identified deletion found in ID was also observed in other states. All specimens were tested for WNV 3'UTR deletion using this assay.

Results: Sequence results from 16 complete genomic sequences revealed 20–48 nucleotide (nt) mutations compared to the prototype WN-NY99. We observed an increase of a nucleotide divergence in the full WNV genomes from 0.18% in 2002 to 0.48% in 2006. It should be noted that 80% of the nt changes in structural regions are transitions (U ' C) and 75% are silent mutations. Twelve new mutations identified in 2005, became fixed in 2006. The 2006 and 2007 isolates shared three amino acid substitutions (Val449Ala, Ala2209Thr and Lys2842Arg), but most nt changes are silent transitions (U ' C, A'G). A 13-nt deletion in the 3'NCR (10414–10426) was identified in isolates from Idaho (ID-Δ13). Further investigation of 47 isolates from 2006 and 2007 for ID-Δ13, showed geographical localization of this variant as observed in 12/25 (48%) of isolates from ID, and in one 2006 isolate from ND. The new ID-Δ13 variant of WNV became fixed in 2007.

Conclusion: In this study we report the emergence of a new genetic variant of WNV carrying a 13-nt deletion at the 3'NCR (WNV-ID-Δ13), found in Idaho. The 3'NCR is known to be critical for WNV replication, however WNV-ID-Δ13 grows well in Vero cell cultures, but preliminary study showed steady replication efficiency and normal plaque in Vero cells. The impact of ID-Δ13 in viral pathogenesis is under investigation. Nucleotide sequence alignments indicate that, most new mutations are not fixed, but WNV has continued to diverge and the number of fixed mutations as well as overall genetic divergence has significantly increased. Surveillance for genetic variation is essential to assure public health since emergence of mutants could potentially decrease sensitivity of screening and diagnostic assays, affect viral pathogenesis, and negatively impact the efficacy of vaccines and the development of specific therapies.

3C-S19-04
SCREENING OF BLOOD DONORS FOR CHIKUNGUNYA VIRUS – DEVELOPMENT AND EVALUATION OF MINIPOOL-NAT AND ANTIBODY TESTS

Schmidt M[1], Hourfar MK[1], Drosten C[2], Seifried E, Panning M[2]
[1]*German Red Cross, Institute Frankfurt, Frankfurt am Main, Germany*
[2]*Institute of Virology, University of Bonn Medical Centre, Bonn, Germany*

Background: The outbreak of Chikungunya fever in the southeastern islands of the Indian Ocean has drawn the attention of the transfusion community to Chikungunya virus. The virus has now spread to India and wide parts of Southeast Asia. Additionally many infections in European travellers returning from these regions to their home countries have been reported. Chikungunya virus can cause a wide spectrum of disease which may range from no or mild symptoms to death. It is known to be spread by blood in symptomatic cases and likely it could be spread by transfusion and transplantation of organs from people with pre-symptomatic or asymptomatic disease. Adequate screening procedures to identify viremic donations, however, were not available until now.

Methods: A real-time minipool NAT assay for the current epidemic strain of Chikungunya virus was used on a total of 29,568 blood donor samples, tested in minipools of up to 96 donations. To validate the sensitivity of the assay, routine donor minipools were spiked with inactivated virus and were used as positive controls. Additional to NAT-testing 9600 blood donations were screened for IgG-antibodies against Chikungunya virus to determine the prevalence of the infection in our blood donor population. Plasma samples from symptomatic Chikungunya virus infected travellers were analyzed for virus-load and antibody status.

Results: By testing 9600 blood donations for Chikungunya-specific IgG-antibodies no reactive donation was detected. Likewise, no viremic donation was identified by screening 29,568 clinically asymptomatic blood donors by minipool-NAT. The minipool-NAT assay provided sufficient sensitivity to detect plasma samples from symptomatic patients infected with the pathogen. It can be expected that the assay is also capable to detect viremic donations from pre-symptomatic or asymptomatic donors. This is because it was found that virus load in Chikungunya virus infected travellers was highest with onset of symptoms (day 0). After day 7 after onset of symptoms no Chikungunya virus RNA was found in symptomatic travellers. Specificity of the assay was 100% because none of the tested blood donors were found to be positive for the reemerged Alphavirus.

Discussion: Although no donation infected with Chikungunya virus has been identified among the donors subject to our study it is accepted that the reemerged pathogen poses a risk for recipients of blood products - in particular for immunocompromized patients. A recent outbreak of Chikungunya virus in Italy has shown that this virus also poses a risk to countries of the western hemisphere if competent vectors are prevalent. With the assay described for the first time highly sensitive screening of blood-donations on a routine basis is feasible. Since as no approved inactivation procedures exist for red blood cells exist, screening for viremic donations may be the method of choice in order to guarantee safe blood products in countries affected by the Chikungunya epidemic.

3C-S19-05
SIMULATING THE IMPACT OF PANDEMIC INFLUENZA ON THE US BLOOD SUPPLY

Walderhaug M, Menis M
US Food & Drug Administration, Rockville, MD, USA

In order to prepare for a possible pandemic influenza event in the US, we investigated the potential for reduced donations and blood-processing staff shortages due to an influenza pandemic to exhaust blood stocks before normal donations and staff levels are restored. Approximately 14.5 million units of blood are collected annually in the US and approximately 5.3 million receive blood transfusions per year. To examine a range of potential scenarios that might occur during a pandemic, we developed a discrete event computer simulation of the estimated aggregate US blood supply, daily blood donations, and daily demand. The simulation used a 'first in, first out' rule with respect to blood units, and kept track of the number of days post collection of each simulated blood unit. During a day's simulation any units older than 42 days were eliminated from the aggregate supply. Daily blood donations were probabilistically simulated based on a normal distribution of means and standard deviations obtained from donation records. Daily blood demand data were estimated in a similar manner based on multiple years of U.S. Centers for Medicare & Medicaid Services (CMS) MedPAR derived data on the daily number of inpatient blood transfusion procedures recorded for elderly patients 65 years old and over. An analysis of daily donations and blood demand showed similar patterns through the week with the least amount of donations and demand on Sunday with peak donations and demand at mid-week. Simulating the daily blood supply for multiple years in simulation showed the estimated aggregate blood supply behavior was similar to observed patterns of blood supply levels in the US specifically, showing a decline in overall levels during the summer followed by a recovery of levels in the winter. To examine the impact of pandemic influenza, a 50% decline in blood donations for 3 months was simulated, and the effect was a depletion most of the aggregate blood supply, if no limitation of blood demand was applied; however, if blood demand is limited to essential uses, then a three month period of reduced donations can be endured despite a significant depletion of aggregate blood stocks. The simulation model provided results that appear to be reasonable with respect to observed estimates of aggregate blood supply and to be useful in exploring a range of possible scenarios expected during pandemic influenza.

© 2008 The Authors
Journal compilation © 2008 Blackwell Publishing Ltd.

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		回	報告日 年　月　日	第一報入手日 2008 年 6 月 4 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	New arenavirus discovered in Bolivia Lancet Infect Dis 2008; 8: 355	公表国 米国	
販売名（企業名）						

研究報告の概要

ボリビア，ペルー及び米国疾病予防管理センター（CDC）の国際チーム（アトランタ，ジョージア州，米国）はボリビアの出血熱の死亡症例において新型のアレナウイルスを発見した。完全ゲノム解析でアレナウイルス属の新型ウイルスであることが認められ，アンデス山脈の山麓にちなんで Chapare virus と名付けられた。当該ウイルスは，系統発生学的には南米で出血熱を自然発生させる他のアレナウイルス，特にサビアウイルスに近いウイルスであった。疾病管理予防センター研究調査員の Stuart Nichol は，「アレナウイルスに関連した出血熱は，アルゼンチン（フニンウイルス），ボリビア（マチュポウイルス及び，現在は Chapare virus），ベネズエラ（グアナリトウイルス）及びブラジル（サビアウイルス）で報告されている。年間の症例数は地域を合わせて数十件から数百件前後まで大きなばらつきがある」とし，また，「Chapere virus がげっ歯類を宿主として長期間存在してきた可能性は非常に高いが，人類への波及はおそらくまれであったと思われる」とも述べている。ハーバード大学医学部（ボストン，マサチューセッツ州，米国）の Michael Farzan 氏は，「南米の野生のげっ歯類において複製するウイルスが人類への感染能を獲得し，重篤な疾患を引き起こすことは容易に起こり得る。これらのげっ歯類の生息環境は様々な形で人類によって破壊されてきていることからも，この点が心配される」と述べている。ウガンダでは，赤オナガザルにおける血清学的検査で新型ポックスウイルスの可能性があるウイルスが発見された。イリノイ大学（Urbana，イリノイ州，米国）主席研究員の Tony Goldberg 氏はこのウイルスは既知のオルソポックスウイルスに類似しているが全く同じものではないとし，さらに「近い将来にこの新型のウイルスが人類に感染する可能性はおそらく低く，また当該研究分野においてポックスウイルスがヒトに感染したエビデンスはない。我々の試験が主に示唆することは，環境において新型であり，また実体の明らかでないポックスウイルスが存在するということである」と述べた。しかしながら，Goldberg は，ポックスウイルスは種のバリアを乗り越えることで悪評が高いことも指摘している。双方の新型ウイルスで懸念されるのは，新たに出現した感染が過去 50 年で約 4 倍に増加しており，野生動物の疾患がこうした疾患の大半を占めているということである。

使用上の注意記載状況・その他参考事項等

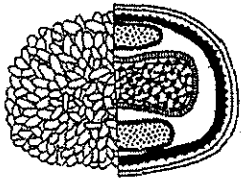
BYL-2008-0336

PLoS Pathog 2008; 4: e1000047; DOI:10.1371/journal.

Emerg Infect Dis 2008; 14: http://www.cdc.gov/eid/content/14/5/801.htm



Arenavirus



報告企業の意見	今後の対応
2 種類の新規ウイルス病原体はどちらもエンベロープウイルスであり，血漿分画製剤の製造工程におけるウイルス除去・不活化工程により除去・不活化されるウイルスである。また，本報告では新たに出現した感染が過去 50 年で約 4 倍に増加していることを強調している。血漿分画製剤の製造工程におけるウイルス除去・不活化工程は，新たに出現するエンベロープウイルスに対しては効果的であるが，非エンベロープウイルスに対しては未だ完全であるとは考えられない。	今後も，新規ウイルス病原体の出現に関する情報収集に努める。

BYL-2008-0336

Highlights from the 18th ECCMID

First European Infection Day

The launch of the first European Day of Fighting Infection took place at the 18th annual European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) in Barcelona, Spain (April 19–23). "We need to make people more aware of infections, and to highlight to the general public in particular that everyone can play a part—for example, in the correct use of antibiotics", Giuseppe Cornaglia (University of Verona, Italy) told *TLID*. "The day will also serve to reinforce collaborations between all players in the field of infectious diseases in Europe and to improve knowledge", he added. The day has been created to mark the 25th anniversary of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID). "An important aim for us now is to work towards fostering greater collaboration between eastern and western Europe, through professional exchange and improving our support to young scientists."

ECCMID research highlights

A key focus of the meeting was around antibiotic resistance in Europe and how best to ensure more rational use of antibiotics by clinicians. In a press conference, Fernando Baquero (Hospital Ramón y Cajal, Madrid, Spain) said clinicians are particularly concerned about resistance to antibiotics commonly used in children. He said: "Innovative antibiotics are not being developed, and industrial research facilities on antimicrobial agents are increasingly being shut down...we therefore cannot use all the antibiotics commonly available for use in adults for the treatment of children".

Sore throats are common in children, yet only 15–30% of them are caused by pathogenic bacteria, most frequently group A streptococci. In an expert session, Paul Little (University of Southampton, UK) warned clinicians against prescribing antibiotics immediately. "There are several alternatives: if rapid streptococcal tests are available it takes just 5 min to exclude or confirm infection. If a rapid test is not available, it's safe to wait 3 days before using antibiotics", he said. Antibiotic therapy should be started after 3–4 days if necessary, "in the meantime you can give anti-inflammatory drugs to control the symptoms".

E Tacconelli and colleagues (Catholic University, Rome, Italy) did a 1-year cohort study to analyse the risk factors for infections by antibiotic-resistant bacteria in hospital admissions. Infections caused by antibiotic-resistant bacteria were diagnosed in 398 patients (seven cases per 1000 admissions). They report an increased risk associated with colonisation in patients aged >60 years with urinary catheters and clinical signs of bacterial infections at admission and in patients previously treated with antibiotics, and conclude that greater recognition of these risk factors may influence the selection of empirical treatment.

Sally Hargreaves

The printed journal includes an image merely for illustration

For more on ESCMID see http://www.escmid.org

New arenavirus discovered in Bolivia

An international team from Bolivia, Peru, and the US Centers for Disease Control and Prevention (CDC, Atlanta, GA, USA) has discovered a new arenavirus in a fatal case of haemorrhagic fever in Bolivia. Complete genome analysis revealed a distinct member of the arenavirus family; named Chapare virus, after a river in the foothills of the Andes. The virus is phylogenetically related to other arenaviruses that naturally cause haemorrhagic fever in South America, particularly Sabia virus.

Study investigator Stuart Nichol (CDC) said that "arenavirus-associated haemorrhagic fever has been described in Argentina (Junin virus), Bolivia (Machupo and now Chapare virus), Venezuela (Guanarito virus), and Brazil (Sabia virus). The number of cases per year varies substantially, from around a few hundred cases down to double digits for the whole region". Nichol added: "It is highly likely that Chapere virus has been present in a rodent reservoir for a long time, although spill-over to human beings is probably infrequent". Michael Farzan (Harvard Medical School, Boston, MA, USA) said: "The discovery underscores the ease with which viruses replicating in South American wild rodents can acquire the ability to infect human beings and cause serious disease. This is especially a concern, since the natural habitats of these rodents are being disrupted in a variety of ways".

A possible new poxvirus has been discovered following serological tests in red colobus monkeys in Uganda. Lead investigator Tony Goldberg (University of Illinois, Urbana, IL, USA) said that the virus is similar, but not identical, to known orthopoxviruses, which includes smallpox virus.

Goldberg added: "The likelihood of the new virus infecting human beings in the near future is probably low; there was no evidence of human poxvirus infection in the study area. One of the main implications of our study is that there are new, as yet unidentified poxviruses in the environment". Nevertheless, Goldberg pointed out that poxviruses are notorious for crossing species barriers.

The concern with both new viruses is that emerging infections have roughly quadrupled over the past 50 years, and that wildlife zoonoses account for the majority of such diseases.

Cathel Kerr

For more on Chapare virus see *PLoS Pathog* 2008; 4: e1000047; DOI:10.1371/journal.ppat.1000047

For more on the novel poxvirus in colobus monkeys in Uganda see *Emerg Infect Dis* 2008; 14: http://www.cdc.gov/eid/content/14/5/801.htm

For more on emerging infectious diseases and wildlife zoonoses see Newsdesk *Lancet Infect Dis* 2008; 8: 218–19

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9月16日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	別紙のとおり		研究報告の公表状況	Proc Natl Acad Sci U S A. 2008;105:14124-14129	公表国 米国	
販売名（企業名）	別紙のとおり					

研究報告の概要

問題点：齧歯類の重症疾患の原因ウイルスとされていた cardiovirus がヒトにおいても存在することが遺伝子学的手法により確認された。

齧歯類の重症疾患の原因となる picornavirus 科に属する cardiovirus は、その罹患率、多様性、ヒトでの症状等についてはあまり知られていない。発熱のある乳児の便検体から 1981 年に培養された Saffold virus は、cardiovirus に分類されている。今回、患者検体から直接ヒト cardiovirus をクローニングしたことについて報告する。これはインフルエンザ様の症状を示した子供の呼吸分泌物から pan-viral microarray 法を用いて発見した最初の報告である。ほぼ全長のウイルスゲノム(7961 bp)の系統樹解析で、ウイルスは cardiovirus のサブグループである Theiler's murine encephalomyelitis virus(TMEV)に属し、Saffold virus と最も密接に関係があった。719 の呼吸器サンプル（急性呼吸器症状を示した患者からは 637 検体(89%)）と神経系疾患患者（無菌性髄膜炎、脳炎及び多発性硬化症）からの髄液検体 400 の RT-PCR によるスクリーニングでは、cardiovirus 感染の痕跡は認められなかった。しかし、胃腸炎患者 498 人の排泄物 751 検体のスクリーニングの結果、6 検体より cardiovirus（1.2%）が検出された。これら Saffold virus を含む 8 つのヒト cardiovirus は、系統樹解析によりすべて同じところにクラスターされたが、VP1 遺伝子にかなりの多様性が認められた(アミノ酸の相同性は 66.9%–100%)。これらの結果は、これまでほとんど確認されていなかったが、現在は主に消化管において確認され、無症候で排出され、そして腸内外の疾患に関連している可能性がある新しいヒト TMEV 様の cardiovirus の多様な集団が存在することを示唆している。

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

使用上の注意記載状況・その他参考事項等

記載なし

MedDRA/J ver.11.1

別紙

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免疫グロブリン、⑤乾燥ペプシン処理人免疫グロブリン、⑥乾燥スルホ化人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン*、⑧乾燥濃縮人活性化プロテインC、⑨乾燥濃縮人血液凝固第Ⅷ因子、⑩乾燥濃縮人血液凝固第Ⅸ因子、⑪乾燥抗破傷風人免疫グロブリン、⑫抗 HBs 人免疫グロブリン、⑬トロンビン、⑭フィブリノゲン加第ⅩⅢ因子、⑮乾燥濃縮人アンチトロンビンⅢ、⑯ヒスタミン加人免疫グロブリン製剤、⑰人血清アルブミン*、⑱人血清アルブミン*、⑲乾燥ペプシン処理人免疫グロブリン*、⑳乾燥人血液凝固第Ⅸ因子複合体*、㉑乾燥濃縮人アンチトロンビンⅢ
販売名(企業名)	①献血アルブミン 20 “化血研”、②献血アルブミン 25 “化血研”、③人血清アルブミン “化血研” *、④ “化血研” ガンマーグロブリン、⑤献血静注グロブリン “化血研”、⑥献血ベニロンーⅠ、⑦ベニロン*、⑧注射用アナクトC2,500 単位、⑨コンファクトF、⑩ノバクトM、⑪テタノセーラ、⑫ヘパトセーラ、⑬トロンビン “化血研”、⑭ボルヒール、⑮アンスロビンP、⑯ヒスタグロビン、⑰アルブミン 20%化血研*、⑱アルブミン 5%化血研*、⑲静注グロブリン*、⑳ノバクトF*、㉑アンスロビンP 1500 注射用
報告企業の意見	cardiovirus は、picornavirus 科に分類される属名の一つである。cardiovirus 属のウイルスはエンベロープを持たず、直径約 30nm で正 20 面体のカプシッドを持ち、核酸は一本のプラス鎖 RNA である。cardiovirus 属には次の 2 つのサブグループがある；脳心筋炎ウイルス (encephalomyocarditis virus；EMCV)、タイラーのマウス脳脊髄炎ウイルス (Theiler's murine encephalomyelitis virus；TMEV)。これらのウイルスは、げっ歯類に感染し消化器官で増殖した後、糞便経口ルートで伝播する。ウイルスが腸管感染しても大抵は軽度か無症状であるが、腸管外に拡がると全身性の疾患を惹き起こす。EMCV 系統のウイルスは脳炎及び心筋炎を惹き起こし、TMEV 系統のウイルスは中枢神経系感染に関連している。ヒトから分離されたとされる cardiovirus 属のウイルスも報告されているが、ヒトから直接クローニングされたことはなく、その罹患率、多様性、ヒトでの症状等についてはあまり知られていない。 本剤の製造工程には、冷エタノール分画工程、ウイルス除去膜ろ過工程あるいは加熱工程等の原理の異なるウイルス除去及び不活化工程が存在しているので、ウイルスクリアランスが期待される。各製造工程のウイルス除去・不活化効果は、「血漿分画製剤のウイルスに対する安全性確保に関するガイドライン (医薬発第 1047 号、平成 11 年 8 月 30 日)」に従い、ウシウイルス性下痢ウイルス (BVDV)、仮性狂犬病ウイルス (PRV)、ブタパルボウイルス (PPV)、A 型肝炎ウイルス (HAV) または脳心筋炎ウイルス (EMCV) をモデルウイルスとして、ウイルスプロセスバリデーションを実施し、評価を行っている。今回報告した cardiovirus 属には、モデルウイルスとして使用している EMCV そのものが属しており、上記バリデーションの結果から、本剤の製造工程が EMCV の除去・不活化効果を有することを確認している。また、これまでに本剤による cardiovirus 感染の報告例は無い。 以上の点から、本剤は cardiovirus に対する安全性を確保していると考える。

*現在製造を行っていない

INF2008-006

Identification of cardioviruses related to Theiler's murine encephalomyelitis virus in human infections

Charles Y. Chiu[†‡], Alexander L. Greninger[†], Kimberly Kanada[†], Thomas Kwok[†], Kael F. Fischer[†], Charles Runckel[†], Janice K. Louie[§], Carol A. Glaser[‡§], Shigeo Yagi[§], David P. Schnurr[§], Tom D. Haggerty[¶], Julie Parsonnet[¶], Don Ganem[‡∥††], and Joseph L. DeRisi[†,††‡‡]

[†]Department of Biochemistry and Biophysics, [∥]Department of Microbiology, [‡]Division of Infectious Diseases, Department of Medicine, and [††]Howard Hughes Medical Institute, University of California, 1700 4th Street, Box 2542, San Francisco, CA 94143; [§]Viral and Rickettsial Disease Laboratory, California Department of Health Services, 850 Marina Bay Parkway, Richmond, CA 94804; and [¶]Division of Infectious Diseases and Geographic Medicine, Department of Medicine, Stanford University School of Medicine, 300 Pasteur Drive, S-169, Stanford, CA 94305

Communicated by Patrick O. Brown, Stanford University School of Medicine, Stanford, CA, July 3, 2008 (received for review March 19, 2008)

Cardioviruses comprise a genus of picornaviruses that cause severe illnesses in rodents, but little is known about the prevalence, diversity, or spectrum of disease of such agents among humans. A single cardiovirus isolate, Saffold virus, was cultured in 1981 in stool from an infant with fever. Here, we describe the identification of a group of human cardioviruses that have been cloned directly from patient specimens, the first of which was detected using a pan-viral microarray in respiratory secretions from a child with influenza-like illness. Phylogenetic analysis of the nearly complete viral genome (7961 bp) revealed that this virus belongs to the Theiler's murine encephalomyelitis virus (TMEV) subgroup of cardioviruses and is most closely related to Saffold virus. Subsequent screening by RT-PCR of 719 additional respiratory specimens [637 (89%) from patients with acute respiratory illness] and 400 cerebrospinal fluid specimens from patients with neurological disease (aseptic meningitis, encephalitis, and multiple sclerosis) revealed no evidence of cardiovirus infection. However, screening of 751 stool specimens from 498 individuals in a gastroenteritis cohort resulted in the detection of 6 additional cardioviruses (1.2%). Although all 8 human cardioviruses (including Saffold virus) clustered together by phylogenetic analysis, significant sequence diversity was observed in the VP1 gene (66.9%–100% pairwise amino acid identities). These findings suggest that there exists a diverse group of novel human Theiler's murine encephalomyelitis virus-like cardioviruses that hitherto have gone largely undetected, are found primarily in the gastrointestinal tract, can be shed asymptomatically, and have potential links to enteric and extraintestinal disease.

DNA microarrays | gastroenteritis | influenza-like illness | picornavirus | virus discovery

Picornaviruses are positive single-stranded RNA viruses that cause a variety of important disease states in humans and animals. Several genera of picornaviruses are recognized, based on genomic sequence and virus biology. The *Cardiovirus* genus of the family Picornaviridae consists of two subgroups: Theiler's murine encephalomyelitis virus (TMEV) and related viruses (Theiler-like virus NGS910 of rats, Vilyuisk virus) (1–3), and encephalomyocarditis virus (EMCV) and related viruses (EMCV, Mengovirus, Columbia SK virus, Maus–Elberfeld virus) (4). All these viruses infect rodents, replicate in the gastrointestinal (GI) tract and are transmitted by the fecal-oral route. Although enteric infection by these viruses is often mild or asymptomatic, extraintestinal spread of these viruses can occur and can lead to systemic disease (1). As their name implies, the EMCV-like agents cause encephalitis and myocarditis, whereas the TMEV family is linked to CNS infection. In experimental settings, intracerebral inoculation of mice with TMEV can produce acute encephalomyelitis and/or a chronic demyelinating disease resembling human multiple sclerosis (MS), depending upon the strain of TMEV used (5). Oral inoculation with TMEV may also result in encephalomyelitis, especially when large inocula are delivered to neonatal mice (6).

Whether authentic human cardioviruses exist has long been debated. The first candidate human cardiovirus was Vilyuisk virus, which was linked to Vilyuisk encephalitis, an unusual neurodegenerative disease found among the Yakuts people of Siberia in the 1950s and still endemic to the region (7, 8). The Vilyuisk virus was initially isolated from the cerebrospinal fluid (CSF) of an affected patient and underwent 41 serial passages in mice before sequencing and characterization as a TMEV-like picornavirus (3, 9). Given its sequence similarity to TMEV and its extensive passage history in mice, questions have arisen as to whether the virus may in fact be of murine origin. In 1981, another TMEV-related cardiovirus was cultured from the stool of an infant who presented with a febrile illness (10). Although early passages appeared to show that the virus was transmissible, long-term continuous propagation of the isolate has been problematic. The nearly complete genomic sequence of this isolate (provisionally called Saffold virus) was recovered from frozen stocks by cloning in 2007 and was found to be much more divergent from TMEV than Vilyuisk virus (10). However, neither Vilyuisk nor Saffold virus was cloned directly from primary clinical specimens, and the diversity, prevalence, and potential clinical manifestations of human cardiovirus infection have remained largely unexplored.

We have previously developed a pan-viral DNA microarray (Virochip; University of California, San Francisco) designed to detect known and novel viruses in clinical specimens on the basis of homology to conserved regions of known viral sequences (11). The current study uses microarrays from the third and fourth generations of this platform (Viro3, Viro4). The Viro3 platform has 19,841 viral oligonucleotides derived from all publicly available viral sequence as of June 2004 (12, 13). The Viro4 platform is a streamlined update of the Viro3 platform consisting of 14,740 viral oligonucleotides derived from all publicly available viral sequence as of June 2006. The Virochip has been used to detect novel pathogens such as the severe acute respiratory syndrome coronavirus (14) and XMRV, a retrovirus identified in prostate tissue of men with germ-line mutations in RNase L (15). The platform has also been successfully used to detect

Author contributions: C.Y.C., D.G., and J.L.D. designed research; C.Y.C., A.L.G., K.K., T.K., and C.R. performed research; C.Y.C., A.L.G., K.F.F., J.K.L., C.A.G., S.Y., D.P.S., T.D.H., J.P., D.G., and J.L.D. contributed new reagents/analytic tools; C.Y.C., A.L.G., T.D.H., J.P., D.G., and J.L.D. analyzed data; and C.Y.C., D.P.S., J.P., D.G., and J.L.D. wrote the paper.

The authors declare no conflict of interest.

Freely available online through the PNAS open access option.

‡‡To whom correspondence should be addressed. E-mail: joe@derisilab.ucsf.edu.

This article contains supporting information online at www.pnas.org/cgi/content/full/0805968105/DCSupplemental.

© 2008 by The National Academy of Sciences of the USA

www.pnas.org/cgi/doi/10.1073/pnas.0805968105

known and divergent viruses in acute respiratory tract infections in several recently published studies (12, 13, 16, 17).

In this study, we used the Virochip to screen respiratory secretions from patients with influenza-like illness who lacked a diagnosis despite extensive microbiological testing. In one such patient, we detected and fully sequenced a cardiovirus in the Saffold group. Related cardioviruses were subsequently found in stool specimens from an additional six individuals collected as part of a study examining household transmission of gastroenteritis (18). We report here the existence and overall phylogeny of a diverse group of human cardioviruses and discuss their potential association with human disease.

Results

Detection of a Cardiovirus in a Patient with Influenza-Like Illness. A total of 460 respiratory secretions from patients meeting a case definition of influenza-like illness were screened for respiratory viruses by culture. In 108 culture-negative specimens selected from elderly and pediatric patients, 16 specimens remained negative after subsequent RT-PCR testing for respiratory syncytial virus (RSV), influenza A/B (Flu A/B), rhinovirus (RV), and enterovirus (EV). These 16 specimens were assayed for the presence of viruses using the Virochip (Viro3), with microarray analysis carried out using E-Predict and ranked z score analysis, as previously described (12, 19).

Four of the 16 specimens yielded a positive microarray hybridization signature suggestive of a virus. Two of the signatures corresponded to metapneumovirus, one signature corresponded to adenovirus, and one signature indicated the presence of a cardiovirus related to TMEV. From the microarray containing the cardiovirus signature, the highest intensity oligonucleotides mapped to the 5′-untranslated region (5′-UTR) and 2C gene of the TMEV genome, the most conserved regions among cardioviruses and picornaviruses in general (Fig. 1*A*, "ARRAY"). To recover viral sequence, we designed primers based on the highest intensity array features and alignment of well conserved sequences from four cardioviruses (TMEV-DA, TMEV-GDVII, Theiler-like NGS910 virus, and EMCV). One set of primers successfully amplified a 224-bp fragment from the viral 5′-UTR. The fragment shared 90% nucleotide identity with the 5′-UTR region of Theiler-like NGS910 virus. This finding established that the virus in question was indeed a cardiovirus and a relative of the TMEV group of viruses. We designated this initial cardiovirus strain UC1.

Complete Genome Sequencing and Analysis of UC1. To clone and sequence the remainder of the UC1 genome, additional short fragments were first obtained from conserved regions in the 2C (helicase) and 3D (polymerase) genes by use of consensus PCR primers derived from alignment of the four cardioviruses mentioned previously. Long-range RT-PCR using specific primers was then used to bridge the gaps. This resulted in PCR amplification of two long overlapping fragments (≈5.3 and 3.7 kB in size) jointly spanning nearly the entire length of the virus genome (Fig. 1*A*, "RT-PCR"). Cloned ends of the genome were recovered and sequenced using a RACE amplification protocol (20, 21).

The nearly complete sequence of UC1 is 7961 nt in length and forms a distinct branch in the *Cardiovirus* genus with Saffold virus (Fig. 1*B*). The overall nucleotide identity to Saffold virus is >90% in the 5′-UTR and the region coding for the nonstructural proteins but only 70% in the region coding for the capsid proteins (Fig. 1*A*, "Saffold"). There is much less overall nucleotide sequence identity to other members of the TMEV subgroup (70–80%) and EMCV (50–55%). A poly(C) tract that has been reported in EMCV but not in TMEV strains is not present in the 5′-UTR of UC1. Similar to other cardioviruses, the ORF of UC1 is predicted to code for a single 2296-amino acid polyprotein that is subsequently cleaved into the L protein, the

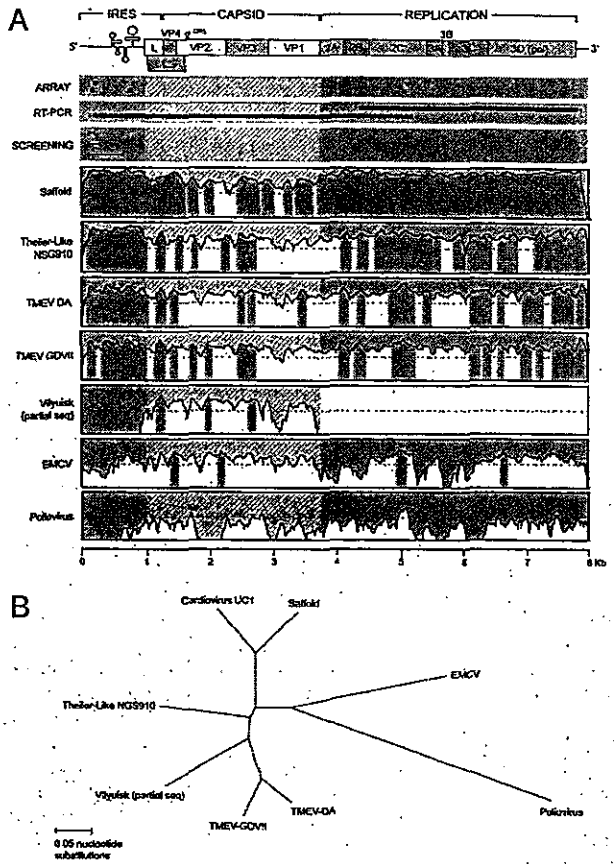


Fig. 1. Genome sequence of UC1. (*A*) Genome sequence similarity plots compare UC1 with Saffold virus, Theiler-Like NGS910 virus, TMEV-DA, TMEV-GDVII, Vilyuisk virus (partial sequence only), EMCV, and poliovirus. The y axis scale for each plot represents percentage of nucleotide identities from 0% to 100%. Regions of the genome with percentage of nucleotide identities of >70% are highlighted in pink. The Virochip oligonucleotides used to detect UC1 ("ARRAY"), the fragments generated by long-range RT-PCR and used to sequence most of the virus ("RT-PCR"), and the cardiovirus primers and resulting PCR fragments used for screening of stool, CSF, and respiratory secretions ("SCREENING") are also shown mapped onto the UC1 genome. The sequences of these primers are provided in Table S1. (*B*) Radial tree depicts the phylogenetic relationships between the genomes of UC1 and the seven aforementioned cardioviruses.

capsid proteins (VP1, VP2, VP3, and VP4), and nonstructural proteins involved in viral replication (2A, 2B, 2C, 3A, 3B, 3C, and 3D) (Fig. 1*A*). Like Saffold virus, UC1 encodes an L protein containing a zinc finger, an acidic domain, and a partially deleted Ser/Thr-rich domain (22, 23) and potentially encodes a severely truncated L* protein that begins with an ACG codon rather than AUG (22, 23) [supporting information (SI) Fig. S1*A*].

In cardioviruses, the surface loops CD of VP1 and EF of VP2 are exposed on the capsid surface and are thought to be involved in host cell tropism and viral pathogenesis (24). These loops are the regions of greatest divergence between UC1 and the other cardioviruses, including Saffold virus (Fig. S1*B*). Between UC1 and Saffold virus, there is 52% and 61% amino acid identity in the exposed surface loops CD and EF, respectively. The corresponding identities (29% and 24%) are much lower between UC1 and the rodent cardioviruses.

Comparison of UC1 Amino Acid Sequence with Other Cardioviruses. The level of divergence between the sequence of UC1 and other cardioviruses is maintained at the amino acid level. Between UC1

Table 1. Amino acid identity of predicted UC1 proteins

Gene	Predicted size, aa	Percent amino acid identity to					
		Saffold	NGS910	Vilyuisk	TMEV-DA	EMCV	Polio
L protein	71	77	61	60	60	26	0
VP4	72	99	72	72	68	62	19
VP2	269	83	69	67	71	64	30
VP3	231	85	80	76	75	68	28
VP1	275	77	56	55	59	48	14
Nonstructural	1389	98	91		83	40	22
Polyprotein	2296	91	76		71	52	22

and Saffold virus, the capsid proteins VP1, VP2, and VP3 are only 77–85% identical, whereas the nonstructural proteins are highly conserved (98% overall identity) (Table 1). The amino acid identities between UC1 and its closest rodent relatives (NGS910 virus and TMEV) are much lower, 56–80% for the capsid proteins and 83–91% for the nonstructural proteins. These comparisons confirm that UC1 is most closely related to Saffold virus, although there is significant sequence divergence in the capsid proteins containing the putative receptor binding sites.

Prevalence of Cardioviruses in Clinical Specimens. To investigate the prevalence of cardiovirus infection in acute human illnesses, we designed PCR primers targeting the 5′-UTR to amplify cardioviruses by real-time one-step RT-PCR. In our initial screen, we ran two RT-PCRs using conserved primers designed to amplify 102-bp and 224-bp fragments from the 5′-UTR of UC1, Saffold virus, or all mouse strains of TMEV. By probit analysis (i.e., the concentration of the target sequence testing positive in 95% of cases) using *in vitro* transcribed UC1 mRNA, the sensitivity of the RT-PCR assay for detection of cardioviruses was 600 copies. Standard curves generated using pooled cardiovirus-negative specimens spiked with UC1 mRNA were linear from 10^4 to 10^{11} copies/ml ($R^2 = 0.9831$–0.9944, Fig. S2). The presence of PCR inhibitors was estimated to be <3% by yeast RNA spiking experiments on randomly selected stool specimens (only 2 of 95 RT-PCRs failed to amplify the yeast positive control). All positives in the initial screen were sequenced and then further confirmed by another RT-PCR using primers designed to amplify an overlapping 608-bp fragment (Fig. 1*A*, "SCREENING").

Since UC1 was first identified in respiratory secretions, we screened 719 respiratory specimens from two large groups of patients: 278 nasopharyngeal aspirates from pediatric patients at a single hospital (190 specimens from patients with an acute respiratory illness) (13) and 441 pooled oropharyngeal and nasopharyngeal swabs from individuals in California with influenza-like illness (25). None of the 719 total respiratory specimens tested was positive for cardioviruses.

We next conducted screening of CSF specimens from patients with aseptic meningitis ($n = 60$), patients with encephalitis ($n = 300$), and patients with MS ($n = 40$) for cardioviruses by RT-PCR. None of the 400 CSF specimens tested was found to be positive.

Given the prominent association of picornaviruses with enteric infection and the known fecal-oral route of transmission, we then sought to assess the prevalence of human cardioviruses in stool. We examined 751 stool specimens from 498 individuals collected as part of a cohort study of household transmission of *Helicobacter pylori* and gastroenteritis (18). The vast majority of subjects were children, with 443 (89%) children younger than 5 years, 30 (6%) children between 5 and 18 years, and 25 (5%) adults. Specimens from 6 children (1.2% of the 498 individuals) were positive for cardioviruses (strains UC2–UC7). All cardiovirus-positive stool specimens were from children <2 years old and from different households. Symptoms in the 6 children included diarrhea and vomiting in 3 (50%) and diarrhea only in 1 (17%); the remaining 2 children were asymptomatic. Of note, from 2 of the symptomatic children, one with diarrhea and vomiting and the other with diarrhea, a cardiovirus was identified not during acute illness but in a specimen obtained months after each child had recovered.

To investigate the possibility of coinfection with additional viruses, we used the Virochip (Viro4) to analyze the nine available specimens collected from the six cardiovirus-positive cases (Table 2). As expected, all six cardiovirus-positive cases were positive for a cardiovirus by Virochip. In three of the cardiovirus-positive stool specimens, there was evidence of coinfection: in two specimens by caliciviruses (norovirus and sapovirus) and in one specimen by a rotavirus. In the other three individuals, viruses other than cardioviruses were detected in the stool at the time of the first visit (adenovirus, norovirus and parechovirus, norovirus and enterovirus), but only cardiovirus

Table 2. Patients with stool positive for cardioviruses

ID	Age at first visit, months	Number ill in household	Days between visits 1 and 2	Clinical symptoms		Virochip/PCR results	
				10 days prior to visit 1	Between visits 1 and 2	Visit 1	Visit 2
UC2	8.4	4/10	—	Diarrhea/ vomiting	—	Cardiovirus, rotavirus	—
UC3	6.1	1/5	139	None	none	—	Cardiovirus, norovirus
UC4	21.4	1/9	91	None	none	Adenovirus	Cardiovirus
UC5	16.3	6/6	95	Diarrhea/ vomiting	none	Norovirus, parechovirus	Cardiovirus
UC6	14.0	1/5	—	Diarrhea/ vomiting	—	Cardiovirus, sapovirus	—
UC7	18.6	3/7	94	Diarrhea	none	Norovirus, enterovirus	Cardiovirus

Dashes indicate entries for which data and/or specimens were not available.

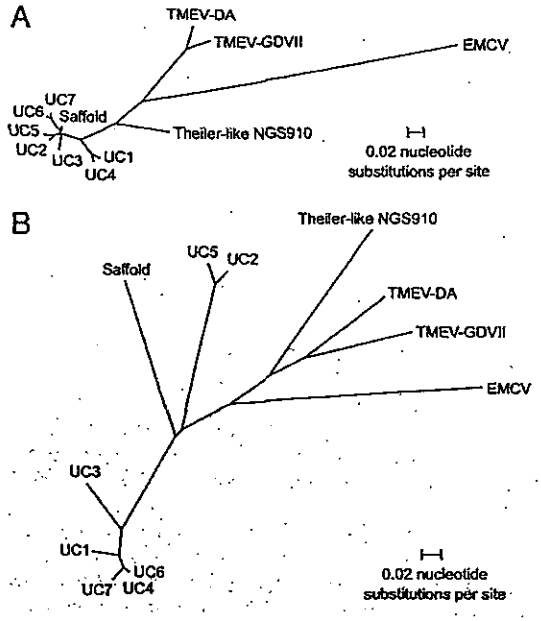


Fig. 2. Strain variation of human cardioviruses. (*A*) Radial tree of a 608-bp region within the 5′-UTR. (*B*) Radial tree of an 819-bp region corresponding to the VP1 gene. Strain designations UC2 to UC7 correspond to patients as listed in Table 2.

was detected in the stool by the second visit. All Virochip results were subsequently confirmed by PCR and sequencing using virus-specific primers.

To assess the sequence variation within different cardiovirus strains, we analyzed a 608-bp region from the 5′-UTR and an 819-bp region corresponding to the VP1 gene for the six positive cardiovirus cases (Fig. 2). The sequence variations within the 608-bp region from the 5′-UTR (2.0–9.1%) and within the 819-bp region corresponding to the VP1 gene (0.3–36.7%) were consistent with infection by independently acquired cardiovirus strains. The amino acid sequence identities in the VP1 gene were lowest between UC2/UC5 and the other cardioviruses (66.9% for Saffold virus, 71.0–72.8% for the other UC strains).

Discussion

Using a pan-viral microarray, we analyzed 16 respiratory specimens from patients with influenza-like illness who still lacked a diagnosis after extensive tests for respiratory viruses. In one specimen, we found a signature for a cardiovirus. Sequence recovery of the genome and phylogenetic analysis revealed that this virus (UC1) is divergent from the rodent cardioviruses and clusters with the Saffold agent. Like Saffold virus, UC1 may code for a truncated L* protein (Fig. S1*A*) that has been implicated in viral persistence and chronic infection of the CNS in TMEV (26). However, because the L* protein of UC1 begins with an ACG codon rather than AUG, it is unclear whether any functional protein is actually expressed, although small amounts of L* protein have been detected in TMEV strains carrying the ACG codon (27).

The binding of sialic acid to TMEV is strongly associated with persistence and neurovirulence, and three amino acids in the VP2 protein are directly involved in this interaction (28, 29). In both UC1 and Saffold virus, there is a substitution or deletion at each of these three positions (Fig. S1*B*), suggesting that sialic acid is unlikely to serve as a receptor for these viruses. Although the cellular receptor is presently unknown, the sequences of UC1 and Saffold virus are most divergent in the capsid region, sharing only 77% and 83% amino acid identity in the VP1 and VP2 proteins, respectively, and 52% and 61% identity in the exposed surface loops CD and EF, respectively. These differences may reflect the use of distinct cellular receptors or may be the result of immune selection during virus evolution (or both); further studies will be required to shed light on these issues.

Cardioviruses were detected in six children out of a total of 498 individuals (1.2%) enrolled in a large gastroenteritis study. Although the initial specimen that was used to culture Saffold virus was collected >25 years ago, cardioviruses UC1 through UC7 were collected from 2000 to 2006, indicating that human cardioviruses continue to circulate in the population. Despite the use of screening RT-PCR assays able to detect all strains of TMEV, cardioviruses detected in human clinical specimens clustered together and were phylogenetically distinct from the rodent cardioviruses (Fig. 2).

Further studies will be required to define the pathogenic role of cardiovirus infection in the intestine fully. Although we did recover a cardiovirus from a number of cases with symptomatic enteritis, other potential GI pathogens were also detected in these cases. Thus, it is presently unclear how frequently enteric cardioviral infection produces clinical illness. Moreover, we detected cardioviruses in stool from subjects without enteritis, suggesting that asymptomatic shedding of cardioviruses in the GI tract can and does occur. In this respect, cardiovirus infection in humans may mimic that of murine TMEV, which is often shed asymptomatically in naturally acquired infections (30).

Cardiovirus infection outside the GI tract is sometimes associated with severe disease in rodents, including encephalomyelitis, demyelinating disease, and myocarditis (1), although only a small percentage of mice naturally infected with TMEV develop systemic disease (1, 5). Our wider screening for cardioviruses indicates that cardiovirus infection is uncommon in the setting of acute respiratory or neurological disease (e.g., aseptic meningitis, encephalitis, MS). However, while this manuscript was under review, Abed and Boivin (31) reported detection of Saffold-like cardioviruses in three children with acute respiratory illness. Moreover, in a case of influenza-like illness reported here, a cardiovirus was the sole agent identified despite comprehensive testing with culture, PCR, and a pan-viral microarray, suggesting that cardioviruses may be pathogenic outside the GI tract in at least some instances.

One remarkable finding from this study was the diversity of the human cardioviruses that have been identified. For the family Picornaviridae, the definition of a new species in a genus is having <70% amino acid identity in the coding regions of either VP1, 2C, 3C, or 3D (32). By this strict definition, cardioviruses UC2 and UC5 would classify as a novel species distinct from Saffold virus, with 66.9% amino acid identity in the VP1 gene. However, since cardioviruses UC1 through UC7 and Saffold virus as a whole clearly define a separate group within the *Cardiovirus* genus by phylogenetic analysis (Figs. 1*B* and 2), we propose a systematic nomenclature for the human cardioviruses, designating all members of this group HTCV, for human TMEV-like cardiovirus, and referring to the strains in this group by a brief suffix (e.g., Saffold agent would be designated HTCV-Saf, UC1 would be designated HTCV-UC1).

Several lines of evidence support the inference that HTCVs are bona fide human viruses and not the products of sporadic viral cross-over events from rodents to humans: (*i*) all seven cardioviruses from humans in this study are strains of HTCV, with no mouse TMEV sequences detected in 1870 total clinical specimens despite screening using two consensus PCR primer sets designed to amplify UC1, Saffold virus, or mouse TMEV; (*ii*) sequence variations within HTCV UC1–7 are most consistent with independent acquisition of different virus strains by patients; and (*iii*) HTCV is substantially diverged from the rodent cardioviruses, especially in the capsid region containing

the putative receptor-binding sites. Taken together, our findings indicate that HTCVs are novel human picornaviruses in the *Cardiovirus* genus that are found primarily in the GI tract, can be shed asymptomatically, and have potential links to self-limited enteric disease and, rarely, to influenza-like illness. Although the full spectrum of clinical diseases linked to HTCV and the mechanisms underlying viral replication remain to be elucidated, the studies reported here now open all these questions to direct experimental scrutiny.

Materials and Methods

Clinical Specimens. ***Respiratory secretions from the California Influenza Surveillance Program study.*** A total of 943 respiratory specimens were sent to the California Department of Health Services (DHS) during the 2005–2006 season (25). Among these 943 specimens, 460 were pooled nasopharyngeal and oropharyngeal swabs collected as part of the California Influenza Surveillance Program (CISP) study under protocols approved by the DHS. Patients enrolled in the CISP study fulfilled a clinical case definition of influenza-like illness (temperature of 37.8°C or greater and a cough and/or sore throat in the absence of a known cause other than influenza). Sixty percent, or 280 specimens, were positive for a virus by culture. Among the remaining 180 culture-negative specimens, a subset of 108 specimens selected from elderly and pediatric patients was then subjected to further screening by RT-PCR to exclude cases of RSV, Flu A/B, RV, and EV (33). Sixteen specimens negative by culture and RT-PCR were then examined using the Virochip. We subsequently screened 441 CISP specimens with remaining available specimen material (96% of the 460 total collected specimens) for cardioviruses by RT-PCR.
Respiratory secretions from the UCSF pediatric respiratory infections study. This collection consisted of 278 consecutive nasopharyngeal aspirates from pediatric patients seen at UCSF from December 2003 to June 2004 (13). All specimens were collected under protocols approved by the UCSF Institutional Review Board. In this group, 190 of the patients (68%) had a respiratory illness, defined as an upper respiratory infection, bronchiolitis, croup, asthma exacerbation, or pneumonia. The remaining 88 patients (32%) were asymptomatic.
Stool from the Stanford Infection and Family Transmission cohort. The Stanford Infection and Family Transmission (SIFT) cohort of 4333 individuals was initiated in 1999 to evaluate the association between *H. pylori* infection and gastroenteritis transmission prospectively (18). Among the 3063 subjects who consented to further use of biological specimens, 774 stool specimens were obtained from 514 individuals; of those, 751 specimens from 498 subjects were available for study. Additional details on the 751 specimens screened for cardioviruses by RT-PCR are described in *SI Text*.
CSF specimens from patients with aseptic meningitis, encephalitis, and MS. A total of 60 CSF specimens from patients with clinically diagnosed aseptic meningitis, 300 CSF specimens from patients with encephalitis (who lacked a diagnosis despite comprehensive testing) (34), and 40 CSF specimens from patients with MS were screened for cardioviruses by RT-PCR. Specimens were collected under protocols approved by the California DHS (encephalitis specimens) or the UCSF Institutional Review Board (aseptic meningitis and MS specimens).

Specimen Preparation and Diagnostic Testing. In the CISP study, routine tube culture or shell vial culture of pooled nasopharyngeal and oropharyngeal swab specimens followed by specific monoclonal antibody testing for viral identification was performed as previously described (33, 35). Total nucleic acid was then extracted from the specimens using the MasterPure Complete DNA and RNA Purification Kit (Epicentre). Real-time one-step RT-PCR assays for RSV, FluA/B, and picornavirus (inclusive of RV and EV) were then performed as previously described (25, 33, 36). In the UCSF pediatric respiratory infections study, 200-μl aliquots of nasopharyngeal lavage were used to extract RNA using the RNeasy Mini Kit (Qiagen Corporation), including on-column DNase digestion. In the SIFT cohort, stool was suspended in 2 ml of PBS at 10% weight per volume and the PureLink96 Viral RNA/DNA Kit (Invitrogen) was used to extract RNA for RT-PCR and Virochip analysis. Cerebrospinal fluid specimens were processed using either a Zymo MiniRNA Isolation Kit (Zymo Research) or the MasterPure Complete DNA and RNA Purification Kit.

Virochip analysis of CISP and SIFT specimens was carried out as previously described (14). Extracted nucleic acid specimens were amplified and labeled using a Round A/B protocol and were hybridized to the Virochip. Microarrays (National Center for Biotechnology Information GEO platforms GPL3429 for Viro3 and GPL6862 for Viro4) were scanned with an Axon 4000B scanner (Axon Instruments). Virochip results were analyzed using cluster analysis, E-Predict, and *z* score analysis as previously described (12, 19, 37). All Virochip microarrays have been submitted to the GEO database (National Center for Biotechnology Information GEO series number GSE11569, accession numbers GSM291246–GSM291254).

Complete Genome Cloning and Sequencing (UC1 strain). Conserved primers from the 5′UTR of cardioviruses were designed based on the highest intensity microarray oligonucleotides and alignment of well conserved sequences from four cardioviruses for which full genome sequences were available: TMEV-DA, TMEV-GDVII, Theiler-like NGS910 virus, and EMCV. After short viral fragments were obtained, six sets of specific primers derived from sequenced fragments and conserved primers were then used to sequence the genome by long-range RT-PCR and 5′/3′ RACE (rapid amplification of cDNA ends). Amplicons for sequencing were cloned into plasmid vectors using the TOPO TA Cloning System (Invitrogen) and sequenced on an ABI3130 Genetic Analyzer (Applied Biosystems) using standard Big Dye terminator (version 3.1) sequencing chemistry. The completed genome sequence of UC1 has been deposited into GenBank (GenBank accession number EU376394).

Phylogenetic Analysis (UC1 strain). Nucleotide and protein sequences associated with the following reference virus genomes were obtained from GenBank: Saffold virus (NC_009448), TMEV-DA (M20301), TMEV-GDVII (NC_001366), Theiler-like NGS910 virus (AB090161), EMCV (NC_001479), poliovirus (NC_002048), and the partially sequenced genome of Vilyuisk virus (M94868). For amino acid analysis, ORFs predicted using ORF Finder (National Center for Biotechnology Information) were used. Multiple sequence alignment was performed using ClustalX (version 1.83). Neighbor-joining trees using the Kimura two-parameter distance correction were generated using 1000 bootstrap replicates and displayed using MEGA (version 3.1). Sequence identities were calculated using BioEdit (version 7.0.9.0).

RT-PCR Screening for Cardioviruses. Real-time quantitative RT-PCR (qRT-PCR) screening for cardioviruses with SYBR Green I (Invitrogen) was performed using conserved PCR primer sets CardioUTR-1F/CardioUTR-2R-A and CardioUTR-1F/CardioUTR-2R-B (Table S1) on a DNA Engine Opticon System (Bio-Rad). To determine limits of sensitivity of the qRT-PCR assay, probit analysis of results from 10 qRT-PCR replicates of eight serial half-log dilutions of *in vitro* transcribed UC1 mRNA (from a starting concentration of ~10^5 copies/ml) was performed using StatsDirect (StatsDirect Ltd.). Standard curves of the qRT-PCR assay were calculated from 3 qRT-PCR replicates of seven serial log dilutions of RNA extracted from pooled respiratory secretions, stool suspensions, and PBS spiked with UC1 RNA (10 specimens per pool). To assess for the presence of PCR inhibitors, RT-PCR for yeast was carried out on 95 randomly selected stool samples, each spiked with 1 ng of *in vitro* transcribed *Saccharomyces cerevisiae* intergenic RNA as a positive control (38).

Positive bands corresponding to the expected 102-bp and 224-bp amplicons were cloned and sequenced in both directions using vector primers M13F and M13R. Secondary confirmation of all positive reactions was performed using RT-PCR with primers CardioUTR-1F and CardioUTR-3R (Table S1), which generated a larger 608-bp amplicon, also in the 5′-UTR. To obtain the full sequences of the VP1 gene in strains UC2 through UC7, RT-PCRs were performed using conserved primers flanking the VP1 region of UC1 and Saffold virus (Table S1). The sequences of the 5′-UTR and VP1 amplicons corresponding to cardiovirus strains UC2 through UC7 have been deposited in GenBank (accession numbers EU604739–EU604750).

PCR Confirmation for Virochip-Positive Stool Specimens. All nine specimens collected from the six positive cardiovirus cases were analyzed using the Virochip as previously described (11, 12). Confirmatory PCR for calicivirus, adenovirus, and parechovirus was carried out using conserved primers as previously reported (39–41). Amplified PCR bands of the expected size were gel extracted and sequenced using standard BigDye chemistry on an ABI3130 (Applied Biosystems).

ACKNOWLEDGMENTS. We thank Silvi Rouskin for expert technical assistance and Amy Kistler, Patrick Tang, Anatoly Urisman, and Yiyang Xu for helpful suggestions on the manuscript. We thank Drs. Stephen Hauser and Jorge Oksenberg for generously providing CSF specimens from patients with MS for cardiovirus screening. These studies were supported by grants from the Doris Duke Charitable Foundation (to J.L.D. and D.G.), Howard Hughes Medical Institute (to J.L.D. and D.G.), and Packard Foundation (to J.L.D.).

1. Brahic M, Bureau JF, Michiels T (2005) The genetics of the persistent infection and demyelinating disease caused by Theiler's virus. *Annu Rev Microbiol* 59:279–298.
2. Ohsawa K, Watanabe Y, Miyata H, Sato H (2003) Genetic analysis of a Theiler-like virus isolated from rats. *Comp Med* 53:191–196.
3. Pritchard AE, Strom T, Lipton HL (1992) Nucleotide sequence identifies Vilyuisk virus as a divergent Theiler's virus. *Virology* 191:469–472.
4. Duke GM, Hoffman MA, Palmenberg AC (1992) Sequence and structural elements that contribute to efficient encephalomyocarditis virus RNA translation. *J Virol* 66:1602–1609.
5. Oleszak EL, Chang JR, Friedman H, Katsetos CD, Platsoucas CD (2004) Theiler's virus infection: A model for multiple sclerosis. *Clin Microbiol Rev* 17:174–207.
6. Ha-Lee YM, *et al.* (1995) Mode of spread to and within the central nervous system after oral infection of neonatal mice with the DA strain of Theiler's murine encephalomyelitis virus. *J Virol* 69:7354–7361.
7. Petrov PA (1970) V. Vilyuisk encephalitis in the Yakut Republic (U.S.S.R). *Am J Trop Med Hyg* 19:146–150.
8. Vladimirtsev VA, *et al.* (2007) Family clustering of Viliuisk encephalomyelitis in traditional and new geographic regions. *Emerg Infect Dis* 13:1321–1326.
9. Lipton HL, Friedmann A, Sethi P, Crowther JR (1983) Characterization of Vilyuisk virus as a picornavirus. *J Med Virol* 12:195–203.
10. Jones MS, Lukashov VV, Ganac RD, Schnurr DP (2007) Discovery of a novel human picornavirus in a stool sample from a pediatric patient presenting with fever of unknown origin. *J Clin Microbiol* 45:2144–2150.
11. Wang D, *et al.* (2002) Microarray-based detection and genotyping of viral pathogens. *Proc Natl Acad Sci USA* 99:15687–15692.
12. Chiu CY, *et al.* (2006) Microarray detection of human parainfluenzavirus 4 infection associated with respiratory failure in an immunocompetent adult. *Clin Infect Dis* 43:e71–e76.
13. Chiu, CY, *et al.* (2008) Utility of DNA microarrays for detection of viruses in pediatric acute respiratory infections. *J Pediatr* 153:76–83.
14. Wang D, *et al.* (2003) Viral discovery and sequence recovery using DNA microarrays. *PLoS Biol* 1:E2.
15. Urisman A, *et al.* (2006) Identification of a novel Gammaretrovirus in prostate tumors of patients homozygous for R462Q RNASEL variant. *PLoS Pathog* 2:E25.
16. Chiu CY, *et al.* (2007) Diagnosis of a critical respiratory illness caused by human metapneumovirus by use of a pan-virus microarray. *J Clin Microbiol* 45:2340–2343.
17. Kistler A, *et al.* (2007) Pan-viral screening of respiratory tract infections in adults with and without asthma reveals unexpected human coronavirus and human rhinovirus diversity. *J Infect Dis* 196:817–825.
18. Perry S, de la Luz Sanchez M, Hurst PK, Parsonnet J (2005) Household transmission of gastroenteritis. *Emerg Infect Dis* 11:1093–1096.
19. Urisman A, *et al.* (2005) E-Predict: A computational strategy for species identification based on observed DNA microarray hybridization patterns. *Genome Biol* 6:R78.
20. Scotto-Lavino E, Du G, Frohman MA (2006) 3' end cDNA amplification using classic RACE. *Nat Protoc* 1:2742–2745.
21. Scotto-Lavino E, Du G, Frohman MA (2006) 5' end cDNA amplification using classic RACE. *Nat Protoc* 1:2555–2562.
22. Kong WP, Ghadge GD, Roos RP (1994) Involvement of cardiovirus leader in host cell-restricted virus expression. *Proc Natl Acad Sci USA* 91:1796–1800.
23. Kong WP, Roos RP (1991) Alternative translation initiation site in the DA strain of Theiler's murine encephalomyelitis virus. *J Virol* 65:3395–3399.
24. Jnaoui K, Michiels T (1998) Adaptation of Theiler's virus to L929 cells: Mutations in the putative receptor binding site on the capsid map to neutralization sites and modulate viral persistence. *Virology* 244:397–404.
25. Louie JK, *et al.* (2007) Creating a model program for influenza surveillance in California: Results from the 2005–2006 influenza season. *Am J Prev Med* 33:353–357.
26. Ghadge GD, Ma L, Sato S, Kim J, Roos RP (1998) A protein critical for a Theiler's virus-induced immune system-mediated demyelinating disease has a cell type-specific antiapoptotic effect and a key role in virus persistence. *J Virol* 72:8605–8612.
27. van Eyll O, Michiels T (2002) Non-AUG-initiated internal translation of the L* protein of Theiler's virus and importance of this protein for viral persistence. *J Virol* 76:10665–10673.
28. Kumar AS, Kallio P, Luo M, Lipton HL (2003) Amino acid substitutions in VP2 residues contacting sialic acid in low-neurovirulence BeAn virus dramatically reduce viral binding and spread of infection. *J Virol* 77:2709–2716.
29. Zhou L, Luo Y, Wu Y, Tsao J, Luo M (2000) Sialylation of the host receptor may modulate entry of demyelinating persistent Theiler's virus. *J Virol* 74:1477–1485.
30. Brownstein D, Bhatt P, Ardito R, Paturzo F, Johnson E (1989) Duration and patterns of transmission of Theiler's mouse encephalomyelitis virus infection. *Lab Anim Sci* 39:299–301.
31. Abed Y, Boivin G (2008) New saffold cardioviruses in 3 children, Canada. *Emerg Infect Dis* 14:834–836.
32. Fauquet, CM, Mayo MA, Maniloff J (2005) *Virus Taxonomy, Classification, and Nomenclature of Viruses* (Elsevier Academic, San Diego, CA).
33. Louie JK, *et al.* (2005) Characterization of viral agents causing acute respiratory infection in a San Francisco University Medical Center Clinic during the influenza season. *Clin Infect Dis* 41:822–828.
34. Glaser CA, *et al.* (2006) Beyond viruses: Clinical profiles and etiologies associated with encephalitis. *Clin Infect Dis* 43:1565–1577.
35. Louie JK, *et al.* (2005) Rhinovirus outbreak in a long term care facility for elderly persons associated with unusually high mortality. *Clin Infect Dis* 41:262–265.
36. Kares S, *et al.* (2004) Real-time PCR for rapid diagnosis of entero- and rhinovirus infections using LightCycler. *J Clin Virol* 29:99–104.
37. Eisen MB, Spellman PT, Brown PO, Botstein D (1998) Cluster analysis and display of genome-wide expression patterns. *Proc Natl Acad Sci USA* 95:14863–14868.
38. Shock JL, Fischer KF, DeRisi JL (2007) Whole-genome analysis of mRNA decay in Plasmodium falciparum reveals a global lengthening of mRNA half-life during the intra-erythrocytic development cycle. *Genome Biol* 8:R134.
39. Echavarria M, Forman M, Ticehurst J, Dumler JS, Charache P (1998) PCR method for detection of adenovirus in urine of healthy and human immunodeficiency virus-infected individuals. *J Clin Microbiol* 36:3323–3326.
40. Farkas T, *et al.* (2004) Genetic diversity among sapoviruses. *Arch Virol* 149:1309–1323.
41. Legay V, Chomel JJ, Lina B (2002) Specific RT-PCR procedure for the detection of human parechovirus type 1 genome in clinical samples. *J Virol Methods* 102:157–160.

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. 7. 23	該当なし	

一般的名称	(製造販売承認書に記載なし)	研究報告の公表状況	ProMED 20080720.2201, 2008 Jul 20. 情報源: HeraldonSunday online, 2008 Jul 20.	公表国 オーストラリア
販売名(企業名)	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

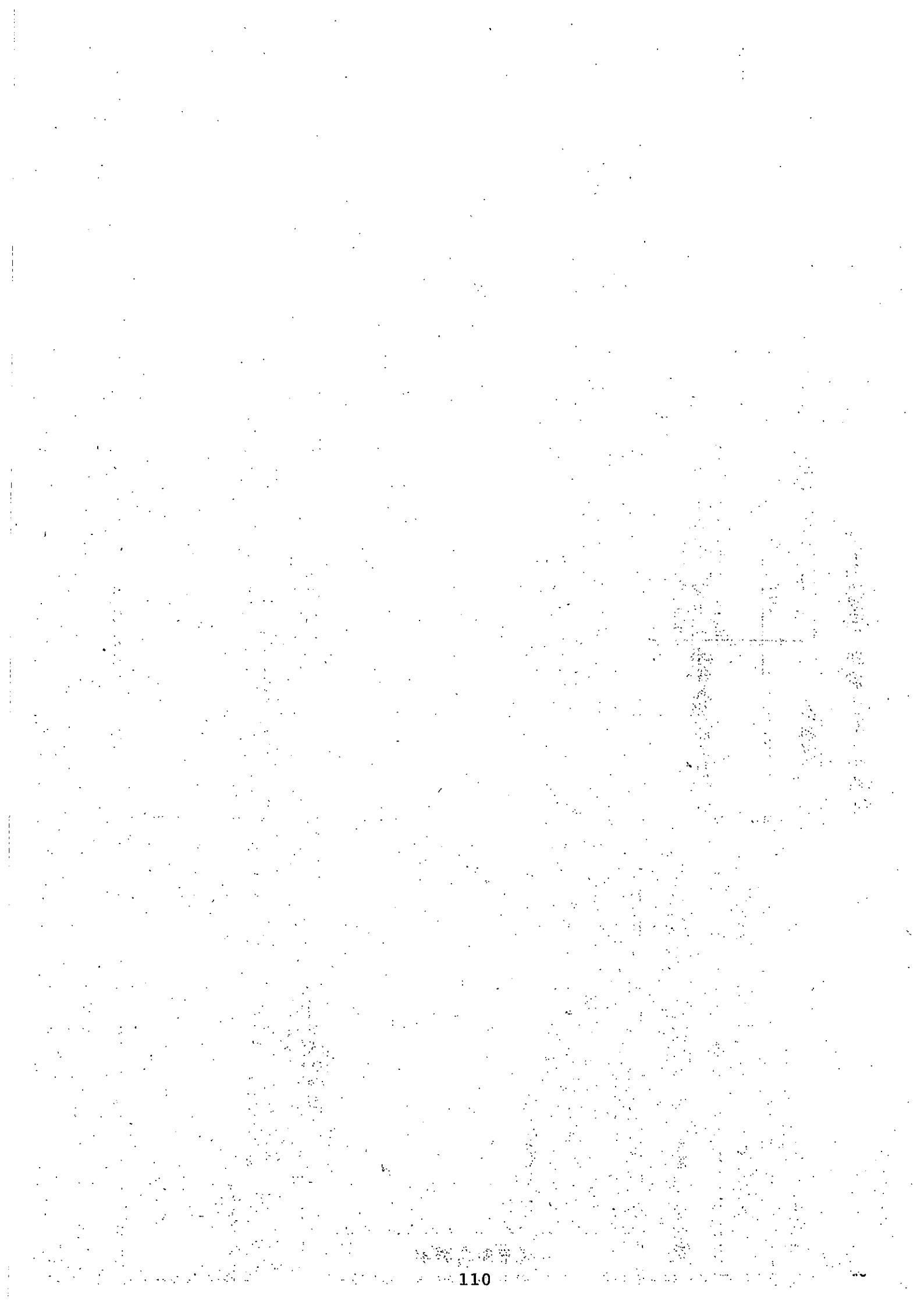
○ヘンドラウイルス感染、ヒト、ウマ―オーストラリア
オーストラリア、ブリスベーンの動物病院スタッフがヘンドラウイルスに感染した。看護師1名と獣医師1名が、感染したウマを治療した後でウイルスに感染したと診断された。患者は2名とも重症である。潜伏期間は最大14日間で、スタッフの間から新たな患者が出ないか監視が続けられている。ウマは感染した3頭中1頭が死亡、1頭を安楽死させたが、1頭は回復に向かっている。
ヘンドラウイルスが以前に流行したのは1994年で、ウマ14頭と調教師1名が死亡した。ヒト-ヒト感染の証拠はなく、広範囲に流行する危険性はない。

報告企業の意見	今後の対応
オーストラリア、ブリスベーンの動物病院スタッフがヘンドラウイルズに感染し、重症となったとの報告である。ヘンドラウイルスはニパウイルスに近縁のウイルスで、現在のところオーストラリア以外での発生はない。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。今後も引き続き、新たなウイルス等による感染症の発生状況等に関する情報の収集に努める。

使用上の注意記載状況・その他参考事項等

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



Archive Number 20080720.2201
Published Date 20-JUL-2008
Subject PRO/AH/EDR> Hendra virus, human, equine - Australia (03): (QLD)

HENDRA VIRUS, HUMAN, EQUINE - AUSTRALIA (03): (QUEENSLAND)
****

A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail is a program of the
International Society for Infectious Diseases
<http://www.isid.org>

Date: Sun 20 Jul 2008
Source: HeraldonSunday online, AAP report [edited]
<http://www.nzherald.co.nz/section/4/story.cfm?c_id=4&objectid=10522474>

Anxious watch over vet staff in virus outbreak

The owner of a Brisbane veterinary clinic is anxiously waiting to see if more of his staff have contracted the potentially fatal Hendra virus. A nurse and a veterinarian at the Redlands Veterinary Clinic were diagnosed with the virus after treating several infected horses. Owner Dr David Lovell said if no more staff were diagnosed this weekend [19-20 Jul 2008], the worst of the crisis should be over. "If we get through this weekend I get the feeling we will be on the road to recovery," Lovell said. "The anticipated maximum incubation period is 14 days and certainly by Tuesday [22 Jul 2008] there would be absolutely no chance of there being a human or horse being exposed or infected because everything would have been shut down and secured for that time."

Lovell said staff had visited the nurse and veterinarian Ben Cunneen in the Princess Alexandra Hospital. "They are no way near being cured but it just means they are not deteriorating and that has to be some cause for optimism. But this is not detracting one bit from the seriousness of the condition."

The veterinarian of 38 years has closed his horse practice during the crisis as 8 other staff who worked closely with affected horses are monitored to see if they are incubating the bug. One of the horses was put down, another died and a 3rd is recovering. Lovell said those horses showed signs of neurological damage such as a staggered gait and falling over.

Cunneen and the nurse suffered flu-like symptoms from the virus, which claimed the life of trainer Vic Rail and 14 horses during the last outbreak in 1994. Brisbane Southside Population Health Unit medical officer Dr Brad McCall said the affected pair would have acquired the infection through close contact with the horses in the late stage of illness or at autopsy. There had been no evidence of person to person transmission of the virus and no risk to the wider community.

Queensland Health continues to monitor 7 people in Proserpine, north Queensland, who have undergone blood tests following a 2nd outbreak of the virus. A virus-affected horse died late last week at a Cannonvale property.

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

[The 1st human case of Hendra virus infection in the outbreak affecting horses at the Redlands Veterinary Clinic in Brisbane was reported on 15 Jul 2008. Now a 2nd person working at the Redlands Veterinary Clinic has been

hospitalised with Hendra virus infection. The condition of these 2 patients appears to be serious but not life-threatening.

The interactive HealthMap/ProMED-mail interactive map of Australia can be accessed at <http://healthmap.org/promed?v=-25.7,134.5,4> to find the location of the city of Brisbane in the state of Queensland. - Mod.CP]

[see also:
Hendra virus, human, equine - Australia (02): (QLD,NSW) 20080717.2168
Hendra virus, human, equine - Australia: (QLD) 20080715.2146
Hendra virus, equine - Australia: (Brisbane) 20080708.2076
2007

Hendra virus, human, equine - Australia (QLD) (04): 2nd corr. 20070903.2902
Hendra virus, human, equine - Australia (QLD) (03): corr. 20070903.2896
Hendra virus, human, equine - Australia (QLD) (02): not 20070831.2871
Hendra virus, human, equine - Australia (QLD): RFI 20070830.2851
2006

Hendra virus, equine - Australia (NSW): susp. 20061109.3222
2004

Hendra virus - Australia (QLD) 20041214.3307
1999

Hendra virus - Malaysia, Singapore: Fact sheet 19990319.0434
Hendra virus, horse - Australia (Queensland) 19990219.0218]

.....cp/ejp/sh

##
****
ProMED-mail makes every effort to verify the reports that are posted, but the accuracy and completeness of the information, and of any statements or opinions based thereon, are not guaranteed. The reader assumes all risks in using information posted or archived by ProMED-mail. ISID and its associated service providers shall not be held responsible for errors or omissions or held liable for any damages incurred as a result of use or reliance upon posted or archived material.
****
Become a ProMED-mail Premium Subscriber at <http://www.isid.org/ProMEDMail_Premium.shtml>
****
Visit ProMED-mail's web site at <http://www.promedmail.org>. Send all items for posting to: promed@promedmail.org (NOT to an individual moderator). If you do not give your full name and affiliation, it may not be posted. Send commands to subscribe/unsubscribe, get archives, help, etc. to: majordomo@promedmail.org. For assistance from a human being send mail to: owner-promed@promedmail.org.
##
##

about ISID | membership | programs | publications | resources
13th ICID | site map | ISID home

©2001,2008 International Society for Infectious Diseases
All Rights Reserved.
Read our privacy guidelines.
Use of this web site and related services is governed by the Terms of Service.

別紙様式第2-1
番号11

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	新医薬品等の区分	厚生労働省処理欄
				2008年7月16日	該当なし	

一般的名称	乾燥濃縮人血液凝固第Ⅷ因子	研究報告の公表状況	TRANSFUSION 2008; 48: 1180-1187	公表国 アメリカ
販売名（企業名）	コンコエイト-HT（ベネシス）			

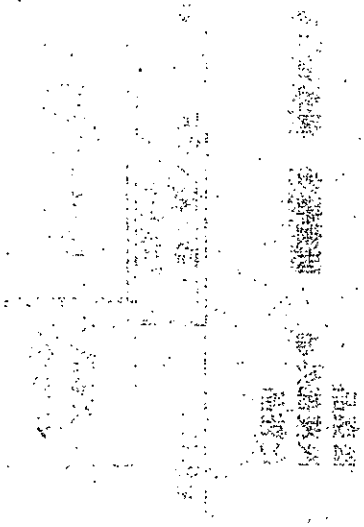
研究報告の概要

＜研究デザイン及び方法＞
ヒトの8つのヘルペスウイルスの同定と定量のために新たに開発された一連のRT-PCRを利用して、テキサス南東部から無作為に抽出した100名の血液ドナーの白血球を豊富に含む血液の陽性率とウイルスDNA量を測定し報告する。
＜結果＞
単純ヘルペスウイルス1及び2型（HSV-1及びHSV-2）、水痘帯状疱疹ウイルス（VZV）、及びHHV-8 DNAは、いずれのドナーにも検出されなかった。対照的に、エプスタインバーウイルス（EBV）（72%）およびHHV-7（65%）は検出頻度が高く、HHV-6（30%）は頻繁に検出され（B型のみ）、サイトメガロウイルス（1%）はめったに検出されなかった。陽性サンプル中のウイルス量の中央値は、血液1mLあたりHHV-6の4237からEBVの46未満の範囲におよんでいた。
＜結論＞
これらの結果から、健康な成人ドナーからの輸血によるヘルペスウイルス感染の可能性は、EBV及びHHV-7で高く、HHV-6で中程度に高く、CMVでは低く、HSV-1、HSV-2、VZV及びHHV-8ではめったにないことが示唆される。本研究で最も注目に値するのは、1人のドナーの血液から6.1×10^7genome equivalent/mLを超えるHHV-6 Type Bが検出されたことである。異常に高いHHV-6 DNAのレベルが健康な成人血液ドナーから検出されたことから、この現象は活動性感染または免疫不全と関係がないようである。

報告企業の意見	今後の対応
健常人血液ドナーからEBV、HHV-7、HHV-6が高頻度に検出され、またHHV-6については異常に高いレベルのウイルスが検出されたとの報告である。 万一、原料血漿にヘルペスウイルスが混入したとしても、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

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、人血液凝固第Ⅷ因子-vWF複合体を濃縮・精製した製剤であり、ウイルス不活化を目的として、製造工程においてリン酸トリ-n-ブチル（TNBP）/ポリソルベート80処理、凍結乾燥の後、60℃、72時間の加熱処理を施しているが、投与に際しては、次の点に十分注意すること。



BENE2008-012

Herpesvirus prevalence and viral load in healthy blood donors by quantitative real-time polymerase chain reaction

S. David Hudnall, Tiansheng Chen, Paul Allison, Stephen K. Tyring, and Ashley Heath

BACKGROUND: After primary infection, human herpesviruses (HHVs) maintain long-term latent persistence, often punctuated years later by sporadic episodes of symptomatic lytic activation. Also, blood-borne herpesvirus from healthy persistently infected blood donors can lead to active primary infection of immunocompromised transfusion recipients.

STUDY DESIGN AND METHODS: Utilizing a set of newly developed real-time polymerase chain reaction assays for detection and quantification of all eight human herpesviruses, the prevalence and viral DNA load of white cell–enriched blood from 100 randomly selected blood donors from the southeast Texas region are reported.

RESULTS: Herpes simplex viruses 1 and 2 (HSV-1 and HSV-2), varicella-zoster virus (VZV), and HHV-8 DNA were not detected in any donor sample. In contrast, Epstein-Barr virus (EBV) (72%) and HHV-7 (65%) were commonly detected, HHV-6 (30%) was often detected (Type B only), and cytomegalovirus (CMV; 1%) was rarely detected. Median viral loads of positive samples (per milliliter of blood) ranged from 4278 for HHV-6 to less than 46 for EBV.

CONCLUSIONS: These results suggest that the potential for transfusion-mediated transmission of herpesviruses from healthy adult blood donors is high for EBV and HHV-7; moderately high for HHV-6; uncommon for CMV; and rare for HSV-1, HSV-2, VZV, and HHV-8. Perhaps the most remarkable finding in this study was the detection of a single donor sample with greater than 6.1×10^7 HHV-6 Type B genome equivalents per mL blood. Given that this extraordinarily high level of HHV-6 DNA was obtained from a healthy adult blood donor, this phenomenon is likely unrelated to active infection or immunodeficiency.

The eight human herpesviruses (herpes simplex virus 1 and 2 [HSV-1, HSV-2], varicella-zoster virus [VZV], Epstein-Barr virus [EBV], cytomegalovirus [CMV], human herpesvirus 6 [HHV-6], human herpesvirus 7 [HHV-7], and human herpesvirus 8 [HHV-8, KSHV]) are large enveloped double-stranded DNA viruses that establish asymptomatic life-long latent persistence in host cells after primary infection.[1] Given the moderate to high seroprevalence rates for all but HHV-8, and the fact that most of the herpesviruses (EBV, CMV, HHV-6, HHV-7, HHV-8) maintain latency in white cells (WBCs), it is likely that a large number of adult blood donors carry herpesvirus DNA in whole blood.

There have been a number of excellent published studies regarding herpesvirus DNA prevalence and virus load in adult donor blood. Many of these studies, however, were performed with relatively few specimens (≤20), many did not determine viral load, and only one previous study[2] of 20 donors assayed for all eight herpesviruses.

A novel nested polymerase chain reaction (PCR) assay with a complex mixture of degenerate and deoxyinosine-substituted primers to the highly conserved herpesvirus DNA polymerase gene was previously developed for the purpose of discovery of novel herpesviruses in animals.[3] Our group adapted this general method for the detection

ABBREVIATIONS: HHV = human herpesvirus; HSV = herpes simplex virus; VZV = varicella-zoster virus.

From the Department of Pathology and Laboratory Medicine, University of Texas Medical Branch, Galveston, Texas; the Gulf Coast Regional Blood Center, Houston, Texas; Department of Dermatology, University of Texas Medical Center, Houston, Texas; and Sigma Genosys, The Woodlands, Texas.

Address reprint requests to: S. David Hudnall, MD, UTMB Department of Pathology and Laboratory Medicine, Galveston, TX 77555-0741; e-mail: shudnall@utmb.edu.

This work was supported by a grant to SDH from the Gulf Coast Regional Blood Center, Houston, Texas.

Received for publication September 28, 2007; revision received December 18, 2007, and accepted December 22, 2007.

doi: 10.1111/j.1537-2995.2008.01685.x

TRANSFUSION 2008;48:1180-1187.

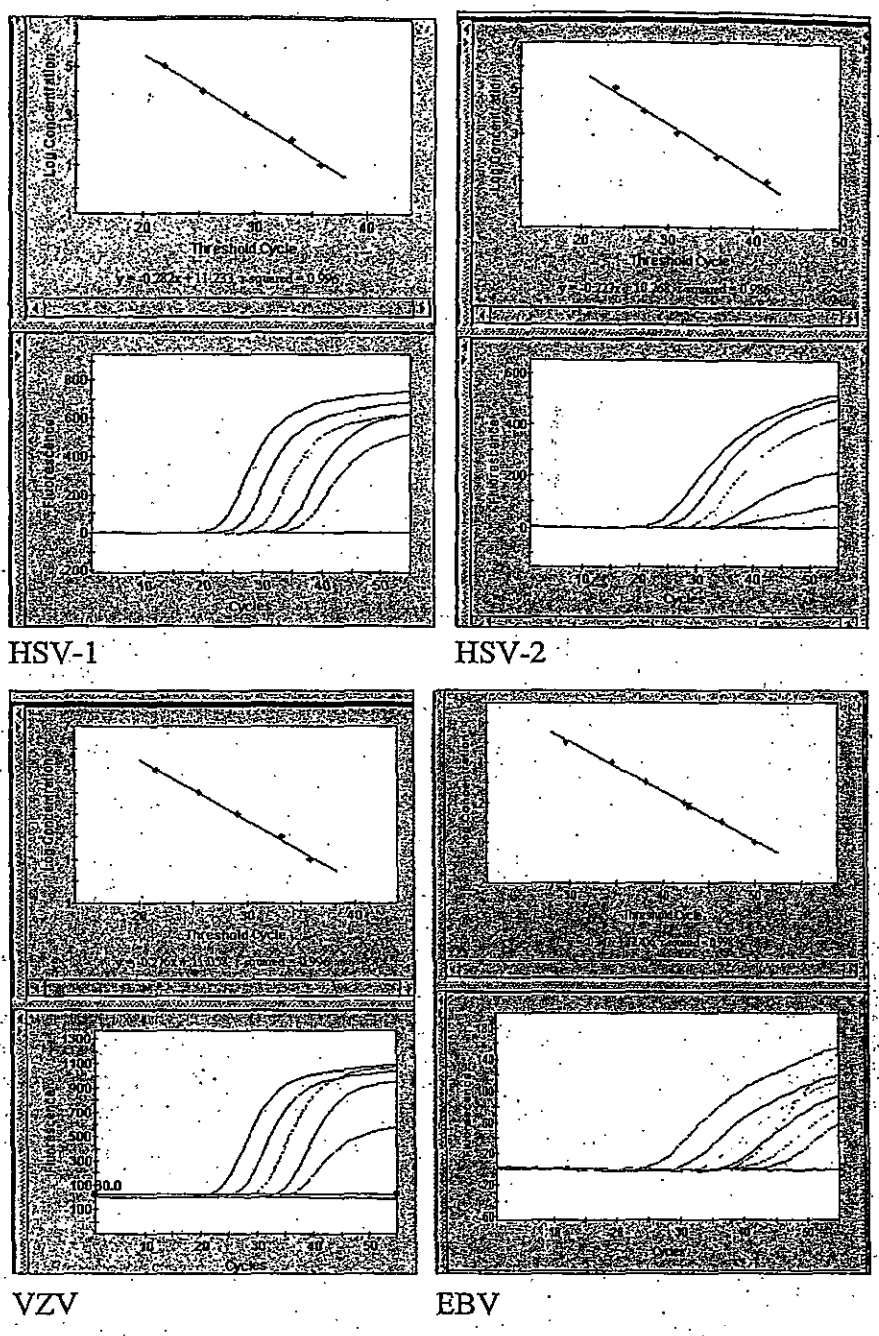


Fig. 1. Real-time PCR standard curves. The top panel displays the linear relationship between log concentration of viral DNA and PCR cycle. The bottom panel demonstrates the relationship between fluorescence signal intensity and PCR cycle. The curves from left to right in the lower panel represent serial dilutions of viral DNA—10^5, 10^4, 10^3, 10^2, and 10^1 viral geq per PCR procedure (per µg). Results for 10^0 geq are shown only for EBV, CMV, and HHV-8.

and differentiation of all eight human herpesviruses by chemiluminescent dot blot nucleic acid hybridization and heteroduplex mobility gel shift assay.[4] While these assays have proven to be excellent tools for herpesvirus detection and differentiation, they do not allow for viral load determination. To address this limitation, we have developed a set of eight real-time PCR assays with TaqMan probes for detection and quantification of the human herpesviruses and have applied these assays to determine the prevalence and viral load of herpesvirus DNA from 100 randomly selected donor blood samples.

MATERIALS AND METHODS

Real-time PCR

Herpesvirus DNA was obtained from the following sources: HSV-1 (ATCC, Rockville, MD), HSV-2 (ATCC), VZV (Ellen strain, ATCC), EBV (B95-8, ATCC), CMV (AD169 strain, ATCC), HHV-6 (U1102 Type A strain and Z29 Type B strain, Advanced Biotechnologies, Columbia, MD), HHV-7 (H7-4 strain, Advanced Biotechnologies), and HHV-8 (BCBL-1, NIH AIDS Reagent Program, Rockville MD). PCR products of each herpesvirus obtained by regular PCR (*Taq* polymerase, Sigma, St Louis, MO) were agarose gel–purified, cloned into the TOPO TA cloning vector (Invitrogen, Carlsbad, CA), and confirmed by DNA sequencing. Herpesvirus plasmid DNA was quantified by ultraviolet (UV) spectrophotometry (DU 640, Beckman, Fullerton, CA) and stored frozen at –20°C until use.

Assay specificity was determined by simultaneously performing two PCR procedures for each set of primers. One reaction was performed with a control sample containing DNA of all eight herpesviruses as template (positive control), and the other reaction was performed with a control sample containing DNA of all but the primer-specific virus (negative control). In each case (data not shown), all primer sets yielded a positive product with the positive control and no product with the negative control. Assay sensitivity was determined with six serial 10-fold dilutions (10^5-10^0 virus genome equivalents [geq]) of each herpesvirus plasmid DNA prepared in TE buffer (10 mmol/L Tris-HCl, 1 mmol/L ethylenediaminetetraacetate, pH 8.0). The standard curves for each virus are displayed in Fig. 1. Linearity of all log standard curves was excellent, with $r^2 > 0.98$ for all eight assays. The limits of detection (sensitivity) of each assay are as follows: HSV-1, 10 geq per µg DNA; HSV-2, 10 geq;

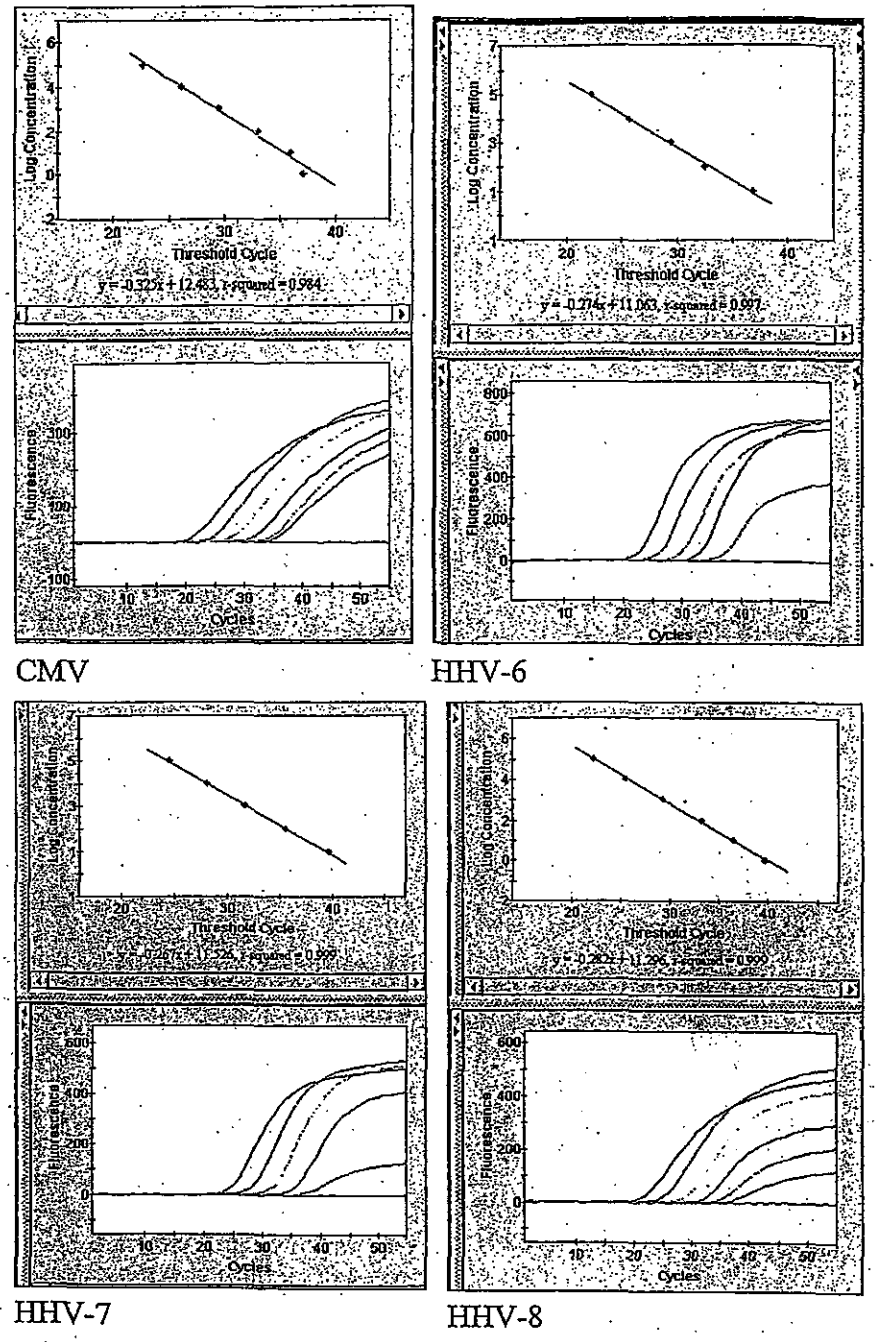


Fig. 1. *Continued*

VZV, 10 geq; EBV, 1 geq; CMV, 1 geq; HHV-6, 5 geq; HHV-7, 10 geq; and HHV-8, 1 geq.

DNA was extracted from 100 samples of WBC-rich whole blood obtained from the Gulf Coast Regional Blood Center (Houston, TX) with a DNA mini kit (QIAamp, Qiagen, Valencia, CA), quantified by UV spectrophotometry (*DU 640, Beckman*), and stored frozen in TE buffer at –20°C until use.

One-step real-time PCR assays for all eight herpesviruses were first developed. These single-step assays proved to be sufficiently sensitive for detection of all herpesviruses except for EBV and HHV-6. Because single-step assays for EBV and HHV-6 proved to be less sensitive in detection of low viral copy number, we developed nested PCR assays for detection of small quantities (<1000 geq/μg) of EBV and HHV-6 (Fig. 1).

To ensure that the nested PCR procedures were quantitative, standard curves for both stages of amplification with high viral load standards were constructed. We were careful to limit the first amplification step (with external primers) to 20 cycles, a cycle number empirically chosen based on results of single-step real-time PCR in which samples with viral loads as high as 2×10^6 copies per mL reverted to positive only after more than 20 cycles of amplification (as shown in Figs. 1 and 2). In addition, standard curves for the nested PCR clearly indicated that the assay was log-linear and quantitative for high viral load samples (Fig. 2).

One microgram of sample DNA (or 2 μL of external EBV and HHV-6 PCR products) was added to a real-time PCR tube containing 12.5 μL of 2× ready mix (JumpStart *Taq*, Sigma), 0.3 μmol per L primers, 0.2 μmol per L dual-labeled probes, 5 mmol per L $MgCl_2$, and ultrapure water up to 25 μL final volume. Real-time PCR was performed in a rapid thermal cycler (Smart Cycler, Cepheid, Sunnyvale, CA) machine under the following conditions: 95°C for 2 minutes, followed by 45 to 55 amplification cycles of 95°C for 15 seconds, 60°C (50°C for HHV-6) for 30 seconds, and 72°C for 30 seconds. All TaqMan primers and probes (see Appendix S1, available online at http://www.blackwell-synergy.com/doi/abs/10.1111/j.0041-1132.2008.01685.x) were produced by Sigma Genosys (The Woodlands, TX) and tested for sensitivity and specificity.

For external EBV PCR, a 1-μg sample of DNA was added to a PCR tube containing 3 μL of 10× reaction buffer (200 mmol/L Tris-HCl, pH 8.8, 100 mmol/L KCl, 100 mmol/L $(NH_4)_2SO_4$, 20 mmol/L MgCl2, 1% Triton X-100, 1 mg/mL bovine serum albumin), 1.2 μL of 25 mmol per L $MgCl_2$, 0.6 μL of 10 mmol per L dNTP mix, 1.5 units of *Taq* polymerase (Orbigen, San Diego, CA), 6 μL of 5× CES (2.7 mol/L betaine, 6.7 mmol/L dithiothreitol,

6.7% dimethyl sulfoxide, and 55 μg per mL bovine serum albumin), 0.3 μmol per L external primers (Set 1), and ultrapure water up to 30 μL final volume. External EBV PCR was performed in a conventional thermal cycler (Peltier, PTC-200, MJ Research, South San Francisco, CA) under the following conditions: 95°C for 2 minutes and 20 amplification cycles of 95°C for 30 seconds, 56°C for 40 seconds, and 72°C for 1 minute, followed by a final 6 minutes' extension at 72°C. EBV internal nested PCR was performed with internal primers (Set 2) and 0.2 μmol per L dual-labeled probe.

For external HHV-6 PCR, 1 μg of sample DNA was added to a preloaded PCR tube (EasyStart micro50, Molecular BioProducts, San Diego, CA) to which was added 5 μL of 1 percent Triton X-100, 2.5 units of *Taq* polymerase (Orbigen), 3 μL of 25 mmol per L $MgCl_2$, 0.32 μmol per L of external primers (Set 1), and ultrapure water up to 50 μL final volume. External HHV-6 PCR was performed in a conventional Peltier thermal cycler (PTC-200, MJ Research) under the following conditions: 94°C for 2 minutes and 20 amplification cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds, followed by a final 5 minutes' extension at 72°C. HHV-6 internal nested PCR was performed with internal primers (Set 2) and both HHV-6A and HHV-6B type–specific probes (0.2 μmol/L each). For extremely high viral loads (as seen with Case 46), our experience indicates that the use of two PCR procedures, each with a single HHV-6 type–specific probe, is preferable.

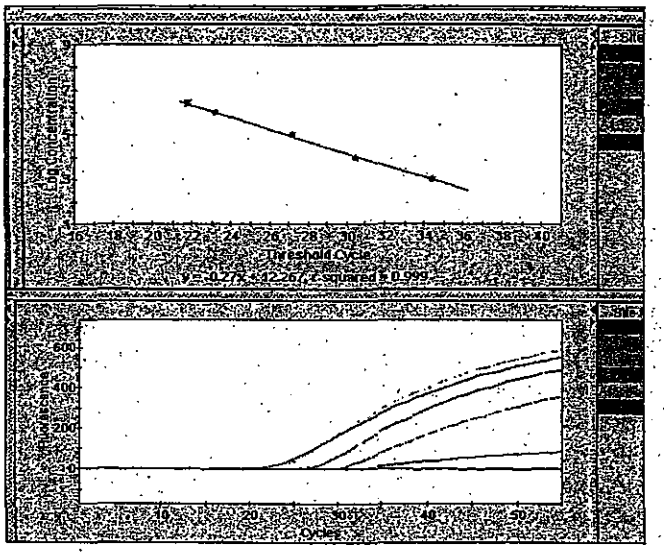


Fig. 2. Real-time PCR standard curve for HHV-6 high-viral-load samples. PCR positivity of extremely high HHV-6 viral loads (>10^6 copies/reaction) was seen only after more than 20 cycles of single-step PCR and yields a highly linear log standard curve with a range of 10^3 to 2.45×10^6 virus copies per reaction. The linearity of the assays allows for viral load quantification of samples with high viral load by one-step PCR.

Virus load calculation

Because each human diploid cell contains approximately 6.6 pg DNA, 1 μg of human genomic DNA from blood was derived from approximately 1.5×10^5 WBCs. One milliliter of whole human blood contains approximately 7×10^6 nucleated cells (WBCs). Thus, the virus copy number (geq) per milliliter of blood is equal to virus copy number per μg of DNA (as determined by the real-time PCR assay) multiplied by 47 μg of DNA per mL blood.

RESULTS

Herpesvirus DNA was commonly detected, with 94 of 100 donor blood samples positive for the presence of at least one herpesvirus (results summarized in Table 1). No herpesvirus DNA was detected in 6 cases. Four herpesviruses (HSV-1, HSV-2, VZV, HHV-8) were undetected in any sample, and CMV was detected in only a single case. In contrast, EBV (72%), HHV-7 (65%), and HHV-6 (30%) were commonly detected. All 30 cases of HHV-6 were Type B; that is, no HHV-6 Type A was identified. Median viral loads of positive samples (virus geq/mL blood) were 4,371 for HHV-6 (range 188-61,610,713), 3,196 for CMV (1 case only), 1,763 for HHV-7 (range 282-27,401), and less than 47 for EBV (range, <47-550,370). A single donor sample containing more than 80×10^6 geq of HHV-6B DNA per mL was identified. Because 1 mL of normal adult blood contains approximately 7×10^6 WBCs, this extremely high viral load translates to approximately 11 virus copies per WBC. Seventeen donor blood samples were positive for the presence of three herpesviruses (16 with EBV, HHV-6, and HHV-7; 1 with EBV, CMV, and HHV-7).

TABLE 1. Prevalence and virus load of herpesviruses in blood donors

	HSV-1	HSV-2	VZV	EBV	CMV	HHV-6	HHV-7	HHV-8
Total samples	100	100	100	100	100	100	100	100
Positive samples	0	0	0	72	1	30	65	0
Median viral load*				<47	3196	4371	1763	
Viral load range				<47-5.5×10^5		188-6.2×10^7	282-2.7×10^4	

* Expressed as virus copy number per mL of whole blood. Each PCR procedure was performed on 1 μg of whole-blood DNA, representing approximately 1.5×10^5 WBCs.

DISCUSSION

Given that acute infection with human herpesviruses may sometimes lead to serious disease, issues regarding the frequency and clinical significance of blood transfusion–mediated transmission of herpesviruses from chronically infected donors to previously uninfected or immunocompromised recipients have been raised. Although these issues have been addressed in the case of CMV, the frequency and significance of infection with the other herpesviruses have not been as thoroughly detailed.

Little information regarding the frequency and virus load of HSV-1–positive blood donors is available. HSV-1 PCR positivity was not detected in healthy adult blood donors from three independent studies.[2,5,6] With a highly sensitive real-time PCR assay, we detected no HSV-1–positive samples from a cohort of 100 adult blood donors. Our results corroborate the earlier negative reports and suggest that HSV-1 transmission by blood transfusion is likely to be a highly unusual event.

Information regarding detection of HSV-2 in healthy adult blood donors is extremely limited. In one small study,[2] HSV-2 PCR positivity was not detected in 20 adult blood donors. In the current study, we detected no HSV-2–positive samples from 100 adult blood donors. Our results corroborate the earlier negative findings and indicate that HSV-2 transmission by blood transfusion is likely to be a highly unusual event.

Relatively little information regarding the incidence of VZV DNA positivity in donor blood is available. Hoang and coworkers[2] detected only 1 VZV-positive sample (virus load 39,029 geq/mL) from a total of 20 samples, whereas de Jong and coworkers[7] detected no positive samples from a total of 20. In our study of 100 donor samples, no positive samples were identified. Thus, these data suggest that VZV transmission by donor blood is likely to be an infrequent event.

Given the very real clinical concerns with transfusion-mediated CMV transmission in immune-compromised recipients, several studies have addressed the issue of CMV positivity in donor blood. Whereas a relatively high frequency of CMV DNA positivity (19%-33%) has been described by some investigators,[5,6,8] other investigators have reported much lower rates of CMV positivity, ranging from 0 to 2.8 percent.[2,9-12] Roback and colleagues[9] identified only 2 positive samples of 1000 samples from the United States whereas Nishiwaki and coworkers[10] identified 27 positive samples of 953 samples from Japan. In the current study, we identified only 1 CMV-positive donor sample of 100 samples from the United States, a result that is consistent with the low prevalence previously reported in US blood donors.[9] In this previous report,[9] the 2 positive samples yielded an estimated 10 to 99 CMV geq per 2.5×10^5 WBCs. In an earlier article,[13] this same group reported donor blood CMV viral loads ranging from 8 to 1560 geq per 2.5×10^5 blood WBCs. Our positive sample contained 3196 CMV geq per mL of blood. Given that 1 mL of blood contains approximately 7×10^6 WBCs, our single positive case contains approximately 114 CMV genomes per 250,000 WBCs, a result remarkably similar to that previously reported.[9]

Give the role of EBV infection in the pathogenesis of posttransplant lymphoproliferative disorders, there has been a great deal of interest in determination of EBV viral load in donor blood. Although EBV infection is very common with greater than 96 percent seroprevalence in adults worldwide,[14] there is a wide range of reported rates for EBV DNA positivity of donor blood, ranging from 5 to 88 percent.[2,5,6,10,15-20] In this study, with a real-time nested PCR method, 72 percent of the donor blood samples contained EBV DNA. The sensitivity of our assay is 1 geq per µg of DNA. We suspect that the lower rates for EBV positivity reported by some investigators were obtained with less sensitive assays. Regarding EBV DNA load in blood, Hoang and colleagues[2] reported 845 geq per mL, Kimura and colleagues[15] reported 585 geq per mL (15.8 geq/µg), and Maurmann and colleagues[19] obtained a range of 3055 to 851,170 geq per mL. The current results indicate that EBV load varies over a wide range, with some donor blood samples containing more than 500,000 geq per mL, a result consistent with those previously reported by Maurmann and colleagues.[19] Qu and coworkers[20] reported the interesting observation that removal of WBCs from 14 EBV DNA–positive whole-blood units rendered all but 1 unit EBV DNA–negative. Thus, although EBV DNA positivity of whole donor blood appears to be quite common, the risk of EBV transmission from red blood cell transfusion is significantly reduced by leukoreduction.

In the current study, HHV-6 DNA was detected in 30 percent of the blood donor samples. At least six previous studies have reported rates of HHV-6 DNA positivity and virus load from adult donor blood samples. In one early study, Wilborn and colleagues[21] reported HHV-6 positivity in only 5.4 percent of donor blood (buffy coat) samples. In four later studies, HHV-6 DNA positivity was detected in 25 to 36 percent of donor blood samples.[2,22-24] Cuende and colleagues[25] made the interesting observation that using 1 µg of DNA, 40 percent of the samples were positive, a rate similar to that reported in the four previously mentioned studies, whereas using 5 µg DNA, 90 percent of the same samples were positive. Assuming that these results are not due to contamination, nonspecificity, or technical error, this finding suggests that detection of extremely low levels of virus may in some cases require amplification of larger amounts of sample DNA. It should be noted, however, that the 30 percent HHV-6 positivity rate obtained in the current study was obtained with an assay with a high sensitivity (5 geq/µg DNA).

Clearly the most surprising finding from the current study was the identification of a single blood donor

sample that contained more than 6.1×10^7 geq of HHV-6 per mL of blood. To ensure the validity of this result the assay was performed four times, with the same result obtained each time. Unusually high levels of HHV-6 DNA were first reported by Luppi and coworkers[26] in peripheral blood mononuclear cells from three patients, two with lymphoproliferative disorders and one with multiple sclerosis. The fact that two of the three patients were HHV-6–seronegative suggested that the virus infection was latent. Luppi and coworkers[26] further demonstrated that the viral genome was integrated into WBC DNA. Clark and colleagues[22] described a single healthy adult with 1.2×10^6 HHV-6 geq per μg DNA (56.4×10^6 geq/mL) in blood that persisted for at least 10 months with no evidence of active disease. These findings have been confirmed and extended by others.[27-31] Tanaka-Taya and coworkers[29] concluded that these levels of viremia translate to more than 1 virus copy per blood WBC. Ward and colleagues[31] identified six patients with a mean of 10^7 geq of HHV-6 per mL of whole blood. These six individuals, ranging in age from newborn to 58 years, presented with a variety of symptoms including neonatal convulsions, EBV-associated encephalitis, and meningitis, while one individual was a healthy adult stem cell donor. Based on demonstration of HHV-6 integration in hair follicle cells and previous reports of vertical transmission of integrated HHV-6,[28,29] Ward and colleagues[31] concluded that the virus was carried by all cells and inherited through the germline.

The current case represents to our knowledge the first report of this unusual phenomenon in a healthy adult blood donor. Because the virus appears latent and unable to provoke a humoral immune response, we believe that this phenomenon likely poses no serious risk to an immunocompetent recipient. It is most likely that in a fully immunocompetent recipient, transfused WBCs carrying latent integrated HHV-6 will be normally cleared from the recipient with no residual infected donor cells. On the other hand, the outcome in immunocompromised recipients or in those who receive stem cell transplants is less certain. In an immunodeficient patient the possibility of viral activation of latent integrated virus leading to acute virus infection cannot be absolutely excluded. Assuming that integrated virus is present in hematopoietic stem cells, it seems likely that recipients of stem cell transplants from donors that carry integrated HHV-6 will permanently carry integrated virus in their hematopoietic cells. The clinical implications of this phenomenon are not known.

HHV-7 infection, like EBV infection, is very common, with a reported seroprevalence of 96 percent.[32] In an early study, no HHV-7 DNA positivity was detected in 20 donor blood samples.[2] In a more recent study,[33] HHV-7 DNA was detected in peripheral blood mononuclear cells from 87 percent of blood donors. In the present study, HHV-7 DNA was detected in 65 percent of donor blood samples, a result similar to the previous study.[33] The earlier negative results[2] were obtained with a nonnested PCR assay coupled with gel detection of product, whereas the current results were obtained with a real time PCR assay. Because the limits of detection of the assays utilized by Hoang and colleagues[2] ranged from 222 (VZV) to 1738 (HSV-2), it is likely that the marked difference in HHV-7 DNA prevalence obtained by these studies is due to the relative insensitivity of the earlier assays.

HHV-8, the most recently discovered human herpesvirus, is also the least commonly encountered in the United States in terms of seroprevalence, with a range of less than 1 to 24 percent depending on geographic region and serologic technique.[1] In terms of HHV-8 DNA positivity of healthy adult blood donors, there is relatively little information. In two independent studies, Hudnall and colleagues[34] and Hoang and colleagues[2] identified no HHV-8 DNA positivity from an aggregate total of 40 donor whole-blood samples, and Broccolo and coworkers[35] identified no HHV-8 DNA positivity from 36 donor plasma samples. The current study extends and corroborates these negative findings by identifying no HHV-8 DNA positivity from 100 donor blood samples with a highly sensitive assay capable of detecting a single virus copy. These results indicate that HHV-8 DNA positivity of adult donor blood in the United States is likely to be a rare phenomenon.

ACKNOWLEDGMENT

The authors thank Ms Linda Beachey (UTMB Hematopathology) for her expert secretarial assistance.

REFERENCES

1. Hudnall SD, Stanberry LR. Chapter 57. Human herpesvirus infections. In: Guerrant RL, Walker DH, Weller PF, editors. Tropical infectious diseases: principles, pathogens, and practice. 2nd ed. Philadelphia (PA): Elsevier Churchill, Livingstone; 2006. p. 590-620.
2. Hoang MP, Rogers BB, Dawson DB, Scheuermann RH. Quantitation of 8 human herpesviruses in peripheral blood of human immunodeficiency virus-infected patients and healthy blood donors by polymerase chain reaction. Am J Clin Pathol 1999;111:655-9.
3. Ehlers B, Borchers K, Grund C, Frölich K, Ludwig H, Buhk HJ. Detection of new DNA polymerase genes of known and potentially novel herpesviruses by PCR with degenerate and deoxyinosine-substituted primers. Virus Genes 1999; 18:211-20.
4. Hudnall SD, Chen T, Tyring SK. Species identification of all eight human herpesviruses with a single nested PCR assay. J Virol Methods 2004;116:19-26.
5. Zhang L, Nikkari S, Skurnik M, Ziegler T, Luukkainen R, Mottonen T, Toivanen P. Detection of herpesviruses by

polymerase chain reaction in lymphocytes from patients with rheumatoid arthritis. Arthritis Rheum 1993;36:1080-6.
6. Alvarez-Lafuente R, Fernandez-Gutierrez B, de Miguel S, Jover JA, Rollin R, Loza E, Clemente D, Lamas JR. Potential relationship between herpes viruses and rheumatoid arthritis: analysis with quantitative real time polymerase chain reaction. Ann Rheum Dis 2005;64:1357-9.
7. de Jong MD, Weel JF, Schuurman T, Wertheim-van Dillen PM, Boom R. Quantitation of varicella-zoster virus DNA in whole blood, plasma, and serum by PCR and electrochemiluminescence. J Clin Microbiol 2000;38:2568-73.
8. 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.
9. 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.
10. Nishiwaki M, Fujimuro M, Teishikata Y, Inoue H, Sasajima H, Nakaso K, Nakashima K, Sadanari H, Yamamoto T, Fujiwara Y, Ogawa N, Yokosawa H. Epidemiology of Epstein-Barr virus, cytomegalovirus, and Kaposi's sarcoma-associated herpesvirus infections in peripheral blood leukocytes revealed by a multiplex PCR assay. J Med Virol 2006;78:1635-42.
11. Greenlee DJ, Fan H, Lawless K, Harrison CR, Gulley ML. Quantitation of CMV by real-time PCR in transfusable RBC units. Transfusion 2002;42:403-8.
12. Thorne LB, Civalier C, Booker J, Fan H, Gulley ML. Analytic validation of a quantitative real-time PCR assay to measure CMV viral load in whole blood. Diagn Mol Pathol 2007;16: 73-80.
13. 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.
14. Sumaya CV, Boswell RN, Ench Y, Kisner DL, Hersh EM, Reuben JM, Mansell PW. Enhanced serological and virological findings of Epstein-Barr virus in patients with AIDS and AIDS-related complex. J Infect Dis 1986;154:864-70.
15. Kimura H, Morita M, Yabuta Y, Kuzushima K, Kato K, Kojima S, Matsuyama T, Morishima T. Quantitative analysis of Epstein-Barr virus load by using a real-time PCR assay. J Clin Microbiol 1999;37:132-6.
16. Jabs WJ, Hennig H, Kittel M, Pethig K, Smets F, Bucsky P, Kirchner H, Wagner HJ. Normalized quantification by real-time PCR of Epstein-Barr virus load in patients at risk for posttransplant lymphoproliferative disorders. J Clin Microbiol 2001;39:564-9.
17. Brengel-Pesce K, Morand P, Schmuck A, Bourgeat MJ, Buisson M, Bargues G, Bouzid M, Seigneurin JM. Routine use of real-time quantitative PCR for laboratory diagnosis of Epstein-Barr virus infections. J Med Virol 2002;66:360-9.
18. Hopwood P, Brooks L, Parratt R, Hunt BJ, Maria B, Alero TJ, Magdi Y, Crawford DH. Persistent Epstein-Barr virus infection: unrestricted latent and lytic viral gene expression in healthy immunosuppressed transplant recipients. Transplantation 2002;74:194-202.
19. Maurmann S, Fricke L, Wagner HJ, Schlenke P, Hennig H, Steinhoff J, Jabs WJ. Molecular parameters for precise diagnosis of asymptomatic Epstein-Barr virus reactivation in healthy carriers. J Clin Microbiol 2003;41:5419-28.
20. Qu L, Xu S, Rowe D, Triulzi D. Efficacy of Epstein-Barr virus removal by leukoreduction of red blood cells. Transfusion 2005;45:591-5.
21. Wilborn F, Schmidt CA, Zimmermann R, Brinkmann V, Neipel F, Siegert W. Detection of herpesvirus type 6 by polymerase chain reaction in blood donors: random tests and prospective longitudinal studies. Br J Haematol 1994; 88:187-92.
22. Clark DA, Ait-Khaled M, Wheeler AC, Kidd IM, McLaughlin JE, Johnson MA, Griffiths PD, Emery VC. Quantification of human herpesvirus 6 in immunocompetent persons and post-mortem tissues from AIDS patients by PCR. J Gen Virol 1996;77:2271-5.
23. Alvarez-Lafuente R, Martin-Estefania C, de Las Heras V, Castrillo C, Picazo JJ, Varela de Seijas E, Gonzalez RA. Active human herpesvirus 6 infection in patients with multiple sclerosis. Arch Neurol 2002;59:929-33.
24. Alvarez-Lafuente R, De Las Heras V, Bartolome M, Garcia-Montojo M, Arroyo R. Human herpesvirus 6 and multiple sclerosis: a one-year follow-up study. Brain Pathol 2006;16: 20-7.
25. Cuende JI, Ruiz J, Civeira MP, Prieto J. High prevalence of HHV-6 DNA in peripheral blood mononuclear cells of healthy individuals detected by nested-PCR. J Med Virol 1994;43:115-8.
26. Luppi M, Marasca R, Barozzi P, Ferrari S, Ceccherini-Nelli L, Batoni G, Merelli E, Torelli G. Three cases of human herpesvirus-6 latent infection: integration of viral genome in peripheral blood mononuclear cell DNA. J Med Virol 1993;40:44-52.
27. Torelli G, Barozzi P, Marasca R, Cocconcelli P, Merelli E, Ceccherini-Nelli L, Ferrari S, Luppi M. Targeted integration of human herpesvirus 6 in the p arm of chromosome 17 of human peripheral blood mononuclear cells in vivo. J Med Virol 1995;46:178-88.
28. Daibata M, Taguchi T, Nemoto Y, Taguchi H, Miyoshi I. Inheritance of chromosomally integrated human herpesvirus 6 DNA. Blood 1999;94:1545-9.
29. Tanaka-Taya K, Sashihara J, Kurahashi H, Amo K, Miyagawa H, Kondo K, Okada S, Yamanishi K. Human herpesvirus 6 (HHV-6) is transmitted from parent to child in an integrated form and characterization of cases with chromosomally integrated HHV-6 DNA. J Med Virol 2004;73: 465-73.
30. Leong HN, Tuke PW, Tedder RS, Khanom AB, Eglin RP, Atkinson CE, Ward KN, Griffiths PD, Clark DA. The preva-

lence of chromosomally integrated human herpesvirus 6 genomes in the blood of UK blood donors. J Med Virol 2007;79:45-51.
31. Ward KN, Leong HN, Nacheva EP, Howard J, Atkinson CE, Davies NW, Griffiths PD, Clark DA. Human herpesvirus 6 chromosomal integration in immunocompetent patients results in high levels of viral DNA in blood, sera, and hair follicles. J Clin Microbiol 2006;44:1571-4.
32. Clark DA, Freeland ML, Mackie LK, Jarrett RF, Onions DE. Prevalence of antibody to human herpesvirus 7 by age. J Infect Dis 1993;168:251-2.
33. Fernandez C, Boutolleau D, Manichanh C, Mangeney N, Agut H, Gautheret-Dejean A. Quantitation of HHV-7 genome by real-time polymerase chain reaction assay using MGB probe technology. J Virol Methods 2002;106: 11-6.
34. Hudnall SD, Chen T, Rady P, Tyring S, Allison P. Human herpesvirus 8 seroprevalence and viral load in healthy adult blood donors. Transfusion 2003;43:85-90.
35. Broccolo F, Locatelli G, Sarmati L, Piergiovanni S, Veglia F, Andreoni M, Buttò S, Ensoli B, Lusso P, Malnati MS. Calibrated real-time PCR assay for quantitation of human herpesvirus 8 DNA in biological fluids. J Clin Microbiol 2002; 40:4652-8.

SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article:

Appendix S1. Real-time PCR reagents (Word document).

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.0041-1132.2008.01685.x
(This link will take you to the article abstract.)

Please note: Blackwell Publishing are not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

番号：2

別紙様式第２－１

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日 平成20年10月20日	新医薬品等の区分 該当なし	機構処理欄
一般的名称	ﾃｸﾈﾁｳﾑ人血清ｱﾙﾌﾞﾐﾝ(99mTc)		研究報告の公表状況	WHO ／ EPR ／ Disease Outbreak News,13 October 2008	公表国 ザンビア、南アフリカ	
販売名（企業名）	テクネアルブミンキット（富士フイルムＲＩファーマ株式会社）					
研究報告の概要	要約：南アフリカ共和国、ザンビア共和国におけるアレナウイルス科の新型ウイルス： 南アフリカ共和国とザンビア共和国で原因不明の疾患で死亡した３名はアレナウイルス科のウイルスによるものであることが検査の結果判明した。					使用上の注意記載状況・その他参考事項等 特になし
報告企業の意見			今後の対応			
アレナウイルス科の新ウイルスにより、３名が死亡したとの報告であり、また、初発症例はおそらくげっ歯類からの感染であるが、その後の症例は、ヒトーヒト感染による感染であること、及び新規かつ重大な感染症の研究報告と判断しております。また詳細は不明であるが、ヒト血液を原料とする血漿分画製剤とは直接関係するものではなく、現時点では、とくに措置等は必要ないと判断する。			現在、アレナウイルスの型を含めて、さらに詳細な調査が進められているところであり、今後とも追加・関連情報に注目し、情報入手次第、必要に応じて、再評価を行う予定とする。			

MedDRA/J Version(11.1)

DRL 2008-002

World Health Organization

New virus from Arenaviridae family in South Africa and Zambia - Update

13 October 2008 -- The results of tests conducted at the Special Pathogens Unit, National Institute for Communicable Diseases (NICD) of the National Health Laboratory Service in Johannesburg, and at the Special Pathogens and Infectious Disease Pathology branches of the Centers for Disease Control in Atlanta, USA, provide preliminary evidence that the causative agent of the disease which has resulted in the recent deaths of 3 people from Zambia and South Africa, is a virus from the Arenaviridae family.

Analysis continues at the NICD and CDC in order to characterize this virus more fully. CDC and NICD are technical partners in the Global Outbreak Alert and Response Network (GOARN).

Meanwhile, a new case has been confirmed by PCR in South Africa. A nurse who had close contact with an earlier case has become ill, and has been admitted to hospital. Contacts have been identified and are being followed-up.

WHO and its GOARN partners continue to support the Ministries of Health of the two countries in various facets of the outbreak investigation, including laboratory diagnosis, investigations, active case finding and follow-up of contacts.

Contacts | E-mail scams | Employment | FAQs | Feedback | Privacy | RSS feeds
© WHO 2008

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9月1日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	別紙のとおり	研究報告の公表状況	ProMED-mail, 20080828.2697	公表国 インド	
販売名（企業名）	別紙のとおり				

研究報告の概要

問題点：インド東部において、原因不明のウイルス感染を原因とする急性脳炎症候群により、この数週間におよそ800人の患者が発生し150人が死亡している。

インド東部のウッタルプラデシ（Uttar Pradesh）州で小児を死亡させている原因不明のウイルスは、インド保健省の専門家らにより「acute encephalitis syndrome；急性脳炎症候群」（PT；感染性脳炎）と診断された。ウッタルプラデシ州の13の地区では、数週間におよそ800人の患者が発生し150人が死亡したと報告され、その数は増加すると見られている。
血液検査で、毎年この地方で大きな流行が発生する日本脳炎が陽性となったのは5%以下である。
通常、感染流行中は、15～20%以上の検体が日本脳炎陽性となる。しかし、2008年は低率であることが混乱を生じている。
2008年、ウッタルプラデシ州27地区の4000万人がJEワクチン接種を受けたが、なぜ発熱が抑えられなかったのかに専門家らはとまどっている。中国製ワクチンSA 14-14-2には問題はなく、WHOにより日本脳炎に対する防御作用が確認されている、と保健省当局者は述べた。
日本脳炎陽性が5%以下であることから、日本脳炎と、2006年以来脳炎の原因となっている水系感染のエンテロウイルスとの混合感染について調査が行われている。

使用上の注意記載状況・その他参考事項等

記載なし

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

MedDRA/J ver.11.0

別紙

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤乾燥ペプシン処理人免疫グロブリン、⑥乾燥スルホ化人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン*、⑧乾燥濃縮人活性化プロテインC、⑨乾燥濃縮人血液凝固第Ⅷ因子、⑩乾燥濃縮人血液凝固第Ⅸ因子、⑪乾燥抗破傷風人免疫グロブリン、⑫抗 HBs 人免疫グロブリン、⑬トロンビン、⑭フィブリノゲン加第ⅩⅢ因子、⑮乾燥濃縮人アンチトロンビンⅢ、⑯ヒスタミン加人免疫グロブリン製剤、⑰人血清アルブミン*、⑱人血清アルブミン*、⑲乾燥ペプシン処理人免役グロブリン*、⑳乾燥人血液凝固第Ⅸ因子複合体*、㉑乾燥濃縮人アンチトロンビンⅢ
販売名(企業名)	①献血アルブミン 20 “化血研”、②献血アルブミン 25 “化血研”、③人血清アルブミン “化血研” *、④ “化血研” ガンマーグロブリン、⑤献血静注グロブリン “化血研”、⑥献血ベニロンーⅠ、⑦ベニロン*、⑧注射用アナクトC2,500 単位、⑨コンファクトF、⑩ノバクトM、⑪テタノセーラ、⑫ヘパトセーラ、⑬トロンビン “化血研”、⑭ボルヒール、⑮アンスロビンP、⑯ヒスタグロビン、⑰アルブミン 20%化血研*、⑱アルブミン 5%化血研*、⑲静注グロブリン*、⑳ノバクトF*、㉑アンスロビンP1500 注射用
報告企業の意見	本感染症については、情報入手時点で病原因子は特定されていない。病原因子が細菌類であれば本剤の製造工程中の「無菌ろ過工程」および、細菌よりも小さいウイルスの除去を目的とした平均孔径 19nm 以下の「ウイルス除去膜ろ過工程」により除去されるものと考えられる。また、病原因子がウイルスであれば、「血漿分画製剤のウイルスに対する安全性確保に関するガイドライン（医薬発第 1047 号、平成 11 年 8 月 30 日）」に従ったウイルスプロセスバリデーションの結果から、病原因子は本剤の製造工程において除去・不活化されることが検証されている。以上のように、病原因子が細菌類あるいは既知のウイルスであれば、今回の感染症に対して本剤は一定の安全性を確保していると考える。また、未知のウイルスであっても、既存のウイルス除去・不活化工程の効果が期待される。 現時点で、感染症の流行はインド国内のみで当該生物由来成分の原産国とは離れているため、本剤への直接の影響はなく、緊急の安全対策の必要性もないと考えられるが、感染症は短期間に爆発的に増加することがあるため、今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。

*現在製造を行っていない

Archive Number 20080828.2697
Published Date 28-AUG-2008
Subject PRO/EDR> Undiagnosed fatal illness - India (04): (UP)

UNDIAGNOSED FATAL ILLNESS - INDIA (04): (UTTAR PRADESH)
****
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]
Date: Tue 26 Aug 2008
Source: The Hindustan Times, online [edited]
<http://www.hindustantimes.com/StoryPage/StoryPage.aspx?sectionName=&id=3825990

The mystery virus striking children dead in eastern Uttar Pradesh (UP) has been diagnosed as "acute encephalitis syndrome" by Union Health Ministry experts. Simply put, they do not know what is causing the acute brain fever.

Within weeks, about 800 cases and 150 deaths were reported from 13 districts in UP, and experts predict that the numbers could rise.

"Less than 5 per cent blood and serum samples have tested positive for Japanese encephalitis (JE), which has seen major outbreaks in the region each year," said Dr Shiv Lal, director of the National Institute of Communicable Diseases.

"Usually, at least 15-20 per cent samples test positive for JE during an outbreak, but the low positivity is causing confusion this year [2008]. With 4 crore [40 million] children in 27 districts in UP being vaccinated against JE this year, experts wonder why the fever refuses to go away. There is no problem with the Chinese vaccine SA 14-14-2," said a health ministry official. The virus, approved by World Health Organization, protects against JE. "We suspect some children could have missed the vaccination drive." All the hospitalised children have reported symptoms of acute encephalitis.

"Since less than 5 per cent have tested positive for JE, we are investigating whether the outbreak is a combination of JE and water-borne enterovirus that caused the disease in 2006," said Dr Lal. The Centre is sending a 4-member team comprising a microbiologist, a pediatrician, an entomologist, and an epidemiologist to Lucknow and Gorakhpur to track the outbreak and collect blood and serum samples from hospitalisd children for viral culture.

"Apart from rapid tests for JE done using kits developed by Pune's National Institute of Virology, we will do virus culture to track the elusive cause of the current outbreak," said Dr Lal, adding that the result could be expected within 2 or 3 days of collection of the samples.

[byline: Sanchita Sharma]

--
communicated by:
ProMED-mail rapporteur Mary Marshall

******
[2]
Date: Wed 27 Aug 2008
From: T Jacob John <vlr_tjjohn@sancharnet.in>

Although the details are skimpy, age distribution and clinical description lacking, yet the available information can be used to propose a provisional diagnosis to be investigated. Heavy rainfall and flooding, febrile illness resembling malaria, and relatively large numbers of death does remind one of leptospirosis. Immediate serological testing for this disease is warranted.

Similar episodes in Orissa and Mumbai a few years ago (all the 3 features above fitted) turned out to be leptospirosis. In Orissa it was for the first time (at least recognized), while in Mumbai the presence of leptospirosis was already known. To add, there is no shortcut to detailed clinical description and elementary epidemiological investigation of cases based on specific diagnostic criteria of the outbreak disease, and exploration of risk factors (to look for transmission pathways). Instead of doing what one can do locally, the complete dependence on experts from elsewhere is not good.

--
Dr T Jacob John
Christian Medical College
Vellore
India
<vlr_tjjohn@sancharnet.in>

[Japanese encephalitis virus infection is an unlikely explanation, but still under investigation. - Mod.CP

ProMED-mail thanks Dr John for his comments and looks forward to more information about this outbreak. - Mod.LL]

[see also:
Undiagnosed fatal illness - India (03): (UP) RFI 20080826.2666
Undiagnosed fatal illness - India (02): (UP) RFI 20080811.2478
Undiagnosed fatal illness - India (Uttar Pradesh): RFI 20080331.1194
2007

Japanese encephalitis - India (02) (Uttar Pradesh) 20071026.3486
Undiagnosed viral disease - India (02): (Uttar Pradesh) 20071026.3485
Undiagnosed viral disease - India: (Uttar Pradesh) 20071022.3440
Japanese encephalitis - India (Uttar Pradesh) 20070930.3233
2006

Japanese encephalitis - India (Uttar Pradesh) (03): vaccine safety 20061222.3583
Leptospirosis - India (Gujarat): not hantavirus 20060831.2476
Leptospirosis - India (Maharashtra) 20060726.2058
Leptospirosis - India (Kerala) 20060609.1612
Leptospirosis - India (Karnataka) 20060123.0226
2005

Undiagnosed deaths - India (Uttar Pradesh) (02) 20051115.3342
Undiagnosed deaths - India (Uttar Pradesh): RFI 20051113.3322
Leptospirosis - India (Maharashtra) 20050811.2348
2004

Leptospirosis - India (South Gujarat) (02) 20040908.2509
Leptospirosis - India (South Gujarat) 20040902.2441
Leptospirosis - India (Tamil Nadu) 20040226.0602]

.....cp/ll/msp/sh

##
****
ProMED-mail makes every effort to verify the reports that are posted, but the accuracy and completeness of the information, and of any statements or opinions based thereon, are not guaranteed. The reader assumes all risks in using information posted or archived by ProMED-mail. ISID and its associated service providers shall not be held responsible for errors or omissions or held liable for any damages incurred as a result of use or reliance upon posted or archived material.
****

Become a ProMED-mail Premium Subscriber at <http://www.isid.org/ProMEDMail_Premium.shtml>
****
Visit ProMED-mail's web site at <http://www.promedmail.org>. Send all items for posting to: promed@promedmail.org (NOT to an individual moderator). If you do not give your full name and affiliation, it may not be posted. Send commands to subscribe/unsubscribe, get archives, help, etc. to: majordomo@promedmail.org. For assistance from a human being send mail to: owner-promed@promedmail.org.
##########
##########

about ISID | membership | programs | publications | resources
13th ICID | site map | ISID home

息2001,2008 International Society for Infectious Diseases
All Rights Reserved.
Read our privacy guidelines.
Use of this web site and related services is governed by the Terms of Service.

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
				2008. 7. 11	該当なし	

一般的名称	(製造販売承認書に記載なし)	研究報告の公表状況		公表国
販売名(企業名)	合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)		Komar N, Clark GG. Rev Panam Salud Publica. 2006 Feb;19(2):112-7.	米国

研究報告の概要

○ラテンアメリカおよびカリブ諸国のウエストナイルウイルスの活動性

目的:ウエストナイルウイルス(WNV)は、2001年に初めてカリブ海地域で検出されて以来、当地で急速に広がった。アメリカ大陸熱帯地域におけるWNV伝播の最近の知見について要約する。

方法:発表された文献のレビューを行い、主要な公衆衛生担当者に意見を求め、未発表データを入手した。

結果:WNV感染症は、ヒトでは2001年に初めてケイマン諸島およびフロリダキーの住民に発症し、2002年早期にジャマイカの健常な鳥類検体に初めて認められた。2002年のWNV感染症の血清学所見は、グアドループ、ドミニカ共和国と東部メキシコでウマ、ニワトリおよび野生鳥類に検出された。2003年には、WNVはメキシコおよび中央アメリカ北部で蔓延し、血清学的エビデンスはバハマ、プエルトリコとキューバで検出された。2004年9月～10月には、コロンビアとトリニダードで南米生態系におけるWNV活動の最初の血清学的エビデンスが表面化し、当地では家畜のWNV中和抗体保有率が高かった。

結論:ラテンアメリカおよびカリブ海地域において、ウマ、ヒトおよびトリでの疾患報告が少ないことは不可解である。熱帯生態系での疾患の低減について、ウイルスの減弱化あるいは他の可能性を検討するため、分離株が必要である。

報告企業の意見	今後の対応
ラテンアメリカおよびカリブ海地域の動物や鳥類において、ウエストナイルウイルスの抗体陽性率は高くなっているが疾患報告は少ない。この不可解な熱帯生態系での疾患の低減について、原因を検討するため分離株が必要で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、ウエストナイルウイルス感染の発生に備え、緊急対応の準備を進めている。今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

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

131

18



Informe especial / Special report

West Nile virus activity in Latin America and the Caribbean

Nicholas Komar[1] *and Gary G. Clark*[2]

Suggested citation Komar N, Clark GG. West Nile Virus activity in Latin America and the Caribbean. Rev Panam Salud Publica. 2006;19(2):112–7.

ABSTRACT **Objectives.** *West Nile virus* (Flavivirus: Flaviviridae; *WNV) has spread rapidly throughout the Caribbean Basin since its initial detection there in 2001. This report summarizes our current knowledge of WNV transmission in tropical America.*
Methods. *We reviewed the published literature and consulted with key public health officials to obtain unpublished data.*
Results. *West Nile virus infections first appeared in human residents of the Cayman Islands and the Florida Keys in 2001, and in apparently healthy Jamaican birds sampled early in 2002. Serologic evidence of WNV infection in 2002 was detected in horses, chickens and resident free-ranging birds in Guadeloupe, the Dominican Republic, and eastern Mexico. In 2003, WNV spread in Mexico and northern Central America, and serologic evidence was detected in the Bahamas, Puerto Rico and Cuba. In 2004, the first serologic evidence of WNV activity in South American ecosystems surfaced in September-October in Colombia and Trinidad, where domestic animals circulated WNV-neutralizing antibodies.*
Conclusions. *The sparse reports of equine, human and avian disease in Latin America and the Caribbean is puzzling. Isolates are needed to evaluate viral attenuation or other possible explanations for reduced disease burden in tropical ecosystems.*

Key words West Nile virus; Latin America; Caribbean region; arboviruses; population surveillance; flavivirus.

INTRODUCTION

Since West Nile virus (*Flavivirus: Flaviviridae;* WNV) first appeared in the Western Hemisphere in New York in 1999, it has spread rapidly across the North American continent, causing large numbers of human cases with neurologic disease and death, and even greater amounts of milder disease characterized principally by fever and rash. Horses and hundreds of species of birds also fell victim to this emerging virus (1). West Nile virus spread southward into the Caribbean Basin and Latin America as well, where its public health impact remains poorly understood and surveillance systems are unprepared to track its spread. The virus was first detected in 2001, in Jamaica and the Cayman Islands. In 2005 WNV activity was reported from many locations in the Caribbean Basin, Mexico, Central America and the northern rim of South America (Figure 1). In order to package our current knowledge of WNV activity and surveillance results from various locations within tropical America, we reviewed published reports and some unpublished data available from public health officials, and provide a summary below. We also comment on the significance of the surveillance findings and on the potential public health threat of WNV in tropical America.

[1] Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases, PO Box 2087 Fort Collins, Colorado 80522, United States of America. Send correspondence and reprint requests to Nicholas Komar, ScD, Arbovirus Diseases Branch, Centers for Disease Control and Prevention, PO Box 2087, Fort Collins CO 80522, United States of America; telephone: (970) 2216496; fax: (970) 2216476; e-mail: nkomar@cdc.gov
[2] Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases, 1324 Calle Cañada, San Juan 00920, Puerto Rico.

METHODS

We reviewed peer-reviewed publications and government reports and consulted with key public health officials within Caribbean Basin countries to obtain unpublished data.

RESULTS

West Nile virus detected in 2001

In the State of Florida (United States of America), Blackmore et al. described surveillance findings for WNV in two epidemic foci in 2001—a northern focus and a southern focus (2). The northern focus was characterized by humid temperate forests typical of the southeastern United States but unlike tropical ecosystems in Latin America. The first evidence for WNV activity here was a dead American Crow (*Corvus brachyrhynchos*) in June, 2001. Nine human cases of West Nile neurologic disease (WNND) were reported between July and October. Entomologic investigations near case residences in July detected WNV in three species of *Culex* (*Culex*) mosquitoes: *Culex quinquefasciatus*, *C. nigripalpus* and *C. salinarius* (3, 4). The first two of these species are common further south in the Caribbean Basin.

The southern epidemic focus in Florida was more typical of Caribbean Island ecology and occurred in the Florida Keys. A human case of WNND with onset in July, 2001, represented the earliest indication of WNV activity there. Two more human cases were reported with onsets in August and September. West Nile virus was isolated from dead corvids (e.g., Fish Crow, *Corvus ossifragus*) and *Streptopelia* doves (probably *Streptopelia decaocto*, Eurasian Collared-Dove, an introduced species that is also abundant in the Bahamas). Entomologic investigations were carried out throughout the Keys during the last quarter of 2001 (5). Infection rates were highest in *Anopheles atropos* (3 of 410), *Deinocerites cancer* (2 of 845) and *Ochlerotatus taeniorhynchus* (2 of 9288). This last species is a ferocious human biter, and abundant in coastal locations throughout the Caribbean Basin. About 20 000 other mosquitoes tested negative.

Follow-up mosquito surveillance studies in the Florida Keys in the following two years yielded no WNV in more than 30 000 mosquitoes tested in 2002, but the virus was detected in 10 pools representing 53 673 mosquitoes in 2003 (6). In 2003, infections were detected from May–September. Infected species included *C. quinquefasciatus* (minimum infection rate 1.7 per 1 000), *C. nigripalpus* (0.9), *O. taeniorhynchus* (0.9), *O. condolescens* (0.6) and *C. erraticus* or *declarator* (0.6). No infections were detected in either *A. atropos* or *D. cancer* even though more than 5 000 of each species were tested. These findings suggest that either WNV became endemic in the Florida Keys but dropped below levels of detection in 2002, or that multiple, temporally dispersed introductions occurred, resulting in transmission activity in both 2001 and 2003.

Although the circumstances of WNV introduction into the Florida Keys are unknown, the likely explanation is that migrating birds served as dispersal hosts, seeding the virus into potential transmission foci during their southward migration in the fall of 2000. By late 2000, WNV activity was reported as far south as North Carolina in the continental United States (7). The virus had probably spread even further south at undetectable levels, to be amplified by resident birds and *Culex* mosquitoes during the warmer spring and early summer months of 2001. While migrating birds are a convenient explanation of WNV dispersal, other possible means of dispersion exist, such as infected mosquitoes that are accidentally transported via surface transportation or airplanes.

South of the Florida Keys, a human WNND case with no history of international travel was reported with onset on August 2, 2001, from tiny Cayman Brac (area 14 square miles [36 square kilometers], population 1 200), in the Cayman Islands, south of Cuba (8). Assuming an incubation period of 2–15 days in people, this infection probably occurred in late July, about the same time that the first human case was infected in the Florida Keys. However, the laboratory diagnosis of this case was not announced until October 15, 2001. Laboratory tests were positive for anti-WNV IgM (indicating recent infection) and a 90% plaque-reduction neutralizing antibody titer ($PRNT_{90}$) of 1:1280, compared with a $PRNT_{90}$ of 1:80 and <1:10 for St. Louis encephalitis virus (SLEV) and Dengue-2 virus, respectively (CDC, unpublished data).

More data supporting WNV transmission activity in the Caribbean Basin in 2001 came from Jamaica, where a Smithsonian Institution-New York State Health Department research team reported 17 seropositive resident birds of 348 collected in 3 of 4 study sites, all on the western side of the island (9). The samples were collected in the first three months of 2002 but probably reflected transmission that had occurred months earlier in 2001. Seropositive bird species included *Turdus aurantius* (n = 4), *Myiopagis cotta* (2), *Coereba flaveola* (2), *Tiaris bicolor* (2), and one each of seven other species. Seropositivity was determined by comparing $PRNT_{90}$ titers for WNV, SLEV and Ilheus virus, a South American flavivirus that is genetically closely related to SLEV, but not in the same antigenic complex as SLEV and WNV (10). All 17 WNV-positive samples were at least four-fold greater in WNV titer than other flavivirus titers. Three samples were positive for SLEV-neutralizing antibodies, which has been previously isolated in Jamaica (11). No samples were positive for Ilheus virus, but five additional samples had similar titers for both SLEV and WNV, and these were classified as undetermined flavivirus infections. The 2001 WNV activity in Jamaica and the Cayman Islands was most likely the result of the same introduction mechanism as postulated for extreme southern Florida: southward dispersal of the virus below limits of detection via migrating birds late in 2000.

Operating under the premise that birds would carry WNV along migration routes, efforts were initiated to detect WNV activity on the southern side

of the Gulf of Mexico, where millions of neotropical migratory birds make landfall each year and spend the winter months. Beginning in 2000, a joint effort by the Universidad Autónoma de Yucatán and Colorado State University blood-sampled and tested migratory and resident birds in Yucatán State, Mexico. The following year, the Smithsonian Institution also began sampling birds on the Yucatán Peninsula. Further south in the Lacandón Forest of Chiapas State, a joint federal Mexico-United States study evaluated blood from about 200 resident domestic animals sampled in July of 2001. From these, a single seropositive cow (*Bos sp.*) with a $PRNT_{90}$ for WNV of 1:80 and a $PRNT_{90}$ for SLEV of 1:20, was considered a probable case of WNV infection (12). However, these authors cautioned against concluding that WNV had reached southern Mexico. They reasoned that a major range extension should be confirmed by a second detection of infection. Also, no evidence of WNV transmission had been detected at that time in the nearby Yucatán Peninsula (9, 13). The Chiapas study demonstrated serologic evidence for infections due to uncharacterized flaviviruses which could have resulted in cross-reaction with WNV. Secondary flavivirus infections are notorious for causing elevated heterologous flavivirus titers (14).

FIGURE 1. Countries of Latin America and the Caribbean with reported activity for West Nile virus (in black) between 2001 and 2004, including Mexico, Belize, Guatemala, El Salvador, Cuba, Bahamas, Cayman Islands, Jamaica, Dominican Republic, Puerto Rico (United States), Guadeloupe (French West Indies), Trinidad and Tobago, and Colombia



Spread of West Nile virus 2002–2004

In 2002, WNV continued to spread in the Caribbean Basin. Guadeloupe (French West Indies) reported numerous subclinical infections in horses and chickens, determined serologically by neutralization (15). In July 2002, 10.4% of the healthy horses in four locations were positive and by January 2003, 61.6% had become positive in these locations. The absence of reported neurologic disease in these horses is mysterious. Subsequent surveillance in 2003 and 2004 failed to detect any transmission (16).

In the Dominican Republic on the Greater Antillean island of Hispaniola, a University of Kansas study team sampled blood and tissues from resident birds captured in November, 2002, for museum collections (17). Five birds of 33 (15.2%) from the Parque Nacional Los Haitises on the northeast coast tested positive for WNV antibodies by neutralization and a specific inhibition-ELISA test. A follow-up study in March, 2003, yielded 12 more WNV-seropositive birds of 58 (20.7%) at the Parque Nacional Monte Cristi in northwest Dominican Republic, along the border with Haiti (18). Positive Dominican bird species included *Phaenicophilus palmarum* ($n = 4$), two each of *Ploceus cucullatus*, *Saurothera longirostris*, *Loxigilla violacea* and *Turdus plumbeus*, and one each of five other species.

Evidence of WNV infection was confirmed in Mexico as of July, 2002. Seropositive horses were reported from six states (Chihuahua, Coahuila, Tamaulipas, Veracruz, Tabasco and Yucatán) (19–21). Seropositive birds were rare and were first detected in the early winter months of 2003 (13, 22).

Mexican authorities began widespread serosurveys in horses and birds in 2003 and found many seropositive horses in 22 states (J. Mendez, personal communication, 4 Feb 2004), with no human cases in 2003 and six human cases (three with encephalitis) in northern Mexico in 2004 (23). The first Mexican isolate came from a dead captive common raven (*Corvus corax*) in Tabasco State (southeast Mexico) in May, 2003 (21). Additional isolates from dead birds were obtained in northwest Mexico later in 2003 and 2004. Phylogenetic analysis of the prM-E region of the WNV genome isolated from the raven in Tabasco linked it to central United States strains from 2002, but revealed slightly greater genetic variation than previous

reports for North American WNV strains (21). Two of the 9 nucleotide mutations resulted in amino acid changes, and one of these altered a glycosylation site within the envelope (E) protein. Virulence testing of plaque-purified subcultures of this isolate revealed variants with reduced virulence in mice (24). Similar observations had been made with a Texas 2002 isolate (25).

The widespread WNV seropositivity among horses observed in Mexico in 2003 was also present in the Central American republics of El Salvador and Guatemala (26, M.E. Morales-Betoulle et al., manuscript in preparation). However, anecdotal reports of fatal or life-threatening neurologic disease in Mexican and Central American horses have rarely been confirmed as due to WNV. One encephalitic horse diagnosed with WNV infection was reported from Belize, with onset October 31, 2003. Interestingly, 2 000 birds sampled in Belize earlier in 2003 and another 2 000 in 2002 all tested negative for WNV antibodies (27).

West Nile virus activity continued in the eastern Caribbean region in 2003. In the Bahamas, a human case of WNND was diagnosed with onset in July, 2003 (28). In early 2004, two seropositive *Turdus plumbeus* (of 734 birds sampled) were detected in Guantanamo Bay Naval Base at the eastern point of Cuba, and in eastern Puerto Rico, one *Coereba flaveola* (of 1 200 birds sampled) was seropositive, probably reflecting transmission in 2003 (29). Three neutralizing antibody- and IgM-positive, healthy horses were also reported in eastern Puerto Rico in May, 2004, and two others were found in central Puerto Rico in July, 2004 (A. Diaz et al., manuscript in preparation). Mosquitoes collected from the locations where seropositive horses resided tested negative for WNV infection. Four seropositive horses from the Havana region and three human WNND cases in central Cuba were announced in January, 2005 (G. Kouri, personal communication, 2 February 2005), reflecting transmission in 2004.

In the fall of 2004, 8 resident unvaccinated horses (of 200 sampled) and 2 domestic Muscovy ducks (of 40 resident birds sampled) were seropositive for WNV in Trinidad (28; R. Salas, personal communication, 17 November 2005), and 12 seropositive equines (of 130 sampled) were reported in northern Colombia (30). These reports mark the first evidence of WNV activity in South American ecosystems (the island of Trinidad is located within sight of the South American mainland off the coast of Venezuela). Efforts to detect WNV-specific antibodies in resident and migrant birds in Brazil in 2002 and 2003 were unsuccessful (31). With the incursion of WNV into northern South America in 2004, it becomes the only zoonotic flavivirus to have been identified in six continents.

DISCUSSION

The failure of efforts to isolate the virus or detect genomic RNA from WNV in Latin America and the Caribbean (with a few exceptions in Mexico) is perplexing and underscores the concern that serologic evidence for WNV activity is at best indirect. Flaviviruses are notorious for their close antigenic relationships and serologic cross-reactivity (10). In spite of strong serologic evidence from cross-neutralization testing against known flaviviruses from the region, the possibility of misdiagnosis due to cross-reaction with an as yet unrecognized "WN-like" virus still exists. In fact, some of the serologic results classified as due to "undifferentiated flavivirus infection" can best be explained by the existence of such a virus. The recent discovery of two strains of WN-like virus in central Europe lends credence to this concern (32). These two WN-like viruses were both identified serologically as WNV, but genetically they are equidistant from both currently recognized WNV lineages and each other and may represent newly discovered WNV lineages or new WN-like flaviviruses.

Another concern is the strong emphasis placed by several research groups on serologic surveillance of migratory birds (9, 13, 22, 29, 31). These studies consume large quantities of valuable resources, yet are unlikely to provide significant results. Given the recent intense transmission of WNV during the summers in temperate North America, the capture of WN-seropositive avian survivors either during migration or on the wintering grounds is to be expected because many of these birds normally migrate to neotropical winter territories where they probably continue to circulate antibodies derived from a WNV infection acquired on their North American breeding grounds. Some studies claim that seropositive migrants are evidence that birds could carry WNV long distances. Unfortunately, although plausible, this conclusion is not valid for two reasons. First, the possibility that WN-seropositive migratory birds were in fact infected locally cannot be disproved. Second, long-distance migration by a healthy, antibody-circulating bird does not indicate that a viremic bird could make the same long-distance flight. More data are needed to support such a hypothesis. However, the observation of infectious WNV at high titers in tissues of convalescent migratory birds (e.g., Killdeer, *Charadrius vociferus*) more than one week post-infection and the demonstration of oral infection in raptors would suggest that recently infected birds that recover from viremia, migrate, and then fall prey to a raptor may still introduce WNV into new distant ecosystems if the raptor becomes infected and circulates sufficient virus in its blood to infect mosquitoes (33).

The most pressing concern regarding the reports of WNV in Latin America and the Caribbean is the absence of data on the disease burden in people, horses or birds. Widespread resistance to virulent strains of WNV in Latin American and Caribbean vertebrates (including people) seems highly unlikely. However, the selection of resistant WNV strains is plausible. If migrating birds are indeed the major mechanism for southward dispersal of WNV, then one could imagine a scenario in which birds infected with highly virulent strains become too sick to migrate, while birds infected with avirulent strains make the long flights

across seas and deserts successfully, spreading avirulent WNV to new transmission foci along their migratory routes. More research is needed to evaluate this hypothesis, but if proven, this bodes well for the future of WNV epidemics in North America, as the avirulent strain might be reintroduced continually from the south by returning migratory birds. South American arboviruses have in fact been isolated from northward-bound birds during the spring migration in Louisiana (34). This scenario may also explain the apparent low virulence for SLEV in birds and horses in North and South America. In fact, South American strains of SLEV are also less viremogenic in birds than are North American strains, and less virulent in mice (35). Whether an avirulent bird and horse strain of WNV will also be less virulent for humans remains to be seen.

Saint Louis encephalitis virus may be responsible for considerable cross-reaction to WNV in serologic tests of serum from Latin America. The virus is expected to cross-react in about 5% of primary WNV infections of birds (36). However, in secondary infections, the proportion of samples that cross-react by PRNT is probably much greater. Secondary flavivirus infections may explain the high rate of flavivirus antibody-positive serum samples in the Caribbean Basin countries that cannot be assigned to a specific infection (because of the presence of similar titers for multiple flaviviruses). Although rarely associated with disease in Latin America, SLEV infections are commonly reported. For example, in Chiapas, Mexico, 20 (10%) of 196 domestic animals (including three of five horses) were diagnosed as positive for SLEV-neutralizing antibodies by PRNT (12). The known range of distribution for SLEV was expanded through the efforts to detect WNV in the Caribbean Basin. For example, two SLE-seropositive birds reported in Puerto Rico provide the first evidence of SLEV activity from that Caribbean location (9, 29).

CONCLUSION AND RECOMMENDATIONS

Although WNV has yet to present a serious disease threat in Latin America and the Caribbean Basin, an outbreak may be pending. The first major outbreak (with >100 human cases of WNND) in the United States was delayed until 2002, three years after initial detection of the virus in 1999. Public health and veterinary authorities in Latin America and the Caribbean should remain vigilant for unusual clusters of severe disease cases. Dead birds (especially corvids) have been particularly useful for the early detection of WNV activity in North America (37). Corvids are less abundant in Latin America, and thus avian mortality may be less useful as a surveillance technique in this region (38). In countries where WNV has already been detected, surveillance efforts should be expanded. Surveillance guidelines for Latin American and Caribbean Basin countries are available (28, 39, 40).

Acknowledgments. We thank the numerous public and veterinary health workers throughout Latin America and the Caribbean and their colleagues in North America and Europe who have worked hard to investigate the status of West Nile virus in tropical America. In particular, we thank Gustavo Kouri, Jorge Mendez, Rosa Alba Salas, María Eugenia Morales-Betoulle and Annette Díaz for sharing unpublished data.

REFERENCES

1. Komar N. West Nile virus: epidemiology and ecology in North America. Adv Vir Res. 2003; 61:185–234.
2. Blackmore CG, Stark LM, Jeter WC, Oliveri RL, Brooks RG, Conti LA, et al. Surveillance results from the first West Nile virus transmission season in Florida, 2001. Am J Trop Med Hyg. 2003;69(2):141–50.
3. Rutledge CR, Day JF, Lord CC, Stark LM, Tabachnick WJ. West Nile virus infection rates in *Culex nigripalpus* (Diptera: Culicidae) do not reflect transmission rates in Florida. J Med Entomol. 2003;40(3):253–8.
4. Godsey MS Jr, Blackmore M, Panella NA, Burkhalter K, Gottfried K, Halsey L, et al. West Nile virus epizootiology in the southeastern United States, 2001. Vector-Borne Zoonotic Dis. 2005;5(1):82–9.
5. Hribar LJ, Vlach JJ, Demay DJ, Stark LM, Stoner RL, Godsey MS, et al. Mosquitoes infected with West Nile virus in the Florida Keys, Monroe County, Florida, USA. J Med Entomol. 2003;40(3):361–3.
6. Hribar LJ, Stark LM, Stoner RL, Demay DJ, Nordholt AL, Hemmen MJ, et al. Isolation of West Nile virus from mosquitoes (Diptera: Culicidae) in the Florida Keys, Monroe County, Florida. Carib J Sci. 2004;40(3):362–7.
7. Marfin AA, Petersen LR, Eidson M, Miller J, Hadler J, Farello C, et al. Widespread West Nile virus activity, eastern United States, 2000. Emerg Infect Dis. 2001;7(4):730–5.
8. Centers for Disease Control and Prevention. West Nile virus activity—United States, 2001. MMWR Morb Mortal Weekly Rep. 2002; 51(23):497–501.
9. Duptuis, AP II, Marra, PP, Kramer, LD. Serologic evidence of West Nile virus transmission, Jamaica, West Indies. Emerg Infect Dis. 2003;9(7):860–3.
10. Calisher CH, Karabatsos N, Dalrymple JM, Shope RE, Porterfield JS, Westaway EG, et al. Antigenic relationships between flaviviruses as determined by cross-neutralization tests with polyclonal antisera. J Gen Virol. 1989; 70(Pt. 1):37–43.
11. Belle EA, King SD, Griffiths BB, Grant LS. Epidemiological investigation for arboviruses in Jamaica, West Indies. Am J Trop Med Hyg. 1980;29(4):667–75.
12. Ulloa A, Langevin SA, Mendez-Sanchez JD, Arredondo-Jimenez JI, Raetz JL, Powers AM, et al. Serologic Survey of Domestic Animals for Zoonotic Arbovirus Infections in the Lacandón Forest Region of Chiapas, Mexico. Vector Borne Zoonotic Dis. 2003;3(1):3–9.
13. Farfán-Ale JA, Blitvich BJ, Lorono-Pino MA, Marlenee NL, Rosado-Paredes EP, Garcia-Rejon JE, et al. Longitudinal studies of West Nile virus infection in avians, Yucatán State, México. Vector Borne Zoonotic Dis. 2004;4(1): 3–14.
14. Inouye S, Matsuno S, Tsurukubo Y. 'Original antigenic sin' phenomenon in experimental flavivirus infections of guinea pigs: studies by enzyme-linked immunosorbent assay. Microbiol Immunol. 1984;28(5):569–74.
15. Quirin R, Salas M, Zientara S, Zeller H, Labie J, Murri S, et al. West Nile virus, Guadeloupe. Emerg Infect Dis. 2004;10(4):706–8.
16. Lefrancois T, et al. West Nile virus Surveillance, Guadeloupe, 2003–2004. Emerg Infect Dis. 2005;11(7):1100–3.
17. Komar O, Robbins MB, Klenk K, Blitvich BJ, Marlenee NL, Burkhalter KL, et al. West Nile

virus transmission in resident birds, Dominican Republic. Emerg Infect Dis. 2003;9(10): 1299–302.
18. Komar O, Robbins MB, Guzman Contreras G, Benz BW, Klenk K, Blitvich BJ, et al. West Nile virus survey of birds and mosquitoes in the Dominican Republic. Vector-borne Zoonotic Dis. 2005;5(2):120–6.
19. Blitvich BJ, Fernandez-Salas I, Contreras-Cordero JF, Marlenee NL, Gonzalez-Rojas JI, Komar N, et al. Serologic evidence of West Nile virus infection in horses, Coahuila State, Mexico. Emerg Infect Dis. 2003;9(7):853–6.
20. Loroño-Pino MA, Blitvich BJ, Farfán-Ale JA, Puerto FI, Blanco JM, Marlenee NL, et al. Serologic evidence of West Nile virus infection in horses, Yucatan State, Mexico. Emerg Infect Dis. 2003;9(7):857–9.
21. Estrada-Franco JG, Navarro-Lopez R, Beasley DW, Coffey L, Carrara AS, Travassos da Rosa A, et al. West Nile virus in Mexico: evidence of widespread circulation since July 2002. Emerg Infect Dis. 2003;9(12):1604–7.
22. Fernandez-Salas I, Contreras-Cordero JF, Blitvich BJ, Gonzalez-Rojas JI, Cavazos-Alvarez A, Marlenee NL, et al. Serologic evidence of West Nile Virus infection in birds, Tamaulipas State, México. Vector Borne Zoonotic Dis. 2003;3(4):209–13.
23. Ramos C, Falcon Lezama JA. 2004. La fiebre del Nilo occidental: una enfermedad emergente en México. Salud Publica Mex. 2004; 46(5):488–90.
24. Beasley DW, Davis CT, Estrada-Franco J, Navarro-Lopez R, Campomanes-Cortes A, Tesh RB, et al. Genome sequence and attenuating mutations in West Nile virus isolate from México. Emerg Infect Dis. 2004;10(12): 2221–4.
25. Davis CT, Beasley DW, Guzman H, Siirin M, Parsons RE, Tesh RB, Barrett AD. Emergence of attenuated West Nile virus variants in Texas, 2003. Virology. 2004;330(1):342–50.
26. Cruz L, Cardenas VM, Abarca M, Rodriguez T, Reyna RF, Serpas MV, et al. Serological evidence of West Nile virus activity in El Salvador. Am J Trop Med Hyg. 2005;72(5):612–5.
27. World Organisation for Animal Health (OIE). West Nile fever in Belize in October 2003. Disease Information 2004;17(10):5 March 2004. Available from: http://www.oie.int/eng/info/hebdo/AIS_55.HTM#Sec5. Accessed 3 February 2005.
28. Caribbean Epidemiology Center (CAREC). Recommendations for West Nile virus surveillance in the Caribbean 2004. Available from: http://www.carec.org/west-nile-2004/. Accessed16 March 2005.
29. Dupuis II AP, Marra PP, Reitsma R, Jones MJ, Louie KL, Kramer LD. Serologic evidence for West Nile virus transmission in Puerto Rico and Cuba. Am J Trop Med Hyg. 2005;73(2): 474–6.
30. Mattar Velilla S, Edwards E, Laguado J, González M, Alvarez J, Komar N. West Nile virus infection in Colombian horses. Emerg Infect Dis. 2005;11(9):1497–8.
31. Alves Araújo FA, Teixeira Vianna RS, Vieira de Andrade Filho G, Lemos Melhado D, Todeschini B, Cavalcanti e Cavalcante G, et al. Segundo inquérito sorológico em aves migratórias e residentes do Parque Nacional da Lagoa do Peixe/RS para detecção do vírus do Nilo Ocidental e outros vírus. SVS Boletim Electronico Epidemiol. 2004;4(5):1–8.
32. Bakonyi T, Hubalek Z, Rudolf I, Nowotny N. Novel flavivirus or new lineage of West Nile virus, central Europe. Emerg Infect Dis. 2005; 11(2):225–31.
33. Komar N, Langevin S, Hinten S, Nemeth N, Edwards E, Hettler D, et al. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. Emerg Infect Dis. 2003;9(3):311–22.
34. Calisher CH, Maness KS, Lord RD, Coleman PH. Identification of two South American strains of eastern equine encephalomyelitis virus from migrant birds captured on the Mississippi delta. Am J Epidemiol. 1971;94(2): 172–8.
35. Bowen GS, Monath TP, Kemp GE, Kerschner JH, Kirk LJ. Geographic variation among St. Louis encephalitis virus strains in the viremic responses of avian hosts. Am J Trop Med Hyg. 1980;29(6):1411–9.
36. Komar N, Panella NA, Burns JE, Dusza SW, Mascarenhas TM, Talbot TO. Serologic evidence for West Nile virus infection in birds in the New York City vicinity during an outbreak in 1999. Emerg Infectious Dis. 2001;7(4): 621–5.
37. Komar N. West Nile virus surveillance using sentinel birds. Annals NY Acad Sci. 2001;951: 58–73.
38. Peterson, AT, Komar N, Komar O, Navarro-Siguenza A, Robbins MB, Martinez-Meyer E. West Nile virus in the New World: Potential impacts on bird species. Bird Conservation International. 2004;14(4):215–32.
39. Pan American Health Organization (PAHO). Guidelines for Surveillance, Prevention and Control of West Nile Virus. Epidemiological Bulletin 23(4):December 2002. Available from: http://www.paho.org/english/dd/ais/be_v23n4-West_Nile.htm. Accessed 16 March 2005.
40. México, Secretaría de Salud. Guía para la vigilancia, prevención y control del virus del oeste del Nilo. Available from: http://www.cenave.gob.mx/von/default.asp?id=30. Accessed 16 March 2005.

Manuscript received on 28 June 2005. Revised version accepted for publication on 9 December 2005.

RESUMEN

La actividad del virus del Nilo occidental en América Latina y el Caribe

Objetivos. El virus del Nilo occidental (VNO, familia Flaviviridae, género *Flavivirus*) se ha propagado rápidamente por toda la cuenca del Caribe desde que se detectó por primera vez en 2001. En este informe se resumen nuestros conocimientos actuales acerca de la transmisión del VNO en zonas tropicales del continente americano.

Métodos. Revisamos todo lo que se ha publicado sobre el tema y consultamos a autoridades de salud clave para obtener datos inéditos.

Resultados. Las infecciones por el virus del Nilo occidental aparecieron por primera vez en seres humanos residentes de las Islas Caimán y de los Cayos de la Florida en 2001, y en pájaros de aspecto sano de los cuales se obtuvieron muestras a principios de 2002. En 2002 se encontraron pruebas serológicas de infección por el VNO en caballos, pollos y aves de corral no estabuladas oriundas de Guadalupe, la República Dominicana y la parte oriental de México. En 2003, el VNO se diseminó dentro de México y por la parte norte de Centroamérica y se encontraron pruebas serológicas en las Bahamas, Puerto Rico y Cuba. En 2004, las primeras pruebas serológicas de actividad vírica en ecosistemas sudamericanos se detectaron en septiembre y octubre en Colombia y Trinidad, donde se observaron anticuerpos neutralizantes contra el VNO en animales domésticos.

Conclusiones. Estos informes esporádicos de enfermedad equina, humana y aviar en América Latina y el Caribe son desconcertantes. Es necesario aislar las cepas para determinar si la atenuación del virus u otro factor explica la carga de enfermedad reducida en ecosistemas tropicales.

Palabras clave Virus del Nilo occidental, América Latina, región del Caribe, arbovirus, vigilancia de la población, flavivirus.

別紙様式第2-1

No. 19

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. 7. 11	該当なし	

一般的名称	(製造販売承認書に記載なし)	研究報告の公表状況		公表国
販売名(企業名)	合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)		ProMED 20080709.2092, 2008 Jul 09. 情報源:Turkish Daily News, 2008 Jul 9.	トルコ

研究報告の概要

○クリミア・コンゴ出血熱―トルコ

2008年7月7日、トルコのブルサ、チャナッカレ、サムスンの病院で3名の患者がダニ媒介性疾患のクリミア・コンゴ出血熱で死亡し、この2ヶ月間での死亡者数は37名になった。

保健省はダニに注意するよう呼びかける声明を発表した。ダニに咬まれた場合は決して手でつぶさずに、皮膚を保護し、医師にピンセットで注意深く取り除いてもらった後、ヨードで消毒することを推奨している。さらに、咬まれた人は10日間医学的観察を行い、発熱、頭痛、吐き気、嘔吐、下痢などの症状が現われた場合は、最寄りの病院を受診するよう、保健省の担当者は話している。

クリミア・コンゴ出血熱は主に動物に感染し、ヒツジや家畜に寄生するダニが、時折人にウイルスを感染させる。迅速に治療しないと出血によって死亡することもある。感染した人の血液や唾液を介して他の人にウイルスが伝播される可能性がある。感染地域はアフリカ、アジア、ヨーロッパの一部だが、近年トルコの気候が温暖になっていることから、ダニの数が増えてより多くの人が感染するようになっていると保健当局では話している。

報告企業の意見	今後の対応
2008年7月7日、トルコの病院で3名の患者がダニ媒介性疾患のクリミア・コンゴ出血熱で死亡し、この2ヶ月間での死亡者数は37名になったとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。今後も引き続き、新たな病原体による感染症の発生状況等に関する情報の収集に努める。

使用上の注意記載状況・その他参考事項等

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

19



Archive Number 20080709.2092
Published Date 09-JUL-2008
Subject PRO/AH/EDR> Crimean-Congo hem. fever - Turkey (11)

CRIMEAN-CONGO HEMORRHAGIC FEVER - TURKEY (11)
****
A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail is a program of the
International Society for Infectious Diseases
<http://www.isid.org>

Date: Wed 9 Jul 2008
Source: Turkish Daily News, Dogan News Agency report [edited]
<http://www.turkishdailynews.com.tr/article.php?enewsid=109351>

On Mon 7 Jul 2008, 3 people were pronounced dead at hospitals in the provinces of Bursa, Canakkale, and Samsun, taking the death toll from tick bites to 37 in the past 2 months. According to the Dogan news agency, a resident of the western province of Bursa went camping 10 days ago and was bitten by a tick. He was hospitalised and diagnosed with the deadly Crimean-Congo hemorrhagic fever (CCHF), and moved to the intensive care unit.

In the western province of Canakkale, a man died in hospital after being treated for suspected CCHF infection. He had told relatives that he had seen a tick on his body. He was buried in a zinc casket with lime spread over the grave as a precaution. Another person had died from CCHF in the same province last month [June 2008].

Another man died from CCHF on Monday [7 Jul 2008] in the northern province of Samsun after he was bitten by a tick and removed it with his hand.

The Health Ministry also issued a statement to warn people against ticks. In case of a tick bite the skin should be covered with [an antiseptic]. The tick should be removed by doctors using tweezers with great care and iodine should be applied to the bite. Health Ministry officials said ticks should never be killed by hand.

Moreover, those people, touched by any tick, should be kept under medical observation for 10 days, and go to the nearest hospital if they have symptoms such as fever, headache, nausea, vomiting, or diarrhea, officials from the Health Ministry said.

CCHF mainly affects animals. Ticks, which live on sheep and cattle, can sometimes pass the virus to people. It is a [haemorrhagic] fever where patients can bleed to death if they are not treated quickly. Those infected can transmit the virus through their blood or saliva. The disease is endemic in parts of Africa, Asia, and Europe. Health authorities said a warmer climate, which Turkey has experienced in recent years, could mean a larger tick population that could in turn infect more people with the disease.

--
Communicated by:
ProMED-mail Rapporteur A-Lan Banks

[The CCHF death toll in Turkey has risen from 33 on 4 Jul 2008, when more than 550 cases were recorded, to the present 37.

The HealthMap/ProMED-mail interactive map of Turkey is available at <http://healthmap.org/promed?v=39.1,35.2,5>.

and a map delineating the administrative provinces of Turkey can be accessed
<http://www.mapsofworld.com/turkey/turkey-political-map.html>. - Mod.CP]

[see also:
Crimean-Congo hem. fever - Turkey (10): treatment 20080704.2038
Crimean-Congo hem. fever - Turkey (09) 20080622.1935
Crimean-Congo hem. fever - Turkey (08) 20080620.1917
Crimean-Congo hem. fever - Turkey (07) 20080616.1892
Crimean-Congo hem. fever - Turkey (06) 20080615.1888
Crimean-Congo hemorrhagic fever - Turkey (05) 20080612.1866
Crimean-Congo hem. fever - Turkey (04) 20080611.1838
Crimean-Congo hem. fever - Turkey (03) 20080522.1686
Crimean-Congo hem. fever - Turkey (02) 20080511.1610
Crimean-Congo hem. fever - Turkey 20080508.1567
2007

Crimean-Congo hemorrhagic fever - Turkey 20070610.1892
2006

Crimean Congo hemorrhagic fever - Turkey (05) 20060822.2359
Crimean-Congo hemorrhagic fever - Turkey (04): WHO 20060809.2230
Crimean-Congo hemorrhagic fever - Turkey (03): comment on tick removal 20060728.2082
Crimean-Congo hemorrhagic fever - Turkey (02) 20060722.2013
Crimean-Congo hemorrhagic fever - Turkey 20060705.1844]
.....cp/mj/dk

*****
ProMED-mail makes every effort to verify the reports that are posted, but the accuracy and completeness of the information, and of any statements or opinions based thereon, are not guaranteed. The reader assumes all risks in using information posted or archived by ProMED-mail. ISID and its associated service providers shall not be held responsible for errors or omissions or held liable for any damages incurred as a result of use or reliance upon posted or archived material.
*****
Become a ProMED-mail Premium Subscriber at
<http://www.isid.org/ProMEDMail Premium.shtml>
*****
Visit ProMED-mail's web site at <http://www.promedmail.org>. Send all items for posting to: promed@promedmail.org (NOT to an individual moderator). If you do not give your full name and affiliation, it may not be posted. Send commands to subscribe/unsubscribe, get archives, help, etc. to: majordomo@promedmail.org. For assistance from a human being send mail to: owner-promed@promedmail.org.
##########
##########

about ISID | membership | programs | publications | resources
13th ICID | site map | ISID home

©2001,2008 International Society for Infectious Diseases
All Rights Reserved.
Read our privacy guidelines.
Use of this web site and related services is governed by the Terms of Service.

別紙様式第2-1

No. 8

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. 7. 11	該当なし	

一般的名称	(製造販売承認書に記載なし)	研究報告の公表状況		公表国
販売名(企業名)	合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)		CDC, Salmonella Saintpaul Outbreak Notices. 2008 Jul 8; Available from: URL: http://www.cdc.gov/salmonella/saintpaul/archive/070808.html	米国

研究報告の概要

○サルモネラ・セントポール流行の調査

CDCは、関係機関と協力して、複数州でのサルモネラ菌血清型セントポールのアウトブレイクを調査している。当初の疫学調査では生のトマトの摂食が関連すると考えられたが、最近、レストランで食事をした患者で多くのクラスターが発生していることが判明した。このため、トマトと同時に摂食されることの多い生のハラペーニョやコリアンダーなども原因となった可能性があるが、現時点では、感染源をこのうちの一つに特定することはできない。

7月7日時点で、41の州、ワシントンD.C.、カナダで991名の患者が同じ遺伝子パターンのサルモネラ・セントポールに感染した。感染が特定された症例は、検査施設が州の衛生研究所にサルモネラ株を送って確認されたものである。患者のうち、情報が得られた711名は、4月10日～6月25日の間に発症し、このうち275名は6月に発症していた。患者の年齢は1歳～99歳で48%が女性だった。最も感染者の割合が高いのは20歳～29歳、最も低いのは10歳～19歳及び80歳以上の年代だった。感染に関連した死亡例が2例報告されている。2007年の4月～6月にこの型のサルモネラ・セントポールに感染した患者は6名しかいなかった。

公衆衛生当局は5月からアウトブレイクの調査を続けているが、患者が食べたものを正確に思い出し、食材を特定することが難しいため、調査は難航している。サルモネラに感染した場合、12～72時間で下痢、発熱、腹痛などの症状を発症し、4～7日間持続する。ほとんどの患者は自然に回復するが、小児、高齢者、免疫不全患者などでは重症化しやすい。FDAは感染源と見られるトマトの摂食を控え、サルサソースなどに使われる生のトマトにも注意するよう呼びかけている。

報告企業の意見	今後の対応
2008年7月7日時点で、米国の41の州、ワシントンD.C.、カナダで991名の患者がサルモネラ・セントポール株に感染したことが確認されたとの報告である。	日本赤十字社では、輸血による細菌感染予防対策として問診時に献血者の健康状態を確認し、発熱を伴う食中毒様の激しい下痢症状がある場合は1ヶ月間献血不適としている。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。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

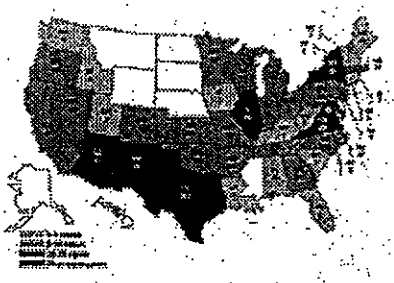
合成血-LR「日赤」
照射合成血-LR「日赤」

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

JRC2008T-046

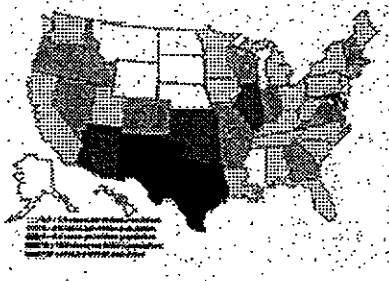
Investigation of Outbreak of Infections Caused by *Salmonella* Saintpaul

Cases infected with the outbreak strain of *Salmonella* Saintpaul, United States, by state, as of July 7, 2008 9pm EDT



Click map to view a larger image.

Incidence of cases of infection with the outbreak strain of *Salmonella* Saintpaul, United States, by state, as of July 7, 2008 9PM EDT



Click map to view a larger image.

Questions and Answers
Related to the Outbreak of *Salmonella* Saintpaul infections associated with tomatoes.

Update for July 8, 2008 - Case count information as of 9 pm EDT, July 7, 2008

Click Here for Advice to Consumers

CDC is collaborating with public health officials in many states, the Indian Health Service, and the U.S. Food and Drug Administration (FDA) to investigate an ongoing multi-state outbreak of human *Salmonella* serotype Saintpaul infections. An initial epidemiologic investigation comparing foods eaten by ill and well persons identified consumption of raw tomatoes as strongly linked to illness. Recently, many clusters of illnesses have been identified in several states among persons who ate at restaurants. These clusters led us to broaden the investigation to be sure that it encompasses food items that are commonly consumed with tomatoes. Fresh tomatoes, fresh hot chili peppers such as jalapeños, and fresh cilantro are the lead hypotheses. However, at this point in the investigation, we can neither directly implicate one of these ingredients as the single source, nor discard any as a possible source.

Since April, 991 persons infected with *Salmonella* Saintpaul with the same genetic fingerprint have been identified in 41 states, the District of Columbia, and Canada. These were identified because clinical laboratories in all states send *Salmonella* strains from ill persons to their State public health laboratory for characterization. One new state, West Virginia, reported an ill person. The number of ill persons identified in each state is as follows: Alabama (2 persons), Arkansas (13), Arizona (47), California (8), Colorado (13), Connecticut (4), Florida (2), Georgia (24), Idaho (4), Illinois (95), Indiana (14), Iowa (2), Kansas (17), Kentucky (1), Louisiana (1), Maine (1), Maryland (29), Massachusetts (24), Michigan (7), Minnesota (10), Missouri (12), New Hampshire (4), Nevada (11), New

Jersey (9), New Mexico (98), New York (28), North Carolina (10), Ohio (8), Oklahoma (24), Oregon (10), Pennsylvania (11), Rhode Island (3), South Carolina (1), Tennessee (8), Texas (382), Utah (2), Virginia (29), Vermont (2), Washington (4), West Virginia (1), Wisconsin (11), and the District of Columbia (1). Four ill persons are reported from Canada; three appear to have been infected while traveling in the United States, and one illness remains under investigation.

Among the 711 persons with information available, illnesses began between April 10 and June 25, 2008, including 275 who became ill on June 1 or later. Many steps must occur between a person becoming ill and the determination that the illness was caused by the outbreak strain of *Salmonella*; these steps take an average of 2-3 weeks. Therefore, an illness reported today may have begun 2-3 weeks ago. Patients range in age from <1 to 99 years; 48% are female. The rate of illness is highest among persons 20 to 29 years old; the rate of illness is lowest in children 10 to 19 years old and in persons 80 or more years old. At least 194 persons were hospitalized. One death in a man in Texas in his eighties has been associated with this outbreak. In addition, a man in his sixties who died in Texas from cancer had an infection with the outbreak strain of *Salmonella* Saintpaul at the time of his death; the infection may have contributed to his death

Only 6 persons infected with this strain of *Salmonella* Saintpaul were identified in the country during April through June of 2007. The previous rarity of this strain and the distribution of illnesses in all U.S. regions suggest that the implicated food is distributed throughout much of the country. Because many persons with *Salmonella* illness do not have a stool specimen tested, it is likely that many more illnesses have occurred than those reported. Some of these unreported illnesses may be in states that are not on today□s map.

Health officials have worked continuously since late May to investigate this outbreak. CDC has sent 17 people to the field to work with other public health officials. The investigation is complex and difficult. One difficult aspect is that people often have difficulty remembering exactly what foods they ate, and remembering specific ingredients is even more difficult. Although laboratory testing of fo ds might help, perishable foods that were consumed by ill persons are often not available to test.

Clinical features of *Salmonella* Infection

Most persons infected with *Salmonella* develop diarrhea, fever, and abdominal cramps 12-72 hours after infection. Infection is usually diagnosed by culture of a stool sample. The illness usually lasts 4-7 days. Although most people recover without treatment, severe infections may occur. Infants, elderly persons, and those with impaired immune systems are more likely than others to develop severe illness. When severe infection occurs, *Salmonella* may spread from the intestines to the bloodstream and then to other body sites, and can cause death. In these severe cases, antibiotic treatment may be necessary.

Advice to consumers

At this time, FDA is advising U.S. consumers to limit their tomato consumption to those that are not the likely source of this outbreak. These include cherry tomatoes; grape tomatoes; tomatoes sold with the vine still attached; tomatoes grown at home; and red plum, red Roma, and round red tomatoes from specific sources listed at: http://www.fda.gov/oc/opacom/hottopics/tomatoes.html*. Consumers should be aware that raw tomatoes are often used in the preparation of fresh salsa, guacamole, and pico de gallo, are part of fillings for tortillas, and are used in many other dishes.

C sumers everywhere are advised to:

- Refrigerate within 2 hours or discard cut, peeled, or cooked tomatoes.
- Avoid purchasing bruised or damaged tomatoes and discard any that appear spoiled.
- Thoroughly wash all tomatoes under running water.
- Keep tomatoes that will be consumed raw separate from raw meats, raw seafood, and raw produce items.
- Wash cutting boards, dishes, utensils, and counter tops with hot water and soap when switching between types of food products.

FDA recommends that U.S. retail outlets, restaurants, and food service operators offer only fresh and fresh cut red plum, red Roma, and round red tomatoes and food products made from these tomatoes from specific sources listed at: http://www.fda.gov/oc/opacom/hottopics/tomatoes.html#retailers*. Cherry tomatoes, grape tomatoes, and tomatoes sold with the vine still attached from any source may be offered.

FDA information on this investigation can be found at: http://www.fda.gov/oc/opacom/hottopics/tomatoes.html*

More information about *Salmonella* and this investigation can be found at:

- *Salmonella* in tomatoes FAQs
- Timeline for Reporting of Cases
- New Mexico Department of Health (PDF – 191 KB)
- Arizona Department of Health Services News Release - Tomatoes: Caution Urged*
- Texas Department of State Health Services - News Update, June 13, 2008*
- Kansas Identifies 3 Cases Linked to Multi-State *Salmonella* Outbreak*

- Kentucky Cabinet for Health and Family Services Press Release
- Indiana State Department of Health Media Update on *Salmonella* Outbreak*
- Maryland Department of Health and Mental Hygiene News Release
- Missouri DHHS: State health department issues cautions about tomatoes*
- New Jersey Department of Health and Human Services: NJ Reports Four *Salmonella* Cases Linked to Multi-State Outbreak
- Utah Department of Health: Health News

nformation on the safe handling of produce can be found at: www.cfsan.fda.gov/~dms/prodsafe.html.*

Previous Updates on this Outbreak

- July 7, 2008
- July 4, 2008
- July 3, 2008
- July 2, 2008
- July 1, 2008
- June 30, 2008
- June 27, 2008
- June 26, 2008
- June 25, 2008
- June 24, 2008

- June 23, 2008
- June 20, 2008
- June 18, 2008
- June 16, 2008
- June 12, 2008
- June 9, 2008
- June 7, 2008
- June 5, 2008
- June 2, 2008

* Links to non-Federal organizations found at this site are provided solely as a service to our users. These links do not constitute an ndorsement of these organizations or their programs by CDC or the Federal Government, and none should be inferred. CDC is not esponsible for the content of the individual organization Web pages found at these links.

Please note: Some of these publications are available for download only as *.pdf files. These files require Adobe Acrobat Reader in rder to be viewed. Please review the information on downloading and using Acrobat Reader software.

Page last modified: July 8, 2008
Content Source: National Center for Zoonotic, Vector-Borne, and Enteric Diseases (ZVED)

Page Located on the Web at http://www.cdc.gov/salmonella/saintpaul/archive/070808.html

DEPARTMENT OF HEALTH AND HUMAN SERVICES
CENTERS FOR DISEASE CONTROL AND PREVENTION
SAFER·HEALTHIER·PEOPLE™

別紙様式第2-1
番号11

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8月1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(7): 1333-1341	公表国 フランス	
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＞
2005年から2007年の間、チクングニヤウイルス（CHIKV）が、2006年2月に症例数の最大ピークとするレユニオン島での大流行を引き起こした。レユニオン島での供血は、2006年1月に中断された。
＜研究デザインおよび方法＞
レユニオン島でウイルス血症の供血がされる平均リスクの推定を異なる流行期について計算した。計算には、定点観測の動向調査（sentinel surveillance）、ウイルス血症の期間、および無症候感染の頻度から割り出したCHIKV予想発現値を用いた。最後のこれら2つのパラメーターのデータは、最初は仮定に基づき、次いでアウトブレイクの期間に実施された検討をもとに出した。この予想リスクを、血小板ドネーションのスクリーニングのために実施したCHIKV核酸増幅検査の結果と比較した。
＜結果＞
アウトブレイクの期間中、リスクの平均値は、ドネーション100,000当たり132と予想された。このリスクは、2006年2月のアウトブレイクの最大期にピークに達し、ドネーション100,000当たり1,500であった。もし採血が中断されていなかったら、全体で47の供血がウイルス血症であったであろう。この期間、757,000人住民のうちの312,500人が蚊を媒介にして感染していたと予想される。2006年1月から5月まで、予想リスク平均値（0.7%）と血小板供血で観察されたリスク（0.4%）は同じ大きさであった。
＜結論＞
この大きなアウトブレイクの間、ウイルス血症の供血の予想リスクは高かったが、蚊媒介のCHIKV感染のリスクに比べ低かった。この予想リスクは、観察されたリスクの結果と一致したことによって裏付けられた。

報告企業の意見	今後の対応
レユニオン島におけるチクングニヤウイルス（CHIKV）の流行時のCHIKV血症献血リスクに関する報告である。血漿分画製剤からのチクングニヤウイルス伝播の事例は報告されていない。また、万一原料血漿にチクングニヤウイルスが混入したとしても、BVD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。

TRANSFUSION COMPLICATIONS

Estimated risk of Chikungunya viremic blood donation during an epidemic on Reunion Island in the Indian Ocean, 2005 to 2007

Cécile Brouard, Pascale Bernillon, Isabelle Quatresous, Josiane Pillonel, Azzedine Assal, Henriette De Valk, and Jean-Claude Desenclos for the workgroup "Quantitative Estimation of the Risk of Blood Donation Contamination by Infectious Agents"

BACKGROUND: Between 2005 and 2007, Chikungunya virus (CHIKV) caused a massive epidemic on Reunion Island with a major peak in the number of cases in February 2006. Blood donation was interrupted on the island in January 2006.

STUDY DESIGN AND METHODS: Estimates of the mean risk of viremic blood donation on Reunion Island were computed for different phases of the epidemic. Calculations used CHIKV incidence estimates derived from sentinel surveillance, duration of viremia, and frequency of asymptomatic infection. Data on these two last parameters were initially based on hypotheses and subsequently obtained from studies carried out during the outbreak. The estimated risk was compared to the results of CHIKV nucleic acid testing (NAT) implemented for platelet (PLT) donations screening.

RESULTS: Over the course of the outbreak, the mean risk was estimated at 132 per 100,000 donations. The risk peaked at 1500 per 100,000 donations at the height of the outbreak in February 2006. In total, 47 blood donations would have been potentially viremic if blood collection had not been interrupted. During this period, an estimated 312,500 of 757,000 inhabitants had been infected by mosquito-borne transmission. From January to May 2006, the estimated mean risk (0.7%) and observed risk on PLT donations (0.4%) were of the same order of magnitude.

CONCLUSION: During this large outbreak, the estimated risk of viremic blood donation was high, but low compared to the risk of mosquito-borne CHIKV transmission. The estimated risk was corroborated by the concordant results with the observed risk.

Chikungunya virus (CHIKV) is an alphavirus that belongs to the Togoviridae family, transmitted by *Aedes* mosquitoes. It was first identified in 1952 during an outbreak in Tanzania.[1,2] Afterward, it caused many outbreaks in Africa[3-7] and in Asia.[3,8-11] In Africa, a sylvatic transmission cycle between wild primates and mosquitoes is thought to maintain the virus, whereas in Asia, it is transmitted from human to human through an urban transmission cycle.[3] CHIKV infection is mainly characterized by sudden onset of fever, arthralgia, myalgia, headache, and edemas.[1,3,8,12,13] Other symptoms like rash, epistaxis, gingivorrhagia, nausea, vomiting, flushed face, or photophobia have also been described. The most typical clinical sign is polyarthralgia that is generally very painful, as suggested by its name Chikungunya meaning in the language of the Tanzanian Makonde plateau "that which bends up" in reference to the stooping posture adopted by patients because of the severity of the joint pains. The symptoms usually resolve within a few days, but in some severe cases, arthralgia may persist for months or years.[3,13] Serosurveys implemented during prior outbreaks have demonstrated that Chikungunya infection can also be asymptomatic.[9]

In early 2005, CHIKV emerged for the first time in the southwest Indian Ocean region (Comoros, Reunion,

ABBREVIATIONS: CHIKV = Chikungunya virus; WNV = West Nile virus.

From the Institut de Veille Sanitaire (InVS) (French Institute of Public Health Surveillance), Saint-Maurice, France; Etablissement Français du Sang (EFS) (French Blood Services), Tours, France.

Address reprint requests to: Cécile Brouard, Institut de Veille Sanitaire, Département des Maladies Infectieuses, 12 Rue du Val d'Osne, 94415 Saint-Maurice, Cedex, France; e-mail: c.brouard@invs.sante.fr.

Received for publication October 1, 2007; revision received November 13, 2007, and accepted November 13, 2007.

doi: 10.1111/j.1537-2995.2008.01646.x

TRANSFUSION 2008;48:1333-1341.

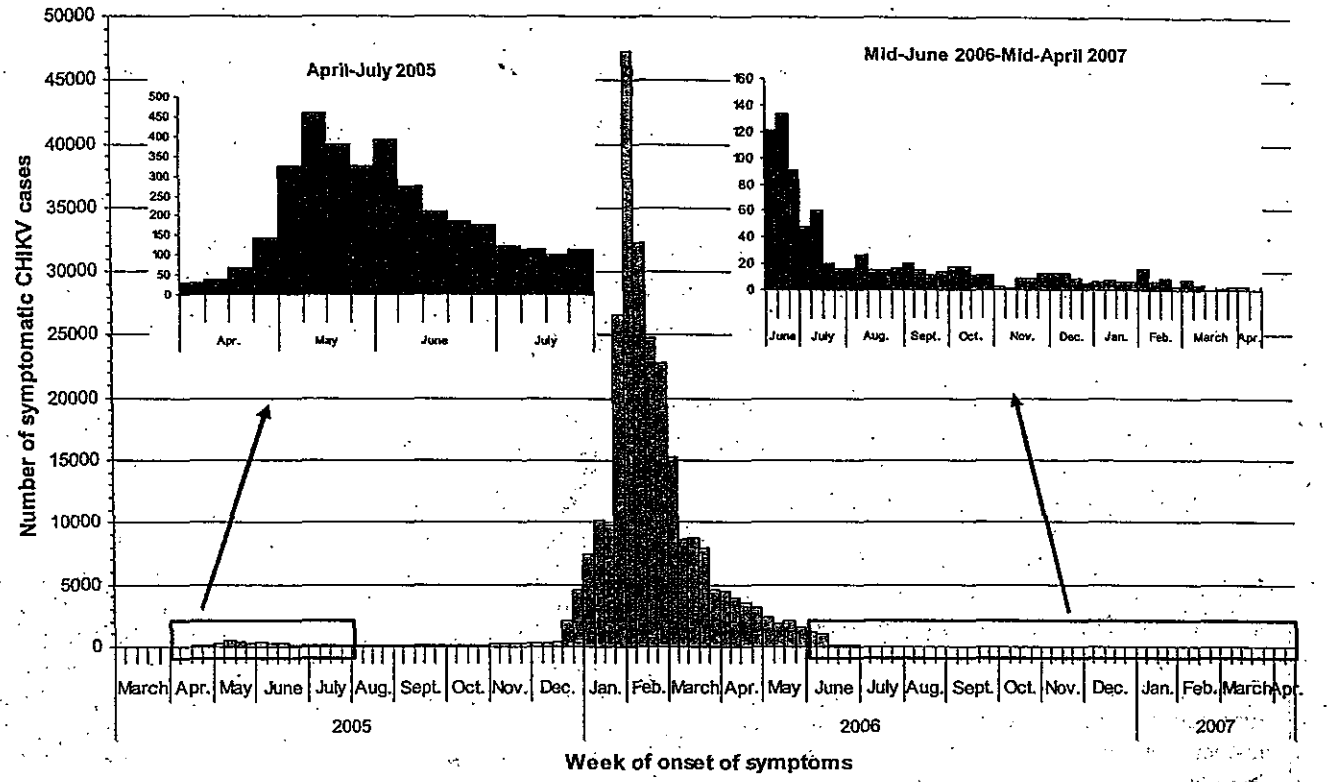


Fig. 1. Distribution of symptomatic cases of CHIKV infection per week of onset of symptoms, Reunion Island, March 28, 2005, through April 15, 2007.

Mayotte, Seychelles, Mauritius, and Madagascar Islands). On Reunion Island, the first cases were identified at the end of April 2005. After a first epidemic peak in May through June 2005 with a maximum of 450 cases during the second week of May, the number of cases decreased during the southern hemisphere winter season. At mid-December, an exponential increase in cases occurred, with almost 10,000 estimated cases at mid-January 2006 (Fig. 1). Because of concerns about the possible transmission of CHIKV by blood transfusion, the French Blood Services (EFS) interrupted blood donations on the island from January 23, 2006, except donations for platelets (PLTs) for which systematic screening for CHIKV genome by nucleic acid amplification testing (NAT) was set up.

At that moment, we estimated the risk of CHIKV viremic blood donation. Afterward, we updated these estimates since more accurate data were available on the incidence of infection and on the frequency of asymptomatic infections. We compared the estimated risk of viremic blood donation to the observed proportion of viremic PLT donations determined by CHIKV NAT screening.

MATERIALS AND METHODS

The estimates were performed by the French Institute of Public Health Surveillance (InVS) in the setting of a work-group including the French Agency for the Safety of Health Products (Afssaps), the French Blood Services (EFS), and the National Institute for Blood Transfusion (INTS). In early 2005, this group initiated a project with the aim of obtaining a priori quantitative risk estimates of contamination of blood donations by infectious agents for various scenarios in terms of incidence and time-space distribution.[14]

General approach

The first estimates performed in January 2006 ("preliminary estimates") concerned the two following periods: Period A, from the detection of the first cases in April 2005 to mid-December 2005 when a large increase of cases occurred (March 28-December 18, 2005; 266 days); and Period B, from mid-December until the interruption of blood collection (December 19, 2005-January 22, 2006; 35 days; Fig. 2).

These estimates were later refined with consolidated incidence data, corrected for delayed care-seeking and delayed reporting and more precise estimates of the proportion of asymptomatic infections obtained through a seroepidemiologic survey carried out at the final phase of the outbreak ("retrospective estimates"). We also estimated the risk of viremic blood donation for five different

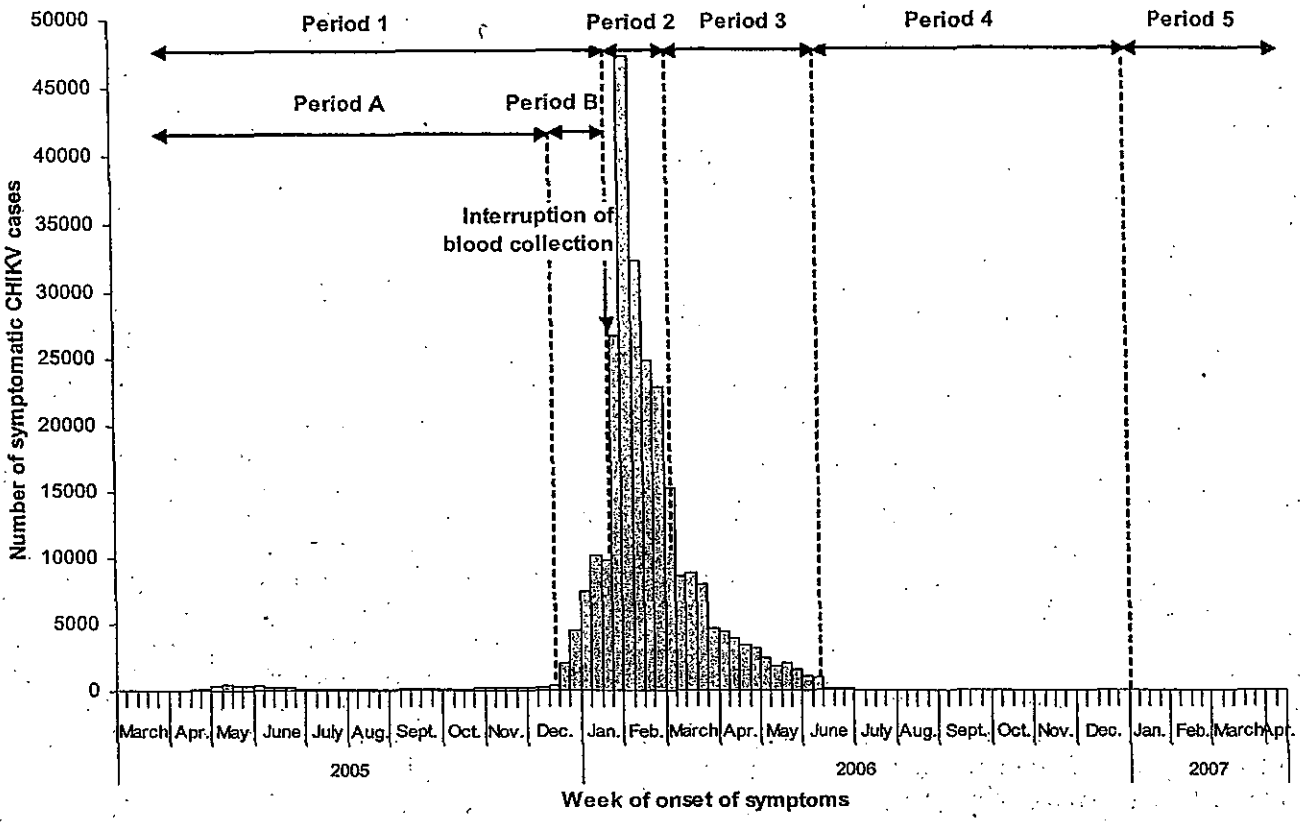


Fig. 2. Periods for risk estimates and distribution of symptomatic cases of CHIKV infection per week of onset of symptoms, Reunion Island, March 28, 2005, through April 15, 2007.

periods of the outbreak with these updated data (Fig. 2).

By use of the quarterly numbers of blood donations collected on Reunion Island in 2005 (unpublished data from EFS), we could then estimate the number of blood donations that would have been collected in 2006 if blood donations had not been interrupted.

To assess the validity of our risk estimates, we compared the estimated risk of viremic blood donation ("estimated risk") to the observed proportion of viremic PLT donations collected and screened for CHIKV genome ("observed risk") over the same period.

Statistical approach

An approximating formula developed by Biggerstaff and Petersen[15] in 2002 for West Nile virus (WNV) was used to estimate the mean risk of viremic blood donation by CHIKV. This formula combines the proportion of asymptomatic (*Pa*) and symptomatic (*Ps*) infections with the duration of viremia among asymptomatic infected individuals (*Va*) and the duration between onset of viremia and onset of symptoms in symptomatic patients (*Vs*). This provides the mean time an infected individual is viremic and asymptomatic. Dividing this mean duration of viremia by the length of the outbreak period (*L*) then provides an estimate of the probability that an individual donates blood during viremia, assuming that a person with symptoms would self-defer or be excluded from donation by the predonation medical examination. Combined with the incidence (*I*) of the infection (including both symptomatic and asymptomatic infection), it gives an estimate of the mean risk of viremic blood donation:

$$\text{Mean risk} = \frac{(Pa \times Va) + (Ps \times Vs)}{L} \times I.$$

As suggested by Biggerstaff and Petersen,[15] risk confidence bounds were obtained by multiplying the confidence bounds of *I* by $[(Pa \times Va) + (Ps \times Vs)]/L$. Confidence intervals (CIs) of *I* were calculated with Fleiss quadratic method.[16]

Data on duration of viremia

In January 2006, few data were available on the duration of CHIKV viremia. In 1964, Sarkar and coworkers[17] described, from virologic studies of hemorrhagic fever in Calcutta, that CHIKV was most frequently isolated from blood within 48 hours after the onset of symptoms, but that it had been isolated as late as 6 days after the onset of illness.

The duration of viremia has been more extensively documented for dengue viruses: 1 or 2 days before the onset of symptoms and between 4 and 6 days and as late as 12 days after the first symptoms.[18-20] We thus used the following parameters for CHIKV: 1.5 days for the mean duration between onset of viremia and onset of symptoms among symptomatic patients (*Vs*) and 1.5 + 6 = 7.5 days for the mean duration of viremia among asymptomatic infected individuals (*Va*) assuming that the whole duration of viremia is similar in symptomatic and asymptomatic infections.

The same estimates of duration of viremia were used for the retrospective estimates since consistent observations were reported during the outbreak on Reunion Island. Thus, during this epidemic, CHIKV has been isolated from blood mostly within 5 days and as late as 12 days after the onset of symptoms. In some cases, CHIKV viremia might have persisted over 12 days since viral loads at 12 days were high.[21]

Data on the proportion of asymptomatic infections

For the preliminary estimates in January 2006, in the absence of data on the proportion of asymptomatic CHIKV infection, two hypotheses were formulated based on the proportion of asymptomatic infections reported during outbreaks of dengue:[22,23] a minimal proportion of asymptomatic infection of 30 percent and a maximal proportion of 70 percent.

Between August and October 2006, a seroprevalence study was conducted among the general population of Reunion Island.[24] This survey showed that 38 percent of the inhabitants of Reunion Island had been infected by CHIKV. The preliminary results indicated that 6 percent of the study population had a positive CHIKV serology without having reported CHIKV symptoms. This suggests that approximately 15 percent of infected individuals during this outbreak may have had an asymptomatic infection. Therefore, this proportion of 15 percent was used for *Pa* for the retrospective estimates.

Incidence of CHIKV infection

We used the incidence data in the general population for the risk estimations assuming that potential blood donors had the same risk of CHIKV infection as the general population. The population of interest was the inhabitants of Reunion Island estimated at 756,745 by a population census conducted in 2004 by the National Institute for Statistics and Economics Studies (INSEE). CHIKV incidence data, by week of onset of symptoms, were obtained from the Reunion-Mayotte Interregional Epidemiology Unit, which had started surveillance for CHIKV infection as soon as the first cases were reported in April 2005. A suspect case of CHIKV infection was defined as a patient with an abrupt onset of fever over 38.5°C associated with incapacitating arthralgia in the absence of any other potential cause of infection. From April to December 2005, surveillance relied on vector control teams, which conducted active and retrospective case-finding around the cases reported by a sentinel physician network, medical laboratories, private practitioners, and patients themselves. The number of cases took into account the symptomatic patients responding to the case definition whether or not they had consulted a general practitioner. During this period, approximately 67 suspect CHIKV cases were identified by active case-finding for every suspect case identified by the sentinel network physicians. From mid-December onward, the number of cases exceeded the capacity of the active surveillance system, and surveillance was then entirely based on the sentinel network. To estimate the total number of cases from the sentinel network data, the multiplier of 67, derived during the phase of active case finding, was used.[25]

For the estimations of the risk of viremic donations, we calculated the estimated incidence of symptomatic and asymptomatic CHIKV infection by multiplying the estimated incidence of suspect cases by 100/(proportion of symptomatic infections).

RESULTS

Preliminary estimates

When the preliminary estimates were performed at the end of January 2006, the number of CHIKV suspect cases was 6500 for Period A and 25,000 for Period B. For Period A, the estimated mean risk of viremic blood donation was 15.2 per 100,000 donations, under the minimal hypothesis of 30 percent asymptomatic infections, and 61.3 per 100,000 donations, under the maximal hypothesis of 70 percent asymptomatic infections (Table 1). For Period B, the mean risk reached 445 per 100,000 donations, under the minimal hypothesis and 1,793 per 100,000 donations, under the maximal hypothesis.

Retrospective estimates

The retrospective estimates used the results of the seroprevalence survey that estimated the proportion of asymptomatic CHIKV infections during this outbreak at 15 percent. The updated estimate of the number of symptomatic cases was 6,864 for Period A and 34,002 for Period B (Table 2). Risk of viremic blood donation was then estimated at 9.6 and 362.5 per 100,000 donations for Periods A and B, respectively. The risk estimates for the five periods of the outbreak are shown in Table 3. Between the identification of the first CHIKV cases and the interruption of blood donations (Period 1), 7 of 14,450 blood donations collected could have been viremic. During

TABLE 1. Preliminary risk estimates of viremic blood donation, Reunion Island, March 28, 2005, through January 22, 2006

	Period A, Mar 28-Dec 18, 2005		Period B, Dec 19, 2005-Jan 22, 2006	
	Minimal hypothesis	Maximal hypothesis	Minimal hypothesis	Maximal hypothesis
Estimated number of symptomatic cases	6,500	6,500	25,000	25,000
Proportion of asymptomatic infections (%)	30	70	30	70
Estimated number of infected cases	9,286	21,667	35,714	83,333
Period length (days)	266	266	35	35
Estimated incidence of CHIKV infection per 100,000	1,227	2,863	4,720	11,012
Estimated risk of viremic blood donation				
Per 100,000 blood donations (95% CI)	15.2 (14.9-15.5)	61.3 (60.6-62.2)	445.0 (440.5-449.5)	1,793.4 (1,781.9-1,804.9)
Per estimated number of blood donations (95% CI)	2.0/12,800 (1.9-2.0)	7.9/12,800 (7.8-8.0)	7.1/1,600 (7.0-7.2)	28.7/1,600 (28.5-28.9)

TABLE 2. Retrospective risk estimates of viremic blood donation, Reunion Island, March 28, 2005, through January 22, 2006

	Period A, Mar 28-Dec 18, 2005	Period B, Dec 19, 2005-Jan 22, 2006
Estimated number of symptomatic cases	6,864	34,002
Proportion of asymptomatic infections (%)	15	15
Estimated number of infected cases	8,075	40,002
Period length (days)	266	35
Estimated incidence of CHIKV infection per 100,000	1,067	5,286
Estimated risk of viremic blood donation		
Per 100,000 blood donations (95% CI)	9.6 (9.4-9.8)	362.5 (359.0-366.0)
Per estimated number of blood donations (95% CI)	1.2/12,800 (1.2-1.3)	5.8/1,600 (5.7-5.9)

Period 2, at the height of the epidemic, the estimated risk of viremic blood donation was 1,500 per 100,000, that is, 29 potentially viremic donations if blood collection had continued. The estimated risk then decreased due to diminishing CHIKV transmission: 210 per 100,000 between March and June 2006 (Period 3), 1.4 per 100,000 for the second semester of 2006 (Period 4), and 0.27 per 100,000 for the first months of 2007 (Period 5), that is, 1 potentially viremic blood donation every 21 years on the basis of 17,500 blood donations collected each year. Finally, over the course of the outbreak, a total of 47 of 35,750 blood donations might have been viremic if blood collection had continued. Simultaneously, an estimated 312,500 of 757,000 inhabitants have been infected by mosquito-borne transmission.

Comparison between estimated risk and observed risk

Between January 23 and May 7, 2006, 2 of the 500 PLT donations screened for CHIKV RNA were positive (0.4%). One donor developed CHIKV symptoms on the day after the blood donation, the other remained asymptomatic. The risk of viremic blood donation over this period was estimated at 720 per 100,000 blood donations, that is, 0.72 percent.

Although an estimated 7 viremic donors had donated blood before the collection was interrupted, no case of transfusion-transmitted CHIKV infection has been identified during this period.

DISCUSSION

During this first and massive epidemic of CHIKV infection on Reunion Island, we computed estimates of the risk of CHIKV viremic blood donation, in real time during the ascending phase of the major epidemic peak, and afterward, we refined these estimates with newly available data. Although we underestimated the incidence of CHIKV infection in our preliminary calculations, we overestimated the proportion of asymptomatic infections. Consequently, the preliminary estimates were 1.2- to 6.4-fold greater than the retrospective calculations. The preliminary estimates, however, provided a right order of magnitude of the risk in real time in an emergency context. The retrospective calculations indicate a mean risk over the course of the outbreak, between April 2005 and April 2007, of 132 per 100,000 donations. The mean risk peaked at approximately 1,500 per 100,000 donations at the height of the outbreak in February 2006. In total, potentially 47 of 35,750 blood donations might have been viremic between April 2005 and April 2007 if blood collection had not been interrupted. We also estimated that 7 blood donations were viremic before the interruption of blood donations on the island. Therefore, this measure enabled the avoidance of 40 potentially viremic donations. By way of comparison, during the outbreak, the total number of individuals infected through mosquito-borne CHIKV transmission is estimated at 312,538 individuals.

This approach has several limitations. The estimates provided relate to a mean risk, which supposes that the risk is constant over the studied period and for the

TABLE 3. Retrospective risk estimates of viremic blood donation, Reunion Island, March 28, 2005, through April 15, 2007

	Period 1, Mar 28, 2005-Jan 22, 2006	Period 2, Jan 23, 2006-Mar 5, 2006	Period 3, Mar 6, 2006-Jun 11, 2006	Period 4, Jun 12, 2006-Dec 31, 2006	Period 5, Jan 1, 2007-Apr 15, 2007	Periods 1-5, Mar 28, 2005-Apr 15, 2007
Estimated number of symptomatic cases	40,866	169,008	54,936	772	75	265,657
Period length (days)	301	42	98	203	105	749
Proportion of asymptomatic infections (%)	15	15	15	15	15	15
Estimated number of infected cases	48,078	198,833	64,631	908	88	312,538
Estimated incidence of CHIKV infection per 100,000	6,353	26,275	8,541	120	12	41,300
Estimated risk of viremic blood donation						
Per 100,000 blood donations (95% CI)	50.7 (50.2-51.1)	1,501.4 (1,495.8-1,507.1)	209.2 (207.6-210.7)	1.4 (1.3-1.5)	0.27 (0.2-0.3)	132.3 (132.0-132.7)
Per estimated number of blood donations (95% CI)	7.3/14,450 (7.3-7.4)	29.1/1,940 (29.0-29.2)	9.9/4,710 (9.8-9.9)	0.14/9,760 (0.13-0.15)	0.01/4,890 (0.01-0.02)	47.3/35,750 (47.2-47.4)

geographic area. Although estimates were performed for several periods selected according to the level of incidence, the number of cases and consequently the risk might have been highly variable during the studied period. In addition, the risk of infection varied by geographic area as later demonstrated by the seroprevalence survey that showed that 29.6 percent of the inhabitants of the North have been infected whereas in the East, this proportion reached 48 percent.[24] Consequently, the mean risk underestimates the maximal risk, corresponding to the peak of the outbreak and to the area where CHIKV transmission was maximal. This maximum risk, however, is highly time and space limited.

To obtain a more dynamic sight of the risk over the course of the epidemic and estimates of the maximal risk, it would have been necessary to develop an approach similar to the one proposed by Biggerstaff and Petersen[15,26] for the WNV epidemic in 2002 in the United States. The latter is a statistical approach based on imputation and resampling techniques providing daily estimates of the risk of blood contamination in an epidemic setting. Conducting such an analysis in the context of this large and long-standing outbreak would have been computationally cumbersome. In our opinion, such a refinement was not essential in regard to the main objectives of the study, that is, providing a right order of magnitude of the risk as an aid for risk management. We considered that providing an approximation of the mean risk over five periods was a suitable alternative. To compute these mean risks, we therefore used the approximating formula proposed by Biggerstaff and Petersen.[15] In 2003, Biggerstaff and Petersen demonstrated for the WNV epidemic in 2002 in the United States that the approximating formula provides a reasonable approximation to the mean risk of transfusion.[15] The same work of comparison of the mean risks estimated by this method and by statistical resampling was carried out, in the setting of our workgroup, for an outbreak of acute hepatitis A in France that occurred in 1996 through 1997.[14,27] It also concluded to a good concordance of the results of both methods. Note that the CIs presented with our mean risk estimates do not take into account the uncertainty on the duration of viremia, the proportion of asymptomatic infections, nor the coefficient of 67, used to estimate incidence of symptomatic infections from the sentinel network data. Even though this limitation led to artificially narrow CIs, point estimates of mean risk should not be affected.

Our incidence data were derived from a sentinel surveillance system. Because a clinical case definition was used, it is possible that other febrile illnesses, not due to CHIKV, were included in the case count. The positive predictive value of a clinical case definition, however, greatly improves if incidence is high. Therefore, the inclusion of noncases in the case count, leading to

overestimation of the incidence and hence the risk of viremic donation, is more likely to occur outside an outbreak setting. The validity of the extrapolated data derived from a sentinel surveillance system estimating the total number of cases in the community should also be questioned. The serosurvey estimated that 38 percent of the inhabitants had been infected and that 32 percent had suffered from symptomatic infections. These data are consistent with the 35 percent of the inhabitants having suffered from symptomatic illness, estimated by the sentinel surveillance system and corroborate our incidence estimates.

We assumed that potential blood donors had the same risk of CHIKV infection as the general population. This assumption was supported by the findings of the serosurvey that showed similar antibody prevalences among adults of both sexes.[24] In addition, when we applied age-specific CHIKV antibody prevalence rates of the serosurvey to the donor population of Reunion Island, the overall seroprevalence among donors was estimated at 37.2 percent, similar to the overall antibody prevalence in the general population (38%).

One major limitation of the validity of our estimates relates to lack of a precise knowledge on the distribution of the duration of asymptomatic viremia in individuals with apparent and inapparent infection. To refine the estimates, further studies are necessary to document the kinetics of CHIKV viremia. This approach also hypothesizes that symptomatic individuals would self-defer or be excluded by the predonation examination. In real life, this may not always be the case. In the United States, among the first 14 identified donors associated with transfusion-related WNV transmission to recipients, 3 were shown to have been symptomatic at the moment of the donation.[15] Nevertheless, for CHIKV infection which is characterized by sudden onset of symptoms, this assumption is more plausible than for WNV which frequently causes paucisymptomatic infection.

Lack of data on the frequency of asymptomatic infection was the most important limiting factor for the preliminary estimates. This variable has a preponderant role in the risk estimate since it contributes both in the computation of the weighted mean of the duration of asymptomatic viremia and in the estimate of the incidence of infection. Valid data were available, however, for the retrospective calculations from the seroprevalence survey. This survey provided an estimate of the proportion of asymptomatic infections obtained directly among the studied population and for the epidemic CHIKV strain circulating.

In spite of the above limitations, the retrospective estimates are likely to give a good approximation of the real risk, as suggested by the observed risk of viremic PLT donations. From January to May 2006, this observed risk was 400 per 100,000 donations, of the same order of magnitude as the risk of 720 per 100,000 donations estimated over the same period.

Up to date, CHIKV infections from transfusion of blood or blood components have not been reported in the literature. On Reunion Island, no case of transfusion-transmitted CHIKV infection has been identified in spite of the estimated seven viremic donations collected before donations were interrupted. Despite the lack of data about transfusion-transmitted CHIKV infection, the high viral load during the acute phase of the infection,[21,28] the fact that several cases of CHIKV transmission have occurred among laboratory personnel handling infected blood,[29] and the fact that CHIKV has been transmitted to a health care worker drawing blood from an infected patient[28] provide evidence that transfusion-related transmission of CHIKV is highly plausible. It is possible that transfusion-related infections have not been recognized or have not been distinguished from infection from mosquito vectors. Also, the true transmission rate from viremic donors to recipients is not known. Several issues may influence the possibility of transmission of CHIKV through transfusion, such as the stability of the virus during storage of blood and the efficiency of virus elimination of blood processing methods, as viral inactivation. Also, the presence of IgM or IgG antibodies in donor blood may neutralize infectivity, as demonstrated for other viruses such as parvovirus[30] and suggested for WNV.[31] In addition, the assessment of the risk of CHIKV transmission from a viremic donor to a recipient would need to take into account the recipient's immune status.

In conclusion, despite the absence of documented cases, blood transfusion–related CHIKV transmission is plausible and the risk of viremic donation can be substantial in an outbreak setting. During this large outbreak, the estimated risk of viremic blood donation was high, but low compared to the risk of mosquito-borne CHIKV transmission. Despite its limitations, this work provided a right order of magnitude of the risk of viremic blood donation in real time during the ascending phase of the epidemic peak. At this moment, the decision of interrupting blood collection relied on the precautionary principle. The low risk estimated for early 2007 was, however, useful to contribute to the decision making process to start again the collection of blood donations on the island from June 14, 2007. This illustrates how this approach may contribute to guiding prevention measures.

ACKNOWLEDGMENTS

The authors thank Reunion-Mayotte Interregional Epidemiology Unit (Reunion-Mayotte Cire) and the Reunion Regional Observatory of Health plus sentinel general practitioners for providing the case onset data and for their important work during this massive and long outbreak.

The workgroup "Quantitative Estimation of the Risk of Blood Donation Contamination by Infectious Agents" consists of (in alphabetical order):

Agence Française de Sécurité Sanitaire des Produits de Santé (Afssaps, French Agency for the Safety of Health Products): J.F. Legras, M. Martin, E. Pouchol, I. Sainte-Marie

Etablissement Français du Sang (EFS; French Blood Services): A. Assal, P. Biagini, MH. Elghouzzi, P. Gallian, P. Morel

Institut National de Transfusion Sanguine (INTS): S. Laperche

Institut de Veille Sanitaire (InVS) (French Institute of Public Health Surveillance): P. Bernillon, F. Biton, C. Brouard, I. Capek, E. Delarocque-Astagneau, H. de Valk, D. Jeannel, A. Mailles, J. Pillonel, I. Quatresous, L. Sanchez, N. Schwarz, V. Vaillant

REFERENCES

1. Robinson MC. An epidemic of virus disease in Southern Province, Tanganyika Territory, in 1952-53. I. Clinical features. Trans R Soc Trop Med Hyg 1955;49:28-32.
2. Lumsden WH. An epidemic of virus disease in Southern Province, Tanganyika Territory, in 1952-53. II. General description and epidemiology. Trans R Soc Trop Med Hyg 1955;49:33-57.
3. Jupp PG, McIntosh BM. Chikungunya virus disease. In: Monath TP, editor. The arboviruses: epidemiology and ecology. Boca Raton (FL): CRC Press; 1988. p. 137-57.
4. Pastorino B, Muyembe-Tamfum JJ, Bessaud M, Tock F, Tolou H, Durand JP, Peyrefitte CN. Epidemic resurgence of Chikungunya virus in democratic Republic of the Congo: identification of a new central African strain. J Med Virol 2004;74:277-82.
5. Thonnon J, Spiegel A, Diallo M, Diallo A, Fontenille D. Chikungunya virus outbreak in Senegal in 1996 and 1997. Bull Soc Pathol Exot 1999;92:79-82.
6. Lanciotti RS, Ludwig ML, Rwaguma EB, Lutwama JJ, Kram TM, Karabatsos N, Cropp BC, Miller BR. Emergence of epidemic O'nyong-nyong fever in Uganda after a 35-year absence: genetic characterization of the virus. Virology 1998;252:258-68.
7. Saluzzo JF, Gonzalez JP, Herve JP, Georges AJ. Epidemiological study of arboviruses in the Central African Republic: demonstration of Chikungunya virus during 1978 and 1979. Bull Soc Pathol Exot Filiales 1980;73:390-9.
8. Carey DE, Myers RM, DeRanitz CM, Jadhav M, Reuben R. The 1964 chikungunya epidemic at Vellore, South India, including observations on concurrent dengue. Trans R Soc Trop Med Hyg 1969;63:434-45.
9. Porter KR, Tan R, Istary Y, Suharyono W, Sutaryo Widjaja S, Ma'Roef C, Listiyaningsih E, Kosasih H, Hueston L, McArdle J, Juffrie M. A serological study of Chikungunya virus transmission in Yogyakarta, Indonesia: evidence for the first outbreak since 1982. Southeast Asian J Trop Med Pub Health 2004;35:408-15.
10. Nimmannitya S, Halstead SB, Cohen SN, Margiotta MR. Dengue and chikungunya virus infection in man in Thailand, 1962-1964. I. Observations on hospitalized patients with hemorrhagic fever. Am J Trop Med Hyg 1969;18:954-71.
11. Thein S, La Linn M, Aaskov J, Aung MM, Aye M, Zaw A, Myint A. Development of a simple indirect enzyme-linked immunosorbent assay for the detection of immunoglobulin M antibody in serum from patients following an outbreak of chikungunya virus infection in Yangon, Myanmar. Trans R Soc Trop Med Hyg 1992;86:438-42.
12. Borgherini G, Poubeau P, Staikowsky F, Lory M, Le Moullec N, Becquart JP, Wengling C, Michault A, Paganin F. Outbreak of chikungunya on Reunion Island: early clinical and laboratory features in 157 adult patients. Clin Infect Dis 2007;44:1401-7.
13. Lambert J, Couturier E, Vaillant V. Infection à chikungunya. Etude descriptive des cas importés en France métropolitaine, 2005-2006. Saint-Maurice: Institut de Veille Sanitaire; 2007.
14. Brouard C, De Valk H, Pillonel J; Groupe "Estimation Quantitative du Risque de Contamination d'un Don de Sang par des Agents Infectieux." Estimation quantitative du risque de contamination d'un don de sang par des agents infectieux. Saint-Maurice: Institut de Veille Sanitaire; 2007.
15. Biggerstaff BJ, Petersen LR. Estimated risk of transmission of the West Nile virus through blood transfusion in the US, 2002. Transfusion 2003;43:1007-17.
16. Fleiss JL. Statistical methods for rates and proportions. 2nd ed. New York: Wiley; 1981.
17. Sarkar JK, Pavri KM, Chatterjee SN, Chakravarty SK, Anderson CR. Virological and serological studies of cases of haemorrhagic fever in Calcutta. Material collected by the Calcutta school of tropical medicine. Indian J Med Res 1964;52:684-91.
18. Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, Rothman AL, Ennis FA, Nisalak A. Dengue in the early febrile phase: viremia and antibody responses. J Infect Dis 1997;176:322-30.
19. Gubler DJ, Suharyono W, Tan R, Abidin M, Sie A. Viraemia in patients with naturally acquired dengue infection. Bull World Health Organ 1981;59:623-30.
20. Guzman MG, Kouri G. Dengue: an update. Lancet Infect Dis 2002;2:33-42.
21. Laurent P, Le Roux K, Grivard P, Bertil G, Naze F, Picard M, Staikowsky F, Barau G, Schuffenecker I, Michault A. Development of a sensitive real-time reverse transcriptase PCR assay with an internal control to detect and quantify chikungunya virus. Clin Chem 2007;53:1408-14.
22. Waterman SH, Novak RJ, Sather GE, Bailey RE, Rios I, Gubler DJ. Dengue transmission in two Puerto Rican communities in 1982. Am J Trop Med Hyg 1985;34:625-32.
23. McBride WJ, Mullner H, LaBrooy JT, Wronski I. The 1993 dengue 2 epidemic in North Queensland: a serosurvey and comparison of hemagglutination inhibition with an ELISA. Am J Trop Med Hyg 1998;59:457-61.

24. Perau J, Catteau C, Michault A, Parain C, Favier F. Fin 2006, 300 000 personnes avaient été atteintes par le chikungunya. Economie de la Réunion 2007;129:16-7.
25. Renault P, Solet JL, Sissoko D, Balleydier E, Larrieu S, Filleul L, Lassalle C, Thiria J, Rachou E, De Valk H, Ilef D, Ledrans M, Quatresous I, Quenel P, Pierre V. A major epidemic of chikungunya virus infection on Reunion Island, France, 2005-2006. Am J Trop Med Hyg 2007;77:727-31.
26. Biggerstaff BJ, Petersen LR. Estimated risk of West Nile virus transmission through blood transfusion during an epidemic in Queens, New York City. Transfusion 2002;42: 1019-26.
27. Sanchez-Garrido L, Bernillon P, Delarocque-Astagneau E, Brouard C, Pillonel J, Santa-Olalla P, De Valk H, Desenclos JC. Modélisation du risque de contamination des dons de sang par le virus de l'hépatite A. Journées de veille sanitaire 2005, 29-30 November; Paris.
28. Parola P, De Lamballerie X, Jourdan J, Rovery C, Vaillant V, Minodier P, Brouqui P, Flahault A, Raoult D, Charrel RN. Novel chikungunya virus variant in travelers returning from Indian Ocean islands. Emerg Infect Dis 2006;12: 1493-9.
29. Centers for Disease Control and Prevention and National Institute of Health. Biosafety in Microbiological and Biomedical Laboratories (BMBL). 5th ed. Washington (DC): US Government; 2007.
30. Parsyan A, Candotti D. Human erythrovirus B19 and blood transfusion—an update. Transfus Med 2007;17:263-78.
31. Busch MP, Caglioti S, Robertson EF, McAuley JD, Tobler LH, Kamel H, Linnen JM, Shyamala V, Tomasulo P, Kleinman SH. Screening the blood supply for West Nile virus RNA by nucleic acid amplification testing. N Engl J Med 2005;353:460-7.

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. 6. 23	該当なし	

一般的名称	(製造販売承認書に記載なし)	研究報告の公表状況	Chuang VW, Wong TY, Leung YH, Ma ES, Law YL, Tsang OT, Chan KM, Tsang IH, Que TL, Yung RW, Liu SH. Hong Kong Med J. 2008 Jun;14(3):170-7.	公表国
販売名(企業名)	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)			中国

研究報告の概要

○1998年～2005年の香港におけるデング熱症例の検討
目的:デング患者の疫学的、臨床的知見、臨床検査知見、並びに転帰の検討。
患者:1998年～2005年に香港の公立病院に入院したデング患者(臨床検査による確定例)全員の医療記録を後方視的に検討した。
結果:合計126名の患者を特定した[デング熱123名(98%)、デング出血熱3名(2%)]。輸血によりデング熱が伝播した患者1名が明らかとなった。合計116名(92%)は「輸入感染」で、10名(8%)は「地域内感染」であった。RT-PCRで確定したデング症例56名のうち、もっとも多かったのはデングウイルス1型(48%)であり、ついで2型(23%)3型(16%)、4型(13%)であった。地域内感染は1、2型のみであった。患者の年齢の中央値は38歳で、入院期間の平均は6日間であった。死亡例はなく、ほぼ全員(98%)が発熱を呈した。入院時のその他の症状は次の通り:筋肉痛(83%)、頭痛(65%)、倦怠感(59%)、皮疹(60%)。3分の1以上の患者が胃腸および上気道の合併症を発現した。もっとも多く認められた身体的所見は斑丘疹状皮疹であった。血小板減少、好中球減少、リンパ球減少は、それぞれ86%、78%、69%の患者に発現した。人口統計学的・臨床的知見、臨床検査知見、ならびに転帰は、4つのデング血清型間で差はなかったが、リンパ球数は、他の型と比べて3型がもっとも低かった(P=0.004)。
結論:発熱、皮疹を呈し、合致する血液学的知見を持ち、流行地への渡航歴のある患者に遭遇した場合には鑑別診断にデング熱を含めるべきである。

使用上の注意記載状況・その他参考事項等

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
1998年～2005年に香港の公立病院に入院したデング患者は合計126名で、うち10名(8%)は「地域内感染」であり1名は輸血による感染だった。ウイルス型は1型が最も多く、地域内感染は1、2型のみであったとの報告である。デングウイルスは東南アジアに定着しており、中国や台湾など日本に近い地域での流行状況を注視していく必要がある。	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。問診でデング熱の既往があった場合には、治癒後1ヶ月間献血不適としている。また、厚生労働科学研究「献血血の安全性確保と安定供給のための新興感染症等に対する検査スクリーニング法等の開発と献血制限に関する研究」班に協力する予定である。今後も引き続き情報の収集に努める。

ORIGINAL ARTICLE

Review of dengue fever cases in Hong Kong during 1998 to 2005

CME

Vivien WM Chuang 莊慧敏
TY Wong 黃天佑
YH Leung 梁耀康
Edmond SK Ma 馬紹強
YL Law 羅育龍
Owen TY Tsang 曾德賢
KM Chan 陳啟明
Iris HL Tsang 曾愷玲
TL Que 郭德麟
Raymond WH Yung 翁維雄
SH Liu 劉少懷

Objective To describe the epidemiology, clinical and laboratory findings, and outcomes of patients presenting locally with dengue.

Design Retrospective review of case records.

Setting Public hospitals, Hong Kong.

Patients Medical records of all laboratory-confirmed dengue patients admitted to public hospitals during 1998 to 2005 were reviewed retrospectively.

Results A total of 126 cases were identified, 123 (98%) being dengue fever and three (2%) dengue haemorrhagic fever. One patient who had blood transfusion–acquired dengue fever was highlighted. A total of 116 (92%) cases were 'imported', while 10 (8%) were local. Among the 56 dengue cases confirmed by reverse transcription–polymerase chain reaction, dengue virus type 1 was the most common accounting for 48% of them, followed by type 2, type 3, and type 4 responsible for 23%, 16%, and 13%, respectively. Only type 1 and type 2 were present in locally acquired infections. The median age of the patients was 38 years and the mean duration of hospitalisation was 6 days. There was no mortality, and nearly all patients (98%) presented with fever. Other symptoms at presentation included: myalgia (83%), headache (65%), fatigue (59%), and skin rash (60%). More than one third of patients had gastro-intestinal and upper respiratory complaints. Maculopapular skin rash was the most common physical finding. Thrombocytopenia, neutropenia, and lymphopenia were present in 86%, 78%, and 69% of the patients, respectively. In only 29% of the patients was dengue fever included in the initial differential diagnosis. The demographic, clinical, and laboratory findings as well as outcomes did not differ significantly among the four dengue serotypes, but the lowest lymphocyte counts of type 3 was lower than the other serotypes (P=0.004).

Conclusion When physicians encounter patients with a relevant travel history, presenting with fever and skin rash, and having compatible haematological findings, dengue fever should be included in the differential diagnosis.

Key words
Dengue; Dengue hemorrhagic fever; Serotyping

Hong Kong Med J 2008;14:170-7

Infection Control Branch, Centre for Health Protection / Infectious Disease Control Training Centre, Hospital Authority, Hong Kong
VWM Chuang, FRCPA, FHKAM (Pathology)
TY Wong, DTM&H, FHKAM (Medicine)
IHL Tsang, BSc, MStat
RWH Yung, MB, BS, FRCPath
Surveillance and Epidemiology Branch; Centre for Health Protection, Department of Health, Hong Kong
YH Leung, MB, BS, MPH
ESK Ma, M Med Sc, PDipID
YL Law, MB, BS
Department of Medicine and Geriatrics, Princess Margaret Hospital, Hong Kong
OTY Tsang, FHKCP, FHKAM (Medicine)
Department of Pathology, Tuen Mun Hospital, Hong Kong
KM Chan, FHKCP, FHKAM (Medicine)
TL Que, FHKAM (Pathology)
Quality and Safety Division, Hospital Authority, Hong Kong
SH Liu, MRCP (UK), MHA (New South Wales)

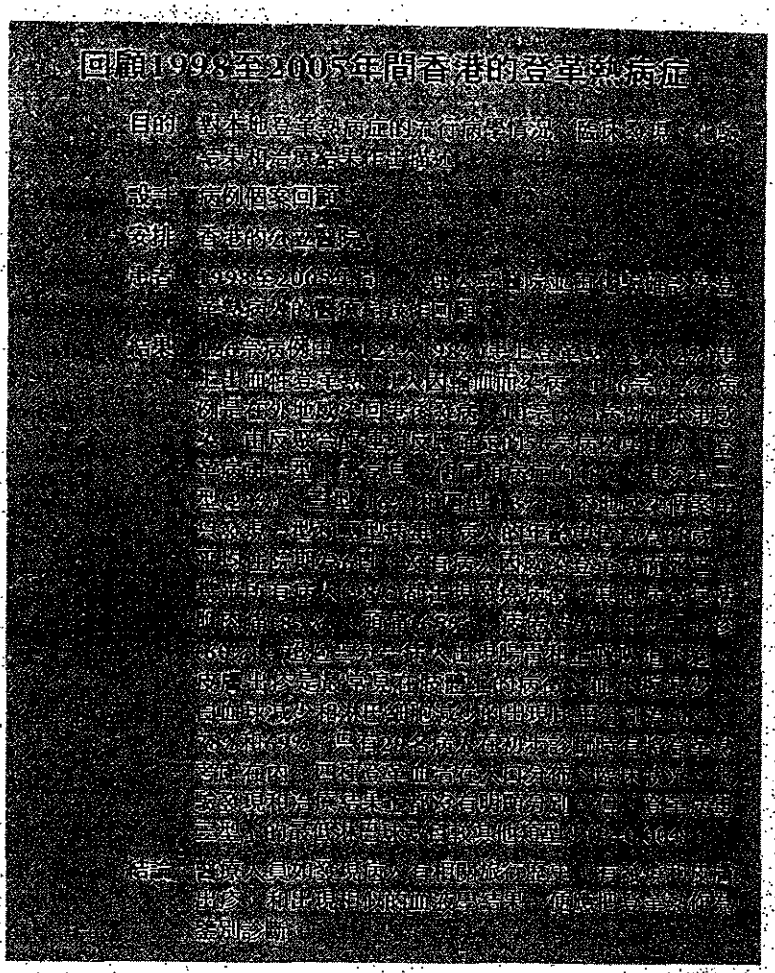
Correspondence to: Dr VWM Chuang
E-mail: chuangwm@ha.org.hk

Introduction

Dengue is the most common and widespread arthropod-borne viral infection in the world today. It is recognised in over 100 countries throughout the tropics and subtropical areas and threatens the health of approximately 40% of the world's population, of nearly 2.5 billion people.[1] The highest burden of disease occurs in South-East Asia and the Western Pacific, where it is one of the 10 leading causes of hospitalisation and childhood mortality.[2]

In Hong Kong, dengue fever was made notifiable since March 1994 and all infections reported to the Department of Health (DH) are investigated to establish their source. The number of cases reported is showing an increasing trend in recent years; the vast majority being imported from other countries. Hong Kong experienced its first local dengue case in September 2002.[3] Thereafter, several others were encountered in Ma Wan and local cases were subsequently identified sporadically in 2002 and 2003.

The epidemiology, clinical manifestations, and laboratory findings of dengue fever infections and its complications have been extensively described in the medical literature,[4,5] but comprehensive review is lacking for our local patients.


回顧1998至2005年間香港的登革熱病症

The objective of this review was to describe the epidemiology and explore the clinical characteristics and laboratory findings of dengue fever and dengue haemorrhagic fever (DHF) cases admitted to Hong Kong public hospitals during the period 1998 to 2005. We also compared the clinical and laboratory features of the four dengue serotypes identified by the polymerase chain reaction (PCR) technique.

Methods

We included patients admitted to public hospitals during 1998 to 2005 by using selective criteria "any diagnosis ICD9CM code" starting with "061 dengue" through the Clinical Data Analysis and Reporting System. A patient list was retrieved and matched with the laboratory-confirmed dengue cases notified to the DH. A case was defined as confirmed by detection of viral genomic sequences in autopsy tissue, serum or cerebrospinal fluid samples by PCR; a four-fold or more rise in immunoglobulin G (IgG) or IgM antibody titres to one or more dengue virus antigens in paired serum samples; or a positive IgM antibody titre in late acute or convalescent phase serum specimens (obtained between September 2003 and July 2004). The epidemiological data and virological results were provided by the Surveillance and Epidemiology Branch, Centre for Health Protection, DH. The clinical presentations, laboratory findings, and outcomes of all the confirmed cases were retrospectively reviewed through medical records.

The dengue cases were categorised into dengue fever, DHF, and dengue shock syndrome. In this paper, the definition of DHF was based on the World Health Organization's criteria and defined as: fever lasting 2 to 7 days, haemorrhagic tendencies (a positive tourniquet test; petechiae, ecchymoses or purpura; bleeding from the mucosa, gastro-intestinal tract, injection sites or other locations; haematemesis or melaena), thrombocytopenia (with platelet counts ≤100 x 10^9 /L) and evidence of plasma leakage due to increased vascular permeability (a rise in haematocrit ≥20% above average for age, sex in the population, a drop in the haematocrit following volume-replacement treatment of ≥20% from baseline, and features consistent with plasma leakage such as pleural effusion, ascites, and hypoproteinaemia). Dengue shock syndrome was defined as DHF together with direct evidence of circulatory failure or indirect evidence manifested as a rapid and weak pulse, narrow pulse pressure (20 mm Hg or hypotension for age) or cold, clammy skin and altered mental status.

Statistical analysis was carried out to compare the epidemiological, clinical, and laboratory findings among the four dengue serotypes. The categorical variables were compared by the Chi squared and Fisher's exact tests. Normally distributed data were compared by analysis of variance and data with skewed distributions by the Kruskal-Wallis test.

Results

Disease trend

In all, 126 patients with laboratory-confirmed dengue fever were admitted to public hospitals from 1998 to 2005. Only three (2%) patients suffered from DHF, while the remaining 123 (98%) had dengue fever; no dengue shock syndrome was reported. The number of patients encountered showed an upward trend from 1998 (2 cases) to 2003 (35 cases), and subsequently remained more or less constant in 2004 (20 cases) and 2005 (24 cases). A total of 116 (92%) were imported, while in 10 (8%) the infection was locally acquired (Fig 1).

No locally acquired disease was reported until in 2002, when nine patients were identified. Among them, six cases were confirmed to be epidemiologically related to the Ma Wan outbreak. Another patient acquired the infection through blood transfusion from one of the Ma Wan cases. The remaining two locally acquired cases in 2002 and one

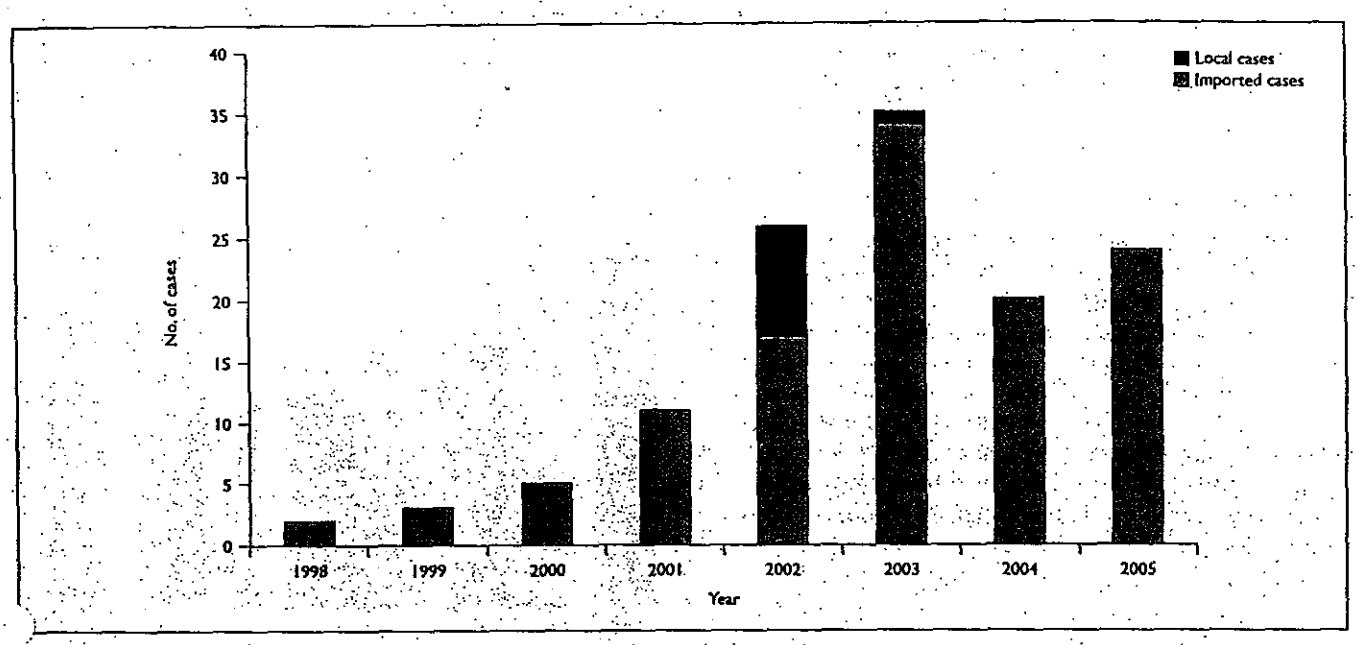


FIG 1. Numbers of dengue fever cases admitted to public hospitals in Hong Kong, 1998-2005

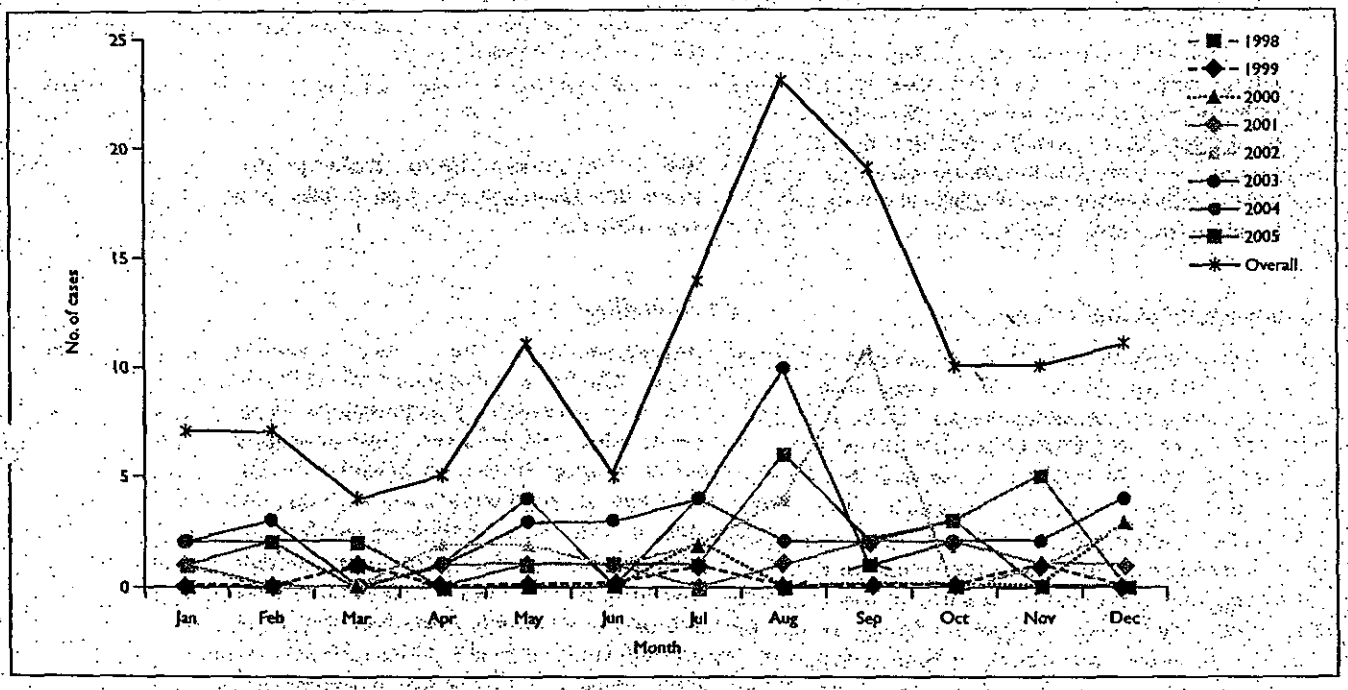


FIG 2. Seasonal variation of dengue fever cases admitted to public hospitals in Hong Kong

in 2003 were sporadic.

Seasonality

In Hong Kong, dengue cases were reported all year round. Figure 2 demonstrates the seasonal variation of cases, with a peak from July to September.

Country of origin for infection

Among the 116 imported cases, 106 (91%) were acquired in South-East Asian countries (Indonesia, Thailand, the Philippines, Vietnam, Singapore, Malaysia, Cambodia, Macau, and the Pacific Islands), eight (7%) originated from South Asia (India, Pakistan, Bangladesh, Sri-Lanka, and Nepal), and one (1%)

from Pitcairn island. Data for one case could not be determined as the patient had recently travelled to more than one country where the infection was endemic.

Patient demographics

The median age of the patients was 38 (range, 5-72) years and the female-to-male ratio was 1:1.2; five (4%) were paediatric patients (aged under 16 years); 114 (90%) were Hong Kong residents. A small proportion of the patients were migrant workers or tourists (4% and 5%, respectively). Among the Hong Kong residents, 86 (75%) were Chinese, 11 (10%) were from other Asian nations (Indonesia, the Philippines, Myanmar, Thailand), three (3%) were White and two (2%) belonged to the Pakistani/Nepalese group. Data on the origin of the remaining 12 patients were missing.

Serotype prevalence

Laboratory data on reverse-transcription PCR serotyping were available since 2002 and the serotypes of the corresponding 56 cases are shown in Figure 3.

All four serotypes, DEN-1, DEN-2, DEN-3 and DEN-4 were present among imported cases; while only DEN-1 (n=6) and DEN-2 (n=1) were present in local cases. Overall, DEN-1 was the most prevalent dengue serotype, responsible for nearly half (48%, 27/56) of all cases, followed by DEN-2 which accounted for about one quarter (23%, 13/56).

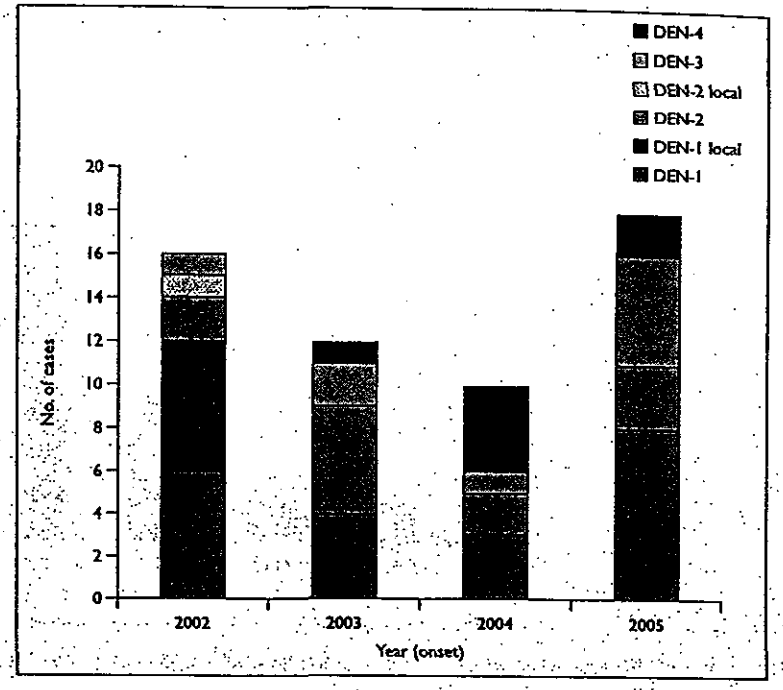


FIG 3. Distribution of serotypes among the dengue fever cases identified from 2002 to 2005.
DEN-1 denotes dengue virus type 1, DEN-2 dengue virus type 2, DEN-3 dengue virus type 3, and DEN-4 dengue virus type 4

Clinical presentations and outcome

Approximately 98% (122/124) of patients presented with fever; the mean value for the highest temperature being 38.2°C (standard deviation, 1.0°C) [Table 1]. The second commonest presenting symptom was myalgia, 83% (75/90). Two thirds of patients had headache, fatigue, and skin rashes. One third of the patients (24/71) complained of retro-orbital pain. The chief presenting complaints in more than one third of the patients were gastro-intestinal (nausea, vomiting and/or diarrhoea) or upper respiratory tract (dry cough and/or sore throat) or both. Over one quarter of patients (28/108, 26%) complained of abdominal pain, and one complained of blurred vision. Except for petechiae which were present in 45% (47/105) of the patients, other spontaneous bleeding was uncommon. Maculopapular skin rash was the commonest physical finding; in 70% of those with a rash it occurred predominately on the trunk. Lymphadenopathy was uncommon, which was only elicited in 16% of the patients. No patient demonstrated biphasic fever. Only one patient had clinical and radiological features of plasma leakage (pleural effusion), and was confirmed to be due to DHF as the final diagnosis. The mean duration of hospitalisation for these patients was 6 days, and there was no mortality.

Laboratory findings

Thrombocytopenia was the most common haematological finding, which affected 107 (86%) of the 124 patients with available platelet counts (Table 1). The mean value of the lowest platelet counts was 64 x 10^9/L. Among those with available results, neutropenia, atypical lymphocytes, and lymphopenia were present in 78%, 75%, and 69% of the patients respectively; half had prolonged activated partial thromboplastin time values. Corresponding proportions with deranged liver function tests and hypoalbuminaemia are also shown in Table 1. Mean values for aspartate aminotransferase and alanine aminotransferase were 212 IU/L and 169 IU/L, respectively.

Clinical differential diagnosis

Dengue infection was included as an initial clinical differential diagnosis in only 29% of the patients. Other differential diagnoses included: viral infection, upper respiratory tract infection, gastroenteritis, typhoid fever, chest infection, malaria, scarlet fever, scrub typhus, influenza, and fever for investigation.

TABLE 1. Recorded clinical symptoms, physical and laboratory findings of dengue cases

Symptoms/findings	No. of patients (%)	Remarks (reference range for laboratory tests)
Clinical symptoms		
Fever	122/124 (98)	
Myalgia	75/90 (83)	
Headache	68/105 (65)	
Skin rash	72/121 (60)	
Fatigue	50/85 (59)	
Dizziness	20/44 (45)	
Retro-orbital pain	24/71 (34)	
Gastro-intestinal tract (nausea, vomiting, and/or diarrhoea)	39/112 (35)	
Upper respiratory tract (non-productive cough, sore throat)	32/112 (29)	
Bleeding manifestations		
Epistaxis	7/67 (10)	
Gum bleeding	8/66 (12)	
Haematemesis	1/65 (2)	Dengue haemorrhagic fever
Tarry stool	1/69 (1)	Dengue haemorrhagic fever
Petechiae	47/105 (45)	
Clinical signs		
Skin rash	86/124 (69)	
Lymphadenopathy	19/116 (16)	
Laboratory findings		
Thrombocytopenia	107/124 (86)	Platelets: 145-370 x 10^9/L
Lymphopenia	79/114 (69)	Lymphocytes: 1.0-3.1 x 10^9/L
Neutropenia	89/114 (78)	Neutrophils: 1.7-5.8 x 10^9/L
Atypical lymphocytes	92/123 (75)	
Prolonged activated partial thromboplastin time	49/97 (51)	Activated partial thromboplastin time: 27.5-40.5 sec
Elevated aspartate aminotransferase	29/32 (91)	Aspartate aminotransferase: <38 IU/L
Elevated alanine aminotransferase	98/123 (80)	Alanine aminotransferase: 3-36 IU/L
Hypoalbuminaemia	34/123 (28)	Albumin: 35-52 g/L

Comparison of epidemiological, clinical, and laboratory findings among the four dengue virus serotypes

There were no statistically significant differences in terms of disease severity between the four virus types, patient gender, age and duration of hospitalisation, headache, myalgia, arthralgia, retro-orbital pain, skin rash, fatigue, gastro-intestinal and respiratory symptoms (Table 2). The percentages of patients with bleeding tendencies were 50%, 67%, 63%, and 33% for DEN-1, DEN-2, DEN-3, and DEN-4 virus type infections, respectively. Further analysis of the haemorrhagic manifestations was conducted by categorisation into epistaxis, gum bleeding, haematuria, and petechiae; 75% of these patients exhibited petechiae only, with no statistically significant difference between virus types (P=0.58). Nor was there any statistically significant difference between patients having different virus subtype infections for laboratory variables, except that the lowest lymphocyte counts of patients infected by serotype 3 was lower than the other serotypes (P=0.004).

Dengue haemorrhagic fever

Of the 126 patients under study, three (2%) were classified as DHF; all were imported from South-East Asian countries, and none could recall a previous history of dengue infection. Their demographic, clinical, and laboratory findings are shown in Table 3. They all received intravenous fluid replacement and platelet transfusions, recovered uneventfully without progression to dengue shock syndrome, and were discharged on day 6 or day 7 after hospital admission. Although these three patients did not recall prior infection, in one it was likely, as evidenced by respective acute and convalescence antibody titres.

Discussion

This is a comprehensive review of dengue fever patients admitted to Hong Kong public hospitals over the past 8 years. Epidemiological data showed that more than 70% of the patients were local Chinese residents with a travel history to neighbouring South-East Asian countries, where dengue fever is more endemic.[6] The most prevalent serotype was DEN-1, followed by DEN-2, DEN-3, and DEN-4, which was consistent with the serotype patterns in the countries from which such infections were imported.[7,8] The outbreak in Ma Wan was the first local one in Hong Kong; only DEN-1 and DEN-2 virus subtypes were encountered in local patients during 2002 and 2003.

Seasonal variations in dengue infections should be interpreted with cautions. Dengue fever is a travel-related arthropod-borne viral disease in Hong Kong; disease activity is closely related to and depends on the seasonal and weather conditions of countries from which the virus is imported. It is difficult to determine the seasonal patterns of dengue fever acquired locally based on the few reported cases. Monthly ovitrap surveillance in Hong Kong showed that the density of *Aedes albopictus* increases from April and peaks in June.[9] It is important to alert the public to keep vigilance against this mosquito-borne viral disease during this peak period.

We report here the first blood transfusion–transmitted dengue in the literature. The patient was a 76-year-old woman, with a history of hypertension and bronchiectasis. She was admitted in 2002 because of progressive malaise. Blood tests revealed

TABLE 2. Comparison of demographic, clinical, and laboratory findings in patients infected with the four dengue serotypes*

	PCR† type 1 (n=27)	PCR type 2 (n=13)	PCR type 3 (n=9)	PCR type 4 (n=7)	Overall (n=56)	P value
Gender (M:F)	13:14	6:7	5:4	3:4	27:29	1.0000
Age, median (IQR)	36.0 (24.0-52.0)	54.0 (33.0-66.0)	28.0 (23.5-61.0)	35.0 (21.0-63.0)	36.0 (26.3-57.8)	0.3559
Duration of hospitalisation, median (IQR) (days)	5.0 (4.0-7.0)	6.0 (3.0-7.5)	7.0 (4.5-8.5)	5.0 (4.0-6.0)	5.0 (4.0-7.0)	0.4589
Retro-orbital pain	9/18 (50)	3/10 (30)	0/4 (0)	1/6 (17)	13/38 (34)	0.2297
Rash—symptom	15/25 (60)	8/13 (62)	2/9 (22)	2/7 (29)	27/54 (50)	0.1332
Rash—sign	17/26 (65)	10/13 (77)	6/9 (67)	1/7 (14)	34/55 (62)	0.0509
Abdominal pain	3/25 (12)	2/12 (17)	1/8 (13)	1/7 (14)	7/52 (13)	1.0000
Diarrhoea	11/25 (44)	5/12 (42)	2/8 (25)	1/6 (17)	19/51 (37)	0.5956
Bleeding manifestation (epistaxis, gum bleeding, petechiae, haematuria)	13/26 (50)	8/12 (67)	5/8 (63)	2/6 (33)	28/52 (54)	0.5775
Hepatomegaly	2/26 (8)	2/13 (15)	0/9 (0)	1/7 (14)	5/55 (9)	0.5883
Leukopenia	25/26 (96)	10/13 (77)	9/9 (100)	5/7 (71)	49/55 (89)	0.0529
Lymphopenia	20/22 (91)	9/13 (69)	8/9 (89)	4/6 (67)	41/50 (82)	0.2550
Atypical lymphocyte	18/26 (69)	10/13 (77)	7/9 (78)	6/7 (86)	41/55 (75)	0.8848
Thrombocytopenia	26/26 (100)	11/13 (85)	8/9 (89)	6/7 (86)	51/55 (93)	0.0931
Elevated aspartate aminotransferase	8/9 (89)	3/4 (75)	4/4 (100)	2/2 (100)	17/19 (89)	1.0000
Elevated alanine aminotransferase	23/26 (88)	11/13 (85)	7/9 (78)	6/7 (86)	47/55 (85)	0.8954
Hypoalbuminaemia	10/26 (38)	5/13 (38)	5/9 (56)	4/7 (57)	24/55 (44)	0.6658
Highest temperature, mean (SD)	38.6 (1.0)	38.2 (1.1)	38.6 (1.3)	38.7 (0.6)	38.5 (1.0)	0.6893
Transfusion	4/23 (17)	2/12 (17)	1/8 (13)	2/6 (33)	9/49 (18)	0.8548

* Data are shown in No. (%), except otherwise stated
† PCR denotes polymerase chain reaction

TABLE 3. Demographic, clinical, and laboratory findings in patients with dengue haemorrhagic fever

Sex/age (years)	Ethnicity	Fever	Haemorrhagic manifestations	Lowest platelet count (x 10^9/L)	Plasma leakage	Laboratory findings	
						Serotype	Serology titer
M/38	Thai	37.2°C	Petechiae, bloody diarrhoea	9	Pleural effusion	Not done	Immunoglobulin M +ve
M/46	Chinese	38.4°C	Petechiae, bruises	9	Ascites	DEN 2	Immunoglobulin M +ve
F/49	Thai	38°C	Coffee ground vomitus, petechiae	8	Hypoalbuminaemia; haemoconcentration	DEN 1	4-fold increase*

* 1st titre: 640 (DEN-1), 5120 (DEN-2), 1280 (DEN-3), 1280 (DEN-4); 2nd titre: 5120 (DEN-1), 10 240 (DEN-2), 10 240 (DEN-3), 10 240 (DEN-4)

macrocytic anaemia and pancytopenia. She was diagnosed to have vitamin B12 deficiency anaemia, which was treated by vitamin B12 replacement and received a blood transfusion on 24 August 2002. On day 2 post-transfusion, she developed low-grade fever, but no skin rash, headache, myalgia, arthralgia, or retro-orbital pain. The patient was treated with antibiotics as for a urinary tract infection, based on the microbiological findings. The fever subsided 3 days later and the patient recovered uneventfully. The blood product she received was donated by a 17-year-old asymptomatic patient living in Ma Wan, during his viremic phase on 17 July 2002. On 24 July 2002, he developed generalised skin rash and attended the Accident and Emergency Department of Yan Chai Hospital. In October, he was subsequently picked up as one of the dengue cases based on serology results during the active case finding exercise in Ma Wan. Molecular testing performed on the donated blood product was positive for dengue virus type 1. The woman who had received the blood transfusion was recalled for blood testing on 7 October 2002, and was found to be positive for corresponding IgM antibodies and had a haemagglutination-inhibition titre of 1:2560. This incident was the first documented cases of such transmission in the literature, and since October

2002, the Hong Kong Red Cross Blood Transfusion Service (BTS) has intensified its donor deferral systems to counter this possibility. Specifically, it now asks about symptoms of dengue fever in the Blood Donor Registration Form (Supplement) by reminding all prospective donors to inform the BTS staff of all instances for flu, fever, headache, eye pain, muscle/joint pain, vomiting, and skin rash experienced 2 weeks before or after blood donation.

In our study, dengue fever was far more common than DHF and dengue shock syndrome, which were rare events. Our patients only manifested mild bleeding with good clinical outcomes and no fatalities. The clinical presentations of dengue fever, such as fever, myalgia, headache, and arthralgia, were comparable to findings reported in other studies.[10-12] Our patients (35%) presented with fewer gastroenteritis symptoms compared to those of others (50-98%).[11,12] Lymphadenopathy was documented in only 16% of our patients, which is much lower than the figure of 50% reported elsewhere.[13] This difference may be accounted for by less-than-adequate physical examination. Gum bleeding and epistaxis were reported in 12% and 10% of our patients respectively, which was also much lower than that reported previously.[11,12] Such differences could be due to the populations studied; patients recruited in endemic countries were mainly encountered during outbreaks in which both dengue fever and DHF were common. Previous studies showed dengue disease severity correlated with high viremia titres, secondary infection, and DEN-2 serotype infection.[14,15] Our findings showed that the haemorrhagic tendencies and duration of hospitalisation were not related to specific serotypes. Although some of our patients did receive platelet transfusions, the efficacy of such treatment in speeding recovery remains controversial. According to Thai experts, platelets are almost immediately destroyed by immune lysis after administration.[16]

Our study had several limitations. First, the target patients were limited to those with laboratory-confirmed dengue admitted to public hospitals. During 1998 to 2005, DH received notification of 203 dengue cases, including 77 who were admitted to private hospitals or consulted general practitioners only. The disease burden might also be underestimated, because some patients might have recovered, without seeking medical attention, while others might not have undergone serological testing. Second, statistical analysis could not be carried out to compare clinical and laboratory parameters in patients with dengue fever and DHF, as there were too few of the latter. Third, laboratory results before 2002 were not available in the Public Health Laboratory Information System. Fourth, not all clinical symptoms and signs listed in Table 2 could be retrieved from the medical records, as some may not have been specifically asked for or looked for.

In conclusion, dengue fever should be considered in the differential diagnosis of febrile patients with or without a travel history. Health care providers should therefore have an understanding of the infection, the spectrum of its clinical features, and methods of diagnosis and appropriate treatment. Until the *Aedes* mosquito can be effectively controlled or a cost-effective vaccine is developed, dengue fever will remain a public health concern, especially in South-East Asia. Control at source is one of the keys to combating dengue fever and requires active participation from all sectors of the community.

Acknowledgements

The members of Dengue Review Working Group gratefully acknowledge the Central Committee on Infectious Disease, Hospital Authority for the provision of patient medical records and Surveillance and Epidemiology Branch, Centre for Health Protection for the epidemiological and laboratory input. Without their support, our review would not have been achieved.

References

1. Dengue/dengue haemorrhagic fever. Wkly Epidemiol Rec 2000;75:193-6.
2. General considerations. In: Dengue haemorrhagic fever: diagnosis, treatment, prevention and control. 2nd ed. Geneva: World Health Organization; 1997:1-11.
3. Auyeung TW, Que TL, Lam KS, Ng HL, Szeto ML. The first patient with locally acquired dengue fever in Hong Kong. Hong Kong Med J 2003;9:127-9.
4. Nimmannitya S. Clinical spectrum and management of dengue haemorrhagic fever. Southeast Asian J Trop Med Public Health 1987;18:392-7.
5. Nimmanitya S, Cardosa J. Dengue haemorrhagic fever. In: Weatherall DJ, Ledingham JGG, Warrell DA, editors. Oxford textbook of medicine. 3rd ed. Oxford, UK: Oxford Medical Publications; 1995.
6. Situation of dengue fever/dengue haemorrhagic fever in the South-East Asia Region. WHO Regional Office for South East Asia website: http://www.searo.who.int/EN/Section10/Section332_1100.htm. Accessed 23 Aug 2007.
7. Weekly Infectious Disease Bulletin [serial online] 2005;31(1). Singapore, Ministry of Health, Singapore. http://www.moh.gov.sg/mohcorp/statisticsweeklybulletins.aspx. Accessed 23 Aug 2007.
8. Limkittikul K, Yingsakmongkon S, Jittmittraphap A, et al. Clinical differences among PCR-proven dengue serotype infections. Southeast Asian J Trop Med Public Health

2005;36:1432-8.
9. Average Monthly Ovitrap Index, 2000-2006. HKSAR Food and Environmental Hygiene Department website: http://www.fehd.gov.hk/safefood/dengue_fever/index.html. Accessed 23 Aug 2007.
10. Kalayanarooj S, Vaughn DW, Nimmannitya S, et al. Early clinical and laboratory indicators of acute dengue illness. J Infect Dis 1997;176:313-21.
11. Singh NP, Jhamb R, Agarwal SK, et al. The 2003 outbreak of Dengue fever in Delhi, India. Southeast Asian J Trop Med Public Health 2005;36:1174-8.
12. Hussin N, Jaafar J, Naing NN, Mat HA, Muhamad AH, Mamat MN. A review of dengue fever incidence in Kota Bharu, Kelantan, Malaysia during the years 1998-2003. Southeast Asian J Trop Med Public Health 2005;36:1179-86.
13. Ryan ET, Wilson ME, Kain KC. Illness after international travel. N Engl J Med 2002;347:505-16.
14. Vaughn DW, Green S, Kalayanarooj S, et al. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. J Infect Dis 2000;181:2-9.
15. Thein S, Aung MM, Shwe TN, et al. Risk factors in dengue shock syndrome. Am J Trop Med Hyg 1997;56:566-72.
16. Alex C, Djatnika S, Ridad A, et al. Thrombocytopenia and platelet transfusions in Dengue Haemorrhagic Fever and Dengue Shock Syndrome. Dengue Bulletin 2003;27:138-43.

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8月11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	フィブリノゲン加第 XIII 因子		研究報告の公表状況	Clearance of dengue virus in the plasma-derived therapeutic proteins. Transfusion. 2008 Jul;48(7):1342-7. 2008 Feb 22.		公表国 中国	
販売名（企業名）	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（血漿分画製剤でのデングウイルスの不活化・除去）

デングウイルスは年間に世界で 5 千万から 1 億人が感染し、感染者の数十万人がより重篤で生命を脅かすデング出血熱やデングショック症候群に進展する。

デングウイルスはフラビウイルス科に属し、直径 50nm のエンベロープを有する RNA ウイルスである。一般に血液などの高蛋白な体液で長期間生存するので、輸血により感染する可能性がある。針刺し事故や骨髄移植、分娩での血液に関連するデングウイルス感染が報告されている。

本研究は血漿分画製剤でのデングウイルス伝播の危険性が、特定のウイルス除去・不活化工程で除去されることを初めて証明するため実施された。

低温エタノール分画、陰イオン交換クロマトグラフィー、パスツリゼーション、S/D 処理とウイルスろ過を含むアルブミンやグロブリンの各製造工程前に、高力価の培養デングウイルスセロタイプ 2 を正常人血漿にスパイクし、各製造工程でのデングウイルスのクリアランスを $TCID_{50}$ アッセイ、RT-PCR で測定した。

デングウイルスの不活化・除去に対して、各製造工程前は全てで有効であった。

また、アルブミンの全製造工程（低温エタノール分画、パスツリゼーション）で少なくとも 10.12 log 減少すること、グロブリンの全製造工程（低温エタノール分画、ウイルスろ過、S/D 処理、クロマトグラフィー）では少なくとも 14.24 log 減少することが証明された。

現在実施されている血漿分画製剤の製造方法は、デングウイルス伝播に関して安全である。

使用上の注意記載状況・その他参考事項等

報告企業の意見	今後の対応
本剤の製造工程（低温エタノール分画、パスツリゼーション、イオン交換樹脂等）でデングウイルスが不活化・除去できると考えられる。	今後とも新しい感染症に関する情報収集に努める所存である。

23

ABT20082006

Clearance of dengue virus in the plasma-derived therapeutic proteins

Yi-Wu Xie, Paul K.S. Chan, Chi Kit Szeto, Sui Yi Kwok, Ida M.T. Chu, Shirley S.L.Chu, Jo L.K. Cheung, Sai Wah Wong, Mahommed B. Ali, and Bing-Lou Wong

BACKGROUND: Viral safety is of paramount importance for human plasma–derived therapeutic proteins. Recent reports of blood-associated transmission and continuous regional outbreaks of dengue fever have prompted a validation of clearance of dengue virus in the manufacture processes of the plasma-derived products.

STUDY DESIGN AND METHODS: A high titer of cultured dengue virus serotype 2 was spiked into process samples before individual steps of albumin and immunoglobulin manufacture processes, including cold ethanol precipitation, cation-exchange chromatography, pasteurization, solvent/detergent treatment, and virus filtration. Clearance of dengue virus was quantified with $TCID_{50}$ assays in the culture of Vero E6 cells and, when appropriate, real-time polymerase chain reaction (RT-PCR) assays.

RESULTS: The individual process steps were all effective in the inactivation and/or removal of dengue virus, and the data obtained clearly demonstrate that the risk of dengue virus transmission was reduced cumulatively by at least 10.12 and at least 14.24 log in the albumin and immunoglobulin manufacture processes, respectively.

CONCLUSION: The dedicated viral inactivation and/or removal approaches currently implemented in the manufacture of plasma-derived products provide a good safety margin with regard to the transmission of dengue virus.

Dengue virus infects 50 to 100 million people worldwide a year; of those infected, several hundred thousand develop the more severe and life-threatening diseases, dengue hemorrhagic fever and dengue shock syndrome. Dengue virus belongs to the family Flaviviridae, which in general is known to survive over long periods in fluids with high protein contents, for example, blood. Therefore, dengue viruses may be transmitted via transfusion of blood or blood components. Albeit rare, it has indeed been documented that blood-associated transmission of dengue virus occurs via routes including needle-stick injuries,[1] marrow transplantation,[2] intrapartum and vertical transmission,[2] and mucocutaneous transmission.[3] This can be a serious public health problem without proper control measures.

Dengue virus is a lipid-enveloped RNA virus, with a diameter of approximately 50 nm.[4] Reportedly, dengue virus has been effectively inactivated by photosensitizers[5,6] and is sensitive to high temperatures and acidic pH.[7] This study aims to demonstrate for the first time that the

ABBREVIATION: BVDV = bovine viral diarrhea virus.

From Advantek Serum Laboratories Limited and the Department of Microbiology, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong.

Address reprint requests to: Bing-Lou Wong, PhD, Advantek Serum Laboratories Limited, 3/F, HKIB, 2 Biotechnology Avenue, 12 Miles, Tai Po Road, Shatin, N.T., Hong Kong; e-mail: blwong@advantekbiologics.com.

The authors are thankful for partial funding support from the Innovation and Technology Fund (S/P 583/03), the Government of the Hong Kong Special Administrative Region. Some results of the present study are used in the U.S. patent application number 11/294,432 and Chinese patent application number 200610150052.2.

Received for publication March 27, 2007; revision received November 12, 2007, and accepted November 19, 2007.

doi: 10.1111/j.1537-2995.2008.01647.x

TRANSFUSION 2008;48:1342-1347.

risk of dengue virus transmission in plasma derivatives is eliminated by specific virus removal and inactivation procedures. Log reduction of dengue virus is investigated at individual steps of the manufacture processes of plasma-derived albumin and immunoglobulins, which include cold ethanol precipitation, cation-exchange chromatography, pasteurization, solvent/detergent (S/D) treatment, and virus filtration. The evaluation of the manufacture processes provides a measure of confidence for eliminating dengue virus.

MATERIALS AND METHODS

Raw materials

Normal human plasma was obtained from the plasma fractionator Shenzhen Weiwu Guangming Biological Products Co. (Shenzhen, China). All chemicals used in this study were of either pharmaceutical grade or analytical grade. Virus filters (Planova 35N, 10 cm^2) were a gift from Asahi Kasei (Tokyo, Japan).

Virus culture and quantification

Dengue virus serotype 2 (S047/00 from Environmental Health Institute, Singapore) was propagated in C6/36 cells (CDC Guangdong, China) in minimal essential medium with 1 percent fetal bovine serum (Gibco, Grand Island, NY). Dengue virus was quantified with $TCID_{50}$ assays in the culture of Vero E6 cells (ATCC, Manassas, VA). Vero E6 cells (2.5×10^5 cells/mL) were seeded in 96-well plates in a volume of 100 µL per well. After 1 day of incubation, 50 µL of medium was added to each well. Each dilution of sample was added at 50 µL per well, and further incubation was carried out at 36 ± 2°C with 5 percent CO_2. Plates were assessed for $TCID_{50}$ endpoint as cytopathic effects developed on the fifth day. The $TCID_{50}$ endpoint was calculated according to the Spearman-Kärber method, and the Poisson distribution was used when no virus was detected in samples. Quantitative real-time polymerase chain reaction (RT-PCR) was used to determine virus titer in the chromatography and cold ethanol precipitation steps. RNA of dengue virus was extracted in duplicate from samples with a viral RNA mini kit (QIAamp, Qiagen, Hilden, Germany) according to the procedure provided by the manufacturer. Dengue virus cDNA was reverse transcribed with random hexamers with reverse transcriptase (Supercript III, Invitrogen, Carlsbad, CA). Quantitative RT-PCR utilizing TaqMan technology (Applied Biosystems, Foster City, CA) was performed on samples and proper controls with specific primers (GTCAACATAGAAGCAGAACCTCCA and CTCTATGATGATGTAGCTGTCTCCG) and SYBR Green fluorescent probes with conditions optimized to detect 4.67 copies of viral RNA for dengue virus. Duplicate PCR procedures were performed for each sample with a sequence detection system (ABI 7900 HT, Applied Biosystems), and the cycling conditions were 50°C for 2 minutes, 95°C for 10 minutes followed by 40 cycles of 95°C for 15 seconds, and 60°C for 1 minute, as well as a dissociation stage of 95°C for 15 minutes, 60°C for 15 minutes, and 95°C for 15 minutes.

Fraction IV precipitation

The supernatant II + III was prepared from frozen human plasma through two consecutive steps of cold ethanol precipitation with 8 percent ethanol at pH 7.1 followed by 19 percent ethanol at pH 5.85.[8] Duplicates of 20 mL of supernatant II + III were spiked with 7.00 log per mL each of dengue virus at a ratio (vol/vol) of 1:10. Ethanol (95%) was added drop by drop into the supernatant II + III to a final ethanol concentration of 40 percent, which was further mixed at –5 to 5.5°C for 1 hour, before being centrifuged at 2300 × *g* to separate the fraction (F)IV from the supernatant IV. The supernatant II+III, the FIV, and the supernatant IV were titrated for quantity of viruses by $TCID_{50}$ assay or RT-PCR assay.

Pasteurization

The purified albumin solution was diafiltrated with 8 volumes of water and then concentrated to a concentration of 22 percent with a 30-kDa cutoff cassette (Millipore, Bedford, MA). Sodium caprylate was added to the concentrated albumin solution to a final concentration of 32 mmol per L, before adjustment of pH to 6.8 to make 20 percent albumin bulk. Two-hundred milliliters of albumin bulk and duplicates of the sterile-filtered 20 percent albumin in a 50-mL bottle was heated to 59°C in a water bath, followed by spiking with dengue virus (6.67, 7.50, or 7.67 log/mL) at a ratio (vol/vol) of 1:20 and 1:25, respectively. Gentle mixing with a mechanical stirrer (stainless steel) was applied to the bulk pasteurization. Samples were taken out for virus titration during the time course of a 10-hour treatment at 59 to 60°C.

FIII precipitation

The FII + III separated from the supernatant II + III above was redissolved, and NaAc-HAc buffer (0.8 mol/L-4 mol/L, pH 3.9) was added dropwise to adjust pH to 5.1. Dengue virus (7.17 or 7.67 log/mL) was spiked at a ratio (vol/vol) of 1:10 into duplicates of 20 mL of the pH-adjusted FII + III. Ethanol (95%) was added drop by drop into the FII + III to a final ethanol concentration of 15 percent, which was further mixed at –5 to 5.5°C for 1.5 hour, before being centrifuged at 2300 × *g* to separate the FIII from the supernatant III. The FII + III, the supernatant III, and the FIII were titrated for quantity of viruses by $TCID_{50}$ assay.

Virus filtration

A quantity of 196 mL of partially purified immunoglobulin was spiked with 7.67 log per mL dengue virus at a ratio (vol/vol) of 1:49, followed by filtration with a 0.22-µm filter (Steritop, Millipore) to remove viral aggregates. The filtered immunoglobulin was subject to virus filtration with the 35N filter in a normal-flow manner, under constant pressure of 80 kPa. Samples were titrated for quantity of viruses by $TCID_{50}$ assay or RT-PCR assay.

S/D treatment

Duplicates of 27 mL of the immunoglobulin purified through virus filtration were heated to 28°C in a water bath, followed by spiking with 7.16 or 7.83 log per mL dengue virus at a ratio (vol/vol) of 1:9. Triton X-100 and tri-*n*-butyl phosphate were added drop by drop into the immunoglobulin to a final concentration of 1 and 0.3 percent, respectively. Gentle mixing was achieved with a mechanical stirrer (stainless steel) for the time course of 16-hour treatment at 28 to 30°C, during which samples were removed for virus titration by $TCID_{50}$ assay.

Cation-exchange chromatography

A chromatography column of 10-mm diameter was packed to a bed height of 11 cm with either new CM Sepharose Fast Flow resin (Pharmacia Biotech, Uppsala, Sweden) or the used resin that had previously been recycled 476 times with the immunoglobulin purification process. The column was equilibrated with 20 mmol per L NaAc buffer, pH 4.0. Adjusted to a pH of 4.0 with 1 M HCl and an ionic strength of 1.4 mS per cm with purified water, duplicates of 75 mL of the S/D-treated immunoglobulin solution were spiked with 7.67 or 7.83 dengue virus at a ratio (vol/vol) of 1:20. The virus-spiked immunoglobulin solution was applied to the column at a linear flow rate of 40 cm/hr at ambient temperature. After washing of the column with 10 column volumes of 10 mmol per L glycine, pH 7.0, immunoglobulins were eluted with 100 mmol per L glycine together with 150 mmol per L sodium chloride, pH 9.0. The column load, the flow-through fraction, and the eluate fraction containing immunoglobulins were titrated for quantity of viruses by $TCID_{50}$ assay or RT-PCR assay.

RESULTS

FIV precipitation

After the 40 percent ethanol precipitation of the supernatant II + III, no dengue virus was detected with the $TCID_{50}$ assay in both the supernatant IV and the FIV (Table 1). Despite its direct cytotoxicity to the virus detector Vero E6 cells, when diluted 500-fold, 40 percent ethanol did not affect the determination of virus titer. Results of quantitative RT-PCR clearly showed that genetic materials of dengue virus were concentrated in the FIV (Table 1), which is discarded during the albumin manufacture. Because chemical inactivation by high concentrations of ethanol is mechanistically different from the physical partitioning effects between fractions, this FIV precipitation step provides an extra safety margin in the effective clearance of dengue viruses.

Pasteurization

The kinetics of inactivation of dengue virus in the 20 percent albumin during the 10-hour pasteurization at 59 to 60°C are shown in Fig. 1. The pasteurization was carried out at 0.5°C below what is normally used in the manufacture, representing a worst-case scenario. Dengue

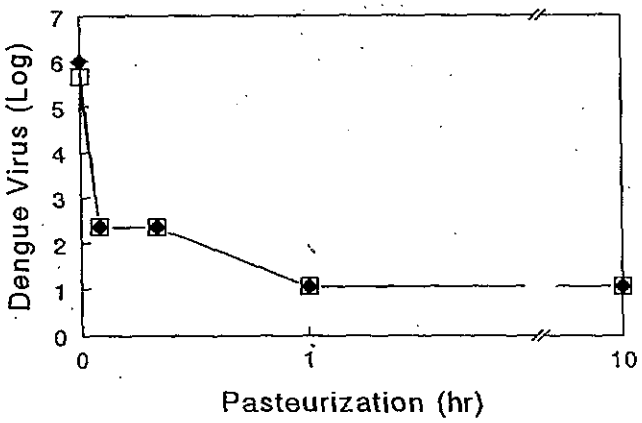


Fig. 1. Inactivation of dengue virus in albumin by pasteurization over time. (□) Bulk pasteurization; (◆) terminal pasteurization. Stock dengue viruses (6.67 or 7.50 log/mL) were spiked at a ratio (vol/vol) of 1:20 and 1:25, respectively, in the bulk pasteurization and terminal pasteurization.

TABLE 1. Clearance of dengue virus in the precipitation of FIV*

Assay (log)	Supernate II + III	FIV	Supernate IV	Log reduction, II + III → supernate IV
$TCID_{50}$	6.83/7.00	2.06†/2.06†	1.65†/1.65†	≥5.18/≥5.35
Quantitative RT-PCR	7.15/8.33	7.40/7.56	3.30/4.98	3.85/3.35

* Data shown are total viral titers (log number multiplied by volume) from duplicate experiments, where stock virus had a titer each of 7.00 log per mL spiked at a ratio of 1:10.
† No dengue virus was detected, and the number was calculated according to the Poisson distribution.

virus was quickly inactivated by the heat treatment, and infectious virus became undetectable within 5 minutes (Fig. 1). Total viral reduction for both the bulk pasteurization and the terminal pasteurization is shown in Table 2.

FIII precipitation

After the 15 percent ethanol precipitation, dengue virus was detectable with the $TCID_{50}$ assay in both the supernatant III and the FIII, with a majority of infectious virus in the FIII. Viral reduction from the FII + III to the supernatant III was calculated and is shown in Table 3.

Virus filtration

The immunoglobulin spiked with dengue virus was processed at 24 to 25°C through the 35N virus filter within 7 hours. No infectious virus was detectable by the $TCID_{50}$ assay in the immunoglobulin filtrate, the sample obtained when the virus filter was reversely flushed with purified water, or the virus-spiked immunoglobulin control standing along the whole virus filtration process. To differentiate physical separation from the chemical inactivation by the low pH, the samples were further quantified for dengue virus with the quantitative RT-PCR assay. The RT-PCR data show that dengue virus was much more concentrated in the back-flush fraction than in the immunoglobulin filtrate. Viral reduction by the virus filtration was calculated and shown in Tables 3 and 4. These results indicate that dengue virus is effectively removed by the 35N virus filtration.

TABLE 2. Clearance of dengue virus in the albumin process*

Process step	Reduction of virus (log)
FIV precipitation	≥5.18†/≥5.35†
Bulk pasteurization	≥4.61
Terminal pasteurization	≥4.94/≥5.44
Cumulative	≥10.12‡/≥10.79‡

* Data are shown from duplicate experiments, except bulk pasteurization, which was conducted once.
† The data from the $TCID_{50}$ assay, but not the RT-PCR assay, are included.
‡ "Bulk pasteurization" is not included in the "cumulative," because it is similar mechanistically to "terminal pasteurization."

TABLE 3. Clearance of dengue virus in the immunoglobulin process*

Process step	Reduction of virus (log)
FIII precipitation	2.16/2.65
Virus filtration	3.37†
S/D treatment	≥5.05/≥5.38
Chromatography	3.66‡/4.18‡
Cumulative	≥14.24/≥15.58

* Data of single virus filtration experiment and duplicate experiments of other processing steps are shown.
† Only RT-PCR data are included.
‡ Virus reduction caused by the presence of S/D is not included.

S/D treatment

The presence of S/D was cytotoxic to the virus detector Vero E6 cells; when diluted 1000-fold, S/D did not affect the determination of virus titer. The kinetics of inactivation of dengue virus in the immunoglobulin during the 16-hour S/D treatment at 28 to 30°C is shown in Fig. 2. Dengue virus was quickly inactivated by the S/D treatment, and infectious virus became undetectable within 1 minute. Total viral reduction for the S/D treatment was shown in Table 3.

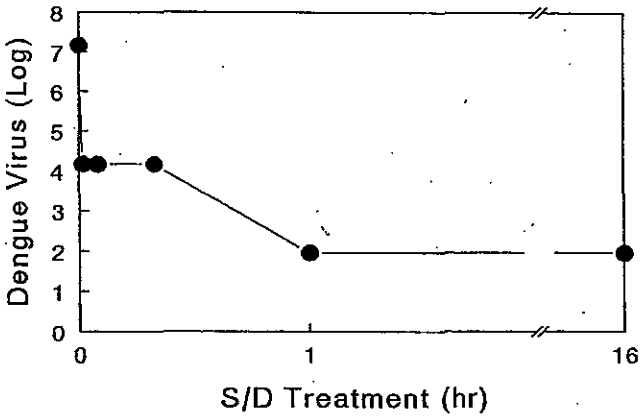


Fig. 2. Inactivation of dengue virus in immunoglobulins by S/D treatment over time. Stock dengue viruses (7.16 log/mL) was spiked at a ratio (vol/vol) of 1:9 in the S/D treatment step; when it was spiked to the immunoglobulin sample neutralized to pH 7.0 a viral titer of 7.00 log was obtained.

TABLE 4. Clearance of dengue virus in the virus filtration*

Assay	Load	Immunoglobulin filtrate	Back-flush	Log reduction, load → filtrate
$TCID_{50}$	8.37	≤2.58†	≤2.51†	≥5.79
Quantitative RT-PCR	8.47	5.10	7.08	3.37

* The numbers shown are total viral titers (log number multiplied by volume), and the stock virus spiked at a ratio of 1:49 had a titer of 7.67 log per mL.
† No dengue virus was detected, and the number was calculated according to the Poisson distribution.

TABLE 5. Clearance of dengue virus in the chromatography*

Assay	Resin	Load	Flow through	Eluate	Log reduction, load → eluate
$TCID_{50}$	New	8.20	≤6.19†	≤1.16†	≥7.04
	Used	8.04	≤6.19†	≤1.11†	≥6.93
Quantitative RT-PCR	New	9.24	8.31	5.58	3.66
	Used	8.99	8.14	4.81	4.18

* The numbers shown are total viral titers (log number multiplied by volume), and the stock virus spiked at a ratio of 1:20 had a titer of 7.67 and 7.83 log per mL for the new and used resins, respectively.
† No dengue virus was detected, and the number was calculated according to the Poisson distribution.

Cation-exchange chromatography

After the chromatography step of the immunoglobulin process, no infectious virus was detectable by the $TCID_{50}$ assay in the column load, the flow-through fraction, the eluate fraction, or the virus-spiked load control standing along the whole chromatography process. Total viral reduction from the column load to the eluate fraction was at least 7.04 and at least 6.93 log for the new resin and the 476-cycled used resin, respectively (Table 5). Because S/D was present in the starting material, the elimination of dengue virus could be a result of inactivation by the chemicals. To differentiate physical removal from chemical inactivation, the samples were further quantified for dengue virus with the quantitative RT-PCR assay. The RT-PCR data show that a majority of dengue virus was observed in the flow-through fraction. Total viral removal by the chromatography process was calculated to be 3.66 and 4.18 log for the new resin and the 476-cycled used resin, respectively (Tables 3 and 5).

DISCUSSION

Viral safety is of paramount importance for human plasma–derived therapeutic proteins such as albumin, α1-proteinase inhibitor, clotting factors, and immunoglobulins. Recent documentation of blood-associated transmission[1-3] and continuous regional outbreaks of dengue fever have prompted a validation of clearance of dengue virus in the manufacture processes of the plasma-derived products. It was the intention of this study to investigate clearance of dengue virus in individual steps of manufacture processes of plasma-derived albumin and immunoglobulins. The results shown in Tables 2 and 3 clearly demonstrate for the first time that specific virus removal and inactivation procedures reduce the risk of dengue virus transmission by more than 10 log cumulatively in plasma-derived albumin and immunoglobulins.

In this study, cold ethanol precipitation is very effective in inactivating dengue virus in the albumin process, but mildly effective in removing dengue virus in the immunoglobulin process. This difference in effectiveness is probably due to the fact that higher concentrations of ethanol were used in the albumin process. It is fairly reasonable to speculate that other therapeutic proteins prepared from plasma by similarly high concentrations of ethanol, for instance, α1-proteinase inhibitor and transferrin purified from the Cohn FIV, would have a good safety margin with regard to transmission of dengue virus.

Pasteurization inactivated dengue virus very quickly and effectively in the albumin process. The presence of a high concentration of albumin or the albumin stabilizing agent sodium caprylate did not seem to protect dengue virus from the heat inactivation. Caprylate has been shown to be an effective virus-inactivating agent at millimolar concentrations under acidic conditions;[9-12] however, caprylate appears unlikely to contribute much to the viral inactivation capacity of the pasteurization step as in the albumin formulation it is used under neutral pH, which do not favor the formation of the active component—the nonionized form of caprylate. As shown by albumin's long history of viral safety in clinical applications, the dedicated viral inactivation step in albumin manufacture processes has been very robust in the inactivation of many different viruses including West Nile virus and bovine viral diarrhea virus (BVDV), both from the same Flaviviridae family as dengue virus.[13,14]

Virus filtration was very effective in separating dengue virus from the immunoglobulin filtrate (Table 4). The data suggest that chemical inactivation by the low pH condition can probably contribute to the viral clearance capacity of this process step. In a separate study with BVDV, which is of similar size but not sensitive to low pH treatment, a majority of the spiked BVDV was trapped in the Planova 35N filter, which was recovered in the back-flush sample (unpublished observation).

Like pasteurization, S/D treatment very quickly and effectively inactivated dengue virus in the immunoglobulin process. This dedicated viral inactivation step in the immunoglobulin manufacture processes has been very robust in the inactivation of many different viruses including West Nile virus and BVDV.[13,14]

The cation-exchange chromatography was originally intended to remove S/D from the immunoglobulin process; however, it was also observed in this study to effectively remove dengue virus by affinity adsorption. In addition, this purification step was mildly effective in the physical removal of BVDV (unpublished observation). Although the chromatographic process may not be a

robust viral removal step in general, it is indeed effective in the clearance of dengue virus.

In summary, this study has shown that effective clearance of dengue virus is achieved in the manufacture processes of albumin and immunoglobulins, providing additional evidence supporting the viral safety of plasma-derived products.

REFERENCES

1. de Wazieres B, Gil H, Vuitton DA, Dupond JL. Nosocomial transmission of dengue from a needlestick injury. Lancet 1998;351:498.
2. Rigau-Perez JG, Vorndam AV, Clark GG. The dengue and dengue hemorrhagic fever epidemic in Puerto Rico, 1994-1995. Am J Trop Med Hyg 2001;64:67-74.
3. Chen LH, Wilson ME. Transmission of dengue virus without a mosquito vector: nosocomial mucocutaneous transmission and other routes of transmission. Clin Infect Dis 2004;39:e56-60.
4. Kuhn RJ, Zhang W, Rossmann MG, Plentnev SV, Corver J, Lenches E, Jones CT. Structure of dengue virus: implications for flavivirus organization, maturation, and fusion. Cell 2002;108:717-25.
5. Lin YL, Lei HY, Wen YY, Luh TY, Chou CK, Liu HS. Light-independent inactivation of dengue-2 virus by carboxyfullerene C3 isomer. Virology 2000;275:258-62.
6. Huang Q, Fu WL, Chen B, Huang JF, Zhang X, Xue Q. Inactivation of dengue virus by methylene blue/narrow bandwidth light system. J Photochem Photobiol B 2004;77:39-43.
7. Manning JS, Collins JK. Effects of cell culture and laboratory conditions on type 2 dengue virus infectivity. J Clin Microbiol 1979;10:235-9.
8. Cohn EJ, Strong LE, Hugles WL, Mulford DJ, Ashworth JN, Melin M, Taylor HL. Preparation and properties of serum and plasma proteins. IV. A system for the separation into fractions of the protein and lipoprotein components of biological tissues and fluids. J Am Chem Soc 1946;68:459-75.
9. Lundblad JL, Seng RL. Inactivation of lipid-enveloped viruses in proteins by caprylate. Vox Sang 1991;60:75-81.
10. Korneyeva M, Hotta J, Lebing W, Rosenthal RS, Franks L, Petteway SR Jr. Enveloped virus inactivation by caprylate: a robust alternative to solvent-detergent treatment in plasma derived intermediates. Biologicals 2002;30:153-62.
11. Lebing W, Remington KM, Schreiner C, Paul HI. Properties of a new intravenous immunoglobulin (IGIV-C, 10%) produced by virus inactivation with caprylate and column chromatography. Vox Sang 2003;84:193-201.
12. Johnston A, Uren E, Johnstone D, Wu J. Low pH, caprylate incubation as a second viral inactivation step in the manufacture of albumin. Parametric and validation studies. Biologicals 2003;31:213-21.
13. Kreil TR, Berting A, Kistner O, Kindermann J. 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 2003;43:1023-8.
14. Remington KM, Trejo SR, Buczynski G, Li H, Osheroff WP, Brown JP, Renfrow H, Reynolds R, Pifat DY. Inactivation of West Nile virus, vaccinia virus and viral surrogates for relevant and emergent viral pathogens in plasma-derived products. Vox Sang 2004;87:10-8.

別紙様式第2-1
番号10

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8月1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: 1348-1354	公表国 プエルトリコ	
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＞
輸血を介したデングウイルス感染が1例報告されている。しかし、流行地域でのデング熱の発生率が高いこと、無症候感染の割合が高いこと、およびウイルス血症の中央値が5日であることから、輸血に関連したデング感染は、報告されているよりもさらに広範囲に拡がっている可能性がある。
＜研究デザインおよび方法＞
2005年9月20日から12月4日までにプエルトリコにある米国赤十字へ提供された全ての血液ドネーションのデングウイルス(DENA) RNAの陽性率を、transcription mediated amplification (TMA) 法と呼ばれる特異タイプの核酸増幅検査を使って測定した。TMAの測定結果が2回くり返して陽性であったものを、TMA法陽性のドネーションと定めた。TMA陽性のドネーションについて、IgM抗体のELISA、RT-PCRおよびウイルス培養による検査を行った。
＜結果＞
検査を行った血液ドネーション16,521検体のうちの12検体（0.07%）が、TMA陽性であった。4検体がRT-PCR陽性（DENVセロタイプ2、3）であった。4つのRT-PCR陽性のうちの3つでウイルスが培養できた。TMA陽性であった12のドネーションのうちの1つがIgM陽性であった。他の輸血ウイルス感染（C型肝炎、WNV）のルーチンのミニプールスクリーニングでされているのと同じように1:16に希釈すると、5つのドネーションだけがTMA陽性であった。
＜結論＞
1,000のドネーション中のほぼ1つがDENV RNAを含み、TMA陽性ドネーションからウイルスが培養できたが、このことは輸血感染のリスクが、WNVについて供血者全員へのスクリーニングがされる前の米国に存在していたリスクに類似していることを示している。WNVと同じく、IgM抗体のスクリーニングは有効でない可能性があり、感染性を有しているドネーションのいくつかはミニプールによって見逃されるであろう。輸血後のデング感染の患者において、輸血感染を考慮すべきである。

報告企業の意見	今後の対応
デングウイルス感染症の流行地域における献血中からデングウイルスが約0.1%の確率で検出されたとの報告である。 血漿分画製剤からのデングウイルス伝播の事例は報告されていない。万一、原料血漿にデングウイルスが混入しても、BVD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。

TRANSFUSION COMPLICATIONS

Dengue virus in blood donations, Puerto Rico, 2005

Hamish Mohammed, Jeffrey M. Linnen, Jorge L. Muñoz-Jordán, Kay Tomashek, Gregory Foster, Amy S. Broulik, Lyle Petersen, and Susan L. Stramer

BACKGROUND: A single instance of transfusion-transmitted dengue infection has been reported. The high incidence of dengue in endemic countries, the high proportion of asymptomatic infection, and the median 5-day viremia, however, suggest that transfusion-associated dengue transmission may be more widespread than documented.

STUDY DESIGN AND METHODS: The prevalence of dengue virus (DENV) RNA was determined in all blood donations to the American Red Cross in Puerto Rico from September 20 to December 4, 2005, using a specific type of nucleic acid amplification test called transcription-mediated amplification (TMA). TMA-positive donations were defined as those having two repeatedly reactive TMA results. TMA-positive donations were tested by enzyme-linked immunosorbent assay for immunoglobulin M (IgM) antibodies, by reverse transcription–polymerase chain reaction (RT-PCR), and by viral culture.

RESULTS: Twelve (0.07%) of 16,521 blood donations tested were TMA-positive. Four were positive by RT-PCR (DENV serotypes 2 and 3). Virus was cultured from 3 of 4 RT-PCR–positive donations. One of the 12 TMA-positive donations was IgM-positive. Only 5 donations remained TMA-positive when diluted 1:16, as is done for routine minipool screening for other transfusion-transmissible viral infections (hepatitis C, human immunodeficiency, West Nile viruses [WNVs]).

CONCLUSION: Nearly 1 in 1000 blood donations contained DENV RNA, and virus could be cultured from TMA-positive donations, suggesting a transfusion transmission risk similar to that which existed in the United States for WNV before universal donation screening. Similar to WNV, IgM antibody screening is likely to be ineffective, and some potentially infectious donations will be missed by minipool screening. Transfusion transmission should be considered in patients with dengue after blood transfusion.

Dengue virus (DENV) is a mosquito-borne flavivirus transmitted by the bite of an infected *Aedes* spp. mosquito. Infection by each of the antigenically distinct serotypes (DENV-1, -2, -3, and -4) confers lifelong serotype-specific immunity. Subsequent infection with another serotype is possible because immunity to heterologous serotypes is short-lived. Most (53%-87%) dengue infections are asymptomatic or mildly symptomatic.[1-3] Dengue infection is characterized by a median 5-day viremia, and in clinically apparent infections, symptom onset occurs 1 day after onset of viremia.[4,5] The clinical spectrum of dengue infection ranges from dengue fever to dengue hemorrhagic fever, dengue shock syndrome, and death. Primary dengue infections often present with features of classic dengue fever including acute onset of fever, arthralgia, myalgia, retroorbital pain, headache, and rash. Subsequent infection with a second dengue serotype increases the risk of developing dengue hemorrhagic fever, which is characterized by fever, thrombocytopenia (platelet count $\leq 100 \times 10^9$/L), hemorrhagic manifestations, and evidence

ABBREVIATIONS: ARC = American Red Cross; DENV = dengue virus; IC = internal control; IR = initially reactive; S/CO ratio = signal-to-cutoff ratio; TMA = transcription-mediated amplification; WNV = West Nile virus.

From the Dengue Branch, Division of Vector-Borne Infectious Disease, Centers for Disease Control and Prevention, San Juan, Puerto Rico; American Red Cross, Gaithersburg, Maryland; Gen-Probe, Inc., San Diego, California; and the Division of Vector-Borne Infectious Disease, Centers for Disease Control and Prevention, Fort Collins, Colorado.

Address reprint requests to: Hamish Mohammed, PhD, Dengue Branch, Division of Vector-Borne Infectious Diseases, National Center for Zoonotic, Vector-Borne, and Enteric Diseases, Centers for Disease Control and Prevention, 1324 Calle Cañada, San Juan, PR 00920; e-mail: hmohammed@cdc.gov.

Conflict of interest: JML and ASB are employees of Gen-Probe, Inc. and are owners of equity stock options in Gen-Probe.

Received for publication December 13, 2007; revision received March 31, 2008, and accepted March 31, 2008.

doi: 10.1111/j.1537-2995.2008.01771.x

TRANSFUSION 2008;48:1348-1354.

of increased vascular permeability and plasma leakage.[6,7] With timely supportive care, dengue hemorrhagic fever case-fatality rates can be reduced to less than 1 percent.[8,9]

The principal dengue vector is *Aedes aegypti*. It is found throughout the tropics and subtropics and in limited areas of some states in the southeastern United States. *Aedes albopictus* is also a competent vector for dengue and has been implicated previously in dengue outbreaks.[10] Although it has not been detected in Puerto Rico, *A. albopictus* exists in some parts of the Americas including more than 20 states in the eastern half of the United States.[11] Autochthonous dengue transmission does sporadically occur in southern Texas along the United States-Mexico border, with the most recent outbreak occurring in the contiguous border towns of Brownsville, Texas, and Matamoros, Tamaulipas (Mexico).[12,13] This suggests the endemicity of dengue in South Texas and the risk of reemergence of dengue in states that border Mexico as well as in southeastern states with competent vector(s) and subtropical climates. Research, however, has found that differences in housing (e.g., use of air conditioning and screens) and lifestyle may prevent this from happening.[12,13]

Although few reports document DENV transmission through receipt of infected blood,[14] tissues,[15] or organs,[16] transfusion-associated dengue transmission may be more common than previously recognized. The high proportion of asymptomatic infections, the median 5-day period of detectable viremia, and the high incidence, especially during outbreaks, suggest that a substantial number of donors could be viremic at the time of donation. In addition, nosocomial transmission of DENV via needle-stick injury[17-21] further indicates the transmissibility of DENV by infected blood. Viremic individuals may unknowingly donate blood before symptom onset or if they remain asymptomatic. West Nile virus (WNV), a related mosquito-borne flavivirus, may provide a useful model for assessing transfusion-associated DENV transmission. Transfusion transmission of WNV is well documented, and all blood donations in the United States are screened using WNV-specific nucleic acid amplification tests (NATs).[22,23]

Dengue was first identified in Puerto Rico in 1963 and is now endemic year-round with occasional islandwide outbreaks. A mean of 5446 (range, 2416-10,048) suspected cases were reported annually during the nonoutbreak years from 1990 to 2004, whereas 6039 cases were reported in 2005 (incidences of 151 versus 159 per 100,000 population/year). Approximately 77,000 blood donations are collected annually by the American Red Cross (ARC) collection centers and blood donation drive sites in Puerto Rico. These donations are used in the continental United States, Puerto Rico, and elsewhere in the Caribbean. To assess the potential for transfusion-associated dengue infection in Puerto Rico, we tested all blood donations to the ARC for dengue viral nucleic acid using a recently developed dengue-specific NAT during an 11-week period of seasonally heightened dengue activity in 2005.

MATERIALS AND METHODS

We analyzed demographic data collected from blood donors and plasma specimens from all blood donations to ARC blood collection centers and blood drives in Puerto Rico from September 20 to December 4, 2005. This study period commenced 2 weeks after the peak of seasonally heightened dengue activity in Puerto Rico (Fig. 1). Plasma specimens containing ethylenediaminetetraacetate as an anticoagulant (BD Vacutainer PPT plasma preparation tubes, BD, Franklin Lakes, NJ) from all blood donations during this study period were retained in a repository at the ARC facility in Gaithersburg, Maryland.

All specimens were first screened for the presence of DENV RNA using a DENV-specific NAT developed by Gen-Probe, Inc. (San Diego, CA) that uses transcription-mediated amplification (TMA). Specimens were tested by TMA at Gen-Probe by trained ARC staff. All initially reactive (IR) specimens were retested and TMA-positive

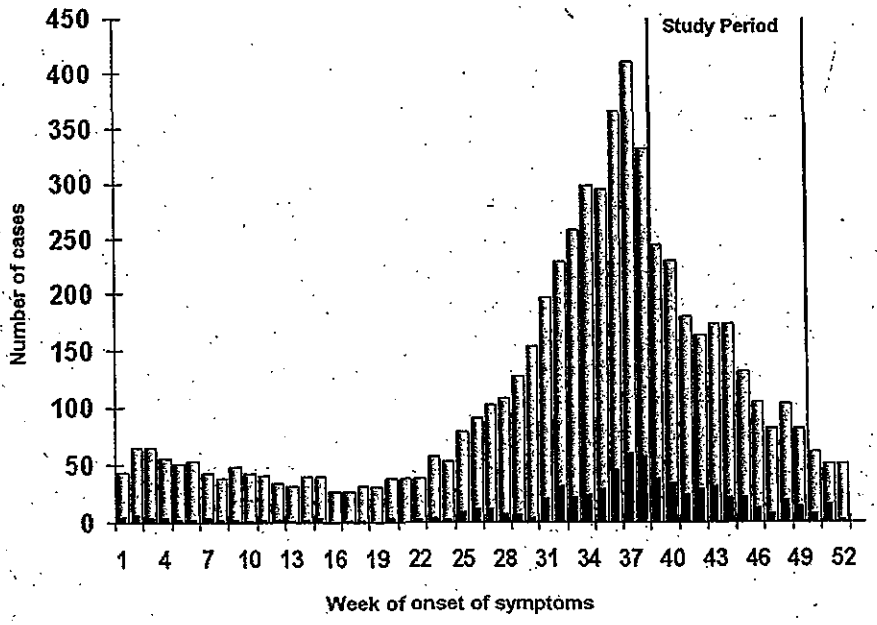


Fig. 1. Number of suspected* (□) and confirmed† (■) dengue cases by week of symptom onset, Puerto Rico, 2005. *Suspected = reported case of dengue with a clinical suspicion of dengue. †Confirmed = laboratory-confirmed (by serology or virology) case of dengue.

specimens were those that were repeatedly reactive; all others were considered to be TMA-negative. Both initial and repeat TMA screening were performed using individual specimens. All IR specimens were sent to the CDC's Dengue Branch Laboratory in San Juan, Puerto Rico, for supplemental testing by reverse transcription–polymerase chain reaction (RT-PCR).[24] Testing of donations was unlinked to donor personal identifiers; thus, subsequent contact with donors or recipients was not possible. Deidentified data from blood donation records were used in the statistical analysis (described below). The data were stored on a single password-protected terminal at the CDC, and no attempt was made to trace the donors. The study protocol was approved by the Institutional Review Board of the ARC.

TMA

Testing was performed using a prototype dengue TMA assay on a fully automated system for NAT blood screen (Procleix Tigris system, Chiron Corp., Emeryville, CA). The assay uses the same chemistry as other human immunodeficiency virus-1/hepatitis C virus and WNV assays (Procleix and Ultrio, respectively, Chiron Corp.)[25-27] and targets sequences that are conserved across all four serotypes. Thus the assay used is capable of detecting all four dengue serotypes. TMA is an isothermal RNA transcription amplification system using bacteriophage T7 RNA polymerase and Moloney murine leukemia virus reverse transcriptase (MMLV RT) to produce RNA amplicons via DNA intermediates. Viral lysis and magnetic-based target capture of viral RNA are followed by amplification and detection with the use of chemiluminescent probes.[26] This technique is able to detect 3.4 West Nile viral copies per mL at a 50 percent detection rate.[28] The analytical sensitivity of the DENV TMA assay used in this study is very similar, with 50 percent detection at 3.5 viral RNA copies per mL and a sample volume of 0.50 mL.[29] Assay results were reported in relative light units, which were used to derive signal-to-cutoff (S/CO) ratios. Cutoff values for the Dengue TMA assay internal control (IC) and analyte signals were calculated using the same formulae used for the Procleix WNV Assay.[30] A sample was considered reactive if the analyte S/CO ratio was at least 1.0, nonreactive if the analyte S/CO ratio was less than 1.0 and the IC signal was above the IC cutoff, and invalid if the analyte S/CO ratio value was less than 1.0 and the IC signal was below the IC cutoff.

Supplemental testing

All TMA-positive specimens were retested at a 1:16 dilution in plasma screened negative for all infectious disease markers including dengue RNA at Gen-Probe to determine the efficacy of testing blood donations by minipooled methods. The TMA-positive and IR specimens were tested using a real-time RT-PCR assay for the detection of NS5 gene sequence (TaqMan, Applied Biosystems, Foster City, CA).[24] This RT-PCR test is multiplexed and detects the four dengue serotypes in one reaction. It can also be used to quantitatively measure viral RNA in blood specimens with a sensitivity of approximately 1×10^3 to 5×10^3 viral RNA copies per mL. The sample volume is 20 µL derived from a 100-µL RNA extract obtained from a 0.24-mL serum specimen. All TMA-positive and IR specimens were also tested for the presence of immunoglobulin M (IgM) and immunoglobulin G (IgG) antibody using IgM MAC–enzyme-linked immunosorbent assay (ELISA) and IgG ELISA, respectively.[31,32] Virus was isolated on C6/36 cells and by mosquito isolation.[33,34]

Statistical analysis

A trend analysis using simple linear regression was performed to determine if there was a change in the number of blood donations collected during the study period. The prevalence of DENV RNA was determined by dividing the number of TMA-positive donations by the number of blood donations collected during the study period. Information about donor characteristics (see Table 1) was obtained from the ARC's electronic donor database and included date of collection, gender, date of birth, zip code of residence, zip code of donation site, donation status (first-time donor or repeat donor), phlebotomy procedure (whole blood, plateletpheresis, or leukapheresis), and donation type (allogeneic, directed, or autologous). Both donor residence and donation site were recoded into the three regions of Puerto Rico set by the United States Postal Service (San Juan Metropolitan Area, west, and east) and then treated as binary variables in the analyses (i.e., San Juan metropolitan area versus other). Age was stratified by its median and considered as a binary categorical variable in analyses.

Differences in TMA positivity by donor characteristics were assessed by the Fisher's exact test and exact logistic regression. Potential covariates identified for inclusion in the final multivariable model included covariates with a p value less than 0.20 on bivariate analysis. Age was added to the model a priori given its association with dengue infection.[2] All comparisons were made with the use of a two-tailed test, and a Type I error rate of 0.05 was used to assess significance.

RESULTS

A total of 16,521 blood donations were collected during the 11-week study period (mean, 1502 donations per week [range, 281-1864] without a significant trend in donation frequency). Twelve donations (0.73 per 1000 donations) were TMA-positive, with two or less identified per week. Eleven of these 12 donations were whole

blood-collections, while the other was a plateletpheresis from an O-donor. Donor and donation characteristics were similar among the TMA-positive and -negative donations ($p > 0.05$ for all variables, Fisher's exact test). In a multivariable model adjusted for age, donors with residence in the San Juan metropolitan area were approximately three times more likely than donors residing outside of the metropolitan area to be TMA-positive (adjusted odds ratio, 3.0; 95% confidence interval, 0.9-10.1).

The five blood donations that had the highest S/CO ratios on initial TMA testing were the only specimens to be TMA-reactive at a dilution of 1:16 (Table 2). Four of these five specimens were positive by RT-PCR and had quantifiable viral loads ranging from 2×10^3 to 8×10^7 viral RNA copies per mL. Three were identified as DENV-2 and the other as DENV-3. DENV was cultured from three of the four specimens, two by mosquito inoculation and one in cell culture. DENV-2 and DENV-3 were the predominant serotypes in circulation in Puerto Rico in 2005.

Serologic testing of the 12 TMA-positive blood donations revealed that only 1 was IgM-positive and 9 were IgG-positive by ELISA (Table 2). The lack of IgG antibody titers in Specimens 1, 4, and 8 indicates no previous dengue infections in these patients. The presence of IgG antibodies in the absence of IgM antibodies could reflect evidence of previous infections in Specimens 2, 3, 5, 7, 9, 10, 11, and 12, and IgG titers equal or greater than 1:163,840 in Specimens 3, 7, 9, and 10 indicate a recent or current secondary infection in those patients.[32] The presence of IgG in the sole donor with IgM antibodies (Specimen 6) could similarly be reflective of recent or current infection.

Other than the 12 TMA-positive specimens, there were an additional three IR specimens with S/CO ratios on initial testing of 1.00, 1.03, and 11.58 and on repeat testing of 0.92, 0.40, and 0.07, respectively. All were negative on PCR, IgM MAC-ELISA, and virus recovery. They were, however, positive on IgG ELISA. In the WNV TMA assay, an S/CO ratio of greater than or equal to 17 has a positive predictive value for confirmation of 95 percent (ARC data, unpublished); it is likely that this relationship is the same for DENV TMA.

TABLE 1. Characteristics of all and TMA-positive blood donors in Puerto Rico, September 20 to December 5, 2005*

Characteristic	All donors (n = 16,521)	TMA-positive donors (n = 12)
Age (years)	37.0 (13-85)	36.5 (16-65)
Male	10,654 (64.5)	8 (67)
Donation status		
First-time donor	5,056 (30.6)	5 (42)
Repeat donor	11,465 (69.4)	7 (58)
Region of residence		
San Juan Metropolitan Area	6,631 (40.1)	8 (67)
East	5,182 (31.4)	3 (25)
West	4,706 (28.5)	1 (8)
Phlebotomy procedure		
Whole blood	15,838 (95.9)	11 (92)
Plateletpheresis	627 (3.8)	1 (8)
Plateletpheresis/RBC pheresis	48 (0.3)	0 (0)
Double RBC pheresis	7 (0.0)	0 (0)
Leukapheresis	1 (0.0)	0 (0)
Donation type		
Allogeneic	16,400 (99.3)	12 (100)
Directed	67 (0.4)	0 (0)
Autologous	54 (0.3)	0 (0)
Region of donation site		
San Juan Metropolitan Area	8,984 (54.4)	8 (67)
East	3,870 (23.4)	4 (33)
West	3,667 (22.2)	0 (0)

* Data are reported as median (range) or number (%).

TABLE 2. Results of supplementary testing of TMA IR specimens (n = 12)

	TMA test Gen-Probe (S/CO ratio)*			Supplementary testing CDC dengue branch					
Specimen	Initial test	Second test	1:16	PCR†	Number viral RNA/mL	IgM‡	IgG	Cell culture	Mosquito inoculation
1	31.96	26.99	27.73	D2	7.14×10^3	0.229	Negative	Negative	D2
2	30.31	31.28	28.78	D3	8.12×10^7	0.337	1:10,240	Negative	D3
3	29.22	27.86	27.12	D2	7.74×10^5	0.409	1:163,840	D2	Negative
4	29.17	24.84	22.92	D2	2.0×10^3	0.229	Negative	Negative	Negative
5	23.89	20.59	8.54	Negative	Undetected	0.469	1:2,560	Negative	Negative
6	21.22	5.28	0.21	Negative	Undetected	8.870	1:160	Negative	Negative
7	17.78	23.10	0.15	Negative	Undetected	0.409	1:655,360	Negative	Negative
8	17.41	18.44	0.31	Negative	Undetected	0.198	Negative	Negative	Negative
9	17.24	21.05	0.33	Negative	Undetected	1.540	1:163,840	Negative	Negative
10	5.97	7.73	0.15	Negative	Undetected	0.440	1:655,360	Negative	Negative
11	4.08	4.15	0.13	Negative	Undetected	0.368	1:10,240	Negative	Negative
12	1.53	5.56	0.60	Negative	Undetected	0.270	1:2,560	Negative	Negative

* S/CO ≥ 1 considered to be reactive.
† D2 = DENV-2, D3 = DENV-3.
‡ >2.000 considered positive.

Nine of the 12 repeat-reactive samples had S/CO values in one or both tests of 17 or greater.

DISCUSSION

This study, and a similar one recently conducted using donations in Honduras, Brazil, and Australia,[29] are the first to document the presence of dengue viral nucleic acid in blood donations. In Puerto Rico, nearly 1 in 1000 donations was positive for the presence of dengue viral nucleic acid by TMA. Furthermore, live virus was recovered from three of the 12 TMA-positive donations, indicating that at least these 3 were capable of transmitting infection to recipients. The prevalence of dengue viral nucleic acid in blood donations in this study was similar to that estimated for WNV in the areas experiencing outbreaks in the continental United States in 2002[35] before universal screening using minipool NAT was implemented in July 2003.[22] Assuming an annual prevalence rate of 0.73 per 1000 (as found in this study) and that each donation is made into a mean of 1.45 transfusable components,[36] there may be as many as 56 potentially viremic donations and 81 components generated from the approximately 77,000 blood donations collected annually by the ARC in Puerto Rico. Dengue incidence is highly seasonal and varies considerably from year to year,[37,38] however, so the prevalence of potentially viremic donors could be considerably higher or lower than this figure at any given time. Furthermore, the three IR specimens lacking reproducible results in repeat TMA testing may have been true-positive specimens but with lower viral loads. If the case, this would underestimate the true prevalence of TMA positivity.

The unlinked study design did not permit contact with the recipients of the TMA-positive donations to assess whether transmission occurred. Nevertheless, virus was cultured from three donations and the viral loads of the four RT-PCR–positive donations indicate that their transfusion would have resulted in inocula orders of magnitude greater than the amount of virus secreted in the saliva of *Aedes* mosquitoes, documented to be as low as 10^2 viral particles per secretion.[39] The RT-PCR assay used in this study had lower sensitivity than the TMA assay, and it was not possible to assess the viral load of the RT-PCR–negative specimens.

Our results indicate the feasibility of NAT as a screening strategy for DENV, as has been successfully used for WNV. Of concern, we found that simulated minipool NAT (dilution 1:16) would not have detected the majority (7 of 12, or 58%) of the TMA-positive specimens; however, the experience with WNV suggests that not all of these donations may be infectious. Approximately 30 percent of WNV NAT–positive donations have viral loads below the limits of detection by minipool NAT and can only be detected by screening of individual donations.[23,40] Although WNV has been transmitted from transfusions detectable only by individual unit screening and with an estimated level of viremia as low as 0.06 plaque-forming units (PFUs) per mL (1 PFU is approximately 400 viral copies),[41] most donations only detectable by individual unit screening had IgM and IgG antibodies and were likely not infectious given the fact that nearly all WNV transfusion transmissions have occurred from antibody-negative donations.[22,25,42] Unfortunately, this same marker of infectivity is not applicable to dengue because of the high prevalence of preexisting, cross-reactive dengue antibodies in the population and the complex and variable serologic response after secondary dengue infection.[32,43]

The global incidence of dengue has risen more than 30-fold in the past 50 years. In areas where dengue is endemic, however, transfusion transmission of the agent is rarely investigated for many reasons, including the fact that this mode of transmission is difficult to prove against a background of endemic dengue. In such cases, the distinction between a recipient infection via mosquito-borne transmission as opposed to transfusion transmission may be too complex to distinguish. Furthermore, many dengue-endemic countries lack hemovigilance systems with sufficient resources to investigate cases of recipient infection that are potentially related to transfusion of blood components. Finally, sophisticated laboratory testing may not be readily available in many dengue-endemic countries and such testing is required to distinguish dengue from other arboviral infections as well as distinguishing current dengue infection from prior infections.

In contrast, when WNV entered the United States, it was against a background of a naïve population. This permitted the laboratory linkage of multiple transfusion recipients with WNV infection to a single infected donor within several clusters of WNV cases. Infectious virus and/or viral RNA could also be recovered from retrieved cocomponent plasma units; in these cases, WNV was readily identified in the absence of competing arboviral infections. The transmissibility of WNV via blood transfusion has been established, and our findings documenting the presence of DENV RNA in the Puerto Rican blood supply, at a level comparable to that which triggered screening of the US blood supply for WNV in 2003, highlight the risks to transfusion safety posed by emerging diseases such as the vector-borne flaviviruses. Further evaluation is required to assess the risk of dengue transmission by TMA-positive donations and the cost and benefit of routine dengue screening in endemic regions.

ACKNOWLEDGMENTS

The authors acknowledge the following individuals for their contribution to this research: Mrs Dilia Magaly Borges and Mr Antonio de Vera (ARC-Puerto Rico) for providing data specific to the PR-region of ARC; Dr Mark Beatty (Pediatric Dengue Vaccine

Initiative) for assisting with protocol development; Mr Gilberto Santiago and Mr Mark Verduin (Dengue Branch, CDC) for laboratory support; Mr Michael Johansson (Dengue Branch, CDC) for statistical support; and Dr Ed Notari (ARC), Dr Mathew Kuehnert (Office of Blood, Organ, and other Tissue Safety (proposed), CDC), Mr Joshua Smith, Dr Kate McElroy, Dr D. Fermín Argüello, and Dr Wellington Sun (Dengue Branch, CDC) for their scientific input in preparing the manuscript.

REFERENCES

1. Endy TP, Chunsuttiwat S, Nisalak A, Libraty DH, Green S, Rothman AL, Vaughn AL, Ennis FA. Epidemiology of inapparent and symptomatic acute dengue virus infection: a prospective study of primary school children in Kamphaeng phet, Thailand. Am J Epidemiol 2002;156:40-51.
2. Rodriguez-Figueroa L, Rigau-Perez JG, Suarez EL, Reiter P. Risk factors for dengue infection during an outbreak in Yanes, Puerto Rico in 1991. Am J Trop Med Hyg 1995;52: 496-502.
3. Burke DS, Nisalak A, Johnson DE, Scott RM. A prospective study of dengue infections in Bangkok. Am J Trop Med Hyg 1988;38:172-80.
4. Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, Endy TP, Raengsakulrach B, Rothman AL, Ennis FA, Nisalak A. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. J Infect Dis 2000;181:2-9.
5. Nishiura H, Halstead SB. Natural history of dengue virus (DENV)-1 and DENV-4 infections: reanalysis of classic studies. J Infect Dis 2007;195:1007-13.
6. Halstead S. Dengue hemorrhagic fever—a public health problem and a field for research. Bull World Health Organ 1980;58:1-21.
7. Haemorrhagic D. Fever: diagnosis, treatment, prevention and control, 2nd ed. Geneva, Switzerland: World Health Organization; 1997.
8. Thang Hung N, Trong Lan N. Improvement of case management—a key factor to reduce case-fatality rate of dengue hemorrhagic fever in Southern Viet Nam. Dengue Bull 2003;27:144-8.
9. Chareonsook O, Foy H, Teeratkul A, Silarug N. Changing epidemiology of dengue hemorrhagic fever in Thailand. Epidemiol Infect 1999;122:161-6.
10. Effler PV, Pang L, Kitsutani P, Vorndam V, Nakata M, Ayers T, Elm J, Tom T, Reiter P, Rigau-Perez JG, Hayes JM, Mills K, Napier M, Clark GG, Gubler DJ; Hawaii Dengue Outbreak Investigation Team. Dengue fever, Hawaii, 2001-2002. Emerg Infect Dis 2005;11:742-9.
11. Moore C. Aedes albopictus in the United States: current status and prospects for further spread. J Am Mosq Control Assoc 1999;15:221-7.
12. Ramos M, Mohammed H, Zielinski-Gutierrez E, Hayden MH, Lopez JL, Fournier M, Trujillo AR, Burton R, Brunkard JM, Anaya-Lopez L, Banicki AA, Morales PK, Smith B, Munoz JL, Waterman SH, The Dengue Serosurvey Working Group. Epidemic dengue and dengue hemorrhagic fever at the Texas-Mexico Border: results of a household-based seroepidemiological survey, December 2005. Am J Trop Med Hyg 2008 Mar;78(3):364-9.
13. Reiter P, Lathrop S, Bunning M, Biggerstaff B, Singer D, Tiwari T, Baber L, Amador M, Thirion J, Hayes J, Seca C, Mendez J, Ramirez B, Robinson J, Rawlings J, Vorndam V, Waterman S, Gubler D, Clark G, Hayes E. Texas lifestyle limits transmission of dengue virus. Emerg Infect Dis 2003; 9:86-9.
14. Tsang C. Local dengue fever cases in 2002. Hong Kong, China: Public Health and Epidemiology Bulletin—Department of Health; 2002.
15. Rigau-Perez JG, Vorndam AV, Clark GG. The dengue and dengue hemorrhagic fever epidemic in Puerto Rico, 1994-1995. Am J Trop Med Hyg 2001;64:67-74.
16. Tan FL-S, Loh DL, Prabhakaran K. Dengue haemorrhagic fever after living donor renal transplantation. Nephrol Dial Transplant 2005;20:447-8.
17. Chen LN, Wilson ME. Nosocomial mucutaneous transmission and other routes of transmission. Emerg Infect Dis 2005;11:775.
18. De Wazieres B, Gil H, Vuitton D, Dupond J. Nosocomial transmission of dengue from a needle stick injury. Lancet 1998;351:498.
19. Nemes Z, Kiss G, Madarassi EP, Peterfi Z, Ferenczi E, Bakonyi T, Ternak G. Nosocomial transmission of dengue [letter]. Emerg Infect Dis 2004;10:1880-1.
20. Wagner D, de With K, Huzly D, Hufert F, Weidmann M, Breisinger S, Eppinger S, Kern WV, Bauer TM. Nosocomial acquisition of dengue. Emerg Infect Dis 2004;10:1872-3.
21. Hirsch J, Deschampes C, Lhuillier M. Metropolitan transmission of dengue by accidental inoculation at a hospital. Ann Intern Med 1990;141:629.
22. Pealer LN, Marfin AA, Petersen LR, Lanciotti RS, Page PL, Stramer SL, Stobierski MG, Signs K, Newman B, Kapoor H, Goodman JL, Chamberland ME; West Nile Virus Transmission Investigation Team. Transmission of West Nile virus through blood transfusion in the United States in 2002. N Engl J Med 2003;349:1236-45.
23. Stramer SL, Fang CT, Foster GA, Wagner AG, Brodsky JP, Dodd RY. West Nile virus among blood donors in the United States, 2003 and 2004. N Engl J Med 2005;353:451-9.
24. Chien LJ, Liao TL, Shu PY, Huang JH, Gubler DJ, Chang GJ. Development of real-time reverse transcriptase PCR assays to detect and serotype dengue viruses. J Clin Microbiol 2006;44:1295-304.
25. Busch MP, Tobler LH, Saldanha J, Caglioti S, Shyamala V, Linnen JM, Gallarda J, Phelps B, Smith RI, Drebot M, Kleinman SH. Analytical and clinical sensitivity of West Nile virus RNA screening and supplemental assays available in 2003. Transfusion 2005;45:492-9.
26. Giachetti C, Linnen JM, Kolk DP, Dockter J, Gillotte-Taylor K, Park M, Ho-Sing-Loy M, McCormick MK, Mimms LT,

McDonough SH. Highly sensitive multiplex assay for detection of human immunodeficiency virus type 1 and hepatitis C virus RNA. J Clin Microbiol 2002;40:2408-19.
27. McCormick MK, Dockter J, Linnen JM, Kolk D, Wu Y, Giachetti C. Evaluation of a new molecular assay for detection of human immunodeficiency virus type 1 RNA, hepatitis C virus RNA, and hepatitis B virus DNA. J Clin Virol 2006;36:166-76.
28. Linnen JM, Deras ML, Cline J, Wu W, Broulik AS, Cory RE, Knight JL, Cass MM, Collins CS, Giachetti C. Performance evaluation of the PROCLEIX West Nile virus assay on semi-automated and automated systems. J Med Virol 2007;79: 1422-30.
29. Linnen JM, Vinelli E, Sabino EC, Tobler LH, Hyland C, Lee TH, Kolk DP, Broulik AS, Collins CS, Lanciotti RS, Busch MP. Dengue viremia in blood donors from Honduras, Brazil and Australia. Transfusion DOI: 10.1111/j.1537-2995.2008.01772.x
30. Linnen JM, Deras ML, Cline J, Wu W, Broulik AS, Cory RE, Knight JL, Cass MM, Collins CS, Giachetti C. Performance evaluation of the PROCLEIX West Nile virus assay on semi-automated and automated systems. J Med Virol 2007;79: 1422-30.
31. Burke DS, Nisalak A, Ussery MA. Antibody capture immunoassay detection of Japanese encephalitis virus immunoglobulin M and G antibodies in cerebrospinal fluid. J Clin Microbiol 1982;16:1034-42.
32. Miagostovich MP, Nogueira RM, dos Santos FB, Schatzmayr HG, Araujo ES, Vorndam V. Evaluation of an IgG enzyme-linked immunosorbent assay for dengue diagnosis. J Clin Microbiol 1999;14:183-9.
33. Rosen L, Gubler D. The use of mosquitoes to detect and propagate dengue viruses. Am J Trop Med Hyg 1974;23: 1153-60.
34. Gubler DJ, Kuno G, Sather GE, Velez M, Oliver A. Mosquito cell cultures and specific monoclonal antibodies in surveillance for dengue viruses. Am J Trop Med Hyg 1984;33:158-65.
35. Biggerstaff BJ, Petersen LR. Estimated risk of transmission of the West Nile virus through blood transfusion in the US, 2002. Transfusion 2003;43:1007-17.
36. Marshall DA, Kleinman SH, Wong JB, AuBuchon JP, Grima DT, Kulin NA, Weinstein MC. Cost-effectiveness of nucleic acid test screening of volunteer blood donations for hepatitis B, hepatitis C and human immunodeficiency virus in the United States. Vox Sang 2004;86:28-40.
37. Rigau-Perez JG. Dengue activity in Puerto Rico during an interepidemic period (1995-1997). Am J Trop Med Hyg 2001;64:75-83.
38. Rigau-Perez JG. The reappearance of dengue-3 and a subsequent dengue-4 and dengue-1 epidemic in Puerto Rico in 1998. Am J Trop Med Hyg 2002;67:355-62.
39. Kraiselburd E, Gubler DJ, Kessler MJ. Quantity of dengue virus required to infect rhesus monkeys. Trans R Soc Trop Med Hyg 1985;79:248-51.
40. Busch MP, Caglioti S, Robertson EF, McAuley JD, Tobler LH, Kamel H, Linnen JM, Shyamala V, Tomasulo P, Kleinman SH. Screening the blood supply for West Nile virus RNA by nucleic acid amplification testing. N Engl J Med 2005;353:460-7.
41. Montgomery SP, Brown JA, Kuehnert M, Smith TL, Crall N, Lanciotti RS, Macedo de Oliveira A, Boo T, Marfin AA; 2003 West Nile Virus Transfusion-Associated Transmission Investigation Team. Transfusion-associated transmission of West Nile virus, United States 2003 through 2005. Transfusion 2006;46:2038-46.
42. Petersen LR, Epstein JS. Problem solved? West Nile virus and transfusion safety. N Engl J Med 2005;353:516-7.
43. Gubler DJ, Sather GE. Laboratory diagnosis of dengue and dengue hemorrhagic fever. In: Homma A, Cunha JF, editors. Proceedings of the international symposium on yellow fever and dengue. Rio de Janeiro, Brazil: Rio de Janeiro; 1988. p. 291-322.

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		回	報告日 年　月　日	第一報入手日 2008 年 6 月 13 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	A clinical trial of a whole-virus H5N1 vaccine derived from cell culture. Ehrlich, H.J. et al, New Engl. J. Med., 358, 2573-2584 (2008)	公表国 オーストリア	
販売名（企業名）						

研究報告の概要

本稿では，抗 H5N1 型鳥インフルエンザワクチンの安全性及び有効性を検討する無作為化用量漸増第 1・2 相試験の試験成績について記載した。Baxter Bioscience 社が同ワクチンを開発し，試験を実施した。当該ワクチンの主な特徴は，サルの腎臓細胞の培養株（ベロ細胞）で作成された自然発生するウイルス株 A/Vietnam/1203/2004 を利用していることである。ミョウバンアジュバントによる作用も検討し，ウイルス全体をワクチンとして使用した。有効性エンドポイントとして，ワクチンの（i）ヘマグルチニン阻害を生じさせる能力，（ii）中和抗体を誘発する能力，（iii）注射 21 日後にセロコンバージョンを生じさせる能力を検討した。各被験者に対し，それぞれ 3.75，7.5，15 又は 30 ug のヘマグルチニン抗原を含有するワクチンをアジュバントとともに，もしくは 7.5 又は 15 ug の抗原を含有するワクチンをアジュバントなしで 21 日の間隔をおいて 2 回投与した。免疫寛容は非常に良好に成立した。いずれのワクチン処方でも，注射部位の軽度疼痛（被験者の 9～27%）及び頭痛（被験者の 6～31%）が最も高頻度に報告された有害事象であった。有効性に関する限り，免疫応答はアジュバントなしの処方を投与した被験者において最も高い割合で認められたが，いずれの処方でも第 21 日目から第 42 日目で中和抗体の抗体価は同程度に増加した。さらに，ウイルス株 A/Indonesia/05/2005 及び A/Hong Kong/156/1997 に対する交差中和が認められた。再度，アジュバントなしの処方が最も高い免疫原性を示した。本試験では重要な用量反応性の関連が示されなかったことから，今後の開発にあたってアジュバントなしの 7.5 ug の処方が選択された。

使用上の注意記載状況・その他参考事項等

BYL-2008-0338

報告企業の意見	今後の対応
抗 H5N1 型鳥インフルエンザワクチンが利用可能となれば、パンデミックの発生及び拡大を防ぐために有効であろう。血漿由来製剤工程におけるウイルス除去は、インフルエンザウイルス除去に対しても有効と考えられる。弊社製品の製造工程に使用されている血漿分画成分は，製造工程中のウイルスバリデーションにおいて，インフルエンザウイルスと同様のエンベロープ RNA ウイルスである HIV（レトロウイルス）の不活化・除去能が確認されている。各成分の製造工程における不活化・除去能は以下のとおり。 • アルブミン・カッター及びコージネイト FS の製造工程培地に使用されているヒト血清アルブミン：17.8 log 以上 • プラスマネート・カッター，コージネイト FS 及びコージネイト FS バイオセットの製造工程培地に使用されている加熱ヒト血漿タンパク：15 log 以上 • コージネイト FS の製造工程に使用されているトランスフェリン：9.1 log 以上 • ベタフェロン皮下注，ゼヴァリン イットリウム (^{90}Y) 静注用セット及びゼヴァリン インジウム (^{111}I) 静注用セットの製造工程に使用されているヒト血清アルブミン：9.98 log 以上	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。

BYL-2008-0338

ORIGINAL ARTICLE

A Clinical Trial of a Whole-Virus H5N1 Vaccine Derived from Cell Culture

Hartmut J. Ehrlich, M.D., Markus Müller, M.D., Helen M.L. Oh, M.D., Paul A. Tambyah, M.B., B.S., Christian Joukhadar, M.D., Emanuele Montomoli, Ph.D., Dale Fisher, F.R.A.C.P., Greg Berezuk, M.S., Sandor Fritsch, Ph.D., Alexandra Löw-Baselli, Ph.D., Nina Vartian, Ph.D., Roman Bobrovsky, Ph.D., Borislava G. Pavlova, Ph.D., Eva Maria Pöllabauer, M.D., Otfried Kistner, Ph.D., and P. Noel Barrett, Ph.D., for the Baxter H5N1 Pandemic Influenza Vaccine Clinical Study Team

ABSTRACT

BACKGROUND

Widespread infections of avian species with avian influenza H5N1 virus and its limited spread to humans suggest that the virus has the potential to cause a human influenza pandemic. An urgent need exists for an H5N1 vaccine that is effective against divergent strains of H5N1 virus.

METHODS

In a randomized, dose-escalation, phase 1 and 2 study involving six subgroups, we investigated the safety of an H5N1 whole-virus vaccine produced on Vero cell cultures and determined its ability to induce antibodies capable of neutralizing various H5N1 strains. In two visits 21 days apart, 275 volunteers between the ages of 18 and 45 years received two doses of vaccine that each contained 3.75 μg, 7.5 μg, 15 μg, or 30 μg of hemagglutinin antigen with alum adjuvant or 7.5 μg or 15 μg of hemagglutinin antigen without adjuvant. Serologic analysis was performed at baseline and on days 21 and 42.

RESULTS

The vaccine induced a neutralizing immune response not only against the clade 1 (A/Vietnam/1203/2004) virus strain but also against the clade 2 and 3 strains. The use of adjuvants did not improve the antibody response. Maximum responses to the vaccine strain were obtained with formulations containing 7.5 μg and 15 μg of hemagglutinin antigen without adjuvant. Mild pain at the injection site (in 9 to 27% of subjects) and headache (in 6 to 31% of subjects) were the most common adverse events identified for all vaccine formulations.

CONCLUSIONS

A two-dose vaccine regimen of either 7.5 μg or 15 μg of hemagglutinin antigen without adjuvant induced neutralizing antibodies against diverse H5N1 virus strains in a high percentage of subjects, suggesting that this may be a useful H5N1 vaccine. (ClinicalTrials.gov number, NCT00349141.)

From the Department of Global Research and Development, Baxter BioScience (H.J.E., G.B., S.F., A.L.-B., N.V., R.B., B.G.P., E.M.P., O.K., P.N.B.), and the Department of Clinical Pharmacology, Medical University of Vienna, Vienna General Hospital (M.M., C.J.) — both in Vienna; Changi General Hospital (H.M.L.O.) and the National University of Singapore and National University Hospital (P.A.T., D.F.) — all in Singapore; and the University of Siena, Siena, Italy (E.M.). Address reprint requests to Dr. Müller at the Department of Clinical Pharmacology, Medical University of Vienna, Vienna General Hospital (AKH), Währinger Gürtel 18-20, 1090 Vienna, Austria, or at markus.mueller@meduniwien.ac.at.

Drs. Ehrlich and Müller contributed equally to this article.

N Engl J Med 2008;358:2573-84.

Copyright © 2008 Massachusetts Medical Society.

Downloaded from www.nejm.org at BAYER-YAKUHIN CO., on August 17, 2008 .
Copyright © 2008 Massachusetts Medical Society. All rights reserved.

THE EMERGENCE OF A NEW HUMAN INfluenza pandemic caused by an avian virus strain is possible. Vaccination against pandemic influenza is considered to be the most effective option to limit its spread. However, the conventional approaches to the manufacture of influenza vaccines have a number of disadvantages and raise concern about whether sufficient quantities of an effective vaccine can be made available early enough at the onset of a pandemic to have a major effect on public health.[1] In addition, clinical studies of conventional split-vaccine formulations without adjuvant have shown poor immunogenicity.[2,3] It has been suggested that whole-virus vaccines have the potential to be more immunogenic than split-virus or subunit vaccines in previously unvaccinated populations.[4,5] The first clinical study of a whole-virus vaccine against avian influenza H5N1 virus showed that a substantially reduced antigen dosage (10 μg) with an alum formulation induced seroconversion in nearly 100% of subjects.[6]

All these studies were carried out with vaccines manufactured by conventional methods (i.e., with the use of embryonated chicken eggs and modified, attenuated reassortant viruses produced by reverse genetics).[7] We have devised a strategy for the development of an H5N1 vaccine that involves the use of a wild-type virus (i.e., the strain circulating in nature) grown in a Vero cell culture. This strategy has the advantage that the lead time for pandemic vaccine production can be reduced, since the generation of attenuated reassortants is not required, although the requirement for the use of enhanced biosafety level 3 (BSL-3) facilities for such a strategy is a relative drawback. In addition, cell culture provides a robust manufacturing platform that eliminates dependence on embryonated chicken eggs, which would be an advantage in the event of limited availability of such eggs during a pandemic caused by a highly pathogenic avian virus. This technique was used to develop a whole-virus vaccine that was highly immunogenic in animal models.[8] We report on the safety and immunogenicity of this vaccine, using formulations with and without alum adjuvant.

METHODS

STUDY DESIGN AND OBJECTIVE

From June 2006 through September 2006, we enrolled a total of 284 men and women between the ages of 18 and 45 years in a randomized, partially blinded (between groups) clinical trial at three sites: one in Austria and two in Singapore. The study was designed by its sponsor, Baxter. Data were collected by the investigators and were held and analyzed by Baxter. The manuscript was written by a subgroup of industry and academic authors; all authors contributed to the content, had full access to the data, and vouch for the completeness and accuracy of the data and data analysis.

The appropriate local review boards and ethics committees approved the protocol for the study, which was conducted in compliance with Good Clinical Practice guidelines and the provisions of the Declaration of Helsinki. The study investigators were unaware of assignments to study groups. (For details of the study design, see the Supplementary Appendix, available with the full text of this article at www.nejm.org.)

The objective was to identify the immunogenicity and safety of various doses of inactivated H5N1 whole-virus vaccine in formulations with and without adjuvant. The primary immunogenicity outcome was the number of subjects with hemagglutination-inhibition and neutralizing antibodies to the vaccine strain (A/Vietnam/1203/2004) 21 days after the first and second doses of vaccine. The primary safety outcome was any systemic reaction after the first and second doses.

VACCINE

The monovalent avian influenza H5N1 whole-virus vaccine (Baxter) was produced with the wild-type strain A/Vietnam/1203/2004, which was obtained from the Centers for Disease Control and Prevention and was inactivated with formalin and ultraviolet light. The vaccine was manufactured in Vero cell culture in an enhanced BSL-3 facility (as required for wild-type H5N1 virus), as described previously.[9]

RANDOMIZATION AND FOLLOW-UP

Subjects were eligible to participate if they were clinically healthy, understood the study procedures, provided written informed consent, and agreed to keep a daily record of symptoms. Women were required to have a negative pregnancy test at screening and before each vaccination.

Subjects were recruited in three study cohorts in a dose-escalating manner and were randomly assigned to receive two 0.5-ml injections into the deltoid muscle at an interval of 21 days (range,

Downloaded from www.nejm.org at BAYER-YAKUHIN CO., on August 17, 2008.
Copyright © 2008 Massachusetts Medical Society. All rights reserved.

19 to 23) with an H5N1 whole-virus formulation containing 3.75 μg, 7.5 μg, 15 μg, or 30 μg of hemagglutinin antigen with a 0.2% alum adjuvant or 7.5 μg or 15 μg of hemagglutinin antigen without adjuvant. There was no placebo group. Subjects and investigators were unaware of the dose of vaccine administered within the subgroups (Fig. 1, and the Supplementary Appendix). Blood samples were taken for serologic testing before the first dose of vaccine and on day 21 after the first and second doses.

Using a diary provided by the investigators, subjects were asked to record daily oral body temperature (using study-issued digital thermometers), local reactions, and systemic adverse events for 7 days after each vaccination. On days 7 and 21 after each vaccination, subjects were asked to return for a review of the diary and assessment for any adverse events.

ASSAYS

We evaluated all immunogenicity outcomes against the influenza-virus strain used in the vaccine (A/Vietnam/1203/2004) according to hemagglutination-inhibition and virus-neutralization assays. To assess cross-reactivity of antibodies, all assays were also conducted with known related influenza strains — for example, an original prototype clade 3 strain (A/Hong Kong/156/1997) and a clade 2 strain (A/Indonesia/05/2005).

Using a hemagglutination-inhibition or virus-neutralization assay, we investigated secondary

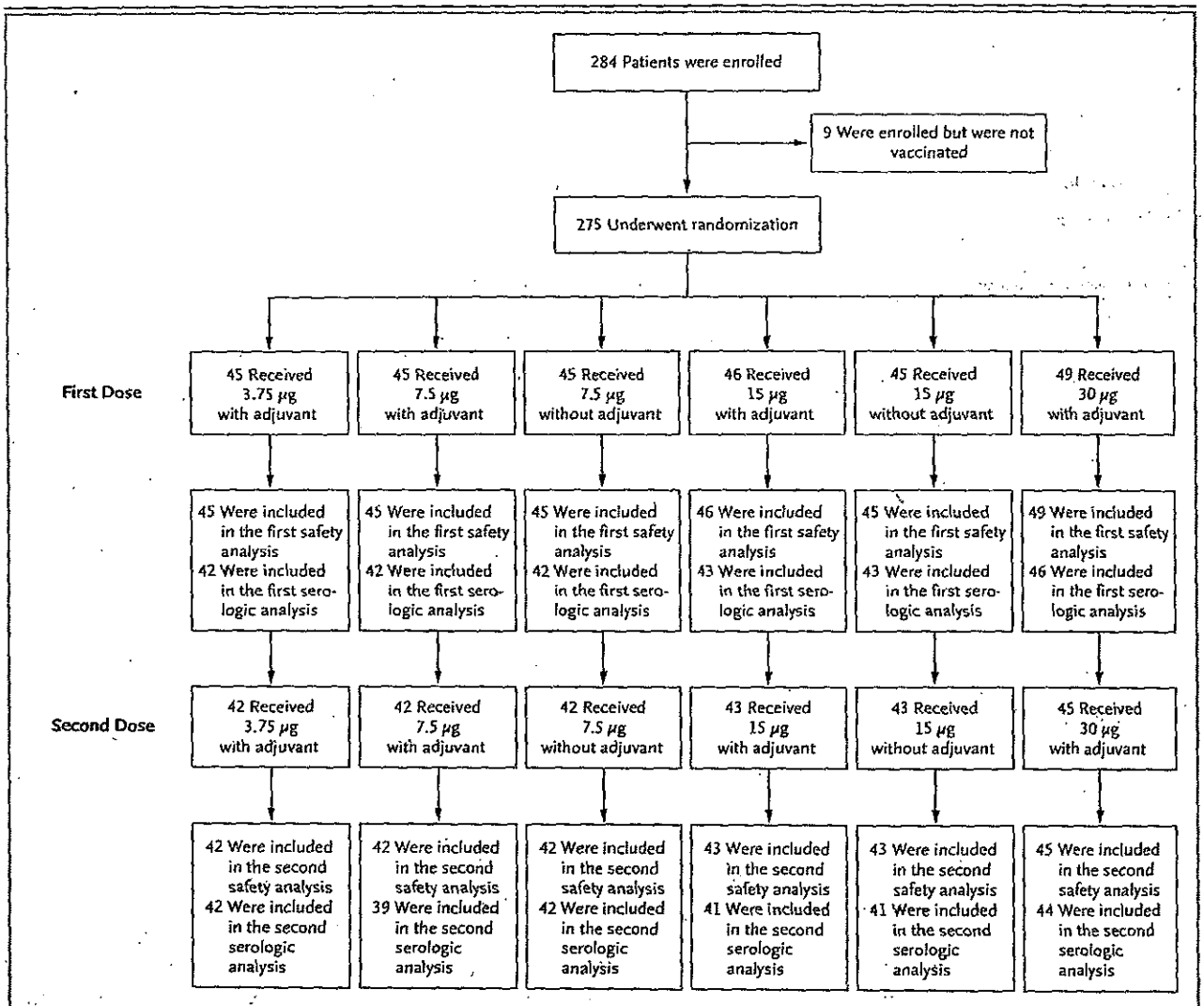


Figure 1. Enrollment and Outcomes.

Downloaded from www.nejm.org at BAYER-YAKUHIN CO., on August 17, 2008.
Copyright © 2008 Massachusetts Medical Society. All rights reserved.

Table 1. Proportion of Subjects with Injection-Site and Systemic Reactions within 7 Days after the First and Second Doses of Vaccine.

Variable	3.75 μg with Adjuvant	7.5 μg with Adjuvant	7.5 μg without Adjuvant	15 μg with Adjuvant	15 μg without Adjuvant	30 μg with Adjuvant
First dose						
No. of subjects	45	45	45	46	45	49
			percent (95% confidence interval)			
Injection-site reaction						
Any	29 (16–44)	22 (11–37)	11 (4–24)	28 (16–43)	20 (10–35)	24 (13–39)
Pain	27 (15–42)	20 (10–35)	9 (2–21)	26 (14–41)	18 (8–32)	24 (13–39)
Erythema*	0 (0–8)	2 (0–12)	2 (0–12)	4 (1–15)	0 (0–8)	0 (0–7)
Swelling*	0 (0–8)	0 (0–8)	0 (0–8)	2 (0–12)	0 (0–8)	2 (0–11)
Induration*	0 (0–8)	2 (0–12)	0 (0–8)	0 (0–8)	4 (1–15)	2 (0–11)
Ecchymosis*	0 (0–8)	0 (0–8)	0 (0–8)	0 (0–8)	2 (0–12)	2 (0–11)
Systemic reaction						
Any	51 (36–66)	31 (18–47)	38 (24–53)	30 (18–46)	47 (32–62)	18 (9–32)
Fever†	2 (0–12)	4 (1–15)	0 (0–8)	4 (1–15)	2 (0–12)	2 (0–11)
Headache	31 (18–47)	18 (8–32)	20 (10–35)	13 (5–26)	24 (13–40)	6 (1–17)
Malaise	13 (5–27)	11 (4–24)	4 (1–15)	13 (5–26)	9 (2–21)	6 (1–17)
Myalgia	9 (2–21)	16 (6–29)	4 (1–15)	9 (2–21)	9 (2–21)	2 (0–11)
Shivering	0 (0–8)	9 (2–21)	7 (1–18)	9 (2–21)	2 (0–12)	0 (0–7)
Second dose						
No. of subjects	42	42	42	43	43	45
			percent (95% confidence interval)			
Injection-site reaction						
Any	17 (7–31)	12 (4–26)	14 (5–29)	19 (8–33)	16 (7–31)	13 (5–27)
Pain	14 (5–29)	10 (3–23)	12 (4–26)	19 (8–33)	16 (7–31)	11 (4–24)
Erythema*	0 (0–8)	2 (0–13)	2 (0–13)	0 (0–8)	0 (0–8)	0 (0–8)
Swelling*	0 (0–8)	2 (0–13)	0 (0–8)	2 (0–12)	0 (0–8)	0 (0–8)
Induration*	5 (1–16)	0 (0–8)	0 (0–8)	2 (0–12)	0 (0–8)	0 (0–8)
Ecchymosis*	0 (0–8)	2 (0–13)	0 (0–8)	0 (0–8)	2 (0–12)	2 (0–12)
Systemic reaction						
Any	31 (18–47)	24 (12–39)	26 (14–42)	28 (15–44)	44 (29–60)	18 (8–32)
Fever†	0 (0–8)	2 (0–13)	5 (1–16)	0 (0–8)	7 (1–19)	2 (0–12)
Headache	19 (9–34)	10 (3–23)	5 (1–16)	9 (3–22)	12 (4–25)	13 (5–27)
Malaise	5 (1–16)	7 (1–19)	5 (1–16)	2 (0–12)	12 (4–25)	9 (2–21)
Myalgia	12 (4–26)	2 (0–13)	2 (0–13)	2 (0–12)	7 (1–19)	0 (0–8)
Shivering	0 (0–8)	2 (0–13)	5 (1–16)	2 (0–12)	7 (1–19)	0 (0–8)

* Listed are injection-site reactions with a diameter of more than 1 cm.
† Fever was defined as an oral temperature of 38°C (100.4°F) or more.

immunogenicity outcomes by analyzing the antibody response 21 days after the first and second doses of vaccine; the increase in the antibody response 21 days after the first and second doses, as compared with baseline; and the number of subjects with seroconversion (which we defined as a minimum increase by a factor of 4 in the titer) 21 days after the first and second doses, as compared with baseline.

The hemagglutination-inhibition assay is the standard test for detection of antibodies against influenza after infection or vaccination. However,

Downloaded from www.nejm.org at BAYER-YAKUHIN CO., on August 17, 2008 .
Copyright © 2008 Massachusetts Medical Society. All rights reserved.

Table 2. Proportion of Subjects with a Virus-Neutralization Antibody Titer of 1:20 or More.

Virus Strain and Day	3.75 μg with Adjuvant	7.5 μg with Adjuvant	7.5 μg without Adjuvant	15 μg with Adjuvant	15 μg without Adjuvant	30 μg with Adjuvant
A/Vietnam/1203/2004 (clade 1)						
Day 0						
No./total no. (%)	0/42	3/42 (7.1)	0/42	1/43 (2.3)	0/43	0/46
95% CI	0.0–8.4	1.5–19.5	0.0–8.4	0.1–12.3	0.0–8.2	0.0–7.7
Day 21						
No./total no. (%)	9/42 (21.4)	11/42 (26.2)	17/42 (40.5)	7/43 (16.3)	17/43 (39.5)	5/46 (10.9)
95% CI	10.3–36.8	13.9–42.0	25.6–56.7	6.8–30.7	25.0–55.6	3.6–23.6
Day 42						
No./total no. (%)	29/42 (69.0)	25/39 (64.1)	32/42 (76.2)	25/41 (61.0)	29/41 (70.7)	29/44 (65.9)
95% CI	52.9–82.4	47.2–78.8	60.5–87.9	44.5–75.8	54.5–83.9	50.1–79.5
A/Indonesia/05/2005 (clade 2)						
Day 0						
No./total no. (%)	1/42 (2.4)	1/42 (2.4)	0/42	1/43 (2.3)	0/43	0/46
95% CI	0.1–12.6	0.1–12.6	0.0–8.4	0.1–12.3	0.0–8.2	0.0–7.7
Day 21						
No./total no. (%)	5/42 (11.9)	5/42 (11.9)	10/42 (23.8)	1/43 (2.3)	7/43 (16.3)	3/46 (6.5)
95% CI	4.0–25.6	4.0–25.6	12.1–39.5	0.1–12.3	6.8–30.7	1.4–17.9
Day 42						
No./total no. (%)	12/42 (28.6)	14/39 (35.9)	19/42 (45.2)	3/41 (7.3)	15/41 (36.6)	13/44 (29.5)
95% CI	15.7–44.6	21.2–52.8	29.8–61.3	1.5–19.9	22.1–53.1	16.8–45.2
A/Hong Kong/156/1997 (clade 3)						
Day 0						
No./total no. (%)	0/42	4/42 (9.5)	2/42 (4.8)	2/43 (4.7)	1/43 (2.3)	1/46 (2.2)
95% CI	0.0–8.4	2.7–22.6	0.6–16.2	0.6–15.8	0.1–12.3	0.1–11.5
Day 21						
No./total no. (%)	9/42 (21.4)	13/42 (31.0)	20/42 (47.6)	9/43 (20.9)	18/43 (41.9)	7/46 (15.2)
95% CI	10.3–36.8	17.6–47.1	32.0–63.6	10.0–36.0	27.0–57.9	6.3–28.9
Day 42						
No./total no. (%)	28/42 (66.7)	25/39 (64.1)	32/42 (76.2)	26/41 (63.4)	32/41 (78.0)	34/44 (77.3)
95% CI	50.5–80.4	47.2–78.8	60.5–87.9	46.9–77.9	62.4–89.4	62.2–88.5

this assay may be insensitive for the detection of anti-H5 antibodies.[10,11] For this reason, immunogenicity analyses focused on a determination of functional neutralizing-antibody responses. Since most licensing authorities typically request data regarding hemagglutination-inhibition assays or single radial hemolysis, these determinations are also reported but only for the vaccine virus strain A/Vietnam/1203/2004. (For details on hemagglutination-inhibition and virus-neutralization assays and single radial hemolysis,[12-14] see the Supplementary Appendix.)

STATISTICAL ANALYSIS

The protocol called for the recruitment of 45 subjects per study group. With this number of subjects, the 95% confidence interval for the percentage of subjects with an antibody response that was associated with protection did not extend more than 15% from the observed rate, assuming a seroprotection rate of approximately 80%.

We used the likelihood-ratio chi-square test to compare the number of subjects with local or systemic reactions within 7 days after vaccination among the various vaccine formulations. For bi-

Downloaded from www.nejm.org at BAYER-YAKUHIN CO., on August 17, 2008 .
Copyright © 2008 Massachusetts Medical Society. All rights reserved.

Table 3. Geometric Mean of the Increase from Baseline (GMI) and Proportion of Subjects with Seroconversion.*

Virus Strain and Day	3.75 μg with Adjuvant		7.5 μg with Adjuvant		7.5 μg without Adjuvant	
	GMI	Seroconversion	GMI	Seroconversion	GMI	Seroconversion
	value (95% CI)	*% (95% CI)*	*value (95% CI)*	*% (95% CI)*	*value (95% CI)*	*% (95% CI)*
A/Vietnam/1203/2004 (clade 1)						
Day 21	2.0 (1.6–2.4)	11.9 (4.0–25.6)	2.0 (1.6–2.5)	9.5 (2.7–22.6)	3.2 (2.4–4.2)	35.7 (21.6–52.0)
Day 42	4.4 (3.5–5.6)	54.8 (38.7–70.2)	4.0 (3.1–5.2)	51.3 (34.8–67.6)	5.3 (4.1–6.9)	69.0 (52.9–82.4)
A/Indonesia/05/2005 (clade 2)						
Day 21	1.7 (1.4–1.9)	4.8 (0.6–16.2)	1.6 (1.3–1.9)	7.1 (1.5–19.5)	2.2 (1.8–2.8)	19.0 (8.6–34.1)
Day 42	2.8 (2.3–3.4)	19.0 (8.6–34.1)	2.7 (2.1–3.4)	28.2 (15.0–44.9)	3.2 (2.5–4.0)	31.0 (17.6–47.1)
A/Hong Kong/156/1997 (clade 3)						
Day 21	2.3 (1.8–2.9)	16.7 (7.0–31.4)	2.3 (1.8–2.8)	14.3 (5.4–28.5)	3.4 (2.5–4.7)	38.1 (23.6–54.4)
Day 42	5.8 (4.4–7.7)	69.0 (52.9–82.4)	5.2 (3.8–7.1)	51.3 (34.8–67.6)	5.9 (4.3–8.1)	66.7 (50.5–80.4)

* Seroconversion was defined as an increase in the virus-neutralization titer by a factor of 4 or more.

nary variables (i.e., seroprotection and seroconversion), response rates and 95% confidence intervals were computed for each strain and time point. The confidence intervals were interpreted in a descriptive manner, and no adjustment for multiplicity was made.[15]

In addition, for the log-transformed values of virus-neutralization titers and single radial hemolysis, a longitudinal analysis was performed within a repeated mixed-model framework of analysis of covariance. Changes from baseline were analyzed, accounting for the fixed effects of vaccine formulation, day, sex, age, baseline titer, interaction between the vaccine formulation and day, and random effects for subjects. Vaccine formulations without adjuvant were compared with formulations with adjuvant within this model. Comparisons were also made between groups receiving 7.5 μg and 15 μg of hemagglutinin antigen without adjuvant. We calculated the proportion of subjects with a virus-neutralization titer of 1:20 or more and that of subjects with results of 25 mm^2 or more on single radial hemolysis, using a generalized linear model with repeated measurements and the general-estimating-equations method (see the Supplementary Appendix).

RESULTS

STUDY POPULATION

A total of 275 subjects between the ages of 18 and 45 years received the first dose of vaccine, and 257 received the second dose. All vaccinated subjects were included in the safety analysis. Two subjects who initially gave their consent withdrew from the study because of nonserious adverse events, including four events in one subject (chills, fatigue, malaise, and insomnia) and one event in the second subject (papular rash); the majority of these symptoms abated within 24 hours. Immunogenicity data were available for 258 subjects for the first dose of vaccine and for 249 subjects for the second dose of vaccine.

SAFETY

The rates of occurrence of injection-site and systemic reactions during the first 7 days after each dose of vaccine are presented in Table 1. No serious, vaccine-related adverse events were recorded. There were two serious adverse events recorded in two subjects: hospitalization due to a contusion of the left foot and hospitalization for an elective abortion.

The most commonly occurring injection-site reaction after vaccination was pain, which occurred in 9 to 27% of subjects; the most frequently reported systemic reaction was headache, which occurred in 6 to 31% of subjects.

There were no significant differences between the vaccine formulations with respect to local reactions after the first dose and the second dose of vaccine ($P=0.32$ and $P=0.97$, respectively, for all comparisons). With respect to systemic reactions, a slight difference was observed between the vaccine formulations after the first dose of vaccine ($P=0.01$), a finding that was largely due

Downloaded from www.nejm.org at BAYER-YAKUHIN CO., on August 17, 2008 .
Copyright © 2008 Massachusetts Medical Society. All rights reserved.

15 μg with Adjuvant		15 μg without Adjuvant		30 μg with Adjuvant	
GMI	Seroconversion	GMI	Seroconversion	GMI	Seroconversion
value (95% CI)	*% (95% CI)*	*value (95% CI)*	*% (95% CI)*	*value (95% CI)*	*% (95% CI)*
1.9 (1.5–2.4)	11.6 (3.9–25.1)	3.1 (2.5–4.0)	34.9 (21.0–50.9)	2.1 (1.8–2.5)	13.0 (4.9–26.3)
3.9 (3.0–5.0)	46.3 (30.7–62.6)	5.7 (4.3–7.5)	68.3 (51.9–81.9)	4.6 (4.0–5.4)	61.4 (45.5–75.6)
1.4 (1.2–1.7)	2.3 (0.1–12.3)	2.3 (1.8–2.9)	16.3 (6.8–30.7)	1.7 (1.5–2.0)	2.2 (0.1–11.5)
2.5 (2.1–2.9)	9.8 (2.7–23.1)	3.6 (2.9–4.5)	43.9 (28.5–60.3)	2.9 (2.5–3.5)	29.5 (16.8–45.2)
2.0 (1.5–2.7)	11.6 (3.9–25.1)	3.3 (2.5–4.3)	30.2 (17.2–46.1)	1.9 (1.6–2.3)	15.2 (6.3–28.9)
4.9 (3.7–6.5)	53.7 (37.4–69.3)	7.8 (5.7–10.6)	75.6 (59.7–87.6)	5.7 (4.6–7.0)	63.6 (47.8–77.6)

to an unexpectedly low rate of headache observed in the group receiving the 30-μg formulation with adjuvant. No difference was shown regarding systemic reactions after the second dose of vaccine (P=0.15).

IMMUNE RESPONSE

At 21 days after the first and second doses, functional neutralizing antibodies against strain A/Vietnam/1203/2004 were detected in patients receiving any of the six formulations. Table 2 shows the rates of response in subjects with a virus-neutralization titer of 1:20 or more, and Table 3 shows the geometric mean increase (GMI) of the titer from baseline and the percentage of seroconversion. Numerically, the formulations without adjuvant induced the highest rates of a virus-neutralization titer of 1:20 or more after the first dose (40.5% and 39.5% for 7.5 μg and 15 μg without adjuvant, respectively) and the second dose (76.2% and 70.7% for 7.5 μg and 15 μg without adjuvant, respectively) (Table 2). Similar results were obtained with respect to GMI (Table 3), since the highest GMIs were obtained for the formulations without adjuvant (5.3 and 5.7 for 7.5 μg and 15 μg without adjuvant, respectively) (Table 3). Among subjects with seroconversion (an increase in the titer by a factor of at least 4 after immunization), the highest rates of response were again seen in subjects who received a 7.5-μg or 15-μg formulation without adjuvant (69.0% and 68.3%, respectively) (Table 3).

Statistical analysis with the use of a mixed model on log-transformed virus-neutralization values confirmed that the formulations without adjuvant induced significantly higher immune responses than did the formulations with adjuvant (P<0.001). There were no significant differences between the two formulations without adjuvant or among the four formulations with adjuvant. All vaccine formulations showed a similar ratio of increase in antibody titer between day 21 and day 42, as shown by the nonsignificant interaction between vaccine formulation and day (Table 4, and Table 4 in the Supplementary Appendix).

Table 5 compares the presumed rates of seroprotection, as measured by hemagglutination-inhibition assay (i.e., the proportion of subjects with a titer ≥40) and single radial hemolysis (i.e., the proportion of subjects with an area of ≥25 mm^2 on single radial hemolysis). Numerically, the formulations without adjuvant again were more immunogenic than those with adjuvant. On single radial hemolysis, the percentage of seroprotection 21 days after the second dose of vaccine without adjuvant was 78.6% for the 7.5-μg dose and 61.0% for the 15-μg dose. Single radial hemolysis for H5N1 antibodies appeared to be more sensitive than hemagglutination-inhibition assay, since the equivalent values for hemagglutination-inhibition assay were 47.6% and 26.8%, respectively.

We also analyzed changes from baseline in results on single radial hemolysis using a mixed-model analysis of covariance for the log-transformed values, and the results were similar to those obtained for the virus-neutralization titers. Again, we observed a significant effect of the

Downloaded from www.nejm.org at BAYER-YAKUHIN CO., on August 17, 2008.
Copyright © 2008 Massachusetts Medical Society. All rights reserved.

Table 4. Mixed-Model Analysis of Log-Transformed Values of Virus-Neutralization Titer.

Effects and Comparison	A/Vietnam/1203/2004 (Clade 1)	A/Indonesia/05/2005 (Clade 2)	A/Hong Kong/156/1997 (Clade 3)
		P Value	
Effect			
Vaccine formulation	0.004	0.001	0.01
Day 21 vs. day 42	<0.001	<0.001	<0.001
Baseline	<0.001	<0.001	<0.001
Sex	0.009	0.08	0.01
Age	0.41	0.18	0.03
Vaccine formulation–day interaction	0.06	0.36	0.01
Comparison			
With adjuvant vs. without adjuvant	<0.001	<0.001	<0.001
Without adjuvant, 7.5 μg vs. 15 μg	0.80	0.97	0.70

vaccine formulations, with formulations without adjuvant showing higher response rates than those with adjuvant. There was no significant difference between the two formulations without adjuvant or among the formulations with adjuvant (Table 4, and Table 5 in the Supplementary Appendix).

CROSS-NEUTRALIZATION

The 7.5-μg and 15-μg formulations without adjuvant showed high levels of cross-reactivity against the A/Hong Kong strain (76.2% and 78.0%, respectively, with a neutralizing titer of ≥1:20) (Table 2). The responses against the clade 2 strain were somewhat lower (with rates of a virus-neutralization titer of ≥1:20 of 45.2% and 36.6% for the 7.5-μg and 15-μg formulations without adjuvant, respectively) (Table 2).

We also analyzed the virus-neutralization response to the heterologous strains using the mixed model. Results were similar to those for the homologous strain. Formulations without adjuvant elicited significantly higher immune responses than those with adjuvant. Antibody titers increased significantly from baseline, independently of the vaccine dose (Table 4, and Tables 3 and 4 in the Supplementary Appendix).

The reverse cumulative distribution curves for antibody titers after the first and second doses of vaccine against all three strains support the finding of higher immunogenicity from the formulations without adjuvant (Fig. 2). Analysis of rates of seroprotection with homologous and heterologous immune responses showed results that were consistent with those obtained by direct analysis of values of virus-neutralization titers and single radial hemolysis (Tables 6 and 7 in the Supplementary Appendix).

DISCUSSION

It has been reported that whole-virus trivalent influenza vaccines are more immunogenic than subvirion vaccines but are also more prone to cause adverse reactions.[5] In our study, a monovalent whole-virus H5N1 vaccine had a side-effect profile similar to that of subvirion H5N1 formulations described previously.[2,3,16] Most important, the low rate of fever among subjects in our study (2 to 7%) compares favorably with that reported both for subvirion H5N1 vaccines and for an egg-derived whole-virus H5N1 vaccine with adjuvant.[2,3,6,16] However, it should be noted that reporting systems and characteristics of the subjects differ among the various studies.

With respect to immunogenicity, the highest neutralizing-antibody response after the second dose of vaccine (76.2%) was obtained with the 7.5-μg formulation without adjuvant, which was equivalent to a rate of seroconversion of 69.0% and represented an increase by a factor of 4 or more in the neutralization titer after two doses of vaccine (Tables 2 and 3). These data are also similar to the levels of immunogenicity reported in a study of an egg-derived whole-virus H5N1 vaccine, in which 96% of subjects who received

Downloaded from www.nejm.org at BAYER-YAKUHIN CO., on August 17, 2008 .
Copyright © 2008 Massachusetts Medical Society. All rights reserved.

Table 5. Antibody Response to the Homologous Virus Strain after the First and Second Doses of Vaccine.*

Dose with or without Adjuvant	Assay	Seroprotection			Seroconversion		GMI	
		Day 0	Day 21	Day 42	Day 21	Day 42	Day 21	Day 42
				percent (95% CI)			*value (95% CI)*	
3.75 μg with adjuvant	HI	2.4 (0.1–12.6)	33.3 (19.6–49.5)	40.5 (25.6–56.7)	33.3 (19.6–49.5)	38.1 (23.6–54.4)	2.7 (1.7–4.4)	4.5 (2.4–8.4)
	SRH	4.8 (0.6–16.2)	26.2 (13.9–42.0)	50.0 (34.2–65.8)	21.4 (10.3–36.8)	47.6 (32.0–63.6)	1.7 (1.2–2.3)	2.9 (2.0–4.2)
7.5 μg with adjuvant	HI	4.8 (0.6–16.2)	35.7 (21.6–52.0)	38.5 (23.4–55.4)	35.7 (21.6–52.0)	35.9 (21.2–52.8)	3.2 (1.9–5.4)	3.6 (1.9–6.8)
	SRH	4.8 (0.6–16.2)	26.2 (13.9–42.0)	35.9 (21.2–52.8)	21.4 (10.3–36.8)	33.3 (19.1–50.2)	1.7 (1.2–2.3)	2.3 (1.5–3.4)
7.5 μg without adjuvant	HI	0.0 (0.0–8.4)	47.6 (32.0–63.6)	47.6 (32.0–63.6)	47.6 (32.0–63.6)	47.6 (32.0–63.6)	4.5 (2.7–7.6)	5.3 (3.0–9.5)
	SRH	7.1 (1.5–19.5)	69.0 (52.9–82.4)	78.6 (63.2–89.7)	61.9 (45.6–76.4)	73.8 (58.0–86.1)	4.8 (3.2–7.2)	6.3 (4.3–9.1)
15 μg with adjuvant	HI	0 (0.0–8.2)	14.0 (5.3–27.9)	14.6 (5.6–29.2)	14.0 (5.3–27.9)	14.6 (5.6–29.2)	1.5 (1.1–2.2)	1.7 (1.1–2.7)
	SRH	4.7 (0.6–15.8)	16.3 (6.8–30.7)	39.0 (24.2–55.5)	11.6 (3.9–25.1)	36.6 (22.1–53.1)	1.4 (1.1–1.8)	2.2 (1.6–3.2)
15 μg without adjuvant	HI	0 (0.0–8.2)	25.6 (13.5–41.2)	26.8 (14.2–42.9)	25.6 (13.5–41.2)	26.8 (14.2–42.9)	2.8 (1.6–4.9)	3.2 (1.7–6.0)
	SRH	2.3 (0.1–12.3)	41.9 (27.0–57.9)	61.0 (44.5–75.8)	39.5 (25.0–55.6)	58.5 (42.1–73.3)	2.8 (1.9–4.2)	4.7 (3.1–7.1)
30 μg with adjuvant	HI	0 (0.0–7.7)	34.8 (21.4–50.2)	36.4 (22.4–52.2)	34.8 (21.4–50.2)	36.4 (22.4–52.2)	3.4 (2.0–5.7)	4.5 (2.4–8.6)
	SRH	2.2 (0.1–11.5)	21.7 (10.9–36.4)	58.1 (42.1–73.0)	19.6 (9.4–33.9)	58.1 (42.1–73.0)	1.5 (1.2–2.0)	3.6 (2.5–5.2)

* GMI denotes geometric mean of the increase, HI hemagglutination-inhibition assay, and SRH single radial hemolysis.

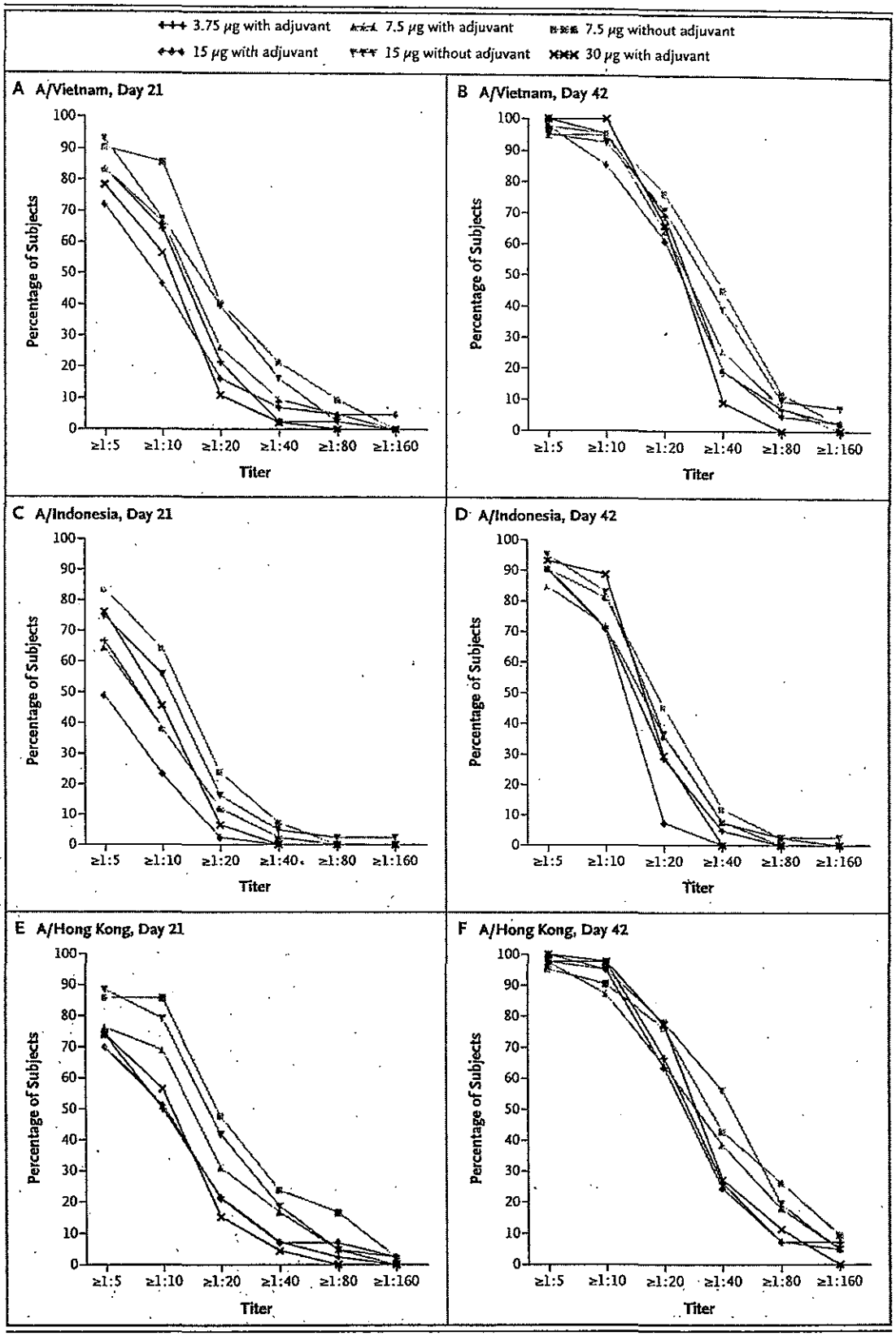
two doses of 5-μg or 10-μg formulations had a neutralization titer of 1:20 or more,[6] although differences in assay systems must be taken into account in making such direct comparisons.

Lower rates of seroprotection and seroconversion (as defined in the guidelines of the Committee for Proprietary Medicinal Products[17]) were obtained with the hemagglutination-inhibition assay than with the virus-neutralization assay, which supports the finding that the hemagglutination-inhibition assay is less sensitive for detection of anti-H5 antibodies, as reported previously.[10,11] In our study, single radial hemolysis, which is considered to have a sensitivity equivalent to that of the hemagglutination-inhibition assay for seasonal influenza strains,[18] was shown to be more sensitive than the hemagglutination-inhibition assay for H5N1.

The lack of enhancement of vaccine immunogenicity by the use of alum adjuvant at the doses studied here was consistent with data from a previous study, which showed that no effect of alum adjuvant was seen with a 15-μg dose of subvirion vaccine, and a 7.5-μg formulation without alum was more immunogenic than the formulation with adjuvant.[3] In the previous study, an enhanced immune response with the use of alum was seen only with the 30-μg formulation. We did not investigate this dose without alum in our study.

However, other studies have described substantial positive effects of other adjuvants on H5N1 immunogenicity. The use of an oil-in-water–based emulsion in a 3.8-μg dose of split-virus vaccine resulted in 82% seroconversion, as compared with 4% seroconversion without adjuvant.[16] The addition of another oil-in-water–based adjuvant (MF-59) to an H5N3 vaccine was also associated with a substantial increase in antibody response.[19]

Downloaded from www.nejm.org at BAYER-YAKUHIN CO., on August 17, 2008 .
Copyright © 2008 Massachusetts Medical Society. All rights reserved.



Downloaded from www.nejm.org at BAYER-YAKUHIN CO., on August 17, 2008 .
Copyright © 2008 Massachusetts Medical Society. All rights reserved.

Figure 2 (facing page). **Reverse Cumulative Distribution Curves for Titers of Neutralizing Antibodies in Six Study Groups after the First and Second Doses of Vaccine against Three Strains of Avian Influenza.**

Shown are the percentages of subjects with specific virus-neutralization titers after the first dose (day 21) and second dose (day 42) of vaccine against A/Vietnam/1203/2004 (clade 1) (Panels A and B, respectively), A/Indonesia/05/2005 (clade 2) (Panels C and D, respectively), and A/Hong Kong/156/1997 (clade 3) (Panels E and F, respectively).

Our data also showed that the whole-virus clade 1–based vaccine can induce a substantial cross-neutralizing response against clade 2 and clade 3 strains. The results described in Table 2 are encouraging: after two doses of 7.5-μg of the formulation without adjuvant, the proportions of subjects with neutralizing titers of 1:20 or more were 45% of those immunized against the clade 2 Indonesia strain and 76% of those immunized against the clade 3 Hong Kong strain. However, there is no available evidence to indicate which neutralizing titer is sufficient to confer protection. Most studies of H5N1 split-virus and whole-virus vaccines have not described attempts to determine the cross-reactivity of antibodies to other H5N1 virus strains. However, a recent study of a novel split-virus vaccine with adjuvant also showed high levels of cross-neutralization against a clade 2 strain.[16] In addition, in a study involving 15 subjects, two doses of an H5N3 vaccine with MF-59 as adjuvant induced intermediate levels of cross-reactivity to antigenically distinct H5N1 strains, and three doses induced high levels of cross-reactivity.[20]

The apparent absence of a dose–response relationship in our study may be surprising. However, it is in agreement with a number of studies of vaccine for pandemic influenza. Leroux-Roels et al. reported no relationship between the dose of antigen and the neutralizing-antibody response for H5N1 formulations with adjuvant,[16] and there appeared to be an inverse dose–response relationship with respect to responses to the clade 2 strain. A number of other studies involving other pandemic-strain vaccines — H9N2,[21] H5N3,[19] and H2N2[22] — have shown no dose–response relationship or even a reduced response at higher doses. The reasons for these findings are unclear, but at least with respect to vaccines with adjuvant, it has been speculated that the ratio of adjuvant to antigen may be critical in determining the immune-enhancing effect rather than the antigen concentration alone.[19] For other viral vaccines, particularly those with soluble proteins, it has been reported that there are distinct dose–response relationships for induction of various cytokines. In many studies, responses similar to those mediated by type 2 helper T cells have been elicited at low doses of vaccine, and responses similar to those mediated by type 1 helper T cells have been elicited at higher doses.[23] Further studies focusing on T-cell responses will be required to investigate this phenomenon. In addition, these studies will be extended by the use of antigen doses lower than 3.75 μg to confirm and extend the results obtained in our study.

Our study provides initial safety and immunogenicity data for a whole-virus H5N1 vaccine produced on Vero cell culture. It also shows that a broadly reactive immune response to clade 2 and clade 3 of H5N1 virus can be obtained with the use of a low-dose clade 1 vaccine without adjuvant. Since we observed no significant dose–response relationship, the 7.5-μg formulation without adjuvant has been chosen for further development.

Supported by Baxter.

Drs. Ehrlich, Berezuk, Fritsch, Löw-Baselli, Vartian, Bobrovsky, Pavlova, Pöllabauer, Kistner, and Barrett report being employed by Baxter and having an equity interest in the company; Drs. Kistner and Barrett, holding patents on influenza vaccines derived from Vero cell cultures; Dr. Müller, receiving consulting and lecture fees and grant support (to the Medical University of Vienna) from Baxter; Dr. Tambyah, serving as a member of the Asia–Pacific Advisory Committee on Influenza and receiving consulting fees from Baxter, Merlion Pharmaceuticals, and Janssen-Cilag, lecture fees from Pfizer, Wyeth and IBC Asia, and grant support from Baxter and Interimmune; and Dr. Montomoli, receiving lecture fees and grant support (to the University of Siena) from Baxter. No other potential conflict of interest relevant to this article was reported.

This study is dedicated to the memory of Dr. Michel Canavaggio, head of bioscience research and development at Baxter and a great supporter of this project, who died in July 2006, about 6 weeks after the initiation of the study.

We thank the following members of the Baxter research and development team for their critical role in this study: L. Grillberger, K. Howard, W. Mundt, M. Reiter, H. Savidis-Dacho, C. Tauer, and W. Wodal; N. Cox and S. Klimov of the Centers for Disease Control and Prevention for providing the H5N1 viruses; and J. Wood of the National Institute for Biological Standards and Control for providing the reference standards.

Downloaded from www.nejm.org at BAYER-YAKUHIN CO., on August 17, 2008 .
Copyright © 2008 Massachusetts Medical Society. All rights reserved.

APPENDIX

In addition to the authors, the following investigators contributed to the trial: *Data Monitoring and Safety Board:* E. Marth, R. Konior, F. Sonnenburg; *Baxter Clinical Study Team:* K. Birthistle, T. Dvorak, S. Geyer, M. Kraft, M.C. Leitgeb, F. Maritsch, L. Phillipson, E. Robotka. Austria: *Medical University of Vienna, Vienna General Hospital (Study Center Management), Vienna:* A. Abrahim, M. Bauer, M. Brunner, A. Cornea, C. Drucker, Z. Erdogan, J. Griss, B. Heinisch, F. Kovar, E. Lackner, C. Lambers, O. Langer, I. Leitner, C. Marsik, W. Poeppl, M. Popovic, R. Sauermann, R. Schaberl, G. Sodeck, C. Thallinger, F. Traunmueller, C. Wagner, M. Zeitlinger; Singapore: *Changi General Hospital (Study Center Management):* S.K. Chua, S. Chuin, R. Fong, A.S. Foo, A.G. Koh, P.K. Lim, S.Y. Yap, L.H. Yew; *National University Hospital (Study Center Management):* J.W.L. Goh, L.Y. Hsu, C.W.P. Loke, J.Y.C. Ng, B.L. Toh, P. Weatherill, Y.P. Zhou.

REFERENCES

1. Wood JM. Selection of influenza vaccine strains and developing pandemic vaccines. Vaccine 2002;20:Suppl 5:40-4.
2. Treanor JJ, Campbell JD, Zangwill KM, Rowe T, Wolff M. Safety and immunogenicity of an inactivated subvirion influenza A (H5N1) vaccine. N Engl J Med 2006;354:1343-51.
3. Bresson JL, Perronne C, Launay O, et al. Safety and immunogenicity of an inactivated split-virion influenza A/Vietnam/1194/2004 (H5N1) vaccine: phase I randomised trial. Lancet 2006;367:1657-64.
4. Wood JM. Developing vaccines against pandemic influenza. Philos Trans R Soc Lond B Biol Sci 2001;356:1953-60.
5. Nicholson KG, Tyrrell DAJ, Harrison P, et al. Clinical studies of monovalent inactivated whole virus and subunit A/USSR/77 (H1N1) vaccine: serological responses and clinical reactions. J Biol Stand 1979;7:123-36.
6. Lin J, Zhang J, Dong X, et al. Safety and immunogenicity of an inactivated adjuvanted whole-virion influenza A (H5N1) vaccine: phase 1 randomised controlled trial. Lancet 2006;368:991-7.
7. Nicolson C, Major D, Wood JM, Robertson JS. Generation of influenza vaccine viruses on Vero cells by reverse genetics: an H5N1 candidate vaccine strain produced under a quality system. Vaccine 2005;23:2943-52.
8. Kistner O, Howard K, Spruth M, et al. Cell culture (Vero) derived whole-virus (H5N1) vaccine based on wild-type virus strain induces cross-protective immune responses. Vaccine 2007;25:6028-36.
9. Kistner O, Barrett PN, Mundt W, Reiter M, Schober-Bendixen S, Dorner F. Development of a mammalian cell (Vero)-derived candidate influenza virus vaccine. Vaccine 1998;16:960-8.
10. Rowe T, Abernathy RA, Hu-Primmer J, et al. Detection of antibody to avian influenza A (H5N1) virus in human serum by using a combination of serologic assays. J Clin Microbiol 1999;37:937-43.
11. Lu BL, Webster RG, Hinshaw VS. Failure to detect hemagglutination-inhibiting antibodies with intact avian influenza virions. Infect Immun 1982;38:530-5.
12. Stephenson I, Wood JM, Nicholson KG, Charlett A, Zambon MC. Detection of anti-H5 responses in human sera by HI using horse erythrocytes following MF59-adjuvanted influenza A/Duck/Singapore/97 vaccine. Virus Res 2004;103:91-5.
13. Reed LJ, Muench H. A simple method of estimating fifty percent endpoints. Am J Hyg 1938;27:493-7.
14. Schild GC, Pereira MS, Chakraverty P. Single-radial-hemolysis: a new method for the assay of antibody to influenza haemagglutinin: applications for diagnosis and seroepidemiologic surveillance of influenza. Bull World Health Organ 1975;52:43-50.
15. Fitting a model that contains a random effect. In: Collett D. Modeling binary data. Boca Raton, FL: Chapman & Hall/CRC Press, 1991:202-12.
16. Leroux-Roels I, Borkowski A, Vanwolleghem T, et al. Antigen sparing and cross-reactive immunity with an adjuvanted rH5N1 prototype pandemic influenza vaccine: a randomised controlled trial. Lancet 2007;370:580-9.
17. Committee for Proprietary Medicinal Products. Note for guidance on harmonisation of requirements for influenza vaccines. London: European Agency for the Evaluation of Medicinal Products, 1997. (Publication no. CPMP/BWP/214/96.)
18. Wood JM, Gaines-Das RE, Taylor J, Chakraverty P. Comparison of influenza serological techniques by international collaborative study. Vaccine 1994;12:167-74.
19. Nicholson KG, Colegate AE, Podda A, et al. Safety and antigenicity of non-adjuvanted and MF59-adjuvanted influenza A/Duck/Singapore/97 (H5N3) vaccine: a randomised trial of two potential vaccines against H5N1 influenza. Lancet 2001;357:1937-43.
20. Stephenson I, Bugarini R, Nicholson KG, et al. Cross-reactivity to highly pathogenic avian influenza H5N1 viruses after vaccination with nonadjuvanted and MF59-adjuvanted influenza A/Duck/Singapore/97 (H5N3) vaccine: a potential priming strategy. J Infect Dis 2005;191:1210-5.
21. Stephenson I, Nicholson KG, Glück R, et al. Safety and antigenicity of whole virus and subunit influenza A/Hong Kong/1073/99 (H9N2) vaccine in healthy adults: phase I randomised trial. Lancet 2003;362:1959-66.
22. Hehme N, Engelmann H, Kuenzel W, Neumeier E, Saenger R. Immunogenicity of a monovalent, aluminum-adjuvanted influenza whole virus vaccine for pandemic use. Virus Res 2004;103:163-71.
23. Constant SL, Bottomly K. Induction of TH1 and TH2 CD4+ T cell responses: the alternative approaches. Annu Rev Immunol 1997;15:297-322.

Copyright © 2008 Massachusetts Medical Society.

FULL TEXT OF ALL *JOURNAL* ARTICLES ON THE WORLD WIDE WEB

Access to the complete text of the *Journal* on the Internet is free to all subscribers. To use this Web site, subscribers should go to the *Journal*'s home page (www.nejm.org) and register by entering their names and subscriber numbers as they appear on their mailing labels. After this one-time registration, subscribers can use their passwords to log on for electronic access to the entire *Journal* from any computer that is connected to the Internet. Features include a library of all issues since January 1993 and abstracts since January 1975, a full-text search capacity, and a personal archive for saving articles and search results of interest. All articles can be printed in a format that is virtually identical to that of the typeset pages. Beginning 6 months after publication, the full text of all Original Articles and Special Articles is available free to nonsubscribers who have completed a brief registration.

Downloaded from www.nejm.org at BAYER-YAKUHIN CO., on August 17, 2008 .
Copyright © 2008 Massachusetts Medical Society. All rights reserved.

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 2008 年 6 月 4 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	Identification of trimeric peptides that bind porcine parvovirus from mixtures containing human blood plasma. Heldt, C.L. et al, Biotechnol. Prog., 24, 554-560 (2008).	公表国 カナダ	
販売名（企業名）						

研究報告の概要

本稿では，ヒト血漿溶液から従来の除去法で十分に取り除けなかったパルボウイルス（粒径が小さい非エンベロープウイルス）を除去する新たな手法について記載する。固相法で三アミノ酸残基よりなるペプチドライブラリーを樹脂上に合成し，種々の溶液に添加したブタパルボウイルス（PPV）への選択的な結合能を有するペプチドをスクリーニングした。カラムから溶出した PPV は，その後の感染性検査で感染能を測定した。その結果，16 種のペプチドを単離し，配列決定したところ，塩基性アミノ酸の存在が PPV 結合に非常に重要であることが判明した。PPV をリン酸緩衝生理食塩水（PBS）に混ぜた場合，すべてのペプチドが最初の 9 カラム体積でウイルスを完全に除去した。著者らは，この方法が浄水への適用に有用と示唆している。それに対してウイルスを 7.5%のヒト血漿（蛋白質 5 mg/mL）を含有する溶液に添加した場合には，アミノ酸配列がトリプトファン－アルギニン－トリプトファン（WRW）の場合のみ，最初の 3 カラム体積で検出可能な PPV を除去することができた。しかし、その後血漿はペプチド樹脂へのウイルスの結合を阻害し始めた。本手法は，改良の余地があるものの，短いペプチドを特定のウイルスの除去プロセスに利用できることを示している。

報告企業の意見	今後の対応
本研究は，まだ対処されていない非エンベロープウイルスに対する効果的な病原体除去法に対応し得る新規の手法が述べられている。	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が，今後も利用可能な本手法に関する情報収集に努める。

使用上の注意記載状況・その他参考事項等

BYL-2008-0333

Identification of Trimeric Peptides That Bind Porcine Parvovirus from Mixtures Containing Human Blood Plasma

Caryn L. Heldt,[†] Patrick V. Gurgel,[†,‡] Lee-Ann Jaykus,[§] and Ruben G. Carbonell*[,†]

Department of Chemical and Biomolecular Engineering and Department of Food, Bioprocessing and Nutrition Science, North Carolina State University, Raleigh, North Carolina 27695, and ProMetic Life Sciences, Montreal, Canada

Virus contamination in human therapeutics is of growing concern as more therapeutic products from animal or human sources come into the market. All biopharmaceutical processes are required to have at least two distinct viral clearance steps to remove viruses. Most of these steps work well for enveloped viruses and large viruses, whether enveloped or not. That leaves a class of small non-enveloped viruses, like parvoviruses and hepatitis A, which are not easily removed by these typical steps. In this study, we report the identification of trimeric peptides that bind specifically to porcine parvovirus (PPV) and their potential use to remove this virus from process solutions. All of the trimeric peptides isolated completely removed all detectable PPV from buffer in the first nine column volumes, corresponding to a clearance of 4.5−5.5 log of infectious virus. When the virus was spiked into a more complex matrix consisting of 7.5% human blood plasma, one of the trimers, WRW, was able to remove all detectable PPV in the first three column volumes, after which human blood plasma began to interfere with the binding of the virus to the peptide resin. These trimer resins removed considerably more virus than weak ion exchange resins. The results of this work indicate that small peptide ligand resins have the potential to be used in virus removal processes where removal of contaminating virus is necessary to ensure product safety.

1. Introduction

The removal of viruses, pathogenic microorganisms, and toxins is an important problem in the growing area of human therapeutics. Every year, more therapeutic products are produced from animal, human, or cell culture sources (*1*), and these sources contain an inherent risk of viral contamination. Therapeutic products from human blood plasma, antibodies, albumin, and factor VIII, just to name a few, could be infected with human immunodeficiency virus (HIV), hepatitis B, B19 virus (formally known as parvovirus B19), SARS coronavirus, or one or more emerging viruses that have yet to be identified (*2*). Cell cultures are often contaminated with retrovirus particles, belonging to the family of viruses that include HIV (*3*). Cell culture lines often used in the production of human antibodies may contain viruses such as murine parvovirus (MVM) or cytomegalovirus (*4*). While this contamination has been greatly reduced since the requirement of strict characterization of cell culture lines and the careful screening of human plasma donors (*3*), the risk of low levels of contamination still exists.

The FDA requires that any process that uses materials from living sources must have two viral clearance steps to lower the risk of contamination (*5*). These steps must demonstrate a distinct mechanism of virus clearance and achieve a minimum of 4 log removal, or 99.99%. There are two broad categories for viral clearance, inactivation and removal (*6*). Inactivation is often performed toward the beginning of the purification of a therapeutic and could involve a lowering of pH or heating of the product. Both of these processes work well against enveloped viruses, but caution must be taken to not harm the desired protein product. Virus removal is often done at the end of a process and most commonly involves nanofiltration of the final product directly before formulation. Nanofiltration works well for viruses of large size. However, small viruses like parvoviruses are often of approximately the same size as the protein product, making it difficult to separate them by filtration (*2*). Complete removal of small non-enveloped viruses with 20 nm pore size filters has been accomplished, but there is significant fouling of these small-pored membranes that can lead to reductions in production rates (*7*, *8*). Filtration of parvoviruses has been improved by flocculation of the virus particles through addition of cationic polymers (*9*) or amino acids (*10*), which allows the use of larger pore membranes that do not foul as quickly. Virus removal can also be accomplished using functionalized membrane surfaces. Quaternary amine groups have been attached to membrane surfaces to facilitate the removal of viruses through an ion exchange mechanism (*11*−*13*). Viral clearance validation may be achieved by conducting spiking experiments on normal process steps used in the purification of a therapeutic (i.e., chromatography columns, precipitation) (*14*), but care should be taken if a chromatography step is to be used concurrently as a viral clearance step and a protein purification step. If both the virus and the protein bind to the resin, it is possible for viruses to accumulate in the column. Without proper cleaning, the virus may elute from the column in subsequent batches and contaminate the therapeutic product (*14*).

Affinity adsorption is rarely used to remove viruses from process streams because the most common affinity ligands for viruses are antibodies. Antibodies are expensive to produce, often cannot withstand the harsh conditions required for the

* To whom correspondence should be addressed. Ph: (919) 515-7054. Fax: (919) 515-5831. Email: ruben@ncsu.edu.
† Department of Chemical and Biomolecular Engineering.
‡ ProMetic Life Sciences.
§ Department of Food, Bioprocessing and Nutrition Science.

10.1021/bp070412c CCC: $40.75 © xxxx American Chemical Society and American Institute of Chemical Engineers
Published on Web 05/10/2008 PAGE EST: 6.1

cleaning of process equipment, and carry an inherent risk of being contaminated with viruses in their own right (*3*). However, affinity adsorption has been applied to the reduction of infectious prions from blood (*15*, *16*), and a small peptide ligand has been found to remove staphylococcal enterotoxin B, a small toxin, from *E. coli* lysate (*17*). Small peptides are more robust than antibodies, and they are also less expensive and can be chemically synthesized, eliminating the risk of virus contamination. Peptides can handle the cycling of production and cleaning much better than antibodies, and by using small peptides, there is no three-dimensional structure that may be destroyed during processing. In this work, several trimeric peptides have been discovered that remove PPV from phosphate buffered saline containing as high as 7.5% human blood plasma. The peptides were found when a synthetic, solid-phase combinatorial library was screened for ligands that bind to porcine parvovirus (PPV). Solid-phase libraries allow screening directly on the chromatographic support that will be used as the separation media and have been successful in the discovery of many affinity peptide ligands (*18*−*21*). The discovered peptides can completely remove any detectable PPV from PBS and completely remove any detectable PPV from the first 3 column volumes when 7.5% human plasma is present. This work demonstrates that small peptides may offer a novel and effective method for removing viruses from complex mixtures.

2. Materials and Methods

2.1. Materials. Phosphate buffered saline (PBS) containing 0.01 M phosphate, 0.138 M NaCl, and 0.0027 M KCl, pH 7.4 was purchased from Sigma (St. Louis, MO), and human blood plasma was a donation from the American Red Cross (Rockville, MD). Amino acids, phenol red, sodium carbonate, sodium phosphate, glucose, calcium chloride, sodium chloride, potassium chloride, and magnesium sulfate were purchased from Sigma-Aldrich (St. Louis, MO) or Fisher Scientific (Waltham, MA). Eagles Minimum Essential Media (EMEM) was purchased from Quality Biologicals (Gaithersburg, MD). MEM vitamins, sterile PBS, trypsin, gentamicin, and glutamine, all for cell culture, were purchased from Invitrogen (Carlsbad, CA).

2.2. Virus Propagation and Titration. The porcine parvovirus (PPV) NADL-2 strain was titrated and propagated on porcine kidney (PK-13) cells, which were a gift from the American Red Cross (Rockville, MD). The PK-13 cells were maintained and the PPV propagated as described in Heldt et al. (*22*) using complete media, which consisted of EMEM supplemented with 2 mM glutamine, 1x gentamicin, and 10% non-heat-inactivated fetal calf serum (Hyclone, Logan, UT). Upon propagation of the virus, the cell culture flasks were frozen at −20 °C and thawed at room temperature. The cells were then scraped from the flask, and the solution was clarified by centrifugation at 3000 rpm for 10 min in an IEC Centra CL2 centrifuge (Thermo Electron, Waltham, MA). This solution was then stored at −80 °C until further use.

Radioactive PPV was prepared by metabolically incorporating a radiolabel during propagation by addition of ^{35}S methionine and cysteine to the cell culture media. This was done by seeding the cells at 6×10^5 cells per 75 cm^2 flask. The next day, the flask was infected with 10^3 MTT units of PPV in 1 mL of PBS. An MTT unit was defined as the concentration of virus where 50% of the cells were considered viable, as determined by the metabolic cleavage of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide salt (MTT) as previously described (*22*). The MTT concentration can be observed optically and quantified by spectrophotometry. The flasks were placed in the incubator at 5% CO_2, 37 °C, and 100% humidity for 1 h. At this time, 5 mL of complete media was added to the flask, which was returned to the incubator. The next day, the media was removed from the flask and starvation media was added, which contained the same amino acids and essential nutrients as the EMEM, except for methionine. The cells were exposed to 5 mL of this starvation media for 1.5 h, and then EasyTag Protein Labeling mixture (Perkin-Elmer, Waltham, MA) was added to a final concentration of 50 μCi/mL. The cells were frozen at −20 °C when approximately 90% cytopathic effect was observed, usually after 4−5 days. Virus purification was done by CsCl gradient centrifugation, as described elsewhere (*22*), after which solutions were dialyzed against PBS for 3 days at 4 °C, stored at 4 °C, and used within 2 weeks.

All infectivity measurements were made using the MTT assay, which previously has been correlated to a $TCID_{50}$ (50% tissue culture infectious dose), a common method for the titration of infectious viruses (*22*).

2.3. Primary Screening of Library. A solid-phase combinatorial trimer library was made by Peptides International (Louisville, KY) using the divide-couple-recombine technique (*23*) on Toyopearl Amino 650 EC (Tosoh Bioscience, Montgomeryville, PA). The library had an alanine and 2 mini-PEG spacer arms [Toyopearl resin-Ala-$(COCH_2$-$(OCH_2CH_2)_2$-$NH)_2$-X-X-X], where X is any naturally occurring amino acid except cysteine or methionine. The library was swelled in 20% methanol in DI water overnight and then buffer exchanged with PBS three times, with the last buffer exchange being overnight. Ten milligrams of dry library was taken and mixed with 50% human blood plasma in PBS for 1 h. ^{35}S-Labeled PPV was added to the library at 10,000 CPM (about 1×10^{-3} μCi) and allowed to equilibrate for about 1.5 h. The library was then placed in a disposable 10 mL fritted column (Bio-Rad Laboratories, Hercules, CA). The beads were washed with PBS followed by PBS containing an additional 1 M NaCl or KCl until no radioactivity could be detected from the wash. The beads were washed again in PBS to remove excess salt and then put into 20 mL of 1% low melt agarose (Bio-Rad Laboratories, Hercules, CA). This was poured onto a 160 mm × 180 mm GelBond (BioWhittaker Inc, Walkersville, MD) and allowed to dry for 3 days. Kodak BioMax MR Film (Kodak, Rochester, NY) was placed onto the dried gel for 10 days and developed with a Konica Medical Film Processor (Tokyo, Japan). A proprietary ligand found by the American Red Cross that binds to PPV (positive control) and a negative control of Amino 650M were used as markers to line up the film and the gel for visualization of radioactive beads. Positive beads were excised from the gel, boiled in water for 10 min each, and vortexed and the water changed for a total of three repeats to remove the agarose and the bound PPV from the beads. The beads then were sent to the Texas A&M Protein Laboratory (College Station, TX) for sequencing by Edman degradation.

2.4. Chromatography to Verify Screening Results. Peptide resins were synthesized on Toyopearl Amino 650M resin (Tosoh Bioscience, Montgomeryville, PA) by Peptides International (Louisville, KY) and were packed into disposable PIKSI columns (ProMetic Biosciences Ltd, Cambridge, England) with a total of 0.5 mL of settled resin in PBS per column. A Rainin (Oakland, CA) 8-channel peristaltic pump was used to add a solution of PPV supernatant in either PBS or 7.5% human blood plasma in PBS, at a rate of 0.1 mL/min. Ten 0.5 mL fractions were collected and tested for infectivity using the MTT assay and compared to the titer of the starting material before addition to the column.

2.5. Acetylated Control. The acetylated control was made by the acetylation of Toyopearl Amino 650M resin. About 50 mL of settled resin was added to a sintered glass funnel and allowed to drain. The resin was washed three times with 100 mL of 0.1 M NaOH. The resin was then washed with deionized water until the pH was below 8. The resin was placed into three separate 50 mL conical tubes and 30 mL of 0.5 M sodium acetate was added to each tube followed by end-over-end rotation for 10 min. A 100% excess of acetic anhydride (Riedel-de Haen, Germany), which amounted to a total of 755 μL, was dissolved into 3 mL of acetone, and 1.2 mL of the solution was added to each conical tube. The tubes were mixed for 2 h. The resin was then returned to the sintered glass funnel and washed three times with 100 mL of DI water, four times with 100 mL of 0.5 M NaOH, and finally at least 10 times with 100 mL of DI water, until the pH of the rinse was below 8. The acetylation was confirmed by taking 50 μL of acetylated resin, 50 μL of Toyopearl Amino 650M resin, and 50 μL of DI water and adding two drops of ninhydrin reagent, 2% solution (Sigma, St. Louis, MO). After 1−2 min the resins were observed for color change; the acetylated resin and the DI water remained yellow, whereas the amino resin turned purple.

3. Results and Discussion

3.1. Library Design. Many hexameric peptide ligands have been found that can purify proteins (*19*, *24*, *25*) and toxins (*17*). Each of these peptide ligands were selected from a hexamer library, which contains over 34 million different combinations, when 18 of the 20 naturally occurring amino acids are used for library production. It would take a tremendous amount of effort to screen all of these sequences, and it is not necessary when purification is the intended use of the ligand. In general, a purification ligand is useful if it can bind over 90% of the target protein and is specific enough to produce an eluted protein that is 80−90% pure, but for virus removal, the goal is reduction of ≥99.99% of a virus, which is at femtomolar to picomolar concentrations. To improve the possibility of finding a ligand that can accomplish this, a trimeric library was designed and screened. This library contained only 5832 different sequences and could be screened many times over to compare different screening conditions. Further, by screening the entire library, there was a greater probability that one or more strongly binding ligands would be found, which would not necessarily occur with a hexamer library.

A spacer arm of two sets of two ethylene glycol units separated by a peptide bond (*26*) (designated AEEA-AEEA by Peptides International) was added to the library to increase the chances of finding a peptide ligand that bound to a conserved area on the virus surface. This spacer arm separated the peptide approximately 15 Å from the undisclosed spacer on the Toyopearl resin. It has also been shown that hydrophilic ethylene glycol does not bind proteins and makes a flexible yet inert spacer arm that allows movement of the ligand, improving binding (*27*−*29*). A surface map of PPV shows that there are canyons on the surface of the virus that are approximately 15 Å in depth (*30*), and so the spacer arm was designed to allow the peptide to reach into the depths of the canyons. For most non-enveloped viruses, it is accepted that the conserved amino acid sequences are located in the depth of these canyons because these are often the location of the receptor binding sites.

3.2. Primary Screening. The library beads were originally blocked with 50% human blood plasma before the virus was added. This blocked any of the peptides that had a high affinity for plasma proteins before the addition of PPV to the library.

Table 1. Peptide Sequences Found from Primary Screening

wash	sequence	
1 M NaCl	KNY	AKL
	WRW	KTF
	KKK	VWR
	KGK	RAA
	KYY	KRR
	FVV	
1 M KCl	FRH	KHR
	KAA	RTG
	RQQ	

After incubation with PPV, the beads were washed to remove any nonspecifically bound virus. One screening run was washed in 1 M NaCl and yielded a total of 24 positive beads from about 10,000; another screening run was washed in 1 M KCl and gave a total of 9 positive beads. Only those positive beads that had a large signal to size ratio (i.e., a small bead that gave a large signal), as determined by visual inspection, were chosen for sequencing. The results of the returned sequences are shown in Table 1. To better determine the significance of the different chemical groups, the amino acids were counted and compared to their probability of random occurrence (Table 2). A random occurrence was determined as the number of amino acids in the chemical group divided by the number of different amino acids in the library and then multiplied by the total number of amino acids found from the sequencing. For example, there are five different aliphatic amino acids, so the random number of aliphatic amino acids is calculated by dividing 5 amino acids the by the 18 different amino acids used in this study, and the result multiplied by the 48 total amino acids in the 16 trimers found by screening. This gives the random occurrence of aliphatic amino acids of 13.3 indicated in Table 2. If the number of amino acids from a certain chemical group was close to the random occurrence number, then it was suspected that the chemical group was just randomly found and may have little to do with the binding of the virus. However, if the number was much higher than that expected to occur randomly, then that group was considered to be significant in the binding of the virus.

Table 2 shows the importance of basic amino acids in the binding of PPV and, to complement this, the lack of acidic groups associated with ligands found to bind to PPV. The results show that positive charges are important for the binding of virus. This is an expected result because canine parvovirus, a related parvovirus, has an isoelectric point of 5.3 (*31*), leaving it negatively charged at physiological pH, which corresponded to the conditions used in the screening studies. There was a random distribution of aromatic and aliphatic groups with seven sequences that contained aromatics and seven sequences that contained aliphatics. The sequences were then categorized into the following: those containing an aromatic amino acid, those containing an aliphatic amino acid, and those containing neither. Since all but one sequence contained a basic group, all sequences chosen for further screening contained a basic amino acid. From these categories, five sequences were chosen for additional screening using column chromatography: WRW and KYY, which contain aromatics; RAA, which contains an aliphatic; and KHR, which contains a histidine. Also, KKK and KRR were combined to form KRK, which contains basic residues.

3.3. Column Chromatography. The resins were packed into disposable columns and tested for breakthrough of PPV in the eluent using infectivity as the enumeration method. First, cell culture supernatant containing PPV was diluted with PBS to a final titer of about 6−7 log (MTT/mL) (approximately a 1:100

Table 2. Chemical Characterization of Sequences

amino acid type	random[a]	actual[b]
aliphatic	13.3	11
cyclic imino	2.7	0
imidazole	2.7	2
basic	5.3	21
aromatic	8.0	9
hydroxy	5.3	2
amide	5.3	3
acidic	5.3	0

[a] The number of amino acids expected from a random distribution of amino acids in 16 trimeric peptides. [b] The number of each type of amino acid that was present in the 16 trimeric peptides.

to 1:10 dilution) and was filtered through a 0.22 μm filter. Virus spiking studies should not be carried out at a dilution more concentrated than 1:10, as the virus solution, which contains contaminants from the cell culture from which it was created, may start to interfere with the virus clearance mechanism (*3*). However, the larger the initial viral load, the better the opportunity to validate a high degree of virus clearance. Virus clearance was calculated in accordance with the expression

$$\log \text{clearance} = -\log\left(\frac{\text{virus detected after clearance step}}{\text{total virus load}}\right) \quad (1)$$

The PPV breakthrough curves were determined by pumping virus-spiked solutions onto the peptide columns at 0.1 mL/min. Fractions equivalent to 1 column volume were collected, for a total of 10 column volumes, and the amount of PPV in the flowthrough fractions was determined. The results are plotted in Figure 1 as the percentage of the detectable PPV as a function of column volume. Presenting the results in terms of a percentage of the detectable clearance automatically accounts for the different initial virus titers of the various batches analyzed in these experiments.

In PBS, all of the resins were able to clear completely the detectable virus available in the solutions, as shown in Figure 1. This is in contrast to the amino resin control (with no peptides, which is considered a weak ion-exchange resin) that was not able to remove any significant amounts of PPV from PBS. Clearly, the peptides were responsible for the binding of the virus, and nonspecific binding to the resin surface was ruled out.

The small peptide resins have the ability to remove viruses from simple solutions such as water, suggesting potential application to water treatment. In fact, microfiltration is being considered as an alternative to chlorine treatment of water supplies (*32*), but the method suffers from many of the same difficulties as nanofiltration for therapeutic processes. Small viruses, like hepatitis A virus, which has a diameter of 27−32 nm (*33*), and norovirus, with a diameter of 30−40 nm (*34*), are able to pass through many nanofiltration and all microfiltration membranes. These viruses are shed in the feces of infected humans and are common contaminants of water supplies. Small peptide ligands theoretically could be placed on microfiltration membranes to improve virus removal without the need to use membranes of small pore size which often cause fouling (*8*) and may require high back pressures.

To challenge the peptides for their ability to remove PPV under therapeutic processing conditions, virus-spiked 7.5% human blood plasma was used. A 7.5% human blood plasma solution contains about 5 mg/mL of protein, which is approximately the amount of protein that can be found in a therapeutic protein product. There are two general viral clearance steps in a monoclonal antibody production process, i.e., a low

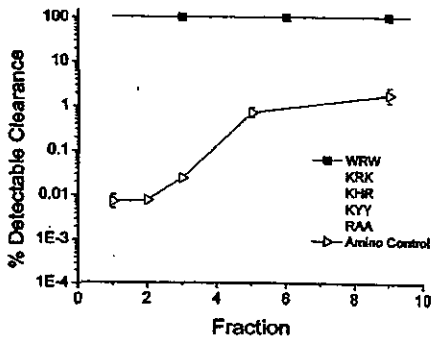


Figure 1. Binding of trimeric peptides to PPV in PBS. All ligands were able to bind 100% of the detectable PPV. The amino control, which is a weak ion-exchange resin, was able to clear less than 1% of the detectable PPV. Columns were run in duplicate, and the error bars represent the detectable clearance of each column.

pH step after cell harvest and a nanofiltration step before or during formulation (*35*). If effective, small peptides theoretically could be used on membranes on the nanofiltration step at the end of the process. All of the peptides were challenged with protein loads similar to those that would be found at this phase of the purification process.

Data on the removal of PPV from this complex mixture using the peptides are presented in Figure 2. All of the resins tested, except for KHR, were able to remove all of the detectable PPV in the first column volume. All of the resins had a breakthrough of PPV before the fifth column volume. It is believed that the proteins found in human blood plasma also began to nonspecifically bind to the peptides at this point, leaving less peptide available for specific binding to PPV. There may be a way to improve the selectivity of the virus over the plasma proteins by optimization of the chromatography process through changes in peptide density or buffer ionic strength.

The amino control resin had the ability to bind to some of the PPV and remove it from solutions. In the first two column volumes in PBS and 7.5% human blood plasma, the amino control achieved about 1 log clearance. This is not surprising, as anion exchange columns are often tested for their ability to clear viruses (*35*). It has been shown that a Q-Sepharose column was able to clear 3 log of PPV when loaded at pH 6.5 (*36*) and as high as 5 log of MVM when loaded in Tris buffer at pH 8.0 (*14*). This follows the trend that increasingly basic solutions will make the virus surface more negatively charged, which would cause increasingly stronger binding of the virus to an anion exchange column. The control in our experiments was a weak ion-exchange resin at pH 7.4, which showed clearance lower than that seen with the Q-Sepharose columns, as would be expected.

An increase in binding of virus over time was found with the amino control resin both in the presence and absence of human blood plasma (Figures 1 and 2). The PPV solution used in the experiments was cell culture supernatant that had been clarified by low-speed centrifugation and filtered through a 0.22 μm filter, and so the solution contained cellular debris. Since the amino control is a weak ion-exchange resin, it is possible that the resin was binding the cellular debris, and the virus then bound to the debris on the resin, as many proteins in host cell proteins are known to be negatively charged and removed by anion exchange (*35*). Since the amino resin has a high positive charge density, it is also possible that these cellular proteins may denature onto the surface, as has been suspected in ion exchange purification (*37*, *38*) and is known to happen when proteins adsorb to surfaces (*39*). Protein denaturation was also

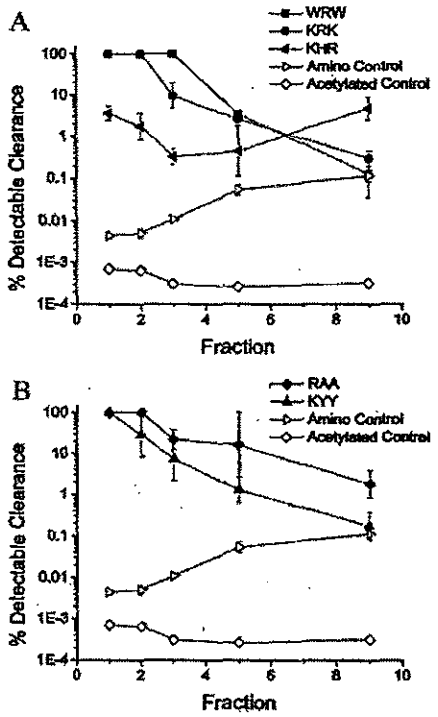


Figure 2. Binding of trimeric peptides to PPV in 7.5% human blood plasma. WRW was able to bind 100% of the detectable PPV in the first 3 column volumes. After 9 column volumes, WRW was still able to bind as much as the amino control. The amino control and acetylated control are the same in each figure as a reference point for comparison of the different peptide resins. Columns were run in duplicate, and the error bars represent the detectable clearance of each column.

a possibility, as this was observed in the purification of tumor necrosis factor-α using an ion exchange column (*37*). The longer these host cell proteins are retained on the column, the greater the potential for protein denaturation, and this may provide different binding sites for the virus. Virus binding to host cell proteins is confirmed by the fact that when highly purified virus suspensions containing less than 100 μg/mL of total protein are used in resin challenge, the amino control resin binds no more than 1 log of PPV even after 10 column volumes (data not shown). Viruses are also known to easily aggregate (*40*), and so the presence of denatured protein could become a new binding surface for the virus.

The trimer KHR was able to achieve 4 logs clearance in the first column volume but still left in solution 3 log (MTT/mL) of virus. This peptide column showed the same decrease in viral clearance in the first three column volumes seen with the other peptide resins, but in subsequent column volumes, it exhibited an increase in detectable clearance. This latter behavior was only seen in the amino control resin and not the other peptide resins. It is suspected that this resin may be causing denaturation of proteins in solution as discussed above, but this issue was not examined further. This resin was just discarded as one of the lead candidates, as its performance as a viral clearance ligand was unacceptable.

The trimers WRW, KRK, RAA, and KYY all exhibited breakthrough of PPV in the presence of plasma proteins after the first three column volumes (Figure 2). There was no detectable cooperative binding observed for these resins in the flowthrough fractions tested. Of these resins, only WRW was able to completely clear all detectable PPV in the first three column volumes from 7.5% human blood plasma. With optimization of the peptide density and spacer length, this resin may be able to clear PPV in all nine column volumes of challenge solution containing 7.5% human blood plasma. Human blood plasma also contains many different proteins, and only one or two may be interfering with the binding of PPV. For example, if albumin is the predominant protein binding to the resin, then the peptide may be able to clear PPV very well from a solution that contains other proteins but not albumin. In this case, WRW has the potential to be used effectively for final purification of a pure protein with excellent removal efficiency.

Chromatographic beads are not the most efficient way to remove large particles from process streams. The viruses, having a diameter on the same order of magnitude as the pore diameter, have small diffusion coefficients in the pores of the beads, and viruses quickly clog the pores. Consequently, the accessible surface area of the beads is mainly associated with the outside surface of the bead, and the inner pore surface is not available for binding. Membranes have a better geometry for binding of particles such as viruses, as there are not any diffusional limitations. However, the screening of a combinatorial library of peptides is difficult to do on a membrane surface. The SPOT method, developed by Ronald Frank (*41*), is used to produce peptide libraries on a cellulose membrane surface, but if done manually, only several hundreds of peptides can be created in 2−3 days (*42*). This is a small library compared to the thousands of peptides that can be screened on chromatographic beads. In addition, the binding to a peptide on cellulose fibers may be quite different from that observed on other membrane materials. There are currently no large ligand libraries on any membrane surface that is likely to be used for large-scale virus removal. This study provides proof-of-concept that peptides have the ability to remove viruses specifically. In the future, it may be beneficial to change the geometry of the support for improved access of all of the ligands to the viral particle, but currently the bead geometry offers a better screening platform.

4. Conclusions

Small trimeric ligands that specifically bind to porcine parvovirus were isolated from a solid-phase peptide library. In PBS, 100% of detectable infectious virus was removed from solution for every fraction that was tested, up to nine column volumes. This demonstrates the potential of these peptides for use in virus removal from samples of relatively simple composition, such as for water purification applications. In more complex mixtures, such as 7.5% human blood plasma, peptide WRW was able to remove all detectable infectious viruses in the first three column volumes. This is impressive for a ligand that contains only three amino acids, as most peptide ligands are a minimum of six amino acids in length. Enhanced specificity and binding affinity may be found using an increased number of amino acids in the ligand, and this is currently being examined. Tethering one or more of the ligands to a membrane with more suitable geometry may improve virus removal efficiency from complex mixtures. The ligands could also be optimized for application to specific process streams, so that a single ligand must only compete with one therapeutic protein, thereby overcoming competitive binding and facilitating use as an efficient virus absorbent.

Acknowledgment

The authors would like to thank Pathogen Removal and Diagnostic Technologies (PRDT), a joint venture between the American Red Cross and ProMetic BioSciences Inc., and the NIH/NCSU Molecular Biotechnology Training Program for

funding. The authors also thank Drs. Dennis T. Brown and Raquel Hernandez for their training in cell culture techniques.

References and Notes

(1) Huang, P. Y.; Peterson, J. Scaleup and virus clearance studies on virus filtration in monoclonal antibody manufacture. In *Membrane Separations in Biotechnology*; Wang, W., Ed.; Marcel Dekker: New York, 2001; pp 327−350.

(2) Kempf, C.; Stucki, M.; Boschetti, N. Pathogen inactivation and removal procedures used in the production of intravenous immunoglobulins. *Biologicals* **2007**, *35* (1), 35−42.

(3) Darling, A. Validation of biopharmaceutical purification processes for virus clearance evaluation. *Mol. Biotechnol.* **2002**, *21* (1), 57−83.

(4) Valdes, R.; Ibarra, N.; Ruibal, I.; Beldarrain, A.; Noa, E.; Herrera, N.; Aleman, R.; Padilla, S.; Garcia, J.; Perez, M. Chromatographic removal combined with heat, acid and chaotropic inactivation of four model viruses. *J. Biotechnol.* **2002**, *96* (3), 251.

(5) Food and Drug Administration. *Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use;6* U.S. Department of Health and Human Services: Rockville, MD, 1997.

(6) Cai, K.; Gierman, T. M.; Hotta, J.; Stenland, C. J.; Lee, D. C.; Pifat, D. Y.; Petteway, S. R., Jr. Ensuring the biological safety of plasma-derived therapeutic proteins. *Biodrugs* **2005**, *19* (2), 79−96.

(7) Furuya, K.; Murai, K.; Yokoyama, T.; Maeno, H.; Takeda, Y.; Murozuka, T.; Wakisaka, A.; Tanifuji, M.; Tomono, T. Implementation of a 20-nm pore-size filter in the plasma-derived factor VIII manufacturing process. *Vox Sang.* **2006**, *91* (2), 119−125.

(8) Lute, S.; Bailey, M.; Combs, J.; Sukumar, M.; Brorson, K. Phage passage after extended processing in small-virus-retentive filters. *Biotechnol. Appl. Biochem.* **2007**, *47*, 141−151.

(9) Wickramasinghe, S. R.; Han, B.; Carlson, J. O.; Powers, S. M. Clearance of minute virus of mice by flocculation and microfiltration. *Biotechnol. Bioeng.* **2004**, *86* (6), 612−621.

(10) Yokoyama, T.; Murai, K.; Murozuka, T.; Wakisaka, A.; Tanifuji, M.; Fujii, N.; Tomono, T. Removal of small non-enveloped viruses by nanofiltration. *Vox Sang.* **2004**, *86* (4), 225−229.

(11) Specht, R.; Han, B.; Wickramasinghe, S. R.; Carlson, J. O.; Czernak, P.; Wolf, A.; Reif, O.-W. Densonucleosis virus purification by ion exchange membranes. *Biotechnol. Bioeng.* **2004**, *88* (4), 465−473.

(12) Zhou, J. X.; Tressel, T.; Gottschalk, U.; Solamo, F.; Pastor, A.; Dermawan, S.; Hong, T.; Reif, O.; Mora, J.; Hutchison, F.; Murphy, M. New Q membrane scale-down model for process-scale antibody purification. *J. Chromatogr. A* **2006**, *1134* (1−2), 66.

(13) Phillips, M.; Cormier, J.; Ferrence, J.; Dowd, C.; Kiss, R.; Lutz, H.; Carter, J. Performance of a membrane adsorber for trace impurity removal in biotechnology manufacturing. *J. Chromatogr. A* **2005**, *1078* (1−2), 74.

(14) Norling, L.; Lute, S.; Emery, R.; Khuu, W.; Voisard, M.; Xu, Y.; Chen, Q.; Blank, G.; Brorson, K. Impact of multiple re-use of anion-exchange chromatography media on virus removal. *J. Chromatogr. A* **2005**, *1069* (1), 79.

(15) Gregori, L.; Lambert, B. C.; Gurgel, P. V.; Gheorghiu, L.; Edwardson, P.; Lathrop, J. T.; MacAuley, C.; Carbonell, R. G.; Burton, S. J.; Hammond, D.; Rohwer, R. G. Reduction of transmissible spongiform encephalopathy infectivity from human red blood cells with prion protein affinity ligands. *Transfusion* **2006**, *46* (7), 1152−1161.

(16) Gregori, L.; Gurgel, P. V.; Lathrop, J. T.; Edwardson, P.; Lambert, B. C.; Carbonell, R. G.; Burton, S. J.; Hammond, D. J.; Rohwer, R. G. Reduction in infectivity of endogenous transmissible spongiform encephalopathies present in blood by adsorption to selective affinity resins. *Lancet* **2006**, *368* (9554), 2226−2230.

(17) Wang, G.; Carbonell, R. G. Characterization of a peptide affinity support that binds selectively to staphylococcal enterotoxin B. *J. Chromatogr. A* **2005**, *1078*, 98−112.

(18) Mondorf, K.; Kaufman, D. B.; Carbonell, R. G. Screening of combinatorial peptide libraries: Identification of ligands for affinity purification of proteins using a radiological approach. *J. Pept. Res.* **1998**, *52*, 526−536.

(19) Yang, H.; Gurgel, P. V.; Carbonell, R. G. Hexamer peptide affinity resins that bind the Fc region of human immunoglobulin G. *J. Pept. Res.* **2005**, *66*, 120−137.

(20) Wang, G.; De, J.; Schoeniger, J. S.; Roe, D. C.; Carbonell, R. G. A hexamer peptide ligand that binds selectively to staphylococcal enterotoxin B: isolation from a solid phase combinatorial library. *J. Pept. Res.* **2004**, *64* (2), 51−64.

(21) Gurgel, P. V.; Carbonell, R. G.; Swaisgood, H. E. Identification of peptide ligands generated by combinatorial chemistry that bind alpha-lactalbumin. *Sep. Sci. Technol.* **2001**, *36* (11), 2411−2431.

(22) Heldt, C. L.; Hernandez, R.; Mudiganti, U.; Gurgel, P. V.; Brown, D. T.; Carbonell, R. G. A colorimetric assay for viral agents that produce cytopathic effects. *J. Virol. Methods* **2006**, *135* (1), 56.

(23) Furka, A.; Sebestyen, F.; Asgedom, M.; Dibo, G. General-method for rapid synthesis of multicomponent peptide mixtures. *Int. J. Pept. Protein Res.* **1991**, *37* (6), 487−493.

(24) Kaufman, D. B.; Hentsch, M. E.; Baumbach, G. A.; Buettner, J. A.; Dadd, C. A.; Huang, P. Y.; Hammond, D. H.; Carbonell, R. G. Affinity purification of fibrinogen using a ligand from a peptide library. *Biotechnol. Bioeng.* **2002**, *77* (3), 278−289.

(25) Gurgel, P. V.; Carbonell, R. G.; Swaisgood, H. E. Fractionation of whey proteins with a hexapeptide ligand affinity resin. *Bioseparations* **2001**, *9*, 385−392.

(26) Ohtsuka, K.; Uemura, K.; Nojima, T.; Waki, M.; Takenaka, S. Immobiliation of RNase S-Peptide on a single-stranded DNA-fixed gold surface and effective masking of its surface by an acridinyl poly(ethylene glycol). *Analyst* **2006**, *131* (1), 55−61.

(27) Leckband, D. E.; Kuhl, T.; Wang, H. K.; Herron, J.; Muller, W.; Ringsdorf, H. 4-4-20 anti-fluorescyl IgG Fab′ recognition of membrane bound hapten: Direct evidence for the role of protein and interfacial structure. *Biochemistry* **1995**, *34*, 11467−11478.

(28) Soltys, P. J.; Etzel, M. R. Equilibrium adsorption of LDL and gold immunoconjugates to affinity membranes containing PEG spacers. *Biomaterials* **2000**, *21*, 37−48.

(29) Weimer, B. C.; Walsh, M. K.; Wang, X. Influence of a polyethylene glycol spacer on antigen capture by immobilized antibodies. *J. Biochem. Biophys. Methods* **2000**, *45* (2), 211.

(30) Simpson, A. A.; Herbert, B.; Sullivan, G. M.; Parrish, C. R.; Zadori, Z.; Tijssen, P.; Rossmann, M. G. The structure of porcine parvovirus: Comparison with related viruses. *J. Mol. Biol.* **2002**, *315*, 1189−1198.

(31) Weichert, W. S.; Parker, J. S. L.; Wahid, A. T. M.; Chang, S.-F.; Meier, E.; Parrish, C. R. Assaying for structural variation in the parvovirus capsid and its role in infection. *Virology* **1998**, *250* (1), 106.

(32) Ali, N. A.; Hasan, A. R.; Wong, L. Y. Development of a novel aymmetric ultra low pressure membranes and a preliniinary study for bacteria and pathogen removal applications. *Desalination* **2007**, *206*, 474−484.

(33) Vaidya, S. R.; Kharul, U. K.; Chitambar, S. D.; Wanjale, S. D.; Bhole, Y. S. Removal of hepatitis A virus from water by polyacrylonitrile-based ultrafiltration membranes. *J. Virol. Methods* **2004**, *119* (1), 7−9.

(34) Sano, D.; Ueki, Y.; Watanabe, T.; Omura, T. Membrane separation of indigenous noroviruses from sewage sludge and treated wastewater. *Water Sci. Technol.* **2006**, *54* (3), 77−82.

(35) Shukla, A. A.; Hubbard, B.; Tressel, T.; Guhan, S.; Low, D. Downstream processing of monoclonal antibodies−Application of platform approaches. *J. Chromatogr. B* **2007**, *848* (1), 28.

(36) Kim, I. S.; Choi, Y. W.; Lee, S. R.; Woo, H. S.; Lee, S. Removal and inactivation of viruses during manufacture of a high purity antihemophilic factor VIII concentrate from human plasma. *J. Microbiol. Biotechnol.* **2001**, *11* (3), 497−503.

(37) Li, M.; Wang, Y. S.; Li, W. J.; Su, Z. G. Stability of tumor necrosis factor-alpha during ion exchange chromatography. *Biotechnol. Lett.* **2003**, *25* (11), 905−908.

(38) Sugihara, A.; Senoo, T.; Enoki, A.; Shimada, Y.; Nagao, T.; Tominaga, Y. Purification and characterization of a lipase from Pichia burtonii. *Appl. Microbiol. Biotechnol.* **1995**, *43* (2), 277−281.

(39) Oscarsson, S. Factors affecting protein interaction at sorbent interfaces. *J. Chromatogr., B: Biomed. Sci. Appl.* **1997**, *699* (1−2), 117.

(40) Berkowitz, S. A.; Philo, J. S. Monitoring the homogeneity of adenovirus preparations (a gene therapy delivery system) using analytical ultracentrifugation. *Anal. Biochem.* **2007**, *362* (1), 16–37.

(41) Frank, R. Spot-synthesis: an easy technique for the positionally addressable, parallel chemical synthesis on a membrane support. *Tetrahedron* **1992**, *48* (42), 9217.

(42) Reichl, U. Antibody epitope mapping using arrays of synthetic peptides. In *Antibody Engineering: Methods and Protocols*; Lo, B. K. C., Ed.; Humana Press Inc: Totowa, NJ, 2004; Vol. 248.

Received October 30, 2007. Accepted April 7, 2008.

BP070412C

医薬品　研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
一般的名称	－		研究報告の公表状況	http://www.fda.gov/cber/gdlns/natparo.pdf	公表国 米国	
販売名（企業名）	－					

研究報告の概要

パルボウイルス B19 DNA を検出するための実施手順に関するガイダンス案が示された。
・全ての血漿由来製剤について、製造プール中のパルボウイルス B19 DNA のウイルス負荷を確実に 10^4IU/mL 以下とするため、製造過程の品質管理検査としてパルボウイルス B19 の NAT を実施すべきである。
・ミニプールサンプルのパルボウイルス B19 の NAT 検査は 10^6IU/mL 以上の個別ユニットを検出できる感度とすべきである。
・個別ユニットのパルボウイルス B19 DNA の力価が 10^6IU/mL 以上であることがわかった場合、又は、製造用プール血漿で 10^4IU/mL のパルボウイルス B19 DNA を上回る可能性がある場合、その後の製造に使用すべきではない。

原料血漿及び回収血漿において、パルボウイルス B19 DNA を検出するため、ならびに製造プールにおけるパルボウイルス B19 DNA のウイルス負荷が 10^4IU/mL 以下であることを示すために用いるパルボウイルス B19 の NAT 検査の精度、感度、特異度、再現性及びその他の性能特性を示すバリデーションデータを維持管理すべきである。

報告企業の意見	今後の対応
血漿由来製剤製造用原料血漿に関するパルボウイルス B19 の NAT 検査に関するガイダンス案の情報である。 当社血漿分画製剤は最終製品において NAT 検査を行い、パルボウイルス B19DNA 陰性であることを確認している。	今後ともパルボウイルス B19 に関する血漿分画製剤の安全性に関する情報に留意していく。

使用上の注意記載状況・その他参考事項等

慎重投与（次の患者には慎重に投与すること）
・溶血性・失血性貧血の患者［ヒトパルボウイルスＢ19 の感染を起こす可能性を否定できない。感染した場合には、発熱と急激な貧血を伴う重篤な全身症状を起こすことがある。］
・免疫不全患者・免疫抑制状態の患者［ヒトパルボウイルスＢ19 の感染を起こす可能性を否定できない。感染した場合には、持続性の貧血を起こすことがある。］

重要な基本的注意
(1)本剤の原材料となる‥［スクリーニング項目、不活化・除去工程］‥投与に際しては、次の点に十分注意すること。
1）血漿分画製剤の現在の製造工程では、ヒトパルボウイルス B19 等のウイルスを完全に不活化・除去することが困難であるため、本剤の投与によりその感染の可能性を否定できないので、投与後の経過を十分に観察すること。

妊婦、産婦、授乳婦等への投与
妊婦又は妊娠している可能性のある婦人には治療上の有益性が危険性を上回ると判断される場合にのみ投与すること。［妊娠中の投与に関する安全性は確立していない。本剤の投与によりヒトパルボウイルス B19 の感染の可能性を否定できない。感染した場合には胎児への障害（流産、胎児水腫、胎児死亡）が起こる可能性がある。］

NIHONSEIYAK
2008-004

Guidance for Industry

Nucleic Acid Testing (NAT) to Reduce the Possible Risk of Parvovirus B19 Transmission by Plasma-Derived Products

DRAFT GUIDANCE

This guidance document is for comment purposes only.

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

Additional copies of this draft guidance are available from the Office of Communication, Training and Manufacturers Assistance (HFM-40), 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448, or by calling 1-800-835-4709 or 301-827-1800, or from the Internet at http://www.fda.gov/cber/guidelines.htm.

For questions on the content of this guidance, contact Mahmood Farshid, Ph.D., at 301-496-0952, or by Fax at 301-402-2780.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
July 2008

Table of Contents

I. INTRODUCTION 1
II. BACKGROUND 1
III. RECOMMENDATIONS 3
IV. REFERENCES 4

Contains Nonbinding Recommendations

Draft – Not for Implementation

Guidance for Industry

Nucleic Acid Testing (NAT) to Reduce the Possible Risk of Parvovirus B19 Transmission by Plasma-Derived Products

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

I. INTRODUCTION

We, FDA, are issuing this guidance to provide you, manufacturers of plasma-derived products, with recommendations for performing parvovirus B19 nucleic acid testing (NAT) as an in-process test for Source Plasma and recovered plasma used in the further manufacturing of plasma-derived products. Such testing will identify and help to prevent the use of plasma units containing high levels of parvovirus B19. This guidance also recommends how to report to the FDA implementation of parvovirus B19 NAT.

We recognize that in the current business practice for parvovirus B19 NAT in-process testing, several weeks can elapse between collection of the units of Source Plasma or recovered plasma and identification of B19 NAT-positive pools or units. We encourage manufacturers of plasma-derived products to employ practices that will reduce the time between product collection and in-process testing to allow for the meaningful notification of blood and plasma collection establishments of positive test results within the dating period of components.

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

II. BACKGROUND

Parvovirus B19 is a small, non-enveloped single strand DNA virus. This virus is highly resistant to all commonly used inactivation methods, including heat and solvent/detergent (S/D) treatment, and is also difficult to remove because of its small size. The parvovirus B19 can be

transmitted by blood components and certain plasma derivatives, and may cause morbidity to susceptible recipients such as pregnant women (and their fetuses exposed in utero), persons with underlying hemolytic disorders, and immune compromised individuals (Refs. 1 and 2). The disease transmission by transfusion of blood components is rare; however, extremely high levels of parvovirus B19, up to 10^{12} IU/mL, in plasma of acutely infected but asymptomatic donors may present a greater risk in plasma derivatives due to pooling of large numbers of plasma units in the manufacture of these products. The virus can be detected by NAT in plasma pools when there are high levels of parvovirus B19 DNA in viremic donations. For example, the parvovirus B19 DNA can be detected in various plasma-derived products, particularly in coagulation factors (Refs. 3 and 4). There have been a few reports of parvovirus B19 infection associated with the administration of coagulation factors (Refs. 5 and 6) and S/D Treated Pooled Plasma (Refs. 1 and 7). Parvovirus B19 DNA is less frequently detected in albumin and immunoglobulin products and, when detected, the levels are usually low. There are no confirmed reports that albumin and immunoglobulin products have transmitted parvovirus B19 infection.

We have held or participated in several meetings to discuss the potential risk of parvovirus B19 infection by plasma-derived products, and the strategy for reducing such risk. The meetings included FDA-sponsored NAT workshops in 1999 and 2001 (Refs. 8 and 9), Blood Products Advisory Committee (BPAC) meetings in 1999, and 2002 (Refs. 10, 11, and 12), the National Heart, Lung, and Blood Institute-sponsored Parvovirus B19 workshop in 1999 (Ref. 1), and an ad hoc Public Health Service (PHS) panel in 2002 (discussed at the 2002 BPAC meeting (Ref. 12)). In these meetings, it was recognized that the scientific data indicate that parvovirus B19 is highly resistant to the available viral inactivation methodologies, and is difficult to remove because of its small size. The viral inactivation/removal steps routinely used in the manufacturing process of plasma-derived products do not alone appear to be sufficient to completely clear the virus if high viral load is present in the starting material. Therefore, in these meetings, a common recommendation for mitigating the risk of parvovirus B19 transmission by plasma derivatives has been to limit the virus load in the manufacturing plasma pool by testing the plasma donations for high titer parvovirus B19 DNA, using a minipool format. This viral load reduction strategy combined with the ability of the manufacturing process to clear the residual virus could greatly reduce the risk of parvovirus B19 infection by plasma-derived products.

The recommended limit in this guidance for viral load of parvovirus B19 DNA in the manufacturing plasma pool (i.e., not to exceed 10^4 IU/mL) was primarily derived from studies that were conducted on the transmission of parvovirus B19 associated with S/D Treated Pooled Plasma (Refs. 1, 7, and 10). In principle, testing in a minipool format to measure the viral load for parvovirus B19 DNA in a manufacturing plasma pool is acceptable in order to exclude only the high-titer plasma donations, thereby avoiding too great a loss of plasma for further manufacturing. Furthermore, during the viremic period for parvovirus B19 infected donors, which can be very lengthy, low levels of parvovirus B19 coexist with parvovirus B19 antibodies (potentially complexing with and neutralizing the virus). Therefore, it is undesirable to remove plasma units with low levels of B19 DNA, because it would diminish the parvovirus B19 antibody levels in plasma pools and in some of the resulting plasma-derived products (Refs. 13 and 14).

III. RECOMMENDATIONS

We recommend that you implement the following procedures to detect the presence of parvovirus B19 DNA:

- For all plasma-derived products, you should perform parvovirus B19 NAT as an in-process quality control test to ensure that the viral load of parvovirus B19 DNA in the manufacturing pools does not exceed 10^4 IU/mL.
- Use parvovirus B19 NAT on minipool samples to screen plasma units intended for further manufacturing into plasma-derived products. The sensitivity of the NAT assay, in any size minipool, should be at least 10^6 IU/mL for detection of any single donation when tested in the minipool (i.e., if the titer of an individual unit is 10^6 IU/mL or higher, the test result on the minipool will be positive). Primers and probes selected for parvovirus B19 NAT should detect all known genotypes of the virus (Ref. 15).
- When identified, you should not use individual plasma units intended for further manufacturing into plasma-derived products, when such units are found to have a titer of parvovirus B19 DNA at or above 10^6 IU/mL, or when use of a positive unit might result in plasma manufacturing pools exceeding a parvovirus B19 DNA titer of 10^4 IU/mL.

You should maintain validation data demonstrating the accuracy, sensitivity, specificity, reproducibility, and other performance characteristics of the parvovirus B19 NAT assay used for the detection of parvovirus B19 DNA in the Source Plasma and recovered plasma, and for demonstrating that the viral load of parvovirus B19 DNA in the manufacturing pool does not exceed 10^4 IU/mL.

If the recommendations are implemented, you must notify FDA of the changes to an approved application under 21 CFR 601.12(c)(5) ("Supplement-Changes Being Effected"), and submit the information required in 21 CFR 601.12(b)(3)(i) through (vii).

IV. REFERENCES

1. Brown, K.E., Young N.S., Alving, B.M., Luiz, H., and Barbosa, L.H. Parvovirus B19 implications for transfusion medicine. Transfusion (2001) 41:130-135.

2. Young, N.S., and Brown, K.E. Parvovirus B19. The New England Journal of Medicine (2004) 350:586-597.

3. Saldanha, J., and Minor, P. Detection of human parvovirus B19 DNA in plasma pools and blood products derived from these pools: implications for efficiency and consistency of removal of B19 DNA during manufacture. British Journal of Haematology (1996) 93:714-719.

4. Schmidt, I., Blumel, J., Seitz, H., Willkommen, H., and Lower, J. Parvovirus B19 DNA in plasma pools and plasma derivatives. Vox Sanguinis (2001) 81:228-235.

5. Blumel, J., et al. Parvovirus B19 transmission by heat-treated clotting factor concentrates. Transfusion (2002) 42:1473-1481.

6. Wu, C.G., et al. Parvovirus B19 transmission by a high-purity factor VIII concentrate. Transfusion (2005) 45:1003-1010.

7. Koenigbauer, U.F., Eastlund, T., and Day, J.W. Clinical illness due to parvovirus B19 infection after infusion of solvent/detergent-treated pooled plasma. Transfusion (2000) 40:1203-1206.

8. Transcript of Workshop on Implementation of Nucleic Acid Testing, December 14, 1999. http://www.fda.gov/cber/minutes/nucleic121499.pdf.

9. Transcript of Application of Nucleic Acid Testing To Blood Borne Pathogens and Emerging Technologies Workshop, December 4, 2001. http://www.fda.gov/cber/minutes/nuclacd1204p1.pdf. http://www.fda.gov/cber/minutes/nuclacd1204p2.pdf.

10. Transcript of FDA's Blood Products Advisory Committee (BPAC) Meeting, September 1999. http://www.fda.gov/cber/advisory/bp/bpmain.htm.

11. Transcript of FDA's Blood Products Advisory Committee (BPAC) Meeting, December 2002. http://www.fda.gov/cber/advisory/bp/bpmain.htm.

12. Transcript of FDA's Blood Products Advisory Committee (BPAC) Meeting, March 14, 2002. http://www.fda.gov/cber/advisory/bp/bpmain.htm.

13. Lefrere, Jean-Jacques, et al. Persistent B19 infection in immunocompetent individuals: implications for transfusion safety. Blood (2005) 106(8):2890-2895.

14. Cassinotti, P., Siegl, G. Quantitative evidence for persistence of human parvovirus B19 DNA in an immunocompetent individual. Eur J Clin Microbiol Infect Dis (2000) 19:886-895.

15. Servant, A., et al. Genetic diversity within human erythroviruses: identification of three genotypes. J Virol (2002) 76:9124-34.

別紙様式第2-1

No. 6

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. 7. 17	該当なし	

一般的名称	(製造販売承認書に記載なし)	研究報告の公表状況	Vrioni G, Pappas G, Priavali E, Gartzonika C, Levidiotou S. Clin Infect Dis. 2008 Jun 15;46(12):e131-6.	公表国
販売名(企業名)	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)			ギリシャ

研究報告の概要

○永続的な微生物:ブルセラDNAは臨床的治癒後何年間も存続する
背景:ブルセラ症の発生率は依然として高いが、病態生理学、診断および治療、特に宿主内でのブルセラ属種の生存能については現在も不明な点が存在している。
方法:定量的リアルタイム・ポリメラーゼ連鎖反応法を用いて、ブルセラ症患者の複数の疾患ステージにおける細菌DNA量をモニターした。39名の急性ブルセラ症患者それぞれから3つ以上の末梢血検体を入手した(診断時1検体、治療終了後1検体、追跡調査時1検体以上)。
結果:大多数の患者(治療終了後では87%、治療終了6ヵ月後77%、治療終了2年以上後70%)は、無症候性であるにもかかわらず、持続的に細菌が検出可能であった。再発を経験した患者3名は、追跡調査中のどの疾患ステージにおいても細菌量に統計的有意差を示さなかった。
結論:適切な治療を行い回復したように見えても、ブルセラ菌DNAは残存する。この知見は、当該疾患の病態生理学に新たな洞察をもたらす。すなわち、ブルセラ菌は除去不可能な持続性の病原体である。

報告企業の意見	今後の対応
ブルセラ症に対して適切な治療を行い回復したように見えても、ブルセラ菌DNAは長期間体内に残存するとの報告である。	日本赤十字社では、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。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

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. 11.0 J

MAJOR ARTICLE

An Eternal Microbe: *Brucella* DNA Load Persists for Years after Clinical Cure

Georgia Vrioni,* Georgios Pappas,* Efthalia Priavali, Constantina Gartzonika, and Stamatina Levidiotou

Department of Microbiology, Medical School, University of Ioannina, Ioannina, Greece

Background. Despite the continuing high incidence of brucellosis, vague aspects of pathophysiology, diagnosis, and treatment continue to exist, particularly with regard to the ability of *Brucella* species to survive inside the host.

Methods. A quantitative real-time polymerase chain reaction assay was used for monitoring bacterial DNA load in brucellosis-affected patients throughout different disease stages. Three or more specimens per patient were obtained (1 at diagnosis, 1 at the end of treatment, and at least 1 during the follow-up period) from 39 patients with acute brucellosis.

Results. The majority of patients (87% at the end of treatment, 77% at 6 months after treatment completion, and 70% at >2 years after treatment) exhibited persistent detectable microbiological load despite being asymptomatic. The 3 patients who experienced relapse did not exhibit any statistically significant difference in their bacterial load at any stage of disease or during follow-up.

Conclusion. *Brucella melitensis* DNA persists despite appropriate treatment and apparent recovery. This finding offers a new insight into the pathophysiology of the disease: *B. melitensis* is a noneradicable, persisting pathogen.

Brucellosis is a zoonosis that is prevalent worldwide [1]. *Brucella* species have recently garnered renewed attention because of their potential for use in biowarfare [2] and their reemergence as a significant cause of travel-related infection [3]. The complex pathophysiology of *Brucella* species [4] is dominated by their ability to manipulate immune response, targeting professional and nonprofessional phagocytes. Therein, *Brucella* species replicate without affecting cellular viability; in fact, the pathogen, by switching off cellular apoptosis, practically renders the cell immortal, thus allowing for its own further survival [5]. This intracellular localization of *Brucella* species in specialized compartments affects both the natural history and the diagnostic and therapeutic principles of brucellosis. The natural history of brucellosis is characterized by a frequently silent, protracted disease evolution. Therapeutically, the disease evolution imposes the need for a prolonged combined treatment that, even when administered in accordance with optimal recommendations, may lead to relapses. Diagnostically, the disease evolution hampers the usefulness of blood cultures and the use of microbiological eradication indexes [6].

Quantification of the microbiological burden may theoretically offer insight into the actual natural history of the disease, and it may allow for the evaluation of when and how the pathogen is eradicated from the human body (the term "microbiological eradication" being questionable for such a disease) [6]. Serological tests are useful for diagnosis [7], but the time required for results after treatment is disappointingly long. In addition, serological test results are usually inadequate in predicting the outcome. The latter may also apply to newer, sophisticated techniques such as ELISA [8]. The development of such novel molecular diagnostic techniques as PCR offered promise—technology preceded clinical application in the context of brucellosis, and even before traditional PCR assays were adequately evaluated clinically [9], novel assays emerged.

Received 8 December 2007; accepted 29 January 2008; electronically published 5 May 2008.

Presented in part: 1st International Meeting on the Treatment of Human Brucellosis, Ioannina, Greece, November 2006.

* Present affiliations: Department of Clinical Microbiology, General University Hospital "Attikon," Medical School, University of Athens, Athens (G.V.); Institute of Continuing Medical Education of Ioannina, Ioannina (G.P.), Greece.

Reprints or correspondence: Dr. Georgios Pappas, Institute of Continuing Medical Education of Ioannina, H. Trikoupi 10, Ioannina 45333, Greece (gpele@otenet.gr).

Clinical Infectious Diseases 2008; 46:e131–6

© 2008 by the Infectious Diseases Society of America. All rights reserved.

1058-4838/2008/4612-00E1$15.00

DOI: 10.1086/588482

Here, we present the results of the application of a real-time PCR assay for the diagnosis and follow-up of brucellosis in a large number of patients, and we discuss the potential pathophysiological significance of the findings.

MATERIALS AND METHODS

Patients. Peripheral blood specimens (both whole blood and serum) were collected from 39 patients who had received a diagnosis of acute brucellosis and from 50 healthy blood donors (control group). A minimum of 3 samples per patient were obtained (1 at diagnosis, 1 at the end of treatment, and at least 1 during the follow-up period). The control group, matched for age and sex, had no antibodies to *Brucella* species. All patients received a diagnosis of acute brucellosis during the period 2001–2004 in the University Hospital of Ioannina, reference center for the district of Epirus in northwestern Greece, where (like in nearby Greek and foreign regions) brucellosis is endemic [1, 10]. Epidemiological, clinical, and microbiological characteristics of the patient population are presented in table 1.

The diagnosis of acute brucellosis was established according to 1 of the following criteria: (1) isolation of *Brucella* species in blood culture or other clinical samples or (2) the presence of suggestive clinical characteristics together with the demonstration of specific antibodies at high titers, seroconversion, or an increase in antibodies in a serum sample obtained 15–20 days after the first sample was obtained.

The posttreatment phase varied from 2 to 36 months, according to the continuity of patient visits. Three patients experienced relapse during the follow-up period. Of the 39 study patients, 30 were treated with the standard regimen of doxycycline plus rifampin, 7 were treated with doxycycline plus ciprofloxacin, 1 was treated with moxalactamm, and 1 was treated with doxycycline plus streptomycin (the latter for 2 weeks). The duration of treatment was 6 weeks for all but 4 patients. Two patients were treated for 12 weeks (1 with doxycycline plus rifampin and 1 with doxycycline plus ciprofloxacin), and 2 patients were treated for 6 months (1 with doxycycline plus ciprofloxacin and 1 with doxycycline plus rifampin). Analysis of the evolution of the bacterial DNA load was performed at the time of initial diagnosis, at the end of treatment, and during the follow-up period (2, 6, 12–24, and 24–36 months after the end of treatment). The study underwent ethics review and approval.

Bacteriological and serological techniques. Serological tests—including rose Bengal plate (RBP) agglutination, Wright seroagglutination, and ELISA (Serion ELISA Classic Brucella IgG/IgM/IgA; Institut Virion\Serion; detecting IgM, IgG, and IgA antibodies)—were performed on all patient and control specimens; blood cultures were performed for 24 of the initial 39 patients.

The RBP agglutination and the Wright seroagglutination tests were performed in accordance with techniques described elsewhere [11]. The ELISA was performed in accordance with the manufacturer's instructions. Blood cultures were processed with BacT/Alert (bioMérieux) in accordance with standard techniques [12, 13] and were monitored for 10 consecutive

Table 1. Demographic characteristics and clinical and microbiological findings for patients with brucellosis.

Variable	Patients
No. of patients/no. of samples studied	39/130
Demographic characteristic	
Male	30 (77)
Female	9 (23)
Age, mean years (range)	41 (16–78)
Clinical characteristic	
Duration of symptoms, mean days (range)	32 (7–270)
Fever	36 (92.3)
Constitutive symptoms	23 (59)
Osteoarticular complications	16 (41)
Hepato/splenomegaly	7 (18)
Orchiepididymitis	3 (10)
CNS disorders	2 (5)
Diagnostic test result	
Titer ≥1:160, by Wright test	36 (92)
RBP test (from 2+ to 4+)	37 (95)
ELISA test	39 (100)
Proportion (%) of patients with positive blood culture results	13/24 (54)

NOTE. Data are no. (%) of patients, unless otherwise indicated. RBP, rose Bengal plate.

days. If the system failed to detect any growth, the vials were transferred to a conventional incubator for 10 additional days. Blind subcultures were performed on days 10 and 20 on *Brucella* agar (BBL; Becton Dickinson) and were incubated at 37°C in a 5%–10% carbon dioxide atmosphere for 3 days [12, 13]. If growth appeared, the suspected colonies were identified by colonial morphology; Gram staining; oxidase, catalase, and urease tests; and positive agglutination with specific antiserum. Identification and biotyping of *Brucella* species were performed in accordance with standard microbiological procedures [14].

Isolation of DNA from clinical blood specimens and bacteria strains. Peripheral blood samples were collected in EDTA tubes. DNA was extracted from whole blood (200 μL) with the QIAamp DNA Blood Mini Kit (Qiagen) in accordance with the manufacturer's instructions. The kit couples the selective binding properties of silica gels with a microcentrifugation step. The specimens were first lysed with protease in a buffer chosen to optimize the DNA-binding capacities on the QIAamp membrane. The use of the silica column allowed, after DNA coating, efficient washing of the samples to eliminate contaminants. After elution, DNA was stored at −80°C until PCR amplification analysis.

Hybridization probe–based quantitative real-time PCR assay. The real-time PCR assay was based on direct amplification of a 207–base pair DNA sequence of a gene that codes for the synthesis of an immunogenetic 31-kilodalton protein specific for the *Brucella* genus (BCSP31). The primer pair used was that published by Baily et al. [15]. The amplification product was detected by using fluorescence technique hybridization probes labeled with LightCycler Red 640 (detected in channel F2). A control amplification reaction in the third channel (F3) acted as an internal run control. A single-tube duplex LightCycler-PCR (LC-PCR) was performed using the FastStart DNA Hybridization Probes kit (Roche Diagnostics). To each LightCycler glass capillary, we added 20 μL reaction mixture containing 6.6 μL PCR-grade water, 2.5 mmol of magnesium chloride, 4.0 μL reagent mix (containing primers and probes), 2.0 μL FastStart mix, and 5.0 μL template (sample or standard). Primers and probes were designed and provided by TIB MOLBIOL. To detect any ampicon contamination or amplification failure, negative controls that contained 5 μL of PCR water instead of DNA and positive controls that contained DNA of *Brucella melitensis* biovar 1 were included in each real-time PCR run. Cycling conditions consisted of initial denaturation at 95°C for 10 min, followed by 55 cycles at 95°C for 10 s, at 55°C for 8 s, and at 72°C for 15 s in a LightCycler instrument (Roche Diagnostics). Fluorescence curves were analyzed with LightCycler software, version 3.5. After amplification, melting-curve analysis was performed to verify the specificity of PCR products (1 cycle consisted of 95°C for 20 s, 40°C for 20 s, and 85°C for 0 s). The BCSP31-derived product was identified by running the melting curve with a specific melting point of 67.5°C (concentration dependent). A standard curve, comprising 10-fold dilutions of *Brucella* BCSP31 DNA of 10^1–10^7 target equivalents, allowed quantification of unknown samples. In the provided standard row (TIB MOLBIOL), the lowest concentration (10 copies) was amplified in 35–36 cycles; 100, 10,000, and 1,000,000 copies were amplified after 32–33, 25–26, and 17–18 cycles, respectively (with the crossing point calculated by the "second derivative maximum"). With the method "fit points," the crossing point values were 32–33, 28–29, 22–23, and 15–16 cycles, respectively. The detection limit of the method spiked with serial dilution of *B. melitensis* DNA was 10 copies/5 μL DNA extract.

RESULTS

Brucella species was initially isolated in blood cultures from 13 (54%) of the 24 patients with available blood specimens. All strains isolated were identified as *B. melitensis* biotype 2. Of the 13 patients who had positive blood culture results, 12 also had positive results for all 3 serological tests used, whereas 1 had positive results only for 2 serological tests (RBP agglutination and ELISA). The remaining 26 (67%) of the 39 patients with acute brucellosis received their diagnoses on the basis of clinical and serological criteria. The RBP agglutination test result was positive for 37 patients (95%). The Wright seroagglutination test titers were within the diagnostic range (titers, >1:160) in 36 patients (92%). For the assessment of the ELISA results, samples with an optical density of 10% greater than the cutoff optical density were considered to be positive; samples from all 39 patients (100%) were positive. The types of antibodies detected by the ELISA were as follows for the 39 ELISA-positive samples: IgG, 27 (69%); IgA, 34 (87%); and IgM, 31 (80%).

All specimens obtained from 39 patients at initial diagnosis had positive real-time PCR assay results, conferring a sensitivity of 100%. All specimens obtained from the control group were negative for *B. melitensis*, conferring a specificity of 100%.

The evolution of the bacterial DNA load is shown in figure 1. The mean *B. melitensis* DNA load (±SD) for the 39 patients at the time of diagnosis was 803 ± 1236 copies/5 μL DNA extract (range, 26–4570 copies/5 μL DNA extract). At the end of treatment, samples from 34 patients (87%) remained positive for *B. melitensis*, with a mean bacterial DNA load (±SD) of 240 ± 314 copies/5 μL DNA extract (range, 0–1230 copies/5 μL DNA extract). Two months after the end of treatment, samples were collected from 34 patients, and the mean bacterial DNA load (±SD) was 192 ± 236 copies/5 μL DNA extract (range, 0–875 copies/5 μL DNA extract; results for 27 patients remained positive). Six months after the end of treatment, samples were collected from 26 patients, and the mean bacterial DNA load (±SD) was 96 ± 135 copies/5 μL DNA extract

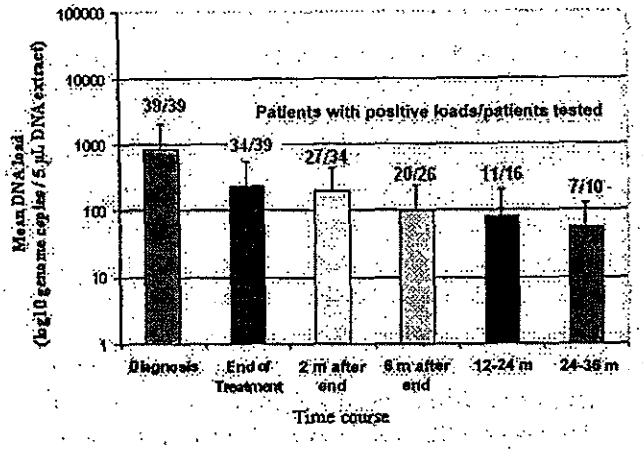


Figure 1. Evolution of *Brucella melitensis* DNA load at initial diagnosis and during the follow-up period. m, Months.

(range, 0–432 copies/5 μL DNA extract; results for 20 patients remained positive). Twelve to 24 months after the end of treatment, samples were collected from 16 patients, and the mean bacterial DNA load (±SD) was 80 ± 126 copies/5 μL DNA extract (range, 0–420 copies/5 μL DNA extract; results for 10 patients remained positive, and 1 patient had negative results for 2 consecutive specimens and then had a positive result but did not experience relapse). Twenty-four to 36 months after the end of treatment, samples were collected from 10 patients, and the mean bacterial DNA load (±SD) was 56 ± 74 copies/5 μL DNA extract (range, 0–220 copies/5 μL DNA extract; 7 patients continued to have positive results). Of 21 patients who were monitored for >1 year after therapy, 13 continued to have positive real-time PCR results but were asymptomatic. One patient had positive real-time PCR results 2 years after infection, although the patient had had negative real-time PCR results at the 1-year follow-up. Serological test results for these patients did not differ significantly between individuals with detectable and undetectable bacterial loads, with a mean Wright agglutination titer of 1:40, the presence of IgG antibodies determined by ELISA in all patients, and the presence of IgA antibodies in a minority of patients. During the follow-up phase of the study, only 3 patients experienced relapse. On relapse, 1 patient had positive blood culture results, whereas the other 2 relapses were diagnosed on the basis of clinical findings. In the 3 patients who experienced relapse, there was no increase in bacterial load during symptom reappearance, compared with their previous follow-up bacterial load measurement, although their titers did not decrease either. There were no statistically significant differences with regard to initial or posttreatment microbiological load between those who did and did not experience relapse. Results of blood cultures, when samples were obtained after treatment completion, were negative for all patients who did not experience relapse.

DISCUSSION

An emerging method for the detection and identification of a variety of infectious agents in the clinical laboratory is real-time PCR [16]. Real-time PCR was developed to improve the sensitivity, specificity, and speed of detecting PCR amplification products [17]. It does not require postamplification handling of PCR products, thereby reducing the risk of laboratory contamination and false-positive results. Moreover, real-time PCR has emerged as a powerful tool for quantification of the microbiological load; this is a concept that is valuable for numerous infectious disorders (e.g., hepatitis and HIV infection); real-time PCR has also blurred the traditional definitions of "eradication." Microbiological eradication (i.e., achievement of negative culture results) has long been used as an end point in clinical trials for several infections. However, these principles do not apply to a potentially chronic intracellular infection such as brucellosis. Therefore, microbiological load could serve as an indirect index of pathogen presence. One has to take into account, however, all of the potential problems of such an application; for example, isolation of DNA particles cannot discriminate between living and "eradicated" microorganisms. Furthermore, microbiological load could theoretically serve as an index of disease burden and thus allow for patient stratification by disease severity, relapse potential, and therapeutic regimens needed.

Our study revealed startling results. A significant number of patients continued to exhibit microbiological load even years after clinical cure and in the absence of any symptom indicative of disease persistence or relapse. We consider our results to be indicative of the long-term presence of viable bacteria in the human body in a cellular reservoir that needs further clarification. Could the results be explained as the outcome of particle shedding by dead bacteria? These dead bacteria could not be the result of antibiotic treatment administered <1 year earlier. Thus, in this case, bacteria indeed persisted for a long period after infection and clinical cure; these bacteria failed to elicit clinical manifestations, possibly because of a robust sustained immune response that eventually eliminates them by switching on the initially cancelled cellular apoptosis [18].

Even if we accept this alternative explanation, the only viable pathophysiological scenario is that, at least in the majority of patients, *Brucella* species persist inside the human body despite apparent clinical cure. The pathogen may replicate at low frequency. It may even cause transient, low-level bacteremia in a manner that can be handled by the body's immune system in such a way as to avoid the evolution of clinical disease. In that case, brucellosis should be considered to be only a chronic infection (much like tuberculosis), the clinical presentation of which depends on the equilibrium between the immune system and the microbial pathogenicity. Certain studies of patients with chronic brucellosis have indeed focused on such a dysregulation

[19]. The level at which this interaction occurs is vague; low-level bacteremia may not be detected easily, and follow-up through cultures of bone marrow specimens, theoretically sound and suggested to be diagnostically superior [20], is not convenient. Moreover, one could hypothesize that, with use of more-sensitive diagnostic techniques, it could be determined that patients with undetectable microbiological loads might actually have microbiological loads.

Contradictory results have emerged from the few relevant studies, with smaller sample numbers, that have recently been reported. Similar findings were elicited in a Spanish study [21] that showed that 4 of 7 individuals who experienced relapse and 3 of 11 who did not experience relapse also exhibited detectable bacterial loads after long-term follow-up. A similar result was also reported from a study in Peru [22] that used plain PCR for follow-up; the majority of the patients had PCR-positive samples even months after treatment completion. These results, however, were not reproduced in another Spanish study [23] that used a slightly different methodology. Two other real-time PCR studies did not evaluate evolution of the microbiological load during disease stages [24, 25].

One could support the hypothesis that patients with detectable microbiological loads were simply inadequately treated and were therefore candidates for relapse. However, the few who experienced relapse did not preferentially belong to the subgroup of patients with continually detected bacterial load. In addition, these patients did not exhibit higher loads or any statistically significant difference than did those of the group that did not experience relapse. Furthermore, relapses usually occur during the first few months after the end of treatment, and 90% of them usually occur during the first follow-up year, a cutoff point surpassed by our patients. No data can be extracted about whether a specific therapeutic regimen was related to long-term detectable microbiological loads because the majority of the patients were treated with the same regimen. In the Spanish study [21], numerous regimens were used, and the small statistical sample did not allow for any conclusions to be drawn.

One might argue that the significance of the study is marred by the fact that all patients with detectable DNA load during the follow-up period had negative results of blood cultures; thus, the PCR results may be considered to be dubious. Yet this fact (i.e., negative blood culture results) underlines the importance of our findings. If these patients had positive results of blood cultures, they would be considered de facto to have experienced relapse (reappearance of positive blood culture results is considered to indicate a relapse even in the absence of symptoms). These patients, however, met absolutely no criteria for relapse or disease in general, despite having detectable DNA load for *Brucella* species. Cases of reappearance of *Brucella* infection years after the initial course of symptoms are not rare, especially in the context of foreign body infection [26].

One might argue that there is selection bias in our results, because bacterial load was evaluated in only 10 patients in the 24–36-month period. However, the other study patients did not experience relapse, as assessed by telephone interview performed by 1 of the authors during this period; thus, the 10 patients for whom bacterial load data were available could be considered to be representative of the entire sample. Furthermore, a similar trend was observed among larger subsets of patients in the various follow-up time frames depicted in figure 1.

Recognizing that brucellosis is a chronic infection means that our understanding of the pathophysiology, diagnosis, and treatment of the disease may be drastically altered. First, one has to understand what suppresses *Brucella* species pathogenicity during the protracted posttreatment period when bacterial load is detectable. Moreover, one has to elucidate which are the critical components of this suppression and whether patients with chronic brucellosis exhibit a defect in these components. Second, one has to define whether the initial bacterial load is related to disease severity, tendency to relapse, or need for enhanced antibiotic treatment. Third, one has to seek other predictors of relapse, because real-time PCR did not exhibit any correlation in the present study. Finally, with regard to therapy, one has to redefine treatment goals. Eradication was never set as an issue in brucellosis, and the "acceptable" percentage of relapses was arbitrary [27]. The future question, however, will be whether to treat aggressively, with monitoring of the bacterial load, or to simply ignore the results and allow the pathogen to parasitize inside the human body.

Acknowledgments

Potential conflicts of interest. All authors: no conflicts.

References

1. Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV. The new global map of human brucellosis. Lancet Infect Dis 2006; 6:91–9.
2. Pappas G, Panagopoulou P, Christou L, Akritidis N. Brucella as a biological weapon. Cell Mol Life Sci 2006; 63:2229–36.
3. Memish ZA, Balkhy HH. Brucellosis and international travel. J Travel Med 2004; 11:49–55.
4. Pappas G, Akritidis N, Bosilkovski M, Tsianos E. Brucellosis. N Engl J Med 2005; 352:2325–36.
5. Gross A, Terraza A, Ouahrani-Bettache S, Liautard JP, Dornand J. In vitro *Brucella suis* infection prevents the programmed cell death of human monocytic cells. Infect Immun 2000; 68:342–51.
6. Pappas G, Papadimitriou P. Challenges in Brucella bacteraemia. Int J Antimicrob Agents 2007; 30(Suppl 1):29–31.
7. Al Dahouk S, Tomaso H, Nöckler K, Neubauer H, Frangoulidis D. Laboratory-based diagnosis of brucellosis—a review of the literature. II. Serological tests for brucellosis. Clin Lab 2003; 49:577–89.
8. Memish ZA, Almuneef M, Mah MW, Qassem LA, Osoba AO. Comparison of the Brucella Standard Agglutination Test with the ELISA IgG and IgM in patients with Brucella bacteremia. Diagn Microbiol Infect Dis 2002; 44:129–32.

9. Navarro E, Casao MA, Solera J. Diagnosis of human brucellosis using PCR. Expert Rev Mol Diagn 2004; 4:115–23.
10. Avdikou I, Maipa V, Alamanos Y. Epidemiology of human brucellosis in a defined area of northwestern Greece. Epidemiol Infect 2005; 133: 905–10.
11. Alton GG, Jones LM, Pietz DE. Laboratory techniques in brucellosis. 2nd ed. Geneva: World Health Organization, 1975.
12. Yagupsky P. Detection of brucellae in blood cultures. J Clin Microbiol 1999; 37:3437–42.
13. Solomon HM, Jackson D. Rapid diagnosis of *Brucella melitensis* in blood: some operational characteristics of the BACT/ALERT. J Clin Microbiol 1992; 30:222–4.
14. Shapiro DS, Wong JD. *Brucella*. In: Murray P, Baron E, Pfaller M, Tenover F, Yolken R, eds. Manual of clinical microbiology. Washington DC: American Society for Microbiology, 1999:625–31.
15. Baily GG, Krahn JB, Drasar BS, Stoker NG. Detection of *Brucella melitensis* and *Brucella abortus* by DNA amplification. J Trop Med Hyg 1992; 95:271–5.
16. Espy MJ, Uhl JR, Sloan LM, et al. Real-time PCR in clinical microbiology: applications for routine laboratory testing. Clin Microbiol Rev 2006; 19:165–256.
17. Tenover FC. Rapid detection and identification of bacterial pathogens using novel molecular technologies: infection control and beyond. Clin Infect Dis 2007; 44:418–23.
18. Tolomeo M, Di Carlo P, Abbadessa V, et al. Monocyte and lymphocyte apoptosis resistance in acute and chronic brucellosis and its possible implications in clinical management. Clin Infect Dis 2003; 36:1533–8.
19. Boura P, Skendros P, Kountouras J, Zacharioudaki E, Tsapas T. Effect of bacterial extracts on the immunologic profile in chronic relapsing brucellosis patients. Int J Immunopathol Pharmacol 1999; 12:103–11.
20. Gotuzzo E, Carrillo C, Guerra J, Llosa L. An evaluation of diagnostic methods for brucellosis—the value of bone marrow culture. J Infect Dis 1986; 153:122–5.
21. Navarro E, Segura JC, Castano MJ, Solera J. Use of real-time quantitative polymerase chain reaction to monitor the evolution of *Brucella melitensis* DNA load during therapy and post-therapy follow-up in patients with brucellosis. Clin Infect Dis 2006; 42:1266–73.
22. Maas KS, Mendez M, Zavaleta M, et al. Evaluation of brucellosis by PCR and persistence after treatment in patients returning to the hospital for follow-up. Am J Trop Med Hyg 2007; 76:698–702.
23. Queipo-Ortuno MI, Colmenero JD, Baeza G, Morata P. Comparison between LightCycler Real-Time Polymerase Chain Reaction (PCR) assay with serum and PCR–enzyme-linked immunosorbent assay with whole blood samples for the diagnosis of human brucellosis. Clin Infect Dis 2005; 40:260–4.
24. Kattar MM, Zalloua PA, Araj GF, et al. Development and evaluation of real-time polymerase chain reaction assays on whole blood and paraffin-embedded tissues for rapid diagnosis of human brucellosis. Diagn Microbiol Infect Dis 2007; 59:23–32.
25. Debeaumont C, Falconnet PA, Maurin M. Real-time PCR for detection of *Brucella* spp DNA in human serum samples. Eur J Clin Microbiol Infect Dis 2005; 24:842–5.
26. Dhand A, Ross JJ. Implantable cardioverter-defibrillator infection due to *Brucella melitensis*: case report and review of brucellosis of cardiac devices. Clin Infect Dis 2007; 44:e37–39.
27. Pappas G, Akritidis N, Tsianos E. Effective treatments in the management of brucellosis. Expert Opin Pharmacother 2005; 6:201–9.

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2008. 7. 3	該当なし	

一般的名称	(製造販売承認書に記載なし)	研究報告の公表状況		公表国
販売名(企業名)	合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)		Pérez-Osorio CE, Zavala-Velázquez JE, Arias León JJ, Zavala-Castro JE. Emerg Infect Dis. 2008 Jul;14(7):1019-23.	メキシコ

研究報告の概要

○*Rickettsia felis*—ヒトへの新興世界的脅威

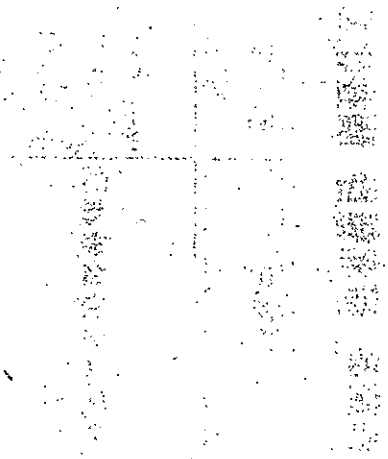
*Rickettsia felis*は、*TRG rickettsiae* (transitional group rickettsiae)に属する新興病原体である。*R. felis*は、イヌ、ネコ、齧歯類など世界中の様々な外部寄生生物から検出され、ネコノミは最も一般的な媒介生物と考えられている。ヒトにおける*R. felis*感染症の臨床症状は発疹熱やデング熱などに類似しており、ヒトの感染症は実際よりも少なく推定されている可能性が高い。ヒト症例は、1994年に第1例が米国で報告された後、ヨーロッパやアジアでも報告されている。*R. felis*は存続のためにノミだけを必要とする可能性があるが、ノミが媒介する紅斑熱リケッチアのライフサイクルにおける動物の役割についてはまだわかっていない。また、鑑別診断においては、ノミが媒介する紅斑熱を考慮すべきである。ヒトにおける*R. felis*感染症の実際の発現率、臨床症状の範囲、重症度を調べ、さらに公衆衛生への影響を評価するために、今後、試験を実施していく必要がある。

報告企業の意見	今後の対応
ネコノミが媒介する*Rickettsia felis*感染症のヒト症例は世界中で報告されているが、症状は発疹熱やデング熱などに類似しており、実際よりも少なく推定されている可能性が高く、今後調査が必要で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、発熱などの体調不良者を献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

使用上の注意記載状況・その他参考事項等

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



Rickettsia felis as Emergent Global Threat for Humans

Carlos E. Pérez-Osorio,* Jorge E. Zavala-Velázquez,* Juan José Arias León,* and Jorge E. Zavala-Castro*

Rickettsia felis is an emergent pathogen belonging to transitional group rickettsiae. First described in 1990, *R. felis* infections have been reported to occur worldwide in fleas, mammals, and humans. Because clinical signs of the illness are similar to those of murine typhus and other febrile illnesses such as dengue, the infection in humans is likely underestimated. *R. felis* has been found throughout the world in several types of ectoparasites; cat fleas appear to be the most common vectors. *R. felis* infection should be considered an emergent threat to human health.

Rickettsia felis is a member of the genus *Rickettsia*, which comprises intracellular pathogens that produce infections commonly called rickettsioses. Although the genus has no recognized subspecies, rickettsiae have traditionally been subdivided into 2 groups: the spotted fever group (SFG) and the typhus group. Infections produced by these 2 groups are clinically indistinguishable; however, groups can be differentiated by outer membrane protein OmpA (absent in the typhus group) and by vector. SFG members are transmitted by ticks; typhus group members, by fleas and lice (*1*,*2*). More recently, Gillespie et al. (*3*) added to this classification by designating the transitional group of rickettsiae and describing an ancestral group of rickettsiae.

In 1990, Adams et al. described a rickettsia-like organism, which resembled *R. typhi*, in the cytoplasm of midgut cells of a colony of cat fleas (*1*). The new rickettsia received the initial name of ELB agent after the company from which the fleas were obtained (El Labs, Soquel, CA, USA) (*4*). The first observations, such as reactivity with antibodies to *R. typhi* (*1*), the type of vector in which it was first discovered (*1*), and the apparent absence of OmpA (*5*), suggested that the new organism belonged to the typhus group of rickettsiae (*4*).

The molecular characterization of the organism described by Adams and reported by Bouyer et al. in 2001 provided sufficient evidence to support the designation of *R. felis* as a member of the SFG (*6*), and in 2002, La Scola et al. provided further characterization (*7*). One noticeable characteristic is the temperature-dependent growth of the bacterium, which requires incubation temperatures of 28°–32°C for optimal growth. However, the most striking characteristic of the novel rickettsia was the plasmid DNA in its genome (*8*).

World Distribution in Potential Host Vectors

Soon after the initial description of the typhus-like rickettsia, Williams et al. (*9*) reported that cat fleas collected from opossums in an urban setting in California were infected with the novel rickettsia, but no organism was detected in the tissues of the opossums. Since this report, this organism has been described in infected vectors from 20 countries on 5 continents (*9*). Not until 2002 did interest in *R. felis* increase, when the United States (*9*), Brazil (*10*), Mexico (*11*), and Spain (*12*) were among the first countries to describe cat fleas (*Ctenocephalides felis*) infected with *R. felis*. During the following 5 years, 28 additional reports appeared from all over the world (Table 1). These reports describe new potential vectors being infected with the emergent rickettsia, including the following: fleas, such as *C. canis* (*13*–*15*), *Anomiopsyllus nudata* (*16*), *Archaeopsylla erinacei* (*15*,*17*), *Ctenophthalmus* sp.

*Universidad Autónoma de Yucatán, Mérida, México

DOI: 10.3201/eid1407.071656

Table 1. Potential vectors infected with *Rickettsia felis* reported worldwide, 1992–2007*

Year	Source of DNA sample	Animal†	Country	Reference
1992	*Ctenocephalides felis*	Opossum	USA	(*9*)
2002	*C. felis*	Cats and dogs	Brazil	(*10*)
2002	*C. felis*	Dogs	Mexico	(*11*)
2002	*C.felis*	Cats and dogs	Spain	(*12*)
2003	*Haemophysalis flava, H. kitaokai,* and *Ixodes ovatus*	Unknown (flagging)	Japan	(*19*)
2003	*C. felis*	Cats	France	(*22*)
2003	*C. felis*	Cats and dogs	UK	(*23*)
2004	*C. felis*	Dogs	Peru	(*24*)
2005	*Anomiopsyllus nudata*	Wild rodents	USA	(*16*)
2005	*C. felis*	Cats and dogs	New Zealand	(*25*)
2005	*C. felis*	Monkey	Gabon	(*26*)
2006	*C. felis* and *C. canis*	Dogs	Brazil	(*13*)
2006	*C. felis* and *C. canis*	Cats and dogs	Uruguay	(*14*)
2006	*Archaeopsylla erinacei* and *C. canis*	Hedgehog and rodents	Algeria	(*15*)
2006	*A. erinacei* and *Ctenophtalmus sp.*	Rodents and hedgehog	Portugal	(*17*)
2006	*Xenopsylla cheopis*	Rodents‡	Indonesia	(*18*)
2006	*C. felis, Rhipicephalus sanguineus,* and *Amblyommma cajennense*	Dogs and horse	Brazil	(*20*)
2006	Unknown flea	Gerbil	Afghanistan	(*27*)
2006	*C. felis*	Cats and dogs	Australia	(*28*)
2006	*C. felis*	Cats	Israel	(*29*)
2006	*C. felis*	Rodents	Cyprus	(*30*)
2007	Mites	Wild rodents	South Korea	(*21*)
2007	*C. felis*	Cats	USA	(*31*)
2007	*C. felis*	Cats	Chile	(*32*)

*PCR was used to detect *R. felis* infection with 1 noted exception.
†Animal host of potential vectors.
‡Quantitative PCR.

(*17*), and *Xenopsylla cheopis* (*18*); ticks, *Haemaphysalis flava* (*19*), *Rhipicephalus sanguineus* (*20*), and *Ixodes ovatus* (*19*); and mites from South Korea (*21*) (Table 1). Despite the large number of potential vectors reported, the only vector currently recognized is *C. felis* because it has been demonstrated that this flea is able to maintain a stable infected progeny through transovarial transmission (*4*). In addition, production of antibody to *R. felis* has been noted in animals after they have been exposed to infected cat fleas (*9*). Other evidence to be considered is the fact that 68.8% of the reports state that the cat flea is the most recurrent vector in which *R. felis* has been detected. These data further support the wide distribution of rickettsiae because they correlate with the worldwide distribution of *C. felis*; this distribution represents a threat to the human population because of lack of host specificity of the cat flea.

R. felis infection is diagnosed by PCR amplification of targeted genes. The genes most commonly amplified by researchers are *gltA* and *ompB*; followed by the 17-kDa gene. Also, 25% of published articles report that *R. felis* was detected by amplifying >2 genes, and all report that amplicons were confirmed as *R. felis* by sequencing. The animal hosts from which the infected ectoparasites were recovered represent a diversity of mammals (Table 1), which included 9 different naturally infested animal species. However, in 16 of 33 articles, ectoparasites were recovered from dogs. Other hosts for ectoparasites were cats (in 13 of 33 reports); rodents (5 of 33 reports); opossums and hedgehogs (2 reports each); and horses, sheep, goats, gerbils, and monkeys (1 report for each animal species).

In summary, the presence of *R. felis* in a diverse range of invertebrate and mammalian hosts represents a high potential risk for public health and the need for further studies to establish the role of ectoparasites other than *C. felis* as potential vectors. To date, whether any vertebrate may serve as the reservoir of this emergent pathogen has not been determined. However, preliminary data from our laboratory suggest that opossums are the most likely candidates.

World Distribution of Human Cases

In 1994, the first human case of infection with the new cat flea rickettsia was reported in the United States (*2*). This became the first evidence of *R. felis'* potential as a human pathogen. *R. felis* infection had a similar clinical manifestation as murine typhus (including high fever [39°–40°C], myalgia, and rash). Although the initial idea was that the murine typhus–like rickettsia had a transmission cycle involving cat fleas and opossums (*2,5,9*), no viable *R. felis* has yet been isolated from a vertebrate host.

Three more cases of *R. felis* infection were reported from southeastern Mexico in 2000. The patients had had contact with fleas or animals known to carry fleas. The clinical manifestations were those of a typical rickettsiosis: all patients had fever and myalgia; but the skin lesions, instead of a rash, were similar to those described for rickettsialpox. In addition, for 3 patients, central nervous system involvement developed, manifested as photophobia, hearing loss, and signs of meningitis (*33*).

As occurred with the fast-growing reports of the worldwide detection of *R. felis* in arthropod hosts, the reports of human cases of *R. felis* infection increased rapidly in the following years (Table 2). But, in contrast, only 11 articles reported human infection by *R. felis* compared with 32 that reported ectoparasite infection with the new rickettsia. Nevertheless, these findings indicate that an effective surveillance system is urgently needed to distinguish *R. felis* rickettsiosis from other rickettsial infections such as murine typhus and Rocky Mountain spotted fever, and from other febrile illnesses such as dengue. Although PCR is still a method of choice for many laboratories, its high cost prevents many from using the technique, particularly in developing countries. Important advances have been achieved in diagnostics, such as the recent establishment of a stable culture of *R. felis* in cell lines that allows its use as antigen in serologic assays differentiating the cat flea rickettsia from others. Use of this culture in the immunofluorecent assay has enabled detection of additional human cases (*38*).

The first autochthonous human case in Europe was reported in 2002, which demonstrated that *R. felis* has a potential widespread distribution and is not confined to the Americas. It also confirmed the risk for human disease anywhere in the world. After the first report in Europe of a human infection of *R. felis*, other human cases have appeared in other countries around the world, including Thailand (*36*), Tunisia (*38*), Laos (*39*), and Spain (*40*); additional cases have been reported in Mexico and Brazil (*34*). All the data support the conclusion that the incidence of *R. felis* rickettsiosis and the simultaneous worldwide distribution of the flea vector plausibly explain its endemicity.

At present, the involvement of domestic animals (e.g., dogs and cats) or wild animals coexisting in urban areas (e.g., opossums) maintains *R. felis* infection in nature. *C. felis* fleas serve as the main reservoir and likely have a central role in transmission of human illness.

Conclusions

R. felis is an emergent rickettsial pathogen with a worldwide distribution in mammals, humans, and ectoparasites. The clinical manifestations of *R. felis* infections resemble those of murine typhus and dengue, which makes them difficult to diagnose without an appropriate laboratory test. For this reason, infections due to this emergent pathogen are likely underestimated and misdiagnosed. Although *R. felis* may require only fleas for its maintenance in nature, we still do not know the role of animals in the life cycle of flea-borne spotted fever rickettsia. In addition, flea-borne spotted fever should be considered in the differential diagnosis of infectious diseases. Further research should be conducted to determine the actual incidence of *R. felis* infection in humans, the spectrum of clinical signs and symptoms, and the severity of this infection and also to assess the impact on public health.

Acknowledgment

We thank Patricia Croquet-Valdes for her comments and helpful advice.

This research was supported by grants from the CONACyT (44064-M) to J. E. Z.-V.

Mr. Perez-Osorio is currently professor of microbiology at the Autonomous University of Yucatan. His research interests focus on rickettsial diseases.

References

1. Adams JR, Schmidtmann ET, Azad AF. Infection of colonized cat fleas, *Ctenocephalides felis* (Bouche), with a rickettsia-like microorganism. Am J Trop Med Hyg. 1990;43:400–9.
2. Schriefer ME, Sacci JB Jr, Dumler JS, Bullen MG, Azad AF. Identification of a novel rickettsial infection in a patient diagnosed with murine typhus. J Clin Microbiol. 1994;32:949–54.

Table 2. Human cases of *Rickettsis felis* infection reported worldwide, 1994–2006*

Year	No. cases	Method	Country	Reference
1994	1	PCR	USA	(*2*)
2000, 2006	5	PCR	Mexico	(*33*)
2001, 2006	3	PCR	Brazil	(*34*)
2002	2	PCR/serology	Germany	(*35*)
2003	1	Serology (seroconversion)	Thailand	(*36*)
2005	3	Serology (Western blot)	South Korea	(*37*)
2006	8	Serology (IFAT/Western blot)	Tunisia	(*38*)
2006	1	Serology (seroconversion)	Laos	(*39*)
2006	33	Serology (IFAT)	Spain	(*40*)
Total	68			

*IFAT, indirect fluorescent antibody test.

3. Gillespie JJ, Beier MS, Rahman MS, Ammerman NC, Shallom JM, Purkayastha A, et al. Plasmids and rickettsial evolution: insight from *Rickettsia felis*. PLoS One. 2007;2:e266. DOI: 10.1371/journal.pone.0000266
4. Azad AF, Sacci JB Jr, Nelson WM, Dasch GA, Schmidtmann ET, Carl M. Genetic characterization and transovarial transmission of a typhus-like rickettsia found in cat fleas. Proc Natl Acad Sci U S A. 1992;89:43–6. DOI: 10.1073/pnas.89.1.43
5. Higgins JA, Radulovic S, Schriefer ME, Azad AF. *Rickettsia felis*: a new species of pathogenic rickettsia isolated from cat fleas. J Clin Microbiol. 1996;34:671–4.
6. Bouyer DH, Stenos J, Crocquet-Valdes P, Moron CG, Popov VL, Zavala-Velazquez JE, et al. *Rickettsia felis*: molecular characterization of a new member of the spotted fever group. Int J Syst Evol Microbiol. 2001;51:339–47.
7. La Scola B, Meconi S, Fenollar F, Rolain JM, Roux V, Raoult D. Emended description of *Rickettsia felis* (Bouyer et al. 2001), a temperature-dependent cultured bacterium. Int J Syst Evol Microbiol. 2002;52:2035–41. DOI: 10.1099/ijs.0.02070-0
8. Ogata H, Renesto P, Audic S, Robert C, Blanc G, Fournier PE, et al. The genome sequence of *Rickettsia felis* identifies the first putative conjugative plasmid in an obligate intracellular parasite. PLoS Biol. 2005;3:e248. DOI: 10.1371/journal.pbio.0030248
9. Williams SG, Sacci JB Jr, Schriefer ME, Andersen EM, Fujioka KK, Sorvillo FJ, et al. Typhus and typhuslike rickettsiae associated with opossums and their fleas in Los Angeles County, California. J Clin Microbiol. 1992;30:1758–62.
10. Oliveira RP, Galvao MA, Mafra CL, Chamone CB, Calic SB, Silva SU, et al. *Rickettsia felis* in *Ctenocephalides* spp. fleas, Brazil. Emerg Infect Dis. 2002;8:317–9.
11. Zavala-Velazquez JE, Zavala-Castro JE, Vado-Solis I, Ruiz-Sosa JA, Moron CG, Bouyer DH, et al. Identification of *Ctenocephalides felis* fleas as a host of *Rickettsia felis*, the agent of a spotted fever rickettsiosis in Yucatan, Mexico. Vector Borne Zoonotic Dis. 2002;2:69–75. DOI: 10.1089/153036602321131869
12. Marquez FJ, Muniain MA, Perez JM, Pachon J. Presence of *Rickettsia felis* in the cat flea from southwestern Europe. Emerg Infect Dis. 2002;8:89–91.
13. Horta MC, Chiebao DP, de Souza DB, Ferreira F, Pinheiro SR, Labruna MB, et al. Prevalence of *Rickettsia felis* in the fleas *Ctenocephalides felis* and *Ctenocephalides canis* from two Indian villages in São Paulo Municipality, Brazil. Ann N Y Acad Sci. 2006;1078:361–3. DOI: 10.1196/annals.1374.071
14. Venzal JM, Perez-Martinez L, Felix ML, Portillo A, Blanco JR, Oteo JA. Prevalence of *Rickettsia felis* in *Ctenocephalides felis* and *Ctenocephalides canis* from Uruguay. Ann N Y Acad Sci. 2006;1078:305–8. DOI: 10.1196/annals.1374.056
15. Bitam I, Parola P, De La Cruz KD, Matsumoto K, Baziz B, Rolain JM, et al. First molecular detection of *Rickettsia felis* in fleas from Algeria. Am J Trop Med Hyg. 2006;74:532–5.
16. Stevenson HL, Labruna MB, Montenieri JA, Kosoy MY, Gage KL, Walker DH. Detection of *Rickettsia felis* in a New World flea species, *Anomiopsyllus nudata* (*Siphonaptera: Ctenophthalmidae*). J Med Entomol. 2005;42:163–7. DOI: 10.1603/0022-2585-(2005)042[0163:DORFIA]2.0.CO;2
17. De Sousa R, Edouard-Fournier P, Santos-Silva M, Amaro F, Bacellar F, Raoult D. Molecular detection of *Rickettsia felis*, *Rickettsia typhi* and two genotypes closely related to *Bartonella elizabethae*. Am J Trop Med Hyg. 2006;75:727–31.
18. Jiang J, Soeatmadji DW, Henry KM, Ratiwayanto S, Bangs MJ, Richards AL. *Rickettsia felis* in *Xenopsylla cheopis*, Java, Indonesia. Emerg Infect Dis. 2006;12:1281–3.
19. Ishikura M, Ando S, Shinagawa Y, Matsuura K, Hasegawa S, Nakayama T, et al. Phylogenetic analysis of spotted fever group rickettsiae based on *gltA*, 17-kDa, and rOmpA genes amplified by nested PCR from ticks in Japan. Microbiol Immunol. 2003;47:823–32.
20. Cardoso LD, Freitas RN, Mafra CL, Neves CV, Figueira FC, Labruna MB, et al. Characterization of *Rickettsia* spp. circulating in a silent peri-urban focus for Brazilian spotted fever in Caratinga, Minas Gerais, Brazil [in Portuguese]. Cad Saude Publica. 2006;22:495–501.
21. Choi YJ, Lee EM, Park JM, Lee KM, Han SH, Kim JK, et al. Molecular detection of various rickettsiae in mites (Acari: Trombiculidae) in southern Jeolla Province, Korea. Microbiol Immunol. 2007;51:307–12.
22. Rolain JM, Franc M, Davoust B, Raoult D. Molecular detection of *Bartonella quintana*, *B. koehlerae*, *B. henselae*, *B. clarridgeiae*, *Rickettsia felis*, and *Wolbachia pipientis* in cat fleas, France. Emerg Infect Dis. 2003;9:338–42.
23. Kenny MJ, Birtles RJ, Day MJ, Shaw SE. *Rickettsia felis* in the United Kingdom. Emerg Infect Dis. 2003;9:1023–4.
24. Blair PJ, Jiang J, Schoeler GB, Moron C, Anaya E, Cespedes M, et al. Characterization of spotted fever group rickettsiae in flea and tick specimens from northern Peru. J Clin Microbiol. 2004;42:4961–7. DOI: 10.1128/JCM.42.11.4961-4967.2004
25. Kelly P, Rolain JM, Raoult D. Prevalence of human pathogens in cat and dog fleas in New Zealand. N Z Med J. 2005;118:U1754.
26. Rolain JM, Bourry O, Davoust B, Raoult D. *Bartonella quintana* and *Rickettsia felis* in Gabon. Emerg Infect Dis. 2005;11:1742–4.
27. Marie JL, Fournier PE, Rolain JM, Briolant S, Davoust B, Raoult D. Molecular detection of *Bartonella quintana*, *B. elizabethae*, *B. koehlerae*, *B. doshiae*, *B. taylorii*, and *Rickettsia felis* in rodent fleas collected in Kabul, Afghanistan. Am J Trop Med Hyg. 2006;74:436–9.
28. Schloderer D, Owen H, Clark P, Stenos J, Fenwick SG. *Rickettsia felis* in fleas, Western Australia. Emerg Infect Dis. 2006;12:841–3.
29. Bauer O, Baneth G, Eshkol T, Shaw SE, Harrus S. Polygenic detection of *Rickettsia felis* in cat fleas (*Ctenocephalides felis*) from Israel. Am J Trop Med Hyg. 2006;74:444–8.
30. Psaroulaki A, Antoniou M, Papaeustathiou A, Toumazos P, Loukaides F, Tselentis Y. First detection of *Rickettsia felis* in *Ctenocephalides felis* fleas parasitizing rats in Cyprus. Am J Trop Med Hyg. 2006;74:120–2.
31. Hawley JR, Shaw SE, Lappin MR. Prevalence of *Rickettsia felis* DNA in the blood of cats and their fleas in the United States. J Feline Med Surg. 2007;9:258–62. DOI: 10.1016/j.jfms.2006.12.005
32. Labruna MB, Ogrzewalska M, Moraes-Filho J, Lep P, Gallegos JL, Lopez J. *Rickettsia felis* in Chile. Emerg Infect Dis. 2007;13:1794–5.
33. Zavala-Velazquez JE, Ruiz-Sosa JA, Sanchez-Elias RA, Becerra-Carmona G, Walker DH. *Rickettsia felis* rickettsiosis in Yucatan. Lancet. 2000;356:1079–80. DOI: 10.1016/S0140-6736(00)02735-5
34. Galvao MA, Zavala-Velazquez JE, Zavala-Castro JE, Mafra CL, Calic SB, Walker DH. *Rickettsia felis* in the Americas. Ann N Y Acad Sci. 2006;1078:156–8. DOI: 10.1196/annals.1374.027
35. Richter J, Fournier PE, Petridou J, Haussinger D, Raoult D. *Rickettsia felis* infection acquired in Europe and documented by polymerase chain reaction. Emerg Infect Dis. 2002;8:207–8.
36. Parola P, Miller RS, McDaniel P, Telford SR III, Rolain JM, Wongsrichanalai C, et al. Emerging rickettsioses of the Thai-Myanmar border. Emerg Infect Dis. 2003;9:592–5.
37. Choi YJ, Jang WJ, Ryu JS, Lee SH, Park KH, Paik HS, et al. Spotted fever group and typhus group rickettsioses in humans, South Korea. Emerg Infect Dis. 2005;11:237–44.
38. Znazen A, Rolain JM, Hammami A, Jemaa MB, Raoult D. *Rickettsia felis* infection, Tunisia. Emerg Infect Dis. 2006;12:138–40.
39. Phongmany S, Rolain JM, Phetsouvanh R, Blacksell SD, Soukkhaseum V, Rasachack B, et al. Rickettsial infections and fever, Vientiane, Laos. Emerg Infect Dis. 2006;12:256–62.

40. Bernabeu-Wittel M, del Toro MD, Nogueras MM, Muniain MA, Cardenosa N, Marquez FJ, et al. Seroepidemiological study of *Rickettsia felis, Rickettsia typhi, and Rickettsia conorii* infection among the population of southern Spain. Eur J Clin Microbiol Infect Dis. 2006;25:375–81. DOI: 10.1007/s10096-006-0147-6

Address for correspondence: Jorge E. Zavala-Castro, Facultad de Medicina, Unidad Interinstitucional de Investigación Clínica y Epidemiológica, Universidad Autónoma de Yucatán, Avenida Itzaes No. 498 x 59 y 59A Centro, CP 97000, Merida, Yucatán, México; email: zcastro@uady.mx

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8月4日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	別紙のとおり	研究報告の公表状況	ProMED-mail, 20080728.2306	公表国 オランダ	
販売名（企業名）	別紙のとおり				

研究報告の概要

問題点：オランダにおける調査において、2008年7月の時点でQ熱症例報告数が急激に増加している。

ブラバント州の公衆衛生局が行った調査では、2008年7月21日付けで491症例が報告されている。オランダ保健省によると、これはブランバント居住者5,000名が感染したことになる。Q熱は、ノールトブラバント州で急速に広がり、ナイメーヘン地域でもある程度広がった。感染症管理センター長であるRoel Coutinhoによると、実際の感染者数は報告された症例数の5倍というより10倍であると思われる。
労働党のブラバント州事務所は、この問題を評議会にかけた。評議会メンバーのNora Kasrioui によると、理事会がどう対処する予定かわからない。Kasrioui は、「Q熱は重篤な疾患であり、地域住民にとって、ますます大きな問題となっていて、政治的な働きかけが必要だと思います。」と述べた。Q熱には不明な点が多く、方針を打ち出し難いと認識しており、「団体は、違和感のある無しにかかわらず、いつでも経済的な援助やそれ以外の援助を受けることができます。」とも述べた。
Countinhoによると、Q熱を根絶することは不可能である。Q熱はヒツジの出産シーズン中からそれ以降に再発することが一般的であり、今のところ、ヤギが主な感染源であると考えられている。RIVM（国立衛生環境研究所）は、獣医学の専門家と共に、どのようにしてQ熱が動物から人に感染するのかを検討中であり、その後、詳細な予防対策が決定される。ちなみに、2007年までオランダにQ熱は存在しないも同然であった。

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

使用上の注意記載状況・その他参考事項等

記載なし

MedDRA/J ver.11.0

30

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤乾燥ペプシン処理人免疫グロブリン、⑥乾燥スルホ化人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン*、⑧乾燥濃縮人活性化プロテインC、⑨乾燥濃縮人血液凝固第Ⅷ因子、⑩乾燥濃縮人血液凝固第Ⅸ因子、⑪乾燥抗破傷風人免疫グロブリン、⑫抗 HBs 人免疫グロブリン、⑬トロンビン、⑭フィブリノゲン加第ⅩⅢ因子、⑮乾燥濃縮人アンチトロンビンⅢ、⑯ヒスタミン加人免疫グロブリン製剤、⑰人血清アルブミン*、⑱人血清アルブミン*、⑲乾燥ペプシン処理人免役グロブリン*、⑳乾燥人血液凝固第Ⅸ因子複合体*、㉑乾燥濃縮人アンチトロンビンⅢ
販売名(企業名)	①献血アルブミン 20 “化血研”、②献血アルブミン 25 “化血研”、③人血清アルブミン “化血研” *、④ “化血研” ガンマーグロブリン、⑤献血静注グロブリン “化血研”、⑥献血ベニロン－Ⅰ、⑦ベニロン*、⑧注射用アナクトC2,500 単位、⑨コンファクトF、⑩ノバクトM、⑪テタノセーラ、⑫ヘパトセーラ、⑬トロンビン “化血研”、⑭ボルヒール、⑮アンスロビンP、⑯ヒスタグロビン、⑰アルブミン 20%化血研*、⑱アルブミン 5%化血研*、⑲静注グロブリン*、⑳ノバクトF*、㉑アンスロビンP 1500 注射用
報告企業の意見	Q 熱はリケッチアの一種コクシエラ・バーネッティ (*Coxiella burnetii*) による人畜共通感染症である。菌の大きさは 0.2～0.4×1.0μm で、球菌の 1/2～1/4 である。感染源はおもに家畜や愛玩動物であるが、自然界では多くの動物やダニが保菌しており感染源となりうる。菌は感染動物の尿、糞、乳汁などに排泄され、環境を汚染する。ヒトは主にこの汚染された環境中の粉塵やエアロゾールを吸入し感染する。ヒトからヒトへの感染はほとんどおこらない。Q 熱の患者は世界中で報告されている。日本では 1999 年 4 月から感染症法による届出が始まり、最近では 2004 年に 7 人、2005 年に 8 人、2006 年に 2 人の患者が報告されている。 Q 熱の潜伏期は一般的には 2～3 週間で、感染量が多いと短くなる。発熱、頭痛、筋肉痛、全身倦怠感、呼吸器症状といったインフルエンザ様症状を示すが、主症状が肺炎、肝炎、あるいはその他の症状であったりと、その臨床像は多彩で Q 熱に特徴的な症状や所見はない。また、患者の 2～10%は心内膜炎を主徴とする慢性型に移行するといわれており、適切な治療をしないと致死率も高くなる。 本剤を含む当所で製造している全ての血漿分画製剤の製造工程には、約 0.2μm の「無菌ろ過工程」および、本菌よりも小さいウイルスの除去を目的とした平均孔径 19nm 以下の「ウイルス除去膜ろ過工程」が導入されているので、仮に製造原料に本菌が混入していたとしても、これらの工程により除去されるものと考えられる。更に、これまでに本剤による Q 熱感染の報告例は無い。 以上の点から、本剤は Q 熱感染に対して一定の安全性を確保していると考える。

*現在製造を行っていない

Archive Number 20080728.2306
Published Date 28-JUL-2008
Subject PRO/AH/EDR> Q fever - Netherlands (02): (NBR)

Q FEVER - NETHERLANDS (02): (NBR) (02)
****
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]
Date: Fri 25 Jul 2008
Source: Agrarisch Dagblad [trans. from Dutch by Mod.AS, edited].
<http://www.agd.nl/1057422/Nieuws/Artikel/Forse-toename-meldingen-Q-koorts.htm>

A substantial increase in the number of reported Q-fever cases

The number of reported cases of Q fever has risen sharply in recent weeks again [For the officially available data, indicating that the 2008 epidemic seems to have peaked by now, see the commentary. - Mod.AS]

The Public Health Service for Brabant had, in their last census on 21 Jul 2008, 491 known cases. That means that 5000 Brabanders have been actually infected, says the Ministry of Health. The disease spread rapidly in Noord-Brabant and, to a lesser extent, in the Nijmegen region. According to Roel Coutinho, head of the Centre for Infectious Disease Control, the actual number of victims is not 5-fold the number of reported cases but rather 10-fold.

The state branch of the Labour Party in Brabant has raised questions about the matter to the Executive Council. According to council member Nora Kasrioui, it is unclear what the directorate intends to do. "The disease is really a serious and growing problem for the population. We believe that the politics should go into action." Kasrioui acknowledges that it is difficult to make policies aimed at Q fever because much remains unclear about the disease. "Uncomfortable or not, organizations can always use help, financial or otherwise." [For the official government policy and background, see item 2].

According to Coutinho, the disease can never be fully eradicated. Normally it reappears during and following the lambing season. At present, goats are seen as the main source of infection. The RIVM (National Institute of Health and Environment), along with veterinary experts, is considering how the transfer from animal to man is established. Thereafter, a decision on further measures for disease prevention will be taken. Until last year [2007], Q fever was almost non-existent in the Netherlands.

[Byline: Jan Cees]

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

******
[2]
Date: 10 Jun 2008
Source: Dutch government official document No VD. 2008/1191, "Measures for Q fever" [Trans. from Dutch by Mod.AS, edited]

<http://www.minlnv.nl/cdlpub/servlet/CDLServlet?p_file_id=28304>

A letter from the Ministers of Agriculture and of Health to the Parliament

Introduction

During the recent weeks, a significant increase in the number of Q-fever infections in humans has been observed again in the north-eastern region of the province Noord (north) Brabant. This has led to unrest among local people. With this letter we will bring you up to date with additional precautionary measures that we will undertake to prevent the spread of Q-fever as much as possible.

Q-fever

Q fever is a disease caused by the bacterium _Coxiella burnetii_. It is a zoonotic disease, which means that spread from animals to humans can take place. Q fever is traditionally present around the world and may affect many species -- not only farm animals but also species such as birds, dogs, cats, rats and wild animals. Ticks can be a vector in the transmission of Q fever between animals.

In particular, small ruminants are regarded as a major source of infection for humans. After excretion, the bacterium can survive a long time in the air and sometimes spread over long distances. People can be infected through various routes, including the inhalation of infectious, airborne particles. Human infection is often manifested by mild symptoms but a more serious course may occur.

The main clinical sign of Q fever in ruminants is abortion in pregnant animals, caused by the bacterium. During and after the abortion the animals excrete large quantities of the bacteria in their manure.

Small ruminants intended for milk production are held mainly in so-called pen barns. A pen barn is a shed where the manure is covered on a regular basis with a new layer of straw. When the mixture of manure and straw reaches a certain height, the shed is emptied. Especially during the manure removal process, bacteria are shed into the air with the consequent risk for both the public and animal health. Possibly, the spreading of manure on land is also a risk factor, but this procedure seems to be of less significance than the removal process of manure from the pen barns. This difference became apparent since manure from Noord Brabant farms has been used as fertilizer in other provinces without harmful results in humans.

Initiatives undertaken

Following the 2007 Q-fever outbreak in Herpen, Noord-Brabant, some steps were agreed between the Ministry of Health, Welfare and Sport (VWS) and the Ministry of Agriculture, Nature and Food Quality (LNV), to obtain better insight regarding the Q-fever problem and to prevent, as far as possible, its spread to man. In this framework, advisory information on the hygiene measures to be applied in small-ruminant farms has been prepared and published on the sites of the Health, Welfare and Sports Ministry, the Agriculture Ministry, and the Animal Health Service (GD).

Research by the Health Services has been undertaken in both large and small ruminant sectors to obtain better understanding of the extent of the problem. This research is funded by both sectors and by the government. There is also research under way into the risk factors for the spread of Q-fever.

The relevant research institutes, namely the National Institute of Health and Environment (RIVM), the Central Veterinary Research Institute (CVI) and the Health Service (GD) are also in the process of development and validation of testing methods suitable for the detection and identification of the bacterium.

Finally, a research initiative is ongoing regarding intervention strategies. Special attention is paid to a vaccine which is currently

being tested in Denmark and France, considering its possible experimental application in the Netherlands as well.

Designating Q fever as an infectious, reportable animal disease

In order to be able to apply preventive and control measures on animal holdings, Q fever should be designated a reportable infectious animal disease. Indeed, this has been carried out by the Minister of Agriculture, adding Q fever to the list of animal diseases (including zoonoses) for which compulsory prevention, control and monitoring are regulated. Holders of small ruminants kept in pen barns are required to report signs which may indicate Q fever. This requirement obliges the veterinarians as well.

Measures regarding manure

Experts agree that manure probably plays an important role in the dissemination of the Q-fever agent in the province of Noord Brabant.

As a meaningful, provisional measure based on the precautionary principle, we plan to ban, for the duration of 3 months, the use of manure from small ruminant holdings in pen barns where serious infection has been established. A period of 3 months is regarded sufficient for a significant reduction of the infection load in the manure. Since the removal of manure from the pen barns is unavoidable as soon as the installation runs full, a practical solution is to be sought and finalized soon.

Other measures and consultations

In addition to the specific measures for the treatment of manure on infected holdings, further sector-related advice will be given in order to prevent future spread of Q fever. One of the ideas is to prescribe an advanced timetable for an earlier-in-season spreading of manure in the fields, preceding the lambing season. The aim is to prevent the utilization of the manure until at least 3 months after the lambing season, allowing significant reduction of its infection load.

Holdings with small ruminants are often frequented by recreation visitors and others interested. Contacts of people with infected premises are also undesirable. Temporarily preventing visits to such holdings seems to us advisable.

There are also a certain number of sheep and goat farms which produce their own cheese. This is often made with raw milk. The consumption of raw products from infected farms is discouraged by the RIVM (National Institute for Healthcare and the Environment). It seems therefore primarily useful to prescribe pasteurization in certain cases. The Minister for Health, Welfare and Sport will take these measures in consultation with RIVM.

With the above mentioned steps we try to limit, as far as possible, the spread of Q fever. The measures are aimed at the earliest possible action to diminish the risk of further spread. The development of the policy is being continued.

[Byline:
G. Verburg, Minister of Agriculture, Nature and Food Quality, and
Dr. A. Klink, Minister of Health, Leisure and Sport]

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

[The above ministerial letter, addressed to the Dutch parliament, provides interesting and useful information on the epidemiology of the disease in the Netherlands and the preventive and control measures undertaken. It is also useful for those engaged in any handling of emergency situations related to zoonotic diseases. Hopefully, action plans and contemplated research will be accomplished according to plan.

In our previous posting (see ProMED archive below), data on the disease incidence from different media sources were inconsistent; we are grateful to Naomi Bryant, National Travel Health Network and

Centre (NaTHNaC), for drawing our attention to that. Official Q fever data for the first 28 weeks of 2008 (1 Jan - 23 Jul 2008) are available on the official website of the Public Health Service for Brabant (GGD Hart voor Brabant). The total number of reported human cases during the said period was 538. The 1st cases appeared during week 3, remaining under 10/week until the 15th week, when it began to rise, peaking during week 22 (72 cases). During the weeks 27-28, the number is again below 10; the outbreak seems to be dying out. The said data can be found (in Dutch) at <http://www.rivm.nl/cib/infectieziekten-A-Z/infectieziekten/Q_koorts/FAQ_Q-koor

According to the said website, prior to 2007 the mean annual number of human Q fever cases, on national level, was 15. Since the disease in animals was not reportable, there is no information on its incidence in animals during the said years. The source indicates that the main animal species responsible for the current outbreak are goats, followed by sheep. - Mod.AS]

[see also:
Q fever - Netherlands: (NBR) 20080725.2267]
.....arn/ejp/jw
##
****
ProMED-mail makes every effort to verify the reports that are posted, but the accuracy and completeness of the information, and of any statements or opinions based thereon, are not guaranteed. The reader assumes all risks in using information posted or archived by ProMED-mail. ISID and its associated service providers shall not be held responsible for errors or omissions or held liable for any damages incurred as a result of use or reliance upon posted or archived material.
****
Become a ProMED-mail Premium Subscriber at <http://www.isid.org/ProMEDMail_Premium.shtml>
****
Visit ProMED-mail's web site at <http://www.promedmail.org>. Send all items for posting to: promed@promedmail.org (NOT to an individual moderator). If you do not give your full name and affiliation, it may not be posted. Send commands to subscribe/unsubscribe, get archives, help, etc. to: majordomo@promedmail.org. For assistance from a human being send mail to: owner-promed@promedmail.org.
##
##

about ISID | membership | programs | publications | resources
13th ICID | site map | ISID home

息2001,2008 International Society for Infectious Diseases
All Rights Reserved.
Read our privacy guidelines.
Use of this web site and related services is governed by the Terms of Service.

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9月9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	乾燥 pH4 処理人免疫グロブリン		研究報告の公表状況	Variant of Mad Cow Disease May Be Transmitted by Blood Transfusions, According to Animal Study http://www.hematology.org/media/08282008.cfm	公表国 英国	
販売名（企業名）	①サングロポール ②サングロポール点滴静注用 2.5g （CSL ベーリング株式会社）					

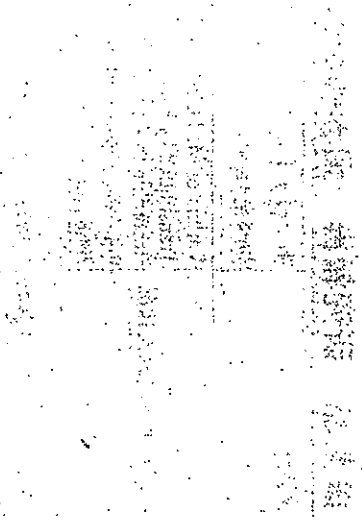
研究報告の概要

問題点（動物実験で輸血により vCJD が感染することが報告）
Blood Online の"Press Releases"に本研究の概要が報告された。しかし概要のため実験系の情報が少ないが、今回報告する。

英国グラスゴー大学獣医学部のヒューストン教授は、BSE とスクレイピーに感染したヒツジの輸血による感染について 9 年間研究している。
その結果、ヒツジ間において BSE とスクレイピーは、輸血により効率的に感染することが示された。特に、疾患の兆候が発現する前のドナーから採取された血液でも感染が伝播し、感染の後期ではより感染していた。
BSE ドナー群の感染した血液を投与されたヒツジ 22 頭のうち、5 頭が TSE の兆候を示し、3 頭は臨床症状の発現なしで、感染のエビデンスを示し、全体で 36%の感染率であった。
スクレイピー感染した血液を投与された 21 頭のうち、9 頭がスクレイピーの症状を発現し、全体で 43%の感染率であった。
これらの結果は、ヒトの輸血により vCJD に感染した 4 症例と一致している。
ドナーの感染期に加え、疾病感受性の遺伝的多様性や輸血成分などの要因が、ヒツジやヒトでの輸血による感染率に影響する。
BSE やスクレイピー感染したヒツジで、輸血による感染率は高い、特にドナーが感染後期の場合は高い。
以上の結果がヒトでの感染と一致していることから、輸血はこれらの疾患が効率的に感染する経路の代表であることが示された。
また、どの血液成分が重篤に感染するかを解明したり、切望されている診断試験を開発するために、BSE やスクレイピーに感染したヒツジの血液は効率的に利用されるであろうことが示された。

使用上の注意記載状況・
その他参考事項等

報告企業の意見	今後の対応
これまで血漿分画製剤によって vCJD が伝播した報告はない。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を添付文書に記載し、注意喚起している。	今後とも新しい感染症に関する情報収集に努める所存である。



ABJ2008-207

American Society of Hematology
1900 M Street, NW, Suite 200, Washington, DC 20036
Phone: 202-776-0544 Fax: 202-776-0545

Press Releases

Variant of Mad Cow Disease May Be Transmitted by Blood Transfusions, According to Animal Study

(WASHINGTON, August 28, 2008) – Blood transfusions are a valuable treatment mechanism in modern medicine, but can come with the risk of donor disease transmission. Researchers are continually studying the biology of blood products to understand how certain diseases are transmitted in an effort to reduce this risk during blood transfusions. According to a study in sheep prepublished online in *Blood*, the official journal of the American Society of Hematology, the risk of transmitting bovine spongiform encephalopathy (BSE, commonly known as "mad cow disease") by blood transfusion is surprisingly high.

BSE is one of a group of rare neurodegenerative disorders called transmissible spongiform encephalopathies (TSEs), and there is no reliable non-invasive test for detecting infection before the onset of clinical disease. In addition to BSE, these diseases include scrapie, a closely related disease in sheep, and Creutzfeldt-Jakob disease (CJD) in humans, which causes neurological symptoms such as unsteadiness and involuntary movements that develop as the illness progresses, rendering late-stage sufferers completely immobile at the time of death.

A new variant of CJD (termed vCJD) was recognized in the United Kingdom in the mid-1990s, apparently as a result of the transmission of BSE to humans. Because the symptoms of this disease can take many years to appear, it was not known how many people might have been infected, and without a reliable test for identifying these individuals, clinicians were very concerned that the infection could be transmitted between people by blood transfusion or contaminated surgical and dental instruments. As a result, costly control measures were introduced as a precautionary measure to reduce the risk of disease transmission, although at the time it was unclear whether there really was a significant risk or whether the control measures would be effective. This sheep study sought to better understand how readily TSEs could be transmitted by blood transfusion in order to help develop more targeted controls.

"It is vitally important that we better understand the mechanisms of disease transmission during blood transfusions so we can develop the most effective control measures and minimize human-to-human infections," said Dr. Fiona Houston, now a Faculty of Veterinary Medicine, University of Glasgow, UK, and lead author of the study.

The nine-year study conducted at the University of Edinburgh compared rates of disease transmission by examining blood transfusions from sheep infected with BSE or scrapie; the BSE donors were experimentally infected, while the scrapie donors had naturally acquired the disease. While scrapie is not thought to transmit to humans, it was included as an infection acquired under field conditions, which could possibly give different results than those obtained from experimentally infected animals. Because of the similarity in size of sheep and humans, the team was able to collect and transfuse volumes of blood equivalent to those taken from human blood donors.

The outcome of the experiment showed that both BSE and scrapie could be effectively transmitted between sheep by blood transfusion. Importantly, the team noted that transmission could occur when blood was collected from donors before they developed signs of disease, but was more likely when they were in the later stages of infection. Of the 22 sheep who received infected blood from the BSE donor group, five showed signs of TSEs and three others showed evidence of infection without clinical signs, yielding an overall transmission rate of 36 percent. Of the 21 infected scrapie recipients, nine developed clinical scrapie, yielding an overall transmission rate of 43 percent.

Investigators noted that the results were consistent with what is known about the four recorded cases of vCJD acquired by blood transfusion in humans. In addition to the stage of infection in the donor, factors such as genetic variation in disease susceptibility and the blood component transfused may influence the transmission rate by transfusion in both sheep and humans.

"The study shows that, for sheep infected with BSE or scrapie, transmission rates via blood transfusion can be high, particularly when donors are in the later stages of infection. This suggests that blood transfusion represents an efficient route of transmission for these diseases," said Dr. Houston. "Since the results are consistent with what we know about human transmission, the work helps justify the control measures put in place to safeguard human

blood supplies. It also shows that blood from BSE- and scrapie-infected sheep could be used effectively in non-human experiments to answer important questions, such as which blood components are most heavily infected, and to develop much-needed diagnostic tests."

Reporters who wish to receive a copy of the study or arrange an interview with lead author, Dr. Houston, may contact Becka Livesay at 202-776-0544 or rlivesay@hematology.org.

The American Society of Hematology (*www.hematology.org*) is the world's largest professional society concerned with the causes and treatment of blood disorders. Its mission is to further the understanding, diagnosis, treatment, and prevention of disorders affecting blood, bone marrow, and the immunologic, hemostatic, and vascular systems, by promoting research, clinical care, education, training, and advocacy in hematology.

Back to Press Release Listing

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7月14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	乾燥濃縮人血液凝固第Ⅷ因子		研究報告の公表状況	Annals of Neurology 2008; 63(6): 697-708	公表国 アメリカ	
販売名 (企業名)	コンコエイト-HT（ベネシス）					

研究報告の概要

<目的>
明確な組織病理および免疫染色所見によって特徴付けられ、また、通常のプリオン病とは異なりプロテアーゼ消化に感受性の高いプリオンたん白（PrP）の異常なアイソフォームに関連した新規プリオン病を報告する。
<方法>
National Prion Disease Pathology Surveillance Center において、11名の被験者の臨床的、組織病理学的および免疫組織化学的特徴、遺伝型ならびにPrPの特徴を調査した。
<結果>
患者らは平均年齢62歳で行動的及び精神医学的症状を示し、その平均罹病期間は20ヶ月であった。海綿状変性のタイプ、PrPの免疫染色パターン、マイクロプラークの存在は、知られたプリオン病のものとは異なっていた。典型的なプロテアーゼ抵抗性PrPは標準的診断では脳の新皮質に検出されなかった。異常PrPは、濃縮すると通常のプリオン病の16倍低い濃度で検出された。それはプロテアーゼ抵抗性PrPが約4倍低く、特徴的な電気泳動像を示した。検査した被験者は、National Prion Disease Pathology Surveillance Centerによって評価された孤発性症例の約3%である。数人の被験者は痴呆の家族歴を有したが、PrP遺伝子のオープン・リーディング・フレームの変異は見つからなかった。
<解釈>
その明確な組織病理学的、PrP免疫組織化学的、物理化学的特徴は、同じ遺伝型と併せて、以前に確認されていない、PrPが関わる疾患であることを示しており、われわれはこれをプロテアーゼ感受性プリオン病（PSPr）と名付けた。PSPrは、プリオン病の中では稀ではなく、われわれのデータが示すよりもさらに多い可能性がある。その理由は、PSPrの症例がアルツハイマー以外の痴呆症のグループに分類されている可能性があるからである。

245

使用上の注意記載状況・その他参考事項等

2. 重要な基本的注意
(1) 略
1) 略
2) 略
3) 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
プロテアーゼ感受性のプリオンたん白と関連した新規プリオン病に関する報告である。 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。

32

ORIGINAL ARTICLE

A Novel Human Disease with Abnormal Prion Protein Sensitive to Protease

Pierluigi Gambetti, MD,[1] Zhiqian Dong, PhD,[1] Jue Yuan, BA,[1] Xiangzhu Xiao, PhD,[1] Mengjie Zheng, PhD,[1] Amer Alshekhlee, MD,[1] Rudy Castellani, MD,[2] Mark Cohen, MD,[1] Marcelo A. Barria, PhD,[3] D. Gonzalez-Romero, PhD,[3] Ermias D. Belay, MD,[4] Lawrence B. Schonberger, MD, MPH,[4] Karen Marder, MD,[5] Carrie Harris, BA,[1] James R. Burke, MD, PhD,[6] Thomas Montine, MD,[7] Thomas Wisniewski, MD,[8] Dennis W. Dickson, MD,[9] Claudio Soto, PhD,[3] Christine M. Hulette, MD,[10] James A. Mastrianni, MD, PhD,[11] Qingzhong Kong, PhD,[1] and Wen-Quan Zou, MD, PhD[1]

Objective: To report a novel prion disease characterized by distinct histopathological and immunostaining features, and associated with an abnormal isoform of the prion protein (PrP) that, contrary to the common prion diseases, is predominantly sensitive to protease digestion.

Methods: Eleven subjects were investigated at the National Prion Disease Pathology Surveillance Center for clinical, histopathological, immunohistochemical, genotypical, and PrP characteristics.

Results: Patients presented with behavioral and psychiatric manifestations on average at 62 years, whereas mean disease duration was 20 months. The type of spongiform degeneration, the PrP immunostaining pattern, and the presence of microplaques distinguished these cases from those with known prion diseases. Typical protease-resistant PrP was undetectable in the cerebral neocortex with standard diagnostic procedures. After enrichment, abnormal PrP was detected at concentrations 16 times lower than common prion diseases; it included nearly 4 times less protease-resistant PrP, which formed a distinct electrophoretic profile. The subjects examined comprised about 3% of sporadic cases evaluated by the National Prion Disease Pathology Surveillance Center. Although several subjects had family histories of dementia, no mutations were found in the PrP gene open reading frame.

Interpretation: The distinct histopathological, PrP immunohistochemical, and physicochemical features, together with the homogeneous genotype, indicate that this is a previously unidentified type of disease involving the PrP, which we designated "protease-sensitive prionopathy" (or PSPr). Protease-sensitive prionopathy is not rare among prion diseases, and it may be even more prevalent than our data indicate because protease-sensitive prionopathy cases are likely also to be classified within the group of non-Alzheimer's dementias.

Ann Neurol 2008;63:697–708

Human prion diseases or transmissible spongiform encephalopathies may be sporadic, inherited, or acquired by infection.[1] Creutzfeldt–Jakob disease (CJD) is the most common phenotype and occurs in all three forms. In the sporadic form, CJD is classified into five subtypes, which can be readily distinguished based on clinical features, type and distribution of brain lesions, and pattern of prion protein (PrP) immunostaining.[2,3] Fatal insomnia, a much rarer phenotype, includes sporadic and inherited forms, and is characterized by loss of ability to sleep and preferential thalamic degeneration.[4] Gerstmann–Sträussler–Scheinker disease (GSS), the third phenotype, occurs exclusively as a heritable disease invariably associated with a mutation in the PrP gene open reading frame (ORF) and is characterized by the presence of prion amyloid plaques.[4]

Despite their heterogeneity, all sporadic human prion diseases described to date have been associated with abnormal PrP (commonly called PrP^{Sc} but henceforth referred to as PrPr), which is resistant to treatment with proteases and is considered the diagnostic hallmark of these diseases.[1] PrPr is derived from normal or cellular PrP (PrP^{C}) via a posttranslational tran-

From the [1]Institute of Pathology, Case Western Reserve University, Cleveland, OH; [2]Department of Pathology, University of Maryland, Baltimore, MD; [3]Department of Neurology, Neuroscience and Cell Biology, George and Cynthia Mitchell Center for Neurodegenerative Diseases, University of Texas Medical Branch, Galveston, TX; [4]Centers for Disease Control and Prevention, Atlanta, GA; [5]Department of Neurology, Columbia University, New York, NY; [6]Department of Medicine, Division of Neurology, Duke University, Durham, NC; [7]Harborview Medical Center, University of Washington, Seattle, WA; [8]Department of Neurology, New York University, New York, NY; [9]Department of Neuropathology, Mayo Clinic College of Medicine, Jacksonville, FL; [10]Department of Pathology, Duke University, Durham, NC; and [11]Department of Neurology, University of Chicago, Chicago, IL.

Received Nov 5, 2007, and in revised form Apr 1, 2008. Accepted for publication Apr 4, 2008.

Address correspondence to Dr Gambetti or Dr. Zou, Institute of Pathology, Case Western Reserve University, 2085 Adelbert Road, Cleveland, OH 44106. E-mail: pxg13@case.edu or wenquan.zou@case.edu

© 2008 American Neurological Association
Published by Wiley-Liss, Inc., through Wiley Subscription Services

sition from α-helical to β-sheet–rich conformations. PrP^C and PrPr are quite different. Whereas PrP^C is soluble in nondenaturing detergents and is completely digested when exposed to appropriate concentrations of proteinase K (PK), PrPr is detergent insoluble and its C-terminal region resists PK treatment.[5] Based on the size of their PK-resistant fragments, at least three major PrPr types are recognized, which codistribute with specific disease phenotypes: (1) PrPr type 1, which on PK treatment generates an approximately 21kDa fragment; (2) PrPr type 2, generating an approximately 19kDa fragment; and (3) PrP7-8, a PrP internal fragment of 7 to 8kDa.[4–6] Both PrPr types 1 and 2 have been observed associated with distinct subtypes of CJD. To date, PrP7-8 has been consistently observed only in GSS. Therefore, the conformational changes, which render PrPr pathogenic and in many but not all cases infectious, may engender different species or strains of PrPr that can be recognized based on their distinct protease-resistant fragments and by their associated clinicopathological phenotype.[5,7–12]

Studies mostly based on experimental models recently have shown that PK-resistant PrP (PrPr) is associated with varying quantities of a PrP isoform that, as PrPr, is detergent insoluble but sensitive to protease digestion (PrPs).[11–15] The relation of PrPs with PrPr and the role that PrPs plays in the pathogenesis of prion diseases remains uncertain.[16–18]

Here we report 11 patients with a human disease characterized by the presence of detergent-insoluble PrP that is predominantly sensitive to protease digestion and forms unusual immunohistochemical patterns. Furthermore, the small amount of PrPr present generates a distinct profile on immunoblot. Several affected patients have family histories of dementia but lack mutations in the PrP gene ORF. We refer to this condition as protease-sensitive prionopathy (PSPr). PSPr broadens the spectrum of human prion diseases and raises several important issues related to the nature of these diseases in light of their association with different PrP isoforms. Among prion diseases, PSPr is not rare. Because the presenting clinical signs often suggest the diagnosis of non-Alzheimer's dementia, PSPr may be even more prevalent than our data indicate because many PSPr cases might currently be classified within this group of dementias. Parts of this study have been presented previously.[19]

Subjects and Methods

Subjects

The 11 (10 autopsy and 1 biopsy) patients and the control subjects were referred to the National Prion Disease Pathology Surveillance Center between May 2002 and January 2006. Consent was obtained to use tissues for research, including genetic analyses.

General Tissue Processing

Fixed and frozen brain tissues were obtained from all subjects and processed as described previously.[20]

Histopathology and Immunohistochemistry

Samples obtained from up to 18 brain regions were processed as described previously.[2,3] Lesion profiles were constructed using semiquantitative evaluation of spongiform degeneration (SD) and astrogliosis in 12 brain regions from 6 subjects, and 4 or 5 regions from 2 subjects. SD and astrogliosis were scored (Fig 1), and the scores from each of the brain regions were summed for each subject separately; values were averaged, and standard deviations were determined and plotted according to the brain region.[2] Vacuoles with larger than 4μm diameter were measured individually on random photomicrographs of frontal neocortex (10/subject, ×180) using Spot-software version 4.6 after calibration (Diagnostic Instruments, Sterling Heights, MI). Sections from the frontal and occipital neocortices, hippocampus, basal ganglia, thalamus, cerebellar hemisphere, and midbrain were processed for PrP immunohistochemistry with the monoclonal antibody (Mab) 3F4 or 1E4 (Cell Sciences, Canton, MA).[2,20–23] Selected brain regions were also immunostained with the Mabs 4G8 to amyloid β.[24]

Electron Microscopy

Formalin-fixed postmortem brain tissue was processed for conventional electron microscopy and for PrP immunohistochemistry according to standard techniques using peroxidase-antiperoxidase Mab 3F4 to PrP.[25]

Molecular Genetics

The entire PrP ORF was amplified by polymerase chain reaction using genomic DNA extracted from unfixed brain tissue or blood and the primers PrPO-F [GTCATYATGGCGAACCTTGG (Y = C + T)] and PrPO-R [CTCATCCCACKATCAGGAAG (K = T + G)]; sequencing was done directly or after cloning into plasmid pSTBlue 1 (Novagen, Madison, WI) by automated sequencing.[22]

Prion Protein Characterization

CONVENTIONAL IMMUNOBLOT.

Five to 20μl 10% wt/vol brain homogenates with or without PK digestion (Sigma Chemical, St. Louis, MO) were loaded onto 15% Tris-HCl Criterion precast gels (Bio-Rad Laboratories, Hercules, CA) for sodium dodecyl sulfate polyacrylamide gel electrophoresis, and immunoblotted with 3F4 and 1E4 to human PrP residues 109 to 112 and 97 to 108, respectively.[23] PrP was deglycosylated with PNGase F (New England Biolabs, Beverly, MA) following manufacturer's instructions.

ENRICHMENT OF THE ABNORMAL PRION PROTEIN.

Two procedures were utilized: (1) capture of the abnormal PrP with the gene 5 protein (g5p), as described previously[13,23]; and (2) abnormal PrP precipitation with sodium phosphotungstate.[26]

SEDIMENTATION OF PRION PROTEIN IN SUCROSE GRADIENTS.

Brain homogenates were incubated with 2% Sarkosyl for 30 minutes on ice, loaded atop a 10 to 60% step sucrose gra-

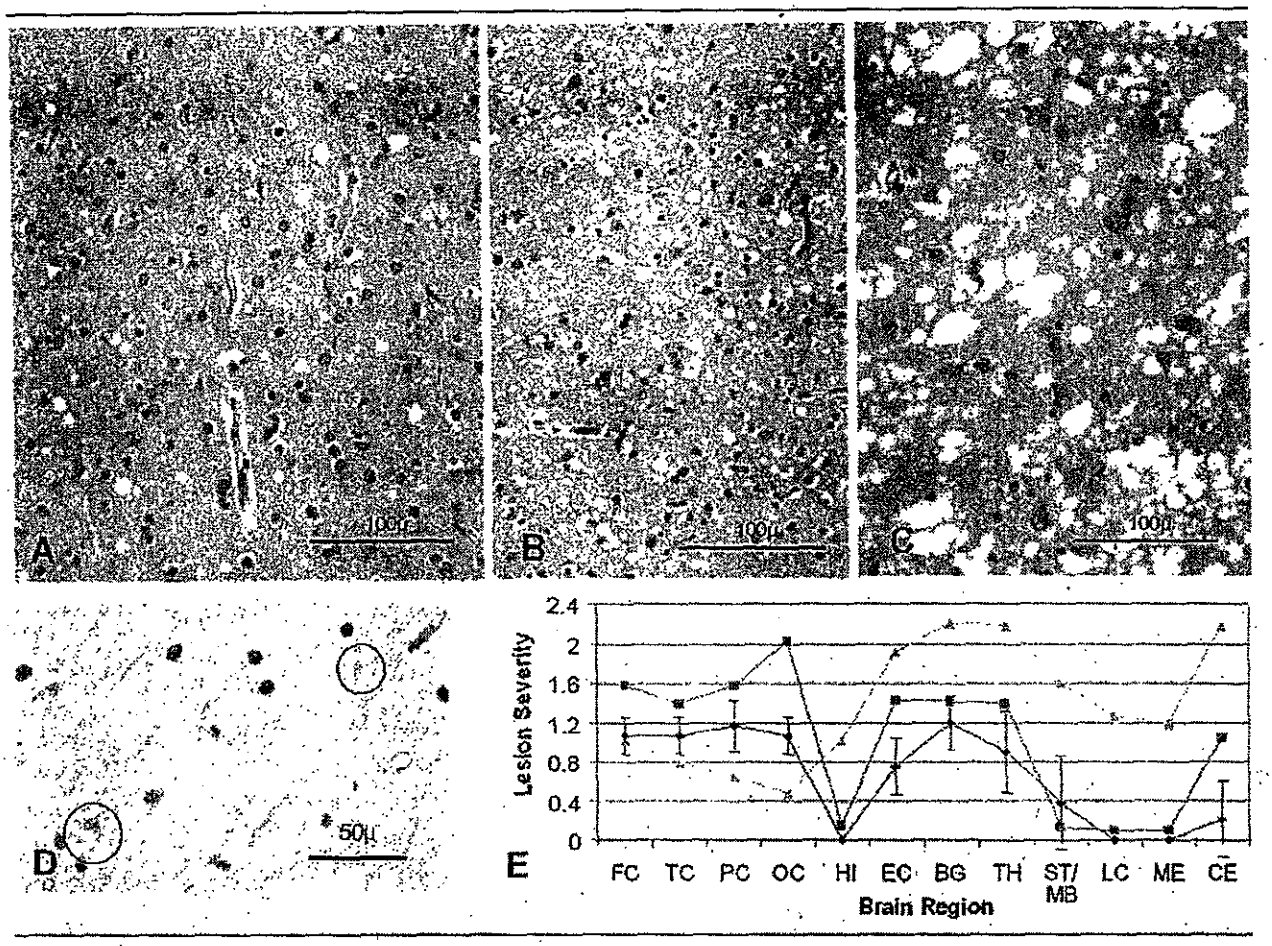


Fig 1. Histopathology and lesion profile. The spongiform degeneration of protease-sensitive prionopathy (PSPr) (A) is characterized by a mixture of small and intermediate size vacuoles, whereas the vacuoles of two subtypes of sporadic Creutzfeldt–Jakob disease (CJD), sCJDMM1 (B) and sCJDMM2 (C), are mostly small (sCJDMM1) or much larger and confluent (sCJDMM2). (D) Eosinophilic microstructures surrounded by a pale halo (circle) *in the cerebellar molecular layer; (A–D) Hematoxylin and eosin staining. (E) Lesion profiles of PSPr* (diamonds), *sCJDMM1* (squares), *and sCJDVV2* (triangles). Vertical bars *refer to standard deviations. In sCJDMM1 and sCJDVV2, for which data were adapted from Parchi and colleagues,[2] standard deviations were omitted for clarity. Spongiform degeneration was scored on a 0 to 4 scale (0 = not detectable; 1 = mild; 2 = moderate; 3 = severe; 4 = confluent); astrogliosis was scored on a 0 to 3 scale (0 = not detectable; 1 = mild; 2 = moderate; 3 = severe). FC = front cortex; TC = temporal cortex; PC = parietal cortex; OC = occipital cortex; HI = CA1 of hippocampus; EC = entorhinal cortex; BG = basal ganglia; TH = thalamus mediodorsal nucleus; MB/ST = midbrain in PSPr, substantia nigra in sCJDMM1 and sCJDVV2; LC = pons; ME = medulla; CE = cerebellar cortex.*

dient and centrifuged 1 hour at 200,000*g* in a SW55 rotor (Beckman Coulter, Fullerton, CA).[16,23,27]

Statistics

Analyses were performed with the two-tail Student's *t* test.

Results

Clinical Features

Mean age of onset and disease duration were 62 years (range, 48–71 years) and 20 months (range, 10–60 months), respectively (Table 1). Presentation and course were dominated by neurobehavioral and psychiatric signs, with progressive motor and cognitive decline. Seven patients were ataxic. Other consistent features included absence of periodic complexes on the electroencephalogram and nondiagnostic 14-3-3 protein test in the cerebrospinal fluid. Magnetic resonance imaging showed diffuse atrophy without restricted diffusion signals in all 10 patients examined. No subject had known history of prion exposure; probable familial occurrence of dementia was reported in 6 of 10 investigated patients (see Table 1).

Neurohistopathology

SD and astrogliosis of moderate severity were present in the cerebral cortex, basal ganglia, and thalamus of

Table 1. Clinical Findings

Case No.	Sex	Age (yr)	Disease Duration (mo)	Symptoms at Onset	Symptoms during Illness Evolution	EEG	MRI Atrophy/ Diffusion[a]	Family History of Dementia	Other Information
1	M	62	60	Behavioral and mood swings, psychosis (patient diagnosed with bipolar illness)	Dementia, aphasia, ataxia, and seizure	Slowing right > left	+/−	Mother died of dementia at age 70	(1) Right hemispheric hypoperfusion on SPECT study; (2) CSF 14-3-3 (not performed)
2	F	71	33	Depression and dementia	Dementia, ataxia, and Parkinsonism	Normal	+/−	Mother with dementia	CSF 14-3-3 (not performed)
3	M	70	12	Dementia and apathy	Aphasia, Parkinsonism, hyperreflexia, and prominent frontal release signs	Normal	+/−	Father with dementia at age 60	(1) Negative CSF 14-3-3; (2) increased CSF proteins 175mg/dl without cells
4	M	50	7 (died in a fall)	Dementia and mood swings	Psychosis, aphasia, patient fell and died of subdural hematoma	Diffuse slowing	NA	NA	Ambiguous CSF 14-3-3
5	F	67	11	Dementia and aphasia	Ataxia and depression	Not performed	+/−	Dementia in a paternal aunt and sister died of dementia at age 69	CSF 14-3-3 (not performed)
6	M	60	13	Dementia	Ataxia, psychosis, and incontinence	NA	+/−	No family history of dementia	CSF 14-3-3 (not performed)
7	F	48	17	Dementia, emotional lability, and outbursts	Motor decline	Diffuse slowing	+/−	Mother with early dementia at age 60	(1) Negative CSF 14-3-3; (2) patient had VP shunt without response
8	F	64	10	Dementia, depression, and psychosis	Ataxia, Parkinsonism, and tremor	Diffuse slowing	+/−	Mother with dementia	Negative CSF 14-3-3
9	M	63	23 (patient alive)	Dementia, personality and behavioral changes	Motor decline, Parkinsonism, and psychosis	Diffuse slowing	+/−	Mother died at age 83 with mild dementia	(1) Global hypoperfusion on SPECT study; (2) negative CSF 14-3-3; (3) increased CSF protein 126mg/dl without cells
10	F	68	17	Insomnia, tremor, and slurred speech	Dementia, ataxia, worsening depression with psychosis and agitation, hyperreflexia	Diffuse slowing	+/−	No family history of dementia	History of bipolar illness with suicidal attempts
11	M	52	13	Decreased verbal output, and progressive motor decline	Dementia, ataxia, and Parkinsonism	Normal	+/−	No family history of dementia	NA

[a]Average disease duration (20.4 ± 15.4) excludes patients #4, who died of subdural hematoma caused by a fall, and #9, still alive at last report. [b]The neurobehavioral and psychiatric manifestations included insomnia, apathy, personality changes, mood swings, emotional outburst, depression, and psychosis. Plus and minus signs, respectively, indicate the presence and absence of atrophy or restricted diffusion signals on brain magnetic resonance imaging (MRI). EEG = electroencephalography; SPECT = single-photon emission computerized tomography; CSF = cerebrospinal fluid; NA = not available; VP = ventriculoperitoneal.

the PSPr cases without severe neuronal loss. SD comprised a mixture of fine vacuoles, comparable with those seen in sCJDMM1 (the most common sCJD subtype), and slightly larger vacuoles that resulted in a mean vacuolar diameter greater than that of sCJDMM1 (7.8 ± 2.7 vs 5.8 ± 1.2μm). But the "larger" vacuoles clearly were smaller than the "coarse" vacuoles characteristic of sCJDMM2 (see Figs 1A–C).[2,3] The hippocampal pyramidal cell layer appeared unaffected; the molecular layer of the dentate gyrus and the stratum lacunosum moleculare showed mild SD, which extended into the subiculum and the entorhinal and inferior temporal neocortices. No kuru plaques or multicore plaques were detected. In some subjects, structures suggestive of microplaques were observed in the molecular layer of the cerebellum (see Fig 1D). Lesion profiling identified the cerebral neocortex, basal ganglia, and thalamus as the regions most severely affected, whereas the brainstem and cerebellum were apparently spared (see Fig

1E). Congo red staining of selected cerebral and cerebellar cortices was negative.

Immunohistochemistry

PrP immunostaining with Mabs 3F4 and 1E4 of the cerebral cortex, basal ganglia, and thalamus from the PSPr cases was strong, and in the hippocampal formation was selective with strong immunoreactivity in the molecular layer of the dentate and stratum lacunosum moleculare, without pyramidal cell layer staining (Figs 2A, B). The staining pattern in the cerebrum was characterized by round, loose clusters of coarse granules quite evenly distributed over a background of smaller granules (see Fig 2C). The size of the cluster-forming granules often increased progressively toward the cluster's center, which generally contained a larger granule or a tight aggregate of small granules (see Fig 2D). Strongly immunostained globular structures were occasionally seen, rarely also in the white matter (see Fig 2D; inset). Immunoreactivity in cerebellum and brainstem was limited to minute, rounded structures or aggregates of a few granules in the cerebellar molecular layer and midbrain colliculi, except for one subject who displayed a large number of these structures (see Fig 2H). The immunostained clusters and globules could not be correlated with histologically detectable lesions except for the intense immunostaining of possible microplaques in the cerebellum of some cases (see Figs 1D and Fig 2H). The pattern of PrP immunostaining of cerebrum and cerebellum in the PSPr cases was readily distinguishable from those of sCJD subtypes and nonprion disease controls (see Figs 2E–J). Furthermore, on paraffin-embedded tissues, PrP immunoreactivity was virtually removed with PK treatment (50μg/ml, 37°C, 1 hour) in these cases, whereas it was only reduced in sCJD (data not shown). Amyloid-β immunostaining showed mostly diffuse plaques apparently compatible with the subject's age.

Electron Microscopy

The ultrastructural examination of the cerebellar molecular layer from the case shown in Figure 1D showed poorly defined, rounded structures with barely detectable filament-like profiles that were embedded in an amorphous-granular matrix. These formations strongly reacted with antibodies to PrP and overall had the features of poorly formed or immature PrP microplaques (Figs 3A, B).

Genetic Findings

All PSPr patients were homozygous for valine at codon 129 of the PrP gene, and none carried mutations in the PrP gene ORF; three subjects had silent polymorphisms (two at codon 117 and one at codon 122).

Prion Protein Characterization: Detergent-Insoluble, Protease-Resistant, and Protease-Sensitive Prion Protein

The total PrP immunoblot profile from all PSPr patients was indistinguishable from that of nonprion disease control subjects (Fig 4A). The glycoform ratios of the three PrP bands from the two groups were similar. Measured by densitometry in arbitrary units, the diglycosylated or upper band was 10.44 ± 1.78 (n = 3) in PSPr versus 7.83 ± 3.64 (n = 5) in nonprion disease control subjects ($p = 0.30$); the monoglycosylated or intermediate band was 4.40 ±1.88 (n = 3) in PSPr versus 3.40 ± 2.74 (n = 5) in control subjects ($p = 0.79$). Under our conditions, the unglycosylated or lower band was not measurable in both PSPr patients and control subjects (see Fig 4A). Furthermore, the mean amount of total PrP present in six subjects apparently did not significantly differ from that of the nonprion disease control subjects (n = 7) (1.69 ± 0.28 vs 1.57 ± 0.39; $p = 0.53$) and from that of cases with prion disease (n = 3) (1.69 ± 0.28 vs 2.03 ± 0.46; $p = 0.20$).

In conventional diagnostic immunoblot procedures using Mab 3F4, classic PrPr (PrP27-30) was undetectable in the brain homogenates from the frontal cortex of all 11 subjects, and from the occipital and cerebellar cortices of the 7 subjects in which these brain regions were tested (see Fig 4A). Treatment with various doses of PK showed no consistent difference between these subjects and nonprion disease control subjects in these brain regions (see Fig 4B). Barely detectable amounts of approximately 6kDa PK-resistant PrP (PrP~6) were present in the temporal cortex of three of the eight tested subjects. Of the eight subjects for whom subcortical regions (substantia nigra, putamen, and thalamus) were available, significant quantity of PK-resistant PrP27-30 was found in one case, and minimal amounts in two others (one showed small amounts of PrP~6 only), whereas no PrPr could be definitely detected in the other five subjects (see Fig 4C). In contrast, probing with Mab 1E4 demonstrated a ladder of PK-resistant PrP fragments ranging from approximately 29 to 6kDa in all PSPr cases examined (see Fig 4D). The ladder-like electrophoretic mobility of the PrPr fragments did not match those associated with common subtypes of CJD, except for an approximately 20kDa fragment, which, after deglycosylation, was tentatively identified as the unglycosylated form of PrPr (see Fig 4C; also data not shown).[2] The approximately 6kDa fragment was also unglycosylated and was reminiscent of the PrP~7 fragment of GSS.[1] These fragments were most obvious at PK concentrations of 5 to 10μg/ml and decreased at greater PK concentrations. The ladder-like electrophoretic profile of PrP treated with PK was highly reproducible and was observed in all 11 PSPr cases examined. In contrast, the PrPr frag-

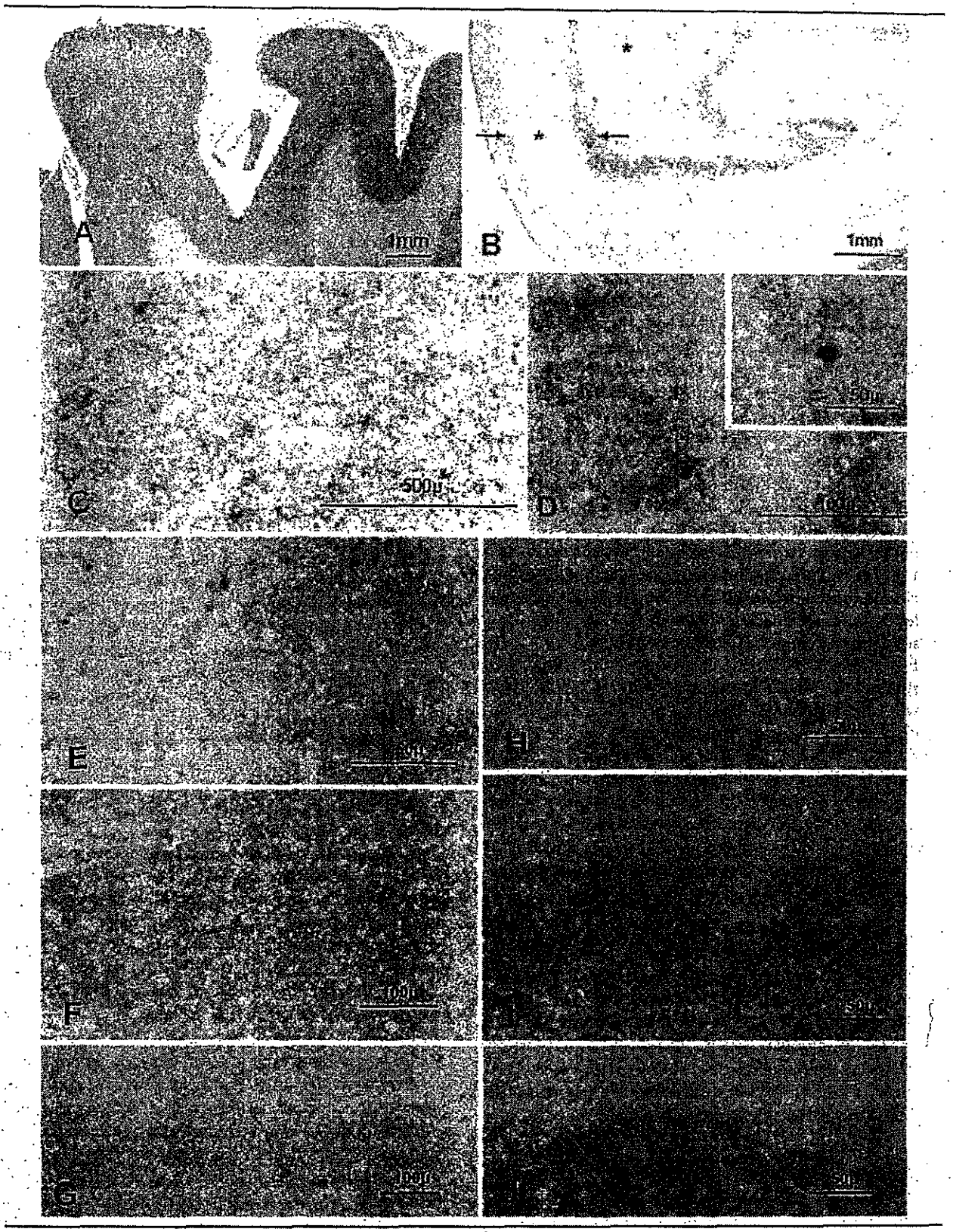


Figure 2.

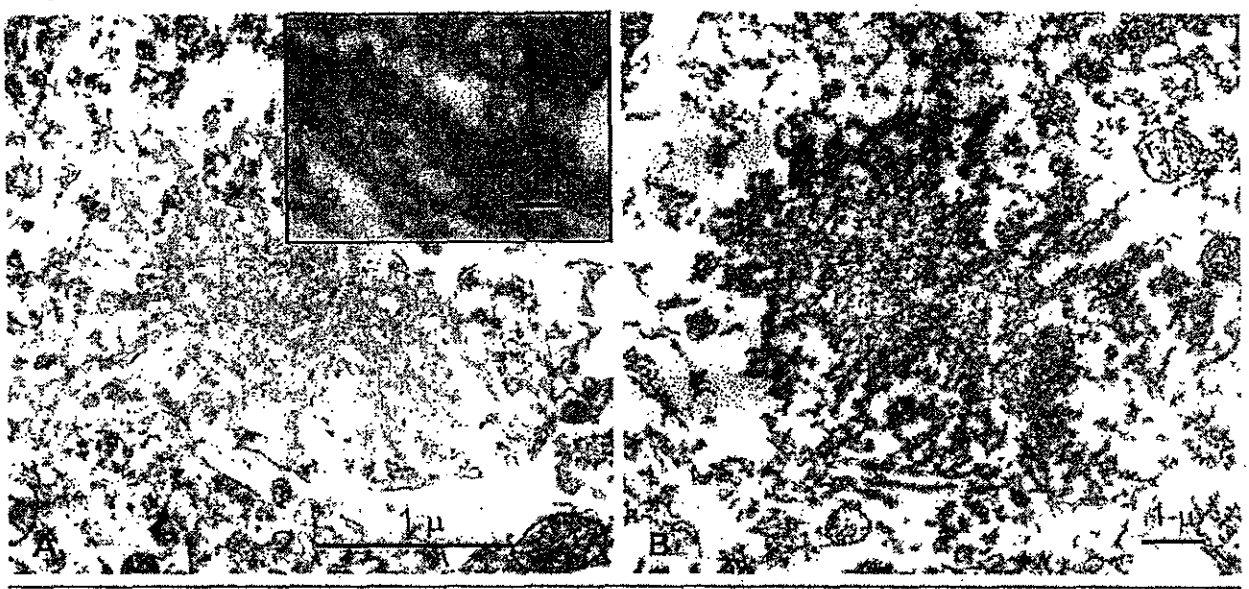


Fig 3. Electron microscopy (EM) of brain microstructures of protease-sensitive prionopathy (PSPr). (A) EM of the eosinophilic microstructures observed at light microscopy (Fig 1D) shows plaquelike formations with fuzzy filamentous appearance (inset). These structures are strongly reactive with antibodies to prion protein (PrP) (B) consistent with PrP microplaques (peroxidase anti-peroxidase with 3F4).

ments from sCJD were clearly detectable with both 3F4 and 1E4 Mab only after treatment with more than 10μg/ml PK, and increased with greater PK concentrations (see Fig 4C; also data not shown). Therefore, a small amount of PrPr detectable with Mab 3F4 is present mostly in the subcortical regions of these subjects. Moreover, most of the PrPr appears to have a different conformation from that of typical PrPr because on PK digestion it generates a unique set of fragments that are detected by 1E4 but not by 3F4.

Fig 2. Prion protein (PrP) immunohistochemistry. (A) Intense and widespread PrP immunostain of the cerebral cortex and (B) distinctive PrP immunostaining pattern in the hippocampal gyrus with staining of the molecular layers (arrows) *but not of the pyramidal cell layer or of the end plate* (asterisk). *(C, D) The cortical staining consists of coarse granules forming loose clusters with larger granules or a tighter aggregate of granules at the center; (D, inset) heavily stained globular structures are also present. (A–D) Protease-sensitive prionopathy (PSPr). (E, F) Immunostaining patterns of the cerebral cortex in sCJDVV2 (E) and sCJDVV1 (F) showing laminar staining and occasional perineuronal staining in sCJDVV2 and weak and fine widespread staining in sCJDVV1. (G) No immunostaining is detectable in the cerebral cortex of a nonprion disease control. (H–J) Cerebellar immunostaining patterns in PSPr (H), sCJDVV2 (I), and sCJDVV1 (J). There is intense and exclusive staining of large granules in the molecular layers in PSPr (H), presumably corresponding to the eosinophilic microstructures surrounded by a pale halo shown in Figure 1D; staining of irregular deposit limited to the granule cell layer in sCJDVV2 (I); no detectable staining in sCJDVV1 (the staining of the granule cell nuclei is nonspecific) (J). (A–I) Monoclonal antibody 3F4.*

Total abnormal PrP and the PrPr conformers were further characterized in abnormal PrP-enriched preparations after the capture of the abnormal PrP with g5p, a single-stranded DNA binding protein with a high affinity for abnormal PrP regardless of its PK resistance.[13,23] The amount of PrP captured by g5p in the PSPr subjects was three times greater than the amount of PrP captured in nonprion disease control subjects (data not shown), but it was nearly 16 times less than the g5p-captured PrP in typical sCJD. As measured by densitometry in arbitrary units, the mean PrP captured by g5p in eight of PSPr subjects was 3.44 ± 2.8% of the total PrP detected by direct gel loading compared with 53.55 ± 24.6% in sCJD ($n = 3$; $p = 0.00015$; Fig 5A). Furthermore, although nearly 90% of the g5p-captured PrP was resistant to PK digestion in sCJD, the PrPr accounted for only 24% of the total abnormal PrP captured in the PSPr subjects (87.59 ± 26.8% in four sCJD cases vs 24.23 ± 14.9% in nine PSPr cases; $p = 0.0001$) (see Fig 5A). The PK-resistant PrP obtained after PrP enrichment from the subjects was distributed in three major bands of approximately 26, 20, and 6kDa, which were detected by both 3F4 and 1E4, and matched the major bands of the immunoblot ladder detected with 1E4 on direct loading (see Fig 5A; also data not shown). A similar PrP banding pattern was obtained after sodium phosphotungstate precipitation, another method of abnormal PrP enrichment.[11,26] It was detected by both 3F4 and 1E4, al-

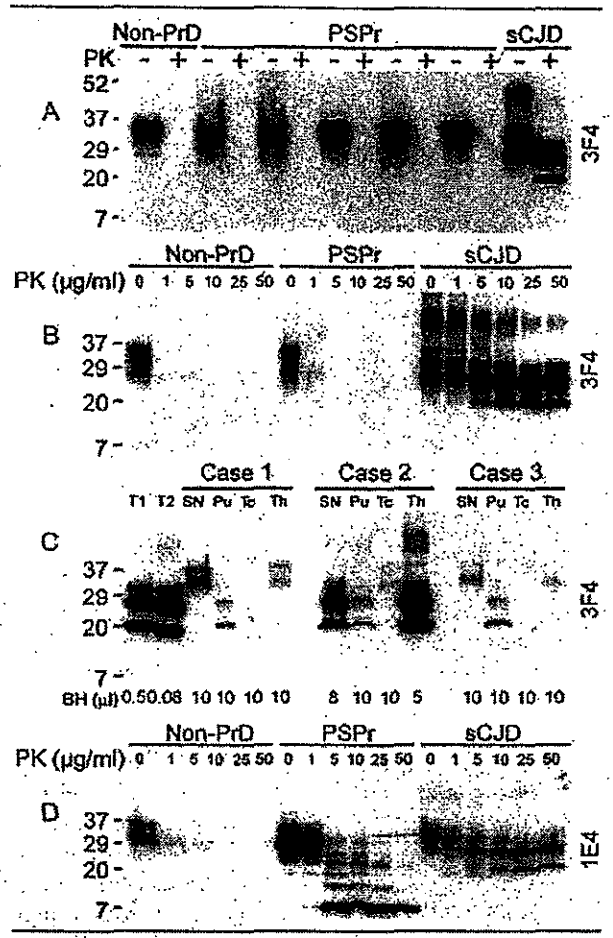


Fig 4. Characterization of prion protein (PrP) in protease-sensitive prionopathy (PSPr). (A) On conventional immunoblots, proteinase K (PK)–resistant PrP is undetectable in nonprion disease control subjects (non-PrD) and PSPr patients, although it is prominent in sporadic Creutzfeldt–Jakob disease (sCJD). (B) PK-resistant PrP from non-PrD and PSPr is not detectable even after treatment with low PK concentrations, but only in sCJD control when probed with the monoclonal antibody 3F4. (C) Subcortical regions of three PSPr cases treated with PK at 50μg/ml before Western blot analysis with 3F4 showed various amounts of PK-resistant PrP in three PSPr cases. Samples from temporal cortex (Tc) were used as controls. (D) When the same samples used in (B) are probed with 1E4, moderately PK-resistant PrP fragments forming a ladder are observed. (A, B, D) Tissues are from the frontal cortex. BH = brain homogenate; Pu = putamen; SN = substantia nigra; T1 = PrPr type 1 control; T2 = PrPr type 2 control; Th = thalamus.

though the bands were much more prominent when probed with 1E4 (see Fig 5B). The abnormal PrP enrichment experiments confirm that, in PSPr patients, there is much less abnormal PrP than in sCJD, and that the proportion of abnormal PrP that is PK resistant is much smaller.

Prion Protein Sedimentation in Sucrose Gradients

After sucrose gradient sedimentation, 30% of the total PrP from the PSPr patients was recovered in fractions 7 to 11 containing large aggregates, whereas these fractions accounted for only 5% of the total PrP in nonprion disease subjects (Figs 6A, B, E). The same fractions contained about 24 and 58% of the total PrP in GSS patients with the A117V mutation and sCJDVV1, respectively (see Figs 6C–E). Also, the percentages of PrP recovered in fractions 2 and 3 differed significantly between PSPr and nonprion disease. PSPr differed from GSS in fractions 7 and 8, and from

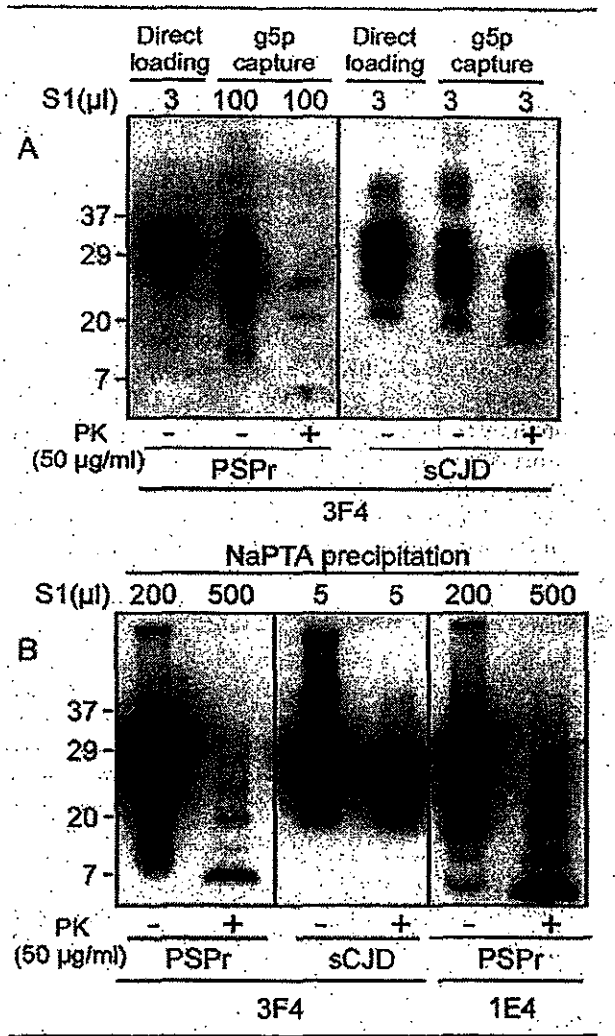


Fig 5. Capture by g5p (A) and sodium phosphotungstate (NaPTA) (B) of prion protein (PrP) from protease-sensitive prionopathy (PSPr) and sCJDMM1 (sporadic Creutzfeldt–Jakob disease). Probing with 3F4 or 1E4 after stripping. The same ladder of proteinase K (PK)–resistant PrP as in Figure 4D is detectable in PSPr preparations after heavy loading of the gel. S1 = supernatant of brain homogenate obtained after low-speed centrifugation (1,000g for 10 minutes).

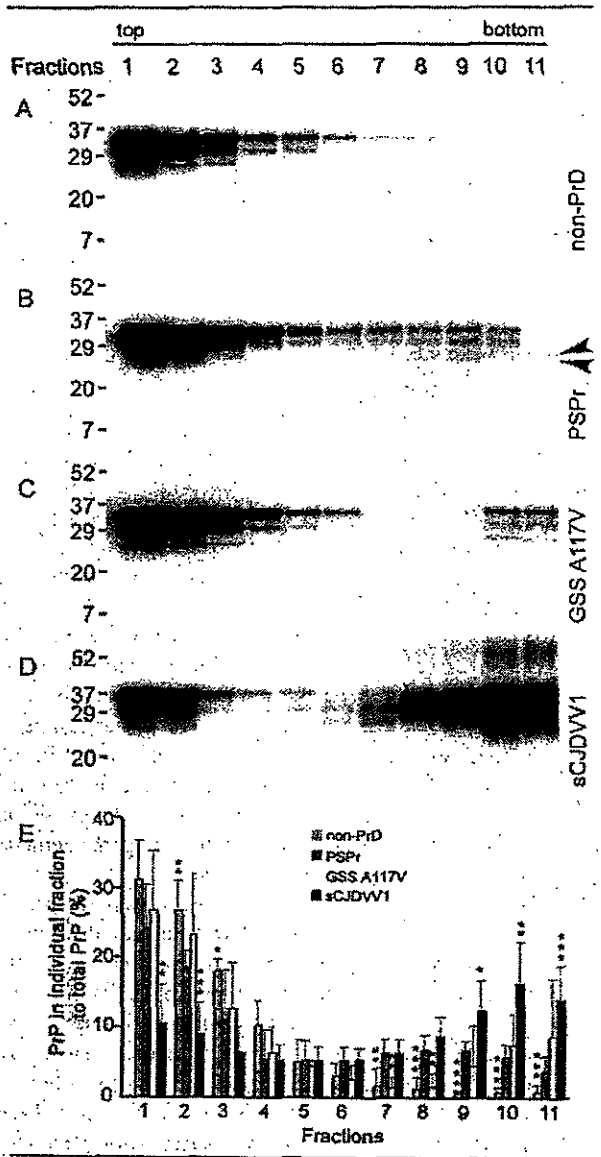


Fig 6. Prion protein (PrP) profiles in sucrose gradient sedimentation. (A) Nonprion disease (non-PrD); (B) protease-sensitive prionopathy (PSPr); (C) Gerstmann–Sträussler–Scheinker disease (GSS) with the A117V mutation (GSSA117V); (D) sCJDVV1; (E) PrP distribution in the fractions plotted as percentages of the total PrP. Although the amounts of PrP from PSPr are similar to those of non-PrD subjects in fractions 1 and 4 to 6, they differ significantly in fractions 2, 3, and 7 to 11, and also clearly differ from GSSA117V in fractions 7 and 8 and sCJDVV1 in fractions 1, 2, and 9 to 11. PSPr fractions 8 to 11 also have a distinctive low double band (B, arrowheads*) not present in the fractions from non-PrD, GSS, and sCJDVV1. n = 6 for non-PrD* (green bars); *n = 6 for PSPr* (red bars); *n = 3 for GSS* (yellow bars); *and n = 7 for sCJDVV1* (blue bars). Vertical bars *refer to standard deviations.* Asterisks *denote PrP fractions from non-PrD, GSS, and sCJDVV1 that by statistical analysis are significantly different from corresponding PSPr fractions.* $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

sCJD in fractions 1, 2, and 9 to 11 (see Fig 6E). In addition to the quantitative differences, the electrophoretic profiles of the high-molecular-weight aggregates from PSPr also differed from those of nonprion disease, GSS, and sCJDVV1 subjects: the lower band was double in PSPr but single in other conditions (see Figs 6A–D). Comparable data were obtained after gel filtration fractionation, which demonstrated that PrP aggregates exceeding 2,000kDa were more abundant in PSPr than in nonprion disease control subjects but much fewer than in sCJD (data not shown).

Discussion

We report 11 patients affected by a disease that involves abnormal PrP and has homogeneous and distinctive features (Table 2). Based on several lines of evidence, we argue that these features allow for the separation of this condition from all known forms of human prion disease. First, the abnormal PrP associated with this disease is predominantly, and in several brain regions almost exclusively, sensitive to protease or PrPs, and the PK-resistant PrP isoform or PrPr has a distinctive electrophoretic profile. The high sensitivity to PK and the distinctive electrophoretic profile of the abnormal PrP clearly distinguish these cases from each of the five subtypes of sCJD and from sporadic fatal insomnia (sFI), the known human sporadic prion diseases.[1] For example, compared with sCJDMM1, the most common and typical sCJD,[2] these cases have 16 times less total abnormal PrP, and the fraction of the total abnormal PrP that is PK resistant is nearly 4 times less. Furthermore, the ladder-like electrophoretic profile of the PrPr associated with this condition has not been observed in either sCJD or sFI, all of which instead are characterized by the presence of the well-known PrPr type 1 or 2.[1] When present, the traditional PrPr, commonly called *PrP27-30*, was located in subcortical regions and was of type 1, another combination not observed in sporadic human prion diseases.[1] Second, these cases are also homogeneous as for the PrP coding genotype because they are all homozygous for valine at codon 129 of the PrP gene, the site of a common methionine/valine polymorphism.[28] Valine homozygosity in white individuals is the rarest 129 genotype, being found only in 12% of people.[28] The sCJD subtypes associated with valine homozygosity, sCJDVV1 and sCJDVV2, have been well characterized and differ from these cases phenotypically and for the characteristics of the abnormal PrP.[1] Third, the pattern of PrP immunostaining and the presence of structures with the features of poorly formed plaques that we observed in the cerebellum are to our knowledge unprecedented. Lastly, the clinical presentation and initial course that prominently features relatively slow cognitive deterioration, occasional gait impairment, and incontinence has evoked the diagnoses of normal pressure hydrocepha-

Table 2. **Summary of Protease-Sensitive Prionopathy Common Features**

Mean Age at Onset (range), yr	Mean Duration (range), mo	Clinical Presentation	Histopathology	PrP IHC	Abnormal PrP	Family History	PrP Genetics
62 (48-71)	20 (10-60)[a]	Cognitive decline (8/11)[b] and mood/behavioral changes (7/11)[b]	Minimal spongiform degeneration with vacuoles larger than typical CJD, and minimal astrogliosis	Intense staining with distinct target pattern in cerebral gray matter; and dot pattern in cerebellar molecular layer	Minimal amount of PK-resistant PrP forming a ladder-like pattern on Western blot	Dementia (8/10)[b] Dementia with age at onset < 61 yr (2/4)[b]	Valine homozygosity at codon 129 No mutation in the PrP gene coding region

[a]One patient alive after 23-month duration and one dead 7 months from onset for other causes excluded. [b]Positive cases/total number of cases. PrP = prion protein; IHC = immunohistochemistry; CJD = Creutzfeldt–Jakob disease; PK = proteinase K.

lus, diffuse Lewy body disease, or frontotemporal dementia, whereas prion disease was suspected only at a later stage based on the relatively short duration.

Although these cases can be easily distinguished from sporadic prion diseases, some of their features such as overrepresentation of PrPs and the multiple PK-resistant PrP fragments, have been reported in GSS.[4] However, all cases of GSS reported to date are associated with a mutation in the coding region of the PrP gene or immediately adjacent to it.[4] None of these cases carried such mutation. Moreover, the ladder-like, PK-resistant, PrP fragments observed in our cases are preferentially detected with 1E4 but not with 3F4, which obviously separates these cases from GSS carrying the multiple PK-resistant PrP fragments. In a recent study, we observed that although 1E4 and 3F4 have adjacent epitopes along human PrP residues 97-112, their accessibility to these epitopes is different because of different neighboring N-terminal residues.[29] It is possible that the 1E4 selectively detected PK-resistant PrP fragments have N-terminal starting sites that are different from those of the well-characterized PrPr types 1 and 2. The earlier evidence clearly indicates that this condition differs from GSS, although the possibility that it represents the long-sought sporadic form of GSS remains to be excluded. Six of the 10 patients with obtainable pedigree had a family history of dementia that cannot be ignored, yet none carried a mutation in the PrP gene ORF. Therefore, at least in some cases, a causative mutation may be located outside the ORF of the PrP gene, a condition never observed in human prion diseases.[1]

All these considerations argue that the 11 patients were affected by a novel condition involving the PrP that cannot be classified within the spectrum of currently known human prion diseases. We suggest the designation of PSPr to emphasize a major distinctive feature (see Table 2).

Compared with other human prion diseases, PSPr is not exceedingly rare, because it accounts for about 3% of all sCJD and 16% of all valine homozygous CJD accessioned by the National Prion Disease Pathology Surveillance Center during the same time period as these 11 patients, making PSPr about as common as some of the well-known sporadic prion diseases (such as sCJDMM2, sFI, and sCJDVV1).[2] Furthermore, because the clinical presentation and the duration of PSPr often do not point to the diagnosis of prion disease, some cases of PSPr may currently be classified within the group of non-Alzheimer's dementias and not be investigated further. Should this be the case, PSPr may be more common than this study suggests.

The small amount of PrPr associated with PSPr and the finding that about 76% of the detectable abnormal PrP is PK sensitive not only hinders the diagnosis but also has implications concerning origin, pathogenicity, infectivity, and classification of PSPr.

The discovery of PrPs has opened a new chapter in prion diseases.[11–15] The demonstration that PrPs forms smaller aggregates than the PrPr counterpart,[16] and that apparently it is competent to convert PrP^C to PrPr in vitro, as well as to seed the polymerization of recombinant PrP into amyloid,[17,18] suggests that PrPs shares defining features with PrPr. However, the pathogenetic mechanisms of PrPs in the absence of PrPr and, therefore, the nature of the prion diseases associated with PrPs currently remain conjectural.

Prion diseases associated with PrPs, in the presence of minimal or no PrPr, have been modeled and studied in detail in a variety of transgenic (Tg) mouse lines carrying mouse homologues of human PrP gene mutants or overexpressing PrP^C.[12,30–33] Two Tg mouse models appear relevant to these cases.

In the first model, Tg mice expressing high levels of mouse PrP carrying the P101L mutation, the mouse equivalent of the human P102L mutation associated with a GSS phenotype,[4,34,35] spontaneously developed a neurodegenerative process characterized by SD and prion plaque formation. After inoculation, they transmitted a disease phenotypically similar to P101L-mutated Tg mice but not to wild-type mice. As in our cases, the affected mice had PrPs but no, or minimal amounts of, PrPr, indicating that PrPs can be associated with a prion disease that is under certain condi-

tions transmissible and has a histopathological phenotype displaying general features of prion diseases.[12]

In the second model, Tg mice carrying the P101L mutation were inoculated with brain homogenate from patients affected by a subtype of GSS P102L characterized by the exclusive presence of an approximately 8kDa PK-resistant fragment reminiscent of the approximately 6kDa fragment observed in small amounts in our cases. The inoculated Tg mice remained largely asymptomatic, but at histological examination, they displayed PrP plaques and had minimal amounts of PrPr.[33] They failed to transmit the disease to wild-type mice, but inoculation to P101L-mutated mice resulted in the formation of PrP plaques in the absence of clinical disease.

These mouse models and now our cases raise issues with the definition of prion diseases. Currently, it is unclear whether PSPr is transmissible because time-consuming transmissibility experiments to different lines of Tg mice and in vitro PrP replication are still ongoing. Should PSPr not be transmissible, the question is whether it is a prion disease. A similar question can be raised for GSS, of which to date only one subtype has been shown to be consistently transmissible.[4] The issue is further compounded by the recent evidence that amyloid β, the pathogenic peptide of Alzheimer's disease, has the propensity to replicate after inoculation into susceptible Tg mice in a conformation-dependent fashion reminiscent of prions.[36] These findings appear to blur the once tight association of prion diseases and transmissibility. It may be more practical to apply the label of prion diseases to all conditions in which the PrP is abnormal and appears to play a central role in the pathology, as in all prion diseases known to date and in PSPr.[37] In contrast, one might reserve the qualification of transmissible to those prion diseases that can be transmitted to recipients expressing relatively normal amounts of wild-type PrP.[36]

The finding that several PSPr patients had first-degree relatives diagnosed with dementia necessitates a search for an underlying genetic cause. In AD, the discovery of mutations outside the gene of the amyloid precursor protein (the central protein in AD, as PrP is in prion diseases) has provided a wealth of information regarding pathogenetic mechanisms of AD.[38] Similarly, the discovery of a mutation outside the PrP gene ORF capable of generating a prion disease may greatly expand our understanding of pathogenetic mechanisms and the role of PrP in prion diseases.

Supported by the NIH grants AG14359 and AG08702, Centers for Disease Control and Prevention (CCU 515004), and the Britton Fund to P.G.; NIH Grant NS049173 to C.S.; and the CJD Foundation to W.Q.Z.

Drs J. McGeehan and G. Kneale kindly provided g5p. Drs J. Hedreen, L. S. Honig, C. S. Calder, L. P. Goldstick, and W. Longstreth helped in obtaining the cases. P. Scalzo and D. Kofskey provided skillful histological and immunohistochemical preparations. B. Chakraborty assisted in the preparation of the manuscript and illustrations.

References

1. Gambetti P, Kong Q, Zou WQ, et al. Sporadic and familial CJD: classification and characterisation. Br Med Bull 2003;66: 213–239.
2. 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;46:224–233.
3. Parchi P, Castellani R, Capellari S, et al. Molecular basis of phenotypic variability in sporadic Creutzfeldt-Jakob disease. Ann Neurol 1996;39:767–778.
4. Kong Q, Surewicz WK, Peterson RB, et al. Inherited prion diseases. In: Prusiner SB, ed. Prion biology and disease. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 2004:673–775.
5. Parchi P, Zou WQ, Wang W, et al. Genetic influence on the structural variations of the abnormal prion protein. Proc Natl Acad Sci USA 2000;97:10168–10172.
6. Parchi P, Chen SG, Brown P, et al. Different patterns of truncated prion protein fragments correlate with distinct phenotypes in P102L Gerstmann-Straussler-Scheinker disease. Proc Natl Acad Sci USA 1998;95:8322–8327.
7. Pattison IH, Millson GC. Scrapie produced experimentally in goats with special reference to the clinical syndrome. J Comp Pathol 1961;71:101–108.
8. Bruce ME, Dickinson AG. Biological evidence that scrapie agent has an independent genome. J Gen Virol 1987;68: 79–89.
9. Kimberlin RH, Cole S, Walker CA. Temporary and permanent modifications to a single strain of mouse scrapie on transmission to rats and hamsters. J Gen Virol 1987;68: 1875–1881.
10. Bessen RA, Marsh RF. Distinct PrP properties suggest the molecular basis of strain variation in transmissible mink encephalopathy. J Virol 1994;68:7859–7868.
11. Safar J, Wille H, Itri V, et al. Eight prion strains have PrPSc molecules with different conformations. Nat Med 1998;4: 1157–1165.
12. Tremblay P, Ball HL, Kaneko K, et al. Mutant PrPSc conformers induced by a synthetic peptide and several prion strains. J Virol 2004;78:2088–2099.
13. Zou WQ, Zheng J, Gray DM, et al. Antibody to DNA specifically detects scrapie but not normal prion protein. Proc Natl Acad Sci USA 2004;101:1380–1385.
14. Safar JG, Geschwind MD, Deering C, et al. Diagnosis of human prion disease. Proc Natl Acad Sci USA 2005;102: 3501–3506.
15. Klingeborn M, Wik L, Simonsson M, et al. Characterization of proteinase K-resistant N- and C-terminally truncated PrP in Nor98 atypical scrapie. J Gen Virol 2006;87:1751–1760.
16. Tzaban S, Friedlander G, Schonberger O, et al. Protease-sensitive scrapie prion protein in aggregates of heterogeneous sizes. Biochemistry 2002;41:12868–12875.
17. Pastrana MA, Sajnani G, Onisko B, et al. Isolation and characterization of a proteinase K-sensitive PrPSc fraction. Biochemistry 2006;45:15710–15717.
18. Colby DW, Zhang Q, Wang S, et al. Prion detection by an amyloid seeding assay. Proc Natl Acad Sci USA 2007;104: 20914–20919.

19. Gambetti P. Prion strains in human prion diseases. NeuroPrion Network of Excellence, Prion 2006 Abstracts. Available at: http://www.neuroprion.com. Accessed last on March 20, 2008.
20. Pastore M, Chin SS, Bell KL, et al. Creutzfeldt-Jakob Disease (CJD) with a mutation at codon 148 of prion protein gene: relationship with sporadic CJD. Am J Pathol 2005;167: 1729–1738.
21. Kascsak RJ, Rubenstein R, Merz PA, et al. Mouse polyclonal and monoclonal antibody to scrapie-associated fibril proteins. J Virol 1987;61:3688–3693.
22. Kong Q, Huang S, Zou WQ, et al. Chronic wasting disease of elk: transmissibility to humans examined by transgenic mouse models. J Neurosci 2005;25:7944–7949.
23. Yuan J, Xiao X, McGeehan J, et al. Insoluble aggregates and protease-resistant conformers of prion protein in uninfected human brains. J Biol Chem 2006;281:34848–34858.
24. Kim KS, Wisniewski HM, Wen GY. Comparison of four staining methods on the detection of Neuritic plaques. Acta Neuropathol (Berl) 1989;79:22–27.
25. Hunter E. Practical electron microscopy. New York: Cambridge University Press, 1993.
26. Wadsworth JD, Joiner S, Hill AF, et al. Tissue distribution of protease resistant prion protein in variant Creutzfeldt-Jakob disease using a highly sensitive immunoblotting assay. Lancet 2001;358:171–180.
27. Pan,T, Change B, Wong P, et al. An aggregation-specific enzyme-linked immunosorbent assay: detection of conformational differences between recombinant PrP protein dimers and PrPSc aggregates. J Virol 2005;79:12355–12364.
28. Collinge J, Palmer MS, Dryden AJ. Genetic predisposition to iatrogenic Creeutzfeldt-Jakob disease. Lancet 1991;337:1441–1442.
29. Yuan J, Dong Z, Guo JP, et al. Accessibility of a critical prion protein region involved in strain recognition and its implications for the early detection of prions. Cell Mol Life Sci 2008; 65:631–643.
30. Hegde RS, Mastrianni JA, Scott MR, et al. A transmembrane form of the prion protein in neurodegenerative disease. Science 1998;279:827–834.
31. Hegde RS, Tremblay P, Groth D, et al. Transmissible and genetic prion diseases share a common pathway of neurodegeneration. Nature 1999;402:822–826.
32. Westaway D, DeArmond SJ, Cayetano-Canlas J, et al. Degeneration of skeletal muscle, peripheral nerves, and the central nervous system in transgenic mice overexpressing wild-type prion proteins. Cell 1994;76:117–129.
33. Piccardo P, Manson JC, King D, et al. Accumulation of prion protein in the brain that is not associated with transmissible disease. Proc Natl Acad Sci USA 2007;104:4712–4717.
34. Hsiao K, Baker HF, Crow TJ, et al. Linkage of a prion protein missense variant to Gertmann-Straussler syndrome. Nature 1989;338:342–345.
35. Hsiao KK, Scott M, Foster D, et al. Spontaneous neurodegeneration in transgenic mice with mutant prion protein. Science 1990;250:1509–1510.
36. Meyer-Luehmann M, Coomaraswamy J, Bolmont T, et al. Exogenous induction of cerebral beta-amyloidogenisis is govered by agent and host. Science 2006;313:1781–1784.
37. Zou WQ. Transmissible spongiform encephalopathy and beyond. Available at: www.sciencemag.org/cgi/eletters/308/5727/1420. September 20, 2007.
38. Selkoe DJ, Podlisny MB. Deciphering the genetic basis of Alzheimer's disease. Annu Rev Genomics Hum Genet 2002;3: 67–99.

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9月8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	乾燥濃縮人アンチトロンビンIII		研究報告の公表状況	Cell 2008; 134: 757-768	公表国 アメリカ	
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

（in vitroでのPMCA増幅によりPrP^{Sc}の異常折り畳み構造が種の壁を超えて伝播し感染性プリオンが生成）
プリオンは異常な折り畳み構造のたん白（PrP^{Sc}）のみから構成される今までにない感染性病原体であり、細胞プリオン蛋白（PrP^{C}）にその異常構造を蔓延させることにより疾患が伝播する。プリオンが有する重要な特質はその種の壁であり、種の壁があることによって1つの種のプリオンは限られた数の別の種にしか感染を起こすことができない。ここで我々は、in vitroにおけるPMCA(protein misfolding cyclic amplification）増幅によって、PrP^{Sc}異常折り畳み構造が種の間で伝播し感染性プリオンが生成されることを報告する。マウスPrP^{Sc}と混合させることによって異常折り畳みが起こったハムスターPrP^{C}は、野生型ハムスターに対して感染性を有する新規なプリオンを生成した。同様の結果は、反対の方向でも得られた。PMCA増幅を繰り返すとin vitro産生プリオンの順応が起こるが、そのプロセスは、in vivoでの連続継代の際に観察される株の安定化を暗示させるものであった。我々の結果から、PMCAが種の間の伝播を調査するための価値のあるツールであることが示された。また、種の壁と株の生成がPrPの異常折り畳み構造の蔓延によって決定されることが示唆された。

使用上の注意記載状況・その他参考事項等

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
in vitroでのPMCA増幅によりPrP^{Sc}の異常折り畳み構造が種の壁を超えて伝播し感染性プリオンが生成されるとの報告である。 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。

Cell

Crossing the Species Barrier by PrP^{Sc} Replication In Vitro Generates Unique Infectious Prions

Joaquín Castilla,[1] Dennisse Gonzalez-Romero,[1,4] Paula Saá,[1,2,4] Rodrigo Morales,[1,3] Jorge De Castro,[1] and Claudio Soto[1,*]
[1]Department of Neurology, University of Texas Medical Branch, Galveston, TX 77555, USA
[2]Centro de Biología Molecular, Universidad Autónoma de Madrid, 28049 Madrid, Spain
[3]University of Chile, Santiago, Chile
[4]These authors contributed equally to this work
*Correspondence: clsoto@utmb.edu
DOI 10.1016/j.cell.2008.07.030

SUMMARY

Prions are unconventional infectious agents composed exclusively of misfolded prion protein (PrP^{Sc}), which transmits the disease by propagating its abnormal conformation to the cellular prion protein (PrP^{C}). A key characteristic of prions is their species barrier, by which prions from one species can only infect a limited number of other species. Here, we report the generation of infectious prions by interspecies transmission of PrP^{Sc} misfolding by in vitro PMCA amplification. Hamster PrP^{C} misfolded by mixing with mouse PrP^{Sc} generated unique prions that were infectious to wild-type hamsters, and similar results were obtained in the opposite direction. Successive rounds of PMCA amplification result in adaptation of the in vitro-produced prions, in a process reminiscent of strain stabilization observed upon serial passage in vivo. Our results indicate that PMCA is a valuable tool for the investigation of cross-species transmission and suggest that species barrier and strain generation are determined by the propagation of PrP misfolding.

INTRODUCTION

Prion diseases also known as transmissible spongiform encephalopathies (TSEs) are infectious neurodegenerative diseases affecting the brain of humans and several species of mammals (Collinge, 2001). Creutzfeldt-Jakob disease (CJD) is the most common TSE in humans, and scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle, and chronic wasting disease (CWD) in cervids are the most prevalent prion diseases in animals. Unlike conventional infectious microorganisms, the TSE agent appears to be devoid of genetic material and instead composed exclusively by a misfolded form of the prion protein (PrP^{Sc}) (Prusiner, 1998). PrP^{Sc} has the unprecedented ability to replicate in the body by inducing the misfolding of the cellular form of the prion protein (PrP^{C}).

One of the characteristics of the agent responsible for prion diseases is its ability to infect some species and not others (Hill and Collinge, 2004; Moore et al., 2005). This phenomenon is known as species barrier. Even between close species, the species barrier is manifested as an incomplete attack rate and a prolongation of the time it takes for animals to develop the clinical disease when injected with another species' infectious material (Hill and Collinge, 2004). Primary interspecies transmission is usually not very efficient, and it takes a long time for the prion replication process to reach the point at which full-blown clinical disease appears. After sequential passages, the PrP^{Sc} in the new host adapts, resulting in a shortage of the incubation period and stabilization of the new strain (Hill and Collinge, 2004).

Compelling evidence indicates that the species barrier is largely controlled by the sequence of PrP (Moore et al., 2005). Unfortunately, we cannot predict the degree of a species barrier simply by comparing the prion proteins from two species. The barrier has to be measured by experimental studies in animals. These studies are long and costly, and in the case of the human species barrier, the studies have to be done with experimental models, the validity of which is not absolutely guarantied. Evaluation of the species barrier is of tremendous medical importance for risk assessment and to implement regulatory measures to avoid spreading of diseases (Moore et al., 2005). At this time, the epidemiological evidence suggests that among animal TSEs only cattle BSE has been transmitted to humans, generating a variant form of CJD (vCJD) (Will et al., 1996). It is unlikely that sheep scrapie is a concern for humans, because the disease has been described for centuries and no increased prevalence of human prion diseases has been found in scrapie-endemic areas (Caramelli et al., 2006; Hunter, 1998). However, the appearance of "atypical" strains of scrapie, as well as the known transmission of BSE to sheep, has generated new concerns of human infections with sheep-derived material (Buschmann and Groschup, 2005; Hunter, 2003). Similarly, the possibility that some of the newly identified animal prion diseases, such as CWD, could be transmitted to humans cannot be ruled out at the present time (Williams, 2005; Xie et al., 2005).

Recently, we reported the generation of infectious prions in vitro by amplification of PrP^{Sc} misfolding in the test tube (Castilla et al., 2005). For these experiments, we used a technology termed PMCA (protein misfolding cyclic amplification) that mimics in vitro some of the fundamental steps involved in PrP^{Sc} replication in vivo at an accelerated rate (Saborio et al., 2001). During PMCA, small quantities of PrP^{Sc} are mixed with excess of PrP^{C}, and through a cyclical process involving incubation and sonication, prion propagation occurs in an autocatalytic way. With this procedure, prions can replicate indefinitely in the test tube and, after successive rounds of dilutions followed by PMCA amplification, PrP^{Sc} used to begin the reaction can be eliminated, and only in vitro-generated misfolded protein remains in the sample (Castilla et al., 2005). Inoculation of PMCA-generated prions into wild-type animals resulted in a disease with the same clinical, neuropathological, and biochemical features as the disease produced by brain-derived infectious material (Castilla et al., 2005). The conclusion drawn from these findings is that all of the information required to propagate the infectious properties is enciphered in the structure of PrP^{Sc}. This is further supported by recent studies from Supattapone and coworkers in which infectious prions were generated in vitro by PMCA with purified PrP^{C} and PrP^{Sc} with the sole addition of synthetic polyanions (Deleault et al., 2007).

The goal of this study was to attempt crossing the species barrier in vitro to generate unique infectious prions in a cell-free system. For these studies, we used mice and hamsters, two experimental rodent systems widely employed in TSE studies and for which several prion strains are available (Bruce, 2003; Kimberlin and Walker, 1988). The PrP sequence shows nine differences between these two animal species (Figure 1A). Infectivity studies have shown that there is a large barrier for prion transmission between these species (Kimberlin et al., 1989; Kimberlin and Walker, 1988; Race et al., 2002). Our findings show that incubation of PrP^{C} from one of the species with PrP^{Sc} from the other resulted in new PrP^{Sc} that was infectious to wild-type animals. Interestingly, a detailed examination of the infectious, neuropathological, and biochemical features of the disease that was produced revealed characteristics that were different from other known prion strains. These results indicate that the prions generated in vitro by crossing of the mouse-hamster barrier represent new strains. Strikingly, studies of the infectious characteristics of these newly generated prions after different rounds of PMCA showed that the procedure not only enabled crossing of the species barrier but also resulted in stabilization of the new strain in vitro by successive rounds of amplification. Our findings show that prions can be propagated in vitro across the species barrier, leading to the generation and adaptation of unique prion strains.

RESULTS

Crossing the Mouse-Hamster Species Barrier to Generate New Hamster Prions

To assess whether prions can be generated in vitro across the species barrier, we used hamsters and mice, two widely studied rodent experimental models of TSEs (Bruce, 2003; Kimberlin and Walker, 1988; Morales et al., 2007). A PMCA experiment done with our standard conditions for amplification of mouse RML prions showed no detectable formation of PrP^{Sc} when hamster PrP^{C} was used as a substrate (Figure 1A). Conversely, a robust PrP^{Sc} generation was observed with mouse PrP^{C} substrate. For this experiment, we mixed a 1000-fold dilution of RML PrP^{Sc}

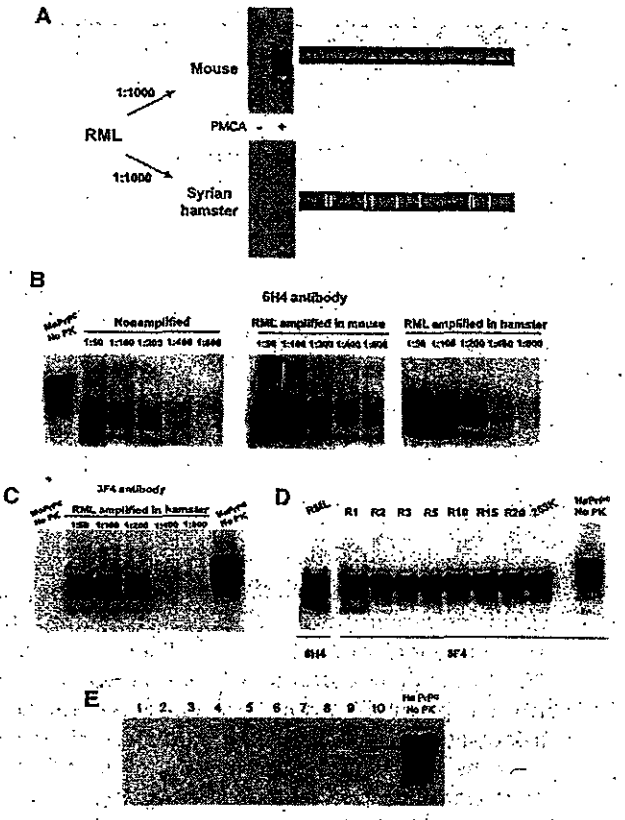


Figure 1. In Vitro Conversion of Hamster PrP^{C} Induced by Mouse RML PrP^{Sc}

(A) RML brain homogenate was diluted 1000-fold into either mouse or hamster normal brain homogenate and subjected to 96 PMCA cycles. The blot shows the results with and without PMCA in each species. At the right side, we show a scheme of PrP indicating the position in which there are amino acid differences between mice and hamsters.

(B) To attempt forcing conversion, we incubated larger quantities (dilutions 1:50 through 1:800) of RML PrP^{Sc} with mouse (central panel) or hamster (right panel) PrP^{C}. All samples (except for the control samples in the left panel labeled "nonamplified") were subjected to 96 PMCA cycles, and PrP^{Sc} signal was detected after PK digestion by western blot with the 6H4 antibody.

(C) The same samples as those in the right panel of (B) were developed with the 3F4 antibody.

(D) The newly generated RML-Ha PrP^{Sc} was serially passed in hamster brain homogenate by a series of 1:10 dilution followed by 48 PMCA cycles. "R" indicates the number of rounds of PMCA; i.e., R5 represent the samples after five serial rounds of PMCA.

(E) For the assessment of spontaneous generation of PrP^{Sc} by PMCA, samples from brain of ten different hamsters were subjected to the same process of serial PMCA as in (D). PrP^{Sc} formation was analyzed by western blot after PK treatment in each PMCA round. The figure shows the results obtained after 20 rounds of PMCA. In the experiments shown in this figure, all samples were treated with PK, except when indicated.

into 10% brain homogenates of healthy hamsters and mice, respectively. We reasoned that if in vivo it takes longer for prions to replicate across species barriers, then in PMCA we should also encounter more difficulties to convert PrP^C when using PrP^{Sc} from a different species. To attempt forcing the in vitro conversion, we added a higher proportion of PrP^{Sc}-containing mouse brain homogenate into the hamster substrate. A range of dilutions from 50- to 800-fold were tested, but the problem with these experiments is that the large concentration of RML PrP^{Sc} used as inoculum makes it difficult to estimate convincingly whether new PrP^{Sc} generation was obtained (Figure 1B). Fortunately, the 3F4 monoclonal antibody can recognize hamster but not mouse PrP (Lund et al., 2007). Using this antibody for western blot, we could clearly observe that protease-resistant hamster PrP^{Sc} was being produced when the reaction was done with low dilutions (from 1:50 to 1:200) of mouse RML PrP^{Sc} (Figure 1C). When the amplification was attempted with 800-fold diluted PrP^{Sc}-containing mouse brain homogenate, only a very faint signal was observed, confirming the results obtained in Figure 1A and the idea that the combination of PrP^C and PrP^{Sc} from different species impairs PMCA efficiency.

Newly generated hamster PrP^{Sc} starting from RML prions was propagated many times in vitro by serial PMCA in order to remove by dilution the initial amount of mouse scrapie brain material added to begin prion replication (Figure 1D). As described before, using this procedure, we can completely remove all molecules of brain-derived PrP^{Sc} from the sample (Castilla et al., 2005). Hamster PrP^{Sc} of RML origin efficiently propagates in vitro at the expense of hamster PrP^C. Interestingly, in the first PMCA round, the glycoform distribution pattern of the in vitro-generated hamster PrP^{Sc} was comparable to the RML profile showing the three glycoform bands (Figure 1D). After further PMCA rounds, this pattern changed to become undistinguishable from PrP^{Sc} associated to the typical hamster strains, such as 263K (Figure 1D) or Hyper (HY), in which the diglycosylated band is highly predominant. This result suggests that the characteristics of the newly generated PrP^{Sc} are being adapted to the new species during successive PMCA cycling, reminiscent of the adaptation process occurring in vivo upon serial passage of the infectious material. After 20 serial rounds of PMCA, representing a dilution equivalent to 10^{-22} with respect to the brain (since the first round contains a 100-fold dilution of the material), our estimation is that no molecules of mouse brain PrP^{Sc} should be present in the sample. This in vitro-generated material was termed RML-Ha PrP^{Sc} to emphasize the RML origin of this new hamster misfolded prion protein. To make sure that newly formed PrP^{Sc} was indeed coming from conversion of hamster PrP^C induced by mouse PrP^{Sc} and not just spontaneous "de novo" formation of PrP^{Sc} in hamsters (Deleault et al., 2007), we did a large experiment to analyze in detail the possibility of spontaneous generation of PrP^{Sc} and infectivity under our experimental conditions. Samples of healthy brain homogenate from ten different hamsters were subjected to serial rounds of PMCA amplification in the absence of PrP^{Sc} seed. After up to 20 serial rounds of PMCA, we did not observe de novo formation of PrP^{Sc} in any of the samples (Figure 1E).

Inoculation of wild-type hamsters with RML-Ha PrP^{Sc} (produced after a 10^{-22} dilution of RML scrapie brain homogenate) produced disease in 100% of the animals by both intracerebral (i.c.) and intraperitoneal (i.p.) routes (Figure 2). The disease exhibits the clinical characteristics typical of hamster scrapie, including hyperactivity, motor impairment, head wobbling, muscle weakness, and weight loss. The incubation time in the first passage was 165 ± 6 days by i.c. inoculation (Figures 2A and 2C). This is longer than the incubation time obtained with hamster scrapie strains, such as 263K and HY, in which a similar quantity of PrP^{Sc} produces disease at around 100 days by this route (Figures 2A and 2C). However, in agreement with our previously reported data (Castilla et al., 2005), when hamster 263K prions were replicated in vitro by PMCA, the newly generated PrP^{Sc} produced disease with a delay similar to that observed with the RML-Ha material (Figures 2A and 2C). The delay in our previous study was eliminated upon a second passage in vivo, in which the new infectious material was stabilized to acquire properties undistinguishable from in vivo-derived 263K (Figures 2B and 2C). Interestingly, in the HY hamster prion strain, PMCA-generated material did not show any statistically significant difference compared to in vivo-produced prions (Figures 2A and 2C). These results suggest that in vitro replication of prions by PMCA maintains the strain characteristics, at least in respect to the incubation periods. To assess the stability of RML-Ha and estimate the stabilized incubation period, we performed a second passage. As shown in Figure 2B, the incubation time of RML-Ha prions was decreased to around 90 days, which is very similar to that obtained with 263K and HY but different from the Drowsy (DY) strain. These results suggest that RML-Ha prions behave similarly to the 263K strain; both in vitro-generated prions show a delay in the first passage that gets corrected upon a second in vivo passage. This feature is not displayed by other hamster prion strains, such as HY, or other species of prions (see below for the results in mice), where PMCA-generated prions exhibited the same incubation period in the first passage as in vivo-produced infectious material. As expected, hamsters inoculated with RML prions did not develop disease during the time of the experiment (>400 days). Animals inoculated with hamster brain homogenate subjected to 20 rounds of PMCA in the absence of PrP^{Sc} (control for the de novo generation of PrP^{Sc}) did not develop disease more than 400 days after inoculation (Figures 2A and 2C). Intraperitoneal inoculations of the infectious material showed a clear difference between the three prion strains used as reference, with 263K being the fastest and DY not producing disease by this route (Figure 2D). The incubation period produced by i.p. inoculation of RML-Ha prions was longer than that of the 263K and HY strains, with an average of 254 days in the first passage. This is also longer than 263K prions amplified in vitro by PMCA, which produced disease after 199 days postinoculation in the first passage (Figures 2D and 2F). A second in vivo passage again stabilized PMCA-generated 263K prions to produce disease at a time indistinguishable from that of brain-derived 263K infectious material. The second passage of RML-Ha prions showed that the stabilized incubation period for the i.p. route was on average around 140 days, which is significantly higher than 263K or 263K-PMCA material but shorter than HY prions (Figures 2E and 2F). The differences remained stable in a third passage (data not shown). These results indicate that in some aspects, RML-Ha prions are similar to the agent in the 263K strain but in other features are intermediate between 263K

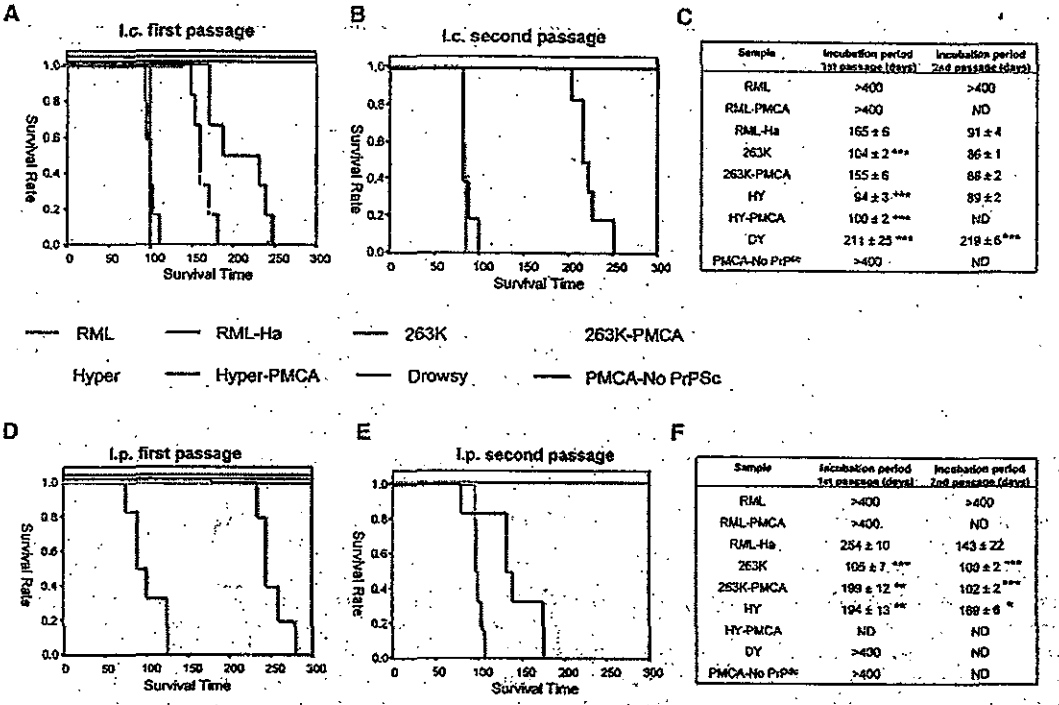


Figure 2. Infectivity of Newly Generated RML-Ha PrP^{Sc} after Crossing the Species Barrier

RML-Ha PrP^{Sc} samples amplified by 20 serial PMCA rounds were inoculated i.c. or i.p. into six wild-type hamsters. For controls, we inoculated similar quantities of PrP^{Sc} from RML or three distinct hamster strains (263K, Hyper, and Drowsy). We also show the data obtained by inoculation of in vitro-generated prions through 20 serial rounds of PMCA by incubation of 263K (263K-PMCA) or Hyper (HY-PMCA) PrP^{Sc} with healthy hamster brain homogenate and RML replicated at expenses of mouse PrP^{C} (RML-PMCA). The figure also show the results obtained by inoculation of the material produced after 20 rounds of PMCA with unseeded normal hamster brain homogenate (PMCA-No PrP^{Sc}). (A) and (D) show the survival curves obtained after i.c. and i.p. inoculation, respectively, of the in vitro-generated RML-Ha after 20 rounds of PMCA. (B) and (E) show the survival curves of the second passage (i.e., animals were inoculated with material obtained from the brain of sick animals in the experiments depicted in [A] and [D]) after i.c and i.p inoculation, respectively. (C) and (F) show the average incubation periods of the experiments done by i.c. and i.p. inoculation of various samples. The values correspond to the average ± standard error. The data was analyzed by ANOVA and the Dunnett multiple comparison post-test. Each set of data was compared to the results obtained with the RML-Ha strain, and significant differences are highlighted with asterisks (* = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$). ND, not done.

and HY prions, providing a first indication that the material obtained by crossing of the mouse-hamster species barrier represents a unique hamster prion strain.

To further assess the characteristics of the disease produced by in vitro-generated RML-Ha prions, we studied in detail the neuropathological and biochemical features of the brain damage. Histopathological studies showed that animals inoculated with RML-Ha prions exhibit the typical brain lesions of scrapie, including spongiform degeneration, astrogliyosis, and PrP^{Sc} deposition (Figures 3A–3C). Quantitative studies of the vacuolation profile in different brain areas showed that RML-Ha-infected hamsters showed the largest extent of spongiosis in medulla and cerebellum and less damage in hippocampus, cortex, and colliculum (Figure 3D). This pattern of brain damage was similar to that observed in 263K-inoculated animals and statistically different from that obtained in hamsters injected with HY and DY (Figure 3D). However, the extent of both astrogliyosis (Figure 3B) and PrP^{Sc} accumulation (Figure 3C) in the medulla of RML-Ha-infected animals was lower than that in 263K-sick animals and similar to that observed in HY-injected hamsters (Figures 3B and 3C). These data suggest again that the RML-Ha prions are a unique strain with properties intermediate between the previously known 263K and HY hamster strains.

Comparative studies of the biochemical characteristics of PrP^{Sc} obtained from the brain of sick animals after inoculation with RML-Ha, 263K, HY, and DY were done by analysis of the electrophoretical pattern of the protein, its susceptibility to proteolytic degradation, and its resistance to denaturation. For comparison of the protease resistance profile, similar quantities of PrP^{Sc} from the new RML-Ha prions and PrP^{Sc} obtained from the brain of sick hamsters inoculated with the prion strains 263K, HY, and DY were treated for 60 min with various concentrations of proteinase K (PK) (Figure 4A). RML-Ha PrP^{Sc} was highly resistant to large PK concentrations. The misfolded protein associated to the newly generated strain was more resistant than HY or DY and similarly (but still significantly more) susceptible to PK digestion than 263K PrP^{Sc} (Figure 4A). The PK concentration in which 50% of the protein was degraded (PK50) was highest for PrP^{Sc} associated to RML-Ha, followed by 263K, HY, DY, and RML (Table S1 available online).

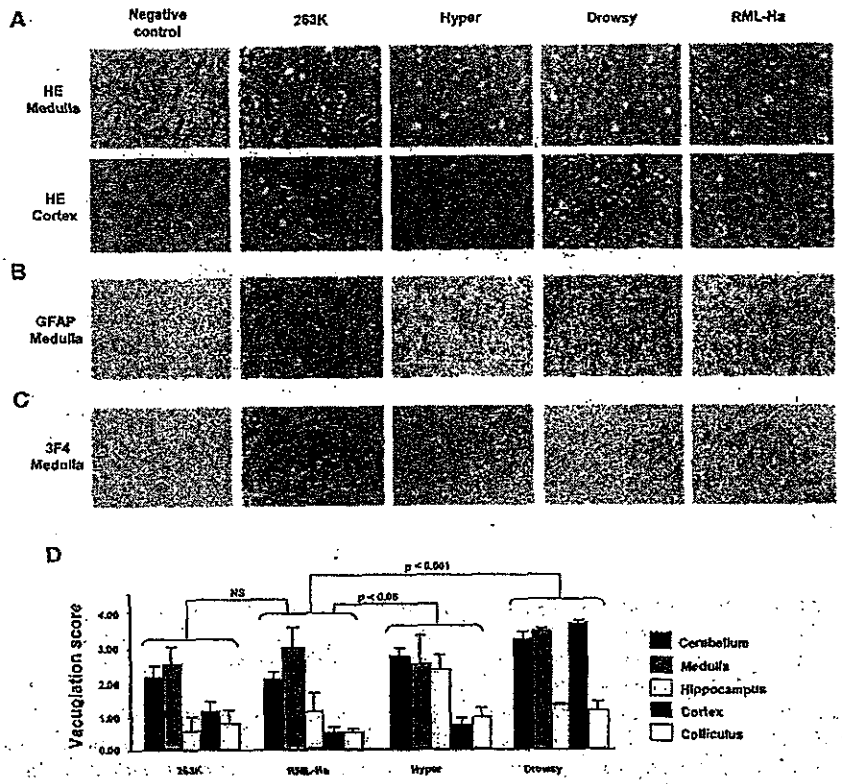


Figure 3. Histopathological Features of the Disease Induced by Inoculation of Hamsters with PMCA-Generated RML-Ha PrP^{Sc}

Brain from sick animals in which disease was produced by inoculation with the in vitro-generated RML-Ha PrP^{Sc} (first passage) or the known hamster strains 263K, Hyper, and Drowsy were analyzed by histological studies. As a control, we used the brain of a hamster inoculated with PBS and sacrificed without disease at 350 days after inoculation.

(A) Spongiform degeneration was evaluated after hematoxilin-eosin staining of medulla and occipital cortex sections and visualized by microscopy at a 40× magnification.

(B) Reactive astrogliosis was evaluated by histological staining with glial fibrillary acidic protein antibody.

(C) PrP accumulation in these animals was evaluated by staining of the tissue with the 3F4 anti-PrP monoclonal antibody.

(D) The vacuolation profile in each brain area was estimated with a semiquantitative scale, as described in the Experimental Procedures. The brain areas used were the following: occipital cortex, cerebellum (mostly white matter), medulla (spinal 5 nucleus, interpolar part), inferior colliculum, and hippocampus (CA1 and CA2 regions). We also included in the analysis brain sections from animals inoculated with the other hamster prion strains. The values represent the average ± standard error of the extent of vacuolation from the five animals analyzed in each set. Statistical analysis by two-way ANOVA with brain regions and prion origin as the variables indicated that differences were highly significant ($p < 0.001$). To assess the significance of the differences between each known prion strain and RML-Ha, we used the Dunnett multiple comparison post-test, and the p values for each combination are shown.

Another characteristic we studied was the electrophoretic mobility and glycosylation pattern of PrP^{Sc} associated to distinct strains. The predominant glycoform for the hamster strains (including the newly generated RML-Ha) is the diglycosylated band, whereas mouse RML PrP^{Sc} shows a more even distribution of the three bands with the main one being the monoglycosylated form. To assess the size of the protein after PK cleavage, we performed endoglycosidase treatment to remove the glycosylated chains (Figure 4B). Whereas PrP^{Sc} associated to the DY strain has a higher electrophoretical mobility, no significant differences were observed among the other proteins. Another biochemical property of misfolded PrP often used to differentiate prion strains is its resistance to chemical denaturation (Safar et al., 1998). Clear differences were observed in the guanidine concentrations required to denature PrP^{Sc} associated to different strains (Figure 4C). The concentration of the chaotropic agent needed to denature 50% of PrP^{Sc} RML-Ha was 1.11 M, substantially different from the 1.69, 1.56, and 1.72 M required for the proteins associated to HY, DY, and RML, respectively (Table S1).

Crossing the Hamster-Mouse Species Barrier to Generate and Stabilize New Mouse Prions

To study the barrier between these rodent species in the opposite direction, we mixed 263K hamster prions with mouse healthy brain homogenate. As before, when a standard PMCA assay was done by dilution of 263K brain homogenate 1000-fold into mouse healthy brain material, we did not see detectable generation of mouse PrP^{Sc} (data not shown). However, when a higher quantity of hamster PrP^{Sc} was added, we were able to generate new mouse PrP^{Sc} (termed 263K-Mo) that could be propagated by serial rounds of PMCA to reach a dilution of the hamster brain homogenate equivalent to 10^{-17} (Figure 5A). Since there are not available antibodies capable of recognizing mouse PrP but not hamster PrP, we could not compare the electrophoretical pattern of PrP^{Sc} generated in the first rounds of PMCA with the profile of PrP^{Sc} typically observed in mouse and hamster strains. However, the western blot pattern of 263K-Mo after 15 rounds of PMCA (when no more molecules of 263K PrP^{Sc} are present) is similar to the one observed for RML and other ovine-derived mouse strains, despite a slightly faster migration (Figure S1A) that will be investigated in more detail later. To assess whether newly generated PrP^{Sc} was indeed coming from conversion of mouse PrP^{C} induced by 263K hamster PrP^{Sc} and not just spontaneous "de novo" formation of PrP^{Sc} in mice, we did an experiment to analyze the possibility of spontaneous generation of PrP^{Sc} and infectivity under our experimental conditions. Samples of healthy brain homogenate from ten different mice were subjected to serial rounds of PMCA amplification in the absence of PrP^{Sc} seed. After up to 20 serial rounds of PMCA, we did not observe de novo formation of PrP^{Sc} in any of the samples (Figure S1B).

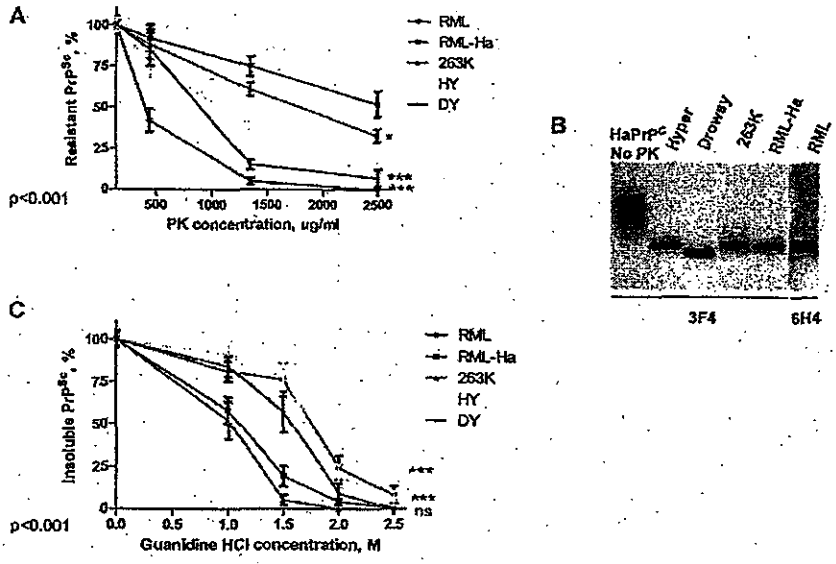


Figure 4. Biochemical Characterization of RML-Ha PrP^{Sc}

Samples from brains of animals inoculated with RML-Ha PrP^{Sc} (first passage in vivo) were used to study the PK resistance profile (A), the relative mobility after deglycosylation and PK treatment (B), and the susceptibility to guanidine denaturation (C). For controls, we used samples from RML or three distinct hamster strains (263K, Hyper, and Drowsy). The results in (A) and (C) correspond to the quantitative evaluation of western blots by densitometric analysis from three independent animals. The data represent the average ± standard error. The data were analyzed by ANOVA and the Dunnett multiple comparison post-test. Each set of data was compared to the results obtained with the RML-Ha strain, and significant differences are highlighted with asterisks (* = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$).

To assess whether mouse PrP^{Sc} generated in vitro from hamster 263K is infectious to wild-type mice and to determine whether the infectious properties are being adapted upon serial PMCA passages, we inoculated several rounds of in vitro-generated material into mice (Figure 5A). Despite the fact that the same amount of PrP^{Sc} was inoculated (as determined by western blot), striking differences in the infectious properties were seen among in vitro-generated prions in distinct rounds of PMCA (Figure 5B). Only two of the six mice inoculated with material produced in the first round of PMCA showed disease symptoms, which appear at a very long time after inoculation (around 500 days) (Figures 5B and 5C). A complete attack rate was observed when animals were inoculated with material produced after three serial rounds of PMCA. However, the incubation period was long (around 310 days on average), and there was a large dispersion among animals (Figures 5B and 5C). The incubation period became stable, short (around 165 days), and there was little dispersion after the six serial rounds of PMCA. These findings indicate that upon successive rounds of PMCA, the newly generated prion, after crossing the species barrier, is becoming adapted and stabilized to the new host, a process very similar to what is seen after several passages in vivo. The large dispersion of incubation times observed in the third round of PMCA suggests that more than one strain has been generated upon crossing of the species barrier and that successive in vitro amplification leads to the selection and cloning of the most efficient of these strains. The incubation time for 263K-Mo after 15 rounds of PMCA (equivalent to a 10^{-17} dilution of the 263K inoculum) was around 165 days, similar to the one produced by scrapie-adapted mouse strains, such as RML, but different from that of the bovine strain 301C (Figure 5D). In vitro replication of the mouse strains RML and 301C at expense of mouse PrP^{C} produced PrP^{Sc} with identical properties as the brain-derived material, reflected as an indistinguishable incubation period (Figure 5D). As expected, mice inoculated with hamster 263K prions did not develop disease during the time of the experiment (>500 days). No disease was also observed in animals inoculated with mouse brain homogenate subjected to 20 rounds of PMCA in the absence of PrP^{Sc}, which corresponds to the control experiment for the de novo generation of PrP^{Sc} (Figure 5D).

To analyze whether the newly generated 263K-Mo infectious material corresponded to a new strain of mouse prions, we studied the histopathological and biochemical features of the brain damage. Animals affected with the disease produced by inoculation of 263K-Mo showed extensive vacuolation in the medulla and hypocampus and moderate but clearly detectable damage in the cerebellum (Figures 6A and 6D). The pattern of spongiform degeneration does not correspond with any of the previously known mouse strains studied and indeed is statistically significantly different to the vacuolation profile produced by RML and 301C prions (Figure 6D). Differences were also detected in the extent of brain inflammation produced by 263K-Mo, since the degree of astrogliosis was less prominent than the one observed in animals inoculated with RML or 301C prions (Figure 6B). The profile of PrP^{Sc} accumulation consisted mostly of diffuse deposition and was not clearly different from the one observed in the other strains (Figure 6C). Then we studied the biochemical characteristics of PrP^{Sc} obtained from the brain of animals infected with 263K-Mo. Electrophoretical migration was assessed after PK digestion and endoglycosidase treatment to remove glycosylation chains. The PK-resistant core of PrP^{Sc} migrated slightly faster than RML but slightly slower than 301C, with an estimated molecular weight of 20 KDa (Figures 7A and 7B). These results indicate that the cleavage site after PK digestion is different from all of the currently known mouse strains. This is important because it is thought that differences in the PK cleavage site reflect disparities in the folding or aggregation of the protein (Chen et al., 2000; Collinge et al., 1996). To further search for biochemical differences, we subjected the protein to proteolytic degradation by using various concentrations of PK. 263K-Mo PrP^{Sc} was much more resistant to PK than to RML (Figure 7C), with a PK_{50} (the PK concentration needed to

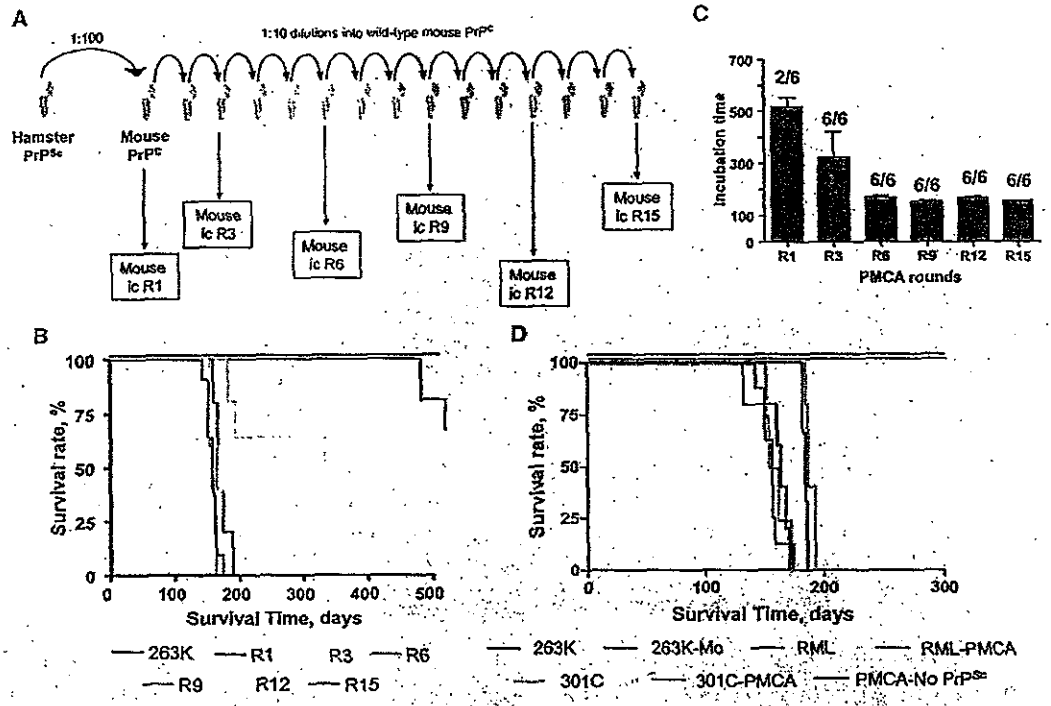


Figure 5. In Vitro Conversion of Mouse PrP^C Induced by Hamster 263K PrP^{Sc} Generates Infectious Prions

(A) Schematic representation of the dilutions done and the PMCA rounds used for our in vivo infectivity experiments.

(B) Survival curve observed after inoculation of six wild-type mice with the material generated after several rounds of PMCA. "R" indicates the number of rounds of PMCA. As a control, the animals were inoculated with 263K hamster prions.

(C) Average and standard error of the incubation times and attack rates observed after inoculation of wild-type mice with the material produced after different rounds of PMCA.

(D) Comparison of survival curves for the stabilized 263K-Mo infectious material (after 15 rounds of PMCA) with those obtained with RML and 301C, two mouse strains of different origin. We also show the data obtained by inoculation of in vitro-generated prions through 20 serial rounds of PMCA by incubation of RML (RML-PMCA) or 301C (301C-PMCA) PrP^{Sc} with healthy mouse brain homogenate. The figure also show the results obtained by inoculation of the material produced after 20 rounds of PMCA with unseeded normal mouse brain homogenate (PMCA-No PrP^{Sc}), which correspond to the control for de novo generation of prions. For all of these experiments, the material was inoculated i.c. as described in the Experimental Procedures.

degrade half of the protein) of 1450 μg/ml (Figure 7D), much larger than the values obtained for RML (240 ug/ml) and 301C (430 ug/ml) (Table S2). Interestingly, the high resistance of PrP^{Sc} is typical of the hamster prions (Table S1), and indeed, 263K, the parental strain of the newly generated mouse prions, has a PK_{50} of around 1700 μg/ml.

DISCUSSION

The phenomenon of the species barrier, by which the agent coming from one species can infect only a limited number of other species, is a typical feature of prion diseases. The molecular basis of this process is not well understood, but it is thought to be controlled by the structure and folding of the prion protein (Moore et al., 2005; Vanik et al., 2004). As with the related phenomenon of prion strains, it is difficult to imagine how an infectious agent lacking genetic material and composed by a single protein can encode the structural diversity and specificity required to control strains variability and species selectivity (Soto and Castilla, 2004).

In addition to the intriguing molecular mechanism behind the species barrier, understanding this phenomenon has profound implications for public health. Indeed, one of the scariest medical problems of the last decades has been the emergence of a new and fatal human prion disease (variant CJD) originated by cross-species transmission of BSE from cattle (Will et al., 1996). BSE has not only been transmitted to humans. The extensive use of cow-derived material for feeding other animals led to the generation of new diseases in exotic felines, nonhuman primates, and domestic cats (Doherr, 2003). Worrisomely, the transmission of BSE into these different species could create new prion strains with unique biological and biochemical characteristics and thus a potentially new hazard for human health. More frightening is perhaps the possibility that BSE has been passed into sheep and goats. Studies have already shown that this transmission is possible and actually relatively easy (Foster et al., 1993). The disease produced is clinically similar to scrapie, but since it comes from BSE it has the potential to be infectious to humans. Another concern is CWD, a disorder affecting farm and wild species of cervids (Sigurdson and Aguzzi, 2006; Williams, 2005). The

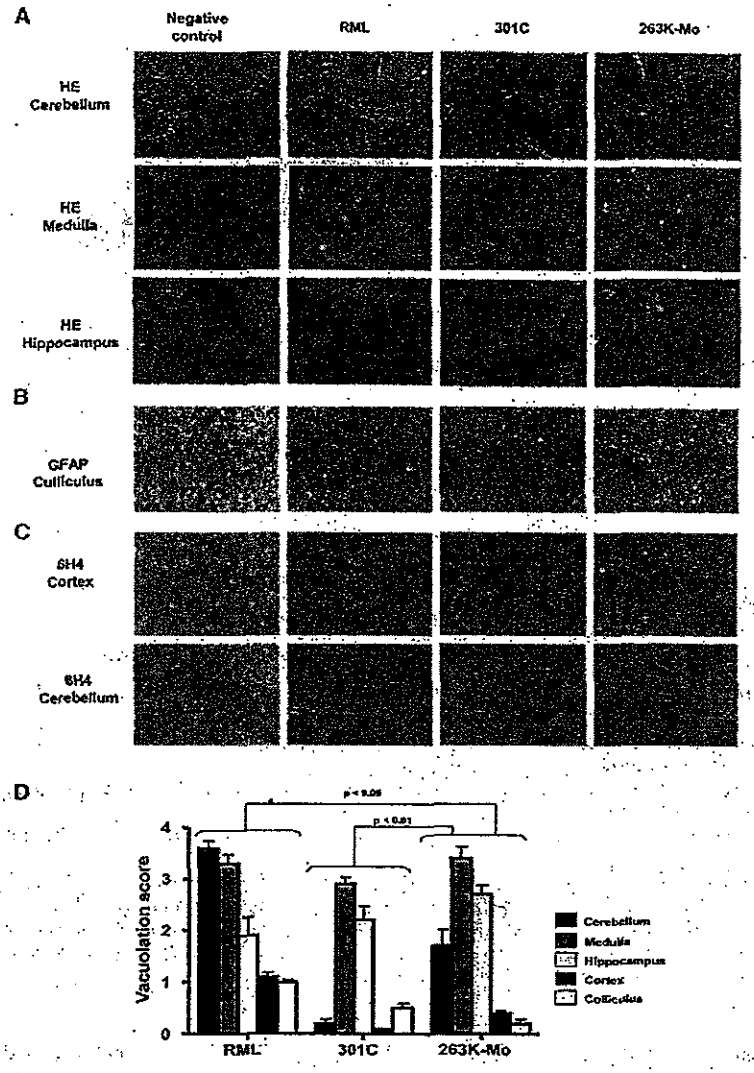


Figure 6. Histopathological Features of the Disease Induced by Inoculation of Mice with PMCA-Generated 263K-Mo PrP^{Sc}

Brains from sick mice in which disease was produced by inoculation with the newly generated 263K-Mo prions after 15 rounds of PMCA (first passage) or the known mouse strains RML and 301C were analyzed by histological studies. As a control, we used brain of a mouse inoculated with PBS and sacrificed without disease at 350 days after inoculation.

(A) Spongiform degeneration was evaluated after hematoxilin-eosin (HE) staining of three different brain areas (cerebellum, medulla, and hippocampus) and was visualized at a 40× magnification.

(B) Reactive astrogliyosis was evaluated in the inferior culliculus by staining with glial fibrillary acidic protein antibody.

(C) PrP accumulation in these animals was evaluated in the occipital cortex and cerebellum by staining of the tissue with the 6H4 antibody.

(D) The vacuolation profile in each brain area was estimated with a semiquantitative scale, as described in the Experimental Procedures. The brain areas used were the following: occipital cortex, cerebellum (mostly white matter), medulla (spinal 5 nucleus, interpolar part), inferior colliculum, and hippocampus (CA1 and CA2 regions). We also included in the analysis brain sections from animals inoculated with RML and 301C. The values represent the average ± standard error of the extent of vacuolation from the five animals analyzed in each set. Statistical analysis by two-way ANOVA with brain regions and prion origin as the variables indicated that differences were highly significant ($p < 0.001$). To assess the significance of the differences between each known prion strain and 263K-Mo, we used the Dunnett multiple comparison post-test, and the p values for each combination are shown.

origin of CWD and its potential to transmit to humans are currently unknown. This is worrisome, considering that CWD has became endemic in some parts of the USA and that the number of cases continues to increase (Williams, 2005). CWD transmissibility studies have been performed in many species in order to predict how this disease could be spread by the consumption of CWD meat (Sigurdson and Aguzzi, 2006). Transmission of CWD to humans cannot be ruled out at present, and a similar infective episode to BSE involving CWD could result in catastrophic consequences.

The exciting scientific problem coupled with the relevant public-health issue prompted us to develop strategies to reproduce the species-barrier phenomenon in the test tube. We reported previously the generation of infectious prions in vitro by cyclic replication of the protein misfolding process featuring the pathogenesis of prion diseases (Castilla et al., 2005). These results were reproduced and extended by other groups to better dissect the elements required for prion replication (Deleault et al., 2007; Weber et al., 2007). The PMCA technology has been adapted to replicate prions from various species (Deleault et al., 2005; Jones et al., 2007; Kurt et al., 2007; Murayama et al., 2007; Safaroff et al., 2005; Soto et al., 2005) and even to use bacterially produced recombinant PrP as substrate (Atarashi et al., 2007). The conclusion drawn from these studies together with the findings reported in this manuscript is that propagation of the PrP^{Sc} misfolding results in formation of infectious material, which maintains the strains and species-barrier properties of the original prions. Qualitatively similar conclusions have been obtained for yeast prions, which are a group of "infectious proteins" that behave as a non-Mendelian genetic element and transmit biological information in the absence of nucleic acid (Wickner et al., 1995). Recent studies showed that bacterially produced N-terminal fragments of the yeast prions Sup35p and Ure2p when transformed into amyloid fibrils were able to propagate the prion phenotype to yeast cells (Brachmann et al., 2005; King and Diaz-Avalos, 2004; Tanaka et al., 2004). Infection of yeast with different conformers led to generation of distinct prion strains in vivo (Brachmann et al., 2005; Tanaka et al., 2004). Remarkably, yeast prions also show the species-barrier phenomenon, and recent data indicate that strain conformation is the critical determinant of cross-species prion transmission (Tanaka et al., 2005).

In the current study, we demonstrate the generation of new infectious prions across the species barrier. For this purpose, we

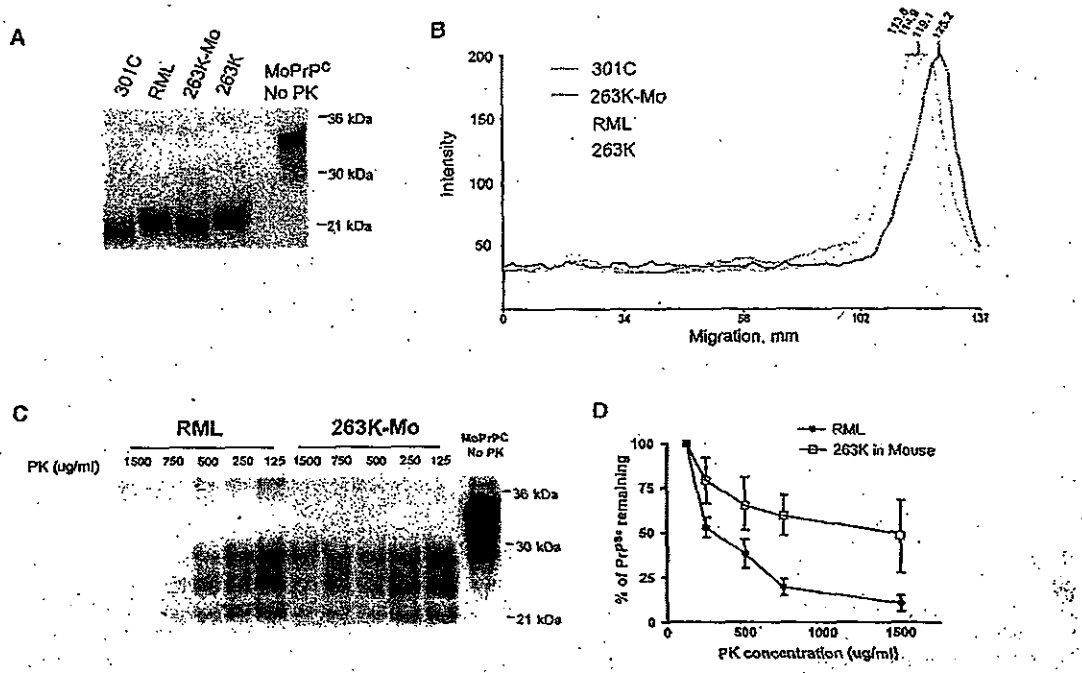


Figure 7. Biochemical Characteristics of 263K-Mo PrP^{Sc}

(A) Samples from brains of mice inoculated with 263K-Mo, RML, or 301C were used to study the electrophoretical migration after deglycosylation and PK treatment.

(B) For assessment of the electrophoretical differences among distinct strains, the blot in (A) was scanned and analyzed by software included in the UVP image analysis system to locate the exact position of the bands.

(C) The PK resistance profile of 263K-Mo PrP^{Sc} was studied and compared with RML.

(D) The results of the experiment shown in (B) were quantitated by densitometric analysis. The data in the figure represent the average ± standard error from three independent animals. The differences were statistically significant as evaluated by one-way ANOVA ($p < 0.01$).

mixed PrP^{Sc} from one species with PrP^{C} from a different animal species and subjected the mixture to serial rounds of PMCA to generate, propagate, and stabilize new prion strains. Hamster PrP^{Sc} generated from mouse RML prions was infectious to wild-type hamsters. Detailed analysis of the disease characteristics and comparison with the illness produced by several known hamster prion strains indicate that the in vitro-generated infectious material across the species barrier corresponds to a new prion strain in hamsters (termed RML-Ha). The main differences of the RML-Ha were on the incubation times after i.p. inoculation, the extremely high resistance to PK degradation, and the pattern of brain damage (Table S1). Similarly, PrP^{Sc} generated by conversion of mouse PrP^{C} with hamster PrP^{Sc} from the 263K strain was shown to be infectious to wild-type mice, with an incubation period comparable to that obtained after inoculation with some of the mouse-adapted scrapie strains, such as RML. Again, the disease produced by the new prions (termed 263K-Mo) was clearly distinguishable from the one produced by some of the currently known mouse prion strains. The major differences were seen in the electrophoretical migration, extremely high resistance to proteolytic degradation, and pattern of brain spongiform degeneration (Table S2). To rule out that newly generated PrP^{Sc} in these experiments was coming from "de novo" spontaneous conversion of PrP^{C} into PrP^{Sc} during PMCA, we used samples of healthy brain homogenate from ten different mice and hamsters that were subjected to serial rounds of PMCA amplification in the absence of PrP^{Sc} seed. After up to 20 serial rounds of PMCA, we did not observe de novo formation of PrP^{Sc} in any of the samples. This material was inoculated into wild-type animals, and no disease was observed more than 400 days after inoculation. These results strongly indicate that the generation of PrP^{Sc} reported in the present study was due to interspecies prion conversion. Nevertheless, we would like to highlight that recently we have been able to generate in vitro PrP^{Sc} de novo without addition of PrP^{Sc} seed (data not shown). However, to reach this aim, the PMCA conditions need to be modified. The modifications include changes on the PMCA parameters (length of incubation and potency of sonication), preincubation, or pretreatment of the normal brain homogenate to induce/stabilize PrP misfolding prior to PMCA. These findings suggest that de novo formation of PrP^{Sc} can be experimentally distinguished from replication of preformed PrP^{Sc}, indicating that the biochemical, conformational, or stability properties of the PrP structures involved in both processes are probably different. Standard PMCA conditions, as those used in the current study, do not result in spontaneous PrP^{Sc} formation.

Interestingly, in our serial PMCA amplifications of RML PrP^{Sc} into hamster PrP^{C}, we observed a progressive change on the western blot profile of the newly generated RML-Ha PrP^{Sc}. Indeed, in the first round of PMCA, the glycoform distribution

pattern was reminiscent of RML and later switched to a profile typical of the hamster strains, characterized by the predomination of the diglycosylated form (Figure 1D). Our interpretation of this result was that consecutive rounds of PMCA may enable the new prion strain to adapt and stabilize. To further study this possibility in our experiments in which mouse prions were generated from 263K hamster prions, we inoculated the material generated after various rounds of PMCA. Strikingly, similar amounts of PrP^{Sc} generated after one and three rounds of PMCA produced disease with incomplete attack rates and/or very long incubation periods (Figures 5B and 5C). Incubation time stabilized after six rounds of serial PMCA, suggesting that at this point the new strain is fully adapted. These findings suggest that PMCA is not only able to reproduce the interspecies transmission of prions but is also able to mimic the strain adaptation process observed in vivo. In vivo adaptation and stabilization of prions generated after crossing the species barrier takes at least four consecutive passages, which requires several years of work (Race et al., 2001, 2002). Conversely, strain adaptation by PMCA takes only 2 or 3 weeks. Importantly, the kinetics of adaptation in vitro and in vivo, as well as the characteristics of the stabilized material, are very similar. Indeed, it has been reported that three serial passages of 263K in mice produce disease in all animals, with an incubation time of around 300 days (Race et al., 2002). This result is very similar to the data obtained with the material generated in vitro after three successive rounds on PMCA replication (Figures 5B and 5C). Moreover, less than three in vivo passages produced an incomplete attack rate, and more than three passages are needed to obtain a stable and low incubation period (Race et al., 2002), which is in the same range of our 263K-Mo infectious material. Finally, similar to our in vitro data, the in vivo cross-species transmission between hamsters and mice also led to the generation of unique prion strains (Race et al., 2001, 2002). Although we are tempted to speculate that each PMCA round has the same effect on strain adaptation as did each in vivo passage, more experiments with other species combinations are needed to reach this conclusion.

In summary, our results show that all elements controlling interspecies transmission of prions are contained in a cell-free system and that new prion strains can be generated, adapted, and stabilized upon crossing the species barrier in vitro by PMCA. These findings provide additional support for the prion hypothesis, suggesting that species-barrier transmission and strain generation are determined by the propagation of PrP misfolding. Furthermore, the data demonstrate that PMCA is a valuable tool for the investigation of the strength of the barrier between diverse species, its molecular determinants, and the expected features of the new infectious material produced. Finally, our findings suggest that the universe of possible prions is not restricted to those currently known but that likely many unique infectious foldings of the prion protein may be produced and that one of the sources for this is cross-species transmission.

EXPERIMENTAL PROCEDURES

Preparation of Tissue Homogenates

Healthy and sick animals were perfused with phosphate-buffered saline (PBS) plus 5 mM ethylenediaminetetraacetic acid (EDTA) before the tissue was harvested. Ten percent brain homogenates (w/v) were prepared in conversion buffer (PBS containing 150 mM NaCl, 1.0% Triton X-100, and the complete cocktail of protease inhibitors from Boehringer Mannheim, Mannheim, Germany). The samples were clarified by a brief, low-speed centrifugation (1500 rpm for 30 s) with an Eppendorf centrifuge (Hamburg, Germany), model 5414.

Serial Replication of Prions In Vitro by PMCA

Aliquots of 10% brain homogenate from clinically sick mice infected with RML or 301C and hamsters infected with 263K, HY, or DY prions were diluted into 10% hamster or mouse healthy brain homogenate. Samples were loaded onto 0.2 ml PCR tubes and positioned on an adaptor placed on the plate holder of a microsonicator (Misonix Model 3000, Farmingdale, NY). Each PMCA cycle consisted of 30 min incubation at 37°C followed by a 20 s pulse of sonication set at potency of 7. Samples were incubated without being shaken immersed in the water of the sonicator bath. After a round of PMCA cycles, a 10 µl aliquot of the amplified material was diluted into 90 µl of more normal brain homogenate, and a new round of PMCA cycles was performed. This procedure was repeated several times to reach the final dilutions indicated in the text. The detailed protocol for PMCA, including reagents, solutions, and troubleshooting, has been published elsewhere (Castilla et al., 2006; Saa et al., 2005).

Proteinase K Degradation Assay

The standard procedure for digestion of PrP^{Sc} consists of subjecting the samples to incubation in the presence of PK (50 µg/ml) for 60 min at 37°C. The digestion was stopped by addition of electrophoresis sample buffer, and the protease-resistant PrP was revealed by western blotting. So that the profile of PK sensitivity for in vitro- and in vivo-generated PrP^{Sc} could be studied, the samples were incubated for 60 min at 37°C with different concentrations of PK ranging from 0 to 2500 µg/ml. The PK_{50} values represent the concentration of PK needed to digest half of the protein, and these values are estimated on the basis of the densitometric analysis of three replicated western blots.

Guanidine Denaturation Assay

Samples were incubated with different concentrations of guanidine hydrochloride for 2 hr at room temperature with shaking. Thereafter, samples were incubated in the presence of 10% sarkosyl for 30 min at 4°C and centrifuged at 100,000 × g for 1 hr in a Biosafe Optima MAX ultracentrifuge (Beckman Coulter, Fullerton, CA). The pellet of the centrifugation was resuspended in conversion buffer and treated with PK as described above. Equivalent aliquots of pellet were analyzed by western blot. The Gdn_{50} value corresponds to the concentration of guanidine hydrochloride required to denaturate 50% of the protein, and these values were estimated on the basis of the densitometric analysis of three replicated western blots.

Protein Deglycosylation Assay

PrP^{Sc} samples were first digested with PK as describe above. After addition of 10% sarkosyl, samples were centrifuged at 100,000 × g for 1 hr at 4°C, supernatant was discarded, and the pellet resuspended in 100 µl of glycoprotein denaturing buffer (New England Biolabs, Beverly, MA) and incubated for 10 min at 100°C. Thereafter, 26 µl of 50 mM sodium phosphate (pH 7.5) containing 1% nonidet P-40 and 3 µl of peptide N-glycosidase F (New England Biolabs, Beverly, MA) were added. Samples were incubated for 2 hr at 37°C, and the reaction was stopped by the addition of electrophoresis buffer and samples were analyzed by western blot.

Western Blot

Proteins were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions, electroblotted into nitrocellulose membrane, and probed with 6H4 (for mouse samples) and 3F4 (for hamster samples) antibodies at a 1:5000 dilution. The immunoreactive bands were visualized by enhanced chemoluminesence assay (Amersham, Piscataway, NJ) with an UVp image analysis system. So that the quantity of PrP^{Sc} in the western blot would be assessed, densitometric analyses were done by triplicate.

PrP^{Sc} Quantification

To inject the same quantity of PrP^{Sc} from each preparation, we compared the samples by western blotting after PK digestion. To obtain a reliable and robust quantification, we ran several different dilutions of the sample in the same gel, to avoid artifacts due to saturation of the signal or to too weak of a signal.

Infectivity Studies

In vivo infectivity studies were done in C57Bl6 female mice or Golden Syrian female hamsters, purchased from Charles river. Animals were 4 to 6 weeks old at the time of inoculation. Anesthesized animals were injected stereotaxically into the right hippocampus with 2 or 4 µl of the mouse or hamster infectious material, respectively. For the i.p. infectivity studies, 100 µl of the sample were injected into the peritoneal cavity. The quantity of infectious material injected corresponds to the plateau portion of the incubation period; therefore, small differences in the amount of infectivity should not change incubation period unless there are strain differences. The onset of clinical disease was measured by scoring of the animals twice a week. For mice, the following scale was used: 1, normal animal; 2, roughcoat on limbs; 3, extensive roughcoat, hunckback, and visible motor abnormalities; 4, urogenital lesions; and 5, terminal stage of the disease in which the animal presented with cachexia and lies in the cage with little movement. For hamsters, the following scoring scale was used: 1, normal animal; 2, mild behavioral abnormalities including hyperactivity and hypersensitivity to noise; 3, moderate behavioral problems including tremor of the head, ataxia, wobbling gait, head bobbing, irritability, and aggressiveness (or lethargy in case of the DY strain); 4, severe behavioral abnormalities including all of the above plus jerks of the head and body and spontaneous backrolls; and 5, terminal stage of the disease in which the animal lies in the cage and is no longer able to stand up. Animals scoring level 4 during two consecutive weeks were considered sick and were sacrificed to avoid excessive pain via exposition to carbonic dioxide. Brains were extracted, the right cerebral hemisphere was frozen and stored at −70°C for biochemical examination of PrP^{Sc} with western blots, and the left hemisphere was used for histology analysis.

Histopathological Studies

Brain tissue was fixed in 10% formaldehyde solution, cut in sections, and embedded in paraffin. Serial sections (6 µm thick) from each block were stained with hematoxylin-eosin, or incubated with monoclonal antibodies recognizing PrP or the glial fibrillary acidic protein, via our previously described protocols (Castilla et al., 2005). Samples were visualized with a Zeiss microscope. The vacuolation profile was estimated by consideration of both number and size of spongiform degeneration in five different brain areas: occipital cortex, cerebellum (mostly white matter), medulla (spinal 5 nucleus, interpolar part), inferior colliculum, and hippocampus (CA1 and CA2 regions). Each analyzed brain area was scored from 0 to 4 according to the extent of vacuolation in slides stained with hematoxilin-eosin and visualized at a 40× magnification. Samples were analyzed blindly by two different persons, and the scores represent the average of the two determinations.

Statistical Analysis

The differences in incubation periods, histopathological profile of brain damage, and biochemical characteristics of PrP^{Sc} were analyzed by ANOVA, followed by the Dunnett Multiple Comparison post-test to estimate the significance of the differences between the newly generated strains and each of the other hamster and mouse prion strains studied. For these studies, the data were analyzed with the GraphPad Instat, version 3.05 software.

SUPPLEMENTAL DATA

Supplemental Data include one figure and two tables and can be found with this article online at http://www.cell.com/cgi/content/full/134/5/757/DC1/.

ACKNOWLEDGMENTS

This research was supported in part by National Institutes of Health grants R01NS049173 and P01AI77774 to C.S. C.S. is a founder of Amprion, Inc., a company focused on the development of a diagnosis for prion diseases through the use of the PMCA technology.

Received: September 18, 2007
Revised: May 8, 2008
Accepted: July 21, 2008
Published: September 4, 2008

REFERENCES

Atarashi, R., Moore, R.A., Sim, V.L., Hughson, A.G., Dorward, D.W., Onwubiko, H.A., Priola, S.A., and Caughey, B. (2007). Ultrasensitive detection of scrapie prion protein using seeded conversion of recombinant prion protein. Nat. Methods *4*, 645–650.

Brachmann, A., Baxa, U., and Wickner, R.B. (2005). Prion generation in vitro: amyloid of Ure2p is infectious. EMBO J. *24*, 3082–3092.

Bruce, M.E. (2003). TSE strain variation. Br. Med. Bull. *66*, 99–108.

Buschmann, A., and Groschup, M.H. (2005). TSE eradication in small ruminants–quo vadis? Berl. Munch. Tierarztl. Wochenschr. *118*, 365–371.

Caramelli, M., Ru, G., Acutis, P., and Forloni, G. (2006). Prion diseases: Current understanding of epidemiology and pathogenesis, and therapeutic advances. CNS Drugs *20*, 15–28.

Castilla, J., Saá, P., Hetz, C., and Soto, C. (2005). In vitro generation of infectious scrapie prions. Cell *121*, 195–206.

Castilla, J., Saa, P., Morales, R., Abid, K., Maundrell, K., and Soto, C. (2006). Protein misfolding cyclic amplification for diagnosis and prion propagation studies. Methods Enzymol. *412*, 3–21.

Chen, S.G., Zou, W., Parchi, P., and Gambetti, P. (2000). PrP(Sc) typing by N-terminal sequencing and mass spectrometry. Arch. Virol. Suppl. 209–216.

Collinge, J. (2001). Prion diseases of humans and animals: Their causes and molecular basis. Annu. Rev. Neurosci. *24*, 519–550.

Collinge, J., Sidle, K.C., Meads, J., Ironside, J., and Hill, A.F. (1996). Molecular analysis of prion strain variation and the aetiology of 'new variant' CJD. Nature *383*, 685–690.

Deleault, N.R., Geoghegan, J.C., Nishina, K., Kascsak, R., Williamson, R.A., and Supattapone, S. (2005). Protease-resistant prion protein amplification reconstituted with partially purified substrates and synthetic polyanions. J. Biol. Chem. *280*, 26873–26879.

Deleault, N.R., Harris, B.T., Rees, J.R., and Supattapone, S. (2007). Formation of native prions from minimal components in vitro. Proc. Natl. Acad. Sci. USA *104*, 9741–9746.

Doherr, M.G. (2003). Bovine spongiform encephalopathy (BSE)–infectious, contagious, zoonotic or production disease? Acta Vet. Scand. Suppl. *98*, 33–42.

Foster, J.D., Hope, J., and Fraser, H. (1993). Transmission of bovine spongiform encephalopathy to sheep and goats. Vet. Rec. *133*, 339–341.

Hill, A.F., and Collinge, J. (2004). Prion strains and species barriers. Contrib. Microbiol. *11*, 33–49.

Hunter, N. (2003). Scrapie and experimental BSE in sheep. Br. Med. Bull. *66*, 171–183.

Hunter, N. (1998). Scrapie. Mol. Biotechnol. *9*, 225–234.

Jones, M., Peden, A.H., Prowse, C.V., Groner, A., Manson, J.C., Turner, M.L., Ironside, J.W., MacGregor, I.R., and Head, M.W. (2007). In vitro amplification and detection of variant Creutzfeldt-Jakob disease PrPSc. J. Pathol. *213*, 21–26.

Kimberlin, R.H., and Walker, C.A. (1988). Pathogenesis of experimental scrapie. Ciba Found. Symp. *135*, 37–62.

Kimberlin, R.H., Walker, C.A., and Fraser, H. (1989). The genomic identity of different strains of mouse scrapie is expressed in hamsters and preserved on reisolation in mice. J. Gen. Virol. *70*, 2017–2025.

King, C.Y., and Diaz-Avalos, R. (2004). Protein-only transmission of three yeast prion strains. Nature *428*, 319–323.

Kurt, T.D., Perrott, M.R., Wilusz, C.J., Wilusz, J., Supattapone, S., Telling, G.C., Zabel, M.D., and Hoover, E.A. (2007). Efficient in vitro amplification of chronic wasting disease PrPRES. J. Virol. *81*, 9605–9608.

Lund, C., Olsen, C.M., Tveit, H., and Tranulis, M.A. (2007). Characterization of the prion protein 3F4 epitope and its use as a molecular tag. J. Neurosci. Methods *165*, 183–190.

Moore, R.A., Vorberg, I., and Priola, S.A. (2005). Species barriers in prion diseases–brief review. Arch. Virol. Suppl. 187–202.

Morales, R., Abid, K., and Soto, C. (2007). The prion strain phenomenon: Molecular basis and unprecedented features. Biochim. Biophys. Acta *1772*, 681–691.

Murayama, Y., Yoshioka, M., Yokoyama, T., Iwamaru, Y., Imamura, M., Masujin, K., Yoshiba, S., and Mohri, S. (2007). Efficient in vitro amplification of a mouse-adapted scrapie prion protein. Neurosci. Lett. *413*, 270–273.

Prusiner, S.B. (1998). Prions. Proc. Natl. Acad. Sci. USA *95*, 13363–13383.

Race, R., Raines, A., Raymond, G.J., Caughey, B., and Chesebro, B. (2001). Long-term subclinical carrier state precedes scrapie replication and adaptation in a resistant species: Analogies to bovine spongiform encephalopathy and variant Creutzfeldt-Jakob disease in humans. J. Virol. *75*, 10106–10112.

Race, R., Meade-White, K., Raines, A., Raymond, G.J., Caughey, B., and Chesebro, B. (2002). Subclinical scrapie infection in a resistant species: Persistence, replication, and adaptation of infectivity during four passages. J. Infect. Dis. *186*, S166–S170.

Saa, P., Castilla, J., and Soto, C. (2005). Cyclic amplification of protein misfolding and aggregation. Methods Mol. Biol. *299*, 53–65.

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.

Safar, J., Wille, H., Itri, V., Groth, D., Serban, H., Torchia, M., Cohen, F.E., and Prusiner, S.B. (1998). Eight prion strains have PrP(Sc) molecules with different conformations. Nat. Med. *4*, 1157–1165.

Sarafoff, N.I., Bieschke, J., Giese, A., Weber, P., Bertsch, U., and Kretzschmar, H.A. (2005). Automated PrPres amplification using indirect sonication. J. Biochem. Biophys. Methods *63*, 213–221.

Sigurdson, C.J., and Aguzzi, A. (2006). Chronic wasting disease. Biochim. Biophys. Acta *1772*, 610–618.

Soto, C., and Castilla, J. (2004). The controversial protein-only hypothesis of prion propagation. Nat. Med. *10*, S63–S67.

Soto, C., Anderes, L., Suardi, S., Cardone, F., Castilla, J., Frossard, M.J., Peano, S., Saá, P., Limido, L., Carbonatto, M., et al. (2005). Pre-symptomatic detection of prions by cyclic amplification of protein misfolding. FEBS Lett. *579*, 638–642.

Tanaka, M., Chien, P., Naber, N., Cooke, R., and Weissman, J.S. (2004). Conformational variations in an infectious protein determine prion strain differences. Nature *428*, 323–328.

Tanaka, M., Chien, P., Yonekura, K., and Weissman, J.S. (2005). Mechanism of cross-species prion transmission: An infectious conformation compatible with two highly divergent yeast prion proteins. Cell *121*, 49–62.

Vanik, D.L., Surewicz, K.A., and Surewicz, W.K. (2004). Molecular basis of barriers for interspecies transmissibility of mammalian prions. Mol. Cell *14*, 139–145.

Weber, P., Giese, A., Piening, N., Mitteregger, G., Thomzig, A., Beekes, M., and Kretzschmar, H.A. (2007). Generation of genuine prion infectivity by serial PMCA. Vet. Microbiol. *123*, 346–357.

Wickner, R.B., Masison, D.C., and Edskes, H.K. (1995). [PSI] and [URE3] as yeast prions. Yeast *11*, 1671–1685.

Will, R.G., Ironside, J.W., Zeidler, M., Cousens, S.N., Estibeiro, K., Alperovitch, A., Poser, S., Pocchiari, M., Hofman, A., and Smith, P.G. (1996). A new variant of Creutzfeldt-Jakob disease in the UK. Lancet *347*, 921–925.

Williams, E.S. (2005). Chronic wasting disease. Vet. Pathol. *42*, 530–549.

Xie, Z., O'Rourke, K.I., Dong, Z., Jenny, A.L., Langenberg, J.A., Belay, E.D., Schonberger, L.B., Petersen, R.B., Zou, W., Kong, Q., et al. (2005). Chronic wasting disease of elk and deer and Creutzfeldt-Jakob disease: Comparative analysis of the scrapie prion protein. J. Biol. Chem. *281*, 4199–4206.

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. 8. 14	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		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)		Gregori L, Kovacs GG, Alexeeva I, Budka H, Rohwer RG. Emerg Infect Dis. 2008 Sep;14(9):1406-12.	米国

研究報告の概要

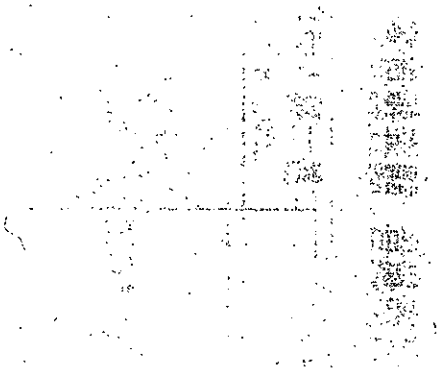
○伝達性海綿状脳症感染性の尿への排出
伝達性海綿状脳症の自然感染経路は未だに仮説段階である。TSE暴露の潜在的要因としての尿を調査するため、高感度検出法とTSE感染性の定量を行った。263Kスクレイピーの臨床症状を呈しているハムスター22匹からプールした尿は、3.8 ± 0.9 感染量/mLの感染性を含んでいた。同じ動物由来の腎臓と膀胱のホモジネートの滴定は2万倍の濃度を示した。これら同じ組織の組織学的、免疫組織化学的分析では、腎臓における疾患関連プリオンタンパク質の散発的な沈着を除いて、炎症あるいは他の病変は見られなかった。尿におけるTSE感染源は未だに解明されていないが、これらの結果は、TSE感染性が尿中に排出されており、その結果自然のTSEの水平感染において何らかの役割を果たしていることを立証している。また、ヒトの尿由来ホルモンや他の医薬品からのTSE伝播の潜在的リスクを示している。

報告企業の意見	今後の対応
263Kスクレイピーの臨床症状を呈しており、腎臓・膀胱に炎症のないハムスター22匹からプールした尿にTSEの感染性がある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

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



RESEARCH

Excretion of Transmissible Spongiform Encephalopathy Infectivity in Urine

Luisa Gregori, Gabor G. Kovacs, Irina Alexeeva, Herbert Budka, and Robert G. Rohwer

The route of transmission of most naturally acquired transmissible spongiform encephalopathy (TSE) infections remains speculative. To investigate urine as a potential source of TSE exposure, we used a sensitive method for detection and quantitation of TSE infectivity. Pooled urine collected from 22 hamsters showing clinical signs of 263K scrapie contained 3.8 ± 0.9 infectious doses/mL of infectivity. Titration of homogenates of kidneys and urinary bladders from the same animals gave concentrations 20,000-fold greater. Histologic and immunohistochemical examination of these same tissues showed no indications of inflammatory or other pathologic changes except for occasional deposits of disease-associated prion protein in kidneys. Although the source of TSE infectivity in urine remains unresolved, these results establish that TSE infectivity is excreted in urine and may thereby play a role in the horizontal transmission of natural TSEs. The results also indicate potential risk for TSE transmission from human urine–derived hormones and other medicines.

Transmissible spongiform encephalopathies (TSEs) are fatal neurologic diseases. In humans, a long asymptomatic incubation period is followed by a progressive clinical course that typically lasts a few months to a year. TSE infectivity and pathologic changes are concentrated in the nervous system; however, much of the transmission risk results from parenteral exposure to the much lower concentrations of infectivity found in tissues outside the nervous system. Thus, despite the very low concentration of TSE infectivity in blood (*1,2*), 4 human cases of transmission of variant Creutzfeldt-Jakob disease through blood transfusions have been documented (*3,4*). If TSE infectivity were excreted, human urine, which is a source of injectible fertility hormones and other drugs (*5,6*), could also pose a risk for transmission. Infected urine might also account for the horizontal transmission of sheep scrapie and might contribute to the natural spread of chronic wasting disease in deer and elk.

Early attempts to transmit Creutzfeldt-Jakob disease by cross-species inoculation of rodents and primates with urine from diseased patients failed (*7,8*). More recent attempts in which urine from infected hamsters was injected back into hamsters have produced variable results (*9,10*). Two other studies have reported infectivity in urine (*11*) and infectivity with disease-specific prion protein (PrP^d) in kidneys of mice with simultaneous scrapie and nephritis but not in those with scrapie alone (*12*). To resolve these discrepancies, we used a highly sensitive and precise method of measuring low concentrations of TSE infectivity, which we have successfully used for quantitation of TSE infectivity in blood (*1,2*), to measure the concentration of TSE infectivity in urine of scrapie-infected hamsters.

Materials and Methods

Urine Collection and Processing

Urine was collected from a cohort of 22 Syrian hamsters (Harlan Sprague-Dawley, Haslet, MI, USA) that had been infected by intracranial injection with 10% (wt/vol) scrapie brain homogenate (263K strain) and from a cohort of 8 age-matched, noninoculated control animals. At the time of urine collection, the scrapie-infected hamsters showed clear clinical evidence of disease but were still able to drink and eat (67–74 days postinoculation). Hamsters were placed 2 at a time for 24 hours in metabolism cages in which they had access to water but not food. Food was

Author affiliations: Veterans Affairs Medical Center, Baltimore, Maryland, USA (L. Gregori, I. Alexeeva, R.G. Rohwer); University of Maryland, Baltimore (L. Gregori, R.G. Rohwer); and Medical University of Vienna, Vienna, Austria (G.G. Kovacs, H. Budka)

DOI: 10.3201/eid1409.080259

withheld to prevent contamination of the urine. Urine was maintained at 4°C during collection. Separate metabolic cages (Rat metabolic cage no. 2100-R; Lab Products, Seaford, DE, USA) were used for each cohort. The urine produced daily was stored at –80°C. The individual collections were then combined into clinical and control pools of ≈60 mL and ≈125 mL, respectively.

Limiting Dilution Titration of Urine

We used the limiting dilution method of titration developed in our laboratory to measure the concentration of TSE infectivity in urine (*1,2*). In this method, a relatively large volume of low-titer sample is injected intracerebrally, 50 µL at a time, into a large cohort of weanling hamsters. Immediately before animal inoculation, aliquots of the clinical and control urine pools were thawed and sonicated on ice with separate sterile ultrasonication probes for each pool. Sonication was for 4 cycles of 15 s on and 10 s off for 1 min of total sonication, using a microtip probe at 40% amplitude (Vibra-Cell 750W; Sonics & Materials, Newtown, CT, USA). Two milliliters of control urine was injected undiluted into 40 hamsters. Clinical urine (urine from hamsters showing clinical signs of disease) was diluted 1:3 with inoculation buffer (phosphate-buffered saline [PBS] supplemented with 1% fetal calf serum and 1× penicillin and streptomycin) to remove concentration-related toxicity. Five milliliters from the clinical urine pool was diluted to 15 mL, and the entire volume was injected into 300 hamsters, 50 µL/animal. Soon after inoculation, 8 animals inoculated with urine from the infected animals died, which left 292 animals in the study. All inoculations were conducted under anesthesia with pentobarbital (40–90 mg/kg). At each step the control urine was processed before the infected urine.

All animals were assessed weekly for early signs of scrapie. At the first signs of disease, animals were separated from their cage mates, observed daily for disease progression, and euthanized after disease was confirmed clinically. After 559 days postinoculation all remaining animals were euthanized. Brains were collected from all animals in the study and assayed for infection-specific, proteinase K–resistant prion protein (PrP^{res}) by Western blot or ELISA, using a dissociation-enhanced lanthanide fluorescent immunoassay (DELFIA) developed in our laboratory (described below). The infection status of each animal was tabulated, and the probabilities of infection and titer were computed as described (*1,2*; Table 1).

Tissue Collection and Processing

Kidneys and urinary bladders were harvested from each of 12 infected animals that donated urine either 71 or 76 days postinoculation. Animals were euthanized by asphyxiation with CO_2. The bladder was removed first and immediately frozen in liquid nitrogen. The kidneys were collected next; the renal capsule was removed before freezing the tissue in liquid nitrogen. Both tissues were dissected aseptically with a clean, sterile set of instruments for each animal and each organ; particular care was taken to not touch other organs or tissues. The tissues (12 bladders and 19 kidneys) were pulverized with a cryomill by using separate cryo-capsules for each tissue (Cryogenic Sample Crusher, Model JFC-300; JAI, Tokyo, Japan). The tissue powder was stored at –80°C until use.

End-Point Dilution Titration of Tissues

Pooled bladder powder (1.65 g) and pooled kidney powder (0.64 g) were separately mixed with homogenization buffer (PBS, pH 7.2) to make 10% (wt/vol) tissue suspensions before sonication at 40% amplitude, using separate sterile microtip probes for each homogenate. The kidney homogenate was prepared according to the same schedule of sonication used for the urine pools. The bladder homogenate was sonicated for 10 s, repeated 2 times (20 s total sonication time) at room temperature. Longer sonication times or delays in the injection of the bladder homogenate caused the sample to solidify, which made it impossible to dilute and inject. Immediately after sonication the homogenates were serially diluted 10-fold in inoculation buffer, and each dilution was injected into hamsters in 1 to 5 cages (4 hamsters/cage) for titration by end-point dilution (Table 2).

All dilutions were by weight. The study was terminated at 426 days postinoculation, and the infection status of each animal was confirmed by Western blot of the brain for PrP^{res}. The titers were calculated by the methods of Reed and Muench (*13*), Pizzi (*14*), and Spearman and Karber (*15*).

PrP^{res} Detection Procedures

Immunoblotting

Individual brains were homogenized in PBS, pH 7.2, to 10% (wt/vol) by using a FASTH homogenizer (Consul AR; Villeneuve, Switzerland) according to the manufacturer's instructions. To test for PrP^{res}, brain homogenate

Table 1. Titer of urine from scrapie-infected hamsters

Hamster	Volume assayed, mL	Fold dilution	Volume inoculated, mL	Total no. hamsters	No. infected hamsters	Titer, ID/mL*	SD†
Infected	4.87	3	14.6	292	18	3.8	0.9
Noninoculated	2	None	2	40	1	–	–

*ID, infectious dose. Titer = –ln(P(0)) × (1/v), where P(0) = (noninfected animals)/(total animals inoculated) and v = inoculation volume, 0.05 mL.
†SD = square root (titer/V), where V = 4.87 mL, the total volume of the undiluted urine inoculated (*1*).

Table 2. End-point dilution titration of urinary bladder and kidney from scrapie-infected hamsters

Dilution	Total/no. infected	
	Bladder	Kidney
10^{-1}	19/19	4/4
$10^{-1.3}$	8/8	20/20
$10^{-1.7}$	8/8	8/8
10^{-2}	4/4	8/8
10^{-3}	4/4	4/3
10^{-4}	4/2	4/1
10^{-5}	4/1	4/0
10^{-6}	4/0	4/0
Titer (log_{10} ID_{50}/g)*	5.5	5.0
Standard error	0.5	0.4

*ID_{50}, 50% infectious dose. Titers calculated by the Reed and Muench method (*13*); standard errors by the Pizzi method (*14*).

was digested with proteinase K at 0.1 mg/mL final concentration as described by Gregori et al. (*1*). Sample buffer containing 2% sodium dodecyl sulfate was added, and the samples were heated at 100°C for 10 min and subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Western blots of the samples were developed by using anti-PrP 3F4 monoclonal antibody (Covance, San Diego, CA, USA) for PrP detection (*1*).

ELISA

After proteinase K digestion and heat denaturation as described for immunoblotting, the samples were diluted 100-fold in assay buffer (DELFIA Assay Buffer; PerkinElmer, Waltham, MA, USA). They were then assayed for PrP concentration by DELFIA by using a Wallac Victor V instrument (PelkinElmer) for signal detection, with purified 3F4 monoclonal antibody (Covance) as the capture antibody and purified 7D9 monoclonal antibody (Covance) labeled with Europium according to the manufacturer's instructions (PerkinElmer) as the detection antibody. The molar ratio of Europium:7D9 antibody was 7.4:1 (*2*).

Histologic and Immunologic Tissue Preparation

Formalin-fixed brains were cut and divided on the midline; 1 hemisphere was cut in the sagittal plane; the other was cut coronally at the anterior basal ganglia, the middle of the thalamus, and the brainstem with cerebellum. Spleens, kidneys, and bladders were divided in the middle. All blocks were embedded in paraffin and processed for conventional staining with hematoxylin and eosin and Luxol fast blue/nuclear fast red (for brain) as well as for immunohistochemical detection of PrP with monoclonal anti-PrP antibody 3F4 (1:1,000; Covance). For detection of PrP^d, sections were pretreated with 30 min of hydrated autoclaving at 121°C followed by 5 min in 96% formic acid. Immunostained sections were counterstained with hematoxylin.

Animal Husbandry and Decontamination Procedures

Animals were maintained in a Biosafety Level 3 (BSL-3) animal facility at the Veterans Affairs Medical Center in Baltimore, Maryland, USA. Standard operating procedures specifically designed for TSEs, including TSE select agents, were followed. The operation of this facility has been described in detail (*16*). Animal cages were changed once a week, and cages and bedding were decontaminated by autoclaving for 1 h at 134°C. The sonicator probes and dissection instruments were decontaminated by autoclaving for 2 h at 134°C immersed in 2 N NaOH, followed by cleaning, repackaging, and sterilizing. All laboratory surfaces were decontaminated before use with either 2 N NaOH or LpH (Steris Corporation, Mentor, OH, USA) (*16*).

Results

Urine Titration

Urine collections from infected and control animals were combined into separate pools. Pools minimized the possibility of an idiosyncratic measurement from an individual and serve as a resource for future experiments once the titer has been determined. Clinically affected animals consumed lower amounts of water and produced 4–5-fold less urine than control animals. This resulted in slightly elevated specific gravity, proteins, glucose, and ketones as measured with a standard urine dipstick. Elevated urine ketones may also have been caused by fasting. The higher concentration of the urine pooled from infected animals resulted in a toxicity that required a 3-fold dilution in buffer before it could be injected.

TSE developed in 18 of the 292 animals that survived the injection of the 3-fold diluted infected pool. Incubation times are shown in Figure 1. As observed in other studies (*1*;*2*), scrapie incubation times for animals infected with low-titer samples begin at ≈150 days and rarely extend past 500 days. None of the animals from either the infected or noninfected cohorts that survived to the end of the experiment were positive by DELFIA. None of the 24 animals that died during incubation without clinical evidence of scrapie were positive for scrapie infection by Western blot. Only those animals with clinical scrapie had the typical PrP^{res} signal in the brain as assessed by Western blot. The infectivity titer of the urine as calculated from the Poisson distribution was 3.8 ± 0.9 infectious doses (ID)/mL (Table 1).

Scrapie developed (at 425 days postinoculation) in 1 of the 40 hamsters inoculated with control urine. Because none of the control donor animals contracted scrapie and because their brains were negative for PrP^{res}, it is clear that this infection resulted from contamination. However, the contamination was unlikely to have been environmental. Our BSL-3 is managed under a strict regimen of continuous decontami-

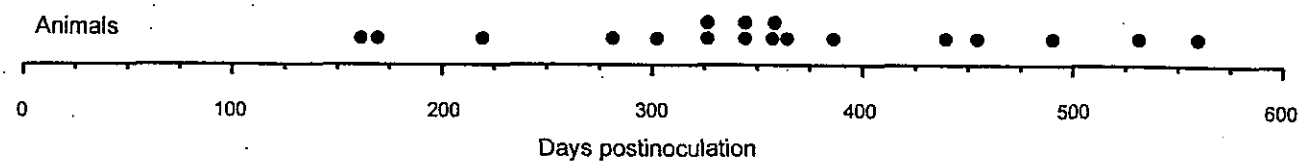


Figure 1. Distribution of incubation times of hamsters infected by injected urine. Each dot represents 1 animal with clinical scrapie that was euthanized at the corresponding day postinoculation. The 22 additional animals that died during the incubation period and the 252 animals that survived to the end of the experiment (559 days) showed no clinical or immunochemical evidence of scrapie and were scored as scrapie negative.

nation and precautionary cleaning (*16*). As evidence of the effectiveness of these measures, we have conducted several titrations, involving hundreds of animals each, in which there were no infections at all during ≥540 days of incubation. One such study was ongoing during the titration of the urine pools reported in this study (*2*). If there are environmental sources of infectivity, the concentration is below the level of detection by the data accumulated in infection-free titrations to date. Instead, after an intensive review of our procedures, we concluded that the most likely source of this contamination was a technical lapse during collection of the urine pools. The level of contamination (1 infection/2 mL of control pool injected vs. 18 infections/4.87 mL of clinical pool injected) is consistent with a pooling error at the time of collection. Nevertheless, had it been an environmental contamination, the associated titer (0.51 ID/mL SD = 0.50 ID/mL) would have had a negligible effect on the value determined for the infected urine.

Tissue Titrations

The concentration of scrapie infectivity in hamster urine is similar to that in plasma of scrapie-infected hamsters at the same stage of disease, which suggests plasma as a possible source of the infectivity. To investigate other possible sources, we also measured the concentration of TSE infectivity in separate pools of kidneys and bladders collected from the same donor animals. The titrations were by the end-point dilution method. The titers calculated by the methods of Reed and Muench (*13*) and Pizzi (*14*) were $10^{5.5\pm0.5}$ 50% infectious doses (ID_{50})/g of bladder and $10^{5.0\pm0.4}$ ID_{50}/g of kidney. The Spearman and Karber method gave almost identical values (*15*).

Histologic and Immunohistochemical Examination of Tissues

Others have reported TSE infectivity in the urine of scrapie-infected mice with nephritis but not in infected mice without nephritis (*11,12*). In contrast, our hamster colony in general, and the animals in this experiment, showed no evidence of inflammation, as indicated by clinical assessments or urine parameters. Nitrates were within normal limits, and no leukocyturia was noted. Proteinuria in the clinical hamsters was likely the consequence of low-volume urine excretion. To further assess whether hamsters infected with scrapie were also affected by kidney inflammation or other abnormalities of the urinary system, we examined the kidneys and the urinary bladders of 8 scrapie-affected hamsters at 84 days postinoculation and 4 preclinically infected hamsters at 49 days postinoculation for PrP^d by immunohistochemical and histologic methods (Figure 2). We also examined control tissues from 10 age-matched uninoculated animals as well as brain and spleen tissues from infected and control animals.

All tissues were evaluated for signs of inflammation and for the pattern of PrP^d immunoreactivity; brains were also examined for spongiform change. No inflammatory changes were found in any tissue examined. In 9 infected animals (clinical and preclinical), we noted nidus formation in the lumina of the bladder with a few neutrophilic granulocytes. However, leukocytes had not invaded the wall of the bladder. Nidus formation is often associated with dehydration.

PrP immunoreactivity was not observed in the bladder wall of scrapie-infected or control animals (data not shown). Spongiform change and deposition of PrP^d was lacking in control animal brains (Figure 2, panel A) and was noted to various extents, according to the stage of the disease, in all scrapie-infected animal brains (Figure 2, panel E). We observed fine synaptic PrP^d immunoreactivity with focal patchy or plaque-like appearance in gray matter structures, but we also noted ependymal, subependymal, perivascular, and white matter PrP^d deposits (data not shown). PrP^d immunoreactivity was observed in the germinal centers of the spleen of all scrapie-infected animals (Figure 2, panel F) but not in those of controls (Figure 2, panel B). None of the control animals exhibited immunoreactivity for PrP^d in the kidneys (Figure 2, panels C, D). PrP^d immunostaining showed fine granular deposits in the collecting tubules of the medulla (Figure 2, panels G, H) in 4 (50%) of 8 animals in the clinical stage of scrapie and in 3 (75%) of 4 animals in the preclinical stage, for a total of 7 (58.3%) of 12 scrapie-infected animals.

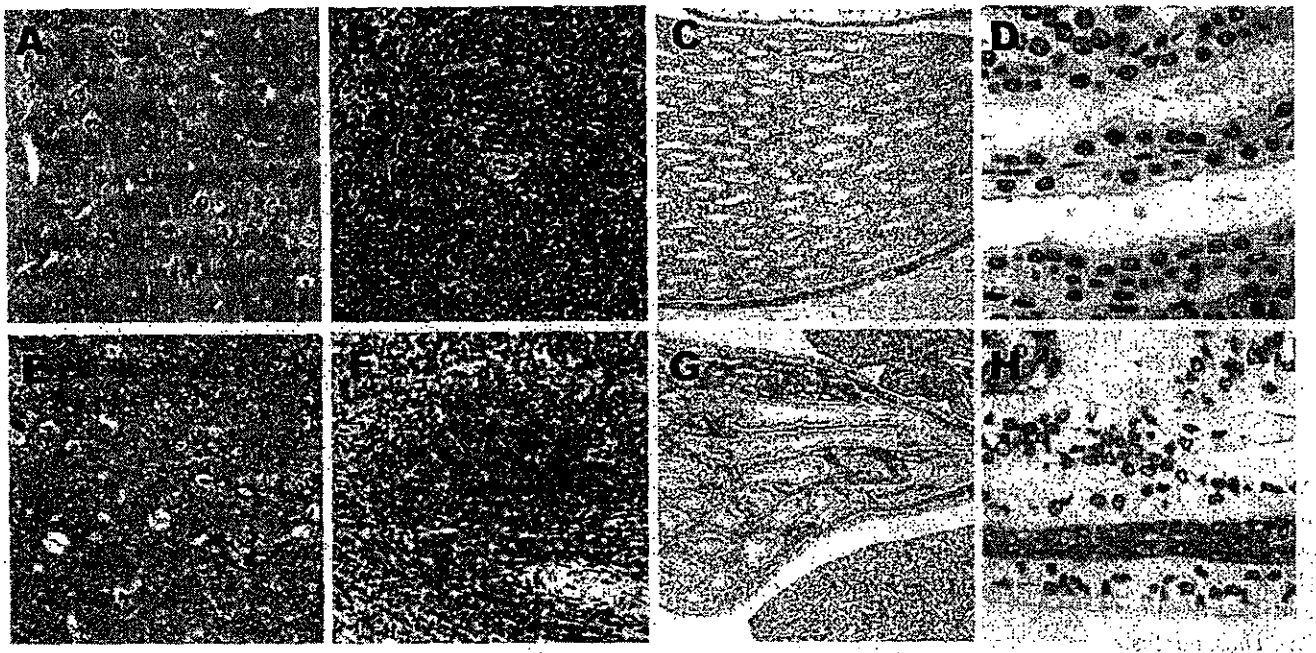


Figure 2. Immunostaining for prion protein (PrP) in control and scrapie-infected hamsters. Deposition of disease-associated PrP is lacking in the brain (A), spleen (B), and kidneys (C,D) of control hamsters. Fine synaptic and plaque-like PrP immunoreactivity in the frontal cortex (E), granular immunoreactivity in the germinal center of spleen (F) and in the collecting tubules of kidneys (G,H) in a representative scrapie-infected animal. Original magnification ×200 for panels A, B, D, E, F, and H and ×40 for panels C and G.

Discussion

Anticipating that the titer of scrapie infectivity in excreted urine would be low, we measured concentration by using limiting dilution titration, a method with which we have extensive experience quantitating TSE infectivity in blood and blood components. In a limiting dilution titration, all animals in the bioassay are inoculated with the highest concentration of inoculum that is tolerated by the intracranial (most efficient) route. Infectivity assorts randomly into the inoculated animals; provided that at least some, but not all, of the animals are infected, the concentration can be calculated from the Poisson distribution of the infections (*1*). The method is highly sensitive and far more precise than other methods of TSE titration. We considered concentrating the urine before bioassay, but to circumvent uncertainties about the recovery of endogenous infectivity, we decided to inject the urine as collected.

We found TSE infectivity in the urine of hamsters that had no evidence of kidney or bladder inflammation. In contrast, Seeger et al. did not detect infectivity in the urine of scrapie-infected mice (*11*) unless the mice were also affected by nephritis, in which case they found low levels of infectivity. Whether the bioassay they used was capable of detecting infectivity at the concentration we observed for hamsters is not clear. If it was not capable, then detection of infectivity in mice with nephritis implies a higher concentration of infectivity in urine excreted by a nephritic kidney. In another study, urine and feces from deer with chronic wasting disease failed to demonstrate infectivity when orally given to the same susceptible species (*17*). Although usually an inefficient route of inoculation, the oral route did successfully transmit chronic wasting disease infectivity in saliva. The authors identified several possible reasons for the unsuccessful transmission by excreta, including incubation time, genotype, or sample size.

In our experiments, cross-contamination by feces can not be excluded as a source of infectivity. Although the metabolism cage effectively separated urine and feces, some contact is possible because of the anatomy of the hamster.

Protein misfolding cyclic amplification uses sonication to generate PrP^{res} and infectivity in vitro. Although we routinely disperse all samples by ultrasonication before injection, our conditions are much harsher than those used to generate PrP^{res} de novo (*18*) and do not support protein misfolding cyclic amplification of PrP^{res}, or presumably infectivity (L. Gregori and R.G. Rohwer, unpub. data).

The kidney and bladder titers were far greater than expected compared with findings of historical studies in which, with only rare exceptions (*19–21*), most attempts at transmission have been unsuccessful. These titers cannot be explained by the infectivity in residual blood (10 ID/mL) (*1,2*). In addition, we observed PrP^d in the kidneys of scrapie-infected animals that had no indications of tissue inflammation. Heikenwalder et al. found PrP^d staining within

follicular infiltrates only in kidneys of mice affected by nephritis and not in control mice with noncomplicated scrapie (*12*). These data together with those by Seeger et al. (*11*) suggested that renal inflammation might be a prerequisite for TSE infectivity in renal tissue and its excretion in urine. In contrast, our results indicate that renal inflammation is not necessary for the deposition of PrP^d in kidneys or for excretion of infectivity. One interpretation is that nephritis enhances the accumulation of PrP^d at sites of inflammation, consistent with the excretion of higher levels of infectivity inferred above for this same condition (*11*).

Two studies of scrapie in naturally and experimentally infected sheep reported PrP^d depositions in the renal papillae (*22*) and in the intraepithelial cortex, medulla, and papillae (*23*). Similar to our findings, both studies indicated that not all scrapie tissues examined were positive for PrP^d. In chronic wasting disease, PrP^d staining was uniquely localized in the ectopic lymphoid follicle of the kidney of a whitetail deer (*24*). All studies indicated either no changes (*22,24*) or mild to no inflammatory changes of the kidney (*23*). Thus, our histologic and immunohistochemical results for scrapie-infected hamsters are consistent with results found for sheep and deer and suggest that under normal conditions TSE diseases do not have concomitant inflammatory changes in the kidney.

That urine titer is similar to that of plasma suggests that urine infectivity may originate from blood (*25*), but how the infectivity would be excreted is not clear. In general, proteins >40 kDa are not excreted and smaller proteins crossing the glomeruli are reabsorbed in the renal tubule and returned to the blood. If TSE infectivity is particulate (>40 kDa), its presence in urine might indicate abnormalities in renal filtration, perhaps related to the accumulation of PrP^d in the collecting tubules of the medulla. The accumulation of immunoglobulins in the urine of TSE-infected hamsters and humans may also indicate malfunction of the urinary system (*9,26*). Excretion of a small C-terminal fragment of the normal cellular form of the prion protein in urine of infected and noninfected animals has been reported (*27*), but PrP^{res} or PrP^d forms can only be inferred from the presence of infectivity. Nevertheless, excretion of proteins similar to PrP^{res} or PrP^d forms has been documented. Follicle-stimulating hormone is a glycosylated protein of 203 amino acids organized mostly as a β-sheet, which bears some remarkable similarities to β-rich forms of the prion protein. Follicle-stimulating and several similar hormones are excreted in urine at great enough concentration to be extracted commercially. Alternatively, TSE infectivity may be excreted by processes analogous to those responsible for the low-level virurias that occur during infections of the nervous system by mumps, measles, and West Nile virus (*28–30*).

To the extent that results from the hamster model can be generalized to other TSE infections (and it has so far proven highly predictive), then even the very low concentrations of infectivity measured here could result in substantial environmental contamination. Several liters of urine and several thousand doses of TSE infectivity may be excreted daily over the course of the illness; even higher titers might be excreted by an animal with nephritis. The high stability of TSE infectivity would account for its persistence in pasture years after infected animals are removed (*31*). Recent studies have shown that infectivity that is adsorbed and immobilized by soil minerals (*32*) can still infect hamsters by oral exposure 29 months later (*33*). Our study also warns of a possible risk from TSE contamination to fertility hormones and other medicinal products extracted from human urine.

Acknowledgments

We thank the BSL-3 animal staff at the Veterans Affairs Medical Center in Baltimore for their excellent animal care.

This work was funded by Department of Defense grant 17-03-1-0756 to R.G.R. and by a National Institutes of Health contract N01-NS-0-2327 for R.G.R. The pathologic investigations were performed within the PrioGen subproject of the European Union Network of Excellence NeuroPrion.

Dr Gregori is deputy director of the Molecular Neurovirology Laboratory in the Veterans Affairs Medical Center in Baltimore and a faculty member of the Department of Neurology at the University of Maryland in Baltimore. Her primary research interest is TSEs, with particular focus on TSE transmission by secondary exposure such as blood transfusion.

References

1. Gregori L, McCombie N, Palmer D, Birch P, Sowemimo-Coker SO, Giulivi A, et al. Effectiveness of leucoreduction for removal of infectivity of transmissible spongiform encephalopathies from blood. Lancet. 2004;364:529–31. DOI: 10.1016/S0140-6736(04)16812-8
2. Gregori L, Gurgel PV, Lathrop JT, Edwardson P, Lambert BC, Carbonell RG, et al. Reduction in infectivity of endogenous transmissible spongiform encephalopathies present in blood by adsorption to selective affinity resins. Lancet. 2006;368:2226–30. DOI: 10.1016/S0140-6736(06)69897-8
3. Hewitt PE, Llewelyn CA, Mackenzie J, Will RG. Creutzfeldt-Jakob disease and blood transfusion: results of the UK Transfusion Medicine Epidemiological Review study. Vox Sang. 2006;91:221–30. DOI: 10.1111/j.1423-0410.2006.00833.x
4. Health Protection Agency. A new case of variant CJD associated with blood transfusion [cited 2008 Jan 2]. Available from http://www.hpa.org.uk/hpa/news/articles/press_releases/2007/070118_vCJD.htm
5. Matorras R, Rodriguez-Escudero FJ. Prions, urinary gonadotrophins and recombinant gonadotrophins. Hum Reprod. 2003;18:1129–30. DOI: 10.1093/humrep/deg176
6. Reichl H, Balen A, Jansen CA. Prion transmission in blood and urine: what are the implications for recombinant and urinary-derived gonadotrophins? Hum Reprod. 2002;17:2501–8. DOI: 10.1093/humrep/17.10.2501
7. Kuroda Y, Gibbs CJ Jr, Amyx HL, Gajdusek DC. Creutzfeldt-Jakob disease in mice: persistent viremia and preferential replication of virus in low-density lymphocytes. Infect Immun. 1983;41:154–61.

8. Gajdusek DC, Gibbs CJ Jr, Alpers M. Transmission and passage of experimenal "kuru" to chimpanzees. Science. 1967;155:212–4.
9. Shaked GM, Shaked Y, Kariv-Inbal Z, Halimi M, Avraham I, Gabizon R. A protease-resistant prion protein isoform is present in urine of animals and humans affected with prion diseases. J Biol Chem. 2001;276:31479–82. DOI: 10.1074/jbc.C100278200
10. Kariv-Inbal Z, Ben-Hur T, Grigoriadis NC, Engelstein R, Gabizon R. Urine from scrapie-infected hamsters comprises low levels of prion infectivity. Neurodegener Dis. 2006;3:123–8. DOI: 10.1159/000094770
11. Seeger H, Heikenwalder M, Zeller N, Kranich J, Schwarz P, Gaspert A, et al. Coincident scrapie infection and nephritis lead to urinary prion excretion. Science. 2005;310:324–6. DOI: 10.1126/science.1118829
12. Heikenwalder M, Zeller N, Seeger H, Prinz M, Klöhn PC, Schwarz P, et al. Chronic lymphocytic inflammation specifies the organ tropism of prions. Science. 2005;307:1107–10. DOI: 10.1126/science.1106460
13. Reed LJ, Muench H. A simple method of estimating fifty per cent endpoints. American Journal of Hygiene. 1938;27:493–7.
14. Pizzi M. Sampling variation of the fifty percent end-point, determined by the Reed-Muench (Behrens) method. Hum Biol. 1950;22:151–90.
15. Spearman C. The method of "right and wrong cases" ("constant stimuli") without Gauss's formulae. British Journal of Psychology 1908;2:227–42.
16. Elliott EJ, MacAuley C, Robins D, Rohwer RG. Working safely with transmissible spongiform encephalopathies. In: Richmond JY, Editor. Anthology of biosafety, vol. 7. Mundelein (IL): American Biological Safety Association; 2005.
17. Mathiason CK, Powers JG, Dahmes SJ, Osborn DA, Miller KV, Warren RJ, et al. Infectious prions in the saliva and blood of deer with chronic wasting disease. Science. 2006;314:133–6. DOI: 10.1126/science.1132661
18. Deleault NR, Harris BT, Rees JR, Supattapone S. Formation of native prions from minimal components in vitro. Proc Natl Acad Sci U S A. 2007;104:9741–6. DOI: 10.1073/pnas.0702662104
19. Hadlow WJ, Race RE, Kennedy RC. Temporal distribution of transmissible mink encephalopathy virus in mink inoculated subcutaneously. J Virol. 1987;61:3235–40.
20. Marsh RF, Burger D, Hanson RP. Transmissible mink encephalopathy: behaviour of the agent in mink. J Infect Dis. 1969;120:713–9.
21. Brown P, Gibbs CJ Jr, Rodgers-Johnson P, Asher DM, Sulima MP, Bacote A, et al. Human spongiform encephalopathy: the National Institutes of Health series of 300 cases of experimentally transmitted disease. Ann Neurol. 1994;35:513–29. DOI: 10.1002/ana.410350504
22. Sisó S, González L, Jeffrey M, Martin S, Chianini F, Steele P. Prion protein in kidneys of scrapie-infected sheep. Vet Rec. 2006;159:327–8.
23. Ligios C, Cancedda GM, Margalith I, Santucciu C, Madau L, Macstrale C, et al. Intraepithelial and interstitial deposition of pathological prion protein in kidneys of scrapie-affected sheep. PLoS One. 2007;2:e859. DOI: 10.1371/journal.pone.0000859
24. Hamir AN, Kunkle RA, Miller J. M, Hall SM. Abnormal prion protein in ectopic lymphoid tissue in a kidney of an asymptomatic white-tailed deer experimentally inoculated with the agent of chronic wasting disease. Vet Pathol. 2006;43:367–9. DOI: 10.1354/vp.43-3-367
25. Brown P, Rohwer RG, Dunstan BC, MacAuley C, Gajdusek DC, Drohan WN. The distribution of infectivity in blood components and plasma derivatives in experimental models of transmissible spongiform encephalopathy. Transfusion. 1998;38:810–6. DOI: 10.1046/j.1537-2995.1998.38998408999.x
26. Head MW, Kouverianou E, Taylor L, Green A, Knight R. Evaluation of urinary PrPSc as a diagnostic test for sporadic, variant, and familial CJD. Neurology. 2005;64:1794–6. DOI: 10.1212/01.WNL.0000161842.68793.8A
27. Narang HK, Dagdanova A, Xie Z, Yang Q, Chen SG. Sensitive detection of prion protein in human urine. Exp Biol Med (Maywood). 2005;230:343–9.
28. Krause CH, Eastick K, Ogilvie MM. Real-time PCR for mumps diagnosis on clinical specimens—comparison with results of conventional methods of virus detection and nested PCR. J Clin Virol. 2006;37:184–9. DOI: 10.1016/j.jcv.2006.07.009
29. Rota PA, Khan AS, Durigon E, Yuran T, Villamarzo YS, Bellini WJ. Detection of measles virus RNA in urine specimens from vaccine recipients. J Clin Microbiol. 1995;33:2485–8.
30. Tonry JH, Brown CB, Cropp CB, Co JK, Bennett SN, Nerurkar VR, et al. West Nile virus detection in urine. Emerg Infect Dis. 2005;11:1294–6.
31. Georgsson G, Sigurdarson S, Brown P. Infectious agent of sheep scrapie may persist in the environment for at least 16 years. J Gen Virol. 2006;87:3737–40. DOI: 10.1099/vir.0.82011-0
32. Johnson CJ, Phillips KE, Schramm PT, McKenzie D, Aiken JM, Pedersen JA. Prions adhere to soil minerals and remain infectious. PLoS Pathog. 2006;2:e32. DOI: 10.1371/journal.ppat.0020032
33. Seidel B, Thomzig A, Buschmann A, Groschup MH, Peters R, Beekes M, et al. Scrapie agent (strain 263K) can transmit disease via the oral route after persistence in soil over years. PLoS One. 2007;2:e435. DOI: 10.1371/journal.pone.0000435

Address for correspondence: Robert G. Rohwer, Veterans Affairs Medical Center, Research Service, 10 North Greene St, Mailstop 151, Baltimore, MD 21201, USA; email: rrohwer@umaryland.edu

EMERGING INFECTIOUS DISEASES *online*

www.cdc.gov/eid

To receive tables of contents of new issues send an email to listserve@cdc.gov with subscribe eid-toc in the body of your message.

Biblioteca Digital

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. 8. 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		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)		Bishop MT, Ritchie DL, Will RG, Ironside JW, Head MW, Thomson V, Bruce M, Manson JC. PLoS ONE. 2008 Aug 6;3(8):e2878.	英国

研究報告の概要

○輸血による二次感染後のvCJD病原体に重大な変化はない
背景:輸血による変異型クロイツフェルトヤコブ病(vCJD)の伝播が同定されたことから、輸血経路による二次伝播後のvCJD病原体に変化があるかを調べることとした。病原性や宿主適応性が増加すれば、vCJDの重大な二次アウトブレイク発生の可能性に関するリスク分析の再評価が必要である。一般集団にvCJDキャリアがいる可能性が高いため、輸血や汚染された外科用器具などの経路から更に感染する可能性がある。
方法:我々は、野生マウスおよびトランスジェニックマウスに、輸血関連vCJD感染第1号症例由来材料を接種した。
主な知見:輸血関連vCJD感染株の伝播の特性は、ウシ海綿状脳症(BSE)からの伝播に関連したvCJD株と著しい類似を見せている。
結論:ヒトにおいて第2の感染経路を通してBSE病原体が適応することにより、ヒトに対する病原性の毒性が増加し、その後の伝播リスクがより大きくなるという仮説が立てられたが、本稿に示した2匹のマウスモデルのデータからは、vCJDのヒト-ヒト伝播後の病原体の伝播効率に重大な変化はないことが示される。

報告企業の意見	今後の対応
野生マウスおよびトランスジェニックマウスに、輸血関連vCJD感染第1号症例由来材料を接種したところ、輸血による二次感染後のvCJD病原体に重大な変化はないことが明らかになったとの報告である。	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人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

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

OPEN ACCESS Freely available online

PLoS one

No Major Change in vCJD Agent Strain after Secondary Transmission via Blood Transfusion

Matthew T. Bishop[1], Diane L. Ritchie[1], Robert G. Will[1], James W. Ironside[1], Mark W. Head[1], Val Thomson[2], Moira Bruce[2], Jean C. Manson[2]*

1 National CJD Surveillance Unit, University of Edinburgh, Western General Hospital, Edinburgh, United Kingdom, 2 Roslin Institute, Neuropathogenesis Division, University of Edinburgh, Edinburgh, United Kingdom

Abstract

Background: The identification of transmission of variant Creutzfeldt-Jakob disease (vCJD) by blood transfusion has prompted investigation to establish whether there has been any alteration in the vCJD agent following this route of secondary transmission. Any increase in virulence or host adaptation would require a reassessment of the risk analyses relating to the possibility of a significant secondary outbreak of vCJD. Since there are likely to be carriers of the vCJD agent in the general population, there is a potential for further infection by routes such as blood transfusion or contaminated surgical instruments.

Methodology: We inoculated both wild-type and transgenic mice with material from the first case of transfusion associated vCJD infection.

Principal Findings: The strain transmission properties of blood transfusion associated vCJD infection show remarkable similarities to the strain of vCJD associated with transmission from bovine spongiform encephalopathy (BSE).

Conclusions: Although it has been hypothesized that adaptation of the BSE agent through secondary passage in humans may result in a greater risk of onward transmission due to an increased virulence of the agent for humans, our data presented here in two murine models suggest no significant alterations to transmission efficiency of the agent following human-to-human transmission of vCJD.

Citation: Bishop MT, Ritchie DL, Will RG, Ironside JW, Head MW, et al. (2008) No Major Change in vCJD Agent Strain after Secondary Transmission via Blood Transfusion. PLoS ONE 3(8): e2878. doi:10.1371/journal.pone.0002878

Editor: Corinne Ida Lasmezas, The Scripps Research Institute, United States of America

Received May 8, 2008; **Accepted** July 9, 2008; **Published** August 6, 2008

Copyright: © 2008 Bishop et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This study was funded by the UK Department of Health and the Medical Research Council. The sponsors had no role in study design, data analysis, or the decision to submit for publication.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: Jean.Manson@roslin.ed.ac.uk

Introduction

Variant Creutzfeldt-Jakob disease (vCJD) is an acquired form of human transmissible spongiform encephalopathy (TSE) caused by infection by the bovine spongiform encephalopathy (BSE) agent that entered the human food chain in the United Kingdom during the 1980s and early 1990s. [1,2] 164 cases of vCJD have been identified in the United Kingdom and a further 41 cases in other countries worldwide. Annual mortality rates indicate that the vCJD outbreak is now in decline in the UK following a peak in 1999/2000. [3] In 2003 the first case of human-to-human secondary transmission of vCJD via blood transfusion was identified through a collaborative study between the UK National Blood Services, the National CJD Surveillance Unit, and the Office of National Statistics (Transfusion Medicine Epidemiology Review, TMER). [4,5] Statistical analysis showed that the possibility of this case being due to BSE infection was in the order of 1:15,000 to 1:30,000. [4] This patient had received a transfusion of non-leucodepleted red cells that had originated from a donor who 3 years 4 months later developed clinical vCJD. The blood recipient was methionine homozygous at codon 129 of the prion protein (PrP) gene (*PRNP*), the same genotype as all tested vCJD cases. [6]

Two further cases of vCJD linked to blood transfusion, in MM genotype individuals, have subsequently been identified through the TMER study. [7,8] Following the discovery of these cases policy changes were made in relation to blood donation in the UK and elsewhere. In 2004 the UK Blood Service deferred transfusion recipients from acting as blood donors.

A fourth case, of asymptomatic infection following blood transfusion, was described in 2004 and this individual was heterozygous (MV) at codon 129. [9] This case was the first indication that individuals with *PRNP* genotypes other than MM could be infected by the vCJD agent. All three codon 129 genotypes are now thought to be susceptible to vCJD infection following the identification of two VV genotype appendix tissues positive for vCJD associated PrP (PrP^{Sc}) in an anonymous screening study, and the successful transmission of vCJD to 'humanised' transgenic mice of each genotype. [10–12]

The implications of these findings are that a significant number of the UK population may be carriers of vCJD infectivity, that some of the individuals may be donating blood, and that not only

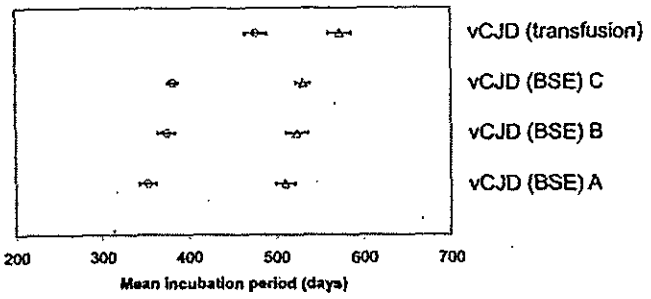


Figure 1. Comparison of incubation periods in wild-type mice. Incubation period plot comparison of vCJD (transfusion) case versus transmissions in wild-type mice of vCJD (BSE) from three sources. (Data shows mean incubation period±standard error of the mean. Open circles RIII line and open triangles VM line.)
doi:10.1371/journal.pone.0002878.g001

those with an MM genotype may be susceptible to infection from this source. Our research in transgenic models indicates that MV and VV individuals are likely to remain in an infectious preclinical state for a significant period of time with incubation periods potentially longer than average lifespan. [12] The identification of four instances of secondary transmission of vCJD infection from a group of 66 individuals known to have received blood products from vCJD donors, including only 28 who survived at least five years post transfusion indicates that blood transfusion is a significant risk factor for vCJD. This is likely to be due to either the route of transmission being more efficient of the agent being more infectious on human-to-human transmission or a combination of both.

TSE transmission by the blood transfusion route has been investigated in a sheep model. [13,14] These studies used intravenous (i.v.) transfusion of whole blood and blood fractions from clinical and preclinical sheep infected with BSE or scrapie. Preliminary data showed that the i.v. route gave relatively short and consistent incubation periods suggesting an efficient transmission route, with success rates of 60% for sheep infected with BSE and 40–45% for natural scrapie. [14,15]

Strain characterisation using a standard panel of inbred lines of wild-type mice originally demonstrated that BSE and vCJD agents had similar biological properties following transmission. [2,16] Similar work in other murine models has also been undertaken to study other human TSEs (genetic and iatrogenic CJD [17], and sporadic CJD [2]), and has been used to examine emerging TSEs (atypical BSE [18] and chronic wasting disease in deer and elk [19]). [20] The development of transgenic mice expressing human PrP has lead to further dissection of the nature of human TSE strains, including transmission of vCJD to gene targeted human transgenic mice. [12,17,21,22] Extensive data from studies in both wild-type and transgenic models at the Neuropathogenesis Division provide an essential background which will allow us to identify any change in the transmission characteristics of vCJD following secondary transmission. [2,12,23]

To investigate the nature of the transmissible agent following secondary transmission from human-to-human following blood transfusion we have examined the biological properties of brain material from the first case of transfusion-associated vCJD inoculated into panels of both wild-type, and transgenic mice expressing human PrP.

Results

Clinical signs of a TSE in the transgenic mice were rare and occurred after long incubation periods (IP) as found in our previous study. [12] Inoculation of the vCJD (transfusion) case produced one clinically positive HuMM mouse (at 659 days post inoculation), two positive HuMV mice (at 596 and 638 dpi) and no positive HuVV mice. Transmission of the vCJD (transfusion) case to the RIII and VM lines showed extended incubation periods compared to the three vCJD (BSE) cases. However, the hierarchy of incubation periods in the two wild-type lines was identical. (Figure 1 and Table 1) These data also show close similarities to previously published vCJD (BSE) transmission to wild-type mice despite different methodologies. These earlier studies used cerebellar material for the inoculum which was injected by simultaneous intracerebral and intraperitoneal routes. [2,23,24]

The frequency of transgenic mice positive for TSE associated vacuolation was similar between the vCJD (transfusion) case and the published vCJD (BSE) case [12], with positive results in 8/15 HuMM, 0/17 HuMV, and 0/17 HuVV mice and 6/16 HuMM, 1/15 HuMV, and 1/15 HuVV mice respectively. Regional distribution of TSE vacuolation in the brain was assessed through lesion profiling. All wild-type and the HuMM transgenic lines had sufficient positive mice to generate a profile (n≥6 mice). The overall pattern of the lesion profiles was the same in the vCJD (transfusion) and vCJD (BSE) cases for all lines of mice, however,

Table 1. Clinical and pathological assessment of wild-type mice.

Inoculum	Mouse Line	Mice Inoculated[a]	Positive for Clinical TSE Signs	Positive for TSE Vacuolation	Incubation Period (days±SEM)
vCJD(BSE) A	RIII	20	17	17	352.76±9.78
vCJD(BSE) B	RIII	20	18	17	374.35±9.98
vCJD(BSE) C	RIII	21	17	16	381.88±6.07
vCJD (transfusion)	RIII	23	18	18	477.33±12.68
vCJD(BSE) A	VM	22	15	22	510.20±10.97
vCJD(BSE) B	VM	22	20	21	523.75±12.57
vCJD(BSE) C	VM	21	13	18	530.69±8.16
vCJD (transfusion)	VM	22	15	18	572.90±12.96

Wild-type mouse lines RIII and VM, inoculated with vCJD(BSE) and vCJD(transfusion) were assessed clinically and pathologically for signs of TSE and mean incubation periods calculated.
[a]The group of 24 was reduced due to unavailability of some brain material for analysis.
doi:10.1371/journal.pone.0002878.t001

for the former case the VM and HuMM mice scores were lower. (Figure 2)

Immunocytochemical (ICC) detection of disease associated abnormal PrP in paraffin sections was also used as a method of assessing whether mice were transmission positive. There were 13/14 HuMM, 8/17 HuMV, 1/17 HuVV positive mice in the vCJD (transfusion) case, which was similar to the frequency of positives in the published vCJD (BSE) case: 11/15 HuMM, 11/13 HuMV, 1/15 HuVV mice. ICC data can be used to show variation in targeting of abnormal PrP deposition in the brain and variation in the nature of deposits. The ICC pattern in transgenic mice inoculated with the vCJD (transfusion) case matched that reported for vCJD (BSE) [12]. The thalamus was specifically targeted with deposition of abnormal PrP, and for the HuMM mice the hippocampus contained many intensely stained plaques including vCJD transmission associated florid plaques. ICC pattern in wild-type mice also showed similarities between the data sets with abnormal PrP deposition targeted to the thalamus and hippocampus, and large aggregates in the white matter of the corpus callosum. (Figure 3)

Biochemical analysis of disease-associated PrP by Western blot can discriminate between human cases of vCJD and sporadic CJD. [25] In the vCJD (transfusion) case the HuMM mice had a type 2B gel mobility and glycoform ratio identical to that found in vCJD (BSE) transmission to HuMM mice, and in vCJD itself. (Figure 4) Brain tissue from both vCJD (transfusion) [4] and published vCJD (BSE) [26] patients showed the type 2B pattern. The levels of PrP^{Sc} seen in the HuMV and HuVV were too low to allow typing by this standard Western blot method.

Discussion

Secondary passage of vCJD infection via blood transfusion in an MM codon 129 genotype individual results in a clinical disease phenotype and pathological characteristics that are similar to vCJD derived from BSE. [4] In this paper we confirm that the agent strain properties of primary and secondary vCJD cases are similar in transmission studies in transgenic and wild-type mice. Strain characteristics can be assessed by the frequency of clinical signs in recipient animals, the incubation period, neuropathological features, and PrP typing. All these parameters were similar in the transmission studies of primary and secondary vCJD in transgenic mice, indicating that the strain properties of the vCJD agent have not changed significantly following secondary passage in humans.

There were some differences in the results of the transmission studies which deserve further comment. The incubation period in wild-type mice was relatively extended in the vCJD(transfusion) case. However, the hierarchy of incubation periods in different inbred mouse strains was unchanged and the most plausible explanation for these findings is that, rather than implicating a change in agent characteristics, the titre of infectivity was less in the brain sample from the vCJD(transfusion) case. The distribution and degree of vacuolation was identical in the RIII mice. (Figure 2) While the distribution was identical in the VM and HuMM mice the degree of vacuolation intensity was lower for the vCJD(transfusion) case. This variability could be due to the much longer incubation times observed in these lines of mice or due to minor changes of the strain properties.

Preliminary investigation of the individuals diagnosed with vCJD following blood transfusion does not indicate a change in the neuropathological characteristics of vCJD following secondary transmission, although further studies are required to confirm this observation.

The level of infectivity in peripheral tissues in secondary cases of vCJD is unknown, although spleen and a lymph node were PrP positive in the sub-clinical case linked to blood transfusion. Evidence from BSE inoculation of primates indicates similar peripheral distribution of disease associated PrP following either oral or intravenous infection. [27] Further studies are required to assess the anatomical distribution, strain properties and level of infectivity in peripheral tissues in secondary vCJD infection. This may be important for accurate assessment of the public health risks associated with the potential for iatrogenic transmission of vCJD, which are not solely defined by the agent characteristics in brain.

Blood transfusion appears to be a relatively efficient means of secondary transmission of vCJD. To date, there have been four such transmissions in a cohort of 28 individuals who survived at least five years following transfusion of blood derived from individuals incubating vCJD. Despite extensive exposure of the UK population to the BSE agent in the food chain, there have been a relatively limited number of primary cases of vCJD (164 in the UK) and the outbreak has been in decline since 1999/2000. An important question is why there should be a disparity in the apparent efficiency of infection between primary and secondary vCJD. Transmission is generally more efficient within species than between species which may explain this observation. [28,29] Inoculation of wild-type mice with material from primary and secondary BSE passage in macaques showed that the BSE agent retained a characteristic lesion profile even though the second passage incubation period in the macaques was reduced by 50%. [30] This suggests that efficiency of transmission may increase without obvious changes to the agent strain.

Another factor is that the intravenous route of infection is very much more efficient than the oral route, as shown in experimental models. [27,31,32] Results from this study suggest the major factor here is likely to be the route of infection rather than any changes in the strain of agent. Future studies, including those using experimental oral exposure to infectivity in transgenic mice, will further address this issue.

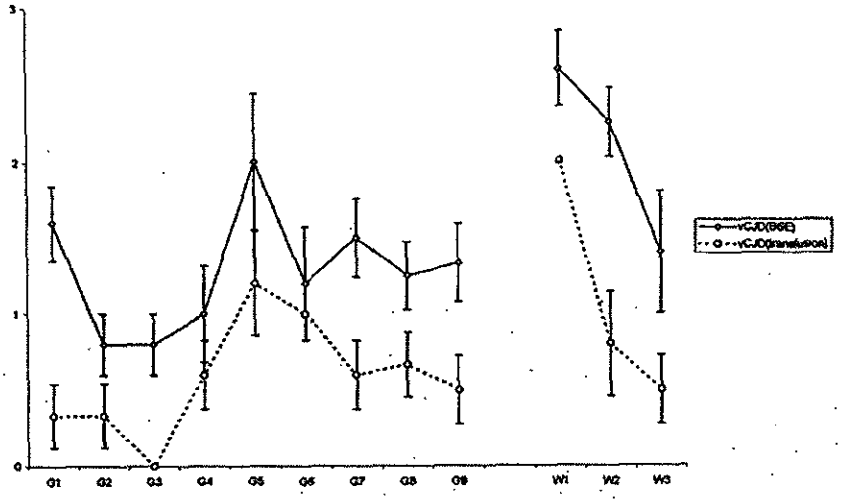
All the primary and secondary clinical cases of vCJD have occurred in individuals with a MM genotype. The sub- or pre-clinical transfusion related infection was in a codon 129 heterozygote and genotyping of positive appendix samples identified in a screening study confirmed valine homozygosity in 2 of 3 samples tested. [10] This indicates that individuals with all codon 129 genotypes may be infected with the vCJD agent and the effect of the MV or VV background on the characteristics of the vCJD agent have not been addressed by the data in this paper.

In conclusion, transmission studies indicate that the strain characteristics of vCJD have not been significantly altered by secondary transmission through blood transfusion. This suggests that the risk of onward transmission of vCJD through other routes, for example contaminated surgical instruments, have not been increased by adaptation of the infectious agent to humans following secondary passage. However the characteristics of the infectious agent in different genetic backgrounds has not yet been defined and the prevalence of vCJD infection in the general population remains uncertain. There is need to continue to implement appropriate policies to protect against the risk of secondary transmission of vCJD until many of the remaining uncertainties are resolved.

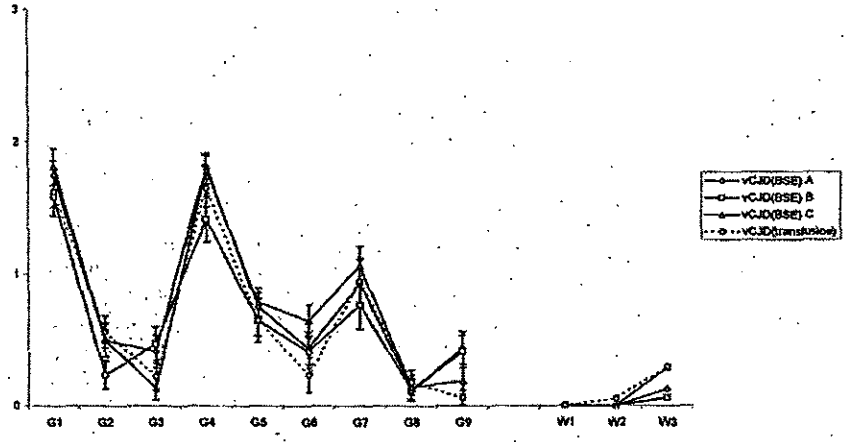
Materials and Methods

The transgenic mice (HuMM, HuMV, HuVV) used in these experiments have been described previously. [12] These mice express human PrP under the regulation of the murine promoter

HuMM


vCJD(BSE)
vCJD(transfusion)
G1
G2
G3
G4
G5
G6
G7
G8
G9
W1
W2
W3

RIII


vCJD(BSE) A
vCJD(BSE) B
vCJD(BSE) C
vCJD(transfusion)
G1
G2
G3
G4
G5
G6
G7
G8
G9
W1
W2
W3

VM

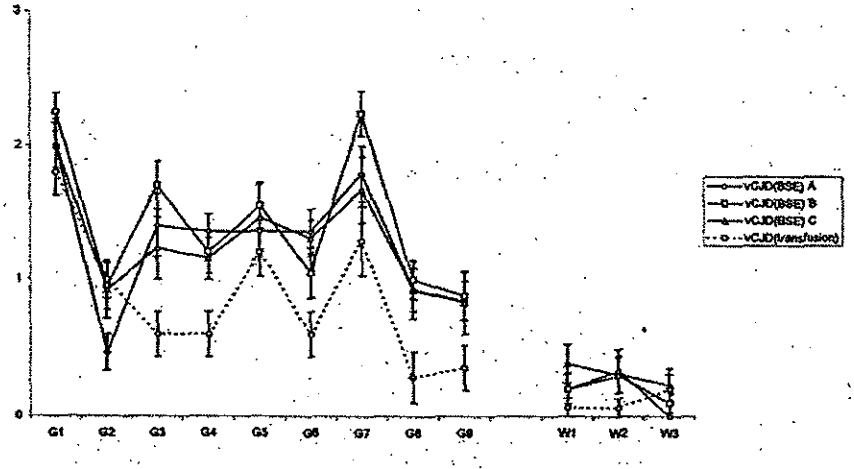

vCJD(BSE) A
vCJD(BSE) B
vCJD(BSE) C
vCJD(transfusion)
G1
G2
G3
G4
G5
G6
G7
G8
G9
W1
W2
W3

Figure 2. Vacuolation scoring in the mouse brain. Lesion profile comparison of vCJD (transfusion) case versus vCJD (BSE) transmissions to identify similarities in vacuolar pathology levels and regional distribution in mouse brains. (mean score ±SEM; dashed line - vCJD (transfusion) case; solid lines – 3x vCJD (BSE) cases for wild-type mice (diamonds – vCJD(BSE) A; squares – vCJD(BSE) B; triangles – vCJD(BSE) C) and published vCJD (BSE) for HuMM transgenic; G1–G9 grey matter scoring regions; W1–W3 white matter scoring regions)
doi:10.1371/journal.pone.0002878.g002

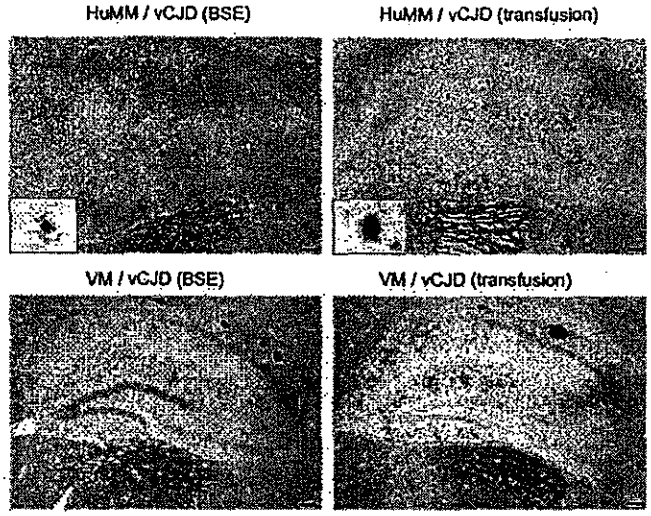


Figure 3. Detection of abnormal PrP in the mouse brain. Immunocytochemical detection of abnormal PrP deposition in hippocampus and thalamus (lateral posterior nucleus) of HuMM transgenic (with additional 40× magnification of florid plaque structure; see box lower left) and VM wild-type mice following inoculation with vCJD (BSE) and vCJD (transfusion) material. (Scale bar 200 μm, anti-PrP antibody 6H4)
doi:10.1371/journal.pone.0002878.g003

sequences, and survive for the same lifespan as non-transgenic mice of the same genetic background (129Ola) with no adverse effects and no features of spontaneous TSE disease. Wild-type mice (lines VM and RIII) are inbred lines used routinely for strain typing of TSEs. RIII is a *Prnp*-a genotype line and VM is a *Prnp*-b genotype line. [33] Use of mice for this work was reviewed and approved by the Neuropathogenesis Division Ethics Committee for Animal Experimentation.

Mice were inoculated as described previously. Groups of 24 wild-type mice received a 0.02 ml dose at 10^{-1} dilution by the intracerebral route, for vCJD (transfusion) and vCJD (BSE). Groups of 18 transgenic mice were injected with inoculum at a higher dilution of 10^{-2} as in previous experiments more concentrated inocula had been found to be toxic to the mice. Inoculum was prepared as a homogenate in sterile saline from frozen frontal cortex (with full consent from the patient's relatives, and approved by the Lothian NHS Board Research Ethics Committee (Reference: 2000/4/157)) to allow accurate comparison with previous data. Cases used for transmission were: the first

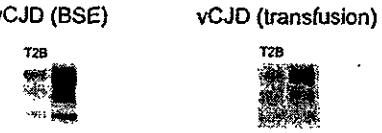


Figure 4. PrP^{Sc} typing by Western blot. Brain homogenates from HuMM mice inoculated with both vCJD (BSE) and vCJD (transfusion) show similar mobility and glycosylation profile (type 2B) as material from vCJD patients. (T2B: control vCJD material; antibody: 6H4)
doi:10.1371/journal.pone.0002878.g004

blood transfusion associated case, designated here as vCJD (transfusion), and three historical vCJD cases designated here as vCJD (BSE) A, B, and C. The historical vCJD cases were not inoculated into the transgenic mice. Data from vCJD (transfusion) inoculation of the transgenic mice was compared with that already published for vCJD (BSE). [12] Data from vCJD (transfusion) inoculation of the wild-type mice was compared with data from the three historical vCJD cases.

Mice were housed in independently ventilated cages in a Category 3 facility, monitored daily and scored for signs of TSE disease weekly from 100 days post inoculation. Mice were culled, when clinical TSE was evident or for animal welfare reasons, by cervical dislocation and the brain bisected sagittally; one half frozen for biochemical analysis of disease-associated prion protein and the other half fixed in formalin for histology.

Vacuolation scoring was performed according to published protocols and lesion profiles generated. [34,35] Immunocytochemical detection of abnormal PrP deposition was performed as published and Western blotting of disease-associated PrP from the frozen half-brain carried out according to Head *et al.* [12,25]

Acknowledgments

We thank Irene McConnell and the Animal Facility staff in the Neuropathogenesis Division, Anne Suttie and the Pathology staff for sectioning the mouse brains and assessing the levels of TSE vacuolation, and Dot Kisielewski for transgenic mouse genotyping.

This study would not be possible without the continued support of the families of those affected by vCJD, and the neurologists and neuropathologists throughout the UK that assist in CJD surveillance.

Author Contributions

Conceived and designed the experiments: MTB RGW MB JCM. Performed the experiments: MTB DLR VT. Analyzed the data: MTB DLR MWH. Contributed reagents/materials/analysis tools: JWI MWH. Wrote the paper: MTB RGW JWI JCM.

References

1. Will RG, Ironside JW, Zeidler M, Cousens SN, Estibeiro K, et al. (1996) A new variant of Creutzfeldt-Jakob disease in the UK. Lancet 347: 921–925.
2. Bruce ME, Will RG, Ironside JW, McConnell I, Drummond D, et al. (1997) Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. Nature 389: 498–501.
3. Andrews NJ, Farrington CP, Ward HJ, Cousens SN, Smith PG, et al. (2003) Deaths from variant Creutzfeldt-Jakob disease in the UK. Lancet 361: 751–752.
4. Llewelyn CA, Hewitt PE, Knight RS, Amar K, Cousens S, et al. (2004) Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. Lancet 363: 417–421.
5. Hewitt PE, Llewelyn CA, Mackenzie J, Will RG (2006) Creutzfeldt-Jakob disease and blood transfusion: results of the UK Transfusion Medicine Epidemiological Review study. Vox Sang 91: 221–230.
6. Zeidler M, Stewart G, Cousens SN, Estibeiro K, Will RG (1997) Codon 129 genotype and new variant CJD. Lancet 350: 668.
7. HPA (2006) New case of blood transfusion-associated variant-CJD. CDR Weekly 16.
8. Wroe SJ, Pal S, Siddique D, Hyare H, Macfarlane R, et al. (2006) Clinical presentation and pre-mortem diagnosis of variant Creutzfeldt-Jakob disease associated with blood transfusion: a case report. Lancet 368: 2061–2067.

9. Peden AH, Head MW, Ritchie DL, Bell JE, Ironside JW (2004) Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. Lancet 364: 527–529.
10. Ironside JW, Bishop MT, Connolly K, Hegazy D, Lowrie S, et al. (2006) Variant Creutzfeldt-Jakob disease: prion protein genotype analysis of positive appendix tissue samples from a retrospective prevalence study. BMJ.
11. Hilton DA, Ghani AC, Conyers L, Edwards P, McCardle L, et al. (2004) Prevalence of lymphoreticular prion protein accumulation in UK tissue samples. J Pathol 203: 733–739.
12. Bishop M, Hart P, Aitchison L, Baybutt H, Plinston C, et al. (2006) Predicting susceptibility and incubation time of human-to-human transmission of vCJD. The Lancet Neurology 5: 393–398.
13. Houston F, Foster JD, Chong A, Hunter N, Bostock CJ (2000) Transmission of BSE by blood transfusion in sheep. Lancet 356: 999–1000.
14. Hunter N, Foster J, Chong A, McCutcheon S, Parnham D, et al. (2002) Transmission of prion diseases by blood transfusion. J Gen Virol 83: 2897–2905.
15. McCutcheon S, Hunter N, Foster JD, Macgregor I, Hornsey V, et al. (2007) Transmission of BSE infection in sheep via blood transfusion. Edinburgh.
16. Hill AF, Desbruslais M, Joiner S, Sidle KCL, Gowland I, et al. (1997) The same prion strain causes vCJD and BSE. Nature 389: 448–450.
17. Taguchi Y, Mohri S, Ironside JW, Muramoto T, Kitamoto T (2003) Humanized knock-in mice expressing chimeric prion protein showed varied susceptibility to different human prions. Am J Pathol 163: 2585–2593.
18. Capobianco R, Casalone C, Suardi S, Mangieri M, Miccolo C, et al. (2007) Conversion of the BASE Prion Strain into the BSE Strain: The Origin of BSE? PLoS Pathogens 3: e31.
19. Kong Q, Huang S, Zou W, Vanegas D, Wang M, et al. (2005) Chronic wasting disease of elk: transmissibility to humans examined by transgenic mouse models. J Neurosci 25: 7944–7949.
20. Manson JC, Cancellotti E, Hart P, Bishop MT, Barron RM (2006) The transmissible spongiform encephalopathies: emerging and declining epidemics. Biochem Soc Trans 34: 1155–1158.
21. Korth C, Kaneko K, Groth D, Heye N, Telling G, et al. (2003) Abbreviated incubation times for human prions in mice expressing a chimeric mouse-human prion protein transgene. Proc Natl Acad Sci U S A 100: 4784–4789.
22. Asante EA, Linehan JM, Gowland I, Joiner S, Fox K, et al. (2006) 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 103: 10759–10764.
23. Bruce ME, McConnell I, Will RG, Ironside JW (2001) Detection of variant Creutzfeldt-Jakob disease infectivity in extraneural tissues. Lancet 358: 208–209.
24. Bruce M, Will R, Ironside J, Fraser H (2006) Evidence for a link between variant Creutzfeldt-Jakob disease and bovine spongiform encephalopathy. In: Hornlimann B, Riesner D, Kretzschmar H, eds. Prions in Humans and Animals Walter de Gruyter. pp 573–578.
25. Head MW, Bunn TJ, Bishop MT, McLoughlin V, Lowrie S, et al. (2004) Prion protein heterogeneity in sporadic but not variant Creutzfeldt-Jakob disease: U.K. cases 1991–2002. Ann Neurol 55: 851–859.
26. Cooper JK, Ladhani K, Minor PD (2007) Reference materials for the evaluation of pre-mortem variant Creutzfeldt-Jakob disease diagnostic assays. Vox Sang 92: 302–310.
27. Herzog C, Sales N, Etchegaray N, Charbonnier A, Freire S, et al. (2004) Tissue distribution of bovine spongiform encephalopathy agent in primates after intravenous or oral infection. Lancet 363: 422–428.
28. Baron T (2002) Mouse models of prion disease transmission. Trends Mol Med 8: 495.
29. Bruce M, Chree A, McConnell I, Foster J, Pearson G, et al. (1994) Transmission of bovine spongiform encephalopathy and scrapie to mice - strain variation and the species barrier. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 343: 405–411.
30. Lasmezas CI, Fournier JG, Nouvel V, Boe H, Marce D, et al. (2001) Adaptation of the bovine spongiform encephalopathy agent to primates and comparison with Creutzfeldt–Jakob disease: implications for human health. Proc Natl Acad Sci U S A 98: 4142–4147.
31. Cervenakova L, Yakovleva O, McKenzie C, Kolchinsky S, McShane L, et al. (2003) Similar levels of infectivity in the blood of mice infected with human-derived vCJD and GSS strains of transmissible spongiform encephalopathy. Transfusion 43: 1687–1694.
32. Bartz JC, Kincaid AE, Bessen RA (2003) Rapid prion neuroinvasion following tongue infection. J Virol 77: 583–591.
33. Barron RM, Baybutt H, Tuzi NL, McCormack J, King D, et al. (2005) Polymorphisms at codons 108 and 189 in murine PrP play distinct roles in the control of scrapie incubation time. J Gen Virol 86: 859–868.
34. Fraser H, Dickinson AG (1968) The sequential development of the brain lesion of scrapie in three strains of mice. Journal of Comparative Pathology 78: 301–311.
35. Bruce ME, McConnell I, Fraser H, Dickinson AG (1991) The disease characteristics of different strains of scrapie in Sinc congenic mouse lines: implications for the nature of the agent and host control of pathogenesis. J Gen Virol 72 (Pt 3): 595–603.

別紙様式第2-1
番号16

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9月16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	乾燥濃縮人アンチトロンビンⅢ	研究報告の公表状況	PLoS ONE 2008; 3(8_e3017): 1-8	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＞
ヒトのvCJDは、古典的BSEに罹った畜殺牛のプリオンを食料として摂取することで感染する。非定型BSEは、高齢牛では殆どが無症候であるが、最近ヨーロッパと北米の畜殺場で確認され、これらの新しいプリオン株に対するヒトの感受性についての問題が提起されている。
＜方法/主な所見＞
古典的BSEと非定型BSEに感染した牛の脳のホモジネートを、以前に古典的BSEのオリジナル株に感受性が高いことを示したヒト以外の霊長動物モデルであるカニクイザルに脳内接種した。こうして発現させた疾患を、臨床兆候、組織学、異常プリオンたん白の生化学の点から比較した。非定型BSEに感染した1頭のサルは生存期間が短く、古典的BSEまたはvCJD接種動物のいずれとも異なる臨床的展開、組織変化、プリオン蛋白(PrPres)パターンを示した。加えて、非定型BSEの接種動物におけるPrPresの生化学的特徴として、octa-repeat領域のプロテイナーゼKへの高い感受性を有していることが判明した。我々は、感染牛と同じ郡に住んでいた孤発性CJDおよびMM type 2 PrP遺伝型の4人の患者のうちの3人に、同じ生化学的特徴があるのを見出した。
＜結論＞
我々の結果は、霊長動物において、古典的BSEよりも非定型BSEの方が病原性が高い可能性があることを示し、加えて外見上孤発性CJDに見えるまれな症例群と結びついている可能性についての問題を提起している。これより、古典的BSEの流行が衰えているにも関わらず、非定型株の発生によって、BSE汚染製品による偶発的汚染から公衆衛生を保護するために現在実施されている措置を緩和することを推進することは抑えるべきである。

報告企業の意見	今後の対応
霊長動物では非定型BSEの方が古典的BSEよりも病原性が高く、孤発性CJDに見える症例と結びついている可能性があるとの報告である。 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。

OPEN ACCESS Freely available online

PLoS one

Atypical BSE (BASE) Transmitted from Asymptomatic Aging Cattle to a Primate

Emmanuel E. Comoy[1]*, Cristina Casalone[2], Nathalie Lescoutra-Etchegaray[1], Gianluigi Zanusso[3], Sophie Freire[1], Dominique Marcé[1], Frédéric Auvré[1], Marie-Magdeleine Ruchoux[1], Sergio Ferrari[3], Salvatore Monaco[3], Nicole Salès[4], Maria Caramelli[2], Philippe Leboulch[1,5], Paul Brown[1], Corinne I. Lasmézas[4], Jean-Philippe Deslys[1]

1 Institute of Emerging Diseases and Innovative Therapies, CEA, Fontenay-aux-Roses, France, 2 Istituto Zooprofilattico Sperimentale del Piemonte, Turin, Italy, 3 Policlinico G.B. Rossi, Verona, Italy, 4 Scripps Florida, Jupiter, Florida, United States of America, 5 Genetics Division, Brigham & Women's Hospital, Harvard Medical School, Boston, Massachusetts, United States of America

Abstract

Background: Human Variant Creutzfeldt-Jakob Disease (vCJD) results from foodborne transmission of prions from slaughtered cattle with classical Bovine Spongiform Encephalopathy (cBSE). Atypical forms of BSE, which remain mostly asymptomatic in aging cattle, were recently identified at slaughterhouses throughout Europe and North America, raising a question about human susceptibility to these new prion strains.

Methodology/Principal Findings: Brain homogenates from cattle with classical BSE and atypical BASE infections were inoculated intracerebrally into cynomolgus monkeys (*Macaca fascicularis*), a non-human primate model previously demonstrated to be susceptible to the original strain of cBSE. The resulting disease was compared in terms of clinical signs, histology, and biochemistry of the abnormal prion protein (PrPres). The BASE monkey, infected with BASE, had a shorter survival and a different clinical evolution, neuropathology, and prion protein (PrPres) pattern than that observed for either classical BSE or vCJD inoculated animals. Use the biochemical signature of PrPres in the BASE-inoculated monkey, from the cBSE-inoculated monkeys, which have a higher transmissibility to the orally-dosed cynomolgus macaque, we found the same biochemical signature in those of four human cases of sporadic CJD and an Italian BASE primary case. [illegible] in the infected brains.

Conclusion/Significance: Our results point to a possibly higher degree of pathogenicity of BASE than classical BSE in primates and also raise a question about a possible link between some cases of apparently sporadic CJD. Thus, despite the waning epidemic of classical BSE, the occurrence of atypical cases of BSE should lead to more stringent measures currently in place to protect public health from accidental contamination by BSE-contaminated products.

Citation: Comoy EE, Casalone C, Lescoutra-Etchegaray N, Zanusso G, Freire S, et al. (2008) Atypical BSE (BASE) Transmitted from Asymptomatic Aging Cattle to a Primate. PLoS ONE 3(8): e3017. doi:10.1371/journal.pone.0003017

Editor: Neil Mabbott, University of Edinburgh, United Kingdom

Received April 24, 2008; **Accepted** August 1, 2008; **Published** August 20, 2008

Copyright: © 2008 Comoy et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work has been supported by the Network of Excellence NeuroPrion.

Competing Interests: CEA owns a patent covering the BSE diagnostic tests commercialized by the company Bio-Rad.

* E-mail: emmanuel.comoy@cea.fr

Introduction

Classical Bovine Spongiform Encephalopathy (cBSE), the first prion disease identified in cattle, was initially reported in 1986 in the UK. Food-borne transmission of cBSE to humans was observed ten years later as a variant form of Creutzfeldt-Jakob Disease (vCJD) [1], leading to a major public health crisis.

This strain of cBSE is now rapidly disappearing as a result of appropriate containment measures. However, atypical forms of BSE have recently been identified in Europe and North America as a consequence of cBSE testing performed in these countries [2–4]. Because these cases are only found sporadically in older animals (≥8 years) coming to slaughter with few or no signs of disease, it would be plausible to suppose that atypical forms of BSE may have a lower virulence than cBSE and be innocuous to humans. However, recent studies suggest that one of the two main forms of atypical BSE, initially discovered in Italy and referred to as the bovine amyloidotic spongiform encephalopathy (BASE), might be at the origin of the cBSE epidemic: inoculation of the BASE strain into transgenic and inbred mice showed an apparent natural evolution towards the typical BSE strain [5,6]. Moreover, a possible link has been suggested between BASE and one subtype (MV2) of human sporadic CJD (sCJD) on the basis of biochemical similarities [2,7]. In contrast to vCJD, sCJD is believed to occur de novo without food-borne transmission. However, specific contaminating events by ingestion are difficult to rule out because human prion diseases can have silent incubation periods exceeding 50 years, as demonstrated for kuru [8].

One strategy to evaluate the risk of BASE for humans consists in assessing the susceptibility to disease transmission and the degree of pathogenicity in a non-human primate model that has already been shown to have characteristic clinical signs, histopathological lesions and PrPres profiles following infections with either BSE or vCJD [9,10]. We therefore inoculated cynomolgus macaque monkeys (*Macacca fascicularis*) intracerebrally with BASE, cBSE and vCJD prion strains. The BASE strain, prepared from brain extract of a 15-

Table 1. Survival times of macaques inoculated intracerebrally with brain homogenates from cattle with BASE or BSE, and from humans with vCJD.

Strain	Source	Dose*	Survival time (months)
BASE	cattle	100 mg	40
BSE	cattle	100 mg	40
vCJD	human	40 mg	25
vCJD	human	40 mg	32
vCJD	human	40 mg	32

*Amount of crude brain in 10% brain suspension inoculated intracerebrally. BSE brain had a 10-fold greater concentration of PrP^{Res} than the BASE brain). Animals inoculated with vCJD also received the equivalent of 8 mg of brain by intra-tonsillar injection.

doi:10.1371/journal.pone.0003017.t001

year-old asymptomatic cow induced a distinctive and more rapidly fatal disease than cBSE, and showed a biochemical signature similar to that of the MM2 cortical subtype of human sCJD.

Methods

Cattle and human samples

The BASE inoculum (mix of brainstem and thalamus) from an asymptomatic 15 year-old Italian Piemontese cow [2]: 250 µl of a 10% brain homogenate in 5% glucose were inoculated intracerebrally (i.c.) to a single macaque monkey. As controls, we used two macaques inoculated i.c. with cBSE (brainstem from infected UK cattle) and 4 macaques inoculated i.c. with human vCJD [9,11]. Twenty-one subjects with a diagnosis of definite sCJD were referred to the Medical Center in Verona, Italy during the period 2000–2004. Tissues were processed 4–18 hours post-mortem according to established guidelines regarding safety and ethics. Brains were cut longitudinally into two halves. Hemi-brains were frozen and stored at −80°C until biochemical studies were performed. The patient group encompassed all of the different

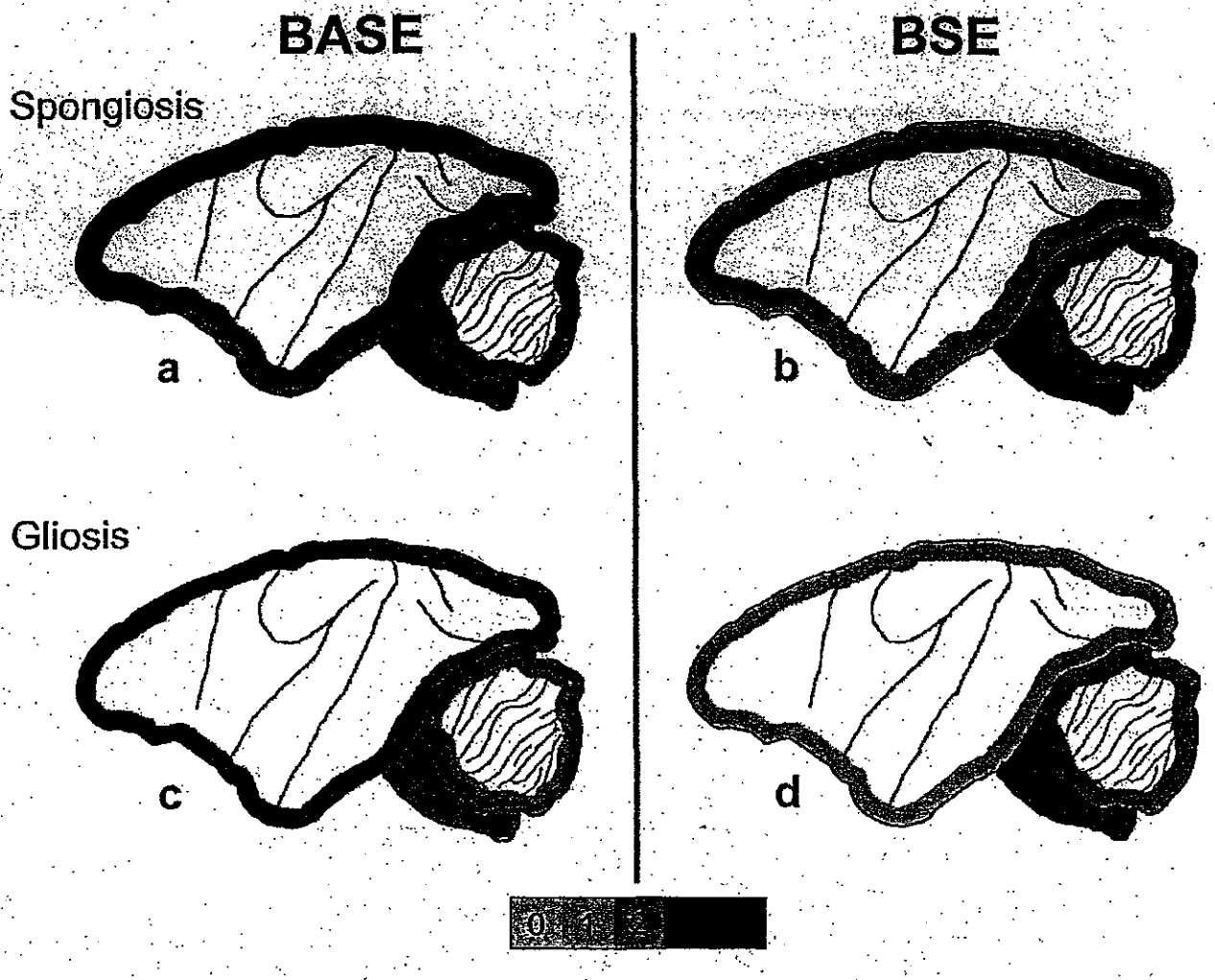


Figure 1. Diagrammatic representation of histologic lesions. Topographic distribution of spongiosis (a and b) or gliosis (c and d) in BASE and cBSE-infected primates. The lesions were scored from 0 to 4 (negative, light, mild, moderate, and severe).

doi:10.1371/journal.pone.0003017.g001

Western blot subtypes of sCJD described by Parchi et al [7]: MM1 (5 cases), MV1 (2), VV1 (1), MM2 (4), MV2 (6) and VV2 (3).

Non-human primate model

Cynomolgus macaques (*Macacca fascicularis*), captive-bred from the Centre de Recherche en Primatologie (Mauritius), were checked for the absence of common primate pathogens before importation and handling in accordance to national guidelines. Animals were maintained in biological security level 3 animal facilities and clinical examinations were performed regularly. They were humanely euthanized at the terminal stage of the disease, and tissues were either fixed in Carnoy's fluid for histological examination or snap-frozen in liquid nitrogen and stored at −80°C for biochemical analyses.

Neuropathology and immunochemistry

Neuropathology and immunochemical detection of proteinase-resistant prion protein (PrPres) and Glial fibrillary acidic protein (GFAP) was performed on brain sections as previously described [12].

PrPres analysis

Tissues were homogenized to 20% (w/v) final concentration in a 5% sterile glucose solution. PrPres was purified according to a protocol optimized for strain discrimination in ruminants [13,14] (Discriminatory kit ref 3551177, BioRad, Marnes la Coquette, France). Briefly, brain homogenates were first subjected to proteolysis using either 0.4 µg ("low" concentration) or 4 µg ("high" concentration) of proteinase K/mg of brain (final concentration) in a special buffer that partially protects the N-terminal part of PrPres in order to increase strain discrimination, and then purified PrPres was concentrated by centrifugation. Purified, non-human primate and human samples were processed for Western blot analysis as previously described: briefly, samples were separated by electrophoresis on a 12% SDS polyacrylamide gel, blotted onto a nitrocellulose membrane and detected by two mouse monoclonal antibodies: the antibody from the BioRad Discriminatory kit, which targets the epitope WGQPHGGX within the N-Terminal octarepeat region at position 57–88, and 3F4, which targets the epitope MKHM in the hydrophobic core at position 109–112. The protein bands were visualized using a peroxidase-conjugated goat anti-mouse antibody and chemiluminescence.

Results

Transmission characteristics of BASE and BSE

Clinical features. The BASE-inoculated macaque developed clinical signs after a 21 months incubation period. Clinical signs evolved slowly during the first four months, being limited to mild tremor and myoclonus, without impairment in coordination or locomotion, and without anxiety or aggressiveness. In the last month, the clinical picture rapidly worsened with evidence of major spatial disorientation (the animal did not recognize its environment and seemed lost in its cage), cognitive troubles (no recall of food location and at intervals unaccountably stopped eating) and the appearance of incoordination and disequilibrium; however, appetite and general fitness were maintained. Euthanasia was performed at the terminal

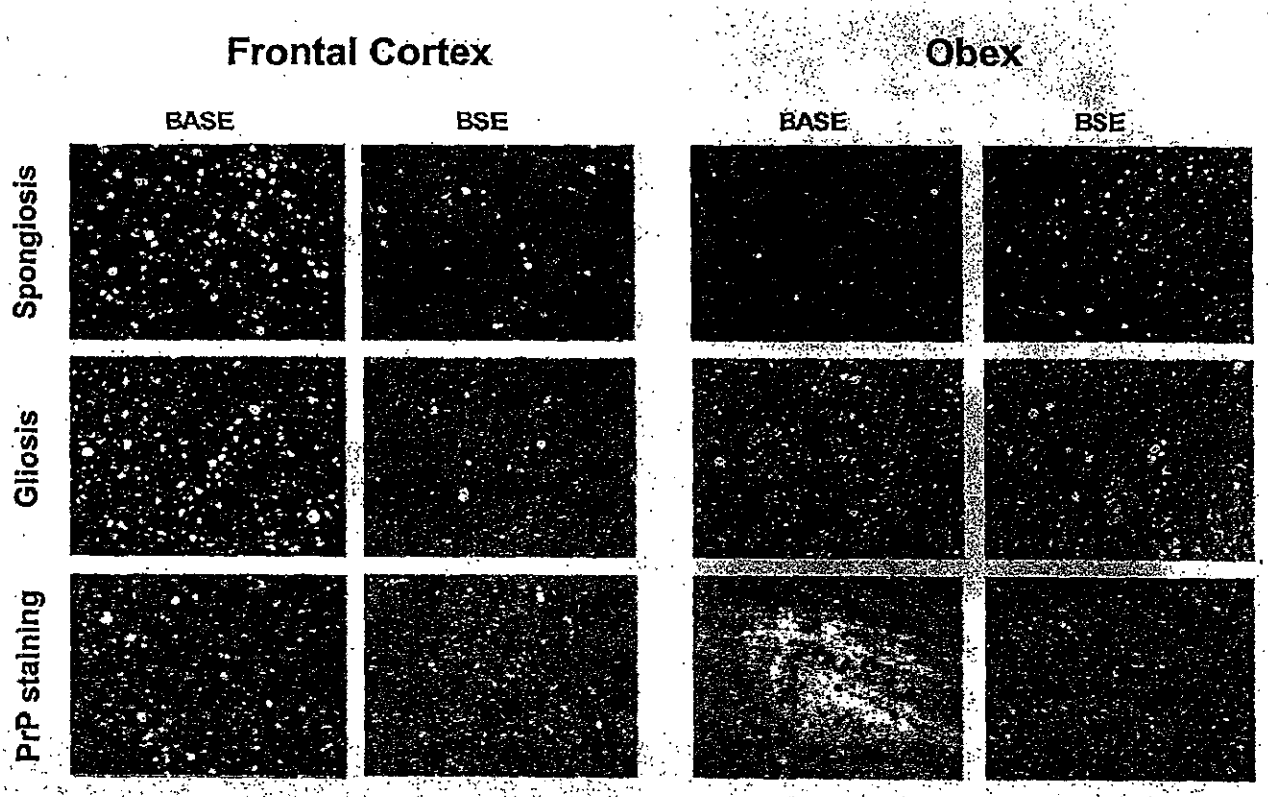


Figure 2. Histopathology and PrPres immunostaining. Spongiosis, gliosis (GFAP staining) and PrPres deposition in frontal cortex and obex in BASE- and cBSE-infected primates (original magnification ×200 for spongiosis and gliosis, ×400 for PrPres staining). Immunostaining of PrPres was performed with 3F4 monoclonal anti-PrP antibody after proteinase K treatment as previously described [11]. No staining was observed in the brain of control healthy primates (data not shown) in these conditions.
doi:10.1371/journal.pone.0003017.g002

stage of illness at 26 months post inoculation (Table 1). The two cBSE-inoculated animals had longer incubations periods (37.5 months) and survivals (40 months) despite a presumably larger infecting dose (100 mg containing a 10-fold higher PrPres concentration). Moreover, the clinical presentation was very different: animals exhibited aggressiveness and anxiety in combination with incoordination, severe ataxic tremor, and loss of appetite to the point of near starvation. The four animals inoculated with human vCJD had a clinical evolution similar to that of animals inoculated with BSE, though with less prolonged survivals (25 to 37 months).

Histopathology (Figures 1 and 2). In the BASE-inoculated animal, the cortex showed widespread spongiosis and gliosis that were especially prominent in the fourth and fifth layers. Spongiosis was intense in the frontal cortex, with a loss of pyramidal cells in the third and fifth layers. Lesions in the parietal cortex were even more severe, with a complete disappearance of neurons in the fourth layer. In the cBSE-inoculated animals, spongiosis and gliosis were more discrete, and mainly affected the occipital cortex. In the obex and cerebellum, the lesions (spongiosis and loss of Purkinje and granular cells) were less pronounced in BASE than cBSE-infected animals.

Immunohistochemistry (Figure 2). In the BASE-infected animal, PrPres was distributed in a diffuse synaptic pattern (either fine and sandy or roughly granular) with laminar enhancement in the parietal cortex but no evidence of plaques, even when stained with thioflavine T (data not shown), whereas cBSE-infected animals had weak diffuse synaptic labeling but multiple intensely-stained PrPres aggregates and characteristic plaques [9].

Strain discrimination by proteinase K sensitivity and antibody reactivity

We made use of a technique developed to discriminate and classify prion strains in small ruminants [14], based on the differential sensitivity of the octapeptide and core regions of PrPres proteins to proteinase K (PK) digestion. Controlled conditions of proteolysis allowed a strain-dependent threshold of removal of the octapeptides. This method, illustrated in Figure S1 (supplementary data), was successfully applied for the diagnosis of the first case of cBSE in a goat [15] and has now been validated by the European Commission for regular use on field. We adapted this test to primate prion strains, using only the higher PK concentration and substituting the monoclonal antibody 3F4 as the anti-core antibody to macaque and human PrP.

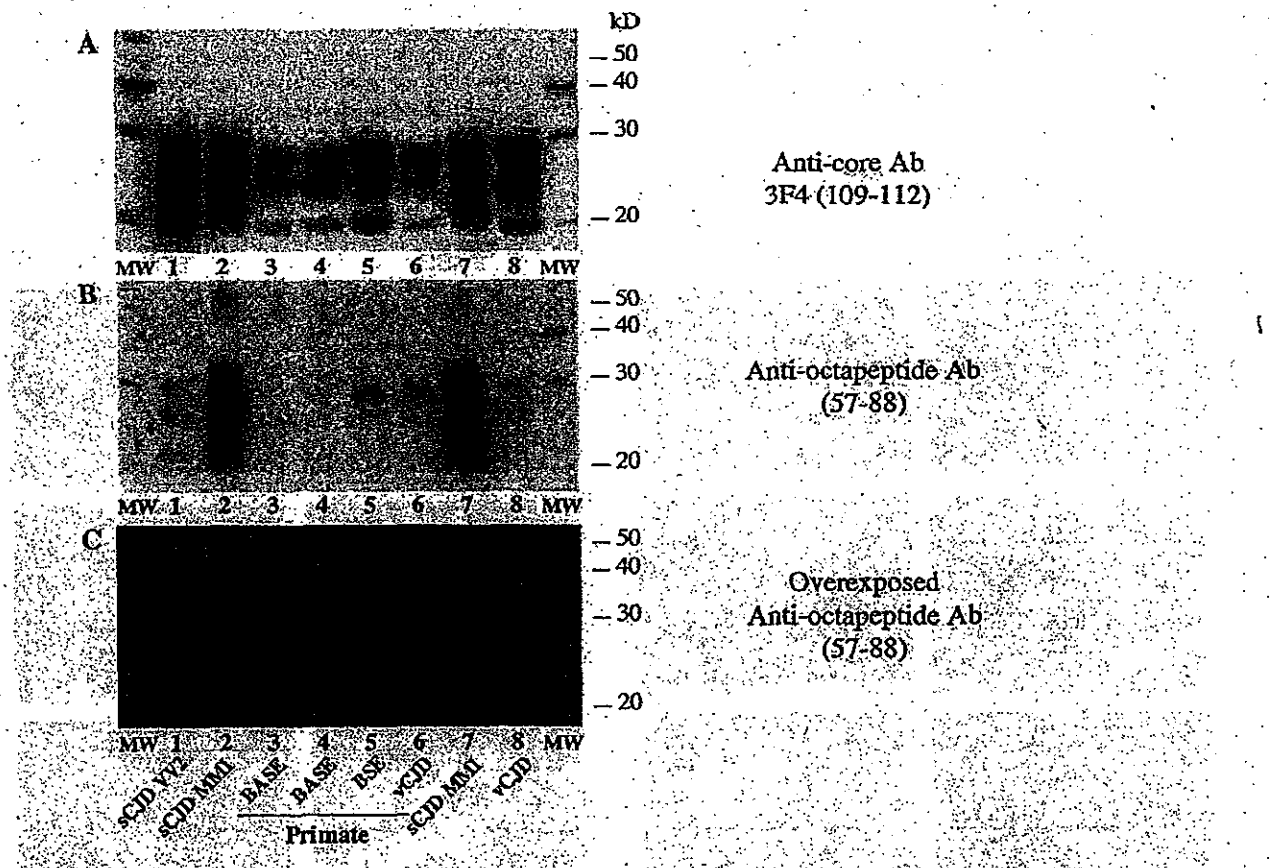


Figure 3. Electrophoretic analysis and differential sensitivity to proteolysis of PrPres in various prion diseases of primates and humans. PrPres from brain homogenates (MM1 or VV2 sCJD, vCJD in humans, or primates experimentally infected with BASE, cBSE or vCJD inocula) were purified under high concentrations of proteinase K, and detected with monoclonal antibodies recognizing either the core (3F4, panel A) or the octapeptide region (panel B and C) of the protein. Frontal cortex and obex regions of BASE-infected primate were both analysed (lanes 3 and 4 respectively). Panel C is an overexposure of the autoradiography of Panel B to detect weak signals. The absence of octarepeat region reactivity in the PrPres of the BASE in Panel C indicates a proportion at least ten fold lower than that of the vCJD or cBSE samples on the basis of a quantitative analysis of chemiluminescence signal intensities.
doi:10.1371/journal.pone.0003017.g003

Banding patterns in Western blots following pre-treatment with a high PK concentration are shown in Figure 3. Both vCJD/cBSE and BASE reacted strongly to anti-core antibody (Panel A). In contrast, vCJD/cBSE also reacted weakly to anti-octapeptide antibody (Panel B), whereas BASE reactivity was abolished (Panels B and C), indicating a gradient of resistance to proteolysis of the N terminal part of the PrPres among these strains. In cattle, the signal was abolished for both cBSE and BASE strains (data not shown).

The method also revealed notable differences of octapeptide sensitivity to PK in different types of human prion disease (figures 3 and 4). Comparisons of the relative signals with both anti-core and anti-octapeptide antibodies for each sample indicated that the N terminal part of PrPres from vCJD and the VV2 subtype of sCJD were far more sensitive than either the MM1 or VV1 subtypes (Panel B). The MV2 subtype showed a strong resistance to proteolysis that was clearly different from the BASE-infected primate; however, three of the four MM2 subtype cases exhibited the same signature as BASE, and the fourth case had a significant proportion of PrPres with an intact octapeptide region, as shown in figure 5, indicating the coexistence of two types of PrPres (the majority being type 2).

All four cases had clinical features consistent with the MM2 subtype as described by Gambetti et al [16]: comparatively long illnesses dominated by cognitive impairment followed by aphasia, and later in the course of illness the appearance of pyramidal and extrapyramidal signs together with myoclonus, but no cerebellar signs. Neuropathology was also typical of the MM2 subtype, with major cortical spongiosis and little or no involvement of the cerebellum (Table 2 summarizes the clinical, laboratory, and neuropathological features of each case).

Discussion

We have shown that BASE, the first identified atypical strain of BSE [2], originating from asymptomatic cattle, is transmissible by i.c. inoculation to a species of non-human primate. Although this observation concerned only one animal, its survival was substantially shorter than for all the macaques inoculated with classical BSE as well as the majority of those inoculated with human vCJD. Moreover, in earlier experiments by others on a total of 6 macaques inoculated i.c. with 50 mg of cBSE brain, none had an incubation period of less than 30 months [17], and humanized transgenic mice have been found to be highly susceptible to infection with BASE, and completely resistant to infection with cBSE [18]. If BASE is more pathogenic than classical BSE for primates, it could indicate a more readily transmissible infection from cattle to humans than previously suspected. A preliminary trial of oral transmission is currently ongoing for alimentary risk assessment: 49 months after oral dosing there is no indication of transmission; however, the incubation period following similar oral challenge with cBSE in an already completed experiment was 60 months.

The disease induced by BASE was different in all respects from that induced by classical BSE. The clinical presentation was characterized by mild tremors and myoclonus, progressing to a marked cognitive disorder, including spatial disorientation but without anxiety, aggressiveness or loss of appetite. In contrast, cBSE presented signs of anxiety and aggressiveness together with progressive difficulties in locomotion as well as cerebellar signs (major ataxia), and severe decrease of appetite with concurrent weight loss. The widespread spongiform lesions and loss of pyramidal cells in the third and fifth layers of the frontal cortex together with the severe parietal lesions could explain the prominent cognitive signs and the spatial disorientation seen in the BASE-infected monkey, contrasting with the severity of lesions in the obex and cerebellum consistent with the incoordination seen in animals inoculated with cBSE. Amyloid plaques, the hallmark of BASE in cattle, are not produced in the Macaque monkey, and conversely, cBSE does not produce plaques in cattle, but does so in the Macaque [9], a clear indication that plaque deposition depends as much on the host as the prion strain.

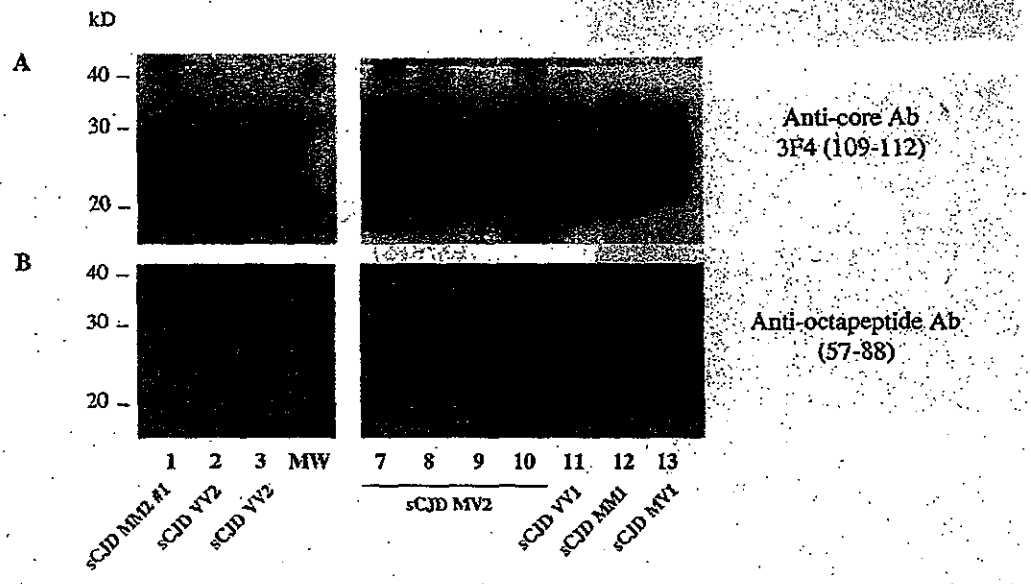


Figure 4. Electrophoretic analysis and differential sensitivity to proteolysis of PrPres in different subtypes of CJD. PrPres from human brain homogenates (MM1, MV1, VV1, MM2, MV2 or VV2 subtypes of sCJD, and vCJD) were purified under high concentrations of proteinase K, and detected with monoclonal antibodies that recognize either the core (3F4, Panel A) or the octapeptide region (Panel B) of the protein. The proportion of PrPres with an intact octarepeat region after PK exposure in VV2 and human (or macaque) vCJD was estimated to be only one-tenth and one-twentieth as high as in an MM1 sub-type of sCJD, respectively.
doi:10.1371/journal.pone.0003017.g004

At the molecular level, under conditions of high proteinase pretreatment and detection using two antibodies reacting with either an epitope in the N terminal octapeptide repeat region or the core of PrP, BASE and cBSE were clearly distinguishable in primate. BASE was detectable only by the core antibody, whereas cBSE was detectable by both antibodies. We estimated that the proportion of octapeptide-resistant PrPres molecules in the BASE brain homogenate was only a small fraction (≤1/10) of that of the cBSE brain homogenate. The difference in octapeptide sensitivity to PK between cBSE and vCJD in macaques on the one hand, and Type 1 sporadic CJD in humans on the other hand, is similar to what was observed between cBSE and classical scrapie in sheep. This method can now be used to test both ruminant and human samples to identify similarities and differences in their molecular protein signatures, and to implement the classification of ruminant and possibly human strains.

Although classical epidemiological studies have not found any link between scrapie in sheep and goats and human CJD, newer molecular biological studies now indicate that about half of all cases of scrapie are due to previously undetected atypical strains [19] that are experimentally transmissible to sheep and mice [20]. Their risk for humans is unknown and is the subject of current studies in experimental models, including primates. cBSE has been shown to be responsible for human cases of vCJD, but the comparative risk for humans of BASE and other atypical strains of BSE is still unknown, and its clarification will require many years of epidemiological surveillance and molecular biological testing of both bovine and human populations.

The first cases of BASE in cattle had PrPres electrophoretic profiles similar to the MV2 subtype of sporadic CJD patients [2] that, together with the presence of amyloid plaques in both the cattle and the patients, suggested a possible link between BASE and this subtype of sCJD. However, our PrPres typing technique has shown that, in the primate, PrPres of other MV2 sCJD patients exhibited a resistance to proteolysis different from the BASE-infected primate, whereas PrPres from vCJD-infected patients and primates behave similarly. This observation, together with the absence of amyloid plaques in the BASE-infected primate, weakens the likelihood of a direct link between BASE and MV2 subtype sCJD patients.

In contrast, the specific signature of PrPres in the BASE-infected primate was similar to that seen in three of four patients with the MM2 cortical subtype of sporadic CJD [7]. It is interesting that an

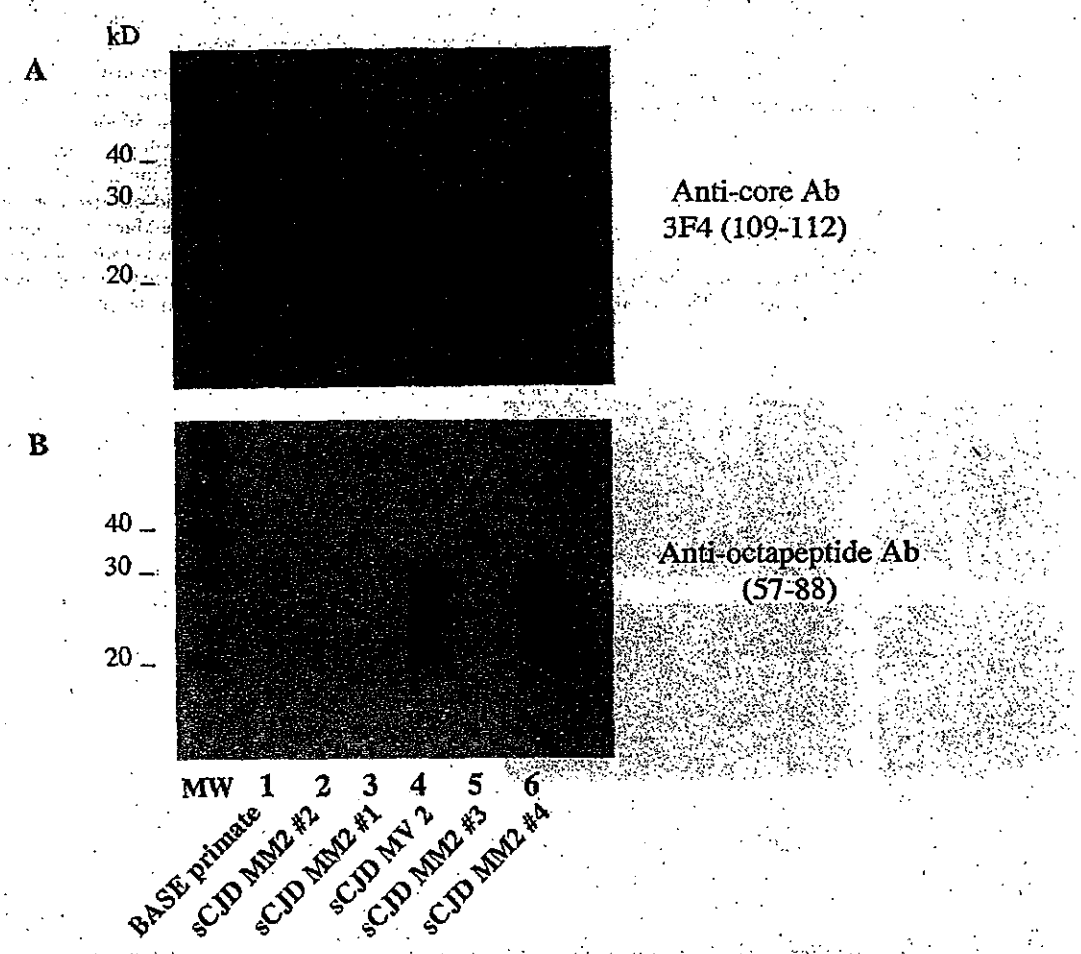


Figure 5. Electrophoretic analysis and differential sensitivity to proteolysis of PrPres in different MM2 CJD patients. PrPres from human brain homogenates (MM2 or MV2 subtypes of sCJD) and from primate experimentally infected with BASE were purified under high concentrations of proteinase K, and detected with monoclonal antibodies that recognize either the core (3F4, Panel A) or the octapeptide region (Panel B) of the protein.
doi:10.1371/journal.pone.0003017.g005

Table 2. Summary of MM2 subtype sporadic CJD patients.

Case #	1	2	3	4
Sex	Female	Male	Male	Female
Age at onset	60 years	59 years	59 years	69 years
Duration of illness	24 months	8 months	6 months	18 months
Onset	Progressive memory impairment beginning with episodic memory disturbance, then attention loss, spatial and temporal orientation	Depression, insomnia, headache. Episodic memory impairment and poor language	Memory disturbance	Depression, memory impairment
Evolution	Progressive cognitive decline marked by episodic memory impairment, apraxia, agnosia, visuo-spatial impairment, executive deficit, akinetic mutism, pyramidal and extrapyramidal signs, myoclonus	Worsening memory impairment, more global cognitive decline, bilateral temporal lobe, frontal lobe, progressive loss of all cortical functions, akinetic mutism, pyramidal and extrapyramidal signs, myoclonus	Confusional state, global impairment of higher cortical functions	Progressive cognitive decline characterized by global impairment of all cortical functions, Myoclonus
Terminal stage	Diffuse spastic rigidity; Pyramidal signs; dystonic movements and sporadic myoclonic jerks.	Akinetic mutism	No information available	Akinetic mutism
MRI	Cortical atrophy with hyperintensity of the basal ganglia (Caudate nucleus and Putamen) in T2-weighted images	Normal at early stage of illness	Normal at early stage of illness	Hyperintensity in T2-weighted images of the temporal cortex (late)
SPECT 99mTc-ECD	Not done	Hypo-perfusion of frontal, parietal and temporal cortices, bilaterally. Normal perfusion of subcortical ganglia and cerebellum	Hypo-perfusion of frontal, parietal and to a lesser extent temporal cortices	Not Done
MMSE	7/30	No data	Not done	Not reported
CSF 14-3-3 protein	Positive	Positive	Not done	Negative
EEG	Diffuse slowing	Diffuse slowing	Diffuse slowing	Periodic sharp waves
Neuropathology	Cortical spongiosis, Cerebellum relatively spared	Cortical spongiosis, Cerebellum relatively spared	Cortical spongiosis, normal cerebellum	Cortical spongiosis, cerebellum relatively spared
Type 2-like	Type 2	Type 2	Type 2	Type 2
Resistance of N-terminal part to proteolysis	No (BASE infected primate-like)	No (BASE infected primate-like)	No (BASE infected primate-like)	Yes

doi:10.1371/journal.pone.0003017.t002

important feature of the clinical-pathological syndrome in this BASE-infected macaque – the absence of cerebellar involvement – is also a common element in patients with the MM2 subtype of human sporadic CJD (Supplementary Figure S2). However, as illustrated by the clinical details of our four tested MM2 cases, there is considerable patient-to-patient variation, just as there can be variation among individual animals experimentally inoculated with a given strain of TSE. [21,22].

It is not known whether atypical strains of BSE have been circulating for years, or represent new forms of disease, and continuing research is clearly needed to answer both this and the equally important question about a possible relationship to at least certain forms of what are presently regarded as sporadic cases of human disease (sCJD) [4,23]. Moreover, the BASE strain has been described to evolve naturally towards BSE after successive transmissions in inbred mice [6]. The stability and pathogenicity of this strain in humans remains to be determined, and it is worth recalling that the stability of the cBSE/vCJD strain, which retains its specific molecular signature in different infected hosts, is the exception rather than the rule. As has been previously observed [24–26], one patient (Case No. 4, cf. figure 5 sample MM2#4) exhibited both types of PrP, i.e. type 2 typical of the MM2 subtype and type 1 observed in the MM1 subtype. On the one hand, this demonstrates the interest of such a simple biochemical test to refine PrP analysis, and on the other hand it raises a question about the existence of different PrPres signatures in the same patient, i.e., different prion strains linked to multiple infections or to variants selected by the host.

In summary, we have transmitted one atypical form of BSE (BASE) to a cynomolgus macaque monkey that had a shorter incubation period than monkeys infected with classical BSE, with distinctive clinical, neuropathological, and biochemical features; and have shown that the molecular biological signature resembled that seen in a comparatively uncommon subtype of sporadic CJD. We cannot yet say whether BASE is more pathogenic for primates (including humans) than cBSE, nor can we predict whether its molecular biological features represent a clue to one cause of apparently sporadic human CJD. However, the evidence presented here and by others justifies concern about a potential human health hazard from undetected atypical forms of BSE, and despite the waning epizoonosis of classical BSE, it would be premature to abandon the precautionary measures that have been so successful in reversing the impact of cBSE. We would instead urge a gradual, staged reduction that takes into account the evolving knowledge about atypical ruminant diseases, and both a permanent ban on the use of bovine central nervous system tissue for either animal or human use, and its destruction so as to eliminate any risk of environmental contamination.

Supporting Information

Figure S1 Resistance to proteolysis of different prion strains in sheep. PrPres from brain homogenates of sheep infected with classical scrapie, experimental cBSE, or atypical Nor-98 scrapie, and of an uninfected control sheep. Samples were purified using low (odd lanes) or high (even lanes) concentrations of proteinase K, and visualized with monoclonal antibodies that recognize either the core region (Panel A) or the octapeptide region (Panel B) of the protein. With the lower concentration of PK used in the purification step (in order to maximize test sensitivity) of one widely utilized BSE screening test [13], all three strains gave a positive result with both the anti-core and anti-octapeptide antibodies (odd lanes). Using a higher concentration of PK (even lanes) did not alter the positivity with either antibody for classical scrapie, but the cBSE strain no longer reacted with the anti-octapeptide antibody while Nor98 did not react with either antibody. Thus, by using the higher concentration of PK and two different antibodies, it is possible to discriminate between all three strains.

Found at: doi:10.1371/journal.pone.0003017.s001 (2.51 MB TIF)

Figure S2 Lesion profiles in cBSE- and BASE-infected macaque, and in MM2 sporadic CJD patients. The lesions were scored from 0 to 4 (negative, light, mild, moderate, and severe) for the different following gray matter regions: frontal (FC), temporal (TC), parietal (PC) and occipital (OC) neocortices, hippocampus (HI), parasubiculum and entorhinal cortex (EC), neostriatum (ST) (nuclei caudatus and putamen), thalamus (TH), substantia nigra (SN), midbrain periventricular gray (PG), locus ceruleus (LC), medulla (ME) (periventricular gray and inferior olive), and cerebellum (CE). Scoring for MME sCJD patient was issued from Parchi et al. [7].

Found at: doi:10.1371/journal.pone.0003017.s002 (1.52 MB TIF)

Acknowledgments

We thank Sebastien Jacquin for careful maintenance of primates, and Aurore Jolit for her precious help for histological studies; we thank Richard Kascsak (New York State Institute for Basic Research in Developmental Disabilities), Jacques Grassi, Eric Quemeneur and their colleagues (CEA/DSV) who provided the anti-PrP antibodies. We thank Sylvie Benestad (National Veterinary Institute, Oslo, Norway) for Nor-98 sheep samples, and Danny Matthews (VLA, Weybridge, UK) for the sheep sample inoculated with experimental cBSE.

Author Contributions

Conceived and designed the experiments: CC CIL. Performed the experiments: NLE SF DM FA. Analyzed the data: EEC NLE MMR SF JPD. Contributed reagents/materials/analysis tools: CC GZ MMR MC. Wrote the paper: EEC GZ PB JPD. Participated to the final reviewing of the manuscript: CC SM NS MC PL CIL.

References

1. Will RG, Ironside JW, Zeidler M, Cousens SN, Estibeiro K, et al. (1996) A new variant of Creutzfeldt-Jakob disease in the UK. Lancet 347: 921–925.
2. Casalone C, Zanusso G, Acutis P, Ferrari S, Capucci L, et al. (2004) Identification of a second bovine amyloidotic spongiform encephalopathy: molecular similarities with sporadic Creutzfeldt-Jakob disease. Proc Natl Acad Sci USA 101: 3065–3070.
3. Biacabe AG, Laplanche JL, Ryder S, Baron T (2004) Distinct molecular phenotypes in bovine prion diseases. EMBO 5: 110–114.
4. Brown P, McShane L, Zanusso G, Detwiler L (2006) On the question of sporadic or atypical bovine spongiform encephalopathy and Creutzfeldt-Jakob disease. Emerg Infect Dis 12: 1816–1821.
5. Buschmann A, Gretzschel A, Biacabe AG, Schiebel K, Corona C, et al. (2006) Atypical BSE in Germany – Proof of transmissibility and biochemical characterization. Vet Microbiol 117: 103–116.
6. Capobianco R, Casalone C, Suardi S, Mangieri M, Miccolo C, et al. (2007) Conversion of the BASE prion strain into the BSE strain: the origin of BSE? PLoS Pathog; 3: e31. Doi:10.1371/journal.ppat.0030031.
7. Parchi P, Giese A, Capellari S, Brown P, Schulz-Schaeffer W, et al. (1999) Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. Ann Neurol 46: 224–233.
8. Collinge J, Whitfield J, McKintosh E, Beck J, Mead S, et al. (2006) Kuru in the 21st century – an acquired human prion disease with very long incubation periods. Lancet 367: 2068–2073.
9. Lasmézas CI, Deslys JP, Demaimay R, Adjou KT, Lamoury F, et al. (1996) BSE transmission to macaques. Nature 381: 743–744.
10. Lasmézas CI, Fournier JG, Nouvel V, Boe H, Marce D, et al. (2001) Adaptation of the bovine spongiform encephalopathy agent to primates and comparison with Creutzfeldt-Jakob disease: implications from human health. Proc Natl Acad Sci USA 98: 4142–4147.
11. Herzog C, Riviere J, Lescoutra-Etchegaray N, Charbonnier A, Leblanc V, et al. (2005) PrP^{TSE} distribution in a primate model of variant, sporadic, and iatrogenic Creutzfeldt-Jakob disease. J Virol 79: 14339–14345.
12. Herzog C, Salès N, Etchegaray N, Charbonnier A, Freire S, et al. (2004) Tissue distribution of bovine spongiform encephalopathy agent in primates after intravenous or oral infection. Lancet 363: 422–428.
13. Deslys JP, Comoy E, Hawkins S, Simon S, Schimmel H, et al. (2001) Screening slaughtered cattle for BSE. Nature 409: 476–477.
14. Deslys JP, Comoy E, Grassi J. Method for diagnosing a transmissible spongiform subacute encephalopathy caused by an unconventional transmissible agent strain in a biological sample. Patent N°99/14242, 12 November 1999.
15. Eloit M, Adjou K, Coulpier M, Fontaine JJ, Hamel R, et al. (2005) BSE agent signatures in a goat. Vet Rec 156: 523–524.
16. Gambetti P, Kong, Zou W, Parchi P, Chen SG (2003) Sporadic and familial CJD: classification and characterization. Brit Med Bull 66: 213–239.
17. Yutzy B, Holznagel E, Coulibaly C, Stuke A, Hahmann U, et al. (2007) Time-course studies of 14-3-3 protein isoforms in cerebrospinal fluid and brain of primates after oral or intracerebral infection with bovine spongiform encephalopathy agent. J Gen Virol 88: 3469–3478.
18. Kong Q, Zheng M, Casalone C, Qing L, Huang S, et al. (2008) Evaluation of the human transmission risk of an atypical bovine spongiform encephalopathy prion strain. J Virol 82: 3697–3701.
19. Benestad SL, Sarradin P, Thu B, Schonheit J, Tranulis MA, et al. (2003) Cases of scrapie with unusual features in Norway and designation of a new type, Nor98. Vet Rec 153: 202–208.
20. Le Dur A, Beringue V, Andreoletti O, Reine F, Lai TL, et al. (2005) A newly identified type of scrapie agent can naturally infect sheep with resistant PrP genotypes. Proc Natl Acad Sci USA 102: 16031–16036.
21. Brown P, Gibbs CJ Jr, Rodgers-Johnson P, Asher DM, Sulima MP, et al. (1994) Human spongiform encephalopathy: the National Institutes of Health series of 300 cases of experimentally transmitted disease. Ann Neurol 35: 513–529.
22. Williams L, Brown P, Ironside J, Gibson S, Will R, et al. (2007) Clinical, neuropathological and immunohistochemical features of sporadic and variant forms of Creutzfeldt-Jakob disease in the squirrel monkey (Saimiri sciureus). J Gen Virol 88: 688–695.
23. Krasnianski A, Meissner B, Schulz-Schaeffer W, Kallenberg K, Bartl M, et al. (2006) Clinical features and diagnosis of the MM2 cortical subtype of sporadic Creutzfeldt-Jakob disease. Arch Neurol 63: 876–880.
24. Puoti G, Giaccone G, Rossi G, Canciani B, Bugiani O, et al. (1999) Sporadic Creutzfeldt-Jakob disease: co-occurrence of different types of PrPSc in the same brain. Neurology 53: 2173–2176.
25. Polymenidou M, Stoeck K, Glatzel J, Vey M, Bellon A, et al. (2005) Coexistence of multiple PrP^{Sc} types in individuals with Creutzfeldt-Jakob disease. Lancet Neurol 4: 805–814.
26. Yull HM, Ritchie DL, Langeveld JPM, van Zijderveld FG, Bruce ME, et al. (2006) Detection of type 1 prion protein in variant Creutzfeldt-Jakob disease. Amer J Pathol 168: 151–157.

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-4

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2008 年 5 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	Estimating the pathogen safety of manufactured human plasma products: application to fibrin sealants and to thrombin. Horowitz, B. and Busch, M. Transfusion, 48, 1739-53 (2008)	公表国 米国	
販売名（企業名）						

研究報告の概要

本稿では，米国で最近上市された 2 種類の血液製剤（トロンビン及びフィブリノゲン）を用いて，パルボウイルス B19（B19V），A 型，B 型及び C 型肝炎，HIV ならびに変異型クロイツフェルト・ヤコブ病（vCJD）関連プリオンの病原体感染リスクを評価した。特に，A 型肝炎ウイルス（HAV）及び B19V は，エンベロープを持たないため不活化がより困難であることから本試験の対象とした。これら血液製剤の製造過程では 2 つの異なるウイルス除去工程が使われている。各病原体の安全域は，出発原料内の最大ウイルス量と製造工程の除去能との比較によって決定した。フィブリノゲン及びトロンビンは，ともに 1 バイアル当たりの病原菌が伝播する残遺リスクが極めて低く，A 型，B 型及び C 型肝炎及び HIV については 10^{15} 分の 1 未満と算出された。B19V については，トロンビンが 1000 万分の 1 未満，フィブリノゲンが 50 万分の 1 未満と算出された。同様に，vCJD の病原体伝播リスクも非常に低いと推定された。新型ウイルス（西ナイルウイルス，H5N1 型インフルエンザウイルス，重症急性呼吸器症候群（SARS）ウイルス又はチクングニヤウイルス―いずれもエンベロープを持つ）の脅威に関しては，現在の製造工程が完全な不活化をもたらすことがわかっている。したがって，血漿製造業界は，血液製剤の安全性を増強するために過去 10～20 年に実施された多くの改善点により，優良な安全性プロファイルをもつ製品の製造し，提供していると著者らは結論付けた。

報告企業の意見	今後の対応
著名な 2 研究機関が発表した本研究では，新たな脅威として現れたエンベロープウイルスに対する完全不活化法や非エンベロープウイルスやプリオンに対する実質的除去法など，病原体不活化法が常に改良され続けてきたことを確認している。したがって，実質的リスクが存在する血液製剤と違い，血漿分画製剤における病原体伝播リスクは極めて低いと考えられる。	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。

使用上の注意記載状況・その他参考事項等

BYL-2008-0323

37

Estimating the pathogen safety of manufactured human plasma products: application to fibrin sealants and to thrombin

Bernard Horowitz and Michael Busch

BACKGROUND: Plasma fractionators have implemented many improvements over the past decade directed toward reducing the likelihood of pathogen transmission by purified blood products, yet little has been published attempting to assess the overall impact of these improvements on the probability of safety of the final product.

STUDY DESIGN AND METHODS: Safety margins for human immunodeficiency virus (HIV), hepatitis C virus (HCV), hepatitis B virus (HBV), hepatitis A virus (HAV), parvovirus B19, and variant form of Creutzfeldt-Jakob disease (vCJD) were calculated for the two fibrin sealants licensed in the United States and for thrombin. These products were selected because their use in a clinical setting is, in most cases, optional, and both were relatively recently approved for marketing by the US Food and Drug Administration (FDA). Moreover, thrombin and fibrinogen both undergo two dedicated virus inactivation steps and/or removal steps in accord with the recommendations of regulatory agencies worldwide. Safety margins were determined by comparing the potential maximum viral loads in contaminated units to viral clearance factors, ultimately leading to the calculation of the residual risk per vial.

RESULTS: The residual risk of pathogen transmission per vial was calculated to be less than 1 in 10^{-15} for HIV, HCV, HBV, and HAV for both fibrinogen and thrombin. Owing to the greater quantities that can be present and its greater thermal stability, the calculated risk for parvovirus transmission was 1 in 500,000 vials for fibrinogen and less than 1 in 10^7 per vial for thrombin. Assuming that vCJD is found to be present in plasma donations, its risk of transmission by these purified and processed plasma derivatives would appear to be very low.

CONCLUSIONS: The pathogen safety initiatives implemented by plasma fractionators over the past 10 to 20 years have resulted in products with excellent pathogen safety profiles. Of the agents examined, parvovirus continues to have the lowest calculated margin of safety. Despite this, parvovirus transmissions should be rare. Manufacturers are encouraged to continue exploring processes to further enlarge parvovirus safety margins and to continue exploring ways of eliminating prions.

Products derived from human plasma have important therapeutic uses, including substitution therapy for hemophilia and primary immune deficiency disorders, plasma expanders after trauma and surgery, and as hemostatic agents.[1-3] Plasma proteins and their functions are so diverse that new applications for currently licensed plasma protein products continue to be investigated[4] and novel plasma protein products continue to be developed.[5-7] Consequently, there has been an increase in the quantity of plasma processed worldwide, and significant improvements have been made in manufacturing procedures and in plant design and operation. Many of these improvements were implemented with the goal of assuring safety of plasma derivatives from transfusion-transmissible pathogens. These include 1) improved selection of donors, 2) use of plasma only from "qualified" donors who repeatedly pass viral screening procedures, 3) use of nucleic acid amplification testing (NAT) methods to detect and eliminate virus before the pooling of donor units, 4) inventory hold policies that allow interdiction of "window-phase"

ABBREVIATIONS: BVDV = bovine viral diarrhea virus; HAV = hepatitis A virus; ID(s) = infectious dose(s); PRV = pseudorabies virus; SARS = severe acute respiratory syndrome; S/D = solvent/detergent (method of virus inactivation); TNBP = tri-(*n*-butyl)phosphate (the solvent in S/D treatment); vCJD = variant form of Creutzfeldt-Jakob disease that infects man and presumptively has arisen from the epidemic of bovine spongiform encephalopathy in cattle; WNV = West Nile virus.

From Horowitz Consultants, LLC, Key Biscayne, Florida; and Blood Systems Research Institute and University of California at San Francisco, San Francisco, California.

Address reprint requests to: Bernard Horowitz, PhD, 785 Crandon Boulevard, Unit 1204, Key Biscayne, FL 33149; e-mail: horowitzconsult@aol.com.

Supported in part by Johnson and Johnson, Inc.

Received for publication November 1, 2007; revision received January 24, 2008, and accepted January 27, 2008.

doi: 10.1111/j.1537-2995.2008.01717.x

TRANSFUSION **;**:**-**.

units before pooling, 5) employment of purification procedures shown to remove virus or prions should they be present, 6) the use of two complementary or "orthogonal" methods of virus inactivation, and 7) the engineering and design of facilities so as to prevent contamination of downstream process streams with upstream fractions.

Products that promote hemostasis and tissue sealing following trauma and surgery are among the more recently licensed human plasma products in the United States. Two fibrin sealants, one from Omrix (New York, NY) and one from Baxter (Deerfield, IL), are licensed in the United States by the Food and Drug Administration (FDA), and Omrix also recently received approval of a topically applied thrombin. While they cannot be used in all surgical settings, such as to control high-pressure (arterial) bleeds, these products have been shown to improve surgical outcomes, reduce the time to hemostasis, reduce blood loss, and reduce surgical complications.[8] Substitutes for these human plasma-derived hemostatic agents have also been developed, including bovine thrombin and recombinant-derived human thrombin. Bovine thrombin is antigenically distinct from human thrombin and has been shown to elicit antibodies when used in man.[9] These antibodies, as well as antibodies elicited to bovine impurities in the product, especially antibodies to coagulation factor (F)V, have resulted in severe bleeding complications due to cross-reaction with their human counterparts.[10-13] Higher purification has reduced this complication, although a recent report[14] indicates that antibody formation still occurs. Products made by recombinant technology have their own, somewhat unique, issues. Depending on the gene construct used and the cell line chosen, the amino acid sequence may differ from that which occurs naturally, and differences in posttranscriptional processing often result in altered patterns of glycosylation or other molecular changes.[15-19] Consequently, immunogenicity is a potential problem that needs to be continually assessed. Also, depending on specific production details, manufacturing procedures must employ steps designed to inactivate and/or remove viral contaminants (and other potential pathogens) known to be present in the cell line and/or in the culture medium employed.[20] Additionally, in many circumstances, the higher cost associated with recombinant proteins limits their use.

In the past decade, many estimates of the viral safety of transfused whole blood and its components (i.e., red blood cells, platelet concentrates, and fresh-frozen plasma) have been published, with each passing year showing improved viral and bacterial safety.[21,22] In the same time frame, aside from monitoring clinical outcomes and despite the aforementioned improvements, little has been published to assess the parallel increase in safety of manufactured plasma products. A recent publication by Janssen and colleagues[23] used a probabilistic, Monte Carlo model to estimate the risk of human immunodeficiency virus (HIV) and hepatitis B virus (HBV) in a hypothetical plasma derivative subjected to what appears to be a single method of viral inactivation. Based on their assumptions, they calculated that the risk per vial approximated 1 in 1 million. Given these improvements, the recent licensure of human plasma-derived topical thrombin, and the frequent surgical use of fibrin sealants, consisting of fibrinogen in addition to thrombin, it is timely to estimate their pathogen safety. These estimates are especially useful since the fibrinogen component of fibrin sealants is among the least processed blood derivatives, while the manufacturing procedures for thrombin, whether part of a fibrin sealant kit or used by itself, are typical of those employed with most newer plasma derivatives. Thrombin and fibrinogen both undergo two dedicated virus inactivation steps and/or removal steps. Fibrinogen and thrombin from each company are each treated by solvent/detergent (S/D). Additionally, Omrix pasteurizes its fibrinogen and nanofilters its thrombin, while Baxter vapor heats both components following lyophilization. The fibrinogen preparation cannot be nanofiltered without suffering large losses in fibrinogen and fibronectin due to their large size. The presence of fibronectin may be important since it contributes to cell adhesion.[24] For HIV, hepatitis C virus (HCV), and HBV, this report updates estimates made by one of us (BH) in 1990[25] using better information on viral loads than was available then and enlarges the pathogen list to include hepatitis A virus (HAV) and parvovirus, both of which are nonenveloped viruses, and the prion that causes variant form of Creutzfeldt-Jakob disease (vCJD). It is anticipated that the method of approach reported here can be applied to other existing or experimental blood protein products.

MATERIALS AND METHODS

Two distinct methods can be used to calculate pathogen safety. The first is to calculate safety margins by comparing the number of infectious units or doses of pathogen in the starting material to the clearance capacity of the manufacturing process. The second is to measure clinical outcomes, comparing the incidence of transmission to the quantity of product infused. Transmissions in a clinical setting should be considered the gold standard since they involve actually measuring what we want to know, and with this information, one can back-calculate clearance capacities of processes for known pathogen burdens. On the other hand, clinical studies of the type required can be extremely lengthy and expensive and the results possibly misleading. The former method has the advantage that estimates of safety can be made in advance of clinical testing. Moreover, the safety margins calculated for a wide range of viruses likely will also be applicable to unstudied and newly emerging viruses.

Product safety margins can be calculated by comparing potential viral loads with the viral clearance capacity by the formulas

$$VL = N \times C,$$

where VL is viral load, N is the number of units in a plasma pool containing infectious virus, and C is the concentration of virus in those units, and

$$\text{Safety Margin} = CC/VL,$$

where CC is the clearance capacity or the ability of the process to remove or inactivate the infectious agent being studied.

Viral load

The pathogens of interest for manufactured plasma products are largely viruses that are present in blood predominantly as cell-free virions (e.g., HBV, HCV, HIV, HAV, and parvovirus B19). Other examples include West Nile virus (WNV) and dengue viruses. The newly described vCJD agent, presumably a prion, is also a potential concern despite the absence of evidence that it is transmitted by purified plasma protein products.[26,27] Cell-associated viruses like cytomegalovirus, Epstein-Barr virus, and human herpes virus 8 are not a concern since infected cells are removed by the apheresis and filtration procedures in common use. Bacteria and fungi are also effectively removed by the terminal sterile filtrations applied to all biologic products, including plasma products, recombinant products, monoclonal antibodies, and so forth, and therefore will not be addressed here.

For the major transfusion-transmitted viral pathogens, the viral loads are typically measured as genome-equivalents (geq) per mL of plasma based on results of quantitative NAT. These loads vary dramatically during the progressive stages of infection with the highest viral loads seen transiently during the acute preseroconversion (i.e., so-called window period) stage of infection; moreover, infectiousness is also highest during this same period.[28-30] Subsequent to antibody seroconversion (and coincident with innate and adaptive cellular immune responses to infection), the agents are 1) eliminated from the body (e.g., eradication of infection, as occurs with WNV, HAV, and dengue); 2) cleared from plasma but with persistence of cell-associated virus in tissues (e.g., latent infections such as herpes viruses, parvovirus B19, and "occult" HBV infections); or 3) persistent at reduced concentrations in plasma (i.e., so-called set-point viremia after establishment of chronic HBV, HCV, and HIV infections). In addition to variations in viral load measured by NAT, as infections evolve the infectivity of viruses change profoundly.[31-39] For HIV (and its model agent simian immunodeficiency virus), HBV, HCV, and WNV, it is now well established that during the acute preseroconversion phase of infection (pre–ramp-up and ramp-up stages), virion particles in plasma are highly infectious, with 10 or fewer geq in the entire volume of plasma sufficient to transmit infection following parenteral injection. In contrast, viral particles present in plasma from the same infected individuals have significantly (10- to 1000-fold) reduced infectious potential weeks to years after seroconversion.[29-35,40-43] The reduced infectivity of plasma virus from postseroconversion phases of chronic infection is attributable to a combination of factors, including presence of endogenous neutralizing antibodies, generation of defective virions (i.e., lacking full genomes or other required infectivity factors), and immune selection of virions with reduced fitness. Hence, viral load distributions observed during acute versus chronic stages of infection need to be adjusted by a factor to account for the relative infectivity of virion particles to derive estimates for the functional viral load during each stage.

All donated blood in the United States, whether for the preparation of components or for use in manufactured plasma products, is screened by serologic assays for HIV-1 and -2, HBV, HCV, and human lymphotropic virus-1 and -2 and by NAT for HIV and HCV. Donors are also excluded if they have certain risk factors that make their exposure to viruses or prions more likely. Additionally, plasma manufacturers screen donated plasma in a minipool format for HBV, HAV, and parvovirus by NAT. The use of NAT greatly reduces viral loads since positive units missed by serologic screening procedures typically have the highest concentrations of virus, which is also highly infectious. Consequently, with very rare exceptions of concordant testing errors in serology and NAT screening, only units that test both serologically negative (i.e., window-phase units) and that have relatively low titers of infectious virus (<500-5000 infectious doses [IDs]/mL) are pooled. Furthermore, manufacturing pools are retested by NAT before fractionation to assure that high-titer viremic units were not missed as a result of erroneous testing. As a result, the probability that a fractionation pool contains a significant level of virus is extraordinarily remote.

Pathogen infectious load estimates are given in Table 1. To estimate the number of positive units missed by the screening procedures employed, currently observed NAT yields, expressed as number of positive samples per million donations, were adjusted to account for the amount the window period is believed to be closed through the use of NAT. From this, we conclude that few plasma pools will contain HIV, HCV, or HAV while contamination by HBV and parvovirus B19 will be considerably more frequent. Based on the analytical sensitivity of the NAT assays, the dilution factors during assay, and the volume of an individual donor unit, we calculated the

TABLE 1. Viral load estimates

Virus	NAT yield (number/million donations)* (A)	Percent NAT closes window period† (B)	Number of positive units missed by NAT/million donations (C) = (A/B – A)	NAT analytic sensitivity‡ (geq/ml)	NAT operational sensitivity‡ (geq/mL in the donor unit)	Maximum genomic load (log geq/ fractionation pool)§	Ratio of IDs to geq¶	Maximum viral load (log ID/pool)
HIV	0.58	48% (11/23)	0.63	1.40	717	5.7	1:1	5.7
HCV	4.08	88% (50/57)	0.56	3.10	1,587	6.0	1:1	6.0
HBV	13	23% (10/43)	44	0.66	338	5.4	1:10	4.4
HAV	0.30	71% (5/7)	0.12	2.0	1,024	5.9	1:1,000	2.9
Parvovirus B19	50.5	71% (5/7)	20.6	22.6	5,120,000	9.6	1:1,000	6.6
vCJD						4.3	1:1	4.3

* For HIV, HCV, and HBV, a NAT yield unit is defined as an antibody- or hepatitis B surface antigen (HBsAg)-negative donation detected by RNA and/or DNA screening using pooled NAT systems. Rates presented are published rates from United States and European whole-blood donor screening programs.[75-78] Although NAT yield rates for these viruses among source plasma donors are higher, this is offset by source plasma policies that stipulate that only plasma from "qualified donors" be released for fractionation and that frozen units be held in inventory enabling interdiction of quarantined potential window-phase units when donors later test reactive for infectious disease markers or are deferred for other reasons.[79] For HAV and parvovirus B19, we use the rate of detection of high titer viremic donations by low sensitivity NAT screening of whole-blood and plasma donors, irrespective of serostatus of viremic units.[80,81]

† The percentage is determined by dividing the number of days NAT detects positive samples by the number of days from when a donor becomes infectious until there is sufficient antibody to be detected serologically (HIV, HCV, HBV) or there is sufficient antibody to render the donation noninfectious (HAV, parvovirus; see Busch et al.[82] for conceptual basis for this approach and Kleinman and Busch[83] for application of this approach to HBV). The residual infectious window periods are defined as the number of days from viremia reaching the minimal infectious threshold (set as 1 copy per 20 mL of plasma; Busch et al.[84]) to the level of viremia detected by pooled-sample NAT, using the viral doubling-times during the acute ramp-up phases established for each agent (20.5 hr for HIV, 10.8 hr for HCV, 2.6 days for HBV, and approx. 1 day for HAV and parvovirus B19). This yielded pre-NAT infectious window periods of 12 days for HIV, 7 days for HCV, 33 days for HBV, and 2 days for HAV and parvovirus B19. The NAT detection windows are based on time from reaching the 50 percent sensitivity of the NAT screening assays to the point of seroconversion for HIV (11 days), HCV (50 days), and HBV (10 days) or the duration of the estimated NAT yield window period for HAV (5 days) and parvovirus B19 (5 days).

‡ We assumed that the 50 percent sensitivity levels for assays used by source plasma donors are in the same range as those reported by the National Genetics Institute (NGI). NGI NAT assays are used by approximately 60 percent of the source plasma sector for all five viruses, as well as by the American Red Cross for HAV and parvovirus B19. For HIV-1, HBV, and HCV, the analytic sensitivity quoted is that of the assay itself without taking sample dilutions or pooling into account (Schreiber et al.[79]). Operational sensitivity takes these dilutions into account and refers to the maximum quantity that could be present in the contaminated donor unit. For parvovirus, Omrix's acceptance requirement for a pool of 512 units is less than 10,000 geq per mL, and thus for operational sensitivity we used $10,000 \times 512$ (the number of units in the minipool).

§ NAT operational sensitivity was multiplied by 700, the assumed volume of the donation. Based on the number of units missed by NAT per million donations and a pool size of 6000 L, we assume that only one positive unit will enter a fractionation pool. For vCJD, we assumed 30 ID per mL in a contaminated unit.

¶ For HIV and HCV, the infectious load is considered to be equivalent to the viral load expressed in geq or copies, given that we are restricting consideration to the acute preseroconversion viremic phase, which is known to be highly infectious (see text), and that seropositive units from other donors, which might contain neutralizing antibodies, have been detected by serologic screening and excluded from the manufacturing pools. For HBV, we similarly assume high-level infectivity of window-phase donations[35,85] but reduce this to a ratio of 1 in 10, in part, because of the likely presence of anti-HBsAg in the plasma pool. We used a ratio of 1:1000 for HAV and parvovirus. We believe this to be justified since the neutralization capacity of anti-HAV is well established. While the ratio of infectious units to geq for parvovirus is unknown, results from tissue culture infectivity studies indicate that the ratio is 1:5000 for genotype 1 and 1:260,000 for genotype 2[86] for products devoid of parvovirus antibody, the lowest ID that has been reported on infusion into a seronegative recipient is 2×10^4 geq,[70] and the infectivity of products containing parvovirus antibody has been shown to be reduced considerably.[83,87,88]

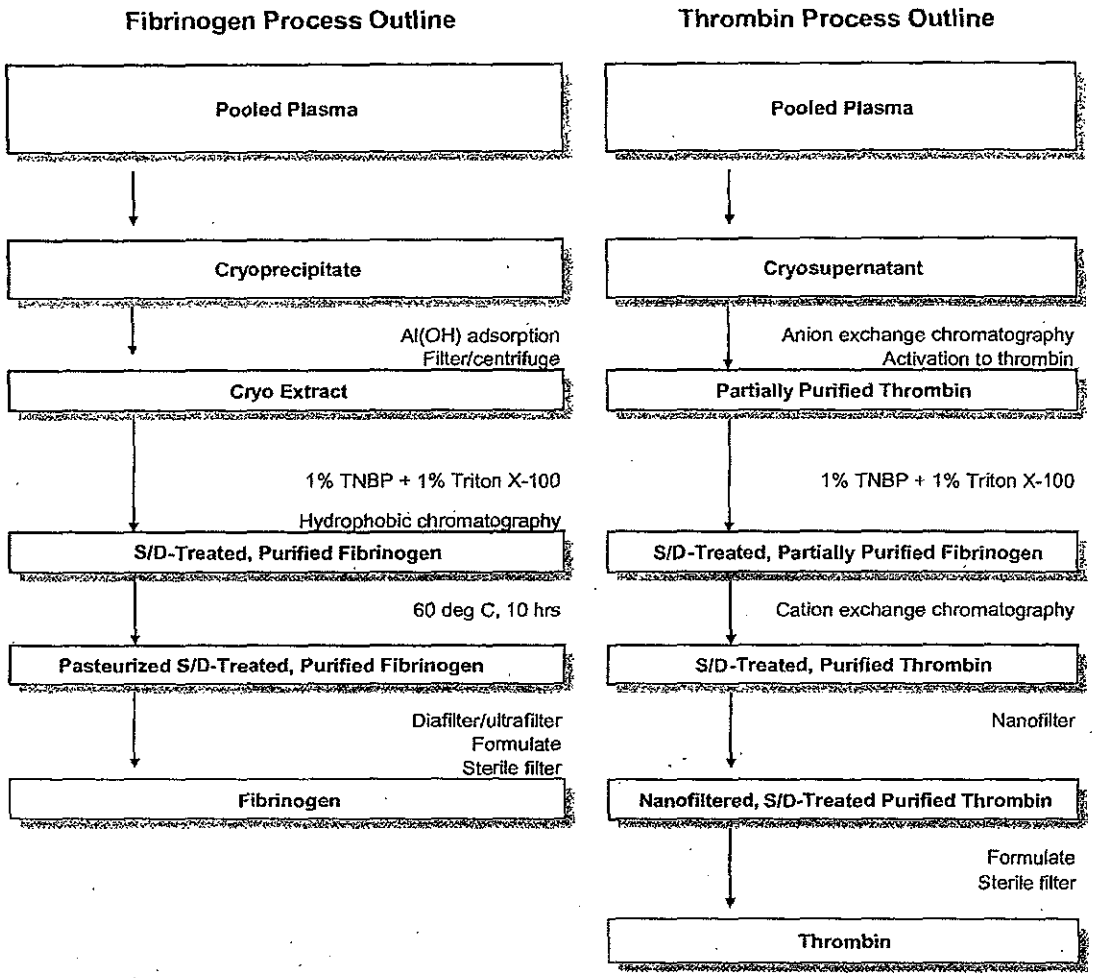


Fig. 1. Process outlines for fibrinogen and thrombin.

maximum genomic load likely to be present in a fractionation pool (Table 1, Column 7). This was adjusted downward for HBV, HAV, and parvovirus B19 to account for the reduced infectivity of virus that occurs as a result of neutralizing antibodies derived from other donors in the pool, since antibody screening is not performed for these prevalent agents. No adjustment was applied to HIV or HCV since later-stage infections with potential neutralizing antibodies are interdicted by the currently deployed serologic tests. Although attempts to transmit vCJD by human plasma have failed,[44] epidemiologic evidence supports its transmission by whole blood and blood components.[45-47] Based on animal models, its concentration is likely to be quite low, estimated at 20 to 30 IDs per mL.[48] With the use of this estimate, the maximum concentration in the plasma pool approximates 0.003 IDs per mL (3 IDs/L), and the total maximum load in a 6000-L plasma pool will approximate $10^{4.3}$ IDs (Table 1).

Clearance capacity

The clearance capacity for pathogens is a function of the extent to which pathogen is removed during steps designed to purify the protein of interest, the inclusion within the manufacturing process of dedicated viral inactivation and removal steps, the presence of neutralizing antibody in final product, and serendipitous inactivation that occurs. The process steps for fibrinogen and thrombin used by Omrix are outlined in Fig. 1. As is typical of modern plasma protein products, each process includes two dedicated, viral elimination steps: fibrinogen is treated with S/D and is pasteurized, and thrombin is treated with S/D and is passed through a purposefully designed, virus removal filter (so-called nanofiltration). Additionally, each chromatographic step and filtration in the presence of filter aid can contribute to pathogen removal. Baxter's Tisseel is processed similarly except, when first introduced in 1998, it utilized vapor heating as

TABLE 2. Validated viral elimination when processing fibrinogen: Omrix process

		Log kill or removal				
	Virus:	HIV-1	BVDV	PRV	HAV	CPV
	Model for:	HIV	HCV	HBV	HAV	Parvovirus B19
Step	Enveloped virus?:	Yes	Yes	Yes	No	No
Cryoprecipitation + Al(OH) treatment		ND	ND	ND	1.5	1.5
S/D treatment		>4.4*	>4.4†	>4.4†	ND	0
Pasteurization		>4.4‡	>5.5‡	ND	>5.8‡	1.3
	Sum:	>8.8	>9.9	>4.0	>7.3	2.8

* No infectivity after 5 minutes. Treatment is for 4 hours.
† No infectivity after 10 minutes, the first time point taken.
‡ 9 to 10 hours were required to achieve reported kills. Treatment is for 10 hours.
Al(OH) = aluminum hydroxide; CPV = canine parvovirus; ND = not done.

TABLE 3. Validated viral elimination when processing thrombin: Omrix process

		Log kill or removal						
	Virus:	HIV-1	BVDV	Sindbis	PRV	EMCV	CPV	MMV
	Model for:	HIV	HCV	HCV	HBV	HAV	Parvovirus B19	Parvovirus B19
Step	Enveloped virus?:	Yes	Yes	Yes	Yes	No	No	No
Cryo removal		ND	ND	ND	ND	ND	ND	ND
Anion-exchange chromatography		ND	ND	ND	ND	ND	ND	ND
S/D treatment		>5.8*	>4.7†	>5.3‡	>4.3†	ND	0	ND
Cation-exchange chromatography		ND	ND	ND	ND	ND	ND	ND
Nanofiltration		>4.4	ND	>5.3	>5.5	7.0	5.9	5.8
	Sum:	>10.2	>4.7	>10.6	>9.8	7.0	5.9	5.8

* No infectivity after 5 minutes. Treatment is for 6 hours for thrombin.
† No infectivity after 10 minutes, the first time point taken.
‡ No infectivity after 15 minutes, the first time point taken.
EMCV = encephalomyocarditis virus; MMV = mouse minute virus; ND = not done.

its sole, dedicated virus inactivation step; S/D treatment has been added recently. Another difference is that its thrombin component is isolated starting with Baxter's activated prothrombin factor complex.

The FDA and other applicable regulatory authorities demand that formal viral inactivation and/or removal studies be performed and that these adhere to international standards as they relate to the selection of viruses to be used, the conduct of these studies under Good Laboratory Practice guidelines and the calculations provided. We need not reiterate those guidelines here, except to say that the model viruses selected were chosen to represent multiple viral types and, in particular, the viruses of concern for products derived from human blood. Thus, viral elimination studies typically use HIV, bovine viral diarrhea virus (BVDV; model for HCV), pseudorabies virus (PRV; model for HBV), HAV or another picornovirus such as encephalomyocarditis virus, and canine parvovirus (or another model for human parvovirus B19).

The results from these formal studies for the fibrinogen and thrombin components of Omrix's and Baxter's fibrin sealant products are given in Tables 2 and 3 and Table 4, respectively (see product package inserts, with updates from manufacturers; see Acknowledgments). The clearance factors for enveloped viruses and the models for HIV, HCV, and HBV exceed the challenge dose for each of the dedicated viral elimination steps (i.e., S/D, pasteurization, nanofiltration, and vapor heating). Consequently, when the same virus has been studied in each of the two dedicated steps, the validated clearance factors exceed 9 log, and where higher doses of virus have been used or more steps validated, clearance factors as large as 18 log have been reported. The validated clearance of nonenveloped viruses is significantly less than for enveloped viruses since only one of the two dedicated viral elimination methods is effective against these viruses. Parvoviruses are a special case since they are especially heat-stable, and only 1 to 2 log of animal parvoviruses are inactivated by either pasteurization or vapor heating. It should be noted, however, that human parvovirus B19 may be more heat-sensitive than the models used here.[49] Nanofiltration is significantly more effective, and Omrix has shown for its thrombin preparation that nanofiltration removes approximately 6 log of parvoviruses.

A more complete estimate of safety margin needs to take into account the contribution of the other steps in the process that contribute to safety despite not being formally validated. It is commonly accepted that immune neutralization contributes to HAV and parvovirus B19 safety and that the neutralization capacity of antibodies to

TABLE 4. Validated viral elimination when processing fibrinogen and thrombin: Baxter process

		Log kill or removal				
	Virus:	HIV-1	BVDV or TBEV	PRV	HAV or ERV	MMV
	Model for:	HIV	HCV	HBV	HAV	Parvovirus B19
Process and step	Enveloped virus?:	Yes	Yes	Yes	No	No
Fibrinogen						
Cryoprecipitation + wash		2.6	1.3	1.5	1.8	ND
Lyophilization + vapor heating		>6.2	>6.8	>7.1	>6.5	1.5
S/D treatment		>6.6	>6.5	>6.7	NA	NA
	Sum:	>15.4	>14.6	>15.3	>8.3	>1.5
Thrombin						
Cryoprecipitation + wash		1.4	ND	1.1	ND	ND
Anion-exchange chromatography		2	ND	3.1	ND	ND
Lyophilization + vapor heating		>5.3	>5.9	>7.0	>4.7	1.0
S/D treatment		>5.2	>6.0	>6.9	NA	NA
	Sum:	>13.9	>11.9	>18.1	>4.7	1.0

MMV = mouse minute virus; NA = not applicable; ND = not done.

TABLE 5. Assignment of additional virucidal activity based on reserve capacity

Time required for complete kill (% of total)	Estimated minimal additional cidal power (log)
>100	0
76-100	1
51-75	2
26-50	3
≤25	4

these viruses is at least 3 to 4 log.[50] Since fibrinogen is purified by simple precipitations, it, like intermediate-purity FVIII preparations, likely benefits from the copresence of antibody in the final preparation.[51] Ion-exchange chromatography typically removes 2 to 3 log of virus.[52-55] Finally, some contribution to the calculation of safety margins should be ascribed to the "reserve capacity" of the viral inactivation method(s) employed, defined as the ability to achieve complete virus kill in a fraction of the treatment time allotted. While numerous publications make clear that linear extrapolation of virus inactivation curves overstates inactivation potential,[56,57] assigning no benefit to reserve capacity when calculating safety margins clearly underestimates inactivation capacity. Unless data indicate otherwise, we propose adopting the scheme described in Table 5. Although seemingly arbitrary, this scheme has the value of simplicity. Its use is supported by the dozens if not hundreds of times results with S/D and heat treatment methods have been reproduced, thereby increasing the quantity of virus subjected to challenge. Also, for S/D treatment methods, viral kill has been shown to be complete even when using tri-(*n*-butyl)phosphate (TNBP) together with sodium cholate, a combination that provides far slower kill kinetics than the more frequently employed TNBP-Tween 80 or TNBP-Triton X-100 combinations (Fig. 2), and the fact that reagent concentration can be halved without affecting viral kill (data not shown). We have not made reserve capacity estimates for vapor heating since much of the loss in viral infectivity occurs before initiation of the heat cycle (Fig. 3).

Taking these factors into account, along with published information on the inactivation of HIV, HCV, and HBV,[58] for fibrinogen, we estimate that 15 to 17 log of enveloped viruses, 10 to 11.5 log of HAV or most other nonenveloped viruses, and 7 log of parvovirus are eliminated. For thrombin, we estimate that 17 to 22 log of enveloped viruses, 11 to 13 log of HAV, and 7 to 10 log of parvoviruses are eliminated (Table 6).

Calculation of safety margin

The calculated margins of safety are given in Table 7. For enveloped viruses, safety margins are exceedingly large, estimated at about 100 billion-fold for fibrinogen and 1 trillion-fold for thrombin. Although lower, the safety margins for HAV for both fibrinogen and thrombin exceed 1 million-fold. Owing to the potentially significantly higher content of parvovirus and its greater resistance to inactivation, fibrinogen enjoys only a small safety margin while that for thrombin is approximately 2000-fold. Even still, when expressed as risk of transmission per vial, a calculation typically required by regulatory authorities, the risk with fibrinogen is calculated at 1 in 500,000 vials and that with thrombin is approximately 1 in 100 million vials or less. Thus, parvovirus transmission should still be an infrequent event.

Clinical experience

Tisseel has been marketed in the United States since 1998, and in Europe it was introduced clinically more than a decade earlier. Evicel and, except for a formulation change, its identical predecessor product Crosseal have been marketed in the United States since 2003 and were available in Europe several years earlier. Throughout this use, there have been no known cases of hepatitis or HIV

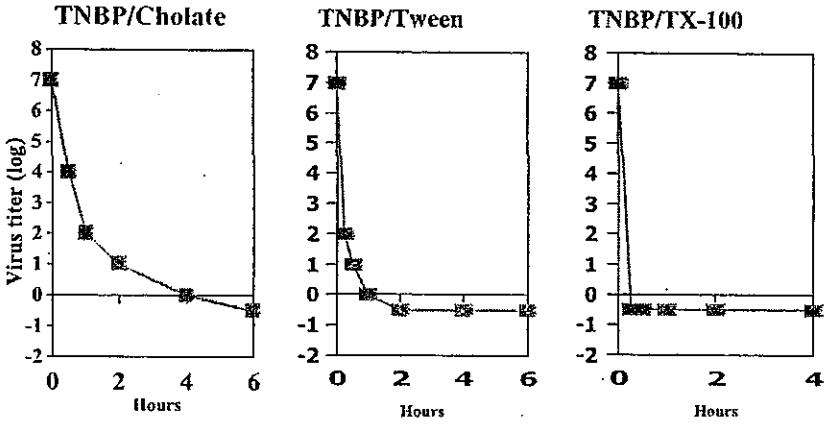


Fig. 2. S/D inactivation of vesicular stomatitis virus added to an antihemophilic factor concentrate.

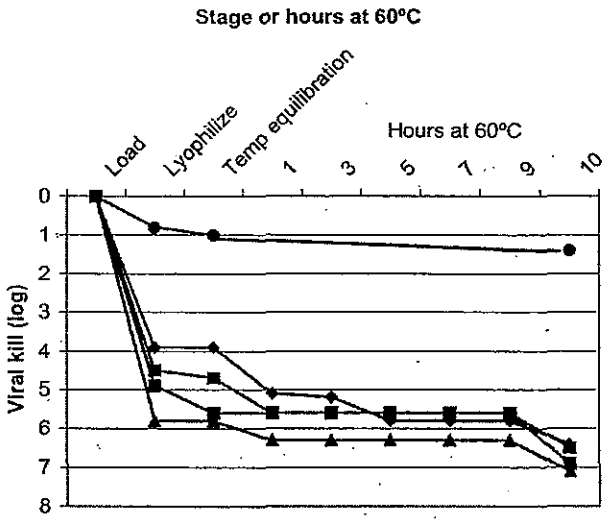


Fig. 3. Validated viral kill on vapor heating of fibrinogen (currently used Baxter process). BVDV (■) and PRV (▲) were undetectable upon reaching 60°C, and HIV (◆) and HAV (■) were undetectable after 5 and 1 hour, respectively, at 60°C. (●) Mouse minute virus.

transmission associated with commercial fibrin sealants.[59] This is notable since, for much of this time, the manufacturing process for Tisseel utilized only one dedicated viral elimination step while modern processes utilize two. On the other hand, epidemiologic evidence suggests that among patients who do not have parvovirus antibody at the time of fibrin sealant application, approximately one-fifth have reduced reticulocyte counts 12 to 20 days after surgery and develop parvovirus antibodies 12 to 48 weeks after surgery.[60] This finding is consistent with the calculations presented above since the study was performed using fibrin sealant prepared from plasma pools that were not screened by NAT for parvovirus, thereby potentially starting with 10,000-fold higher parvovirus loads. Experience with S/D-plasma indicates that reducing the quantity of parvovirus DNA to no more than 10^4 geq per mL (10^6 geq/patient exposure) eliminates parvovirus transmission as measured by DNA replication or seroconversion when the patient also receives product containing parvovirus antibody.[61] Additional clinical studies are needed to show whether the fibrin sealants manufactured today can still transmit parvovirus B19 or one of the newly described, human blood-borne parvoviruses.[62]

New viral threats

In the past 5 to 8 years, three new pathogens, WNV, Chikungunya virus, and vCJD, have emerged as potential threats to the blood supply. Other infectious agents, like H5N1 influenza virus, the strain of corona virus that causes severe acute respiratory syndrome (SARS); dengue virus; and vaccinia virus are potentially transmissible by blood and blood products. With the exception of vCJD, all are enveloped viruses and would be expected to be completely cleared by the processes now in place for manufactured blood products like fibrin sealant or thrombin. As shown in Table 8, WNV, H5N1 influenza virus, SARS-associated corona virus, and Chikungunya virus were all inactivated completely to the extent of challenge by the methods of viral inactivation discussed above.

The infectious agent of vCJD is believed to be a protein that resists most methods of inactivation including all that are applied to manufactured plasma products. Although there is evidence that it can be found in blood (see above), despite years of surveillance there have been no reported transmissions by manufactured plasma products. Model studies indicate that significant quantities would be removed by the purification processes now in use, including cryoprecipitation, depth filtration with filter aids, nanofiltration, and ion-exchange or affinity chromatography.[25,63-69] Based mostly on published findings, compared with a total maximum load of about 4 log of vCJD per plasma pool, the fibrinogen and thrombin processes should remove greater than 7.6 and greater than 13 log, respectively (Table 9), providing large safety margins.

DISCUSSION

The safety of modern plasma-derived products with respect to HBV, HCV, and HIV has been proven clinically over the past decade or more, mostly using manufacturing procedures employing only one dedicated method of

TABLE 6. Estimated viral elimination when processing fibrinogen and thrombin: based on both Omrix's and Baxter's processes

Step		Log reduction HIV	HCV	HBV	HAV	Parvovirus B19
Fibrinogen						
Cryoprecipitation + Al(OH) or wash		1.5	1.5	1.5	1.5	1.5
Immune neutralization					3*	3*
S/D treatment		>6	>6	>6	0	0
Heat treatment		>6	>6	>6	>5.8 to >6.5†	1.3
Greater heat sensitivity of B19 than CPV						1
Reserve capacity of virucidal methods‡		4	4	2	0	0
	Sum:	17.5	17.5	15.5	10.3-11.5	6.8
Thrombin						
Cryo removal		1	1	1	1	1
Immune neutralization		0	0	0	0	0
Initial fractionation (Baxter)§		2	2	2	2	2
Chromatographic purification		3	3	3	3	3
S/D treatment		>6	>6	>6	0	0
S/D reserve capacity		4	4	2	NA	NA
Heat treatment (Baxter)		>6	>6	>6	6.9	1.3
Nanofiltration (Omrix)		>4.4	>5.3	>5.5	7	5.9
	Sum:	18-22	19-22	17.5-20	11-13	7.3-9.9¶

* Virus neutralization is predicated on the fibrinogen containing antibody.
† The lower number applies to Omrix's fibrinogen and the higher number applies to Baxter's fibrin.
‡ The reserve capacity of vapor treating is estimated at zero since most of the reported viral kill takes place prior to initiating the heat cycle. The estimate of HBV reserve capacity with S/D treatment comes from studies with duck HBV added to whole plasma.
§ A mean of 2 log removal during cold alcohol fractionation is assumed.
¶ The lower number applies to Baxter's thrombin and the higher number applies to Omrix's thrombin.
NA = not applicable.

TABLE 7. Calculation of viral safety margins

	HIV	HCV	HBV	HAV	Parvovirus
Viral load (log; from Table 1)	5.7	6.0	4.4	2.9	6.6
Fibrinogen					
Viral clearance capacity (log)	17.5	17.5	15.5	10.3	6.8
Safety margin (fold)	6.3×10^{11}	3.2×10^{11}	1.3×10^{11}	2.5×10^{7}	1.6
Risk/vial (with virus at maximum load)*	3×10^{-16}	5×10^{-16}	1×10^{-15}	7×10^{-12}	1×10^{-4}
Adjusted risk/vial (all lots)†	1×10^{-19}	3×10^{-19}	5×10^{-17}	2×10^{-16}	2×10^{-5}
Thrombin					
Viral clearance capacity (log)	18	19	17	11	7.3-9.9
Safety margin (fold)	2×10^{12}	1×10^{13}	4×10^{12}	1×10^{9}	2×10^{3}
Risk/vial (with virus at maximum load)‡	8×10^{-18}	2×10^{-18}	4×10^{-18}	1×10^{-13}	3×10^{-5}-8×10^{-6}§
Adjusted risk/vial (all lots)†	4×10^{-21}	8×10^{-22}	2×10^{-19}	3×10^{-19}	7×10^{-6}-2×10^{-10}§

* Assumes 1 vial per L of plasma.
† The risk was adjusted to include lots without virus (calculated from Table 1, Column 4) and further assumes that the average viral load of contaminated lots is 1 log lower than the maximum load.
‡ Assumes 10 vials per L of plasma.
§ The larger number applies to Baxter's thrombin and the smaller number applies to Omrix's thrombin.

virus inactivation.[70] The safety record of fibrin sealant products, composed of both fibrinogen and thrombin, matches the safety record of other manufactured plasma products.[57] This suggests that for these and other enveloped viruses, the safety margins of fibrin sealant or stand-alone thrombin should be much higher than required since both employ an additional, dedicated method of viral elimination and, indeed, our calculated safety margins for enveloped viruses are extremely high. As a consequence, when new threats from enveloped viruses (e.g., WNV, pandemic influenza, and dengue viruses) are identified, the procedures in place for manufactured blood products are sufficient to ensure safety. This contrasts with the record of so-called labile blood components that have been shown to transmit, for example, WNV, at least until new screening tests are developed and deployed. From a patient safety perspective, it is also important to note that the safety margins for fibrin sealant and thrombin exceed those for labile blood components by many orders of magnitude. This is a direct consequence of the multiple improvements adopted by manufacturers of purified blood products over the past 20 years including deploying robust methods of virus inactivation.

TABLE 8. Inactivation of new viral threats

Virus	Preparation	Treatment	Log kill	First time point where infectious virus was not detected	Reference
WNV	α1-proteinase inhibitor	Pasteurization at 60°C for 10 hr	≥6.5	5 hr	Remington et al.[59]
	Antihemophilic factor concentrate	S/D (0.3% TNBP/1.0% Tween 80) at 28°C for 6 hr	≥5.9	1 hr	
WNV	FEIBA	Vapor heating (60°C for 10 hr and 80°C for 1 hr)	>7.6	6 hr	Kreil et al.[60]
	Antihemophilic factor concentrate	S/D (0.3% TNBP and 1% Triton X-100) at 20°C for 60 min	>6.0	<1 min	
H5N1 influenza virus	FEIBA	Vapor heating (60°C for 10 hr and 80°C for 1 hr)	>5.3	10 hr	Kreil et al.[61]
	IVIG	S/D (0.3% TNBP, 1% Triton X-100 and 0.3% Tween 80) at 18°C for 60 min	>4.7	<2 min	
SARS-associated corona virus	Haptoglobin, AT III, or IVIG	Pasteurization at 60°C for 10 hr	>3.3 to >6.5	1 hr	Yunoki et al.[62]
Chikungunya virus	IVIG	Pasteurization at 60°C for 10 hr	>5.2	1 hr	Uemura et al.[63]

AT III = antithrombin III; FEIBA = factor VIII bypassing activity produced by CSL Behring; H5N1 = the strain of influenza virus that causes SARS; IVIG = intravenous immune globulin.

Calculated safety margins for nonenveloped viruses are smaller since the manufacturing procedures for many plasma proteins, including both fibrinogen and thrombin, typically employ only one dedicated viral inactivation and/or removal method effective against these viruses, and parvoviruses are especially stable to thermal inactivation. There are no reports of HAV transmission by fibrin sealants even before adoption of NAT screening procedures. This is in accord with the finding that coagulation FVIII preparations did not transmit HAV provided they were either heat-treated or affinity-purified.[71] Additionally, given the modest processing fibrinogen undergoes and the known presence of immunoglobulin G in cryoprecipitate, it is reasonable to assume that fibrinogen is further protected by anti-HAV, present as a "contaminant."

Numerous reports describe the transmission of parvovirus B19 by coagulation factor concentrates[72] and its transmission by fibrin sealant has also been reported.[58] Frequent transmission results from the high concentration of virus that can be present in plasma pools containing units from donors with acute-phase viremia[73] and because parvovirus is not inactivated by S/D and is relatively stable to heat treatment methods. Beginning around 2001, commercial manufacturers of plasma products began employing NAT to screen incoming plasma units in a minipool format to limit viral loads. Originally, testing was performed to ensure that titers did not exceed 10^5 geq per mL; for Omrix's and Baxter's fibrin sealant products, a standard of not more than 10^4 geq per mL has been adopted for the minipool being tested. A recent article by Geng et al.[51] confirms the benefits of screening incoming plasma for parvovirus B19 by NAT. Despite this improvement, the maximum load of infectious virus that might be present remains considerable, and it would appear that the fibrinogen component might still transmit parvovirus B19, albeit at very low frequency. Clinical studies will be required to confirm this since the antibody content of fibrinogen might provide adequate protection, or parvovirus B19 might be more sensitive to heat treatment than the animal parvovirus models used, as has been suggested.[74] The greater safety margin calculated for the thrombin component arises from the use of more vigorous purification procedures and the overall effectiveness of nanofilters in removing parvovirus. Because of its size and shape, fibrinogen cannot be nanofiltered successfully.

All evidence to date indicates that vCJD is not transmitted by manufactured plasma products. Safety may result from the geographic restrictions that have been instituted to eliminate individuals who are at high risk of exposure, the very low levels in blood, and its removal by steps in common use including precipitations, filtrations, and column chromatography. If a vCJD contaminated unit was included in the plasma pool, the calculated safety margin for fibrinogen and thrombin is very high. Unlike viruses used in spiking studies, however, the structure of

TABLE 9. Clearance of prions: Omrix process

	Prion load (log; from Table 1): 4.3 Log removal	Reference*
Fibrinogen		
Cryoprecipitation	1.6 mean (0.6-2.6)	Foster, 2000[56] and 2006[59]
Al(OH) extraction and filtration	>2	Omrix validated study
Oil extraction of S/D reagents	2	Omrix preliminary study
Hydrophobic chromatography	2	Foster, 1999[53]
Clearance capacity (log)	>7.6	
Safety margin (fold)	1995	
Adjusted risk/vial†	7×10^{-10}	
Thrombin		
Cryo removal	1	Foster, 2000[56] and 2006[59]
Filtrations	2	Foster, 2000[56] and 2006[59]
Anion-exchange chromatography	3	Foster, 2000[56] and 2006[59]
Cation-exchange chromatography	3	Foster, 2000[56] and 2006[59]
Nanofiltration	4.4 mean (1.6 to >5.9)	Foster, 2000[56] and 2006[59]
Clearance capacity (log 10)	13.4	
Safety margin (fold)	1.3×10^9	
Adjusted risk/vial†	1×10^{-16}	

* See also additional Foster references.[51,54,55,57] It should be noted that the form that the infectious vCJD agent takes in plasma is unknown and that, should it be present in plasma pools, its behavior may differ from the materials used.

† We employed the same assumptions as used in Table 7 plus assumed risk of vCJD presence was the same as for CJD, i.e., 1 per 1 million donations, and mean load was same as maximum load.

the causative agent of vCJD is unknown and may differ significantly from the models in use. Because of this uncertainty and the devastating nature of the disease, the authorities in the United Kingdom have concluded that recipients of plasma-derived FVIII, F IX, and antithrombin prepared using donations from individuals who subsequently developed vCJD should be told that they may be at increased risk for developing the disease.[75] This emphasizes the importance of donor exclusion criteria implemented for all products licensed in the United States or Europe. With these exclusions taken into account, the risk for vialed product should remain well less than 1 in 1 million for fibrinogen and less than 1 in 1 trillion for thrombin.

Finally, we should comment about the methods employed in making these calculations. In addition to the validated studies presented to the FDA and other regulatory agencies, we examined other steps in the manufacturing process that were likely to contribute to safety together with published information from other related processes. We also have taken into account the benefit of using viral inactivation methods that have a large reserve capacity. Our intent in employing this approach was not to replace the stricter approach taken by regulatory authorities, but simply to more completely assess safety. Nonetheless, we acknowledge that our calculations are estimates and actual findings may differ. Acknowledging these shortcomings but based on our calculations, we encourage manufacturers and other interested parties to continue seeking ways to enlarge the safety margin, especially for nonenveloped viruses and to address the theoretical risk posed by vCJD

ACKNOWLEDGMENTS

The authors thank Drs Israel Nur of Omrix and Thomas Kreil of Baxter for providing pathogen elimination data to us for our use in this article.

REFERENCES

1. Key NS, Negrier C. Coagulation factor concentrates: past, present and future. Lancet 2007;370:439-48.
2. Abusriwil H, Stockley RA. Alpha-1-antitrypsin replacement therapy: current status. Curr Opin Pulm Med 2006;12:125-31.
3. Toubi E, Etzioni A. Intravenous immunoglobulin in immunodeficiency states: state of the art. Clin Rev Allergy Immunol 2005;29:167-72.
4. Solomon B. Intravenous immunoglobulin and Alzheimer's disease immunotherapy. Curr Opin Mol Ther 2007;9:79-85.
5. Laursen I, Houen G, Hojrup P, Brouwer N, Krogsoe LB, Blou L, Hansen PR. Second-generation nanofiltered plasma-derived mannan-binding lectin product: process and characteristics. Vox Sang 2007;92:338-50.
6. Lariviere B, Rouleau M, Picard S, Beaulieu AD. Human plasma fibronectin potentiates the mitogenic activity of platelet-derived growth factor and complements its wound healing effects. Wound Repair Regen 2003;11:79-89.
7. Poulle M, Burnouf-Radosevich M, Burnouf T. Large-scale preparation of highly purified human C1-inhibitor for therapeutic use. Blood Coagul Fibrinolysis 1994;5:543-9.
8. Jackson MR. Fibrin sealants in surgical practice: an overview. Am J Surg 2001;182(2 Suppl):1S-7S.

9. Dorion RP, Hamati HF, Landis B, Frey C, Heydt D, Carey D. Risk and clinical significance of developing antibodies induced by topical thrombin preparations. Arch Pathol Lab Med 1998;122:887-94.
10. Ortel TL, Mercer MC, Thames EH, Moore KD, Lawson JH. Immunologic impact and clinical outcomes after surgical exposure to bovine thrombin. Ann Surg 2001;233: 88-96.
11. Schoenecker JG, Johnson RK, Lesher AP, Day JD, Love SD, Hoffman MR, Ortel TL, Parker W, Lawson JH. Exposure of mice to topical bovine thrombin induces systemic autoimmunity. Am J Pathol 2001;159:1957-69.
12. Su Z, Izumi T, Thames EH, Lawson JH, Ortel TL. Antiphospholipid antibodies after surgical exposure to topical bovine thrombin. J Lab Clin Med 2002;139:349-56.
13. Streiff MB, Ness PM. Acquired FV inhibitors: a needless iatrogenic complication of bovine thrombin exposure. Transfusion 2002;42:18-26.
14. Lawson JH, Lynn KA, Vanmatre RM, Domzalski T, Klemp KF, Ortel TJ, Niklason LE, Parker W. Antihuman factor V antibodies after use of relatively pure bovine thrombin. Ann Thorac Surg 2005;79:1037-8.
15. Kamoda S, Ishikawa R, Kakehi K. Capillary electrophoresis with laser-induced fluorescence detection for detailed studies on N-linked oligosaccharide profile of therapeutic recombinant monoclonal antibodies. J Chromatogr A 2006; 1133:332-9.
16. Hepner F, Cszasar E, Roitinger E, Lubec G. Mass spectrometrical analysis of recombinant human growth hormone (Genotropin® reveals amino acid substitutions in 2% of the expressed protein. Proteome Sci 2005;3:1.
17. Brand CM, Leadbeater L, Bellati G, Marotta F, Ideo G. Antibodies developing against a single recombinant interferon protein may neutralize many other interferon-alpha subtypes. J Interferon Res 1993;13:121-5.
18. Oberg K, Alm G, Magnusson A, Lundqvist G, Theodorsson E, Wide L, Wilander E. Treatment of malignant carcinoid tumors with recombinant interferon alfa-2b: development of neutralizing interferon antibodies and possible loss of antitumor activity. J Natl Cancer Inst 1989;81:531-5.
19. Berrini A, Borromeo V, Secchi C. Monoclonal antibodies can reveal immunoreactivity differences between pituitary and recombinant bovine growth hormone. Hybridoma 1994;13:485-9.
20. US Food and Drug Administration. Guidance for industry: Q5A viral safety evaluation of biotechnology products derived from cell lines of human or animal origin. September 1998. Available from: URL: http://www.fda.gov/cder/guidance/q5a-fnl.pdf
21. Schreiber GB, Busch MP, Kleinman SH, Korelitz JJ. The risk of transfusion-transmitted viral infections. The Retrovirus Epidemiology Donor Study. N Engl J Med 1996;334:1685-90.
22. 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.
23. Janssen MP, Over J, Vanderpoel CL, Cuijpers HT, Vanhout BA. A probabilistic model for analyzing viral risks of plasma-derived medicinal products. Transfusion 2008;48: 153-62.
24. Mosesson MW. Fibrinogen and fibrin structure and functions. J Thromb Haemost 2005;3:1894-904.
25. Horowitz B. Blood protein derivative viral safety: observations and analysis. Yale J Biol Med 1990;63:361-9.
26. Hewitt PE, Llewelyn CA, Mackenzie J, Will RG. Creutzfeldt-Jakob disease and blood transfusion: results of the UK Transfusion Medicine Epidemiological Review study. Vox Sang 2006;91:221-30.
27. Burnouf T, Padilla A. Current strategies to prevent transmission of prions by human plasma derivatives. Transfus Clin Biol 2006;13:320-8.
28. Cohen MS, Pilcher CD. Amplified HIV transmission and new approaches to HIV prevention. J Infect Dis 2005;191: 1391-3.
29. Wawer MJ, Gray RH, Sewankambo NK, Serwadda D, Li X, Laeyendecker O, Kiwanuka N, Kigozi G, Kiddugavu M, Lutalo T, Nalugoda F, Wabwire-Mangen F, Meehan MP, Quinn TC. Rates of HIV-1 transmission per coital act, by stage of HIV-1 infection, in Rakai, Uganda. J Infect Dis 2005;191:1403-9.
30. Pilcher CD, Joaki G, Hoffman IF, Martinson FE, Mapanje C, Stewart PW, Powers KA, Galvin S, Chilongozi D, Gama S, Price MA, Fiscus SA, Cohen MS. Amplified transmission of HIV-1: comparison of HIV-1 concentrations in semen and blood during acute and chronic infection. AIDS 2007;21: 1723-30.
31. Fiebig EW, Wright DJ, Rawal BD, Garrett PE, Schumacher RT, Peddada L, Heldebrant C, Smith R, Conrad A, Kleinman SH, Busch MP. Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. AIDS 2003; 17:1871-9.
32. 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.
33. Busch MP, Caglioti S, Robertson EF, McAuley JD, Tobler LH, Kamel H, Linnen JM, Shyamala V, Tomasulo P, Kleinman SH. Screening the blood supply for West Nile virus RNA by nucleic acid amplification testing. N Engl J Med 2005;353:460-7.
34. Glynn SA, Wright DJ, Kleinman SH, Hirschkorn D, Tu Y, Heldebrant C, Smith R, Giachetti C, Gallarda J, Busch MP. Dynamics of viremia in early hepatitis C virus infection. Transfusion 2005;45:994-1002.
35. Yoshikawa A, Gotanda Y, Minegishi K, Taira R, Hino S, Tadokoro K, Ohnuma H, Miyakawa K, Tachibana K, Mizoguchi H; Japanese Red Cross NAT Screening Research

Group. Lengths of hepatitis B viremia and antigenemia in blood donors: preliminary evidence of occult (hepatitis B surface antigen-negative) infection in the acute stage. Transfusion 2007;47:1162-71.
36. Satake M, Taira R, Yugi H, Hino S, Kanemitsu K, Ikeda H, Tadokoro K. Infectivity of blood components with low hepatitis B virus DNA levels identified in a lookback program. Transfusion 2007;47:1197-205.
37. Komiya Y, Katayama K, Yugi H, Mizui M, Matsukura H, Tomoguri T, Miyakawa Y, Tabuchi A, Tanaka J, Yoshizawa H. Minimum infectious dose of hepatitis B virus in chimpanzees and difference in the dynamics of viraemia between genotype A and C. Transfusion 2008;48:286-84.
38. Busch MP, Murthy KK, Hirschkorn DF, Herring BL, Delwart EL, Racanelli V, Rehermann B, Alter HJ. Infectivity of donations from eclipse and ramp-up stages of HCV in chimpanzees. Abstract S39-030H. Amer Assn of Blood Banks Annual Meeting 2007, Anaheim, CA., Oct 2007. Transfusion 2007;47(9 Suppl):17A.
39. Ma M, Piatak M, Fritts L, Lu D, Lifson J, Busch MP, Miller CJ. Transmission of simian immunodeficiency virus (SIV) by plasma collected prior to detectable viremia, and infectivity of ramp-up versus chronic stages. Abstract S40-030H. Amer Assn of Blood Banks Annual Meeting 2007, Anaheim, CA, Oct 2007. Transfusion 2007;47(9 Suppl):17A-18A.
40. Hijikata M, Shimizu YK, Kato H, Iwamoto A, Shih JW, Alter HJ, Purcell RH, Yoshikura H. Equilibrium centrifugation studies of hepatitis C virus: evidence for circulating immune complexes. J Virol 1993;67:1953-8.
41. Brummelhuis HG, Over J, Duivis-Vorst CC, Wilson-de Sturler LA, Ates G, Hoek PJ, Reerink-Brongers EE. Contributions to the optimal use of blood. IX. Elimination of hepatitis B transmission by (potentially) infectious plasma derivatives. Vox Sang 1983;45:205-16.
42. Prince AM, Horowitz B, Baker L, Shulman RW, Ralph H, Valinsky J, Cundell A, Brotman B, Boehle W, Rey F, Piet M, Reesink H, Lelie N, Tersmette M, Miedema F, Barbosa L, Nemo G, Nastala CL, Allan JS, Lee DR, Eichberg JW. Failure of a human immunodeficiency virus (HIV) immune globulin to protect chimpanzees against experimental challenge with HIV. Proc Natl Acad Sci U S A 1988;85:6944-8.
43. Prince AM, Pawlotsky JM, Soulier A, Tobler L, Brotman B, Pfahler W, Lee DH, Li L, Shata MT. Hepatitis C virus replication kinetics in chimpanzees with self-limited and chronic infections. J Viral Hepat 2004;11:236-42.
44. Bruce ME, McConnell I, Will RG, Ironside JW. Detection of variant Creutzfeldt Jakob disease infectivity in extraneural tissues. Lancet 2001;358:208-9.
45. Llewelyn CA, Hewitt PE, Knight RS, Amar K, Cousens S, Mackenzie J, Will RG. Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. Lancet 2004;363:417-21.
46. Peden AH, Head MW, Ritchie DL, Bell JE, Ironside JW. Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. Lancet 2004;364:527-9.
47. 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.
48. Cervenakova LO, Yakovleva O, McKenzie C, Kolchinsky S, McShane L, Drohan WN, Brown P. Similar levels of infectivity in the blood of mice infected with human-derived vCJD and GSS strains of transmissible spongiform encephalopathy. Transfusion 2003;43:1687-94.
49. 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.
50. Terpstra FG, Parkkinen J, Tolo H, Koenderman AH, Ter Hart HG, von Bonsdorff L, Torma E, van Engelenburg FA. Viral safety of Nanogam, a new 15 nm-filtered liquid immunoglobulin product. Vox Sang 2006;90:21-32.
51. Geng Y, Wu CG, Bhattacharyya SP, Tan D, Guo ZP, Yu MY. Parvovirus B19 DNA in Factor VIII concentrates: effects of manufacturing procedures and B19 screening by nucleic acid testing. Transfusion 2007;47:883-9.
52. Einarsson M, Morgenthaler JJ. Removal of viruses from plasma fractions by chromatography. Curr Stud Hematol Blood Transfus 1989;56:138-45.
53. Burnouf T. Chromatography in plasma fractionation: benefits and future trends. J Chromatogr B Biomed Appl 1995; 664:3-15.
54. Lemon SM, Murphy PC, Smith A, Zou J, Hammon J, Robinson S, Horowitz B. Removal/neutralization of hepatitis A virus during manufacture of high purity, solvent/detergent factor VIII concentrate. J Med Virol 1994;43:44-9.
55. Griffith M. Ultrapure plasma factor VIII produced by anti-F VIII c immunoaffinity chromatography and solvent/detergent viral inactivation. Characterization of the Method M process and Hemofil M antihemophilic factor (human). Ann Hematol 1991;63:131-7.
56. Woese C. Thermal inactivation of animal viruses. Ann N Y Acad Sci 1960;83:741-51.
57. Tersmette M, deGoede RE, Over J, deJonge E, Radema H, Lucas CJ, Huisman HG, Miedema F. Thermal inactivation of human immunodeficiency virus in lyophilised blood products evaluated by ID50 titrations. Vox Sang 1986;51: 239-43.
58. Horowitz B. Specific inactivation of viruses which can potentially contaminate blood products. Dev Biol Stand 1991;75:43-52.
59. Joch C. The safety of fibrin sealants. Cardiovasc Surg 2003; 11(Suppl 1):23-8.
60. Kawamura M, Sawafuji M, Watanabe M, Horinouchi H, Kobayashi K. Frequency of transmission of human parvovirus B19 infection by fibrin sealant used during thoracic surgery. Ann Thorac Surg 2002;73:1098-100.
61. Brown KE, Young NS, Alving BM, Barbosa LH. Parvovirus B19: implications for transfusion medicine. Summary of a workshop. Transfusion 2001;41:130-5.

62. Fryer JF, Delwart E, Hecht FM, Bernardin F, Jones MS, Shah N, Baylis SA. Frequent detection of the parvoviruses, PARV4 and PARV5, in plasma from blood donors and symptomatic individuals. Transfusion 2007;47:1054-61.
63. Foster PR. Assessment of the potential of plasma fractionation processes to remove causative agents of transmissible spongiform encephalopathy. Transfus Med 1999;9:3-14.
64. Reichl HE, Foster PR, Welch AG, Li Q, MacGregor IR, Somerville RA, Fernie K, Steele PJ, Taylor DM. Studies on the removal of a bovine spongiform encephalopathy-derived agent by processes used in the manufacture of human immunoglobulin. Vox Sang 2002;83:137-45.
65. Foster PR, Griffin BD, Bienek C, McIntosh RV, MacGregor IR, Somerville RA, Steele PJ, Reichl HE. Distribution of a bovine spongiform encephalopathy-derived agent over ion-exchange chromatography used in the preparation of concentrates of fibrinogen and factor VIII. Vox Sang 2004; 86:92-9.
66. Foster PR, McLean C, Welch AG, Griffin BD, Hardy JC, Bartley A, MacDonald S, Bailey A. Removal of abnormal prion protein by plasma fractionation. Transfus Sci 2000;22 (1-2):53-6.
67. Foster PR, Welch AG, McLean C, Griffin BD, Hardy JC, Bartley A, MacDonald S, Bailey AC. Studies on the removal of abnormal prion protein by processes used in the manufacture of human plasma proteins. Vox Sang 2000;78:86-95.
68. Cervenakova L, Brown P, Hammond DJ, Lee CA, Saenko EL. Factor VIII and transmissible spongiform encephalopathy: the case for safety. Haemophilia 2002;8:63-75.
69. Foster PR. Plasma products. In: Turner ML, editor. Creutzfeldt-Jakob disease: managing the risk of transmission by blood, plasma, and tissues. Bethesda (MD): AABB Press; 2006. p. 188-213.
70. Tabor E. The epidemiology of virus transmission by plasma derivatives: clinical studies verifying the lack of transmission of hepatitis B and C viruses and HIV type 1. Transfusion 1999;39:1160-8.
71. Horowitz B, Ben-Hur E. Efforts in minimizing risk of viral transmission through viral inactivation. Ann Med 2000;32: 475-84.
72. Wu CG, Mason B, Jong J, Erdman D, McKernan L, Oakley M, Soucie M, Evatt B, Yu MY. Parvovirus B19 transmission by a high-purity factor VIII concentrate. Transfusion 2005; 45:1003-10.
73. 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.
74. Blumel J, Schmidt I, Willkommen H, Lower J. Inactivation of parvovirus B19 during pasteurization of human serum albumin. Transfusion 2002;42:1011-8.
75. CJD Incidents Panel. Fourth Annual Report 1st September 2003 to 31st August 2004 to the Advisory Committee on Dangerous Pathogens Working Group on Transmissible Spongiform Encephalopathies. Available from: http://www.hpa.nhs.uk/infections/topics_az/cjd/report03-04.pdf
76. Roth WK, Weber M, Buhr S, Drosten C, Weichert W, Sireis W, Hedges D, Seifried E. Yield of HCV and HIV-1 NAT after screening of 3.6 million blood donations in central Europe. Transfusion 2002;42:862-8.
77. Stramer SL, Glynn SA, Kleinman SH, Strong DM, Caglioti S, Wright DJ, Dodd RY, Busch MP; National Heart, Lung, and Blood Institute Nucleic Acid Test Study Group. Detection of HIV-1 and HCV infections among antibody-negative blood donors by nucleic acid-amplification testing. N Engl J Med 2004;351:760-8.
78. 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 L, Niederhauser C, Pastila S, Pillonel J, Pineau J, van der Poel CL, Politis C, Roth WK, Sauleda S, Seed CR, Sondag-Thull D, Stramer SL, 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.
79. Schreiber GB, Glynn SA, Zerlauth G, Wright DJ, McEntire R. Estimated HIV, HCV, and HBV residual risks of source-plasma starting material for plasma derived medicinal products. Vox Sang 2008 (in press).
80. Parsyan A, Candotti D. Human erythrovirus B19 and blood transfusion—an update. Transfus Med 2007;17:263-78.
81. Schmidt M, Themann A, Drexler C, Bayer M, Lanzer G, Menichetti E, Lechner S, Wessin D, Prokoph B, Allain JP, Seifried E, Kai Hourfar M. Blood donor screening for parvovirus B19 in Germany and Austria. Transfusion 2007;47: 1775-82.
82. 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.
83. 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-S29.
84. Busch MP, Tobler LH, Gerlich WH, Schaefer S, Giachetti C, Smith R. Very low level viremia in HCV infectious unit missed by NAT. Transfusion 2003;43:1173-4.
85. 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.
86. Modrof J, Berting A, Tille B, Klotz A, Forstner C, Rieger S, Aberham C, Gessner M, Kreil TR. Neutralization of human parvovirus B19 by plasma and intravenous immunoglobulins. Transfusion 2008;48:178-86.
87. Davenport R, Geohas G, Cohen S, Beach K, Iazo A, Lucchesi K, Pehta J. Phase IV study of Plas+SD: hepatitis A

(HAV) and parvovirus B19 safety results. Blood 2000;96: 451a.
88. Doyle S, Corcoran A. The immune response to parvovirus B19 exposure in previously seronegative and seropositive individuals. J Infect Dis 2006;194:154-8.
89. Remington KM, Trejo SR, Buczynski G, Li H, Osheroff WP, Brown JP, Renfrow H, Reynolds R, Pifat DY. Inactivation of West Nile virus, Vaccinia virus, and viral surrogates for relevant and emergent viral pathogens in plasma-derived products. Vox Sang 2004;87:10-8.
90. Kreil TR, Berting A, Kistner O, Kindermann J. 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 2003;43:1023-8.
91. Kreil TR, Unger U, Orth SM, Petutschnig G, Kistner O, Berting A. H5N1 influenza virus and the safety of plasma products. Transfusion 2007;47:452-9.
92. Yunoki M, Yrayama T, Yamamoto I, Abe S, Ikuta K. Heat sensitivity of a SARS-associated coronavirus introduced into plasma products. Vox Sang 2004;87:302-3.
93. Uemura YY, Yang H, Heldebrant CM, Takechi K, Yokoyama K. Inactivation and elimination of viruses during preparation of human intravenous immunoglobulin. Vox Sang 1994;67:246-54.

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. 6. 23	該当なし	

一般的名称	(製造販売承認書に記載なし)	研究報告の公表状況		公表国
販売名(企業名)	合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)		Cazenave JP. XXIIXth Congress of the International Society of Blood Transfusion; 2008 Jun 7-12; Macao.	フランス

研究報告の概要

○輸血用血液製剤の病原体不活化についての欧州の見解
血液、血液成分および血漿由来製剤の安全性および有効性の向上は、現在も重要な問題である。医療技術の進歩と寿命の延長に伴い、血液需要は増加し続けている。現在、供血者の選別によって、輸血用血液製剤の安全性が確保されているが、ウイルス、細菌、寄生虫感染の残存リスクに加えて、新たなウイルスによる感染の危険が存在する。化学的、光化学的なゲノム修飾による病原体不活化は、広範囲の病原体に対応する予防的アプローチである。多くの不活化技術が利用され、血漿由来製剤の不活化に成功した。有機溶媒・界面活性剤とメチレンブルーを用いた方法はヨーロッパの多くの国で利用され、副作用の発生もなく製剤の安全性を向上させているが、赤血球と血小板製剤(PC)には適用できない。アモトサレン(Intercept)、リボフラビン(Mirasol)を用いた新しい方法は、CEマークを受けヨーロッパで導入されている。MirasolはPCおよび血漿用に開発されており、赤血球にも適用される可能性がある。血小板減少症患者を対象としたMirasol PCの第III相臨床試験の結果は、近いうちに報告される予定である。Intercept PCは、ヨーロッパの複数施設で既に15,000単位以上が輸血されている。フランスでは、レユニオン島でチクングニヤウイルスの流行時(2006年)に、またマルティニークとグアドループ-ギアナではデングおよびシャーガス病対策として導入された。アルザス(不活化のパイロット実施地域)では、Intercept PCが2006年5月に、Intercept血漿が2007年7月に導入された。2008年1月時点で、Intercept PCは22,000本以上、Intercept血漿は8,000本が輸血された。Intercept PCの臨床的止血効果は従来の未処理PCと同等(血小板量同等)であり、輸血副作用は約50%減少した。Intercept血漿は、貯留保管を経た血漿の現行適応症に対して使用され、有効性は同等であった。2007年のトロントのコンセンサス会議では、すべての輸血用血液製剤の不活化完全実施を求める声明が発表された。将来、全ての輸血用血液製剤の不活化を可能とするべきである。

報告企業の意見	今後の対応
輸血用血液製剤の病原体不活化について、ヨーロッパでは有機溶媒・界面活性剤、メチレンブルー、アモトサレン、リボフラビンなどを用いた方法が開発され、フランスのアルザス地方ではこれを導入し、3万本の使用実績が報告された。	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。厚生労働省の薬事・食品衛生審議会へ不活化技術導入にかかる基本的考え方を報告したところである。

使用上の注意記載状況・その他参考事項等

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

JRC2008T-037

Monday: Parallel Sessions S1 – Pathogen Reduction/ Inactivation

2A-S01-01

PATHOGEN REDUCTION: AN AMERICAN VIEW

Klein H
NIH, Bethesda, MD, USA

Blood transfusion is extremely safe in the United States. The risks of known viral infections are now so low that they must be calculated from donor data rather than measured directly. Nevertheless, measures for interdicting bacterial contamination remain imperfect, a variety of known pathogens, including viruses and parasites, are not screened out of the blood supply, and the risk of emerging infections transmitted by blood remains a concern of the public, the regulatory agencies, and the medical establishment. Following the HIV epidemic of the early 1980's, the plasma fractionation industry adopted pathogen reduction technology and has improved the process continuously; no transmission of major pathogens has since been reported when proper validated plasma fraction production has been performed, and transmission of some newly recognized agents, such as West Nile virus has been prevented. The blood collection services and the regulatory agencies have remained wedded to the reactive strategy of surveillance, screening, and testing as an approach to new infectious threats. The result has been an accepted disease burden prior to introduction of screening methods and a continued loss of blood donors. Barriers to adopting pathogen reduction technology include concerns about product safety, reduced therapeutic dose, absence of a single technique to treat all blood components, recognition that no technology inactivates all pathogens, and the added cost and complexity of the inactivation process. In January 2008, the Advisory Committee on Blood Safety and Availability recommended to the US Secretary of Health and Human Services that the potential benefits of pathogen reduction warrant a commitment and concerted effort to add this technology as a broadly applicable safeguard to the nation's blood supply. Pathogen reduction was seen as a pro-active and pre-emptive strategy to address the residual risk of known agents and to prevent emerging agents from becoming transfusion risks. The Committee recognized that to achieve this goal, government, industry, blood organizations, and public stakeholders must work in concert to commit the required financial and technical resources.

2A-S01-02

EUROPEAN VIEW ABOUT PATHOGEN INACTIVATION IN LABILE BLOOD PRODUCTS

Cazenave JP
EFS-Alsace, Strasbourg, France

Increased safety and efficiency of blood, blood components and drugs derived from plasma remain a major concern. Blood transfusion in Europe is tightly regulated. The demand for blood has continually increased as health care and life expectancy have increased. The safety of labile blood products [red blood cell concentrates (RBCC), platelet concentrates (PC) and plasma] is currently ensured by medical and biological donor selection measures. Nonetheless, in addition to the residual risk of viral, bacterial and parasitic infection, there is the emerging danger associated with new viruses. PI based on chemical or photochemical genomic modifications is a broad-spectrum and pro-active approach. A number of PI techniques have been use with success to inactivate plasma derived products. The solvent-detergent (SD) and the methylene blue (MB) methods are used in many countries in Europe, increasing the safety of the products and without side effects. Unfortunately, SD and PI technologies cannot be applied to RBCC and PC. New PI methods, amotosalen (Intercept, Cerus) and riboflavin (Mirasol, Gambro) have received CE marking and are being implemented in Europe. A PI process (Mirasol PRT, Gambro) is being developed for PC, plasma and possibly RBCC, using riboflavin, UV and visible light. The procedure inactivates a wide range of pathogens. Toxicity is reduced. A phase III clinical study to evaluate the efficacy and safety of Mirasol PC in thrombocytopenic patients is to be reported. Amotosalen hydrochloride and UVA (Intercept, Cerus) inactivate a broad spectrum of pathogens in PC and plasma. Intercept PC (both apheresis and buffy-coat derived) have been implemented in several centres in Europe (more than 15, 000 units transfused). In France Intercept PC have been implemented during an epidemic of Chikungunya virus in the Ile de la Réunion in 2006 and in EFS-Martinique and EFS-Guadeloupe-Guyane in 2007 (dengue and Chagas disease). EFS-Alsace, a pilot region, has introduced Intercept PI for PC (40% apheresis and 60% buffy coat derived PC, about 15 000 units/year) in May 2006 and Intercept PI for plasma (about 15 000 units/year) in July 2007. The distribution of both products is universal to patients. As of January 2008 more than 22 000 Intercept PC and 8,000 Intercept plasma have been transfused. For all patients, clinical haemostasis provided by Intercept PC is equivalent (same platelet dose) to conventional non treated PC and transfusion adverse reactions are reduced by about 50%. Intercept plasma has been used for current indications with equivalent effects as quarantine plasma. Inactivation of RBCC is a major undertaking. The use of FRALE S-303 (Cerus) is in the more advanced stage of development. In 2007, the Consensus Conference of Toronto concluded with statements that will guide the ultimate implementation of PI for all labile blood products: (1) active surveillance cannot account for the risk of an emerging transfusion-transmitted pathogen; (2) such risks require a proactive approach; (3) PI should be implemented when feasible and safe methods are available; and (4) costs and benefits should be assessed. Universal inactivation of all labile blood products should be possible in future.

2A-S01-03

A RANDOMIZED, CONTROLLED, 2-PERIOD CROSSOVER STUDY OF RECOVERY AND LIFESPAN OF RADIOLABELED AUTOLOGOUS 35-DAY-OLD RED BLOOD CELLS PREPARED WITH A MODIFIED S-303 TREATMENT FOR PATHOGEN INACTIVATION

Cancelas JA[1], Dumont L[2], Herschel L[2], Roger J[2], Rugg N[1], Garratty G[3], Arndt P[3], Propst M[4], Laurence L[4], Sundin D[4], AuBuchon J[2]
[1]*University of Cincinnati, Cincinnati, USA* [2]*Dartmouth-Hitchcock Medical Center, Lebanon, USA* [3]*American Red Cross Blood Services, Pomona, USA* [4]*Cerus Corporation, Concord, USA*

Background: The S-303 Treatment System for Red Blood Cell concentrates (RBC) developed by Cerus Corporation uses S-303, a frangible anchor-linker-effector compound, to irreversibly inactivate contaminating bacteria, viruses, protozoa, and leukocytes. Following observations of antibodies specific for S-303 treated RBCs in a Phase three trial the treatment process was modified to reduce S 303 binding to treated RBCs.
Aims: The present study was conducted to evaluate recovery/lifespan of 35-day old autologous RBCs, prepared with the modified S-303 process.
Study Design: This was a proof-of-concept, radiolabeled, crossover Phase I study conducted in 28 healthy subjects (10 male, 18 female). The study was divided into three periods: Screening and enrollment, Treatment Period 1, and Treatment Period 2. In each treatment period, subjects underwent autologous blood donation on Day 0 and infusion of double-label (51Cr/99mTc) autologous RBCs on Day 35. All whole blood units were processed into AS-3 solution, and leukocyte reduced. In random sequence, one unit (Test) from each subject was treated with the modified pathogen inactivation process (0.2 mM S-303 and 20 mM GSH) and stored at 4°C for 35 day. The other unit (Control) was prepared as conventional RBC and stored at 4°C for 35 day. Following infusion, blood samples were obtained over a 24 hour period (for single and double radio-isotope determinations of post-transfusion recovery). Additional samples were collected for 35 day post-infusion to determine lifespan. Biochemical assessments of study units (e.g. ATP, 2,3-DPG, PCV) were performed on days 0 and 35 of storage. Crossmatch reactivity to S303 treated RBC was conducted during the study using conventional gel cards.

© 2008 The Authors
Journal compilation © 2008 Blackwell Publishing Ltd.

別紙様式第 2-1
番号 13

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 8 月 27 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	ProMED/20080826.2660	公表国 中国	
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

中国の新しい研究が、狂犬病感染が劇的に急増していることを報告した。この報告によると、中国のいくつかの省においてヒトの狂犬病の症例数が、2000 年以降急に跳ね上がったという。
報告者らが調査したのは、中国保健省のサーベイランス・データベースから得た、1990 年 1 月から 2007 年 7 月までの 22,527 のヒト狂犬病症例のデータである。報告者らは、ヒトの狂犬病は 1990-1996 年に下火になり、このときはわずか 159 の症例が報告されただけであったが、この数字は、2006 年に 3,279 症例に跳ね上がったことを見出した。
さらに、狂犬病に遭遇する頻度が多いのは、中国の南西部および南部の省、特に人口密度の高い地域であることを見出した。
報告者の 1 人は、「狂犬病流行のこの 4 つの省では、イヌの狂犬病を排除する厳しい強制的措置が欠けているか、またはヒトへ投与する最新技術による細胞培養の狂犬病ワクチンがないのです」と述べた。報告者らによると、最も影響が大きかった広東省では、患者の 62.5%が、受けた傷への適切な治療を受けておらず、92.5%が曝露後に十分なワクチン接種を受けていなかったという。また 91.25%が抗狂犬病免疫グロブリンの投与を受けなかった。
この報告者らは、現在の狂犬病の管理プログラムを、監督を強化することによって改善し、これによって地方と政府との人的交流を改善し、狂犬病への意識を高め、都市の計画立案と開発を変更してヒトと動物とのふれ合いのバランスを図るべきであると勧告している。
（本研究は in press であり、"Rabies trend in China (1990-2007) and post-exposure prophylaxis in the Guangdong province" と題され、BMC Infectious Diseases に掲載される予定である。）

報告企業の意見	今後の対応
中国における狂犬病が 2006 年に急増したとの報告である。 血漿分画製剤からの狂犬病ウイルス伝播の事例は報告されていない。また、万一原料血漿に狂犬病ウイルスが混入したとしても、BVD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。

321

39

Archive Number 20080826.2660
Published Date 26-AUG-2008
Subject PRO/AH/EDR> Rabies - China: increased incidence

RABIES - CHINA: INCREASED INCIDENCE
****
A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail is a program of the
International Society for Infectious Diseases
<http://www.isid.org>

Date: Fri 22 Aug 2008
Source: Science Daily [edited]
<http://www.sciencedaily.com/releases/2008/08/080820194839.htm>

A new Chinese study has reported a dramatic spike in rabies infections. The research shows that in some provinces of China the number of human rabies cases has jumped since the new millennium.

Jia-Hai Lu, from the School of Public Health at Sun Yat-Sen University, China, led a team of researchers who studied the rabies trend in China between 1990 and 2007. Lu describes how things have changed in the last 8 years: "In China, human rabies was largely under control during the years 1990-1996, via nation-wide rabies vaccination programmes. Since the end of the century, however, cases of human rabies have jumped high enough to trigger a warning sign for control and prevention."

Rabies, an infection of the nervous system transmitted by animal bites, causes over 50 000 deaths each year around the world. During recent years, most of the research on control of rabies has concentrated on the development of post-exposure prophylaxis (preventative treatment -- in this case, preventing the worsening of an infection). According to the researchers, "The use of human and equine rabies immunoglobulins (HRIG/ERIG) has saved the lives of countless patients who would have died if treated with vaccine alone. However, both products are often in short supply worldwide and are virtually unaffordable in developing countries." [See ProMED post 20080826.2659 Announcements (03): Rabies vaccine supply limited - USA (CDC)].

Data from 22 527 human rabies cases from January 1990 to July 2007 were obtained from a surveillance database from the Ministry of Health of China. The authors found that human rabies was under control from 1990 to 1996, when only 159 cases of rabies were reported, but this figure had leapt to 3279 cases in 2006.

The authors found that rabies was most frequently encountered in the southwestern and southern territories of China, especially in highly populated areas. Lu said, "The 4 rabies-endemic provinces lacked strictly enforced measures to eliminate dog rabies or an ample supply

of modern cell culture rabies vaccines for humans." Most of the patients were children or teenagers, and most contracted the disease after being bitten by a dog, usually on the head and neck. According to the authors, "In the worst-affected province, Guangdong, 62.5 percent of patients did not receive proper treatment on their wounds, 92.5 percent did not receive adequate post-exposure vaccination, and 91.25 percent did not receive any anti-rabies immunoglobulin."

The authors recommend that the current rabies control programme be improved by increasing supervision, improving the interaction between local and national authorities, increasing rabies awareness, and altering urban planning and development to balance the interaction between humans and animals.

Reference

Han Si, Zhong-Min Guo, Yuan-Tao Hao, Yu-Ge Liu, Ding-Mei Zhang, Shao-Qi Rao, and Jia-Hai Lu: Rabies trend in China (1990-2007) and post-exposure prophylaxis in the Guangdong province. BMC Infectious Diseases, (in press) [available at <http://www.biomedcentral.com/content/pdf/1471-2334-8-113.pdf>].

Adapted from materials provided by BMC Infectious Diseases (<http://www.biomedcentral.com/bmcinfectdis/>) via EurekAlert!, a service of AAAS (<http://www.eurekalert.org>).

—

Communicated by:
Shamsudeen Fagbo, DVM
<oloungbo@yahoo.com>

[It is useful to read the full article, not so much for the summary of incidence trends or methods but to fully appreciate the application of potential control mechanisms. The authors emphasize the need for improved availability and timely application of anti-rabies biologicals and the undertaking of dog vaccination programs for the control of rabies in dogs as critical elements for success in reducing the rate of occurrence of rabies in China. Such strategies have worked in other countries around the world and have even previously worked in China in the 1990s. The failure of effective dog vaccination programs in China is a step back.

CDC's (US Centers for Disease Control and Prevention) Advisory Council on the subject agrees with the importance of vaccination in dogs in the following introduction:

"As a result of improved canine vaccination programs and stray animal control, a marked decrease in domestic animal rabies cases in the United States occurred after World War II. This decline led to a substantial decrease in indigenously acquired rabies among humans. In 1946, a total of 8384 indigenous rabies cases were reported among dogs and 33 cases in humans. In 2006, a total of 79 cases of rabies were reported in domestic dogs, none of which was attributed to enzootic dog-to-dog transmission, and 3 cases were reported in

humans. The infectious sources of the 79 cases in dogs were wildlife reservoirs or dogs that were translocated from localities where canine rabies virus variants still circulate. None of the 2006 human rabies cases was acquired from indigenous domestic animals. Thus, the likelihood of human exposure to a rabid domestic animal in the United States has decreased substantially."

See "Human Rabies Prevention – United States, 2008, Recommendations of the Advisory Committee on Immunization Practices" at <http://www.cdc.gov/mmwr/pdf/rr/rr57e507.pdf>.

WHO's introduction to their section on rabies from the "WHO recommended standards and strategies for surveillance, prevention, and control of communicable diseases" includes 3 main control strategies: post-exposure prophylaxis, pre-exposure immunization in high risk groups, and control of the disease in dogs.

WHO provides further information in the introduction as follows:
"Rabies is a vaccine-preventable disease, and it is still a significant public health problem in many countries of Asia and Africa, even though safe, effective vaccines for both human and veterinary use exist. Most of the 55 000 deaths from rabies reported annually around the world occur in Asia and Africa, and most of the victims are children: 30–50 percent of the reported cases of rabies — and therefore deaths — occur in children under 15 years of age. The main route of transmission is the bite of rabid dogs. Most of the children who die from rabies were not treated or did not receive adequate post-exposure treatment. Although the efficacy and safety of modern cell culture vaccines have been recognized, some Asian countries still produce and use nervous tissue vaccines, which are less effective, require repeated visits to the hospital, and often have severe side-effects. Moreover, these patients do not receive the necessary rabies immunoglobulin, because of a perennial global shortage and because of its high price, so that it is unaffordable in countries where canine rabies is endemic.

"Due to complete absence of any successful medical treatment for clinical rabies and the horrific nature of the disease, most rabies victims die at home rather than being admitted to a hospital in abysmal conditions. These circumstances add to the notorious lack of surveillance data. Underestimating the health implications of rabies leads many high ranking decision-makers in public health and animal health to perceive rabies as a rare disease of humans resulting from a bite of an economically unimportant animal (the dog). Therefore, rabies usually falls between 2 stools and is not dealt with appropriately either by the Ministry of Health or the Ministry of Agriculture."
See "Human and Animal Rabies" at <http://www.who.int/rabies/en/>. – Mod.PC]

[see also:
Rabies, canine – China: compulsory vaccination 20080120.0254
2007
—
Rabies, human, canine – China (02) 20070725.2390

Rabies, human, canine – China 20070612.1917
Rabies, human, canine – China (Hunan) 20070305.0782
Rabies, human, canine – China (Beijing) 20070204.0449
2006
———
Rabies, human, canine – China (04) 20061213.3504
Rabies, human, canine – China (03) 20061111.3240
Rabies, human, canine – China (03) 20061110.3225
Rabies, human, canine – China (02) 20061010.2905
Rabies, human, canine – China (Beijing, Shanghai) 20060827.2443
Rabies, human, canine – China (Beijing) (02) 20060826.2424
Rabies, canine, human – China (Shandong) 20060805.2174
Rabies, canine, human – China (Guangdong) 20060608.1605
Rabies, canine, human – China (Beijing) 20060226.0632
2005
———
Rabies, canine, human – China (Beijing) 20050814.2380
Rabies, canine, human – China (Guangdong): correction 20050319.0799
Rabies, canine – China (Guangdong) 20050318.0784]
.....pc/mj/jw

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. 7. 3	該当なし	

一般的名称	(製造販売承認書に記載なし)	研究報告の公表状況	Dujardin JC, Campino L, Cañavate C, Dedet JP, Gradoni L, Soteriadou K, Mazeris A, Ozbel Y, Boelaert M. Emerg Infect Dis. 2008 Jul;14(7):1013-8.	公表国
販売名(企業名)	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)			ヨーロッパ

研究報告の概要

○ヨーロッパにおける生物媒介性疾患の拡大とリーシュマニア症に対する軽視(ネグレクト)
リーシュマニア症は南ヨーロッパ各国に定着しており、毎年700例近く、トルコを含めると3,950例の地域内感染症例が報告される。
ヒトでのリーシュマニア症の発現率は100,000人当り0.02～0.49(トルコを含むと8.53/100,000)である。無症候症例は、臨床症例1例に対して30～100例程度発生すると見られている。これは、血液事業に重大な影響を及ぼす可能性がある。南フランスとギリシャの流行地域に住む供血者由来の血液の血清陽性率は、それぞれ3.4%、15%であった。スペインの高流行地域の供血者の22.1%は、PCR法でリーシュマニア症陽性であった。また、無症候感染は、エイズ患者などの免疫不全者で重度の臨床型に進行する場合がある。飼い犬のリーシュマニア症血清陽性率は最高25%と推定されている。薬剤耐性*L. infantum*は、イヌを介してヨーロッパ国外に輸出されるかもしれない。
薬剤耐性の出現などの問題があるにもかかわらず、全ヨーロッパレベルでの協調的な疾患調査は行われていない。リーシュマニア症は、睡眠病やシャーガス病などと同様に、発展途上国で最も軽視された疾患の1つであり、有効で安価で使用簡便な薬剤の開発、調査や対策は行われてこなかった。この主な理由の1つには、リーシュマニア症が発展途上国の貧しい者の疾患であるということがある。
2001年以降、複数の研究チームが欧州一地中海諸国から科学者を集め、リーシュマニア症研究者のネットワークが形成された。今後研究者は、基礎研究を進めると共に、結果を発表することで政策決定に影響を与え、生物媒介性疾患の1つとして対策が行われるよう働きかけなくてはならない。

報告企業の意見	今後の対応
リーシュマニア症は南ヨーロッパ各国に定着しており、毎年700例近くの症例が報告されているが、全ヨーロッパレベルでの調査や対策が行われていないとの報告である。リーシュマニア症をはじめとするダニ媒介性疾患の対策は難しく、流行状況に注意が必要である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努めるとともに、ヨーロッパにおける輸血感染症の動向等に注意する。

使用上の注意記載状況・その他参考事項等

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.11.0

Spread of Vector-borne Diseases and Neglect of Leishmaniasis, Europe

Jean-Claude Dujardin,* Lenea Campino,† Carmen Cañavate,‡ Jean-Pierre Dedet,§ Luigi Gradoni,¶ Ketty Soteriadou,# Apostolos Mazeris,** Yusuf Ozbel,†† and Marleen Boelaert*

The risk for reintroduction of some exotic vector-borne diseases in Europe has become a hot topic, while the reality of others is neglected at the public health policy level. Leishmaniasis is endemic in all southern countries of Europe, with ≈700 autochthonous human cases reported each year (3,950 if Turkey is included). Asymptomatic cases have been estimated at 30–100/1 symptomatic case, and leishmaniasis has up to 25% seroprevalence in domestic dogs. Even though leishmaniasis is essentially associated with *Leishmania infantum* and visceral leishmaniasis, new species, such as *L. donovani* and *L. tropica*, might colonize European sand fly vectors. Drug-resistant *L. infantum* strains might be exported outside Europe through dogs. Despite this possibility, no coordinated surveillance of the disease exists at the European level. In this review of leishmaniasis importance in Europe, we would like to bridge the gap between research and surveillance and control.

In August through September of 2007, a chikungunya outbreak occurred in the province of Ravenna, Italy (*1*). The risk for reintroduction of vector-borne diseases in Europe as a consequence of global warming was highlighted, although long-distance tourism, travel, and trade could also play major roles in the transcontinental transport of microorganisms (*2*). The European Centre for Disease Control is currently assessing the magnitude and importance of vector-borne diseases in Europe, focusing on Lyme disease, tick-borne encephalitis, leptospirosis, malaria, plague, tularemia, viral hemorrhagic fevers, hantavirus, and West Nile fever. Concern about the impact of global warming and the spread of arthropod-borne diseases and other infectious agents in Europe is justifiable. However, existing autochthonous vector-borne infections should not be forgotten or ignored, which may be the case, as illustrated here for leishmaniasis.

*Instituut voor Tropische Geneeskunde, Antwerp, Belgium; †Instituto de Higiene e Medicina Tropical, Lisbon, Portugal; ‡Instituto de Salud Carlos III, Madrid, Spain; §Université Montpellier 1, Montpellier, France; ¶Istituto Superiore di Sanità, Rome, Italy; #Hellenic Pasteur Institute, Athens, Greece; **National Reference Laboratory for Animal Health, Nicosia, Cyprus; and ††Ege University Medical School, Bornova, Izmir, Turkey

DOI: 10.3201/eid1407.071589

Leishmaniasis in Europe

Leishmaniasis is a major vector-borne disease, which is endemic to 88 countries and is the only tropical vector-borne disease that has been endemic to southern Europe for decades. In southern Europe, most of the reported cases are due to zoonotic visceral leishmaniasis (VL), which is the most dangerous form and is lethal when untreated. Cutaneous leishmaniasis (CL), which is more benign than VL, is also present. Incidence of leishmaniasis in humans is relatively low, ranging from 0.02/100,000 to 0.49/100,000 (8.53/100,000 including Turkey). We estimate that this corresponds to a total of ≈700 reported new cases per year for southern European countries (3,950 if Turkey is included; Table and Figure). However, autochthonous leishmaniasis appears not to be limited to the Mediterranean region anymore. It has spread northward, as shown by the recent reports of indigenous VL cases in northern Italy and southern Germany (*8*,*9*).

However, these numbers are misleading for several reasons. First, data from patients infected in southern Europe, but diagnosed elsewhere, are not taken into consideration. For instance, a leishmaniasis reference center established on a voluntary basis in Germany identified within 2 years 70 cases of leishmaniasis. Of the 27 VL case-patients, most

Table. Leishmaniasis situation in 7 disease-endemic countries of Europe (including Turkey)*

Country	Human leishmaniasis				Canine leishmaniasis
	Notification status	Current information from reference centers (2000–2006)	VL + CL incidence x 100,000†	Imported cases (VL + CL)	
Portugal‡	Compulsory for VL	≈22 VL cases/y recorded at IHMT	0.07–0.17	≈2 cases/y recorded at IHMT	Average 20% seroprevalence in disease-endemic areas (3)
Spain§	Compulsory in 12/17 autonomous communities; 20%–45% underreporting for VL, ≈100% for CL (4).	≈100 VL cases/y recorded by National Epidemiologic Surveillance Network, RENAVE	0.18–0.29	≈5 cases/y recorded at ISCIII	Average 8.5% seroprevalence (5)
France¶	Not compulsory, but spontaneous reports at UMON	≈24 VL + CL cases/y reported at UMON	0.02–0.19	≈65 cases/y recorded at UMON	Seroprevalence in disease-endemic areas of southern France 4%–20%#
Italy**	Compulsory for both VL and CL, but CL underreported	≈200 VL cases/y recorded at ISS; ≈300 CL cases/y estimated by ISS	0.15–0.38	≈8 cases/y recorded at ISS	Average 15% seroprevalence in peninsular Italy; average 2% seroprevalence in continental Italy (6)
Greece††	Compulsory for both VL and CL, but underreported	≈21 VL cases/y notified	0.06–0.49	Unknown	Average seroprevalence 25% in disease-endemic areas (7)
Cyprus‡‡	Compulsory for both VL and CL, but underreported	5 VL + CL cases recorded in 2006	0.25–0.47	Unknown	Average seroprevalence 20% in disease-endemic areas
Turkey§§	Compulsory for both VL and CL	≈37 VL cases/y and ≈2,300 CL cases/y notified	1.6–8.53	Unknown	Average 15.7% seroprevalence

*Authors' institutions are national reference laboratories for leishmaniasis diagnosis and surveillance and rely on consolidated countrywide networks of collaborating clinical health centers. Diagnosis records are cross-checked with case notifications to provide more realistic figures and estimates. VL, visceral leishmaniasis; CL, cutaneous Leishmaniasis; WHO, World Health Organization.
†WHO-EURO, WHO Europe, 1996–2005; http://data.euro.who.int/CISID.
‡Instituto de Higiene e Medicina Tropical (IHMT), Lisbon, Portugal.
§Instituto de Salud Carlos III (ISCIII), Madrid, Spain.
¶Université de Montpellier (UMON), data from Centre National de Référence des Leishmania, Montpellier, France.
#Source: retrospective canine leishmaniasis database, Centre National de Référence des Leishmania.
**Istituto Superiore di Sanità (ISS), Rome, Italy.
††Hellenic Pasteur Institute (HPI), Athens, Greece.
‡‡National Reference Laboratory for Animal Health (VS), Nicosia, Cyprus.
§§Ege University (EUMS-DP), Izmir, Turkey.

(17) had been infected within European Union boundaries: Spain, Portugal, Greece, or France (*10*). Five cases were in children. Similarly, a retrospective study in the Hospital for Tropical Diseases in London showed that most of the imported VL case-patients in the United Kingdom were adult men touring the Mediterranean (*11*). Second, in the absence of public health surveillance at the European level, underreporting is common (see the Leishmaniasis and the Globalization of Neglect section). Third, asymptomatic infections may be common in some regions: for 1 clinical case of VL, there may be 30–100 subclinical infections (*12*). This underreporting can have major consequences for blood banks: blood from donors living in areas of endemicity in southern France and Greece had 3.4% and 15% seropositivity, respectively (*13,14*). In addition, 22.1% of blood donors in a highly disease-endemic area from Spain were PCR positive for leishmaniasis (*15*). Furthermore, asymptomatic infections may progress to severe clinical forms in immunocompromised persons, for example, in AIDS patients (*16*). Fourth, the etiologic agent of southern European VL, *Leishmania infantum*, is also infecting dogs (with a seroprevalence of up to 34% in areas of Spain where the disease is highly endemic) (Table). Dogs with leishmaniasis infections are generally very sick, causing a major problem in southern Europe (e.g., ≈5,000 clinical cases occur each year in France) (Table). However, sick as well as asymptomatic dogs also represent a risk for humans, as they constitute the major reservoir of the parasite on which sand fly vectors may feed and transmit the infection.

Import–Export Balance of European Leishmaniasis

In addition to the reality of autochthonous leishmaniasis in Europe, the risk for introduction of new species through travelers or immigrants from countries where

non-European species are endemic should also be considered. However, the probability that these species could enter in a transmission cycle is relatively low. The probability depends on contact between infected persons and sand flies, the capacity of the infected person to act as reservoir, and the susceptibility of European sand flies to the different *Leishmania* species. For most species, humans are generally a transmission dead-end. However, for 2 species, the risk might theoretically be higher: *L. tropica*, which is causing CL in Africa, the Middle East, and Southwest Asia, and *L. donovani*, the etiologic agent of VL in East Africa and the Indian subcontinent. These 2 species are indeed associated with an anthroponotic transmission cycle. On one hand, *L. donovani*, which is transmitted by a different species of sand fly outside Europe, might be hosted by most European sand flies, except *Phlebotomus papatasi* and *P. sergenti* (*17*). On the other hand, *L. tropica*, which has more stringent requirements in terms of vector, would need *P. sergenti*, which was reported in several places in southern Europe, from Portugal (*18*) to Cyprus (*19*). *L. tropica* was indeed encountered in Greece (*20*), and according to a very recent report, the first autochthonous cases of *L. donovani* in Europe have been detected in Cyprus (*21*). The clinical phenotype associated with both species needs also to be considered for an exhaustive risk evaluation. *L. tropica* causes lesions that are generally more difficult to treat with antimonial drugs (*22*), whereas *L. donovani* is considered to be more aggressive than *L. infantum* and often does not respond to treatment with first-line drugs (*23*).

In addition to being concerned about importation and spread of exotic *Leishmania* species in Europe, exportation should also be considered. The best known historical example of the spread of leishmaniasis is the migration of *L. infantum* from Europe to Latin America, where it colonized in *Lutzomyia longipalpis* and is now causing a serious public health problem (>3,500 cases of VL per year in Brazil) (*24*). This spread is thought to have been caused by conquistadores' dogs (*25*). Another and current example concerns the *L. major/L. infantum* hybrids recently described in HIV-positive VL patients from Portugal (*26*). Indeed, these hybrids were shown to be able to develop in *P. papatasi* (*27*), a vector that is widespread in Europe, Africa, and Asia. Considering the reservoir role of HIV–co-infected patients and the peridomestic and anthropophilic nature of *P. papatasi*, these hybrid strains might circulate by using this sand fly vector, thereby increasing the risk of their spreading into new foci throughout the broad range of *P. papatasi* distribution (*27*). Finally, the way Europe deals with its leishmaniasis public and animal health problem can still have major consequences for the rest of the world. Miltefosine, one of the few available antileishmania drugs, has been recently launched in the market for canine leishmaniasis treatment in Portugal, Spain, Italy, Greece, and Cyprus. Because dogs are never cured parasitologically and given the long half-life of the drug, the lack of European policy might contribute to the emergence of parasites resistant to miltefosine. This resistance could be a problem for European human patients, as miltefosine is being used on a compassionate basis in several European AIDS co-infected patients unresponsive to amphotericin B or pentavalent antimonials (*28*,*29*). Furthermore, if dogs infected with miltefosine-resistant strains were to migrate to Latin America, where several countries have registered the drug for human use (currently Colombia, Guatemala, Argentina, Venezuela, Paraguay, Ecuador, and Honduras; *30*), the impact might be greater.

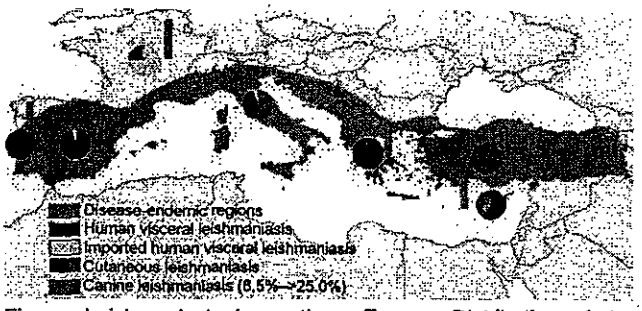


Figure. Leishmaniasis in southern Europe. Distribution of the endemic disease; relative proportion of autochthonous (visceral, cutaneous) and imported human cases and seroprevalence in dogs (from data reported in Table).

Leishmaniasis and the Globalization of Neglect

Twelve million persons have leishmaniasis, and 500,000 new cases of VL occur each year. More than 50,000 die of this disease each year. The disease is spreading because of several risk factors, climate being only one. Humanmade changes to the environment and population movements (for economic or political reasons) may lead to alterations in the range and densities of the vectors and reservoirs, increasing human exposure to infected sand flies. Urbanization of leishmaniasis becomes more common and in conjunction with the ruralization of HIV/AIDS, it contributes to increase the problem of co-infections in contexts where access to highly active antiretroviral therapy is not the same as in industrialized countries. *Leishmania* spp. have already become resistant to antimonial drugs (the first-line drug in many developing countries) in some regions and may soon become resistant to miltefosine (*23*). Despite this increasing resistance, leishmaniasis is one of the most neglected diseases in developing countries, along with others like sleeping sickness or Chagas disease. Leishmaniasis is a disease for which we lack effective, affordable, and easy to use drugs, and the pharmaceutical industry has had few incentives to engage

in their development. In addition, leishmaniasis surveillance and control are also neglected. One of the main reasons for this neglect is that in developing countries, leishmaniasis is a disease of the poor. Risk for infection and clinical development are mediated by poverty, while leishmaniasis diagnosis and treatment are expensive and may lead to further impoverishment and reinforcement of the vicious cycle of disease and poverty (*31*).

In Europe, physicians are sometimes ill-informed on the diagnosis and treatment of leishmaniasis. In France, a telephone advice line was created in 2006 by the National Reference Centre of Leishmania to help physicians in their therapeutic diagnosis. A study in Germany, a non–disease-endemic country, showed that the median time between symptom onset and correct diagnosis was 85 and 61 days in case-patients of VL and CL, respectively (*32*). This value was lower in a leishmaniasis-endemic area, such as southern Italy (35 days, [*33*]). VL, which was initially a pediatric disease in Europe (hence the name of *L. infantum*), only began to gain attention when the co-infection of HIV/AIDS was documented. Between the late 1980s and early 2001, >1,900 cases were reported in southwestern Europe (*16*). Even though it was reported that both pathogens could be transmitted through sharing of needles among intravenous drug users (*34*), in many cases of co-infection, the parasite was already present at the time of HIV infection, which indicates that HIV infection would have an unmasking effect on the true endemicity of *Leishmania* infection. In other words, the wave of *Leishmania*/HIV co-infection showed that *L. infantum* could behave as an opportunistic parasite, with many asymptomatic carriers (*12*), and with the clinical syndromes being only the tip of the iceberg. Because of the highly active antiretroviral therapy, cases of co-infection generally decreased in the region, with the exception of Portugal (*35*).

Notification of VL varies according to the country. It does not belong to the list of 30 notifiable diseases in France. However, notification is compulsory in Greece, Italy, and Portugal, though only obligatory in 12 of 17 autonomous communities of Spain. Underreporting is common. In Portugal, for instance, 76 cases of autochthonous VL were officially reported at the country level from 2000 through 2005. During the same period, 127 cases (+67%) were observed in the Institute of Tropical Medicine of Lisboa (Table). In the case of autochthonous cutaneous leishmaniasis, consolidated data are lacking, but this clinical form is definitely underreported because of its benign nature and the fact that it usually does not require hospitalization. Nonetheless, leishmaniasis is not a disease placed under public health surveillance at the European level. It does not even belong to the package of rare diseases considered as a priority in the Public Health Programme 2003–2008. (Rare diseases, including those of genetic origin, are life-threatening or chronically debilitating diseases that are of such low prevalence [<5/10,000 persons] that special combined efforts are needed to address them so as to prevent significant illness or perinatal or early deaths or a considerable reduction in a person's quality of life or socioeconomic potential.) At the regional level, the only dedicated network of surveillance was the one launched by the World Health Organization and the Joint United Nations Programme on HIV/AIDS in 1993 for the surveillance of *Leishmania*/HIV co-infections, which essentially involved European countries as well as some developing countries.

The low-profile perception seen for human leishmaniasis differs dramatically from the veterinary world's perception. The high incidence of canine leishmaniasis in southern Europe makes *Leishmania* one of the main dog killers in the region, and private veterinarians are well aware of it. Dogs are treated individually to protect from sand fly bites, and those diagnosed as infected are considered extremely difficult to treat. Specific web sites are available for owners of infected dogs to discuss and compare treatment regimens and pose questions to veterinarians. Several pharmaceutical companies are investing in research and development of vaccines, drugs, and topical insecticides for specific cure and prevention of canine leishmaniasis. This high-profile perception, however, drops when dogs must be treated as the reservoir of human leishmaniasis. For instance, the issue of notification is treated differently in various leishmaniasis–endemic countries, but even where notification is compulsory (i.e., Italy and Spain), it is not a common practice. In Italy, the network Leishmap is currently monitoring the spread of canine leishmaniasis and vectors in northern Italy. Leishmap is a scientific network, supported by a private company (*36*). Furthermore, private interests are sometimes at odds with public health goals. Drugs for leishmaniasis are not regulated in the veterinary market, and medications intended for use in humans, such as Ambisome, are used in domestic pets, with the potential risk that they might be a source for the emergence and spreading of resistant strains.

Countering the Neglect

Since 2001, several research consortia gathered scientists from Euro-Mediterranean countries (www.leishrisk.net). These consortia and other research groups generated knowledge, tools, and education packages and led to a solid European research network dedicated to the study of leishmaniasis. Bridging research with surveillance and control is an issue of dialogue and advocacy. On one hand, health professionals need to be in close contact with scientists to help translate basic research into relevant and applicable tools. For instance, sequencing the whole genome of *Leishmania* represented a technologic challenge, but the next challenge is to exploit this sequencing for the benefit of the patient (www.leishrisk.net). On the other hand, scientists

must market their results to influence health policy. Changes in health policy are being made; during manuscript revision, we were informed of the selection of leishmaniasis among the priority zoonoses addressed by the Episouth network (www.leishrisk.net).

Deciding health policy is a complex social, economic, and political interrelationship that is much broader than leishmaniasis alone (or even infectious diseases generally). However, if Europe justifiably wants to invest more in surveillance of vector-borne diseases, the time has come to recognize its real impact on both animal and human health and include leishmaniasis as one of these diseases.

Acknowledgments

We thank Kirami Olgen for the preparation of the map on leishmaniasis.

LEISHMED (coordination action) and LEISHRISK (specific support action) are funded by the 6th Frame Programme of European Union (Programme INCO): contracts INCO-CT-2004-509086 and INCO 043490, respectively.

Dr Dujardin is the head of the Molecular Parasitology Unit at the Institute of Tropical Medicine of Antwerp, Belgium. He specializes in the molecular epidemiology of leishmaniasis and in natural drug resistance.

References

1. Watson R. Europe witnesses first local transmission of chikungunya fever in Italy. BMJ. 2007;335:532–3. DOI: 10.1136/bmj.39332.708738.DB
2. Lines J. Chikungunya in Italy. Globalisation is to blame, not climate change. BMJ. 2007;335:576. DOI: 10.1136/bmj.39342.563310.80
3. Cortes S, Afonso MO, Alves-Pires C, Campino L. Stray dogs and leishmaniasis in urban areas, Portugal. Emerg Infect Dis. 2007;13:1431–2.
4. Alvar J. *Las leishmaniasis: de la biología al control, 2001. Salamanca*: Laboratorios Intervet S.A.; 2001.
5. Miró G, Molina R. Leishmaniosis canina: Manejo clínico y situación actual en España, 2006. Spain: Bayer Health Care; 2006
6. Gradoni L. Epizootiology of canine leishmaniasis in southern Europe. In: R. Killick-Kendrick, editor. Canine leishmaniasis: an update. Wiesbaden (Germany): Hoechst Roussel Vet; 1999. p. 32–9.
7. Papadopoulou C, Kostoula A, Dimitriou D, Panagiou A, Bobojianni C, Antoniades GJ. Human and canine leishmaniasis in asymptomatic and symptomatic population in Northwestern Greece. J Infect. 2005;50:53–60. DOI: 10.1016/j.jinf.2004.05.004
8. Maroli M, Rossi L, Baldelli R, Capelli G, Ferroglio E, Genchi C, et al. The northward spread of leishmaniasis in Italy: evidence from retrospective and ongoing studies on the canine reservoir and phlebotomine vectors. Trop Med Int Health. 2008;13:256–64.
9. Bogdan C, Schonian G, Banuls AL, Hide M, Pratlong F, Lorenz E, et al. Visceral leishmaniasis in a German child who had never entered a known endemic area: case report and review of the literature. Clin Infect Dis. 2001;32:302–6. DOI: 10.1086/318476
10. Harms G, Schönian G, Feldmeier H. Leishmaniasis in Germany. Emerg Infect Dis. 2003;9:872–5.
11. Malik ANJ, John L, Bryceson ADM, Lockwood DNJ. Changing pattern of visceral leishmaniasis, United Kingdom, 1985–2004. Emerg Infect Dis. 2006;12:1257–9.
12. Pampiglione S, Manson-Bahr PE, La Placa M, Borgatti MA, Musumeci S. Studies in Mediterranean leishmaniasis. 3. The leishmanin skin test in kala-azar. Trans R Soc Trop Med Hyg. 1975;69:60–8. DOI: 10.1016/0035-9203(75)90012-7
13. Le Fichoux Y, Quaranta JF, Aufcuvre JP, Lelievre A, Marty P, Suffia I, et al. Occurrence of *Leishmania infantum* parasitemia in asymptomatic blood donors living in an area of endemicity in southern France. J Clin Microbiol. 1999;37:1953–7.
14. Kyriakou DS, Alexandrakis MG, Passam FH, Kourelis TV, Foundouli P, Matalliotakis E, et al. Quick detection of *Leishmania* in peripheral blood by flow cytometry. Is prestorage leucodepletion necessary for leishmaniasis prevention in endemic areas? Transfus Med. 2003;13:59–62. DOI: 10.1046/j.1365-3148.2003.00420.x
15. Riera C, Fisa R, Udina M, Gállego M, Portus M. Detection of *Leishmania infantum* cryptic infection in asymptomatic blood donors living in an endemic area (Eivissa, Balearic Islands, Spain) by different diagnostic methods. Trans R Soc Trop Med Hyg. 2004;98:102–10. DOI: 10.1016/S0035-9203(03)00015-4
16. Desjeux P, Alvar J. *Leishmania*/HIV co-infections: epidemiology in Europe. Ann Trop Med Parasitol. 2003;97(Suppl 1):3–15. DOI: 10.1179/000349803225002499
17. Myskova J, Svobodova M, Beverley SM, Volf P. A lipophosphoglycan-independent development of *Leishmania* in permissive sand flies. Microbes Infect. 2007;9:317–24. DOI: 10.1016/j.micinf.2006.12.010
18. Afonso MO, Campino L, Cortes S, Alves-Pires C. The phlebotomine sandflies of Portugal. XIII–Occurrence of *Phlebotomus sergenti* Parrot, 1917 in the Arrabida leishmaniasis focus. Parasite. 2005;12:69–72.
19. Depaquit J, Léger N, Ferté H, Rioux JA, Gantier JC, Michaelides A, et al. Phlebotomines of the Isle of Cyprus. III. Species inventory. Parasite. 2001;8:11–20.
20. Gariffallou A, Schnur LF, Stratigos JD, Hadziandoniou M, Savigos M, Stavrianeas N, et al. Leishmaniasis in Greece II. Isolation and identification of the parasite causing cutaneous leishmaniasis in man. Ann Trop Med Parasitol. 1984;78:369–75.
21. Antoniou M, Haralambous C, Mazeris A, Pratlong F, Dedet J-P, Soteriadou K. *Leishmania donovani* leishmaniasis in Cyprus. Lancet Infect Dis. 2008;8:6–7. DOI: 10.1016/S1473-3099(07)70297-9
22. Hadighi R, Mohebali M, Boucher P, Hajjaran H, Khamesipour A, Ouellette M. Unresponsiveness to Glucantime treatment in Iranian cutaneous leishmaniasis due to drug-resistant *Leishmania tropica* parasites. PLoS Med. 2006;3:e162. DOI: 10.1371/journal.pmed.0030162
23. Croft SL, Sundar S, Fairlamb AH. Drug resistance in leishmaniasis. Clin Microbiol Rev. 2006;19:111–26. DOI: 10.1128/CMR.19.1.111-126.2006
24. Miles MA, Vexenat JA, Furtado Campos JH, Fonseca de Castro JA. Canine leishmaniasis in Latin America: control strategies for visceral leishmaniasis. In: R. Killick-Kendrick, editor. Canine leishmaniasis: an update. Barcelona: Hoechst Roussel Vet;1999. p. 46–53.
25. Mauricio IL, Stothard JR, Miles MA. The strange case of *Leishmania chagasi*. Parasitol Today. 2000;16:188–9. DOI: 10.1016/S0169-4758(00)01637-9
26. Ravel C, Cortes S, Pratlong F, Morio F, Dedet JP, Campino L. First report of genetic hybrids between two very divergent *Leishmania* species: *Leishmania infantum* and *Leishmania major*. Int J Parasitol. 2006;36:1383–8. DOI: 10.1016/j.ijpara.2006.06.019
27. Volf P, Benkova I, Myskova J, Sadlova J, Campino L, Ravel C. Increased transmission potential of *Leishmania major*/*Leishmania infantum* hybrids. Int J Parasitol. 2007;37:589–93. Epub 2007 Feb 15. DOI: 10.1016/j.ijpara.2007.02.002
28. Schraner C, Hasse B, Hasse U, Baumann D, Faeh A, Burg G, et al. Successful treatment with miltefosine of disseminated cutaneous leishmaniasis in a severely immunocompromised patient infected with HIV-1. Clin Infect Dis. 2005;40:e120–4. DOI: 10.1086/430444

29. Troya J, Casquero A, Refoyo E, Fernández-Guerrero ML, Górgolas M. Long term failure of miltefosine in the treatment of refractory visceral leishmaniasis in AIDS patients. Scand J Infect Dis. 2008;40:78–80. DOI: 10.1080/00365540701466215
30. World Health Organization. Eighteenth programme report/progress 2005–2006. Geneva: The Organization; 2007.
31. Alvar J, Yactayo S, Bern C. Leishmaniasis and poverty. Trends Parasitol. 2006;22:552–7. DOI: 10.1016/j.pt.2006.09.004
32. Weitzel T, Mühlberger N, Jelinek T, Schunk M, Ehrhardt S, Bogdan C, et al. Imported leishmaniasis in Germany 2001–2004: data of the SIMPID surveillance network [in German]. Eur J Clin Microbiol Infect Dis. 2005;24:471–6. DOI: 10.1007/s10096-005-1363-1
33. Pagliano P, Rossi M, Rescigno C, Altieri S, Coppola MG, Gramiccia M, et al. Mediterranean visceral leishmaniasis in HIV-negative adults: a retrospective analysis of 64 consecutive cases (1995–2001). J Antimicrob Chemother. 2003;52:264–8. DOI: 10.1093/jac/dkg340
34. Alvar J, Jimenez M. Could infected drug-users be potential *Leishmania infantum* reservoirs? AIDS. 1994;8:854. DOI: 10.1097/00002030-199406000-00024
35. World Health Organization. Report of the fifth Consultative Meeting on HIV-*Leishmania* Co-Infection, Addis Ababa 20–22 March 2007. Geneva: The Organization; 2007.
36. Capelli G, Baldelli R, Ferroglio E, Genchi C, Gradoni L, Gramiccia M, et al. Monitoring of canine leishmaniasis in northern Italy: an update from a scientific network. Parassitologia. 2004;46:193–7.

Address for correspondence: Jean-Claude Dujardin, Instituut voor Tropische Geneeskunde, Molecular Parasitology, Nationalestraat 155, B-2000 Antwerp, Belgium; email: jcdujardin@itg.be

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. 5. 26	該当なし	

一般的名称	人血清アルブミン	研究報告の公表状況	Patel KK, Andrzejewski C Jr, Webley WC. American Society for Microbiology 108th General Meeting; 2008 Jun 1-5; Boston.	公表国 米国
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社)			

研究報告の概要

○市販アルブミン製剤におけるクラミジアの存在

目的:人血清アルブミン(HSA)は、多数の健康なボランティアドナーから採血された全血由来のプールから製造され、一般的には病院で、特に治療的プラズマフェレーシスを行う患者の補液療法に用いられている。我々の研究室他における以前の調査から、C. pneumoniae(Cp)組織体が、これらの健常ドナー及び採血された血液細胞製剤から分離された。これらの知見に基づき、無細胞性の血液製剤であるHSAについてクラミジアが存在するか分析することにした。

方法:様々なHSA製剤のボトルをApheresis Medicine Service at Baystate Medical Centerから入手した。これらの素材は品質管理目的に使用された廃棄製剤や期限切れロット、アフェレーシス療法を中断した際の補液の残りである。クラミジアの検出は標準PCR、ウエスタンブロット、組織培養、免疫蛍光染色を用いて行った。

結果:メーカー4社が製造しているHSA製剤20種類を検討した。クラミジア特異的DNAがPCRで20本全て(100%)から検出された。種特異的プライマーを使用したところ、17検体(90%)がCP DNA陽性となったが、Chlamydia trachomatis(Ct)DNAが陽性となったものはなかった。クラミジア特異的ポリクローナル抗体を用いたウエスタンブロットで、PCRのデータが裏付けられた。培養では、クラミジア生菌が11(55%)のHSA製剤で認められた。

結論:PCR及びウエスタンブロットを用いて、20本のHSA製剤全てにおいて、クラミジアの存在が確認された。予期せぬ事だったが、これら無細胞性HSA製剤において、in vitro培養を行ったところ、11検体でクラミジア生菌が生育した。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25

血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
PCR及びウエスタンブロットを用いて、20本の市販人血清アルブミン製剤全てにおいて、クラミジアの存在が確認され、in vitro培養を行ったところ、11検体でクラミジア生菌が生育したとの報告である。	これまで、本製剤によるC. pneumoniae感染の報告はない。クラミジアは熱感受性で56℃30分で不活化されるとの報告もあるが、日本赤十字社が製造販売している赤十字アルブミンははるかに厳しい、60℃10時間の液状加熱で不活化を行なっている。また最終製剤についてPCR法で試験したところC. pneumoniaeは検出されなかったことから、本製剤の安全性は確保されていると考えるが、今後も情報収集に努める。

(4)

別紙

Print this Page for Your Records Close Window

Abstract Title: *Chlamydia* Presence in Commercial Albumin Preparations

Author Block: K. K. Patel[1], **C. Andrzejewski, Jr.**[2], W. C. Webley[1];
[1]Univ. of Massachusetts, Amherst, MA, [2]Baystate Med. Ctr., Springfield, MA.

Presentation Number: C-260

Poster Board Number: 0089

Keywords: Chlamydia,Human Albumin,transfusion

Purpose: Human serum albumin (HSA) prepared from large pools of whole blood collected from normal volunteer donors, is commonly used in hospitals for fluid replacement therapy, especially in patients undergoing therapeutic plasmapheresis. From previous investigations by our lab as well as others, *C. pneumoniae* (Cp) organisms have been isolated from these normal blood donors and cellular blood products collected from them. Based on these previous findings we elected to assess HSA, an acellular derivative of donor blood, for the presence of *Chlamydia*. ***Methods***: Bottles of various preparations of HSA were obtained from the Apheresis Medicine Service at Baystate Medical Center, Springfield MA. These materials were obtained from discarded stocks used for quality control purposes, outdated lots and from residual replacement fluids from truncated apheresis procedures. Detection of *Chlamydia* was accomplished through standard PCR, Western blotting, tissue culture, and immunofluorescence techniques. ***Results***: Twenty different HSA preparations from four different manufacturers were examined. *Chlamydia*-specific DNA was detected by PCR in all 20 [100%] HSA preparations examined. Using genus-specific primers, 17 samples [90%] were positive for *Cp* DNA, while zero samples were positive for *Chlamydia trachomatis* (Ct) DNA. Western blotting analysis uing *Chlamydia*-specific polyclonal antibody supported the PCR data. Culture analysis demonstrated that viable *Chlamydia* was present in 11 [55%] of these commercial HSA preparations. ***Conclusions***: The presence of *Chlamydia* was evidenced by both PCR and western blotting techniques in all 20 HSA preparations examined. Unexpectedly, in these acellular HSA preparations, in vitro culture analysis revealed that 11 [55%] of the samples tested harbored viable *Chlamydia*.

OASIS - Online Abstract Submission and Invitation System™ ©1996-2008, Coe-Truman Technologies, Inc.

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. 8. 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	Polizzotto MN, Neo H, Spelman D, Shortt J, Cole-Sinclair MF, Borosak M, Wong P, Wood EM. Transfusion. 2008 Jul;48(7):1520-1.	公表国 オーストラリア	
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

○赤血球輸血に関連した*Streptococcus pneumoniae*菌血症

我々の知る限り初めての赤血球(RBC)輸血による*Streptococcus pneumoniae*感染の1例を報告する。受血者は骨髄異形成症候群と汎血球減少症を発症した79歳男性である。輸血前の診察では平熱だったが、血小板輸血に続いて赤血球1単位を輸血した40分後に39.6℃の発熱、悪寒、背部痛、血圧低下、低酸素血症を発症した。輸血を中止し、抗菌薬による治療を行ったところ回復した。

受血者及びRBCバッグの残存血液検体の培養により*S. pneumoniae*血清型4が生育した。付属セグメントとRBCバッグ本体に色調の違いはなかった。また、RBCの前に輸血した血小板製剤の残りは得られなかった。輸血されたRBC製剤は採血後10日間保存された物で、採血、製造工程での異常はなかった。供血者は53歳の男性で、脾臓摘出術、喫煙、呼吸器感染症など*S. pneumoniae*感染のリスク要因はなく、これまで30年以上供血を続けてきた。血液、鼻腔、咽喉検体の培養は、尿の肺炎球菌抗原検査と共に陰性だった。

*S. pneumoniae*が冷蔵保存されたRBC製剤中で生存、増殖するというデータはこれまでなかったが、今回、RBC製剤1単位に1×10^3の*S. pneumoniae*を接種し、4℃で10日間保存後に採取した検体を培養したところ、持続的な増殖が認められた。ただ、21日後の検体では増殖は認められなかった。

感染の原因は供血者の一時的な菌血症と考えられるが、先に輸血された血小板製剤の汚染の可能性も否定できない。本症例は、通常考えられない細菌によるRBCの汚染も疑う必要があることとヘモビジランスの重要性を物語る。輸血後の敗血症の迅速な診断と抗生物質療法、原因の追及と、随伴製剤の回収が重要である。

報告企業の意見	今後の対応
赤血球輸血による*Streptococcus pneumoniae*敗血症の初の報告例である。本症例では、患者とバッグ内の残存血液から細菌が検出され、再現実験も行われているが、通常4℃の低温下では増殖しないとされている菌であり、因果関係を断定するにはなお十分な検証が必要と考える。	日本赤十字社では、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を喚起している。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)における「本ガイドライン対象以外の病原体の取扱い イ.細菌」に準じ細菌感染が疑われる場合の対応を医療機関に周知している。今後も細菌やウイルスの検出や不活化の方法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

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

337

A2



LETTERS TO THE EDITOR

Streptococcus pneumoniae septicemia associated with red blood cell transfusion

Bacterial contamination of blood components, including red blood cells (RBCs), remains an important risk of transfusion. Bacteria responsible for clinically significant contamination of RBCs include both skin commensals and organisms that are able to proliferate even in refrigerated storage conditions.[1] We describe a case of transfusion-transmitted *Streptococcus pneumoniae* infection caused by contaminated RBCs. This highly pathogenic, but fragile, noncommensal organism has not to our knowledge been reported previously as a cause of transfusion-transmitted bacterial infection.[2]

The recipient was a 79-year-old man with myelodysplastic syndrome and pancytopenia. He had neither history of splenectomy nor evidence of functional hyposplenism. He was afebrile with a normal physical examination before blood transfusion. He received a transfusion of pooled platelets (PLTs) without complication which was followed by transfusion of a unit of RBCs suspended in additive solution and citrate-phosphate-dextrose additive (Adsol, Baxter Healthcare, Deerfield, IL). After approximately 40 minutes, the recipient developed a fever of 39.6°C, rigors, back pain, hypotension, and hypoxia. The transfusion was discontinued and he was treated with intravenous piperacillin-tazobactam, vancomycin, and gentamicin.

Blood samples from the recipient at the time of the fever, together with the residual contents of the container of the RBCs, were available for further testing, but the container of the previously transfused unit of PLTs was not retrievable. The residual contents of the unit of RBCs were not discolored compared with the attached segments. A Gram stain was negative. Cultures were performed separately on the blood samples from the recipient and the residual contents of the unit of RBCs (BacT/ALERT, bioMérieux, Durham, NC). These cultures flagged positive within 12 hours, and subcultures demonstrated Gram-positive α-hemolytic diplococci that were optochin-sensitive and bile-soluble, characteristic of *S. pneumoniae*. Automated identification and susceptibility testing (Vitek 2, bioMérieux) confirmed these findings and demonstrated β-lactam sensitivity. The organisms from both the unit and the recipient were serotype 4.

The recipient made a gradual clinical recovery. Subsequent blood cultures were negative. He became afebrile within 24 hours. He completed a 5-day course of piperacillin-tazobactam (vancomycin and gentamicin were withdrawn after availability of sensitivities) and was discharged with a 10-day course of oral amoxicillin. He remained well at last review.

We assessed possible sources of this unusual contamination of RBCs. The implicated unit of RBCs had been collected 10 days before transfusion. The phlebotomy and processing had been uncomplicated, with no apparent breach of aseptic technique or evidence of respiratory droplet contamination. Plasma from the same blood collection was discarded for an unrelated reason; no PLTs had been made. Components associated with the previously transfused unit of PLTs were also cultured and showed no growth.

The donor was a 53-year-old man, with no history of splenectomy, tobacco use, or respiratory tract infection. He had not been immunized against *S. pneumoniae*. He had donated on numerous occasions more than 30 years before the implicated donation without complication. He had no symptoms suggesting infection at the time of blood collection, but he did report a dental infection 3 months previously. This infection had been treated by tooth extraction and oral antibiotics. There was no evidence of a persistent infection. There had been no other procedures in the intervening period. Physical examination was normal. Cultures of blood and of swabs from the donor's antecubital fossae, nose, and throat were negative, as was urinary testing for pneumococcal antigen.

S. pneumoniae has been reported as a rare contaminant of PLTs,[3] but unlike commensal streptococci (which have infrequently been implicated in cases of contamination of RBCs[4]), there has been no evidence that this organism survives and proliferates outside a host in refrigerated storage of RBCs.[2] Its ability to survive under these conditions was further explored (though without direct evidence of proliferation in RBCs). A unit of RBCs was inoculated with 1×10^3 organisms of *S. pneumoniae* from the original cultures. The inoculated unit of RBCs was maintained at 4°C for 10 days, after which samples were cultured and identified as above. These again demonstrated sustained growth of *S. pneumoniae*. Further cultures performed on separate samples taken after 21 days of incubation at 4°C showed no growth.

This previously unreported cause of contamination of RBCs and transfusion-transmitted *S. pneumoniae* infection had significant adverse clinical consequences and has implications for clinicians and blood services. This case illustrates the importance of a high index of clinical suspicion for RBC contamination, even where the organism is atypical, and the role of robust hemovigilance systems. Prompt recognition of the source of sepsis enabled timely institution of antibiotic therapy and liaison with the blood service to review the donor's health status, collection, and production processes and to enable immediate recall of associated blood components. The source of contamination could not be established definitively. We postulate that it may have originated from a transient bacteremia. While

unlikely, the possibility of retrograde contamination from the previously transfused PLTs cannot be excluded.[5] This episode was not preventable, given the absence of symptoms that might have led to donor deferral and the lack of visual evidence of contamination.

Mark N. Polizzotto, MBBS, BMedSc
Hing Neo, BSc
Denis Spelman, MBBS, MPH
Jake Shortt, MB ChB, BMedSc
Merrole F. Cole-Sinclair, MBBS, BSc
Alfred Pathology Service
The Alfred Hospital
Melbourne
e-mail: m.csinclair@alfred.org.au
Marija Borosak, MBBS
Ping Wong, MBBS, MPH
Erica M. Wood, MBBS
Australian Red Cross Blood Service
Melbourne, Victoria, Australia

REFERENCES

1. Brecher ME, Hay SN. Bacterial contamination of blood components. Clin Microbiol Rev 2005;18:195-204.
2. AlonsoDeVelasco E, Verheul AF, Verhoef J, Snippe H. Streptococcus pneumoniae: virulence factors, pathogenesis, and vaccines. Microbiol Rev 1995;59:591-603.
3. Schuetz AN, Kost CB, Roback JD. Transfusion-transmitted *Streptococcus pneumoniae* from a single donor apheresis platelet unit. J Clin Apher 2005;20:86-7.
4. Perez P, Salmi LR, Folléa G, Schmit JL, de Barbeyrac B, Sudre P, Salamon R; BACTHEM Group; French Haemovigilance Network. Determinants of transfusion-associated bacterial contamination: results of the French BACTHEM case-control study. Transfusion 2001;41:862-71.
5. Engstrand M, Engstrand L, Högman CF, Hambraeus A, Branth S. Retrograde transmission of Proteus mirabilis during platelet transfusion and the use of arbitrarily primed polymerase chain reaction for bacteria typing in suspected cases of transfusion transmission of infection. Transfusion 1995;35:871-3.

Unusually strong, but transient, nonspecific human immunodeficiency virus types 1 and 2 antibody reactivity

In low-risk populations, such as nonremunerated voluntary blood donors, a high proportion of reactive screening immunoassay (IA) and indeterminate immunoblot results represent nonspecific reactivity due to cross-reacting antibodies. To reduce the number of donors with nonspecific indeterminate immunoblot results the Australian Red Cross Blood Service uses a dual IA strategy for human immunodeficiency virus types 1 and 2 antibody (anti-HIV-1 and -2), hepatitis C virus antibody (anti-HCV), and human T-lymphotropic virus types I and II (anti-HTLV-I and -II) screening whereby blood donors' samples that test reactive on a primary screening IA are retested on a secondary IA and further tested by immunoblot only if reactive on both IAs.[1] All donations are screened also by nucleic acid testing for HIV-1 and HCV RNA using a dedicated tube. We report the case of a donor with apparent transient nonspecific reactivity on primary and secondary anti-HIV-1 and -2 IAs and HIV Western blot.

Between July 2002 and August 2006, blood samples from six donations by a female donor all tested nonreactive by the anti-HIV-1 and -2 primary screening IA (PRISM HIV O Plus chemiluminescent immunoassay [ChLIA], Abbott Diagnostic Laboratories, Delkenheim, Germany). A sample from the donor's seventh donation in November 2006, however, tested strongly reactive by the ChLIA with a mean sample-to-cutoff (S/CO) ratio of 6.15. The sample was further tested in duplicate on the secondary IA (Genscreen Plus HIV Ag-Ab EIA, Bio-Rad, Marnes La Coquette, France) with a mean S/CO ratio of 24.2. The Western blot (HIV blot 2.2, MP Biomedicals, Irvine, CA) result showed 1+ reactivity to p24 and gp160 antigen bands and therefore was interpreted as indeterminate according to the criteria of the National Serology Reference Laboratory, Australia. The donor was recalled 3 weeks later, and a blood sample was retested by the same IAs and found to be nonreactive by both (PRISM S/CO, 0.69; Genscreen S/CO, 0.05) with no band reactivity on the Western blot. All seven donations (tested in pools of 16) and the recall sample (tested individually) were nonreactive by the HIV-1 and HCV assay for HIV-1 and HCV RNA (Procleix, Chiron Blood Testing, Emeryville CA). At a recall interview, the 48-year-old donor did not report any risk factors for HIV infection, but indicated a history of rheumatoid arthritis and a current pruritus.

Although the donor's blood sample gave unusually high S/CO ratios on both the PRISM and Genscreen IAs and an indeterminate Western blot result, follow-up testing indicated that these results were due to nonspecific reactivity. None of the donations had detectable HIV-1 RNA and the serologic reactivity was transient, becoming undetectable within 3 weeks. In addition, the transient serologic reactivity was associated with a clinical condition involving an underlying immune response, consistent with previous studies reporting an association between nonspecific reactivity with evidence of a concomitant immune response.[2-4] We believe that it is unlikely that the sample that showed reactivity on two IAs and the Western blot was contaminated because there were no anti-HIV–positive donor samples in the laboratory at the time. As well, instrument carryover from a positive control is unlikely because testing is fully automated and a new disposable tip is used to sample from each tube.

B　個別症例報告概要

○　総括一覧表
○　報告リスト

個別症例報告のまとめ方について

個別症例報告が添付されているもののうち、個別症例報告の重複を除いたものを一覧表の後に添付した（国内症例については、資料3において集積報告を行っているため、添付していない）。

感染症定期報告の報告状況(2008/9/1～2008/11/30)

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
90001	2008/09/16	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)注射液	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
90002	2008/09/25	バイエル薬	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	無	有	無
90003	2008/09/26	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	無	無
90004	2008/09/26	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	有効成分	有	無	無
90005	2008/09/26	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
90006	2008/09/26	ベネシス	人血清アルブミン 乾燥濃縮人アンチトロンビンIII 人ハプトグロビン 乾燥濃縮人血液凝固第VIII因子	ヘパリン	ブタ小腸粘膜	中国	製造工程	有	無	無
90007	2008/09/26	ベネシス	①ポリエチレングリコール処理人免疫グロブリン ②人免疫グロブリン	人免疫グロブリンG	人血液	①日本、 ②米国	有効成分	有	有	無
90008	2008/09/26	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	①有効成分 ②③添加物	有	有	無
90009	2008/09/26	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
90010	2008/09/26	化学及血清療法研究所	乾燥人血液凝固第IX因子複合体 乾燥濃縮人血液凝固第IX因子	血液凝固第IX因子	ヒト血液	日本	有効成分	有	無	有
90011	2008/09/26	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	有
90012	2008/09/26	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	有
90013	2008/09/29	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
90014	2008/09/29	日本赤十字社	ー	合成血	人血液	日本	有効成分	有	無	無
90015	2008/09/29	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
90016	2008/09/29	バクスター	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	人血漿	米国	有効成分	無	有	無
90017	2008/09/29	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
90018	2008/09/29	CSLベーリング	①人血清アルブミン ②人血液凝固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、ドイツ、オーストリア	①有効成分 ②③添加物	有	有	有
90019	2008/09/29	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	有	有
90020	2008/09/29	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	有
90021	2008/09/29	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	有
90022	2008/09/29	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	有
90023	2008/10/10	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	有
90024	2008/10/21	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
90025	2008/10/21	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
90026	2008/10/21	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	有
90027	2008/10/21	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	有
90028	2008/10/21	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
90029	2008/10/21	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	有
90030	2008/10/23	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	有

90031	2008/10/23	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
90032	2008/10/23	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
90033	2008/10/23	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	無
90034	2008/10/23	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
90035	2008/10/23	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
90036	2008/10/24	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	有
90037	2008/10/24	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	有
90038	2008/10/24	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	①③有効成分、 ②製造工程	有	無	有
90039	2008/10/24	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	有
90040	2008/10/24	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
90041	2008/10/24	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
90042	2008/10/24	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
90043	2008/10/24	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
90044	2008/10/27	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(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
90045	2008/10/29	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
90046	2008/10/29	バイエル薬品	①加熱人血漿たん白 ②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	①有効成分 ②製造工程	有	有	無
90047	2008/10/29	バイエル薬	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	有	無
90048	2008/10/29	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	有
90049	2008/10/29	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	有
90050	2008/10/29	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	有
90051	2008/10/30	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
90052	2008/11/14	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	有
90053	2008/11/14	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	有	無	有
90054	2008/11/14	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	ヒト血液	日本	有効成分	有	無	無
90055	2008/11/19	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
90056	2008/11/19	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
90057	2008/11/19	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
90058	2008/11/19	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
90059	2008/11/19	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	有	無
90060	2008/11/25	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
90061	2008/11/25	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
90062	2008/11/26	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	有
90063	2008/11/26	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	ブタ胃粘液	米国、カナダ	製造工程	無	無	有

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11回	11-1	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	2008/05/15、3-08000006
第6回	6-1	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4回	4-1	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
第3回	3-1	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
	2-1	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
第2回	2-1	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

MedDRAバージョン11.0により集計した。

第10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9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7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5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3回症例番号2-1は，第2回症例番号2-1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)。

90002	2008/09/25	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

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８回	8-1	全身障害および投与局所様態	発熱	日本	男	11ヵ月	2006年７月８日	回復	症例報告	当該製品	未完了報告①：2006年９月19日 未完了報告②：2006年10月３日 未完了報告③：2006年10月18日 識別番号：A-06000119 ウイルス感染の可能性も完全に否定できないとのことにより感染症として取り扱う。
		皮膚および皮下組織障害	発疹				2006年７月11日	回復			
		肝胆道系障害	肝障害				2006年７月10日	回復			
	8-2	感染症および寄生虫症	伝染性紅斑	日本	女	6	2006年11月13日	回復	症例報告	当該製品	
	8-3	皮膚および皮下組織障害	全身紅斑	日本	男	1	2007年１月２日	回復	症例報告	当該製品	未完了報告：2007年１月31日 識別番号：A-06000214 ウイルス感染の可能性も完全に否定できないとのことにより感染症として取り扱う。
第７回	6-1	感染症および寄生虫症	伝染性紅斑	日本	男	1	2005年10月11日	回復	症例報告	当該製品	取下げ報告：2006年３月24日 識別番号：A-05000194
第６回	6-1	感染症および寄生虫症	伝染性紅斑	日本	男	1	2005年10月11日	回復	症例報告	当該製品	未完了報告：2005年12月１日 識別番号：A-05000194
	5-1	肝胆道系障害	肝機能異常	日本	男	11ヵ月	2005年４月５日	回復	症例報告	当該製品	完了報告：2005年９月26日 識別番号：A-05000095 第５回報告の「ウイルス性肝炎」から副作用・感染症名が「肝機能障害」に変更になった。
第５回	5-1	感染症および寄生虫症	ウイルス性肝炎	日本	男	11ヵ月	2005年４月５日	回復	症例報告	当該製品	未完了報告：2005年７月27日 識別番号：A-05000095

MedDRA/J Version11.0 使用

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11回	11-1	感染症および寄生虫症	伝染性紅斑	日本	男	58	2008年4月11日	不明	症例報告	当該製品	
	11-2	感染症および寄生虫症	B型肝炎	日本	男	69	2002年11月5日	軽快	症例報告	当該製品	完了報告　：2008年6月19日 未完了報告：2008年6月16日 識別番号　：A-08000289
	11-3	感染症および寄生虫症	B型肝炎	日本	男	53	2004年12月	未回復	症例報告	当該製品	完了報告　：2008年6月27日 未完了報告：2008年6月16日 識別番号　：A-08000290
	11-4	感染症および寄生虫症	C型肝炎	日本	男	81	2005年3月30日	未回復	症例報告	当該製品	完了報告　：2008年6月17日 識別番号　：A-08000294
第9回	9-1	感染症および寄生虫症	伝染性紅斑	日本	男	10	2007年1月24日	回復	症例報告	当該製品	
	8-1	全身障害および投与局所様態	発熱	日本	男	11ヵ月	2006年7月8日	回復	症例報告	当該製品	完了報告：2007年2月2日 識別番号：A-06000119 ウイルス感染の可能性も完全に否定できないとのことにより感染症として取り扱う。
		皮膚および皮下組織障害	発疹				2006年7月11日	回復			
		肝胆道系障害	肝障害				2006年7月10日	回復			
	8-3	皮膚および皮下組織障害	全身紅斑	日本	男	1	2007年1月2日	回復	症例報告	当該製品	取下げ報告：2007年2月15日 識別番号：A-06000214

MedDRA/J Version11.0 使用

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３回	3-1	感染症および寄生虫症	ロタウイルス胃腸炎	日本	女	1	2004年４月20日	軽快	症例報告	当該製品	

MedDRA/J Version11.0使用

90007	2008/09/26	80498	ベネシス	①ポリエチレングリコール処理人免疫グロブリン ②人免疫グロブリン	人免疫グロブリンG

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11回	11-1	臨床検査	B型肝炎表面抗原陽性	日本	不明	不明	不明	不明	症例報告	当該製品	未完了報告日：2008年7月22日 識別番号：A-08000410 2008年8月21日に、「当該患者はHBVに感染していなかった」との情報を入手した
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

90008	2008/09/26	80499	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

MedDRA/J Version11.0使用

Alb

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11回	11-1	感染症および寄生虫症	C型肝炎	ドイツ	女	60	2007/04/13	不明	自発報告	外国製品	08000001、3回(完了) 平成20年7月25日 MedDRA ver.11.0
	11-1	感染症および寄生虫症	医薬品を介する感染因子の伝播	ドイツ	女	60	不明	不明	自発報告	外国製品	08000001、3回(完了) 平成20年7月25日 MedDRA ver.11.0
	11-1	感染症および寄生虫症	C型肝炎	ドイツ	女	60	2007/04/13	不明	自発報告	外国製品	08000001、2回(追加) 平成20年5月20日 MedDRA ver.11.0
	11-1	感染症および寄生虫症	医薬品を介する感染因子の伝播	ドイツ	女	60	不明	不明	自発報告	外国製品	08000001、2回(追加) 平成20年5月20日 MedDRA ver.11.0
	11-1	感染症および寄生虫症	C型肝炎	ドイツ	女	不明	2007/04/13	不明	自発報告	外国製品	08000001、1回(未完了) 平成20年4月15日 MedDRA ver.11.0
	11-1	感染症および寄生虫症	医薬品を介する感染因子の伝播	ドイツ	女	不明	不明	不明	自発報告	外国製品	08000001、1回(未完了) 平成20年4月15日 MedDRA ver.11.0
	10-1	感染症および寄生虫症	B型肝炎	ドイツ	男	24	2008/01/10	不明	自発報告	外国製品	07000022、3回(完了;同一症例をアルブミンの番号10-1で報告) 平成20年3月31日 MedDRA ver.10.1
	10-1	感染症および寄生虫症	B型肝炎	ドイツ	男	24	2008/01/10	不明	自発報告	外国製品	07000022、2回(完了;同一症例をアルブミンの番号10-1で報告) 平成20年3月14日 MedDRA ver.10.1

	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	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

90011	2008/09/26	80502	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（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第11回	11-1	臨床検査	Ｃ型肝炎ウイルス	ブラジル	男	不明	不明	不明	症例報告	外国製品	登録番号：05000274 報告日：2008年4月21日 MedDRA: Version(11.0)
	11-1	感染症および寄生虫症	ＨＩＶ感染	ブラジル	男	不明	不明	死亡	症例報告	外国製品	登録番号：05000274 報告日：2008年4月21日 MedDRA: Version(11.0)
	11-2	臨床検査	Ｃ型肝炎ウイルス	ブラジル	男	不明	不明	死亡	症例報告	外国製品	登録番号：07000030 報告日：2008年3月5日 MedDRA: Version(11.0)
	11-2	感染症および寄生虫症	急性ＨＩＶ感染	ブラジル	男	不明	不明	死亡	症例報告	外国製品	登録番号：07000030 報告日：2008年3月5日 MedDRA: Version(11.0)
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	

別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第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。

90015	2008/09/29	80520	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

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11回	5-231	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000274	2008/4/21	11.0	第11回症例番号5-231は第5回症例番号5-231において報告したものの追加報告
第11回	5-231	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000274	2008/4/21	11.0	第11回症例番号5-231は第5回症例番号5-231において報告したものの追加報告
第10回	10-1	臨床検査	C型肝炎ウイルス	ブラジル	男性	小児	2004/5/25	不明	症例報告	外国製品	07000015	2007/10/29	10.1	
第10回	10-1	臨床検査	C型肝炎ウイルス	ブラジル	男性	小児	2004/5/25	不明	症例報告	外国製品	07000015	2007/12/28	10.1	追加報告
第10回	10-2	感染症および寄生虫症	急性HIV感染	アメリカ	男性	34	不明	不明	症例報告	外国製品	07000017	2007/12/6	10.1	
第10回	10-2	臨床検査	C型肝炎ウイルス	アメリカ	男性	34	不明	不明	症例報告	外国製品	07000017	2007/12/6	10.1	
第10回	10-3	感染症および寄生虫症	C型肝炎	ベルギー	男性	不明	1991	未回復	症例報告	外国製品	07000028	2008/2/25	10.1	
第9回		0*	0	0	0	0	0	0	0	0	0	0	0	* 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	

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	7-008	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	06000011	2006/5/10	9.0	
第7回	7-007	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	06000013	2006/5/15	9.0	
第7回	7-061	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	06000014	2006/5/15	9.0	
第7回	7-010	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	死亡	症例報告	外国製品	06000015	2006/5/15	9.0	
第7回	7-005	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	06000016	2006/5/15	9.0	
第7回	7-062	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	06000017	2006/5/15	9.0	
第7回	7-006	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	06000018	2006/5/22	9.0	
第7回	7-012	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	11	2006/5/2	不明	症例報告	外国製品	06000019	2006/6/16	9.0	
第7回	5-130	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000065	2006/3/30	9.0	第7回症例番号5-130は第5回症例番号5-130と重複症例のため報告破棄
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	

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	7-058	臨床検査	C型肝炎ウイルス	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000684	2006/3/13	8.1	
第7回	5-139	感染症および寄生虫症	HIV感染	香港	男性	不明	1985	死亡	症例報告	外国製品	05000104	2006/3/2	8.1	第7回症例番号5-139は第5回症例番号5-139において報告したものの追加報告
第7回	7-001	感染症および寄生虫症	HIV感染	イギリス	男性	24	1985	不明	症例報告	外国製品	06000007	2006/5/1	9.0	
第7回	7-002	感染症および寄生虫症	HIV感染	イギリス	男性	9	1985	不明	症例報告	外国製品	06000009	2006/5/10	9.0	
第7回	7-003	感染症および寄生虫症	HIV感染	イギリス	男性	不明	1985/10/4	不明	症例報告	外国製品	06000010	2006/5/10	9.0	
第7回	7-004	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1986	不明	症例報告	外国製品	05000646	2006/3/3	8.1	
第7回	7-005	感染症および寄生虫症	HIV感染	イギリス	男性	不明	1986	不明	症例報告	外国製品	06000016	2006/5/15	9.0	
第7回	7-006	感染症および寄生虫症	HIV感染	イギリス	男性	不明	1986	不明	症例報告	外国製品	06000018	2006/5/22	9.0	
第7回	7-007	感染症および寄生虫症	HIV感染	イギリス	男性	不明	1986/3	不明	症例報告	外国製品	06000013	2006/5/15	9.0	
第7回	7-008	感染症および寄生虫症	HIV感染	イギリス	男性	不明	1986/4/9	不明	症例報告	外国製品	06000011	2006/5/10	9.0	
第7回	7-009	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1988	不明	症例報告	外国製品	05000637	2006/3/3	8.1	
第7回	7-010	感染症および寄生虫症	HIV感染	イギリス	男性	不明	1988/5	死亡	症例報告	外国製品	06000015	2006/5/15	9.0	
第7回	7-011	感染症および寄生虫症	HIV感染	台湾	男性	不明	1997/4/17	不明	症例報告	外国製品	05000635	2006/3/2	8.1	
第7回	5-130	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000065	2006/3/30	9.0	第7回症例番号5-130は第5回症例番号5-130と重複症例のため報告破棄
第7回	7-013	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000638	2006/3/3	8.1	
第7回	7-020	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000645	2006/3/3	8.1	
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	

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6回	6-163	感染症および寄生虫症	B型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000591	2006/2/16	8.1	
第6回	6-166	感染症および寄生虫症	B型肝炎	ペルー	男性	不明	不明	不明	症例報告	外国製品	05000598	2006/2/16	8.1	
第6回	6-176	感染症および寄生虫症	B型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000613	2006/2/22	8.1	
第6回	6-007	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	1985	不明	症例報告	外国製品	05000571	2006/2/13	8.1	
第6回	6-015	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	1986	不明	症例報告	外国製品	05000619	2006/2/22	8.1	
第6回	6-025	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	1990	不明	症例報告	外国製品	05000536	2006/2/8	8.1	
第6回	6-026	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	1990	不明	症例報告	外国製品	05000537	2006/2/8	8.1	
第6回	6-032	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	14	1994	不明	症例報告	外国製品	05000458	2005/10/28	8.1	
第6回	6-035	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	32	1995/5/24	不明	症例報告	外国製品	05000607	2006/2/22	8.1	
第6回	6-037	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	2001	不明	症例報告	外国製品	05000562	2006/2/13	8.1	
第6回	6-038	感染症および寄生虫症	C型肝炎	アルゼンチン	女性	不明	2003	不明	症例報告	外国製品	05000628	2006/2/24	8.1	
第6回	4-06	感染症および寄生虫症	C型肝炎	イギリス	男性	11	不明	不明	症例報告	外国製品	04000081	2005/9/16	8.1	第6回症例番号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	

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6回	6-129	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000539	2006/2/8	8.1
第6回	6-131	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000543	2006/2/10	8.1
第6回	6-132	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000544	2006/2/10	8.1
第6回	6-133	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000545	2006/2/10	8.1
第6回	6-134	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000546	2006/2/10	8.1
第6回	6-135	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000547	2006/2/10	8.1
第6回	6-136	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000548	2006/2/10	8.1
第6回	6-144	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000556	2006/2/13	8.1
第6回	6-145	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000557	2006/2/13	8.1
第6回	6-146	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000558	2006/2/13	8.1
第6回	6-147	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000573	2006/2/14	8.1
第6回	6-148	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	未回復	症例報告	外国製品	05000559	2006/2/13	8.1
第6回	6-149	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000560	2006/2/13	8.1
第6回	6-150	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000561	2006/2/13	8.1
第6回	6-152	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000564	2006/2/13	8.1
第6回	6-153	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000565	2006/2/13	8.1
第6回	6-154	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000566	2006/2/13	8.1
第6回	6-013	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

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6回	6-002	感染症および寄生虫症	HIV感染	アルゼンチン	不明	不明	1984	不明	症例報告	外国製品	05000569	2006/2/13	8.1	
第6回	6-003	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1984	不明	症例報告	外国製品	05000585	2006/2/16	8.1	
第6回	6-004	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1984	不明	症例報告	外国製品	05000595	2006/2/16	8.1	
第6回	6-005	感染症および寄生虫症	HIV感染	台湾	男性	不明	1984/10/18	不明	症例報告	外国製品	05000620	2006/2/22	8.1	
第6回	6-006	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1985	不明	症例報告	外国製品	05000570	2006/2/13	8.1	
第6回	6-008	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1985	不明	症例報告	外国製品	05000608	2006/2/22	8.1	
第6回	6-009	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1985	不明	症例報告	外国製品	05000610	2006/2/22	8.1	
第6回	6-010	感染症および寄生虫症	HIV感染	台湾	男性	不明	1985/1/3	不明	症例報告	外国製品	05000630	2006/2/24	8.1	
第6回	6-011	感染症および寄生虫症	HIV感染	台湾	男性	不明	1985/11/13	不明	症例報告	外国製品	05000633	2006/2/24	8.1	
第6回	6-012	感染症および寄生虫症	HIV感染	台湾	男性	不明	1985/5/1	不明	症例報告	外国製品	05000629	2006/2/24	8.1	
第6回	6-013	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1986	不明	症例報告	外国製品	05000567	2006/2/13	8.1	
第6回	6-014	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1986	不明	症例報告	外国製品	05000592	2006/2/16	8.1	
第6回	6-015	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	1986	不明	症例報告	外国製品	05000619	2006/2/22	8.1	
第6回	5-136	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1986	不明	症例報告	外国製品	05000100	2005/10/27	8.0	第6回症例番号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	

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6回	6-078	感染症および寄生虫症	HIV感染	パナマ	男性	不明	不明	死亡	症例報告	外国製品	05000481	2005/12/2	8.1	
第6回	6-080	感染症および寄生虫症	HIV感染	チリ	男性	不明	不明	死亡	症例報告	外国製品	05000483	2005/12/2	8.1	
第6回	6-081	感染症および寄生虫症	HIV感染	チリ	男性	不明	不明	死亡	症例報告	外国製品	05000484	2005/12/2	8.1	
第6回	6-082	感染症および寄生虫症	HIV感染	パナマ	男性	不明	不明	死亡	症例報告	外国製品	05000485	2005/12/2	8.1	
第6回	6-083	感染症および寄生虫症	HIV感染	パナマ	男性	不明	不明	死亡	症例報告	外国製品	05000486	2005/12/2	8.1	
第6回	6-084	感染症および寄生虫症	HIV感染	パナマ	男性	不明	不明	死亡	症例報告	外国製品	05000487	2005/12/2	8.1	
第6回	6-085	感染症および寄生虫症	HIV感染	パナマ	男性	不明	不明	死亡	症例報告	外国製品	05000488	2005/12/2	8.1	
第6回	6-086	感染症および寄生虫症	HIV感染	チリ	男性	不明	不明	死亡	症例報告	外国製品	05000489	2005/12/2	8.1	
第6回	6-090	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000498	2006/2/6	8.1	
第6回	6-101	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000509	2006/2/8	8.1	
第6回	6-105	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000513	2006/2/8	8.1	
第6回	6-107	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000515	2006/2/8	8.1	
第6回	6-108	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000516	2006/2/8	8.1	
第6回	6-111	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000519	2006/2/8	8.1	
第6回	6-112	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000520	2006/2/8	8.1	
第6回	6-117	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000525	2006/2/8	8.1	
第6回	6-118	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000526	2006/2/8	8.1	
第6回	6-144	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000556	2006/2/13	8.1	
第6回	6-162	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000590	2006/2/16	8.1	
第6回	6-176	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000613	2006/2/22	8.1	
第6回	4-06	臨床検査	HIV検査陽性	イギリス	男性	11	1981/11/23	不明	症例報告	外国製品	04000081	2005/9/16	8.1	第6回症例番号4-06は前回報告における第4回症例番号4-06において報告したものの追加報告
第6回	6-020	肝胆道系障害	肝炎	ブラジル	男性	不明	1988	不明	症例報告	外国製品	05000576	2006/2/16	8.1	
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

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6回	6-039	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000433	2005/9/1	8.0	
第6回	6-040	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000434	2005/9/1	8.0	
第6回	6-041	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000435	2005/9/1	8.0	
第6回	6-042	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000436	2005/9/1	8.0	
第6回	6-043	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000437	2005/9/1	8.0	
第6回	6-044	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000438	2005/9/9	8.0	
第6回	6-046	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000440	2005/9/9	8.0	
第6回	6-047	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000441	2005/9/9	8.0	
第6回	6-065	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000463	2005/11/2	8.1	
第6回	6-067	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000465	2005/11/2	8.1	
第6回	6-068	臨床検査	C型肝炎ウイルス	ペルー	男性	不明	不明	不明	症例報告	外国製品	05000466	2005/11/2	8.1	
第6回	6-073	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000473	2005/11/21	8.1	
第6回	6-074	臨床検査	C型肝炎ウイルス	パナマ	男性	不明	不明	不明	症例報告	外国製品	05000476	2005/11/30	8.1	
第6回	6-090	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000498	2006/2/6	8.1	
第6回	6-091	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000499	2006/2/6	8.1	
第6回	6-092	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000500	2006/2/6	8.1	
第6回	6-094	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000502	2006/2/6	8.1	
第6回	6-095	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000506	2006/2/6	8.1	
第6回	6-098	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000505	2006/2/6	8.1	
第6回	6-099	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000507	2006/2/8	8.1	
第6回	6-100	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000508	2006/2/8	8.1	
第6回	6-101	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000509	2006/2/8	8.1	
第6回	6-102	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000510	2006/2/8	8.1	
第6回	6-103	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000511	2006/2/8	8.1	
第6回	6-104	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000512	2006/2/8	8.1	
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	

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6回	6-029	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000578	2006/2/16	8.1	
第6回	6-167	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000599	2006/2/16	8.1	
第6回	6-168	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000600	2006/2/21	8.1	
第6回	6-170	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000602	2006/2/21	8.1	
第6回	6-171	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000603	2006/2/21	8.1	
第6回	6-172	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000604	2006/2/21	8.1	
第6回	6-177	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000614	2006/2/22	8.1	
第6回	6-178	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000615	2006/2/22	8.1	
第6回	6-179	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000616	2006/2/22	8.1	
第6回	6-180	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000617	2006/2/22	8.1	
第6回	6-181	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000621	2006/2/22	8.1	
第6回	6-182	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000622	2006/2/22	8.1	
第6回	6-186	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000631	2006/2/24	8.1	
第6回	6-187	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000632	2006/2/24	8.1	
第5回	5-001	感染症および寄生虫症	A型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	04000118	2005/3/18	8.0	
第5回	5-002	感染症および寄生虫症	A型肝炎	ベネズエラ	男性	不明	不明	死亡	症例報告	外国製品	05000244	2005/7/15	8.0	
第5回	5-003	感染症および寄生虫症	A型肝炎	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000245	2005/7/15	8.0	
第5回	5-004	感染症および寄生虫症	B型肝炎	ベネズエラ	男性	不明	1994	不明	症例報告	外国製品	05000225	2005/7/11	8.0	
第5回	5-001	感染症および寄生虫症	B型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	04000118	2005/3/18	8.0	
第5回	5-002	感染症および寄生虫症	B型肝炎	ベネズエラ	男性	不明	不明	死亡	症例報告	外国製品	05000244	2005/7/15	8.0	
第5回	5-003	感染症および寄生虫症	B型肝炎	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000245	2005/7/15	8.0	
第5回	5-005	感染症および寄生虫症	B型肝炎	アメリカ	男性	53	不明	不明	症例報告	当該製品	04000114	2005/3/15	7.1	
第5回	5-006	感染症および寄生虫症	B型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	04000119	2005/3/18	8.0	
第5回	5-007	感染症および寄生虫症	B型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	05000118	2005/6/9	8.0	
第5回	5-008	感染症および寄生虫症	B型肝炎	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000147	2005/6/20	8.0	
第5回	5-004	感染症および寄生虫症	C型肝炎	ベネズエラ	男性	不明	1994	不明	症例報告	外国製品	05000225	2005/7/11	8.0	
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	

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5回	5-022	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000071	2005/6/15	8.0	
第5回	5-023	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000072	2005/6/1	8.0	
第5回	5-023	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000072	2005/6/15	8.0	
第5回	5-024	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000073	2005/6/1	8.0	
第5回	5-024	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000073	2005/6/15	8.0	
第5回	5-025	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000074	2005/6/1	8.0	
第5回	5-025	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000074	2005/6/15	8.0	
第5回	5-026	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000075	2005/6/1	8.0	
第5回	5-027	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000076	2005/6/1	8.0	
第5回	5-028	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000092	2005/6/1	8.0	
第5回	5-029	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000093	2005/6/1	8.0	
第5回	5-030	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000094	2005/6/1	8.0	
第5回	5-031	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000095	2005/6/1	8.0	
第5回	5-032	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000096	2005/6/1	8.0	
第5回	5-033	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000097	2005/6/1	8.0	
第5回	5-034	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000077	2005/6/1	8.0	
第5回	5-035	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000078	2005/6/1	8.0	
第5回	5-035	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000078	2005/6/15	8.0	
第5回	5-036	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000079	2005/6/1	8.0	
第5回	5-037	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000080	2005/6/1	8.0	
第5回	5-038	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000081	2005/6/1	8.0	
第5回	5-039	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000082	2005/6/1	8.0	
第5回	5-040	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000083	2005/6/1	8.0	
第5回	5-041	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000084	2005/6/1	8.0	
第5回	5-042	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000088	2005/6/1	8.0	
第5回	5-043	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000089	2005/6/1	8.0	
第5回	5-043	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000089	2005/6/15	8.0	
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	

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5回	5-065	感染症および寄生虫症	C型肝炎	ドミニカ共和国	男性	不明	不明	不明	症例報告	外国製品	05000234	2005/7/12	8.0	
第5回	5-066	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000238	2005/7/15	8.0	
第5回	5-067	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000239	2005/7/15	8.0	
第5回	5-068	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000243	2005/7/15	8.0	
第5回	5-069	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000246	2005/7/15	8.0	
第5回	5-070	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000253	2005/7/19	8.0	
第5回	5-070	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000253	2005/8/2	8.0	
第5回	5-071	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000262	2005/7/22	8.0	
第5回	5-072	感染症および寄生虫症	C型肝炎	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000263	2005/7/22	8.0	
第5回	5-073	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000281	2005/7/26	8.0	
第5回	5-074	感染症および寄生虫症	C型肝炎	アルゼンチン	女性	不明	不明	不明	症例報告	外国製品	05000310	2005/7/27	8.0	
第5回	5-075	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000311	2005/7/27	8.0	
第5回	5-076	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000312	2005/7/27	8.0	
第5回	5-077	感染症および寄生虫症	C型肝炎	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000324	2005/7/27	8.0	
第5回	5-078	感染症および寄生虫症	C型肝炎	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000325	2005/7/27	8.0	
第5回	5-079	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000327	2005/7/29	8.0	
第5回	5-080	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000328	2005/7/29	8.0	
第5回	5-081	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000347	2005/8/2	8.0	
第5回	5-082	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000348	2005/8/2	8.0	
第5回	5-083	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000349	2005/8/2	8.0	
第5回	5-084	感染症および寄生虫症	C型肝炎	コスタリカ	男性	不明	不明	不明	症例報告	外国製品	05000352	2005/8/2	8.0	
第5回	5-085	感染症および寄生虫症	C型肝炎	パナマ	男性	不明	不明	不明	症例報告	外国製品	05000361	2005/8/2	8.0	
第5回	5-086	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000363	2005/8/2	8.0	
第5回	5-087	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000364	2005/8/2	8.0	
第5回	5-088	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000365	2005/8/2	8.0	
第5回	5-089	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000366	2005/8/2	8.0	
第5回	5-090	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000367	2005/8/3	8.0	
第5回	5-091	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000369	2005/8/3	8.0	
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	

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5回	5-021	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000070	2005/6/1	8.0
第5回	5-021	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000070	2005/6/15	8.0
第5回	5-022	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000071	2005/6/1	8.0
第5回	5-022	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000071	2005/6/15	8.0
第5回	5-023	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000072	2005/6/1	8.0
第5回	5-023	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000072	2005/6/15	8.0
第5回	5-024	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000073	2005/6/1	8.0
第5回	5-024	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000073	2005/6/15	8.0
第5回	5-025	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000074	2005/6/15	8.0
第5回	5-025	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000074	2005/6/1	8.0
第5回	5-026	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000075	2005/6/1	8.0
第5回	5-027	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000076	2005/6/1	8.0
第5回	5-028	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000092	2005/6/1	8.0
第5回	5-029	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000093	2005/6/1	8.0
第5回	5-030	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000094	2005/6/1	8.0
第5回	5-031	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000095	2005/6/1	8.0
第5回	5-032	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000096	2005/6/1	8.0
第5回	5-033	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000097	2005/6/1	8.0
第5回	5-034	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000077	2005/6/1	8.0
第5回	5-035	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000078	2005/6/1	8.0
第5回	5-035	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000078	2005/6/15	8.0
第5回	5-036	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000079	2005/6/1	8.0
第5回	5-037	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000080	2005/6/1	8.0
第5回	5-038	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000081	2005/6/1	8.0
第5回	5-039	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000082	2005/6/1	8.0
第5回	5-040	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000083	2005/6/1	8.0
第5回	5-041	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000084	2005/6/1	8.0
第5回	5-042	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000088	2005/6/1	8.0
第5回	5-053	感染症および寄生虫症	HIV感染	ドミニカ共和国	男性	不明	不明	不明	症例報告	外国製品	05000155	2005/6/27	8.0
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

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5回	5-107	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000012	2005/5/12	8.0
第5回	5-108	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	死亡	症例報告	外国製品	05000013	2005/5/12	8.0
第5回	5-109	感染症および寄生虫症	HIV感染	香港	男性	不明	不明	不明	症例報告	外国製品	05000015	2005/5/23	8.0
第5回	5-110	感染症および寄生虫症	HIV感染	香港	男性	不明	不明	不明	症例報告	外国製品	05000016	2005/5/23	8.0
第5回	5-111	感染症および寄生虫症	HIV感染	香港	男性	不明	不明	不明	症例報告	外国製品	05000017	2005/5/23	8.0
第5回	5-112	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000018	2005/5/23	8.0
第5回	5-113	感染症および寄生虫症	HIV感染	香港	男性	不明	不明	不明	症例報告	外国製品	05000019	2005/5/23	8.0
第5回	5-114	感染症および寄生虫症	HIV感染	香港	男性	不明	不明	不明	症例報告	外国製品	05000020	2005/5/23	8.0
第5回	5-115	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000021	2005/5/23	8.0
第5回	5-116	感染症および寄生虫症	HIV感染	香港	男性	不明	不明	不明	症例報告	外国製品	05000022	2005/5/23	8.0
第5回	5-117	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000023	2005/5/23	8.0
第5回	5-118	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000025	2005/5/26	8.0
第5回	5-119	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000026	2005/5/26	8.0
第5回	5-120	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	死亡	症例報告	外国製品	05000027	2005/5/26	8.0
第5回	5-121	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000053	2005/5/30	8.0
第5回	5-122	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000054	2005/5/30	8.0
第5回	5-123	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000056	2005/5/30	8.0
第5回	5-124	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000057	2005/5/30	8.0
第5回	5-125	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000058	2005/5/30	8.0
第5回	5-126	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000061	2005/5/30	8.0
第5回	5-127	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000062	2005/5/30	8.0
第5回	5-128	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000063	2005/5/30	8.0
第5回	5-129	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000064	2005/5/30	8.0
第5回	5-130	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000065	2005/5/30	8.0
第5回	5-131	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000066	2005/5/30	8.0
第5回	5-132	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000067	2005/5/30	8.0
第5回	5-133	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000068	2005/5/30	8.0
第5回	5-134	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000098	2005/6/1	8.0
第5回	5-135	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	死亡	症例報告	外国製品	05000099	2005/6/1	8.0
第5回	5-136	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000100	2005/6/1	8.0
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

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	
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	

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5回	5-193	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000182	2005/8/8	8.0	
第5回	5-194	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000185	2005/7/4	8.0	
第5回	5-195	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000186	2005/7/4	8.0	
第5回	5-196	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000187	2005/7/4	8.0	
第5回	5-197	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000189	2005/7/4	8.0	
第5回	5-198	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000190	2005/7/4	8.0	
第5回	5-199	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000191	2005/7/4	8.0	
第5回	5-200	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000194	2005/7/4	8.0	
第5回	5-201	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000202	2005/7/8	8.0	
第5回	5-202	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000203	2005/7/8	8.0	
第5回	5-203	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000204	2005/7/8	8.0	
第5回	5-204	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000206	2005/7/8	8.0	
第5回	5-205	感染症および寄生虫症	HIV感染	コロンビア	男性	不明	不明	不明	症例報告	外国製品	05000209	2005/7/8	8.0	
第5回	5-206	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000212	2005/7/8	8.0	
第5回	5-207	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000213	2005/7/11	8.0	
第5回	5-208	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000214	2005/7/11	8.0	
第5回	5-209	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000219	2005/7/11	8.0	
第5回	5-210	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000230	2005/7/11	8.0	
第5回	5-211	感染症および寄生虫症	HIV感染	コスタリカ	男性	不明	不明	不明	症例報告	外国製品	05000232	2005/7/12	8.0	
第5回	5-212	感染症および寄生虫症	HIV感染	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000233	2005/7/12	8.0	
第5回	5-213	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000235	2005/7/12	8.0	
第5回	5-214	感染症および寄生虫症	HIV感染	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000237	2005/7/12	8.0	
第5回	5-215	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000240	2005/7/15	8.0	
第5回	5-216	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000241	2005/7/15	8.0	
第5回	5-217	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000242	2005/7/15	8.0	
第5回	5-218	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000248	2005/7/15	8.0	
第5回	5-219	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000249	2005/7/15	8.0	
第5回	5-220	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000250	2005/7/15	8.0	
第5回	5-221	感染症および寄生虫症	HIV感染	ベネズエラ	男性	不明	不明	死亡	症例報告	外国製品	05000251	2005/7/15	8.0	
第5回	5-222	感染症および寄生虫症	HIV感染	アルゼンチン	女性	不明	不明	死亡	症例報告	外国製品	05000252	2005/7/19	8.0	
第5回	5-223	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000254	2005/7/19	8.0	
第5回	5-224	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000256	2005/7/19	8.0	
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	

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
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

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
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

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5回	5-158	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000116	2005/6/9	8.0
第5回	5-159	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000041	2005/5/30	8.0
第5回	5-161	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000043	2005/5/30	8.0
第5回	5-162	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000044	2005/5/30	8.0
第5回	5-162	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000044	2005/6/27	8.0
第5回	5-163	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000045	2005/5/30	8.0
第5回	5-163	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000045	2005/6/15	8.0
第5回	5-164	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000046	2005/5/30	8.0
第5回	5-165	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000047	2005/5/30	8.0
第5回	5-166	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000048	2005/5/30	8.0
第5回	5-167	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000049	2005/5/30	8.0
第5回	5-167	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000049	2005/6/15	8.0
第5回	5-168	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000050	2005/5/30	8.0
第5回	5-168	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000050	2005/6/15	8.0
第5回	5-169	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000051	2005/5/30	8.0
第5回	5-169	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000051	2005/6/15	8.0
第5回	5-170	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000052	2005/5/30	8.0
第5回	5-170	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000052	2005/6/15	8.0
第5回	5-173	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000121	2005/6/13	8.0
第5回	5-174	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000122	2005/6/13	8.0
第5回	5-177	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000129	2005/6/15	8.0
第5回	5-180	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000148	2005/6/20	8.0
第5回	5-181	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000156	2005/6/27	8.0
第5回	5-182	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000159	2005/6/27	8.0
第5回	5-185	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000164	2005/6/27	8.0
第5回	5-186	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000165	2005/6/27	8.0
第5回	5-187	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000167	2005/6/27	8.0
第5回	5-187	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000167	2005/8/18	8.0
第5回	5-188	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000171	2005/6/30	8.0
第5回	5-189	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000173	2005/6/30	8.0
第5回	5-190	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000175	2005/6/30	8.0
第5回	5-191	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000176	2005/6/30	8.0
第5回	5-192	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000181	2005/6/30	8.0
第5回	5-194	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000185	2005/7/4	8.0
第5回	5-195	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000186	2005/7/4	8.0
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

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
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

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5回	5-277	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000423	2005/8/30	8.0
第5回	5-278	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000424	2005/8/30	8.0
第5回	5-279	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000425	2005/8/30	8.0
第5回	5-280	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000427	2005/8/30	8.0
第5回	5-282	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000429	2005/8/30	8.0
第5回	5-283	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000430	2005/8/30	8.0
第5回	5-284	臨床検査	C型肝炎ウイルス	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000431	2005/8/30	8.0
第5回	5-287	臨床検査	C型肝炎ウイルス	イギリス	男性	不明	不明	不明	症例報告	外国製品	04000107	2005/3/3	7.1
第5回	5-288	臨床検査	C型肝炎ウイルス	イギリス	男性	25	不明	不明	症例報告	外国製品	05000003	2005/4/18	8.0
第5回	5-289	臨床検査	C型肝炎ウイルス	イギリス	男性	57	不明	不明	症例報告	外国製品	04000102	2005/3/3	7.1
第5回	5-290	臨床検査	C型肝炎ウイルス	イギリス	男性	58	不明	不明	症例報告	外国製品	04000104	2005/3/3	7.1
第5回	5-291	臨床検査	C型肝炎ウイルス	イギリス	男性	不明	不明	不明	症例報告	外国製品	04000120	2005/3/18	8.0
第5回	5-292	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000024	2005/5/26	8.0
第5回	5-293	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000055	2005/5/30	8.0
第5回	5-294	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000059	2005/5/30	8.0
第5回	5-295	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000060	2005/5/30	8.0
第5回	5-296	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000101	2005/6/1	8.0
第5回	5-297	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000108	2005/6/7	8.0
第5回	5-298	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000110	2005/6/9	8.0
第5回	5-299	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000115	2005/6/9	8.0
第5回	5-300	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000117	2005/6/9	8.0
第5回	5-301	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000123	2005/6/13	8.0
第5回	5-302	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000127	2005/6/15	8.0
第5回	5-303	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000128	2005/6/15	8.0
第5回	5-304	臨床検査	C型肝炎ウイルス	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000130	2005/6/15	8.0
第5回	5-305	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000131	2005/6/15	8.0
第5回	5-306	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000132	2005/6/15	8.0
第5回	5-307	臨床検査	C型肝炎ウイルス	コスタリカ	男性	不明	不明	不明	症例報告	外国製品	05000138	2005/6/20	8.0
第5回	5-308	臨床検査	C型肝炎ウイルス	コスタリカ	男性	不明	不明	不明	症例報告	外国製品	05000139	2005/6/20	8.0
第5回	5-309	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000140	2005/6/20	8.0
第5回	5-310	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000141	2005/6/20	8.0
第5回	5-311	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000142	2005/6/20	8.0
第5回	5-312	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000143	2005/6/20	8.0
第5回	5-313	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000146	2005/6/20	8.0
第5回	5-314	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000149	2005/6/27	8.0
第5回	5-315	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000151	2005/6/27	8.0
第5回	5-316	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000152	2005/6/27	8.0
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

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5回	5-329	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000179	2005/6/30	8.0
第5回	5-330	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000180	2005/6/30	8.0
第5回	5-331	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000184	2005/7/4	8.0
第5回	5-332	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000188	2005/7/4	8.0
第5回	5-333	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000192	2005/7/4	8.0
第5回	5-334	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000193	2005/7/4	8.0
第5回	5-335	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000197	2005/7/8	8.0
第5回	5-336	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000198	2005/7/8	8.0
第5回	5-337	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000199	2005/7/8	8.0
第5回	5-338	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000200	2005/7/8	8.0
第5回	5-339	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000201	2005/7/8	8.0
第5回	5-340	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000205	2005/7/8	8.0
第5回	5-341	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000207	2005/7/8	8.0
第5回	5-342	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000208	2005/7/8	8.0
第5回	5-343	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000210	2005/7/8	8.0
第5回	5-344	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000211	2005/7/8	8.0
第5回	5-345	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000216	2005/7/11	8.0
第5回	5-346	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000217	2005/7/11	8.0
第5回	5-347	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000220	2005/7/11	8.0
第5回	5-348	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000222	2005/7/11	8.0
第5回	5-349	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000224	2005/7/11	8.0
第5回	5-350	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000229	2005/7/11	8.0
第5回	5-351	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000236	2005/7/12	8.0
第5回	5-352	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000247	2005/7/15	8.0
第5回	5-353	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000255	2005/7/19	8.0
第5回	5-354	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000260	2005/7/22	8.0
第5回	5-355	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000265	2005/7/22	8.0
第5回	5-356	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000266	2005/7/22	8.0
第5回	5-357	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000268	2005/7/22	8.0
第5回	5-358	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000269	2005/7/22	8.0
第5回	5-359	臨床検査	C型肝炎ウイルス	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000270	2005/7/22	8.0
第5回	5-360	臨床検査	C型肝炎ウイルス	コスタリカ	男性	不明	不明	不明	症例報告	外国製品	05000271	2005/7/22	8.0
第5回	5-361	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000275	2005/7/26	8.0
第5回	5-362	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000277	2005/7/26	8.0
第5回	5-363	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000278	2005/7/26	8.0
第5回	5-364	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000298	2005/7/26	8.0
第5回	5-365	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000300	2005/7/26	8.0
第5回	5-366	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000301	2005/7/26	8.0
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

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
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

378

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5回	5-427	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000432	2005/8/30	8.0	
第5回	5-001	臨床検査	HIV検査陽性	イギリス	男性	不明	1986/3	不明	症例報告	外国製品	04000118	2005/3/18	8.0	
第5回	5-288	臨床検査	HIV検査陽性	イギリス	男性	25	1985/10/25	不明	症例報告	外国製品	05000003	2005/4/18	8.0	
第5回	5-006	臨床検査	HIV検査陽性	イギリス	男性	不明	1985	不明	症例報告	外国製品	04000119	2005/3/18	8.0	
第5回	5-287	臨床検査	HIV検査陽性	イギリス	男性	不明	1984/2/16	不明	症例報告	外国製品	04000107	2005/3/3	7.1	
第5回	5-285	臨床検査	HIV検査陽性	イギリス	男性	不明	1983/12	不明	症例報告	外国製品	04000128	2005/3/31	8.0	
第4回	4-01	感染症および寄生虫症	A型肝炎	イギリス	男性	29	不明	不明	症例報告	外国製品	04000077	2004/10/8	7.1	
第4回	4-01	感染症および寄生虫症	B型肝炎	イギリス	男性	29	不明	不明	症例報告	外国製品	04000077	2004/10/8	7.1	
第4回	4-02	感染症および寄生虫症	B型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	04000078	2004/10/8	7.1	
第4回	4-03	感染症および寄生虫症	B型肝炎	イギリス	男性	11	不明	不明	症例報告	外国製品	04000081	2004/10/8	7.1	
第4回	4-04	感染症および寄生虫症	C型肝炎	イギリス	男性	15	1990/7	不明	症例報告	外国製品	04000076	2004/10/8	7.1	
第4回	4-01	感染症および寄生虫症	C型肝炎	イギリス	男性	29	不明	不明	症例報告	外国製品	04000077	2004/10/8	7.1	
第4回	4-02	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	04000078	2004/10/8	7.1	
第4回	4-03	感染症および寄生虫症	C型肝炎	イギリス	男性	11	不明	不明	症例報告	外国製品	04000081	2004/10/8	7.1	
第4回	4-05	感染症および寄生虫症	C型肝炎	イギリス	男性	31	不明	不明	症例報告	外国製品	04000080	2004/10/8	7.1	
第4回	4-06	感染症および寄生虫症	C型肝炎	イギリス	男性	34	不明	不明	症例報告	外国製品	04000079	2004/10/8	7.1	
第4回	4-07	感染症および寄生虫症	C型肝炎	アメリカ	男性	25	不明	不明	症例報告	当該製品	04000100	2005/2/24	7.1	
第4回	4-08	感染症および寄生虫症	C型肝炎	アメリカ	男性	41	不明	不明	症例報告	当該製品	04000099	2005/2/24	7.1	
第4回	4-09	感染症および寄生虫症	C型肝炎	アメリカ	男性	27	不明	不明	症例報告	外国製品	04000098	2005/2/24	7.1	
第4回	4-10	感染症および寄生虫症	C型肝炎	アメリカ	女性	22	不明	不明	症例報告	外国製品	04000095	2005/2/21	7.1	
第4回	4-11	感染症および寄生虫症	C型肝炎	アメリカ	男性	17	不明	不明	症例報告	外国製品	04000094	2005/2/21	7.1	
第4回	4-12	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	04000075	2004/10/8	7.1	
第4回	4-13	感染症および寄生虫症	HIV感染	イギリス	男性	32	1988/8	不明	症例報告	外国製品	04000074	2004/10/8	7.1	
第4回	4-06	感染症および寄生虫症	HIV感染	イギリス	男性	34	1985/1/9	不明	症例報告	外国製品	04000079	2004/10/8	7.1	
第4回	4-01	感染症および寄生虫症	HIV感染	イギリス	男性	29	1985	不明	症例報告	外国製品	04000077	2004/10/8	7.1	
第4回	4-04	感染症および寄生虫症	HIV感染	イギリス	男性	15	1984/12/14	不明	症例報告	外国製品	04000076	2004/10/8	7.1	
第4回	4-05	臨床検査	HIV検査陽性	イギリス	男性	31	1987	不明	症例報告	外国製品	04000080	2004/10/8	7.1	
第4回	4-03	臨床検査	HIV検査陽性	イギリス	男性	11	1981/11/23	不明	症例報告	外国製品	04000081	2004/10/8	7.1	
第3回	3-1	感染症および寄生虫症	C型肝炎	トルコ	男性	44	2001/11/22	不明	症例報告	当該製品	04000001	2004/4/1	7.0	

90016	2008/09/29	80521	バクスター	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

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11回	5-231	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000274	2008/4/21	11.0	第11回症例番号5-231は第5回症例番号5-231において報告したものの追加報告
第11回	5-231	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000274	2008/4/21	11.0	第11回症例番号5-231は第5回症例番号5-231において報告したものの追加報告
第10回	10-1	臨床検査	C型肝炎ウイルス	ブラジル	男性	小児	2004/5/25	不明	症例報告	外国製品	07000015	2007/10/29	10.1	
第10回	10-1	臨床検査	C型肝炎ウイルス	ブラジル	男性	小児	2004/5/25	不明	症例報告	外国製品	07000015	2007/12/28	10.1	追加報告
第10回	10-2	感染症および寄生虫症	急性HIV感染	アメリカ	男性	34	不明	不明	症例報告	外国製品	07000017	2007/12/6	10.1	
第10回	10-2	臨床検査	C型肝炎ウイルス	アメリカ	男性	34	不明	不明	症例報告	外国製品	07000017	2007/12/6	10.1	
第10回	10-3	感染症および寄生虫症	C型肝炎	ベルギー	男性	不明	1991	未回復	症例報告	外国製品	07000028	2008/2/25	10.1	
第9回		0*	0	0	0	0	0	0	0	0	0	0	0	* 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	

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	7-008	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	06000011	2006/5/10	9.0	
第7回	7-007	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	06000013	2006/5/15	9.0	
第7回	7-061	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	06000014	2006/5/15	9.0	
第7回	7-010	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	死亡	症例報告	外国製品	06000015	2006/5/15	9.0	
第7回	7-005	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	06000016	2006/5/15	9.0	
第7回	7-062	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	06000017	2006/5/15	9.0	
第7回	7-006	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	06000018	2006/5/22	9.0	
第7回	7-012	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	11	2006/5/2	不明	症例報告	外国製品	06000019	2006/6/16	9.0	
第7回	5-130	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000065	2006/3/30	9.0	第7回症例番号5-130は第5回症例番号5-130と重複症例のため報告破棄
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	

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	7-058	臨床検査	C型肝炎ウイルス	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000684	2006/3/13	8.1	
第7回	5-139	感染症および寄生虫症	HIV感染	香港	男性	不明	1985	死亡	症例報告	外国製品	05000104	2006/3/2	8.1	第7回症例番号5-139は第5回症例番号5-139において報告したものの追加報告
第7回	7-001	感染症および寄生虫症	HIV感染	イギリス	男性	24	1985	不明	症例報告	外国製品	06000007	2006/5/1	9.0	
第7回	7-002	感染症および寄生虫症	HIV感染	イギリス	男性	9	1985	不明	症例報告	外国製品	06000009	2006/5/10	9.0	
第7回	7-003	感染症および寄生虫症	HIV感染	イギリス	男性	不明	1985/10/4	不明	症例報告	外国製品	06000010	2006/5/10	9.0	
第7回	7-004	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1986	不明	症例報告	外国製品	05000646	2006/3/3	8.1	
第7回	7-005	感染症および寄生虫症	HIV感染	イギリス	男性	不明	1986	不明	症例報告	外国製品	06000016	2006/5/15	9.0	
第7回	7-006	感染症および寄生虫症	HIV感染	イギリス	男性	不明	1986	不明	症例報告	外国製品	06000018	2006/5/22	9.0	
第7回	7-007	感染症および寄生虫症	HIV感染	イギリス	男性	不明	1986/3	不明	症例報告	外国製品	06000013	2006/5/15	9.0	
第7回	7-008	感染症および寄生虫症	HIV感染	イギリス	男性	不明	1986/4/9	不明	症例報告	外国製品	06000011	2006/5/10	9.0	
第7回	7-009	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1988	不明	症例報告	外国製品	05000637	2006/3/3	8.1	
第7回	7-010	感染症および寄生虫症	HIV感染	イギリス	男性	不明	1988/5	死亡	症例報告	外国製品	06000015	2006/5/15	9.0	
第7回	7-011	感染症および寄生虫症	HIV感染	台湾	男性	不明	1997/4/17	不明	症例報告	外国製品	05000635	2006/3/2	8.1	
第7回	5-130	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000065	2006/3/30	9.0	第7回症例番号5-130は第5回症例番号5-130と重複症例のため報告破棄
第7回	7-013	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000638	2006/3/3	8.1	
第7回	7-020	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000645	2006/3/3	8.1	
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	

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6回	6-163	感染症および寄生虫症	B型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000591	2006/2/16	8.1	
第6回	6-166	感染症および寄生虫症	B型肝炎	ペルー	男性	不明	不明	不明	症例報告	外国製品	05000598	2006/2/16	8.1	
第6回	6-176	感染症および寄生虫症	B型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000613	2006/2/22	8.1	
第6回	6-007	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	1985	不明	症例報告	外国製品	05000571	2006/2/13	8.1	
第6回	6-015	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	1986	不明	症例報告	外国製品	05000619	2006/2/22	8.1	
第6回	6-025	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	1990	不明	症例報告	外国製品	05000536	2006/2/8	8.1	
第6回	6-026	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	1990	不明	症例報告	外国製品	05000537	2006/2/8	8.1	
第6回	6-032	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	14	1994	不明	症例報告	外国製品	05000458	2005/10/28	8.1	
第6回	6-035	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	32	1995/5/24	不明	症例報告	外国製品	05000607	2006/2/22	8.1	
第6回	6-037	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	2001	不明	症例報告	外国製品	05000562	2006/2/13	8.1	
第6回	6-038	感染症および寄生虫症	C型肝炎	アルゼンチン	女性	不明	2003	不明	症例報告	外国製品	05000628	2006/2/24	8.1	
第6回	4-06	感染症および寄生虫症	C型肝炎	イギリス	男性	11	不明	不明	症例報告	外国製品	04000081	2005/9/16	8.1	第6回症例番号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	

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6回	6-129	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000539	2006/2/8	8.1
第6回	6-131	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000543	2006/2/10	8.1
第6回	6-132	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000544	2006/2/10	8.1
第6回	6-133	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000545	2006/2/10	8.1
第6回	6-134	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000546	2006/2/10	8.1
第6回	6-135	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000547	2006/2/10	8.1
第6回	6-136	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000548	2006/2/10	8.1
第6回	6-144	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000556	2006/2/13	8.1
第6回	6-145	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000557	2006/2/13	8.1
第6回	6-146	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000558	2006/2/13	8.1
第6回	6-147	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000573	2006/2/14	8.1
第6回	6-148	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	未回復	症例報告	外国製品	05000559	2006/2/13	8.1
第6回	6-149	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000560	2006/2/13	8.1
第6回	6-150	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000561	2006/2/13	8.1
第6回	6-152	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000564	2006/2/13	8.1
第6回	6-153	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000565	2006/2/13	8.1
第6回	6-154	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000566	2006/2/13	8.1
第6回	6-013	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

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6回	6-002	感染症および寄生虫症	HIV感染	アルゼンチン	不明	不明	1984	不明	症例報告	外国製品	05000569	2006/2/13	8.1	
第6回	6-003	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1984	不明	症例報告	外国製品	05000585	2006/2/16	8.1	
第6回	6-004	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1984	不明	症例報告	外国製品	05000595	2006/2/16	8.1	
第6回	6-005	感染症および寄生虫症	HIV感染	台湾	男性	不明	1984/10/18	不明	症例報告	外国製品	05000620	2006/2/22	8.1	
第6回	6-006	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1985	不明	症例報告	外国製品	05000570	2006/2/13	8.1	
第6回	6-008	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1985	不明	症例報告	外国製品	05000608	2006/2/22	8.1	
第6回	6-009	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1985	不明	症例報告	外国製品	05000610	2006/2/22	8.1	
第6回	6-010	感染症および寄生虫症	HIV感染	台湾	男性	不明	1985/1/3	不明	症例報告	外国製品	05000630	2006/2/24	8.1	
第6回	6-011	感染症および寄生虫症	HIV感染	台湾	男性	不明	1985/11/13	不明	症例報告	外国製品	05000633	2006/2/24	8.1	
第6回	6-012	感染症および寄生虫症	HIV感染	台湾	男性	不明	1985/5/1	不明	症例報告	外国製品	05000629	2006/2/24	8.1	
第6回	6-013	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1986	不明	症例報告	外国製品	05000567	2006/2/13	8.1	
第6回	6-014	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1986	不明	症例報告	外国製品	05000592	2006/2/16	8.1	
第6回	6-015	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	1986	不明	症例報告	外国製品	05000619	2006/2/22	8.1	
第6回	5-136	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	1986	不明	症例報告	外国製品	05000100	2005/10/27	8.0	第6回症例番号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	

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6回	6-078	感染症および寄生虫症	HIV感染	パナマ	男性	不明	不明	死亡	症例報告	外国製品	05000481	2005/12/2	8.1	
第6回	6-080	感染症および寄生虫症	HIV感染	チリ	男性	不明	不明	死亡	症例報告	外国製品	05000483	2005/12/2	8.1	
第6回	6-081	感染症および寄生虫症	HIV感染	チリ	男性	不明	不明	死亡	症例報告	外国製品	05000484	2005/12/2	8.1	
第6回	6-082	感染症および寄生虫症	HIV感染	パナマ	男性	不明	不明	死亡	症例報告	外国製品	05000485	2005/12/2	8.1	
第6回	6-083	感染症および寄生虫症	HIV感染	パナマ	男性	不明	不明	死亡	症例報告	外国製品	05000486	2005/12/2	8.1	
第6回	6-084	感染症および寄生虫症	HIV感染	パナマ	男性	不明	不明	死亡	症例報告	外国製品	05000487	2005/12/2	8.1	
第6回	6-085	感染症および寄生虫症	HIV感染	パナマ	男性	不明	不明	死亡	症例報告	外国製品	05000488	2005/12/2	8.1	
第6回	6-086	感染症および寄生虫症	HIV感染	チリ	男性	不明	不明	死亡	症例報告	外国製品	05000489	2005/12/2	8.1	
第6回	6-090	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000498	2006/2/6	8.1	
第6回	6-101	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000509	2006/2/8	8.1	
第6回	6-105	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000513	2006/2/8	8.1	
第6回	6-107	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000515	2006/2/8	8.1	
第6回	6-108	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000516	2006/2/8	8.1	
第6回	6-111	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000519	2006/2/8	8.1	
第6回	6-112	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000520	2006/2/8	8.1	
第6回	6-117	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000525	2006/2/8	8.1	
第6回	6-118	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000526	2006/2/8	8.1	
第6回	6-144	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000556	2006/2/13	8.1	
第6回	6-162	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000590	2006/2/16	8.1	
第6回	6-176	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000613	2006/2/22	8.1	
第6回	4-06	臨床検査	HIV検査陽性	イギリス	男性	11	1981/11/23	不明	症例報告	外国製品	04000081	2005/9/16	8.1	第6回症例番号4-06は前回報告における第4回症例番号4-06において報告したものの追加報告
第6回	6-020	肝胆道系障害	肝炎	ブラジル	男性	不明	1988	不明	症例報告	外国製品	05000576	2006/2/16	8.1	
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

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6回	6-039	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000433	2005/9/1	8.0
第6回	6-040	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000434	2005/9/1	8.0
第6回	6-041	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000435	2005/9/1	8.0
第6回	6-042	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000436	2005/9/1	8.0
第6回	6-043	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000437	2005/9/1	8.0
第6回	6-044	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000438	2005/9/9	8.0
第6回	6-046	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000440	2005/9/9	8.0
第6回	6-047	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000441	2005/9/9	8.0
第6回	6-065	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000463	2005/11/2	8.1
第6回	6-067	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000465	2005/11/2	8.1
第6回	6-068	臨床検査	C型肝炎ウイルス	ペルー	男性	不明	不明	不明	症例報告	外国製品	05000466	2005/11/2	8.1
第6回	6-073	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000473	2005/11/21	8.1
第6回	6-074	臨床検査	C型肝炎ウイルス	パナマ	男性	不明	不明	不明	症例報告	外国製品	05000476	2005/11/30	8.1
第6回	6-090	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000498	2006/2/6	8.1
第6回	6-091	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000499	2006/2/6	8.1
第6回	6-092	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000500	2006/2/6	8.1
第6回	6-094	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000502	2006/2/6	8.1
第6回	6-095	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000506	2006/2/6	8.1
第6回	6-098	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000505	2006/2/6	8.1
第6回	6-099	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000507	2006/2/8	8.1
第6回	6-100	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000508	2006/2/8	8.1
第6回	6-101	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000509	2006/2/8	8.1
第6回	6-102	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000510	2006/2/8	8.1
第6回	6-103	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000511	2006/2/8	8.1
第6回	6-104	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000512	2006/2/8	8.1
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

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6回	6-029	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000578	2006/2/16	8.1
第6回	6-167	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000599	2006/2/16	8.1
第6回	6-168	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000600	2006/2/21	8.1
第6回	6-170	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000602	2006/2/21	8.1
第6回	6-171	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000603	2006/2/21	8.1
第6回	6-172	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000604	2006/2/21	8.1
第6回	6-177	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000614	2006/2/22	8.1
第6回	6-178	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000615	2006/2/22	8.1
第6回	6-179	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000616	2006/2/22	8.1
第6回	6-180	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000617	2006/2/22	8.1
第6回	6-181	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000621	2006/2/22	8.1
第6回	6-182	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000622	2006/2/22	8.1
第6回	6-186	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000631	2006/2/24	8.1
第6回	6-187	臨床検査	C型肝炎ウイルス	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000632	2006/2/24	8.1
第5回	5-001	感染症および寄生虫症	A型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	04000118	2005/3/18	8.0
第5回	5-002	感染症および寄生虫症	A型肝炎	ベネズエラ	男性	不明	不明	死亡	症例報告	外国製品	05000244	2005/7/15	8.0
第5回	5-003	感染症および寄生虫症	A型肝炎	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000245	2005/7/15	8.0
第5回	5-004	感染症および寄生虫症	B型肝炎	ベネズエラ	男性	不明	1994	不明	症例報告	外国製品	05000225	2005/7/11	8.0
第5回	5-001	感染症および寄生虫症	B型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	04000118	2005/3/18	8.0
第5回	5-002	感染症および寄生虫症	B型肝炎	ベネズエラ	男性	不明	不明	死亡	症例報告	外国製品	05000244	2005/7/15	8.0
第5回	5-003	感染症および寄生虫症	B型肝炎	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000245	2005/7/15	8.0
第5回	5-005	感染症および寄生虫症	B型肝炎	アメリカ	男性	53	不明	不明	症例報告	当該製品	04000114	2005/3/15	7.1
第5回	5-006	感染症および寄生虫症	B型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	04000119	2005/3/18	8.0
第5回	5-007	感染症および寄生虫症	B型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	05000118	2005/6/9	8.0
第5回	5-008	感染症および寄生虫症	B型肝炎	アメリカ	男性	不明	不明	不明	症例報告	外国製品	05000147	2005/6/20	8.0
第5回	5-004	感染症および寄生虫症	C型肝炎	ベネズエラ	男性	不明	1994	不明	症例報告	外国製品	05000225	2005/7/11	8.0
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

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5回	5-022	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000071	2005/6/15	8.0	
第5回	5-023	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000072	2005/6/1	8.0	
第5回	5-023	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000072	2005/6/15	8.0	
第5回	5-024	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000073	2005/6/1	8.0	
第5回	5-024	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000073	2005/6/15	8.0	
第5回	5-025	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000074	2005/6/1	8.0	
第5回	5-025	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000074	2005/6/15	8.0	
第5回	5-026	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000075	2005/6/1	8.0	
第5回	5-027	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000076	2005/6/1	8.0	
第5回	5-028	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000092	2005/6/1	8.0	
第5回	5-029	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000093	2005/6/1	8.0	
第5回	5-030	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000094	2005/6/1	8.0	
第5回	5-031	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000095	2005/6/1	8.0	
第5回	5-032	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000096	2005/6/1	8.0	
第5回	5-033	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000097	2005/6/1	8.0	
第5回	5-034	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000077	2005/6/1	8.0	
第5回	5-035	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000078	2005/6/1	8.0	
第5回	5-035	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000078	2005/6/15	8.0	
第5回	5-036	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000079	2005/6/1	8.0	
第5回	5-037	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000080	2005/6/1	8.0	
第5回	5-038	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000081	2005/6/1	8.0	
第5回	5-039	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000082	2005/6/1	8.0	
第5回	5-040	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000083	2005/6/1	8.0	
第5回	5-041	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000084	2005/6/1	8.0	
第5回	5-042	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000088	2005/6/1	8.0	
第5回	5-043	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000089	2005/6/1	8.0	
第5回	5-043	感染症および寄生虫症	C型肝炎	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000089	2005/6/15	8.0	
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	

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5回	5-065	感染症および寄生虫症	C型肝炎	ドミニカ共和国	男性	不明	不明	不明	症例報告	外国製品	05000234	2005/7/12	8.0
第5回	5-066	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000238	2005/7/15	8.0
第5回	5-067	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000239	2005/7/15	8.0
第5回	5-068	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000243	2005/7/15	8.0
第5回	5-069	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000246	2005/7/15	8.0
第5回	5-070	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000253	2005/7/19	8.0
第5回	5-070	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000253	2005/8/2	8.0
第5回	5-071	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000262	2005/7/22	8.0
第5回	5-072	感染症および寄生虫症	C型肝炎	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000263	2005/7/22	8.0
第5回	5-073	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000281	2005/7/26	8.0
第5回	5-074	感染症および寄生虫症	C型肝炎	アルゼンチン	女性	不明	不明	不明	症例報告	外国製品	05000310	2005/7/27	8.0
第5回	5-075	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000311	2005/7/27	8.0
第5回	5-076	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000312	2005/7/27	8.0
第5回	5-077	感染症および寄生虫症	C型肝炎	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000324	2005/7/27	8.0
第5回	5-078	感染症および寄生虫症	C型肝炎	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000325	2005/7/27	8.0
第5回	5-079	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000327	2005/7/29	8.0
第5回	5-080	感染症および寄生虫症	C型肝炎	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000328	2005/7/29	8.0
第5回	5-081	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000347	2005/8/2	8.0
第5回	5-082	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000348	2005/8/2	8.0
第5回	5-083	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000349	2005/8/2	8.0
第5回	5-084	感染症および寄生虫症	C型肝炎	コスタリカ	男性	不明	不明	不明	症例報告	外国製品	05000352	2005/8/2	8.0
第5回	5-085	感染症および寄生虫症	C型肝炎	パナマ	男性	不明	不明	不明	症例報告	外国製品	05000361	2005/8/2	8.0
第5回	5-086	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000363	2005/8/2	8.0
第5回	5-087	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000364	2005/8/2	8.0
第5回	5-088	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000365	2005/8/2	8.0
第5回	5-089	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000366	2005/8/2	8.0
第5回	5-090	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000367	2005/8/3	8.0
第5回	5-091	感染症および寄生虫症	C型肝炎	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000369	2005/8/3	8.0
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

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5回	5-021	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000070	2005/6/1	8.0
第5回	5-021	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000070	2005/6/15	8.0
第5回	5-022	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000071	2005/6/1	8.0
第5回	5-022	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000071	2005/6/15	8.0
第5回	5-023	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000072	2005/6/1	8.0
第5回	5-023	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000072	2005/6/15	8.0
第5回	5-024	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000073	2005/6/1	8.0
第5回	5-024	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000073	2005/6/15	8.0
第5回	5-025	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000074	2005/6/15	8.0
第5回	5-025	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000074	2005/6/1	8.0
第5回	5-026	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000075	2005/6/1	8.0
第5回	5-027	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000076	2005/6/1	8.0
第5回	5-028	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000092	2005/6/1	8.0
第5回	5-029	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000093	2005/6/1	8.0
第5回	5-030	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000094	2005/6/1	8.0
第5回	5-031	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000095	2005/6/1	8.0
第5回	5-032	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000096	2005/6/1	8.0
第5回	5-033	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000097	2005/6/1	8.0
第5回	5-034	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000077	2005/6/1	8.0
第5回	5-035	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000078	2005/6/1	8.0
第5回	5-035	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000078	2005/6/15	8.0
第5回	5-036	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000079	2005/6/1	8.0
第5回	5-037	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000080	2005/6/1	8.0
第5回	5-038	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000081	2005/6/1	8.0
第5回	5-039	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000082	2005/6/1	8.0
第5回	5-040	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	不明	症例報告	外国製品	05000083	2005/6/1	8.0
第5回	5-041	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000084	2005/6/1	8.0
第5回	5-042	感染症および寄生虫症	HIV感染	南アフリカ	男性	不明	不明	死亡	症例報告	外国製品	05000088	2005/6/1	8.0
第5回	5-053	感染症および寄生虫症	HIV感染	ドミニカ共和国	男性	不明	不明	不明	症例報告	外国製品	05000155	2005/6/27	8.0
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

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5回	5-107	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000012	2005/5/12	8.0	
第5回	5-108	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	死亡	症例報告	外国製品	05000013	2005/5/12	8.0	
第5回	5-109	感染症および寄生虫症	HIV感染	香港	男性	不明	不明	不明	症例報告	外国製品	05000015	2005/5/23	8.0	
第5回	5-110	感染症および寄生虫症	HIV感染	香港	男性	不明	不明	不明	症例報告	外国製品	05000016	2005/5/23	8.0	
第5回	5-111	感染症および寄生虫症	HIV感染	香港	男性	不明	不明	不明	症例報告	外国製品	05000017	2005/5/23	8.0	
第5回	5-112	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000018	2005/5/23	8.0	
第5回	5-113	感染症および寄生虫症	HIV感染	香港	男性	不明	不明	不明	症例報告	外国製品	05000019	2005/5/23	8.0	
第5回	5-114	感染症および寄生虫症	HIV感染	香港	男性	不明	不明	不明	症例報告	外国製品	05000020	2005/5/23	8.0	
第5回	5-115	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000021	2005/5/23	8.0	
第5回	5-116	感染症および寄生虫症	HIV感染	香港	男性	不明	不明	不明	症例報告	外国製品	05000022	2005/5/23	8.0	
第5回	5-117	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000023	2005/5/23	8.0	
第5回	5-118	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000025	2005/5/26	8.0	
第5回	5-119	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000026	2005/5/26	8.0	
第5回	5-120	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	死亡	症例報告	外国製品	05000027	2005/5/26	8.0	
第5回	5-121	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000053	2005/5/30	8.0	
第5回	5-122	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000054	2005/5/30	8.0	
第5回	5-123	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000056	2005/5/30	8.0	
第5回	5-124	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000057	2005/5/30	8.0	
第5回	5-125	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000058	2005/5/30	8.0	
第5回	5-126	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000061	2005/5/30	8.0	
第5回	5-127	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000062	2005/5/30	8.0	
第5回	5-128	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000063	2005/5/30	8.0	
第5回	5-129	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000064	2005/5/30	8.0	
第5回	5-130	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000065	2005/5/30	8.0	
第5回	5-131	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000066	2005/5/30	8.0	
第5回	5-132	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000067	2005/5/30	8.0	
第5回	5-133	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000068	2005/5/30	8.0	
第5回	5-134	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000098	2005/6/1	8.0	
第5回	5-135	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	死亡	症例報告	外国製品	05000099	2005/6/1	8.0	
第5回	5-136	感染症および寄生虫症	HIV感染	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000100	2005/6/1	8.0	
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	

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	
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	

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5回	5-193	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000182	2005/8/8	8.0	
第5回	5-194	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000185	2005/7/4	8.0	
第5回	5-195	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000186	2005/7/4	8.0	
第5回	5-196	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000187	2005/7/4	8.0	
第5回	5-197	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000189	2005/7/4	8.0	
第5回	5-198	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000190	2005/7/4	8.0	
第5回	5-199	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000191	2005/7/4	8.0	
第5回	5-200	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000194	2005/7/4	8.0	
第5回	5-201	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000202	2005/7/8	8.0	
第5回	5-202	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000203	2005/7/8	8.0	
第5回	5-203	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000204	2005/7/8	8.0	
第5回	5-204	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000206	2005/7/8	8.0	
第5回	5-205	感染症および寄生虫症	HIV感染	コロンビア	男性	不明	不明	不明	症例報告	外国製品	05000209	2005/7/8	8.0	
第5回	5-206	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000212	2005/7/8	8.0	
第5回	5-207	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000213	2005/7/11	8.0	
第5回	5-208	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000214	2005/7/11	8.0	
第5回	5-209	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000219	2005/7/11	8.0	
第5回	5-210	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000230	2005/7/11	8.0	
第5回	5-211	感染症および寄生虫症	HIV感染	コスタリカ	男性	不明	不明	不明	症例報告	外国製品	05000232	2005/7/12	8.0	
第5回	5-212	感染症および寄生虫症	HIV感染	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000233	2005/7/12	8.0	
第5回	5-213	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000235	2005/7/12	8.0	
第5回	5-214	感染症および寄生虫症	HIV感染	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000237	2005/7/12	8.0	
第5回	5-215	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000240	2005/7/15	8.0	
第5回	5-216	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000241	2005/7/15	8.0	
第5回	5-217	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000242	2005/7/15	8.0	
第5回	5-218	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000248	2005/7/15	8.0	
第5回	5-219	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000249	2005/7/15	8.0	
第5回	5-220	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000250	2005/7/15	8.0	
第5回	5-221	感染症および寄生虫症	HIV感染	ベネズエラ	男性	不明	不明	死亡	症例報告	外国製品	05000251	2005/7/15	8.0	
第5回	5-222	感染症および寄生虫症	HIV感染	アルゼンチン	女性	不明	不明	死亡	症例報告	外国製品	05000252	2005/7/19	8.0	
第5回	5-223	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000254	2005/7/19	8.0	
第5回	5-224	感染症および寄生虫症	HIV感染	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000256	2005/7/19	8.0	
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	

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	
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	

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
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

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5回	5-158	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000116	2005/6/9	8.0	
第5回	5-159	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000041	2005/5/30	8.0	
第5回	5-161	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000043	2005/5/30	8.0	
第5回	5-162	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000044	2005/5/30	8.0	
第5回	5-162	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000044	2005/6/27	8.0	
第5回	5-163	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000045	2005/5/30	8.0	
第5回	5-163	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000045	2005/6/15	8.0	
第5回	5-164	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000046	2005/5/30	8.0	
第5回	5-165	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	不明	症例報告	外国製品	05000047	2005/5/30	8.0	
第5回	5-166	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000048	2005/5/30	8.0	
第5回	5-167	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000049	2005/5/30	8.0	
第5回	5-167	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000049	2005/6/15	8.0	
第5回	5-168	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000050	2005/5/30	8.0	
第5回	5-168	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000050	2005/6/15	8.0	
第5回	5-169	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000051	2005/5/30	8.0	
第5回	5-169	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000051	2005/6/15	8.0	
第5回	5-170	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000052	2005/5/30	8.0	
第5回	5-170	臨床検査	C型肝炎ウイルス	香港	男性	不明	不明	死亡	症例報告	外国製品	05000052	2005/6/15	8.0	
第5回	5-173	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000121	2005/6/13	8.0	
第5回	5-174	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000122	2005/6/13	8.0	
第5回	5-177	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000129	2005/6/15	8.0	
第5回	5-180	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000148	2005/6/20	8.0	
第5回	5-181	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000156	2005/6/27	8.0	
第5回	5-182	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000159	2005/6/27	8.0	
第5回	5-185	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000164	2005/6/27	8.0	
第5回	5-186	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000165	2005/6/27	8.0	
第5回	5-187	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000167	2005/6/27	8.0	
第5回	5-187	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000167	2005/8/18	8.0	
第5回	5-188	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000171	2005/6/30	8.0	
第5回	5-189	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000173	2005/6/30	8.0	
第5回	5-190	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000175	2005/6/30	8.0	
第5回	5-191	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000176	2005/6/30	8.0	
第5回	5-192	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000181	2005/6/30	8.0	
第5回	5-194	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000185	2005/7/4	8.0	
第5回	5-195	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000186	2005/7/4	8.0	
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	

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	
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	

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5回	5-277	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000423	2005/8/30	8.0	
第5回	5-278	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000424	2005/8/30	8.0	
第5回	5-279	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000425	2005/8/30	8.0	
第5回	5-280	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000427	2005/8/30	8.0	
第5回	5-282	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000429	2005/8/30	8.0	
第5回	5-283	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000430	2005/8/30	8.0	
第5回	5-284	臨床検査	C型肝炎ウイルス	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000431	2005/8/30	8.0	
第5回	5-287	臨床検査	C型肝炎ウイルス	イギリス	男性	不明	不明	不明	症例報告	外国製品	04000107	2005/3/3	7.1	
第5回	5-288	臨床検査	C型肝炎ウイルス	イギリス	男性	25	不明	不明	症例報告	外国製品	05000003	2005/4/18	8.0	
第5回	5-289	臨床検査	C型肝炎ウイルス	イギリス	男性	57	不明	不明	症例報告	外国製品	04000102	2005/3/3	7.1	
第5回	5-290	臨床検査	C型肝炎ウイルス	イギリス	男性	58	不明	不明	症例報告	外国製品	04000104	2005/3/3	7.1	
第5回	5-291	臨床検査	C型肝炎ウイルス	イギリス	男性	不明	不明	不明	症例報告	外国製品	04000120	2005/3/18	8.0	
第5回	5-292	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000024	2005/5/26	8.0	
第5回	5-293	臨床検査	C型肝炎ウイルス	アルゼンチン	男性	不明	不明	不明	症例報告	外国製品	05000055	2005/5/30	8.0	
第5回	5-294	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000059	2005/5/30	8.0	
第5回	5-295	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000060	2005/5/30	8.0	
第5回	5-296	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000101	2005/6/1	8.0	
第5回	5-297	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000108	2005/6/7	8.0	
第5回	5-298	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000110	2005/6/9	8.0	
第5回	5-299	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000115	2005/6/9	8.0	
第5回	5-300	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000117	2005/6/9	8.0	
第5回	5-301	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000123	2005/6/13	8.0	
第5回	5-302	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000127	2005/6/15	8.0	
第5回	5-303	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000128	2005/6/15	8.0	
第5回	5-304	臨床検査	C型肝炎ウイルス	ブラジル	女性	不明	不明	不明	症例報告	外国製品	05000130	2005/6/15	8.0	
第5回	5-305	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000131	2005/6/15	8.0	
第5回	5-306	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000132	2005/6/15	8.0	
第5回	5-307	臨床検査	C型肝炎ウイルス	コスタリカ	男性	不明	不明	不明	症例報告	外国製品	05000138	2005/6/20	8.0	
第5回	5-308	臨床検査	C型肝炎ウイルス	コスタリカ	男性	不明	不明	不明	症例報告	外国製品	05000139	2005/6/20	8.0	
第5回	5-309	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000140	2005/6/20	8.0	
第5回	5-310	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000141	2005/6/20	8.0	
第5回	5-311	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000142	2005/6/20	8.0	
第5回	5-312	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000143	2005/6/20	8.0	
第5回	5-313	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000146	2005/6/20	8.0	
第5回	5-314	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000149	2005/6/27	8.0	
第5回	5-315	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000151	2005/6/27	8.0	
第5回	5-316	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000152	2005/6/27	8.0	
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	

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5回	5-329	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000179	2005/6/30	8.0	
第5回	5-330	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000180	2005/6/30	8.0	
第5回	5-331	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000184	2005/7/4	8.0	
第5回	5-332	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000188	2005/7/4	8.0	
第5回	5-333	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000192	2005/7/4	8.0	
第5回	5-334	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000193	2005/7/4	8.0	
第5回	5-335	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000197	2005/7/8	8.0	
第5回	5-336	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000198	2005/7/8	8.0	
第5回	5-337	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000199	2005/7/8	8.0	
第5回	5-338	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000200	2005/7/8	8.0	
第5回	5-339	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000201	2005/7/8	8.0	
第5回	5-340	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	死亡	症例報告	外国製品	05000205	2005/7/8	8.0	
第5回	5-341	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000207	2005/7/8	8.0	
第5回	5-342	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000208	2005/7/8	8.0	
第5回	5-343	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000210	2005/7/8	8.0	
第5回	5-344	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000211	2005/7/8	8.0	
第5回	5-345	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000216	2005/7/11	8.0	
第5回	5-346	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000217	2005/7/11	8.0	
第5回	5-347	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000220	2005/7/11	8.0	
第5回	5-348	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000222	2005/7/11	8.0	
第5回	5-349	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000224	2005/7/11	8.0	
第5回	5-350	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000229	2005/7/11	8.0	
第5回	5-351	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000236	2005/7/12	8.0	
第5回	5-352	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000247	2005/7/15	8.0	
第5回	5-353	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000255	2005/7/19	8.0	
第5回	5-354	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000260	2005/7/22	8.0	
第5回	5-355	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000265	2005/7/22	8.0	
第5回	5-356	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000266	2005/7/22	8.0	
第5回	5-357	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000268	2005/7/22	8.0	
第5回	5-358	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000269	2005/7/22	8.0	
第5回	5-359	臨床検査	C型肝炎ウイルス	ベネズエラ	男性	不明	不明	不明	症例報告	外国製品	05000270	2005/7/22	8.0	
第5回	5-360	臨床検査	C型肝炎ウイルス	コスタリカ	男性	不明	不明	不明	症例報告	外国製品	05000271	2005/7/22	8.0	
第5回	5-361	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000275	2005/7/26	8.0	
第5回	5-362	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000277	2005/7/26	8.0	
第5回	5-363	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000278	2005/7/26	8.0	
第5回	5-364	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000298	2005/7/26	8.0	
第5回	5-365	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000300	2005/7/26	8.0	
第5回	5-366	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000301	2005/7/26	8.0	
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	

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
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

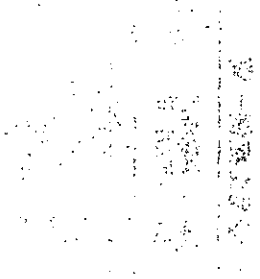
別紙様式第4

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5回	5-427	臨床検査	C型肝炎ウイルス	ブラジル	男性	不明	不明	不明	症例報告	外国製品	05000432	2005/8/30	8.0
第5回	5-001	臨床検査	HIV検査陽性	イギリス	男性	不明	1986/3	不明	症例報告	外国製品	04000118	2005/3/18	8.0
第5回	5-288	臨床検査	HIV検査陽性	イギリス	男性	25	1985/10/25	不明	症例報告	外国製品	05000003	2005/4/18	8.0
第5回	5-006	臨床検査	HIV検査陽性	イギリス	男性	不明	1985	不明	症例報告	外国製品	04000119	2005/3/18	8.0
第5回	5-287	臨床検査	HIV検査陽性	イギリス	男性	不明	1984/2/16	不明	症例報告	外国製品	04000107	2005/3/3	7.1
第5回	5-285	臨床検査	HIV検査陽性	イギリス	男性	不明	1983/12	不明	症例報告	外国製品	04000128	2005/3/31	8.0
第4回	4-01	感染症および寄生虫症	A型肝炎	イギリス	男性	29	不明	不明	症例報告	外国製品	04000077	2004/10/8	7.1
第4回	4-01	感染症および寄生虫症	B型肝炎	イギリス	男性	29	不明	不明	症例報告	外国製品	04000077	2004/10/8	7.1
第4回	4-02	感染症および寄生虫症	B型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	04000078	2004/10/8	7.1
第4回	4-03	感染症および寄生虫症	B型肝炎	イギリス	男性	11	不明	不明	症例報告	外国製品	04000081	2004/10/8	7.1
第4回	4-04	感染症および寄生虫症	C型肝炎	イギリス	男性	15	1990/7	不明	症例報告	外国製品	04000076	2004/10/8	7.1
第4回	4-01	感染症および寄生虫症	C型肝炎	イギリス	男性	29	不明	不明	症例報告	外国製品	04000077	2004/10/8	7.1
第4回	4-02	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	04000078	2004/10/8	7.1
第4回	4-03	感染症および寄生虫症	C型肝炎	イギリス	男性	11	不明	不明	症例報告	外国製品	04000081	2004/10/8	7.1
第4回	4-05	感染症および寄生虫症	C型肝炎	イギリス	男性	31	不明	不明	症例報告	外国製品	04000080	2004/10/8	7.1
第4回	4-06	感染症および寄生虫症	C型肝炎	イギリス	男性	34	不明	不明	症例報告	外国製品	04000079	2004/10/8	7.1
第4回	4-07	感染症および寄生虫症	C型肝炎	アメリカ	男性	25	不明	不明	症例報告	当該製品	04000100	2005/2/24	7.1
第4回	4-08	感染症および寄生虫症	C型肝炎	アメリカ	男性	41	不明	不明	症例報告	当該製品	04000099	2005/2/24	7.1
第4回	4-09	感染症および寄生虫症	C型肝炎	アメリカ	男性	27	不明	不明	症例報告	外国製品	04000098	2005/2/24	7.1
第4回	4-10	感染症および寄生虫症	C型肝炎	アメリカ	女性	22	不明	不明	症例報告	外国製品	04000095	2005/2/21	7.1
第4回	4-11	感染症および寄生虫症	C型肝炎	アメリカ	男性	17	不明	不明	症例報告	外国製品	04000094	2005/2/21	7.1
第4回	4-12	感染症および寄生虫症	C型肝炎	イギリス	男性	不明	不明	不明	症例報告	外国製品	04000075	2004/10/8	7.1
第4回	4-13	感染症および寄生虫症	HIV感染	イギリス	男性	32	1988/8	不明	症例報告	外国製品	04000074	2004/10/8	7.1
第4回	4-06	感染症および寄生虫症	HIV感染	イギリス	男性	34	1985/1/9	不明	症例報告	外国製品	04000079	2004/10/8	7.1
第4回	4-01	感染症および寄生虫症	HIV感染	イギリス	男性	29	1985	不明	症例報告	外国製品	04000077	2004/10/8	7.1
第4回	4-04	感染症および寄生虫症	HIV感染	イギリス	男性	15	1984/12/14	不明	症例報告	外国製品	04000076	2004/10/8	7.1
第4回	4-05	臨床検査	HIV検査陽性	イギリス	男性	31	1987	不明	症例報告	外国製品	04000080	2004/10/8	7.1
第4回	4-03	臨床検査	HIV検査陽性	イギリス	男性	11	1981/11/23	不明	症例報告	外国製品	04000081	2004/10/8	7.1
第3回	3-1	感染症および寄生虫症	C型肝炎	トルコ	男性	44	2001/11/22	不明	症例報告	当該製品	04000001	2004/4/1	7.0

403

90017 2008/09/29 80522 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



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11回	1	感染症および寄生虫症	B型肝炎	ドイツ	男	24	2008/1/10	不明	症例報告	外国製品	識別番号3-07000031 完了報告提出日2008年3月12日
	1	感染症および寄生虫症	B型肝炎	ドイツ	男	24	2008/1/10	不明	症例報告	外国製品	識別番号3-07000031 追加報告提出日2008年3月25日
	2	臨床検査	C型肝炎抗体陽性	日本	女	37	2007/9/11	不明	症例報告	当該製品	識別番号1-07000251 未完了報告提出日2008年3月12日
	2	臨床検査	C型肝炎抗体陽性	日本	女	37	2007/9/11	不明	症例報告	当該製品	識別番号1-07000251 未完了報告提出日2008年4月10日
	2	臨床検査	C型肝炎抗体陽性	日本	女	37	2007/9/11	不明	症例報告	当該製品	識別番号1-07000251 完了報告提出日2008年4月30日
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

90018	2008/09/29	80523	CSLベーリング	①人血清アルブミン ②人血液凝固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

別紙(4)

感染症発生症例一覧

	番号	感染症の種類		発生国	性別	年齢	発現時期	転帰	出典	区分	備考
		器官別大分類	基本語								
第11回	1	臨床検査	C型肝炎抗体陽性	日本	女	37	2007/9/11	不明	症例報告	当該製品	識別番号1-07000251 未完了報告提出日2008年3月12日
	1	臨床検査	C型肝炎抗体陽性	日本	女	37	2007/9/11	不明	症例報告	当該製品	識別番号1-07000251 未完了報告提出日2008年4月10日
	1	臨床検査	C型肝炎抗体陽性	日本	女	37	2007/9/11	不明	症例報告	当該製品	識別番号1-07000251 完了報告提出日2008年4月30日
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

90019	2008/09/29	80524	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

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11回	1	臨床検査	C型肝炎抗体陽性	日本	女	37	2007/9/11	不明	症例報告	当該製品	識別番号1-07000251 未完了報告提出日2008年3月12日
	1	臨床検査	C型肝炎抗体陽性	日本	女	37	2007/9/11	不明	症例報告	当該製品	識別番号1-07000251 未完了報告提出日2008年4月10日
	1	臨床検査	C型肝炎抗体陽性	日本	女	37	2007/9/11	不明	症例報告	当該製品	識別番号1-07000251 完了報告提出日2008年4月30日
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

90020	2008/09/29	80525	CSLベーリング	フィブリノゲン加第ＸＩＩＩ因子		トロンビン末

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11回	1	感染症および寄生虫症	B型肝炎	ドイツ	男	24	2008/1/10	不明	症例報告	外国製品	識別番号3-07000026 追加報告提出日2008年3月19日 第10回報告症例番号1と同一症例
	1	感染症および寄生虫症	B型肝炎	ドイツ	男	24	2008/1/10	不明	症例報告	外国製品	識別番号3-07000026 追加報告提出日2008年4月1日 第10回報告症例番号1と同一症例
	2	臨床検査	C型肝炎抗体陽性	日本	女	37	2007/9/11	不明	症例報告	当該製品	識別番号1-07000251 未完了報告提出日2008年3月12日
	2	臨床検査	C型肝炎抗体陽性	日本	女	37	2007/9/11	不明	症例報告	当該製品	識別番号1-07000251 未完了報告提出日2008年4月10日
	2	臨床検査	C型肝炎抗体陽性	日本	女	37	2007/9/11	不明	症例報告	当該製品	識別番号1-07000251 完了報告提出日2008年4月30日
	3	感染症および寄生虫症	C型肝炎	ドイツ	女	60	2007/4/13	不明	症例報告	外国製品	識別番号3-08000005 未完了報告提出日2008年4月24日
	3	感染症および寄生虫症	C型肝炎	ドイツ	女	60	2007/4/13	不明	症例報告	外国製品	識別番号3-08000005 完了報告提出日2008年5月29日
第10回	1	感染症および寄生虫症	B型肝炎	ドイツ	男	24	2008/1/10	不明	症例報告	外国製品	識別番号3-07000026 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

90021	2008/09/29	80526	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11回	1	臨床検査	C型肝炎抗体陽性	日本	女	37	2007/9/11	不明	症例報告	当該製品	識別番号1-07000251 未完了報告提出日2008年3月12日
	1	臨床検査	C型肝炎抗体陽性	日本	女	37	2007/9/11	不明	症例報告	当該製品	識別番号1-07000251 未完了報告提出日2008年4月10日
	1	臨床検査	C型肝炎抗体陽性	日本	女	37	2007/9/11	不明	症例報告	当該製品	識別番号1-07000251 完了報告提出日2008年4月30日
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

90022	2008/09/29	80527	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

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11回	1	感染症および寄生虫症	C型肝炎	日本	女	37	2007/9/11	未回復	症例報告	当該製品	識別番号1-07000251 未完了報告提出日2008年3月12日
	1	臨床検査	C型肝炎抗体陽性	日本	女	37	2007/9/11	不明	症例報告	当該製品	識別番号1-07000251 未完了報告提出日2008年4月10日
	1	臨床検査	C型肝炎抗体陽性	日本	女	37	2007/9/11	不明	症例報告	当該製品	識別番号1-07000251 完了報告提出日2008年4月30日
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

90026	2008/10/21	80611	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

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	B型肝炎	日本	女	33	2008/8/11	③未回復	症例報告	当該製品	2008/9/1提出、識別番号1-08000567　未完了報告
2	感染症および寄生虫症	C型肝炎	日本	女	31	2008/7/16	②軽快	症例報告	当該製品	2008/8/13提出、識別番号1-08000512　未完了報告
3	感染症および寄生虫症	敗血症性ショック	日本	女	74	2008/7/10	⑤死亡	症例報告	当該製品	2008/7/28提出、識別番号1-08000433　未完了報告
4	感染症および寄生虫症	敗血症性ショック	日本	女	74	2008/7/10	⑤死亡	症例報告	当該製品	2008/8/29提出、識別番号1-08000433　完了報告（4番と同一症例）
5	感染症および寄生虫症	B型肝炎	日本	女	75	2008/7/10	⑤死亡	症例報告	当該製品	2008/8/12提出、識別番号1-08000511　未完了報告
6	感染症および寄生虫症	B型肝炎	日本	女	71	2008/6/25	②軽快	症例報告	当該製品	2008/7/28提出、識別番号1-08000430　未完了報告
7	感染症および寄生虫症	B型肝炎	日本	女	71	2008/6/25	②軽快	症例報告	当該製品	2008/8/13提出、識別番号1-08000430　未完了報告（6番と同一症例）
8	感染症および寄生虫症	C型肝炎	日本	男	77	2008/6/18	⑥不明	症例報告	当該製品	2008/7/10提出、識別番号1-08000399　未完了報告
9	感染症および寄生虫症	B型肝炎	日本	男	59	2008/6/10	③未回復	症例報告	当該製品	2008/6/27提出、識別番号1-08000382　未完了報告
10	感染症および寄生虫症	B型肝炎	日本	男	59	2008/6/10	③未回復	症例報告	当該製品	2008/7/17提出、識別番号1-08000382　未完了報告（9番と同一症例）
11	感染症および寄生虫症	C型肝炎	日本	男	65	2008/4/17	③未回復	症例報告	当該製品	2008/7/3提出、識別番号1-08000390　未完了報告
12	感染症および寄生虫症	C型肝炎	日本	男	65	2008/4/17	③未回復	症例報告	当該製品	2008/7/25提出、識別番号1-08000390　未完了報告（11番と同一症例）
13	感染症および寄生虫症	B型肝炎	日本	男	60	2008/4/17	⑥不明	症例報告	当該製品	2008/7/18提出、識別番号1-08000407　未完了報告
14	感染症および寄生虫症	B型肝炎	日本	男	60	2008/4/17	①回復	症例報告	当該製品	2008/8/6提出、識別番号1-08000407　完了報告（13番と同一症例）
15	感染症および寄生虫症	C型肝炎	日本	男	1	2008/3/10	⑥不明	症例報告	当該製品	2008/5/2提出、識別番号1-08000080　未完了報告
16	感染症および寄生虫症	B型肝炎	日本	女	59	2008/3/7	③未回復	症例報告	当該製品	2008/4/9提出、識別番号1-08000026　未完了報告
17	感染症および寄生虫症	C型肝炎	日本	女	67	2008/2/28	⑥不明	症例報告	当該製品	2008/5/7提出、識別番号1-08000083　未完了報告
18	感染症および寄生虫症	C型肝炎	日本	女	67	2008/2/28	⑥不明	症例報告	当該製品	2008/6/2提出、識別番号1-08000083　未完了報告（17番と同一症例）
19	感染症および寄生虫症	B型肝炎	日本	女	25	2008/2/25	③未回復	症例報告	当該製品	2008/4/16提出、識別番号1-08000031　未完了報告
20	感染症および寄生虫症	C型肝炎	日本	女	72	2008/1/23	②軽快	症例報告	当該製品	2008/3/21提出、識別番号1-07000277　未完了報告
21	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	2008/6/9提出、識別番号1-07000178　未完了報告 第11回症例番号21は前回報告における第10回症例番号3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	C型肝炎	日本	男	58	2007/10/26	①回復	症例報告	当該製品	2008/4/22提出、識別番号1-07000123　完了報告 第11回症例番号22は前回報告における第10回症例番号11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	C型肝炎	日本	男	58	2007/10/26	①回復	症例報告	当該製品	2008/6/24提出、識別番号1-07000123　完了報告（22番と同一症例） 第11回症例番号23は前回報告における第10回症例番号11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	30	2007/10/15	③未回復	症例報告	当該製品	2008/3/31提出、識別番号1-07000136　完了報告 第11回症例番号24は前回報告における第10回症例番号14、15において報告したものの追加報告
25	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	2008/3/31提出、識別番号1-07000160　取り下げ 第11回症例番号25は前回報告における第10回症例番号17において報告したものの追加報告

別紙5

MedDRA/J Ver. 11.0J

別紙様式第4

感染症発生症例一覧

第11回	26	感染症および寄生虫症	B型肝炎	日本	女	47	2007/4/24	⑥不明	症例報告	当該製品	2008/7/14提出、識別番号1-08000404　未完了報告
	27	感染症および寄生虫症	B型肝炎	日本	女	39	2006/11/29	①回復	症例報告	当該製品	2008/8/20提出、識別番号1-06000204　完了報告 第11回症例番号27は前々々回報告における第8回症例番号4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	サイトメガロウイルス感染	日本	男	1	2006/11/15	②軽快	症例報告	当該製品	2008/8/29提出、識別番号1-06000191　完了報告 第11回症例番号28は前々々回報告における第8回症例番号7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	女	34	2006/10/27	①回復	症例報告	当該製品	2008/6/30提出、識別番号1-06000195　完了報告 第11回症例番号29は前々々回報告における第8回症例番号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	男	44	2006/6/30	⑥不明	症例報告	当該製品	2008/8/18提出、識別番号1-06000083　完了報告 第11回症例番号30は前々々々回報告における第7回症例番号35、36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	C型肝炎	日本	男	10	2006/6/27	⑥不明	症例報告	当該製品	2008/7/2提出、識別番号1-06000080　完了報告 第11回症例番号31は前々々々回報告における第7回症例番号34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	男	77	2006/6/20	②軽快	症例報告	当該製品	2008/7/7提出、識別番号1-06000072　完了報告 第11回症例番号32は前々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
	33	感染症および寄生虫症	B型肝炎	日本	男	59	2006/6/20	⑥不明	症例報告	当該製品	2008/7/28提出、識別番号1-06000086　完了報告 第11回症例番号33は前々々々回報告における第7回症例番号37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	男	85	2006/6/7	②軽快	症例報告	当該製品	2008/6/27提出、識別番号1-06000061　完了報告 第11回症例番号34は前々々々回報告における第7回症例番号26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	78	2006/6/6	⑥不明	症例報告	当該製品	2008/7/22提出、識別番号1-06000069　完了報告 第11回症例番号35は前々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
	36	感染症および寄生虫症	C型肝炎	日本	男	48	2006/6/1	①回復	症例報告	当該製品	2008/6/20提出、識別番号1-06000057　完了報告 第11回症例番号36は前々々々回報告における第7回症例番号25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	56	2006/5/23	⑥不明	症例報告	当該製品	2008/6/27提出、識別番号1-06000066　完了報告 第11回症例番号37は前々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
	38	感染症および寄生虫症	B型肝炎	日本	男	57	2006/5/19	②軽快	症例報告	当該製品	2008/8/29提出、識別番号1-06000087　完了報告 第11回症例番号38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
	39	感染症および寄生虫症	B型肝炎	日本	男	81	2006/5/17	⑥不明	症例報告	当該製品	2008/3/24提出、識別番号1-06000039　完了報告 第11回症例番号39は前々々々回報告における第7回症例番号20において報告したものの追加報告

MedDRA/J Ver. 11.0J

別紙様式第4

感染症発生症例一覧

	40	感染症および寄生虫症	B型肝炎	日本	女	80	2006/5/2	②軽快	症例報告	当該製品	2008/4/22提出、識別番号1-06000043　完了報告 第11回症例番号40は前々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
	41	感染症および寄生虫症	C型肝炎	日本	男	78	2006/1/30	③未回復	症例報告	当該製品	2008/6/20提出、識別番号1-05000261　完了報告 第11回症例番号41は前々々々々回報告における第6回症例番号3、第7回症例番号10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	B型肝炎	日本	男	34	2006/1/5	②軽快	症例報告	当該製品	2008/6/20提出、識別番号1-05000243　完了報告 第11回症例番号42は前々々々々回報告における第6回症例番号6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	C型肝炎	日本	男	72	2005/12/29	⑥不明	症例報告	当該製品	2008/5/19提出、識別番号1-05000242　完了報告 第11回症例番号43は前々々々々回報告における第6回症例番号8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	細菌感染	日本	男	82	2005/9/29	①回復	症例報告	当該製品	2008/3/27提出、識別番号1-05000202　完了報告 第11回症例番号44は前々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
	45	感染症および寄生虫症	B型肝炎	日本	女	1	2005/7/11	⑥不明	症例報告	当該製品	2008/8/20提出、識別番号1-06000042　完了報告 第11回症例番号45は前々々々回報告における第7回症例番号21において報告したものの追加報告
	46	感染症および寄生虫症	B型肝炎	日本	男	58	2005/4/1	①回復	症例報告	当該製品	2008/4/10提出、識別番号1-05000047　完了報告 第11回症例番号46は前々々々々々回報告における第5回症例番号20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	79	2005/1/26	③未回復	症例報告	当該製品	2008/8/5提出、識別番号1-08000462　未完了報告
	48	感染症および寄生虫症	B型肝炎	日本	男	80	2003/11/5	①回復	症例報告	当該製品	2008/4/10提出、識別番号1-05000031　完了報告 第11回症例番号48は前々々々々々回報告における第5回症例番号47において報告したものの追加報告
第10回	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
	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
	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
	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
第8回	4	感染症および寄生虫症	B型肝炎	日本	女	39	2006/11/29	③未回復	症例報告	当該製品	2007/1/5 提出、識別番号 1-06000204 未完了報告
	7	感染症および寄生虫症	サイトメガロウイルス感染	日本	男	1	2006/11/15	⑤死亡	症例報告	当該製品	2006/12/18 提出、識別番号 1-06000191 未完了報告
	10	感染症および寄生虫症	B型肝炎	日本	女	34	2006/10/27	③未回復	症例報告	当該製品	2006/12/22 提出、識別番号 1-06000195 未完了報告
	10	感染症および寄生虫症	C型肝炎	日本	男	78	2006/1/30	③未回復	症例報告	当該製品	2006/3/13 提出、識別番号 1-05000261 未完了報告
	20	感染症および寄生虫症	B型肝炎	日本	男	81	2006/5/17	③未回復	症例報告	当該製品	2006/6/5 提出、識別番号 1-06000039 未完了報告
	21	感染症および寄生虫症	B型肝炎	日本	女	1	2005/7/11	③未回復	症例報告	当該製品	2006/6/9 提出、識別番号 1-06000042 未完了報告
	22	感染症および寄生虫症	B型肝炎	日本	女	80	2006/5/2	②軽快	症例報告	当該製品	2006/6/9 提出、識別番号 1-06000043 未完了報告
	25	感染症および寄生虫症	C型肝炎	日本	男	48	2006/6/1	③未回復	症例報告	当該製品	2006/6/23 提出、識別番号 1-06000057 未完了報告
	26	感染症および寄生虫症	B型肝炎	日本	男	85	2006/6/7	③未回復	症例報告	当該製品	2006/7/6 提出、識別番号 1-06000061 未完了報告

別紙様式第4

感染症発生症例一覧

第7回	28	感染症および寄生虫症	B型肝炎	日本	女	56	2006/5/23	③未回復	症例報告	当該製品	2006/7/12 提出、識別番号 1-06000066 未完了報告
	29	感染症および寄生虫症	C型肝炎	日本	女	78	2006/6/6	③未回復	症例報告	当該製品	2006/7/18 提出、識別番号 1-06000069 未完了報告
	31	感染症および寄生虫症	B型肝炎	日本	男	77	2006/6/20	③未回復	症例報告	当該製品	2006/7/18 提出、識別番号 1-06000072 未完了報告
	34	感染症および寄生虫症	C型肝炎	日本	男	10	2006/6/27	⑥不明	症例報告	当該製品	2006/7/24 提出、識別番号 1-06000080 未完了報告
	35	感染症および寄生虫症	B型肝炎	日本	男	44	2006/6/30	⑥不明	症例報告	当該製品	2006/7/31 提出、識別番号 1-06000083 未完了報告
	36	感染症および寄生虫症	B型肝炎	日本	男	44	2006/6/30	⑥不明	症例報告	当該製品	2006/8/18 提出、識別番号 1-06000083 未完了報告
	37	感染症および寄生虫症	B型肝炎	日本	男	59	2006/6/20	③未回復	症例報告	当該製品	2006/8/2 提出、識別番号 1-06000086 未完了報告
	38	感染症および寄生虫症	B型肝炎	日本	男	57	2006/5/19	②軽快	症例報告	当該製品	2006/8/2 提出、識別番号 1-06000087 未完了報告
第6回	3	感染症および寄生虫症	C型肝炎	日本	男	78	2006/1/30	③未回復	症例報告	当該製品	2006/3/1 提出、識別番号 1-05000261 未完了報告
	6	感染症および寄生虫症	B型肝炎	日本	男	34	2006/1/5	②軽快	症例報告	当該製品	2006/2/2 提出、識別番号 1-05000243 未完了報告
	8	感染症および寄生虫症	C型肝炎	日本	男	72	2005/12/29	②軽快	症例報告	当該製品	2006/1/26 提出、識別番号 1-05000242 未完了報告
	13	感染症および寄生虫症	細菌感染	日本	男	82	2005/9/29	①回復	症例報告	当該製品	2005/11/28 提出、識別番号 1-05000202 未完了報告
第5回	20	感染症および寄生虫症	B型肝炎	日本	男	58	2005/04/01	①回復又は軽快	症例報告	当該製品	2005/06/14 提出、識別番号 1-05000047 未完了報告
	47	感染症および寄生虫症	B型肝炎	日本	男	80	2003/11/05	⑧不明	症例報告	当該製品	2005/05/13 提出、識別番号 1-05000031 未完了報告

90030	2008/10/23	80632	日本赤十字社	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	B型肝炎	日本	女	73	2008/7/18	③未回復	症例報告	当該製品	2008/8/5提出、識別番号1-08000463　未完了報告
2	感染症および寄生虫症	B型肝炎	日本	女	73	2008/7/18	③未回復	症例報告	当該製品	2008/8/27提出、識別番号1-08000463　未完了報告（1番と同一症例）
3	感染症および寄生虫症	敗血症	日本	女	76	2008/7/16	②軽快	症例報告	当該製品	2008/7/31提出、識別番号1-08000435　未完了報告
4	感染症および寄生虫症	C型肝炎	日本	女	31	2008/7/16	②軽快	症例報告	当該製品	2008/8/13提出、識別番号1-08000512　未完了報告
5	感染症および寄生虫症	敗血症性ショック	日本	女	74	2008/7/10	⑤死亡	症例報告	当該製品	2008/7/28提出、識別番号1-08000433　未完了報告
6	感染症および寄生虫症	敗血症性ショック	日本	女	74	2008/7/10	⑤死亡	症例報告	当該製品	2008/8/29提出、識別番号1-08000433　完了報告（5番と同一症例）
7	感染症および寄生虫症	B型肝炎	日本	女	75	2008/7/10	⑤死亡	症例報告	当該製品	2008/8/12提出、識別番号1-08000511　未完了報告
8	感染症および寄生虫症	細菌感染	日本	女	66	2008/7/7	①回復	症例報告	当該製品	2008/7/22提出、識別番号1-08000411　未完了報告
9	感染症および寄生虫症	ブドウ球菌性毒素ショック症候群	日本	女	64	2008/6/30	⑥不明	症例報告	当該製品	2008/7/11提出、識別番号1-08000403　未完了報告
10	感染症および寄生虫症	B型肝炎	日本	女	71	2008/6/25	②軽快	症例報告	当該製品	2008/7/28提出、識別番号1-08000430　未完了報告
11	感染症および寄生虫症	B型肝炎	日本	女	71	2008/6/25	②軽快	症例報告	当該製品	2008/8/13提出、識別番号1-08000430　未完了報告（10番と同一症例）
12	感染症および寄生虫症	C型肝炎	日本	男	58	2008/6/20	③未回復	症例報告	当該製品	2008/7/10提出、識別番号1-08000401　未完了報告
13	感染症および寄生虫症	細菌感染	日本	女	49	2008/6/19	③未回復	症例報告	当該製品	2008/7/3提出、識別番号1-08000389　未完了報告
14	感染症および寄生虫症	細菌感染	日本	女	49	2008/6/19	①回復	症例報告	当該製品	2008/8/13提出、識別番号1-08000389　完了報告（13番と同一症例）
15	感染症および寄生虫症	C型肝炎	日本	男	77	2008/6/18	⑥不明	症例報告	当該製品	2008/7/10提出、識別番号1-08000399　未完了報告
16	感染症および寄生虫症	B型肝炎	日本	女	56	2008/6/18	①回復	症例報告	当該製品	2008/7/22提出、識別番号1-08000413　未完了報告
17	感染症および寄生虫症	B型肝炎	日本	女	56	2008/6/18	①回復	症例報告	当該製品	2008/8/6提出、識別番号1-08000413　未完了報告（16番と同一症例）
18	感染症および寄生虫症	細菌感染	日本	男	62	2008/6/12	①回復	症例報告	当該製品	2008/6/27提出、識別番号1-08000381　未完了報告
19	感染症および寄生虫症	細菌感染	日本	男	62	2008/6/12	①回復	症例報告	当該製品	2008/7/22提出、識別番号1-08000381　完了報告（18番と同一症例）
20	感染症および寄生虫症	C型肝炎	日本	男	61	2008/6/4	⑥不明	症例報告	当該製品	2008/8/18提出、識別番号1-08000515　未完了報告
21	感染症および寄生虫症	B型肝炎	日本	男	56	2008/6/4	③未回復	症例報告	当該製品	2008/8/27提出、識別番号1-08000541　未完了報告
22	感染症および寄生虫症	B型肝炎	日本	女	52	2008/5/30	②軽快	症例報告	当該製品	2008/6/23提出、識別番号1-08000355　未完了報告
23	感染症および寄生虫症	B型肝炎	日本	女	52	2008/5/30	②軽快	症例報告	当該製品	2008/7/17提出、識別番号1-08000355　完了報告（22番と同一症例）
24	感染症および寄生虫症	B型肝炎	日本	女	63	2008/5/9	⑥不明	症例報告	当該製品	2008/5/28提出、識別番号1-08000172　未完了報告
25	感染症および寄生虫症	B型肝炎	日本	女	63	2008/5/9	⑥不明	症例報告	当該製品	2008/6/11提出、識別番号1-08000172　未完了報告（24番と同一症例）
26	感染症および寄生虫症	細菌感染	日本	男	86	2008/5/7	①回復	症例報告	当該製品	2008/5/20提出、識別番号1-08000124　未完了報告
27	感染症および寄生虫症	細菌感染	日本	男	86	2008/5/7	①回復	症例報告	当該製品	2008/6/18提出、識別番号1-08000124　完了報告（26番と同一症例）
28	感染症および寄生虫症	細菌感染	日本	男	75	2008/5/2	①回復	症例報告	当該製品	2008/5/20提出、識別番号1-08000123　未完了報告
29	感染症および寄生虫症	細菌感染	日本	男	75	2008/5/2	①回復	症例報告	当該製品	2008/6/18提出、識別番号1-08000123　完了報告（28番と同一症例）
30	感染症および寄生虫症	B型肝炎	日本	男	87	2008/5/1	③未回復	症例報告	当該製品	2008/6/11提出、識別番号1-08000222　未完了報告
31	感染症および寄生虫症	B型肝炎	日本	男	66	2008/4/17	③未回復	症例報告	当該製品	2008/5/2提出、識別番号1-08000081　未完了報告
32	感染症および寄生虫症	B型肝炎	日本	男	66	2008/4/17	③未回復	症例報告	当該製品	2008/6/2提出、識別番号1-08000081　未完了報告（31番と同一症例）
33	感染症および寄生虫症	細菌感染	日本	女	51	2008/4/17	⑥不明	症例報告	当該製品	2008/5/7提出、識別番号1-08000082　未完了報告
34	感染症および寄生虫症	細菌感染	日本	女	51	2008/4/17	①回復	症例報告	当該製品	2008/5/30提出、識別番号1-08000082　完了報告（33番と同一症例）

別紙様式第4

感染症発生症例一覧

35	感染症および寄生虫症	敗血症	日本	男	48	2008/3/25	②軽快	症例報告	当該製品	2008/4/15提出、識別番号1-08000028　未完了報告
36	感染症および寄生虫症	細菌感染	日本	男	55	2008/3/4	③未回復	症例報告	当該製品	2008/3/18提出、識別番号1-07000254　未完了報告
37	感染症および寄生虫症	細菌感染	日本	男	11	2008/2/19	③未回復	症例報告	当該製品	2008/3/6提出、識別番号1-07000246　未完了報告
38	感染症および寄生虫症	C型肝炎	日本	女	39	2008/2/19	⑥不明	症例報告	当該製品	2008/3/25提出、識別番号1-07000279　未完了報告
39	感染症および寄生虫症	C型肝炎	日本	女	39	2008/2/19	③未回復	症例報告	当該製品	2008/4/8提出、識別番号1-07000279　未完了報告（38番と同一症例）
40	感染症および寄生虫症	細菌感染	日本	女	63	2008/2/18	②軽快	症例報告	当該製品	2008/3/4提出、識別番号1-07000243　未完了報告
41	感染症および寄生虫症	C型肝炎	日本	女	28	2008/2/16	④回復したが後遺症あり	症例報告	当該製品	2008/3/28提出、識別番号1-07000290　未完了報告
42	感染症および寄生虫症	B型肝炎	日本	男	81	2008/2/4	⑤死亡	症例報告	当該製品	2008/3/24提出、識別番号1-07000231　未完了報告 第11回症例番号42は前回報告における第10回症例番号2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	C型肝炎	日本	女	85	2008/1/29	③未回復	症例報告	当該製品	2008/3/11提出、識別番号1-07000250　未完了報告
44	感染症および寄生虫症	C型肝炎	日本	女	72	2008/1/23	②軽快	症例報告	当該製品	2008/3/21提出、識別番号1-07000277　未完了報告
45	感染症および寄生虫症	B型肝炎	日本	男	74	2008/1/21	③未回復	症例報告	当該製品	2008/4/9提出、識別番号1-08000025　未完了報告
46	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	2008/6/9提出、識別番号1-07000202　未完了報告 第11回症例番号46は前回報告における第10回症例番号5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	女	53	2007/12/17	③未回復	症例報告	当該製品	2008/6/9提出、識別番号1-07000178　未完了報告 第11回症例番号47は前回報告における第10回症例番号8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	男	80	2007/11/30	⑤死亡	症例報告	当該製品	2008/6/9提出、識別番号1-07000231　未完了報告 （42番と同一症例） 第11回症例番号48は前回報告における第10回症例番号2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	60	2007/7/28	⑥不明	症例報告	当該製品	2008/6/27提出、識別番号1-08000380　未完了報告
50	感染症および寄生虫症	C型肝炎	日本	男	58	2007/5/31	⑥不明	症例報告	当該製品	2008/6/30提出、識別番号1-07000039　完了報告 第11回症例番号50は前々回報告における第9回症例番号11、12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	C型肝炎	日本	男	58	2007/5/31	⑥不明	症例報告	当該製品	2008/6/30提出、識別番号1-07000039　完了報告 （50番と同一症例） 第11回症例番号51は前々回報告における第9回症例番号11、12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	女	47	2007/4/24	⑥不明	症例報告	当該製品	2008/7/14提出、識別番号1-08000404　未完了報告
53	感染症および寄生虫症	C型肝炎	日本	男	70	2006/12/14	⑥不明	症例報告	当該製品	2008/5/19提出、識別番号1-06000209　完了報告 第11回症例番号53は前々々回報告における第8回症例番号8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	B型肝炎	日本	女	39	2006/11/29	①回復	症例報告	当該製品	2008/8/20提出、識別番号1-06000204　完了報告 第11回症例番号54は前々々回報告における第8回症例番号11において報告したものの追加報告

第11回

MedDRA/J Ver. 11.0J

別紙様式第4

感染症発生症例一覧

55	感染症および寄生虫症	B型肝炎	日本	男	65	2006/9/22	②軽快	症例報告	当該製品	2008/7/28提出、識別番号1-06000136　完了報告　第11回症例番号55は前々々回報告における第8回症例番号23、24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	女	43	2006/8/10	⑥不明	症例報告	当該製品	2008/6/30提出、識別番号1-06000110　完了報告　第11回症例番号56は前々々々回報告における第7回症例番号1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	B型肝炎	日本	男	53	2006/7/25	⑥不明	症例報告	当該製品	2008/7/28提出、識別番号1-06000101　完了報告　第11回症例番号57は前々々々回報告における第7回症例番号3において報告したものの追加報告
58	感染症および寄生虫症	細菌感染	日本	女	26	2006/7/20	①回復	症例報告	当該製品	2008/9/2提出、識別番号1-06000090　完了報告　第11回症例番号58は前々々々回報告における第7回症例番号4において報告したものの追加報告
59	感染症および寄生虫症	C型肝炎	日本	男	10	2006/6/27	⑥不明	症例報告	当該製品	2008/7/2提出、識別番号1-06000080　完了報告　第11回症例番号59は前々々々回報告における第7回症例番号5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	B型肝炎	日本	男	77	2006/6/20	②軽快	症例報告	当該製品	2008/7/7提出、識別番号1-06000072　完了報告　第11回症例番号60は前々々々回報告における第7回症例番号6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	敗血症	日本	女	63	2006/6/2	①回復	症例報告	当該製品	2008/8/18提出、識別番号1-06000052　完了報告　第11回症例番号61は前々々々回報告における第7回症例番号7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	細菌感染	日本	男	26	2006/5/29	①回復	症例報告	当該製品	2008/8/4提出、識別番号1-06000044　完了報告　第11回症例番号62は前々々々回報告における第7回症例番号10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	76	2006/5/22	⑥不明	症例報告	当該製品	2008/7/2提出、識別番号1-06000079　完了報告　第11回症例番号63は前々々々回報告における第7回症例番号11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	B型肝炎	日本	男	57	2006/5/19	②軽快	症例報告	当該製品	2008/8/29提出、識別番号1-06000087　完了報告　第11回症例番号64は前々々々回報告における第7回症例番号12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	男	46	2006/5/12	⑥不明	症例報告	当該製品	2008/4/22提出、識別番号1-06000041　完了報告　第11回症例番号65は前々々々回報告における第7回症例番号13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	男	77	2006/5/10	②軽快	症例報告	当該製品	2008/3/24提出、識別番号1-06000036　完了報告　第11回症例番号66は前々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
67	感染症および寄生虫症	B型肝炎	日本	女	80	2006/5/2	②軽快	症例報告	当該製品	2008/4/22提出、識別番号1-06000043　完了報告　第11回症例番号67は前々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
68	感染症および寄生虫症	C型肝炎	日本	男	21	2006/5/2	⑥不明	症例報告	当該製品	2008/6/20提出、識別番号1-06000045　完了報告　第11回症例番号68は前々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

別紙様式第4

感染症発生症例一覧

	69	感染症および寄生虫症	細菌感染	日本	女	77	2006/4/18	①回復	症例報告	当該製品	2008/7/14提出、識別番号1-06000018　完了報告　第11回症例番号69は前々々々回報告における第7回症例番号25において報告したものの追加報告
	70	感染症および寄生虫症	バシラス感染	日本	女	63	2006/4/8	②軽快	症例報告	当該製品	2008/6/26提出、識別番号1-06000013　完了報告　第11回症例番号70は前々々々回報告における第7回症例番号26において報告したものの追加報告
	71	感染症および寄生虫症	クレブシエラ感染	日本	女	17	2006/3/7	②軽快	症例報告	当該製品	2008/6/12提出、識別番号1-05000273　完了報告　第11回症例番号71は前々々々回報告における第7回症例番号32において報告したものの追加報告
	72	感染症および寄生虫症	エンドトキシンショック	日本	女	69	2006/3/3	①回復	症例報告	当該製品	2008/6/20提出、識別番号1-05000272　完了報告　第11回症例番号72は前々々々回報告における第7回症例番号33において報告したものの追加報告
	73	感染症および寄生虫症	敗血症性ショック	日本	男	59	2006/2/23	⑤死亡	症例報告	当該製品	2008/6/26提出、識別番号1-06000022　完了報告　第11回症例番号73は前々々々回報告における第7回症例番号35において報告したものの追加報告
	74	感染症および寄生虫症	C型肝炎	日本	男	78	2006/1/30	③未回復	症例報告	当該製品	2008/6/20提出、識別番号1-05000261　完了報告　第11回症例番号74は前々々々々回報告における第6回症例番号1、前々々々回報告における第7回症例番号37において報告したものの追加報告
	75	感染症および寄生虫症	B型肝炎	日本	男	34	2006/1/5	②軽快	症例報告	当該製品	2008/6/20提出、識別番号1-05000243　完了報告　第11回症例番号75は前々々々々回報告における第6回症例番号4において報告したものの追加報告
	76	感染症および寄生虫症	C型肝炎	日本	男	72	2005/12/29	⑥不明	症例報告	当該製品	2008/5/19提出、識別番号1-05000242　完了報告　第11回症例番号76は前々々々々回報告における第6回症例番号5において報告したものの追加報告
	77	感染症および寄生虫症	エンドトキシンショック	日本	男	70	2005/12/28	②軽快	症例報告	当該製品	2008/3/24提出、識別番号1-05000237　完了報告　第11回症例番号77は前々々々々回報告における第6回症例番号6において報告したものの追加報告
	78	感染症および寄生虫症	C型肝炎	日本	男	46	2005/9/17	③未回復	症例報告	当該製品	2008/7/22提出、識別番号1-06000073　完了報告　第11回症例番号78は前々々々回報告における第7回症例番号44において報告したものの追加報告
	79	感染症および寄生虫症	B型肝炎	日本	女	1	2005/7/11	⑥不明	症例報告	当該製品	2008/8/20提出、識別番号1-06000042　完了報告　第11回症例番号79は前々々々回報告における第7回症例番号45において報告したものの追加報告
	80	感染症および寄生虫症	B型肝炎	日本	女	82	2005/4/18	②軽快	症例報告	当該製品	2008/3/24提出、識別番号1-05000027　完了報告　第11回症例番号80は前々々々々々回報告における第5回症例番号23において報告したものの追加報告
第10回	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
	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
	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

MedDRA/J Ver. 11.0J

別紙様式第4

感染症発生症例一覧

第9回	11	感染症および寄生虫症	C型肝炎	日本	男	58	2007/5/31	③未回復	症例報告	当該製品	2007/6/15 提出、識別番号 1-07000039 未完了報告
	12	感染症および寄生虫症	C型肝炎	日本	男	58	2007/5/31	③未回復	症例報告	当該製品	2007/6/15 提出、識別番号 1-07000039 未完了報告
第8回	8	感染症および寄生虫症	C型肝炎	日本	男	70	2006/12/14	③未回復	症例報告	当該製品	2007/1/10 提出、識別番号 1-06000209 未完了報告
	11	感染症および寄生虫症	B型肝炎	日本	女	39	2006/11/29	③未回復	症例報告	当該製品	2007/1/5 提出、識別番号 1-06000204 未完了報告
	23	感染症および寄生虫症	B型肝炎	日本	男	66	2006/9/22	②軽快	症例報告	当該製品	2006/10/13 提出、識別番号 1-06000136 未完了報告
	24	感染症および寄生虫症	B型肝炎	日本	男	65	2006/9/22	⑥不明	症例報告	当該製品	2006/11/6 提出、識別番号 1-06000136 未完了報告
第7回	1	感染症および寄生虫症	B型肝炎	日本	女	43	2006/8/10	⑥不明	症例報告	当該製品	2006/8/28 提出、識別番号 1-06000110 未完了報告
	3	感染症および寄生虫症	B型肝炎	日本	男	53	2006/7/25	③未回復	症例報告	当該製品	2006/8/15 提出、識別番号 1-06000101 未完了報告
	4	感染症および寄生虫症	細菌感染	日本	女	26	2006/7/20	①回復	症例報告	当該製品	2006/8/8 提出、識別番号 1-06000090 未完了報告
	5	感染症および寄生虫症	C型肝炎	日本	男	10	2006/6/27	③未回復	症例報告	当該製品	2006/7/24 提出、識別番号 1-06000080 未完了報告
	6	感染症および寄生虫症	B型肝炎	日本	男	77	2006/6/20	③未回復	症例報告	当該製品	2006/7/18 提出、識別番号 1-06000072 未完了報告
	7	感染症および寄生虫症	敗血症	日本	女	63	2006/6/2	①回復	症例報告	当該製品	2006/6/20 提出、識別番号 1-06000052 未完了報告
	10	感染症および寄生虫症	細菌感染	日本	男	26	2006/5/29	③未回復	症例報告	当該製品	2006/6/13 提出、識別番号 1-06000044 未完了報告
	11	感染症および寄生虫症	B型肝炎	日本	男	76	2006/5/22	③未回復	症例報告	当該製品	2006/7/24 提出、識別番号 1-06000079 未完了報告
	12	感染症および寄生虫症	B型肝炎	日本	男	57	2006/5/19	②軽快	症例報告	当該製品	2006/8/2 提出、識別番号 1-06000087 未完了報告
	13	感染症および寄生虫症	B型肝炎	日本	男	46	2006/5/12	③未回復	症例報告	当該製品	2006/6/5 提出、識別番号 1-06000041 未完了報告
	14	感染症および寄生虫症	B型肝炎	日本	男	77	2006/5/10	③未回復	症例報告	当該製品	2006/6/1 提出、識別番号 1-06000036 未完了報告
	16	感染症および寄生虫症	B型肝炎	日本	女	80	2006/5/2	②軽快	症例報告	当該製品	2006/6/9 提出、識別番号 1-06000043 未完了報告
	17	感染症および寄生虫症	C型肝炎	日本	男	21	2006/5/2	③未回復	症例報告	当該製品	2006/6/13 提出、識別番号 1-06000045 未完了報告
	25	感染症および寄生虫症	細菌感染	日本	女	77	2006/4/18	②軽快	症例報告	当該製品	2006/5/8 提出、識別番号 1-06000018 未完了報告
	26	感染症および寄生虫症	桿菌感染	日本	女	63	2006/4/8	②軽快	症例報告	当該製品	2006/4/26 提出、識別番号 1-06000013 未完了報告
	32	感染症および寄生虫症	クレブシエラ感染	日本	女	17	2006/3/7	③未回復	症例報告	当該製品	2006/3/24 提出、識別番号 1-05000273 未完了報告
	33	感染症および寄生虫症	エンドトキシンショック	日本	女	69	2006/3/3	①回復	症例報告	当該製品	2006/3/20 提出、識別番号 1-05000272 未完了報告
	35	感染症および寄生虫症	敗血症性ショック	日本	男	59	2006/2/23	⑤死亡	症例報告	当該製品	2006/5/17 提出、識別番号 1-06000022 未完了報告
	37	感染症および寄生虫症	C型肝炎	日本	男	78	2006/1/30	③未回復	症例報告	当該製品	2006/3/13 提出、識別番号 1-05000261 未完了報告
	43	感染症および寄生虫症	C型肝炎	日本	男	46	2005/9/17	③未回復	症例報告	当該製品	2006/7/20 提出、識別番号 1-06000073 未完了報告
	44	感染症および寄生虫症	B型肝炎	日本	女	1	2005/7/11	③未回復	症例報告	当該製品	2006/6/9 提出、識別番号 1-06000042 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	78	2006/1/30	③未回復	症例報告	当該製品	2006/3/1 提出、識別番号 1-05000261 未完了報告
	4	感染症および寄生虫症	B型肝炎	日本	男	34	2006/1/5	②軽快	症例報告	当該製品	2006/2/2 提出、識別番号 1-05000243 未完了報告
	5	感染症および寄生虫症	C型肝炎	日本	男	72	2005/12/29	②軽快	症例報告	当該製品	2006/1/26 提出、識別番号 1-05000242 未完了報告
	6	感染症および寄生虫症	エンドトキシンショック	日本	男	70	2005/12/28	②軽快	症例報告	当該製品	2006/1/11 提出、識別番号 1-05000237 未完了報告
第5回	23	感染症および寄生虫症	B型肝炎	日本	女	82	2005/04/18	⑧不明	症例報告	当該製品	2005/05/09 提出、識別番号 1-05000027 未完了報告

90033	2008/10/23	80635	日本赤十字社	人血小板濃厚液		人血小板濃厚液

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 11 回	11-1	肝胆道系障害	肝機能異常	日本	男	63	2004/02/13	不明	自発報告	当該製品	08000292、2 回(完了) 平成 20 年 7 月 1 日 MedDRA ver.11.0
	11-1	肝胆道系障害	肝機能異常	日本	男	63	2004/02/13	不明	自発報告	当該製品	08000292、1 回(未完了) 平成 20 年 6 月 16 日 MedDRA ver.11.0
	10-1	感染症および寄生虫症	B 型肝炎	ドイツ	男	24	2008/01/10	不明	自発報告	外国製品	07000022、3 回(完了;同一症例をアンスロビン P 番号 10-1 で報告) 平成 20 年 3 月 31 日 MedDRA ver.10.1
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 14 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

	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								
	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
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

428

90039	2008/10/24	80641	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

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11回	11-1	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	2008/05/15、3-08000006
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11.0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)。

900451	2008/10/29	80668	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

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11回	11-1	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	2008/05/15、3-08000006
第6回	6-1	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4回	4-1	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
	4-2	感染症および寄生虫症	パルボウイルス感染	日本	不明	不明	不明	不明	その他	当該製品	2005/01/27、5-04000014
	4-3	感染症および寄生虫症	パルボウイルス感染	日本	不明	不明	不明	不明	その他	当該製品	2005/01/27、5-04000014
	4-4	感染症および寄生虫症	パルボウイルス感染	日本	不明	不明	不明	不明	その他	当該製品	2005/01/27、5-04000014
第3回	3-1	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
	2-1	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
第2回	2-1	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

MedDRAバージョン11.0により集計した。

第10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9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7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5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3回症例番号2-1は，第2回症例番号2-1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)。

90046	2008/10/29	80670	バイエル薬品	①加熱人血漿たん白 ②オクトコグ アルファ(遺伝子組換え)	加熱人血漿たん白

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11回	11-1	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	2008/05/15、3-08000006
第6回	6-1	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4回	4-1	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
第3回	3-1	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
	2-1	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
第2回	2-1	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

MedDRAバージョン11.0により集計した。

第10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9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7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5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3回症例番号2-1は，第2回症例番号2-1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)。

90047	2008/10/29	80671	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

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	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11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	07000029	2008年4月4日	10.1	2008年2月28日に報告したものの追加報告
第11回	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	07000032	2008年4月7日	11.0	2008年3月21日に報告したものの追加報告
第11回	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	07000252	2008年6月27日	10.1	2008年3月12日に報告したものの追加報告
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	07000029	2008年2月28日	10.1	
第10回	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	07000245	2008年3月5日	10.1	
第10回	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	07000245	2008年3月21日	10.1	2008年3月5日に報告したものの追加報告
第10回	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	07000252	2008年3月12日	10.1	
第10回	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	07000032	2008年3月21日	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	07000048	2007年6月29日	10.0	
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	07000048	2007年8月29日	10.0	2007年6月29日に報告したものの追加報告
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	07000048	2007年9月4日	10.0	2007年6月29日に報告したものの完了報告
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	外国製品	06000021	2006年8月1日	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	04000186	2004年12月16日	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	04000023	2004年4月27日	7.0	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
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	A03-51	2003/8/8（未完了報告）	6.1	

90051|2008/10/30|80680|バクスター|人血清アルブミン　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11回	11-1	感染症および寄生虫症	B型肝炎	日本	男	69	2002年11月5日	軽快	症例報告	当該製品	完了報告日：2008年6月19日 未完了報告日：2008年6月16日 識別番号：A-08000289
第6回	6-1	感染症および寄生虫症	C型肝炎	日本	男	78	2004年9月27日	死亡	症例報告	当該製品	取下げ報告日：2006年3月31日 未完了報告日：2006年3月28日 識別番号：A-05000275

90059	2008/11/19	80718	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

MedDRA/J Version11.1 使用

ATIII