資料2

感染症定期報告に関する今後の対応について

平成１６年度第５回
運営委員会確認事項
（平成１６年９月１７日）

１ 基本的な方針

運営委員会に報告する資料においては、

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

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

２ 具体的な方法

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

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

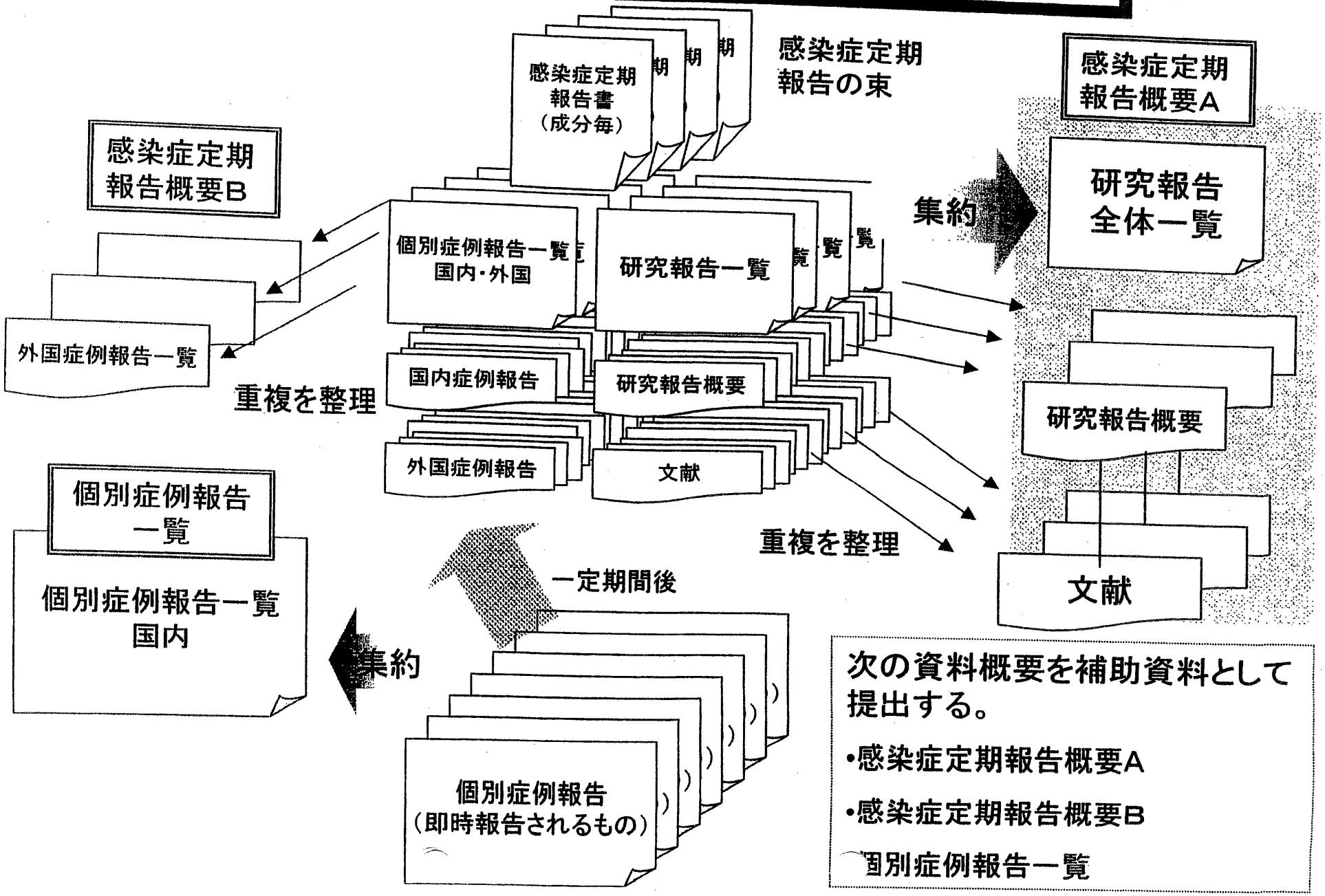
② 感染症発生症例報告のうち、発現国が「外国」の血漿分画製剤の使用による症例は、同一製品毎に報告期間を代表する感染症発生症例一覧（別紙様式第4）をまとめた**「資料概要B」**を事務局が作成し、送付する。

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

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

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

感染症定期報告・感染症個別症例報告の取り扱い



次の資料概要を補助資料として提出する。

- 感染症定期報告概要A
- 感染症定期報告概要B
- 個別症例報告一覧

感染症定期報告概要

（平成２０年１０月２９日）

平成１９年６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６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（2008/6/1～2008/8/31）

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
80103	2008/06/17	80246	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。	
80103	2008/06/17	80246	BSE	OIE／World animal health situation 2008年4月17日	2008年3月までに、英国から国際獣疫事務局(OIE)に報告されたBSE数である。1987年以前は英国全体で446頭であったが、1992年には37280頭となった。その後、減少し、2007年には67頭となった。2008年は3月31までに10頭報告されている。	
80101	2008/06/06	80226	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。	
80103	2008/06/17	80246	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。	
80103	2008/06/17	80246	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。	1
80103	2008/06/17	80246	C型肝炎	American Society for the Study of Liver Diseases 2007年11月2-6日	慢性HCV感染患者1930名(感染群)とHCV陰性患者1941名（対照群）とを比較し、リスク因子を検討した。静注薬物使用、1992年以前の輸血および2つ以上の入れ墨は感染群の方が対照群より有意に高かった。入れ墨はHCV感染リスク要因のない患者群においてもHCV感染と強く相関していた。	
80103	2008/06/17	80246	C型肝炎	J Med Virol 2008; 80: 261-267	2003年4～10月にイタリアの血液透析施設で患者4名にHCV抗体セロコンバージョンが認められた。この4名と以前からHCV抗体陽性であった10名のHCV RNAおよびHCV遺伝子型を検査し、系統遺伝学的解析をした結果、新規感染患者4名のHCVは遺伝子型2cで、2c型慢性感染患者1名から分離されたウイルスと近縁であった。感染制御手段の不備と装置による伝播が疑われた。	

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
80103	2008/06/17	80246	E型肝炎	J Med Virol 2008; 80: 283-288	英国サウスハンプシャーの単一施設において2005年6月から13ヶ月間にE型肝炎13例が発生した。これらの患者はルーチンのE型肝炎血清検査を導入開始後に特定された。同一期間中A型肝炎は2例、B型肝炎は4例であったことから、原因不明の急性肝疾患を発症し、関連する渡航歴のない患者全員にルーチンのE型肝炎検査を実施することが重要と考えられる。	
80101	2008/06/06	80226	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。	
80103	2008/06/17	80246	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。	2
80103	2008/06/17	80246	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。	
80103	2008/06/17	80246	HTLV	American Society of Hematology 2007年12月8-11日	1999年1月～2006年12月に長崎で献血を行った初回献血者の年齢別、出生年別および期間別HTLV-1血清陽性率の傾向分析を行った。血清陽性率は年齢が高くなるにつれ有意に増加した。また1987～1990年に生まれた献血者では1985～1986年に生まれた献血者と比較して有意に低かった。ウイルスキャリアの母親の授乳を避ける事を指導した県の対応が陽性率の低下に貢献していることが示された。	
80120	2008/07/29	80316	アメリカ・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。	
80103	2008/06/17	80246	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。	

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
80103	2008/06/17	80246	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。	
80133	2008/08/27	80378	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。	3
80113	2008/07/24	80288	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。	
80103	2008/06/17	80246	ウイルス感染	CDC/MMWR 2007; 56(45): 1181-1184	米国4州における2006-2007年のアデノウイルス血清型14(Ad14)に関連した急性呼吸器疾患に関する報告である。Ad14は稀にしか報告されないが、全ての年齢層の患者に重症で致死的な呼吸器疾患を起こす可能性がある。2006年5月にニューヨーク州で生後12日目の乳児がAd14感染により死亡し、07年3-6月にオレゴン州、ワシントン州およびテキサス州で計140名の感染患者が確認された。これらの患者から新規のAd14変異種が分離された。	
80101	2008/06/06	80226	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。	4
80113	2008/07/24	80288	ウイルス感染	J Med Virol 2008; 80: 365-371	定期的に輸血を受けるサラセミア患者で、Torque Teno virus (TTV)の有無を調べたところ、2-20歳の患者の約10%(118名中12名)がTTV陰性であった。フェリチン、ASTおよびALT値はTTV陽性群より陰性群の方が低かった。TTV-HCV共感染群ではフェリチンおよびALT値がTTV単独感染群より高かった。輸血による高頻度かつ継続的なTTV感染はサラセミア患者における肝機能障害と相関することが示唆された。	
80101	2008/06/06	80226	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。	5

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
80103	2008/06/17	80246	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Asuncion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。	
80103	2008/06/17	80246	ウイルス感染	Transfusion 2007; 47: 1972-1983	供血者血漿検体中のサイトメガロウイルス(CMV) DNA陽性率を検討した。過去にCMV血清陰性で初めて抗CMV IgG陽性を示した供血者82名の血漿検体44%が反復的にCMV DNA陽性であった。1年以上血清反応陽性または血清反応陰性供血者はいずれもCMV DNA陰性であった。白血球除去の実施にもかかわらず、新規血清反応陽性供血者のウイルス血症は輸血伝搬性CMVの残存リスクの重要な原因と考えられる。	
80120	2008/07/29	80316	ウイルス感染	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。	6
80110	2008/07/03	80278	ウイルス感染	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。	7
80113	2008/07/24	80288	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。	
80107	2008/06/24	80255	エボラ出血	CDC 2008年1月8日	CDCとウガンダ保健省は、2007年8月から始まったウガンダ西部に位置するBundibugyo地区におけるエボラ出血熱のアウトブレイクを報告した。2008年1月3日までに148人が罹患し、37人が死亡した。患者検体の遺伝子解析により、既知の4つのエボラウイルス株と異なる、新たなウイルス株である可能性が示唆された。確定には更なる研究が必要である。	
80103	2008/06/17	80246	エボラ出血	ProMED-mail20071130.3869	保健当局は、ウガンダ西部において16名が死亡し、他に50人が罹患したエボラウイルスは、新規の株であると2007年11月30日に発表した。最初の症例はコンゴ民主共和国と国境を接するBundibugyo地区において11月10日に報告された。この株では出血はあまり見られず、患者は高熱の後、死亡する。	
80105	2008/06/17	80248	クロイツフェルト・ヤコブ病	2007年プリオン研究会 Poster-20	日本の人口動態統計では、CJDによる死亡は過去20年以上に渡り増加傾向を示し、2005年は人口100万対1.23人であった。CJDサーベイランス委員会による調査では過去8年間に918例がプリオン病と判定された。病型別では、孤発性CJD 716例、遺伝性プリオン病128例、感染性(獲得性)CJD 72例(変異型CJD 1例、硬膜移植後CJD 71例)、および分類不能 2例であった。	

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
80103	2008/06/17	80246	チクングニヤウイルス感染	Lancet 2007; 370: 1840-1846	イタリア北東部の隣接する2つの村で原因不明の発熱性疾患患者が多数報告され、ヒトおよび蚊由来の検体を分析した結果、チクングンヤウイルス(CHIKV)が原因であることが明らかとなった。2007年7月4日から9月27日の間に205例のCHIKV感染症例を同定した。村の親戚を訪問した時に発症したインド出身男性が初発症例と推定された。系統遺伝学的分析により、イタリアのCHIKV株はインド洋諸島での初期のアウトブレイクで分離された株と高い相同性を示した。	
80107	2008/06/24	80255	チクングニヤウイルス感染	PLoS Pathogens 2007; 3: 1895-1906	2005～2006年にレユニオン諸島でアウトブレイクしたチクングニヤウイルス(CHIKV)感染は、エンベロープ蛋白遺伝子の変異株(E1-A226V) が関係していた。この変異の、ネッタイシマカおよびヒトスジシマカにおけるCHIKV適合性に対する影響を調べた。その結果、CHIKVのヒトスジシマカに対する感染性が有意に増加し、哺乳マウスへの伝播がより効率的になることが明らかとなった。通常のベクターであるネッタイシマカがいない同地域でCHIVKが大流行したのはこの変異が原因と考えられる。	
80113	2008/07/24	80288	バベシア症	Blood 2007; 110: 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。	8
80105	2008/06/17	80248	パルボウィルス	Transfusion 2007; 47: 1756-1764	米国の血液センター7施設において2000-2003年の期間に採取した5020名の供血者由来の保存血漿検体を高感度PCRスクリーニング法を用いてパルボウイルスB19 DNAについて検査した。B19 DNA陽性率は0.88%であった。DNA陽性検体の全てがIgG陽性で、23%がIgM陽性であった。IgM血清陽性率はDNA値と相関した。	
80120	2008/07/29	80316	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。	9
80105	2008/06/17	80248	パルボウィルス	Transfusion 2008; 48: 178-186	B19V IgG力価に関係したB19V中和の役割を検討するため、製造血漿プール1000以上について酵素免疫測定法による検査を実施した。血漿プールは平均33±9IU/mL(最小値11IU/mL)のB19V IgG力価を含有し、これらの11IU/mLのB19V IgGは、B19V遺伝子型1の感染性を4.6 log、遺伝子型2の感染性を3.9 log以上を中和した。このため、このようなプール由来の10%静注用免疫グロブリン製剤(IVIG)は、さらに高いB19V中和活性を含有することが明らかとなった。	
80102	2008/06/10	80231	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蒸気加熱処理装置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。	10
80101	2008/06/06	80226	パルボウィルス	Vox Sanguinis 2007; 93: 341-347	過去30～35年間に製造された第Ⅷ因子製剤中にヒトパルボウイルスが存在するかを調べた。175ロットのうち28ロットがPARV4シークエンスを含み、その内2ロットにジェノタイプ1型及び2型の両方が存在した。最大ウイルス量は10^5copies/mL以上であった。PARV4陽性の第Ⅷ因子製剤の大部分は1970年代及び1980年代に製造されていた。B19Vは175ロット中70ロットで陽性であった。	

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
80120	2008/07/29	80316	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。	11
80101	2008/06/06	80226	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。	
80103	2008/06/17	80246	ブルセラ症	J Travel Med 2007; 14: 343-345	64歳の日本人男性が6週間続く発熱で1998年6月2日に都内の病院に入院した。入院時の血液培養からグラム陰性桿菌が検出され、Brucella melitensis2型と同定された。患者は同年3月にイラクに滞在し、ヒツジのチーズを摂取したことが明らかとなった。患者の妻(60歳)が同年5月31日から発症し、Brucella melitensisが血液と関節液の培養で検出された。イラクの帰国者からその妻へ、ブルセラ症が性感染した可能性がある。	
80103	2008/06/17	80246	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。	
80118	2008/07/28	80298	ﾘﾝﾊﾟ性脈絡髄膜炎	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。	12
80101	2008/06/06	80226	ﾘﾝﾊﾟ性脈絡髄膜炎	N Engl J Med 2008; 358: 991-998	オーストラリアで一人のドナーから臓器移植を受けた3例が移植後4-6週後に死亡した。他のいかなる方法でも原因不明であったが、2例のレシピエントの移植肝および腎から得られたRNAを偏りのない迅速シークエンシングで解析することにより、ﾘﾝﾊﾟ性脈絡髄膜炎に関係する新規のアレナウイルスが原因であることが明らかとなった。レシピエントの腎、肝、血液および脳脊髄液からこのウイルスが検出され、また免疫組織学的および血清学的に確認された。この方法は病原体発見の強力な手段である。	
80107	2008/06/24	80255	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。	13

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
80105	2008/06/17	80248	異型クロイツフェルト・ヤコブ病	2007年プリオン研究会 Poster-38	BSE感染ウシ由来の脳乳剤を用いてPrPresのin vitro感染系の確立を試みた。感染させたヒト由来グリオーマ細胞株から抗プリオン抗体に反応する約30KのPK耐性のバンドが検出された。このバンドは非感染細胞には存在しなかった。また、9ヶ月継代した感染細胞の培養上清に伝達性があることが明らかとなった。さらに20nmのウイルス除去膜によって培養上清の伝達性が減少することが認められた。	
80103	2008/06/17	80246	異型クロイツフェルト・ヤコブ病	Arch Neurol 2007; 64: 1780-1784	運動失調や記憶障害などを呈し、発症後14ヶ月で死亡した患者(39歳女性)の剖検を行ったところ、白質の広汎な変性と皮質および白質におけるPrP沈着を示す非定型孤発性CJDであった。小脳組織由来のPrPScを分子分析した結果、vCJDでみられるPrPSc 4型と似た新規のPrPScであることが示された。典型的vCJDとはEDTA存在下でのプロテアーゼ開裂部位が異なった。この患者のPRNPコドン129はホモバリンであった。	
80102	2008/06/10	80231	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。	14
80101	2008/06/06	80226	異型クロイツフェルト・ヤコブ病	J Biol Chem 2007; 282: 35878-35886	トランスジェニックマウス(101LL)を用いた感染性実験の結果、TSE疾患の臨床症状と脳の空胞化という徴候を示すがPrPScのレベルが低いかもしくはイムノブロット法では検出されない動物の脳組織内に、高力価のTSE感染性が存在しうることが明らかとなった。この結果はPrPScのレベルと感染価との間の相関性に疑問を投げかけるものであり、プロテアーゼK抵抗性のPrPをほとんどもしくは全く含まない組織が感染性となりうること、および高力価のTSE感染性を有しうることを示すものである。	
80103	2008/06/17	80246	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。	
80102	2008/06/10	80231	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。	15
80103	2008/06/17	80246	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。	

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
80103	2008/06/17	80246	異型クロイツフェルト・ヤコブ病	ProMED-mail20080107.0087	英国National CJD Surveillance Unitに報告された2008年1月7日現在のCJD数は、vCJD診断確定死亡症例(確定例)114名、vCJD可能性死亡症例(神経病理学的確定診断がない)48名、vCJD可能性死亡症例(神経病理学的確定診断待ち)1名で、vCJD診断確定または可能性例の死亡総数163名であった。生存中のvCJD可能性症例数は3名であった。英国におけるvCJD流行は減少しつつあるという見解に一致する。	
80101	2008/06/06	80226	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。	
80101	2008/06/06	80226	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。	
80103	2008/06/17	80246	感染	ABC Newsletter 2008年1月11日	血液安全・安定供給諮問委員会は、米国保健社会福祉省事務局に対し、安全で効果的な輸血用血液製剤の病原体低減技術(不活化)の早急な開発を優先して進め、開発され次第実施するよう勧告した。病原体低減の効果と安全性を示すエビデンスの蓄積は、今後蔓延する可能性のある感染症に対し広く適応できるセーフガードとして、この技術の導入を保証するという決議を採択した。	
80103	2008/06/17	80246	感染	Transfusion 2007; 47: 2338-2347	2007年3月29-30日、カナダのトロントで行われた病原体不活化(PI)技術に関するコンセンサス会議の報告である。近年の検査技術の発達により、現状の輸血感染症リスクは非常に低く、PIを直ちに導入する事は推奨しない。しかし新興感染症のリスクは未知数であり、PIは予防手段として重要である。広範囲の病原体を不活化できる安全な方法が確立されれば実施すべきである。	
80103	2008/06/17	80246	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。	16
80103	2008/06/17	80246	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。	17

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
80103	2008/06/17	80246	感染	Vox Sanguinis 2007; 93(Suppl.2): 31	日本赤十字社(JRC)が全国的ヘモビジランス体制を導入してから14年が経過した。報告された輸血副作用症例数は年間約2000例で、過去3年間はほぼ一定である。非溶血性輸血副作用は報告症例の約80%を占め、輸血関連急性肺障害などが含まれる。輸血感染症の報告数は年々減少している。JRCのヘモビジランスは病院の自発報告に基づいており、病院と血液センターとの協力が不可欠である。	
80120	2008/07/29	80316	寄生虫感染	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。	18
80103	2008/06/17	80246	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。	
80103	2008/06/17	80246	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。	19
80107	2008/06/24	80255	鳥インフルエンザ	China View, www.chinaview.cn 2008-01-10	2007年12月に江蘇省南京で発生した52歳男性の鳥インフルエンザ感染患者は、患者であった息子との濃厚な接触により感染したものであり、ウイルスの変異は認められていない。しかし、息子と父親はいずれも死亡した家禽との接触がないため、息子の感染源は明らかになっていない。息子は11月24日に発症し、12月2日に死亡し、父親は12月3日に発症したが回復した。ヒト用トリインフルエンザワクチンは臨床試験Phase IIの段階にある。	
80103	2008/06/17	80246	鳥インフルエンザ	WHO/CSR 2007年12月9日	中国におけるトリインフルエンザの状況(update5):2007年12月9日、中国衛生省は同ウイルスの新規ヒト感染症例を報告した。Jiangsu省の52才の男性で、12月2日に同ウイルス感染で死亡した24才男性の父親で、現在入院中である。中国での確定例は27例で、うち17例が死亡している。	
80103	2008/06/17	80246	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。	20

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. 4. 15	該当なし	

一般的名称	人赤血球濃厚液	研究報告の公表状況	平力造, 伊藤綾香, 沼本高志, 五井薫, 後藤直子, 百瀬俊也, 日野学. 第56回日本輸血・細胞治療学会総会; 2008 Apr 25-27; 福岡.	公表国
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)			日本

研究報告の概要

○2007年輸血関連感染症報告症例の解析

【はじめに】日本赤十字社では、薬事法に基づき収集した副作用・感染症症例を独立行政法人医薬品医療機器総合機構へ報告している。2007年に全国の医療機関から報告された輸血関連感染症例(疑い例を含む)の現状とその解析結果について報告する。

【対象と方法】2007年に医療機関から報告された症例を対象とし、ウイルス感染(疑)症例は当該献血者の保管検体の個別NAT等により、細菌感染(疑)症例は当該製剤(使用済みバッグ)又は同一製造番号の凍結血漿の無菌試験等により調査を行い評価した。

【結果と考察】2007年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(2005年229例<年間265例>、2006年162例<年間191例>)に比べ減少傾向にある。その内訳は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。HBVの10例は献血者と患者のHBV塩基配列の比較により因果関係が高いと評価した。残る2例は、患者のセロコンバージョンによりウイルス遺伝子を確認できなかった症例と、献血者と患者のウイルス遺伝子型が異なっていた症例であった。HCVの1例は医療機関からの自発報告より判明した症例で、献血者と患者のHCVは遺伝子型III(2a)で塩基配列の比較により因果関係が高いと評価した。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。細菌感染(疑)例で医療機関での患者血培実施例は24例中22例(91.7%)で、陽性は14例(63.6%)であった。日赤における調査結果は全て適合(陰性)であり、患者血培陽性14例中9例(64.3%)は使用済みバッグにて調査した症例であった。輸血後感染症は種々の安全対策により減少傾向にあるが、残存リスクを考慮しつつ、ヘモビジランスの一環として輸血関連感染症の動向を今後も注視し、解析結果をフィードバックすることにより輸血用血液の安全性向上に資することとしたい。

報告企業の意見	今後の対応
2007年に全国の医療機関から報告された輸血関連感染症例の現状とその解析結果についての報告である。	日本赤十字社では、HBV、HCV、HIV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、「血液製剤等に係る遡及調査ガイドライン」(平成17年3月10日付薬食発第0310009号)に基づき、輸血感染症の調査を行っている。輸血感染症に関する新たな知見等について今後も情報の収集に努める。検査精度向上のため、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の導入を順次進めている。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

P-033　2007年輸血関連感染症報告症例の解析

日本赤十字社血液事業本部
平　力造，伊藤綾香，沼本高志，五井　薫，後藤直子，百瀬俊也，日野　学
TEL：03-5534-7503（5406）FAX：03-5534-3774　E-mail：taira@bs.jrc.or.jp

【はじめに】日本赤十字社では，薬事法に基づき収集した副作用・感染症症例を独立行政法人医薬品医療機器総合機構へ報告している．2007年に全国の医療機関から報告された輸血関連感染症例(疑い例を含む)の現状とその解析結果について報告する．【対象と方法】2007年に医療機関から報告された症例を対象とし，ウイルス感染（疑）症例は当該献血者の保管検体の個別NAT等により，細菌感染（疑）症例は当該製剤（使用済みバッグ）又は同一製造番号の凍結血漿の無菌試験等により調査を行い評価した．【結果と考察】2007年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（2005年229例<年間265例>，2006年162例<年間191例>）に比べ減少傾向にある．その内訳はHBVが61例，HCV 32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．HBVの10例は献血者と患者のHBウイルス塩基配列の比較により因果関係が高いと評価した．残る2例は，患者のセロコンバージョンによりウイルス遺伝子を確認できなかった症例と，献血者と患者のウイルス遺伝子型が異なっていた症例であった．HCVの1例は自発報告より判明した症例で，献血者と患者のHCウイルスは遺伝子型III (2a) で塩基配列の比較により因果関係が高いと評価した．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．細菌感染（疑）例で医療機関での患者血培実施例は24例中22例（91.7%）で，陽性は14例（63.6%）であった．日赤における調査結果は全て適合（陰性）であり，患者血培陽性14例中9例(64.3%)は使用済みバッグにて調査した症例であった．輸血後感染症は種々の安全対策により減少傾向にあるが，残存リスクを考慮しつつ，ヘモビジランスの一環として輸血関連感染症の動向を今後も注視し，解析結果をフィードバックすることにより輸血用血液の安全性向上に資することとしたい．

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. 4. 15	該当なし	

一般的名称	人赤血球濃厚液	研究報告の公表状況	松林圭二, 坂田秀勝, 今絵未, 武田尋美, 阿部生馬, 佐藤進一郎, 加藤俊明, 池田久實. 第56回日本輸血・細胞治療学会総会; 2008 Apr 25-27; 福岡.	公表国
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)			日本

研究報告の概要

○HEV NAT陽性献血者におけるHEV感染のNatural course

【目的】E型肝炎の多くは無症候性で経過するといわれている。しかし、感染初期のウイルス動態や不顕性感染症例の自然経過についてはほとんど知られていない。今回HEV NAT陽性献血者を追跡調査することによりHEV感染のNatural courseについて新しい知見が得られたので報告する。

【方法】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(過去6ヵ月間)を行い、喫食歴や自覚症状の有無等のアンケート調査、HEV抗体測定(Viragent HEV Ab IgM、IgG)、HEV-RNA定量、生化学検査、分子系統樹解析等を行なった。

【成績】HEV NAT陽性者のほぼ全員がHEV感染の自覚症状を認めなかった。男性:女性=2:1で、平均年齢は41.2歳であり、GenotypeはG3がG4の16倍と圧倒的に多かった。また、アンケートに回答した陽性者の7割に過去2ヵ月以内の動物内臓肉の喫食歴が認められた。献血時、7割の陽性者がIgM、IgG抗体とも陰性のウインドウ期の献血であり、献血時点から過去6ヵ月以内の献血からはHEV RNAおよび抗体は検出されなかった。一方、追跡調査できたすべてのHEV RNA陽性者においてIgG抗体の陽転が認められたが、その一部については陽性判明時から1年以上経過すると陽性基準を下回る例も認められた。詳細に追跡できた陽性献血者19例のうち9例に軽度のALT上昇が見られた。HEV RNA陽性者のHEV血症状態は献血後最長55日間持続し、HEV(G3)の推定倍加時間は50±12.4時間でHBVとほぼ同等であった。

【結論】HEV NAT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あった。感染後、HEVはHBVと同様の倍加速度で緩やかに増殖し、ウイルス血症状態は比較的長期間(約8週間)持続する例もあるが、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。

報告企業の意見	今後の対応
北海道のHEV NAT陽性献血者を追跡調査したところ、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あり、典型的な無症候性一過性感染の経過をたどったとの報告である。	日本赤十字社では、厚生労働科学研究「E型肝炎の感染経路・宿主域・遺伝的多様性・感染防止・診断・治療に関する研究班」と共同して、献血者におけるHEV感染の疫学調査を行っている。また、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。加えて、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。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

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

(N)

O-026　HEV NAT 陽性献血者における HEV 感染の Natural course

北海道赤十字血液センター検査部[1]，日本赤十字社血漿分画センター品質管理部検査課[2]
松林圭二[1]，坂田秀勝[1]，今　絵未[1]，武田尋美[1]，阿部生馬[2]，佐藤進一郎[1]，加藤俊明[1]，池田久實[1]
TEL：011-613-6634　FAX：011-613-6221　E-mail：kmatsu@hokkaido.bc.jrc.or.jp

【目的】E 型肝炎の多くは無症候性で経過するといわれている．しかし，感染初期のウイルス動態や不顕性感染症例の自然経過についてはほとんど知られていない．今回 HEV NAT 陽性献血者を追跡調査することにより HEV 感染の Natural course について新しい知見が得られたので報告する．
【方法】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（過去 6 ヵ月間）を行い，喫食歴や自覚症状の有無等のアンケート調査，HEV 抗体測定（Viragent HEV Ab IgM，IgG），HEV-RNA 定量，生化学検査，分子系統樹解析等を行なった．
【成績】HEV NAT 陽性者のほぼ全員が HEV 感染の自覚症状を認めなかった．男性：女性＝2：1 で，平均年齢は 41.2 歳であり，Genotype は G3 が G4 の 16 倍と圧倒的に多かった．また，アンケートに回答した陽性者の 7 割に過去 2 ヶ月以内の動物内臓肉の喫食歴が認められた．献血時，7 割の陽性者が IgM，IgG 抗体とも陰性のウインドウ期の献血であり，献血時点から過去 6 ヵ月以内の献血からは HEV RNA および抗体は検出されなかった．一方，追跡調査できたすべての HEV RNA 陽性者において IgG 抗体の陽転が認められたが，その一部については陽性判明時から 1 年以上経過すると陽性基準を下回る例も認められた．詳細に追跡できた陽性献血者 19 例のうち 9 例に軽度の ALT 上昇が見られた．HEV RNA 陽性者の HEV 血症状態は献血後最長 55 日間持続し，HEV（G3）の推定倍加時間は 50±12.4 時間で HBV とほぼ同等であった．
【結論】HEV NAT 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あった．感染後，HEV は HBV と同様の倍加速度で緩やかに増殖し，ウイルス血症状態は比較的長期間（約 8 週間）持続する例もあるが，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．

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6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	人C1-インアクチベータ		研究報告の公表状況	Contemporary North American influenza H7 viruses possess human receptor specificity: Implications for virus transmissibility Proceedings of the National Academy of Sciences USA (PNAS) May 27, 2008; 105 (21) 7558-7563	公表国 米国	
販売名（企業名）	ベリナートP（CSLベーリング株式会社）					

研究報告の概要 19

問題点（北米のH7型インフルエンザウイルスはヒトの受容体に特異性があり、ウイルス伝播に影響）

ユーラシアや北米でH7型トリインフルエンザウイルス感染が2002年から発生し、オランダ、カナダのブリティッシュコロンビア州、英国で人での感染が確認された。H7型トリインフルエンザのヒト感染はほとんどが結膜炎で、ヒトからヒトへの感染は稀であった。

H7N7型は2003年にオランダで80人以上が感染し、1人が死亡し、H7N3型は2004年にブリティッシュコロンビア州で2例の結膜炎、H7N2型は2007年に英国で多数のインフルエンザ様症状や結膜炎、2003年にニューヨークで1例の気道感染が発生した。

著者らはglycan microarray法でH7型のトリインフルエンザウイルスの受容体結合する構造を調べ、またフェレットを用いて感染性を調べた。その結果、2003年にオランダで発生した高病原性H7N7型は、α2-3結合シアル酸に対する古典的な結合選択性は維持しており、高病原性H5N1型と同様にフェレットでの感染は認められなかった。

しかし2004年にカナダで分離されたH7N3型と2002-2003年に米国で分離されたH7N2型は、ヒト気管上皮細胞に傑出して見られる結合型のα2-6結合シアル酸に対する親和性を高めたHAを保有している。

著者らは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能率的に増殖していて、直接接触で感染する能力があることを確認した。

以上のことから、H7型トリインフルエンザウイルスは、1918年（H1N1）、1957年(H2N2)、1968年(H3N2)に世界的に大流行したヒトインフルエンザウイルスのように、ヒトの間で感染する可能性があることを確認した。

使用上の注意記載状況・その他参考事項等

報告企業の意見	今後の対応
トリインフルエンザウイルスを用いたバリデーションテストで、ウイルスが60℃10時間の液状加熱で不活化された報告がある。 万一原料血漿に混入しても本剤の製造工程の60℃10時間の液状加熱で不活化されると考えられる。	今後とも新しい感染症に関する情報収集に努める所存である。

(3)

Contemporary North American influenza H7 viruses possess human receptor specificity: Implications for virus transmissibility

Jessica A. Belser*†, Ola Blixt‡, Li-Mei Chen*, Claudia Pappas*, Taronna R. Maines*, Neal Van Hoeven*, Ruben Donis*, Julia Busch‡, Ryan McBride‡, James C. Paulson‡, Jacqueline M. Katz*, and Terrence M. Tumpey*§

*Influenza Division, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30333; †Emory University, Atlanta, GA 30322; and ‡Departments of Physiological Chemistry and Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037

Edited by Peter Palese, Mount Sinai School of Medicine, New York, NY, and approved March 21, 2008 (received for review February 7, 2008)

Avian H7 influenza viruses from both the Eurasian and North American lineage have caused outbreaks in poultry since 2002, with confirmed human infection occurring during outbreaks in The Netherlands, British Columbia, and the United Kingdom. The majority of H7 infections have resulted in self-limiting conjunctivitis, whereas probable human-to-human transmission has been rare. Here, we used glycan microarray technology to determine the receptor-binding preference of Eurasian and North American lineage H7 influenza viruses and their transmissibility in the ferret model. We found that highly pathogenic H7N7 viruses from The Netherlands in 2003 maintained the classic avian-binding preference for α2–3-linked sialic acids (SA) and are not readily transmissible in ferrets, as observed previously for highly pathogenic H5N1 viruses. However, H7N3 viruses isolated from Canada in 2004 and H7N2 viruses from the northeastern United States isolated in 2002–2003 possessed an HA with increased affinity toward α2–6-linked SA, the linkage type found prominently on human tracheal epithelial cells. We identified a low pathogenic H7N2 virus isolated from a man in New York in 2003, A/NY/107/03, which replicated efficiently in the upper respiratory tract of ferrets and was capable of transmission in this species by direct contact. These results indicate that H7 influenza viruses from the North American lineage have acquired sialic acid-binding properties that more closely resemble those of human influenza viruses and have the potential to spread to naïve animals.

hemagglutinin | transmission receptor binding animal model

Avian influenza viruses within the H5 and H7 subtype continue to pose a major public health threat. Since 2004, highly pathogenic avian influenza (HPAI) H5N1 viruses have resulted in >380 cases of laboratory-confirmed human infection in 14 countries (1). Despite the high virulence of H5N1 viruses observed in humans and mammalian models (2), human-to-human transmission has been only rarely documented (3–5). Additionally, influenza H7 subtype viruses within both Eurasian and North American lineages have been responsible for multiple outbreaks and human infections since 2002. These include outbreaks of HPAI H7N7 in The Netherlands in 2003 that resulted in >80 cases of human infection and one fatality; HPAI H7N3 in British Columbia, Canada, in 2004 that resulted in two cases of conjunctivitis; a cluster of human infections of low pathogenic avian influenza (LPAI) H7N2 in the United Kingdom in 2007 that resulted in multiple cases of influenza-like illness and conjunctivitis; and a single case of human respiratory infection in New York in 2003 (6–11). The majority of human infections with H7 influenza viruses have resulted in conjunctivitis, but similar to H5N1 viruses, probable human-to-human transmission among family contacts has been rarely documented through molecular diagnosis (7). Representative viruses isolated from these outbreaks were found to replicate efficiently in the mouse and ferret models, and one virus isolated from a fatal respiratory case during the H7N7 Netherlands outbreak, A/NL/219/03, was highly lethal in both mammalian models (12, 13). However, further study is needed to assess the pandemic potential of H7 influenza viruses within this subtype.

Influenza virus attachment to host cells is mediated by the virus HA binding to sialic acid (SA) glycans present on host cell surfaces. Avian influenza viruses predominantly bind α2–3-linked SA, whereas human influenza viruses preferentially bind to α2–6 SA (14). The three influenza pandemic viruses of the last century, causing the pandemics of 1918 (H1N1), 1957 (H2N2), and 1968 (H3N2), each possessed an HA with a human α2–6 SA-binding preference yet are thought to have originated from an avian virus possessing the α2–3 SA-binding preference (15, 16). With few exceptions, avian H5N1 influenza viruses isolated from humans have maintained the classic α2–3 SA binding (17–20). However, the SA-binding preference of recent H7 influenza viruses associated with disease in humans has not been well studied.

The ferret model has been used successfully to study the transmission of human and avian influenza viruses (21–23), because ferrets exhibit a similar distribution of SA as reported in humans with a higher proportion of α2–6 SA glycans on upper respiratory tract epithelial cells and α2–3 SA in the lower respiratory tract (24–27). These studies have shown that avian H5N1 viruses, despite replicating to high titers in the respiratory tract, are not readily transmissible by either respiratory droplet or contact transmission (21). To date, the transmissibility of viruses within the H7 subtype has not been examined experimentally. Here, we use glycan microarray technology to determine the receptor-binding preference of H7 influenza viruses of both Eurasian and North American lineages and assess the transmissibility of selected H7 influenza viruses using the ferret model. Surprisingly, we found that recently isolated H7N2 and H7N3 viruses of the North American lineage possess increased binding to α2–6 SA, with several strains exhibiting preferential binding characteristic of human influenza viruses. One of these was an H7N2 virus, A/NY/107/03, associated with respiratory disease in an adult male, which we found to be capable of efficient direct contact transmission in the ferret model.

Results

Receptor-Binding Preference of Eurasian H7 Influenza Viruses. Previous studies have elucidated the molecular basis for the receptor-binding preference of influenza viruses of multiple subtypes, in-

Author contributions: J.A.B., T.R.M., J.C.P., J.M.K., and T.M.T. designed research; J.A.B., O.B., C.P., T.R.M., N.V.H., J.B., and R.M. performed research; L.-M.C., R.D., and J.C.P. contributed new reagents/analytic tools; J.A.B., O.B., T.R.M., J.C.P., J.M.K., and T.M.T. analyzed data; and J.A.B., J.C.P., J.M.K., and T.M.T. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

§To whom correspondence should be addressed. E-mail: tft9@cdc.gov.

This article contains supporting information online at www.pnas.org/cgi/content/full/0801259105/DCSupplemental.

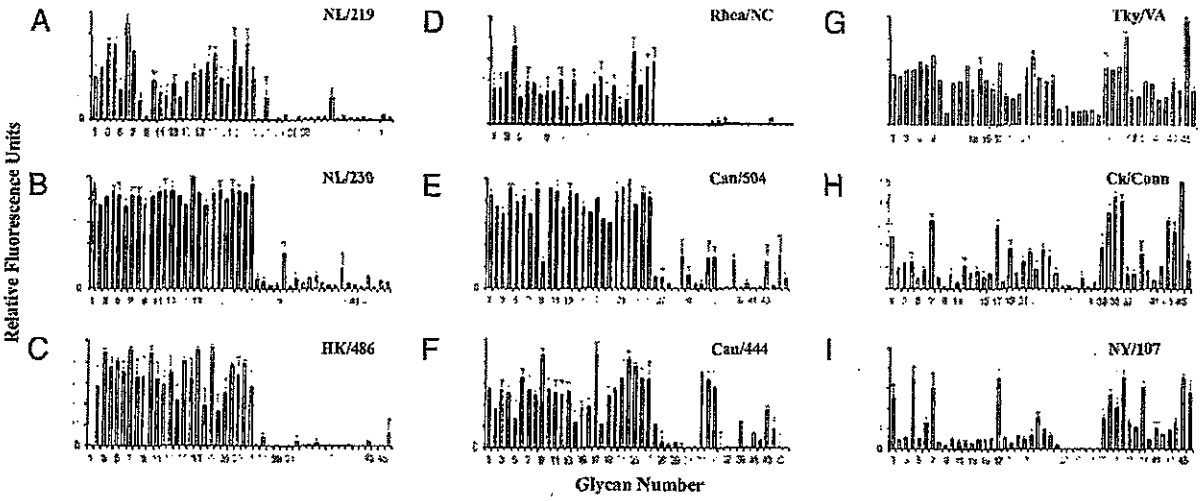


Fig. 1. Glycan microarray analysis of Eurasian and North American lineage H7 influenza viruses. Analysis was performed on the following viruses: NL/219 (*A*), NL/230 (*B*), HK/486 (H5N1) (*C*), Rhea/NC (*D*), Can/504 (*E*), Can/444 (*F*), Tky/VA (*G*), Ck/Conn (*H*), and NY/107 (*I*). The glycan microarray was performed by using whole virus with antisera raised against homologous or cross-reactive virus as a primary antibody. Colored bars highlight glycans that contain α2–3 SA (yellow) and α2–6 SA (green). Error bars reflect the standard deviation in the signal for six independent replicates on the array. Structures of each of the numbered glycans are found in Table S1 (*SI Text*) and for selected glycans in Table 2.

cluding H1, H2, H3, H5, and H9 viruses (15, 16, 28–30). However, recently isolated H7 influenza viruses have not been comprehensively analyzed for their HA-binding preference. We used a glycan microarray with whole virus to determine the α2–3 and α2–6 SA-binding preference of Eurasian or North American lineage H7 influenza viruses associated with disease in humans or related viruses isolated from birds. Two HPAI H7N7 Eurasian lineage viruses isolated from an outbreak in The Netherlands in 2003 were tested, A/NL/219/03 (NL/219) and A/NL/230/03 (NL/230). NL/219 was isolated from a human with fatal respiratory disease, whereas NL/230 was isolated from an individual with conjunctivitis (6). Both H7N7 viruses exhibited preferential binding specificity toward α2–3 SA (Fig. 1 *A* and *B*). This pattern of binding closely resembles the strong α2–3 SA-binding preference observed with HPAI H5N1 viruses isolated from humans, as has been reported and is demonstrated here with A/HK/486/97 (HK/486) virus (Fig. 1*C*) (29). These results were confirmed by hemagglutination assay, with NL/219, NL/230, and HK/486 viruses binding to turkey red blood cells (RBCs) resialylated with α2–3- but not α2–6-linked sialosides (Table 1). These findings suggest that HPAI H7N7 Eurasian lineage viruses, similar to HPAI H5N1 viruses, have maintained classic avian specificity for α2–3 SA despite causing productive infections in humans.

Table 1. Hemagglutination assay of H7 influenza viruses using differentially sialylated turkey RBCs

	Presence or absence of hemagglutination			
Virus	TRBC	α2–6 RBC	α2–3 RBC	desial RBC
NL/219	+	−	+	−
NL/230	+	−	+	−
Rhea/NC	+	−	+	−
Can/504	+	+	+	−
Can/444	+	+	+	−
NY/107	+	+	+	−
Tky/VA	+	+	+	−
Ck/Conn	+	+	+	−
HK/486	+	−	+	−
Tx/91	+	+	−	−
PBS	−	−	−	−

Receptor-Binding Preference of North American H7 Influenza Viruses. H7N2 subtype viruses have been routinely isolated from the live-bird market system in the northeastern United States since 1994 (31). Glycan-binding analysis of A/Rhea/NC/39482/93 (Rhea/NC), a LPAI H7N1 virus isolated in 1993, exhibited a classic avian α2–3 SA receptor-binding preference (Fig. 1*D*). However, the more recent H7N2 viruses A/Tky/VA/4529/02 (Tky/VA), which caused a major outbreak among commercial poultry in Virginia and was associated with serologic evidence of human infection (32), and a 2003 H7N2 poultry isolate A/Ck/Conn/260413-2/03 (Ck/Conn), exhibited significantly increased binding to glycans with α2–6 SA (Fig. 1 *G* and *H*). A genetically related H7N2 virus isolated from a single case of human respiratory infection in 2003, A/NY/107/03 (NY/107), also exhibited a marked increase in α2–6 SA binding and reduced binding to glycans with α2–3 SA (Fig. 1*I*). Two H7N3 viruses (A/Canada/504/04 and A/Canada/444/04), associated with human conjunctivitis during an outbreak of HPAI in British Columbia (Fig. 1 *E* and *F*), also revealed increased binding to α2–6 SA compared with Eurasian lineage viruses (Fig. 1 *A–C*). An assay using resialylated erythrocytes independently documented the dual α2–6 and α2–3 SA binding of all H7N3 and H7N2 viruses (Table 1).

More detailed analysis of glycan microarray data revealed that the specificity differences among the H7 viruses was more striking for subclasses of α2–6 and α2–3 glycans as summarized in Table 2. Although neither of the Eurasian viruses nor the Rhea/NC virus bound glycans with α2–6 SA, all of the post-2002 North American viruses exhibited moderate to strong binding to the α2–6 SA of the biantennary N-linked glycans (nos. 34 and 35). Three viruses, Tky/VA, Ck/Conn, and NY/107, exhibited moderate to strong binding to most glycans with α2–6 SA, including a glycan with an internal sialic acid (no. 45) not recognized by the other viruses. Although these three viruses were similar in binding α2–6 SA, they exhibited significant differences in their binding of glycans with α2–3 SA. Tky/VA bound as well to glycans with α2–3 SA as the Eurasian viruses. In contrast, Ck/Conn and NY/107 exhibited strong binding to only 4 of the 32 glycans with α2–3 SA, including two sulfated (nos. 1 and 4), one branched (no. 7), and one linear (no. 17) glycan (Fig. 2 and Table 2). Binding to the remaining glycans with α2–3 SA was significantly reduced, especially for NY/107. The reduced binding to glycans with α2–3 SA is notable, because this was a characteristic of influenza viruses with H1, H2, and H3 HAs when first introduced into the human population (15, 16, 28, 33).

Table 2. Comparison of the detailed sialoside receptor specificity of H7 influenza viruses

	α 2–3 sialosides						α 2–6 sialosides		
	Sulfated		Branched	Linear		Fucosylated	Branched	Linear	Internal
Virus	1,4	3,5	7	17	10–12,15,16, 18,19	20–25	34,35	40–44	45
NL/219	+++	+++	+++	+++	+++	+++	–	–	–
NL/230	+++	+++	+++	–++	+++	–++	+/–	+/–	+/–
Rhea/NC	+++	+++	+++	+++	+++	–++	–	–	–
Can/504	+++	+++	+++	+++	+++	+++	++	+	–
Can/444	+++	+++	+++	+ +	+++	++	++	+	
Tky/VA	+++	+++	+++	+++	+++	+	+++	+++	+++
Ck/Conn	++	+	++	++	+	++	–++	++	+++
NY/107	+++	+	+++	+–+	+	+	++	+	+++

Structures shown in symbol form (see key below) are structures of single glycans or composite structures for chemically related glycans represented by the numbers underneath, which correspond to the numbers in the complete structure list (Table S1) and the glycan microarray data in Fig. 2. Error bars reflect the standard deviation in the signal for six independent replicates on the array. The relative binding of the virus to each glycan subclass is qualitatively estimated based on relative strength of the signal for the data shown in Figs. 1 and 2. Strong (+++), moderate (++), weak (+) detectable (+/–), absent (–). ◆, NeuAc; ○, Gal; □, GalNAc; ●, Glc; ■, GlcNAc; ○, Man; △, Fuc

Transmissibility of Eurasian H7 Influenza Viruses in Ferrets. To assess the impact of enhanced α2–6 SA specificity on the transmissibility of the North American H7 viruses, both respiratory droplet and contact transmission experiments were performed as described (21) by using the ferret transmission model. Six ferrets were inoculated intranasally with 10^7 50% egg infectious doses (EID_{50}), a dose reported to consistently infect ferrets with human or avian influenza viruses (34). Twenty-four hours postinoculation (p.i.), three of the inoculated ferrets were placed in modified cages with a perforated side wall adjacent to a naïve ferret, allowing air exchange between ferrets while preventing direct contact of animals or indirect contact with food or bedding (respiratory droplet transmission). The remaining three inoculated ferrets were each co-housed with a naïve ferret to assess direct contact transmission. Criteria for efficient transmission included detection of virus in nasal washes (NW) of contact ferrets and seroconversion of convalescent sera from contact ferrets, both of which occur during the efficient transmission of human influenza H3N2 viruses as shown in this model system (21).

NL/219 virus has been shown to be highly virulent in the ferret

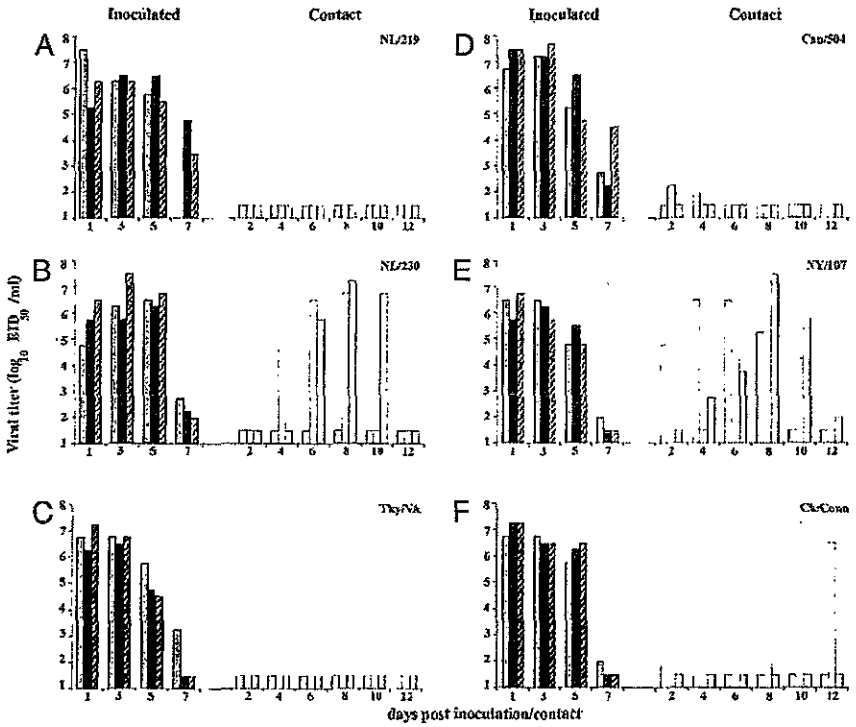


Fig. 2. Direct contact transmissibility of H7 influenza viruses. Three ferrets were inoculated with 10^7 EID_{50} of NL/219 (*A*), NL/230 (*B*), Tky/VA (*C*), Can/504 (*D*), NY/107 (*E*), or Ck/Conn (*F*) virus, and nasal washes were collected from each ferret on the indicated days p.i. (dark bars). A naïve ferret was placed in the same cage as each inoculated ferret 24 h p.i., and nasal washes were collected from each contact ferret on indicated days p.c. (light bars). The limit of virus detection was $10^{1.5}$ EID_{50}/ml.

Table 3. Clinical signs, virus replication, seroconversion, and direct contact transmission in ferrets inoculated with H7 influenza viruses

		No. of inoculated ferrets/total number					
		Clinical signs				No. of contact ferrets/total number	
Virus	Subtype	Weight loss, %*	Respiratory symptoms, day p.i.	Peak mean $\log_{10}$ nasal wash titer, day p.i.	Seroconversion (HI titer range)†	Virus detected in nasal wash	Seroconversion (HI titer range)†
NL/219	H7N7	3/3 (18.3)	3/3 (3)	6.3 (1,3)	1/1 (320)‡	0/3	0/3
NL/230	H7N7	3/3 (6.7)	1/3 (5)	6.5 (3,5)	3/3 (320–640)	2/3	2/3 (320, 640)
Can/504	H7N3	3/3 (17.2)	1/3 (5)	7.4 (3)	3/3 (320–640)	2/3	0/3
Tky/VA	H7N2	3/3 (6.6)	2/3 (3)	6.75 (1)	3/3 (320–640)	0/3	0/3
NY/107	H7N2	3/3 (6.3)	1/3 (5)	6.3 (1)	3/3 (640–1,280)	3/3	3/3 (1,280–2,560)
Ck/Conn	H7N2	2/3 (3.8)	1/3 (7)	7.1 (1)	3/3 (320–640)	1/3	1/3 (160)

*The percentage mean maximum weight loss is shown.
†HI assays were performed with homologous virus and horse RBCs.
‡Only one ferret survived and was tested.

model (13). In the current study, both NL/219 and NL/230 viruses replicated efficiently in the upper respiratory tract of inoculated ferrets, with peak mean nasal wash virus titers of 6.3 ± 0.2 and 6.5 ± 0.9 $\log_{10}$ EID_{50}/ml detected on day 3 p.i., respectively (Fig. 2*A* and *B*). Two of three animals inoculated with NL/219 virus in each experiment were humanely killed 5–7 days p.i. because of severe weight loss or development of hind-limb paralysis. NL/219 virus did not transmit by either direct contact or respiratory droplets, because virus was not isolated from nasal washes of contact ferrets, and seroconversion of contact animals for hemagglutination inhibition (HI) antibody did not occur (Fig. 2*A*, Table 3, data not shown). Respiratory droplet transmission of NL/230 virus was not observed (data not shown); however, in the direct contact experiment, NL/230 virus was detected in the nasal washes of two of three contact ferrets, with peak NW virus titers $>10^{6.5}$ EID_{50}/ml by day 8 postcontact (p.c.) in these animals (Fig. 2*B*). Both NL/230 contact ferrets that had virus isolated from NW seroconverted by the end of the experiment (Table 3). The third NL/230 contact ferret did not have detectable virus in NW and did not seroconvert (Fig. 2*B*, Table 3). This pattern of NL/230 virus transmission by direct contact was confirmed in a duplicate experiment that resulted in seroconversion of only two of three ferrets. Taken together, these results indicate that, despite similar receptor-binding properties as measured by glycan array and resialyation assay, NL/230 virus exhibited an enhanced ability to transmit in the ferret model by direct contact compared with NL/219 virus.

Transmissibility of North American H7 Influenza Viruses in Ferrets. Next, we assessed the ability of the H7 viruses of the North American lineage to spread to naïve ferrets by either respiratory droplet or contact transmission. Tky/VA virus replicated efficiently in the upper respiratory tract of inoculated ferrets, with peak mean virus titers reaching 6.75 ± 0.5 $\log_{10}$ EID_{50}/ml on day 1 p.i. (Fig. 2*C*). However, Tky/VA virus did not transmit by direct contact, because virus was not isolated from nasal washes of contact ferrets, and seroconversion of contact ferrets was not detected (Fig. 2*C*, Table 3). Can/504, a HPAI H7N3 virus, was also found to replicate efficiently in the upper respiratory tract of inoculated ferrets, with peak mean nasal wash virus titers reaching 7.4 ± 0.3 $\log_{10}$ EID_{50}/ml on day 3 p.i. (Fig. 2*D*). Additionally, substantial weight loss was observed in ferrets inoculated with Can/504 virus (Table 3). Low levels of virus were detected in the nasal washes of two ferrets in direct contact with inoculated animals (1.98–2.25 $\log_{10}$ EID_{50}/ml); however, seroconversion of these contact ferrets did not occur (Table 3). These low virus titers are most likely due to the presence of residual virus on the noses of contact ferrets that was acquired from the environment or from the inoculated ferrets and therefore does not constitute efficient virus transmission, because sustained high titers of virus in the upper respiratory tract were not detected, and seroconversion did not occur. Respiratory droplet transmission of Tky/VA or Can/504 virus was not detected (data not shown).

As discussed above, two H7N2 viruses, NY/107 and Ck/Conn, exhibited enhanced α2–6 SA binding with decreased binding to α2–3 SA in the glycan microarray, with NY/107 showing the most significant decrease. Similar to all other H7 viruses tested, NY/107 and Ck/Conn viruses were detected at high titers in nasal washes of inoculated ferrets, with peak mean virus titers of 6.3 ± 0.5 $\log_{10}$ EID_{50}/ml and 7.1 ± 0.3 $\log_{10}$ EID_{50}/ml, respectively, on day 1 p.i. (Fig. 2 *E* and *F*). In contrast with Tky/VA or Can/504 viruses, NY/107 virus transmitted efficiently to three of three ferrets by direct contact, with peak virus titers in nasal washes from each contact ferret reaching $\geq 10^{5.25}$ EID_{50}/ml and seroconversion occurring in all contact animals (Fig. 2*E*, Table 3). Transmission by direct contact occurred by day 2 p.c. in one ferret and by day 6 p.c. in the remaining two contact animals (Fig. 2*E*). In comparison, Ck/Conn virus transmitted by direct contact in one of three contact ferrets, with peak NW virus titer reaching $10^{7.25}$ EID_{50}/ml on day 10 p.c. (Fig. 2*F*). Seroconversion of the remaining two contact ferrets did not occur, indicating that Ck/Conn virus, unlike NY/107 virus, did not transmit efficiently by direct contact (Table 3). Similar to other H7 viruses in this study, respiratory droplet transmission was not observed with either virus (data not shown). These findings demonstrate the ability of an H7 influenza virus isolated from a human, NY/107, to transmit efficiently by direct contact in the ferret model.

Discussion

Like other avian influenza viruses, those within the H7 subtype fall into two geographically distinct lineages, Eurasian and North American (35, 36). H7 viruses within these lineages have caused outbreaks and human infection in recent years and continue to pose a public health threat. To better assess the pandemic potential of H7 influenza viruses, we examined the receptor-binding preference and transmissibility of selected H7 viruses associated with disease in humans. We found that Eurasian lineage HPAI H7 influenza viruses tested in this study closely resemble recent HPAI H5N1 viruses with respect to their binding preference for α2–3 SA receptors. Conversely, we observed an increase in α2–6 binding among North American lineage H7 viruses isolated between 2002 and 2004. Several of these also showed reduced binding of α2–3 SA receptors characteristic of human influenza viruses. The most dramatic shift in receptor specificity was observed for a human H7 influenza virus that was also transmitted efficiently between animals by direct contact.

Previous studies have suggested that Eurasian lineage H7 influenza viruses share receptor-binding properties similar to H5 vi-

ruses, because analysis of the HA crystal structure derived from the H7 virus A/Tky/Italy/02 demonstrated specific binding to avian receptor and not human receptor analogues (37). Recent advances in glycan microarray technology allowed us to more closely analyze the fine differences in receptor specificity of viruses between both Eurasian and North American H7 viruses. Here, we used a whole-virus assay that allowed for examination of the binding properties of influenza viruses without the need for generation of recombinant HA. We were particularly interested in the North American H7 viruses, because some of these avian viruses appear to be adapted to the upper respiratory tract of chickens, which have been shown to express more α2–6 SA receptors compared with wild aquatic birds (38); *in vivo* studies have demonstrated that North American lineage LPAI H7N2 viruses replicate to high titer in the upper respiratory tract of chickens and turkeys compared with the gastrointestinal tract (39–41).

All H7 viruses tested replicated to high titer in the upper respiratory tract in inoculated ferrets as shown (13); nevertheless, most isolates tested failed to transmit despite the high titers of virus shed by inoculated animals. Respiratory symptoms such as sneezing and nasal discharge were observed in some ferrets inoculated with each H7 virus (Table 3). However, the frequency and duration of these symptoms in this model more closely resembled those observed in ferrets inoculated with H5N1 viruses, rather than the more pronounced respiratory symptoms observed in ferrets infected with human H3N2 or H1N1 viruses (21, 23). With the exception of NL/219-inoculated ferrets, which exhibit substantial lethargy after infection (13), ferrets inoculated with H7 viruses in this study remained alert and playful for the duration of the experiment, suggesting that frequent interaction between inoculated and contact ferrets is not sufficient for virus transmission to occur. Additionally, the results of this study indicate that increased virus binding to α2–6 SA is not sufficient for transmission of avian influenza viruses to occur, supporting previous studies demonstrating the lack of transmission of an H5N1 virus with an increased α2–6-binding preference (21, 22). Recent studies have highlighted increased complexity of the structural topology among α2–3 and α2–6 SA and suggest that conformational features of the linkage contribute to virus binding and could play a role in virus transmissibility (42). This diversity of SA receptors could in part contribute to the enhanced ability of NL/230 virus to transmit in the ferret model by direct contact compared with NL/219 virus. Although the Eurasian lineage H7N7 viruses analyzed in this study displayed similar receptor-binding properties as measured by glycan array and hemagglutination assay, Munster *et al.* (43) found differential attachment of NL/219 virus and a virus closely related to NL/230 to tissues in the lower respiratory tract of humans. The enhanced transmissibility observed with NL/230 virus in this study compared with NL/219 virus would additionally suggest subtle differences in the receptor-binding properties between these H7N7 viruses that have yet to be identified.

Unlike most subtypes of influenza, infection with H7 influenza viruses frequently results in conjunctivitis in humans and not respiratory disease (6, 7, 9). Unlike the upper respiratory tract in humans, which contains a high distribution of α2–6 SA, corneal, conjunctivial, and lacrimal duct epithelial cells of the human eye express predominantly α2–3 SA (27, 44, 45). Additionally, the sialylated secretions (mucins) of both surfaces differ in their SA content; mucins in the airway epithelium contain α2–3 SA, whereas ocular secreted mucins contain α2–6 SA (46–48). The high α2–3 SA content of the human ocular surface suggests that avian influenza viruses would be well suited to use this surface as a portal of entry. However, although human infection with H7 influenza viruses frequently results in conjunctivitis, documented cases of ocular disease after H5N1 infection are rare (7, 49, 50). The heterogeneity of SA-binding preference observed between H7 influenza virus lineages suggests that, similar to virus transmissibility, ocular tropism is a complex property that cannot be explained by SA receptor binding alone.

We identified a LPAI H7N2 virus, NY/107, which was associated with human respiratory infection and not ocular disease and was effectively transmitted in the ferret model by direct contact (10). Among all H7 viruses analyzed by glycan microarray, NY/107 displayed the most dramatic increase in α2–6 SA binding along with decreased α2–3 SA binding avidity. Strong α2–6 SA binding appears to be an essential component of conferring transmissibility in human influenza viruses, because H1N1 variant viruses exhibiting the classic avian α2–3-binding preference or dual α2–3- and α2–6-binding preference were unable to transmit efficiently (23). These results suggest that a decrease in α2–3 SA binding may also be needed in addition to α2–6 SA-binding avidity. However, despite similar α2–3 and α2–6 SA binding observed by glycan array with Ck/Conn virus, this virus was transmitted only by direct contact in one of three animals. Efficient contact transmission was also not observed with Tky/VA virus, despite this virus sharing 98.4% HA amino acid identity with NY/107 virus (40). Future studies will allow for a better understanding of the genetic determinants responsible for the heightened transmissibility observed with this virus. NY/107 virus, like all H7 viruses tested in this study, did not transmit by respiratory droplets in the ferret model. However, the efficient NY/107 virus transmission observed by direct contact in ferrets has not been observed with HPAI H5N1 viruses (21, 22) and may indicate the capacity of a NY/107-like virus to acquire properties that would confer efficient transmission by respiratory droplets; this underscores the importance of studying virus transmissibility by both routes.

LPAI H7N2 viruses have been acquiring additional basic amino acids at the HA cleavage site since 1994, resulting in a cleavage site that more closely resembles HPAI viruses (51). These viruses are also characterized by a deletion of 8 aa in the HA1 proximal to the receptor-binding site (31); further study will help elucidate whether this deletion contributes to the enhanced α2–6 SA binding observed among these viruses. The classic avian specificity for α2–3-linked SA observed with Rhea/NC could suggest a possible correlation between the acquisition of α2–6 SA binding and the introduction of LPAI H7N2 viruses into the live bird markets of the northeastern U.S. The finding of enhanced α2–6 SA binding of North American H7 viruses underscores the necessity for continued surveillance and study of these viruses as they continue to resemble viruses with pandemic potential.

Materials and Methods

Viruses. Virus stocks were grown in the allantoic cavity of 10-day-old embroynated hens' eggs as described (13). The 50% EID_{50} titer for each virus stock was calculated by the method of Reed and Muench (52), after serial titration in eggs. A/Texas/36/91 (Tx/91) stock was grown on Madin–Darby canine kidney cells containing DMEM, 0.025 M Hepes, 0.3% BSA (Gibco Invitrogen), and *N*-p-tosyl-L-phenylalanine chloromethyl ketone (TPCK)-treated trypsin (Sigma–Aldrich). All experiments with HPAI viruses were conducted under biosafety level 3 containment, including enhancements required by the U.S. Department of Agriculture and the Select Agent Program (53).

Glycan Microarray Analysis. Analysis of the receptor specificity of influenza virus using glycan microarrays was done largely as described (33, 54). Custom arrays for influenza research were produced for the Centers for Disease Control and Prevention on National Health Service-activated glass slides (Schott Nexterion) by using a glycan library provided by the Consortium for Functional Glycomics [www.functionalglycomics.org; see supporting information (SI) Table S1 for a list of glycan structures]. Viruses were inactivated by treatment with β-propiolactone (0.001%) overnight at 4°C with virus inactivation confirmed by two rounds of passage in eggs. Virus preparations were diluted to 1 ml into PBS buffer containing 3% (wt/vol) BSA (PBS-BSA) to HA titers of 256–512. Virus suspensions were applied to slides and the slides were incubated in a closed container and subjected to gentle agitation for 1 h. Unbound virus was washed off by dipping slides sequentially in PBS with 0.05% Tween-20 (PBS-T) and PBS. While still wet, slides were overlaid with corresponding primary antibodies diluted in PBS-BSA, either goat antiserum A/FPV/Rostock/34 (H7N1) (1:500) (for NL/219, NL/230, and Ck/

Conn viruses), ferret anti-A/Canada/444/04 (H7N3) (1:500) (for Can/504 and Can/444 viruses), ferret anti-A/Turkey/VA/4529/02 (H7N2) (1:500) (for Tky/VA virus), ferret anti-A/NY/107/03 (H7N2) (1:500) (for NY/107 virus), chicken anti-A/Rhea/NC/39482/93 (H7N1) (1:500) (for Rhea/NC virus), or sheep anti-A/Vietnam/1203/04 (H5N1) (1:1,000) (for HK/486 virus) (1 h). Slides were washed briefly with PBS-T/PBS as above followed by application of the appropriate secondary antibody conjugates, either anti-ferret-IgG FITC (1:200), anti-goat-IgG FITC (1:200), goat anti-chicken-IgY-FITC (1:200) (Genway Biotechnology), or anti-sheep-IgG-FITC (1:200) in PBS-BSA were subsequently incubated (1 h) followed by PBS-T/PBS washes and a final wash step in deionized water. After the slides were dried in a steam of nitrogen, they were immediately scanned (ProScanArray HT slide scanner with Autoloader, Perkin–Elmer) followed by image analysis with ImaGene 6.1 software (Biodiscovery).

Ferret Transmission Experiments. Male Fitch ferrets, 7–10 months of age (Triple F Farms) and serologically negative by HI assay for currently circulating influenza A H1N1, H3N2, and B viruses were used in this study. Ferrets were housed for the duration of each experiment in a Duo-Flo Bioclean mobile clean room (Lab Products). Ferrets were inoculated with 10^7 EID_{50} of each virus, and nasal washes were collected on indicated days p.i. as described (2). Respiratory droplet and contact transmission experiments were conducted as described (21), with a total of six ferrets used for each experiment.

Hemagglutination Assays. Convalescent sera were collected from all ferrets on days 18–21 p.i./p.c. and tested for H7 specific antibodies by HI by using homologous virus and 1% horse RBCs as described (55). Hemagglutination assays using resialyated turkey RBC were performed as described (56, 57) with minor modifications. Turkey RBC were enzymatically desialyated, followed by resialylation using either α2–6-(*N*)-sialyltransferase (Japan Tobacco) or α2–3-(*N*)-sialyltransferase (Calbiochem). Assays were performed by using both 4 and 8 hemagglutination units of virus yielding identical results.

ACKNOWLEDGMENTS. We thank David Swayne (Southeast Poultry Research Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Athens, GA), Ron Fouchier (Erasmus Medical Center, Rotterdam, The Netherlands), and Yan Li (Canadian Center for Human and Animal Health) for providing some of the viruses used in this study. We also thank Julia Hoffman for early help with the glycan microarray and Anna Crie for assistance with generating the figures. The glycan microarray was produced for the Centers for Disease Control by using a glycan library generously provided by the Consortium for Functional Glycomics funded by National Institute of General Medical Sciences Grant GM62116. J.A.B. received financial support for this work from the Oak Ridge Institute for Science and Education, Oak Ridge, TN. Glycan microarray data presented here will be made available on-line through the Consortium for Functional Glycomics web site upon publication (www.functionalglycomics.org).

1. World Health Organization (2007) *Cumulative Number of Confirmed Human Cases of Avian Influenza A/(H5N1) Reported to WHO* (World Health Organization, Geneva).
2. Maines TR, *et al.* (2005) Avian influenza (H5N1) viruses isolated from humans in Asia in 2004 exhibit increased virulence in mammals. *J Virol* 79:11788–11800.
3. Ungchusak K, *et al.* (2005) Probable person-to-person transmission of avian influenza A (H5N1). *N Engl J Med* 352:333–340.
4. Olsen SJ, *et al.* (2005) Family clustering of avian influenza A (H5N1). *Emerg Infect Dis* 11:1799–1801.
5. Kandun IN, *et al.* (2006) Three Indonesian clusters of H5N1 virus infection in 2005. *N Engl J Med* 355:2186–2194.
6. Fouchier RA, *et al.* (2004) Avian influenza A virus (H7N7) associated with human conjunctivitis and a fatal case of acute respiratory distress syndrome. *Proc Natl Acad Sci USA* 101:1356–1361.
7. Koopmans M, *et al.* (2004) Transmission of H7N7 avian influenza A virus to human beings during a large outbreak in commercial poultry farms in the Netherlands. *Lancet* 363:587–593.
8. Hirst M, *et al.* (2004) Novel avian influenza H7N3 strain outbreak, British Columbia. *Emerg Infect Dis* 10:2192–2195.
9. Tweed SA, *et al.* (2004) Human illness from avian influenza H7N3, British Columbia. *Emerg Infect Dis* 10:2196–2199.
10. Centers for Disease Control (2004) Update: influenza activity–United States and worldwide, 2003–04 season, and composition of the 2004–05 influenza vaccine. *MMWR Morb Mortal Wkly Rep* 53:547–552.
11. ●●● (2007) Avian influenza A/(H7N2) outbreak in the United Kingdom. *Euro Surveill* 12, E070531 2.
12. de Wit E, *et al.* (2005) Protection of mice against lethal infection with highly pathogenic H7N7 influenza A virus by using a recombinant low-pathogenicity vaccine strain. *J Virol* 79:12401–12407.
13. Belser JA, *et al.* (2007) Pathogenesis of Avian Influenza (H7) Virus Infection in Mice and Ferrets: Enhanced Virulence of Eurasian H7N7 Viruses Isolated from Humans. *J Virol* 81:11139–11147.
14. Matrosovich MN, *et al.* (1997) Avian influenza A viruses differ from human viruses by recognition of sialyloligosaccharides and gangliosides and by a higher conservation of the HA receptor-binding site. *Virology* 233:224–234.
15. Matrosovich M, et al. (2000) Early alterations of the receptor-binding properties of H1, H2, and H3 avian influenza virus hemagglutinins after their introduction into mammals. *J Virol* 74:8502–8512.
16. Connor RJ, Kawaoka Y, Webster RG, Paulson JC (1994) Receptor specificity in human, avian, and equine H2 and H3 influenza virus isolates. *Virology* 205:17–23.
17. Matrosovich M, Zhou N, Kawaoka Y, Webster R (1999) The surface glycoproteins of H5 influenza viruses isolated from humans, chickens, and wild aquatic birds have distinguishable properties. *J Virol* 73:1146–1155.
18. Shinya K, *et al.* (2005) Characterization of a human H5N1 influenza A virus isolated in 2003. *J Virol* 79:9926–9932.
19. Gambaryan A, *et al.* (2006) Evolution of the receptor binding phenotype of influenza A (H5) viruses. *Virology* 344:432–438.
20. Yamada S, *et al.* (2006) Haemagglutinin mutations responsible for the binding of H5N1 influenza A viruses to human-type receptors. *Nature* 444:378–382.
21. Maines TR, *et al.* (2006) Lack of transmission of H5N1 avian-human reassortant influenza viruses in a ferret model. *Proc Natl Acad Sci USA* 103:12121–12126.
22. Yen HL, *et al.* (2007) Inefficient transmission of H5N1 influenza viruses in a ferret contact model. *J Virol* 81:6890–6898.
23. Tumpey TM, *et al.* (2007) A two-amino acid change in the hemagglutinin of the 1918 influenza virus abolishes transmission. *Science* 315:655–659.
24. Baum LG, Paulson JC (1990) Sialyloligosaccharides of the respiratory epithelium in the selection of human influenza virus receptor specificity. *Acta Histochem Suppl* 40:35–38.
25. Leigh MW, Connor RJ, Kelm S, Baum LG, Paulson JC (1995) Receptor specificity of influenza virus influences severity of illness in ferrets. *Vaccine* 13:1468–1473.
26. van Riel D, *et al.* (2006) H5N1 Virus Attachment to Lower Respiratory Tract. *Science* 312:399.
27. Shinya K, *et al.* (2006) Avian flu: influenza virus receptors in the human airway. *Nature* 440:435–436.
28. Rogers GN, D'Souza BL (1989) Receptor binding properties of human and animal H1 influenza virus isolates. *Virology* 173:317–322.
29. Stevens J, *et al.* (2006) Structure and receptor specificity of the hemagglutinin from an H5N1 influenza virus. *Science* 312:404–410.
30. Matrosovich MN, Krauss S, Webster RG (2001) H9N2 influenza A viruses from poultry in Asia have human virus-like receptor specificity. *Virology* 281:156–162.
31. Suarez DL, Garcia M, Latimer J, Senne D, Perdue M (1999) Phylogenetic analysis of H7 avian influenza viruses isolated from the live bird markets of the Northeast United States. *J Virol* 73:3567–3573.
32. Centers for Disease Control (2004) Update: influenza activity - United States, 2003–04 season. *MMWR Morb Mortal Wkly Rep* 53:284–287.
33. Stevens J, et al. (2006) Glycan microarray analysis of the hemagglutinins from modern and pandemic influenza viruses reveals different receptor specificities. *J Mol Biol* 355:1143–1155.
34. Hinshaw VS, Webster RG, Easterday BC, Bean WJ, Jr (1981) Replication of avian influenza A viruses in mammals. *Infect Immun* 34:354–361.
35. Rohm C, Horimoto T, Kawaoka Y, Suss J, Webster RG (1995) Do hemagglutinin genes of highly pathogenic avian influenza viruses constitute unique phylogenetic lineages? *Virology* 209:664–670.
36. Banks J, Speidel EC, McCauley JW, Alexander DJ (2000) Phylogenetic analysis of H7 haemagglutinin subtype influenza A viruses. *Arch Virol* 145:1047–1058.
37. Russell RJ, Stevens DJ, Haire LF, Gamblin SJ, Skehel JJ (2006) Avian and human receptor binding by hemagglutinins of influenza A viruses. *Glycoconj J* 23:85–92.
38. Gambaryan A, Webster R, Matrosovich M (2002) Differences between influenza virus receptors on target cells of duck and chicken. *Arch Virol* 147:1197–1208.
39. Tumpey TM, Kapczynski DR, Swayne DE (2004) Comparative susceptibility of chickens and turkeys to avian influenza A H7N2 virus infection and protective efficacy of a commercial avian influenza H7N2 virus vaccine. *Avian Dis* 48:167–176.
40. Pappas C, Matsuoka Y, Swayne DE, Donis RO (2007) Development and evaluation of an influenza subtype H7N2 vaccine candidate for pandemic preparedness. *Clin Vaccine Immunol* 14:1425–1432.
41. Swayne DE, Pantin-Jackwood M (2006) Pathogenicity of avian influenza viruses in poultry. *Dev Biol (Basel)* 124:61–67.
42. Chandrasekaran A, *et al.* (2008) Glycan topology determines human adaptation of avian H5N1 virus hemagglutinin. *Nat Biotechnol* 26:107–113.
43. Munster VJ, *et al.* (2007) The molecular basis of the pathogenicity of the Dutch highly pathogenic human influenza A H7N7 viruses. *J Infect Dis* 196:258–265.
44. Terraciano AJ, *et al.* (1999) Sialyl Lewis X, Lewis X, and N-acetyllactosamine expression on normal and glaucomatous eyes. *Curr Eye Res* 18:73–78.
45. Paulsen F, *et al.* (1998) Functional anatomy of human lacrimal duct epithelium. *Anat Embryol (Berl)* 198:1–12.
46. Couceiro JN, Paulson JC, Baum LG (1993) Influenza virus strains selectively recognize sialyloligosaccharides on human respiratory epithelium; the role of the host cell in selection of hemagglutinin receptor specificity. *Virus Res* 29:155–165.
47. Berry M, Ellingham RB, Corfield AP (1996) Polydispersity of normal human conjunctival mucins. *Invest Ophthalmol Vis Sci* 37:2559–2571.
48. Thale A, et al. (2001) The efferent lacrimal ducts of the human. Morphological and biochemical studies. *Ophthalmologe* 98:67–73.
49. Chan PK (2002) Outbreak of avian influenza A(H5N1) virus infection in Hong Kong in 1997. *Clin Infect Dis* 34 Suppl 2:S58–64.
50. Tam JS (2002) Influenza A (H5N1) in Hong Kong: an overview. *Vaccine* 20 Suppl 2:S77–81.
51. Spackman E, Senne DA, Davison S, Suarez DL (2003) Sequence analysis of recent H7 avian influenza viruses associated with three different outbreaks in commercial poultry in the United States. *J Virol* 77:13399–13402.
52. Reed LJ, Muench HA (1938) A simple method of estimating fifty per cent endpoints. *Am J Hyg* 27:493–497.
53. Richmond JY, McKinney RW (2007) in *Biosafety in microbiological and biomedical laboratories*, eds Richmond JY, McKinney RW (Centers for Disease Control and Prevention, Atlanta), 5th ed.
54. Blixt O, *et al.* (2004) Printed covalent glycan array for ligand profiling of diverse glycan binding proteins. *Proc Natl Acad Sci USA* 101:17033–17038.
55. Stephenson I, Wood JM, Nicholson KG, Charlett A, Zambon MC (2004) Detection of anti-H5 responses in human sera by HI using horse erythrocytes after MF59-adjuvanted influenza A/Duck/Singapore/97 vaccine. *Virus Res* 103:91–95.
56. Glaser L, et al. (2005) A single amino acid substitution in 1918 influenza virus hemagglutinin changes receptor binding specificity. *J Virol* 79:11533–11536.
57. Glaser L, *et al.* (2006) Sequence analysis and receptor specificity of the hemagglutinin of a recent influenza H2N2 virus isolated from chicken in North America. *Glycoconj J* 23:93–99.

別紙様式第 2-1
番号 9

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 4 月 4 日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	ウロキナーゼ注射剤		研究報告の公表状況	Emerging Infectious Diseases 2008; 14(5): 834-836	公表国 カナダ	
販売名 （企業名）	①ウロキナーゼ 6 万-Wf（ベネシス） ②ウロキナーゼ 12 万-Wf（ベネシス） ③ウロキナーゼ 24 万-Wf（ベネシス）					

研究報告の概要

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 名の子供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 aa 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。

ピコルナウイルス科は 9 つの属に分類され、エンテロウイルス、ヘパトウイルス、ライノウイルス、コブウイルス及びパレコウイルスはヒトに感染するが、アフトウイルス、エルボウイルス、テスコウイルス及びカルジオウイルスは動物の病原体である。カルジオウイルス属は、タイラーウイルスと脳心筋炎ウイルス（EMCVs）の 2 つの種に分けられる。ラットやマウスは EMCVs の自然宿主であるが、これらのカルジオウイルスはこれまで、ブタ、げっ歯類、ゾウ、マカク及びヒトに感染することがわかっている。最近、Saffold ウイルス（SAF-V）と暫定的に名付けられた新規カルジオウイルスが、発熱した 8 歳の女子の排便サンプルから分離された。このウイルスは、新規のカルジオウイルスの種と考えられ、知られている他のカルジオウイルスよりも遺伝的にタイラー様ウイルスに関連していた。
SAF-V と、今回の研究で示した SAF-V に関連するカナダの株が、新種のヒトカルジオウイルスの種と分類されるべきか、あるいはタイラーウイルス種の中の新しいクレードとして分類されるべきかについて、今後決定される必要がある。

使用上の注意記載状況・その他参考事項等

感染症に関連する記載はない。

報告企業の意見	今後の対応
新規の 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 名の子供の鼻咽頭吸引物から検出されたとの報告である。 カルジオウイルスは、ピコルナウイルス科に属する直径 22～30nm のエンベロープを有しない RNA ウイルスである。血漿分画製剤からのカルジオウイルス感染に関する報告は、入手していない。 万一、添加剤のアルブミンの原料血漿にカルジオウイルスが混入したとしても、EMCおよびCPVをモデルウイルスとしたウイルスバリデーション試験成績から、製造工程において十分に不活化・除去されると考えている。	新規カルジオウイルスに関する追加情報の入手に努める。

④

New Saffold Cardioviruses in 3 Children, Canada

Yacine Abed*† and Guy Boivin*†

In Canada, cardiovirus isolates related to Saffold virus were detected in nasopharyngeal aspirates from 3 children with respiratory symptoms. Polyprotein sequence of the Can112051-06 isolate had 91.2% aa identity with Saffold virus; however, EF and CD loops of the viral surface varied substantially.

The family *Picornaviridae* contains 9 genera: *Enterovirus, Hepatovirus, Rhinovirus, Kobuvirus,* and *Parechovirus* infect humans, whereas *Aphtovirus, Erbovirus, Teschovirus,* and *Cardiovirus* are animal pathogens (*1*). The genus *Cardiovirus* is divided into 2 species: Theiler viruses and the encephalomyocarditis viruses (EMCVs) (*2–5*). Although rats and mice are the natural hosts for EMCVs, these cardioviruses have been found to infect many animal species including pigs, rodents, elephants, macaques, and humans (*6–9*). Recently, a new cardiovirus provisionally named Saffold virus (SAF-V) was isolated from a stool sample of an 8-month-old girl with fever (*10*). This virus is believed to constitute a novel cardiovirus species and is more genetically related to Theiler-like virus than to other known cardioviruses (*10*). We report the identification and characterization of 3 SAF-V isolates recovered from children with respiratory symptoms.

The Patients

The first patient was a 23-month-old girl who was referred on March 6, 2006, to a tertiary hospital for bilateral otitis media that had not responded to amoxicillin or later to cefprozil. She also had cough, rhinorrhea, and fever of 39°C. Her 5-month-old brother had similar clinical signs. Blood cultures were negative, as were antigen detection tests for influenza A and B viruses, the respiratory syncytial virus, and adenoviruses. After 24 hours, the girl was discharged with a diagnosis of bilateral acute otitis media secondary to a viral infection. A nasopharyngeal aspirate collected at the time of admission was inoculated onto different continuous cell lines including human lung adenocarcinoma (A-549); human rhabdosarcoma (RD); transformed human kidney (293); human colon adenocarcinoma (HT-29); human laryngeal carcinoma (Hep-2); human foreskin fibroblast; mink lung; and Vero, MDCK, and rhesus monkey kidney (LLC-MK2) cells. Cultures were incubated for 3 weeks at 37°C in 5% CO_2. A viral isolate (Can112051-06) with cytopathic effects (round cells) suggestive of a picornavirus was observed only in LLC-MK2 cells after 6 days of incubation (Figure 1). An immunofluorescent assay that used the Pan-Enterovirus Blend kit (Light Diagnostics, Levingston, UK) gave a moderate fluorescent signal. Nucleic acid extracts from Can112051-06 were further analyzed with a multiplex real-time reverse transcription–PCR (RT-PCR) assay for common respiratory viruses (influenza A and B viruses,

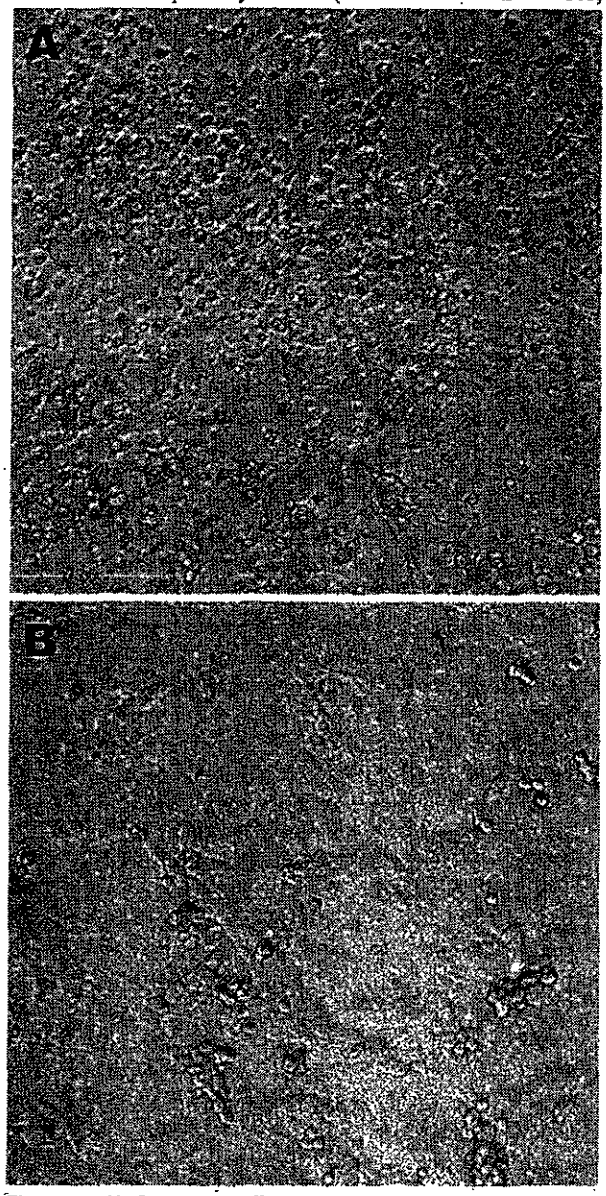


Figure 1. A) Cytopathic effects (round cells) observed 6 days after infection of rhesus monkey kidney (LLC-MK2) cells (second passage) with the Can112051-06 Saffold virus–like cardiovirus strain. B) Uninfected LLC-MK2 cells. Magnification ×10.

*Centre Hospitalier Universitaire de Québec, Quebec City, Quebec, Canada; and †Infectious Disease Research Centre, Quebec City, Quebec, Canada

human respiratory syncytial virus, and human metapneumovirus) (*11*) as well as RT-PCR assays for enteroviruses and parechoviruses (*12*); results were negative.

The supernatant from LLC-MK2–infected cells was treated with DNase and divided into 2 aliquots for DNA and RNA extractions by using the QIAamp Blood Mini Kit and QIAamp Viral RNA extraction kits (QIAGEN, Mississauga, Ontario, Canada), respectively. Nucleic acids were then used in the sequence-independent single-primer amplification method as described (*13*). Amplicons of 800–1,200 bp obtained from RNA samples were cloned and sequenced.

Sequence determination of cloned amplicons followed by tBLASTx analysis showed similarity of Can112051-06 sequences with the SAF-V VP4 and 2C sequences (data not shown). Subsequent PCR amplifications and sequencing reactions that used primers selected from our clones and the complete SAF-V genome sequence (GenBank accession no. EF165067) enabled us to determine the complete polyprotein encoding region of the Can112051-06 isolate (GenBank accession no. AM922293). This region was 6,879 nt long compared with 6,888 nt for the SAF-V polyprotein sequence; nucleotide identity between the 2 strains was 82.5%. The Can112051-06 polyprotein comprised 2,293 aa compared with 2,296 aa for the SAF-V polyprotein; amino acid identity between the 2 strains was 91.2%. Deletions of 1 aa in the VP2 and 2 in the VP1 proteins were found in Can112051-06 with regard to the prototype SAF-V strain. As expected, the Can112051-06 and SAF-V polyproteins contained 11 putative cleavage sites. The 8 aa flanking these sites were conserved; 6 sites were identical in the 2 strains, whereas the remaining sites had 1- or 2-aa differences (Table 1). The resulting 12 proteins of Can112051-06 and SAF-V had 76.1%–100% aa identities (Table 2). The highest difference level was seen in the L peptide. In addition to the L peptide, some cardioviruses, in particular Theiler's murine encephalomyelitis virus strains that are associated with persistent infections, contain an alternate open reading frame (ORF), the so-called L* (*14*). As for the prototype SAF-V strain, the Can112051-06 putative L* ORF is unlikely to encode a protein because it has an ACG (instead of ATG) start codon (data not shown). In addition, contrasting with the SAF-V L*, which contained 57 aa, the Can112051-06 L* sequence contained only 34 aa. Comparison of the L* sequence of Can112051-06 with the first 34 aa of the SAF-V L* sequence showed 60.6% identity (data not shown). Four small loops are exposed on the virion surface of cardioviruses; 2 are part of the VP2 EF loop structure, and 2 are part of the VP1 CD loop structure. The EF loop structure of Can112051-06, which contained 55 aa (residues 274-328 of the polyprotein), had 61.8% aa identity with that of SAF-V (Figure 2, panel A). Similarly, the CD loop structure of Can112051-06, which contained 40 aa (residues 712-751 of the polyprotein), had 67.5% aa identity with the SAF-V counterpart (Figure 2, panel B).

Table 1. Cleavage sites of Can112051-06 and prototype Saffold virus cardiovirus polyproteins*

Cleavage site	Can112051-06	Saffold virus
L / VP4	MEPQ / GNSN	MEPQ / GNSN
VP4 / VP2	PLLM / DQNT	PLLM / DQNT
VP2 / VP3	LEDQ / SPIP	LEAD / SPIP
VP3 / VP1	YTPH / GVDN	YTPQ / GVDN
VP1 / V2A	LELQ / NPIS	LELQ / DPIS
2A / 2B	FQLQ / GGVL	FQLQ / GGVL
2B / 2C	LQQQ / SPVR	LQQQ / SPIR
2C / 3A	LVAQ / SPGN	LVAQ / SPGN
3A / 3B	EGEQ / AAYS	EGEQ / AAYS
3B / 3C	LDVQ / GGGK	LDVQ / GGGK
3C / 3D	LIPQ / GAIV	LTPQ / GAIV

*Can112051-06 GenBank accession no. AM922293; Saffold virus GenBank accession no. EF165067.

Other respiratory samples with picornavirus-like cytopathic effects on LLC-MK2 cells and weakly immunofluorescent signals according to the Pan-Enterovirus Blend Kit were screened for cardiovirus SAF-V by using a specific RT-PCR assay targeting a 2A–2C encoding region (1,407 nt, 469 aa). With use of this strategy, 2 more cases were noted in September 2006: 1 in a 19-month-old child hospitalized for suspected bacteremia and a cold and 1 in a 4-year-old child hospitalized for right lung pneumonia and otitis media. The 2A-2C aa sequences of these additional isolates were identical and shared 96.6% and 97.2% aa identities with the corresponding regions of Can112051-06 and the prototype SAF-V, respectively.

Conclusions

Our findings suggest a pathogenic role for SAF-V-like viruses in humans. Although the polyprotein sequences of the Can112051-06 strain and the original US strain were related, the EF and CD loop structures varied substantially (61.8% and 67.5% aa identities, respectively). For com-

Table 2. Amino acid identities between Can112051-06 and prototype Saffold virus proteins*

Protein	% Identity
L	76.1
VP4	97.2
VP2	83.9
VP3	85.2
VP1	76.7
2A	95.8
2B	97.6
2C	96.6
3A	100
3B	95.0
3C	96.8
3D	97.0

*Can112051-06 GenBank accession no. AM922293; Saffold virus GenBank accession no. EF165067.

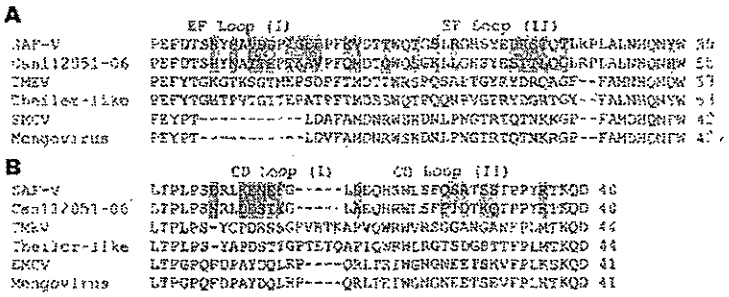


Figure 2. Comparison of amino acid sequences of the A) EF loop structure (part of the VP2 protein) and B) the CD loop structuré (part of the VP1 protein) between Can112051-06 and other cardioviruses including Saffold virus (SAF-V), Theiler's murine encephalomyelitis virus (TMEV), Theiler-like virus, encephalomyocarditis virus (EMCV), and Mengovirus. Amino acid differences between Can112051-06 and SAF-V are shaded.

parison, the EF and CD loop structure sequences of EMCV and Mengovirus (2 members of the EMCV species) have 95.2% and 95.1% aa identities, respectively. The difference between time of isolation of SAF-V (1981) and the Can112051-06 strain (2006) is unlikely to be responsible for such a high level of sequence variation. We previously showed that the amino acid sequences of the VP0-VP1 capsid region of Canadian human parechovirus 1 strains isolated from 1985 through 2004 had 89.2% to 97.5% identities (*12*). Because the EF and CD loop structures are exposed on the viral surface of cardioviruses and thus constitute an important site for recognition by neutralizing antibodies (*15*), Can112051-06 and the original SAF-V might represent different serotypes, although further serologic studies are needed to confirm this hypothesis. The implication of the weak immunofluorescent signal seen in cardiovirus-infected cells stained with an enterovirus antibody is uncertain because of the considerable difference between the capsid proteins of cardioviruses and enteroviruses, which constitute 2 separate picornavirus genera.

In contrast to the initial recovery of this virus from a stool sample (*10*), our 3 strains were recovered from nasopharyngeal aspirate samples of children with fever and some other respiratory signs. The cardioviruses were the only pathogens identified in these samples. Whether SAF-V and the related Canadian strains described in this study should be classified as a new human *Cardiovirus* species or as a new clade within the *Theilovirus* species remains to be determined.

Dr Abed is an associate professor in the Department of Medical Biology at Laval University, Quebec City, Canada. His research interests include the study of influenza and emerging respiratory viruses.

Dr Boivin is the holder of the Canada Research Chair on Emerging Viruses and Antiviral Resistance at Laval University. His research interests include antiviral resistance mechanisms for herpes and influenza viruses and characterization of emerging viruses.

References

1. King AMQ, Brown F, Christian P, Hovi T, Hyypia T, Knowles NJ, et al. Family *Picornaviridae*. In: Van Regenmortel MHV, Fauquet CM, Bishop DHL, Carstens EB, Estes MK, Lemon SM, et al., editors. Virus taxonomy. San Diego: Academic Press; 2000. p. 657–78.
2. Ohsawa K, Watanabe Y, Miyata H, Sato H. Genetic analysis of a Theiler-like virus isolated from rats. Comp Med. 2003;53:191–6.
3. Lipton HL, Friedman A, Sethi P, Crowther JR. Characterization of Vilyuisk virus as a picornavirus. J Med Virol. 1983;12:195–203.
4. Craighead JE, Huber SA, Haynes MK. Diverse patterns of immune and non-immune-mediated disease in EMC M-variant-infected mice. J Autoimmun. 1990;3:27–9.
5. Martin LR, Neal ZC, McBride MS, Pamenberg AC. Mengovirus and encephalomyocarditis virus poly(C) tract lengths can affect virus growth in murine cell culture. J Virol. 2000;74:3074–81.
6. Knowles NJ, Dickinson ND, Wilsden G, Carra E, Brocchi E, De Simone F. Molecular analysis of encephalomyocarditis viruses isolated from pigs and rodents in Italy. Virus Res. 1998;57:53–62.
7. Grobler DG, Raath JP, Braak LE, Keet DF, Gerdes GH, Barnard BJ, et al. An outbreak of encephalomyocarditis-virus infection in free-ranging African elephants in the Kruger National Park. Onderstepoort J Vet Res. 1995;62:97–108.
8. Emerson CL, Wagner JL. Antibody responses to two encephalomyocarditis virus vaccines in rhesus macaques (*Macaca mulatta*). J Med Primatol. 1996;25:42–5.
9. Kirkland PD, Gleeson AB, Hawkes RA, Naim HM, Broughton CR. Human infection with encephalomyocarditis virus in New South Wales. Med J Aust. 1989;151:176–8.
10. Jones MS, Lukashov VV, Ganac RD, Schnurr DP. Discovery of a novel human picornavirus in a stool sample from a pediatric patient presenting with fever of unknown origin. J Clin Microbiol. 2007;45:2144–50.
11. Boivin G, De Serres G, Cote S, Gilca R, Abed Y, Rochette L, et al. Human metapneumovirus infections in hospitalized children. Emerg Infect Dis. 2003;9:634–40.
12. Abed Y, Boivin G. Human parechovirus infections in Canada. Emerg Infect Dis. 2006;12:969–75.
13. Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A. Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proc Natl Acad Sci U S A. 2005;102:12891–6.
14. Michiels T, Jarousse N, Brahic M. Analysis of the leader and capsid coding regions of persistent and neurovirulent strains of Theiler's virus. Virology. 1995;214:550–8.
15. Jnaoui K, Minet M, Michiels T. Mutations that affect the tropism of DA and GDVII strains of Theiler's virus in vitro influence sialic acid binding and pathogenicity. J Virol. 2002;76:8138–47.

Address for correspondence: Guy Boivin, Research Center in Infectious Diseases, CHUQ-CHUL, Rm RC-709, 2705 Laurier Blvd, Quebec City, Quebec, Canada G1V 4G2; email: guy.boivin@crchul.ulaval.ca

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 4 月 24 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	ウロキナーゼ注射剤	研究報告の公表状況	PLos Pathogens 2008; 4(4): e1000047	公表国 ボリビア
販売名 (企業名)	①ウロキナーゼ 6 万-Wf（ベネシス） ②ウロキナーゼ 12 万-Wf.（ベネシス） ③ウロキナーゼ 24 万-Wf（ベネシス）			

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
出血熱症例の小さな流行が、2003 年 12 月と 2004 年 1 月にボリビアのコチャバンバの近くで発生した。検体は死亡した 1 症例のみから入手できたが、症例の臨床経過は発熱、頭痛、関節痛、筋肉痛、嘔吐を含み、その後悪化と多くの出血の徴候が現れた。非細胞変性のウイルスが患者血清サンプルのうちの 2 つから単離され、出血熱を伴うことが知られている南米のアレナウイルス（Guanarito、Machupo と Sabia）に対するウサギ多価抗血清を用いた IFA 染色によってアレナウイルスと同定した。RT-PCR 分析、並びにすべての S 及び L RNA セグメント配列の解析から、このウイルスが、全ての病原性の南アメリカアレナウイルスを含む新世界クレード B アレナウイルスに属することが確認された。このウイルスはサビアウイルスに最も近いことが示されたが、ヌクレオチド S 及び L セグメントにおいて 26%及び 30%の違いがあり、L、Z、N 及び GP 蛋白のアミノ酸に 26%、28%、15%及び 22%の違いがあった。このことは、このウイルスが新規のウイルスであることを示しており、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。	感染症に関連する記載はない。

報告企業の意見	今後の対応
ボリビアの致死性出血熱症例から新規アレナウイルスの Chapare ウイルスが分離されたとの報告である。 アレナウイルスは直径50～300nmのエンベロープを有するRNAウイルスである。万一、新規アレナウイルスが添加剤のアルブミンの原料血漿に混入しても、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、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。	新規アレナウイルスに関する情報に注視する。

(57)

OPEN ACCESS Freely available online PLoS PATHOGENS

Chapare Virus, a Newly Discovered Arenavirus Isolated from a Fatal Hemorrhagic Fever Case in Bolivia

Simon Delgado[1], Bobbie R. Erickson[2], Roberto Agudo[3], Patrick J. Blair[4], Efrain Vallejo[3], César G. Albariño[2], Jorge Vargas[5], James A. Comer[2], Pierre E. Rollin[2], Thomas G. Ksiazek[2], James G. Olson[4], Stuart T. Nichol[2]*

1 Centro de Salud de Eterazama, Cochabamba, Bolivia, 2 Special Pathogens Branch, Division of Viral and Rickettsial Disease, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America, 3 Servicio Departamental de Salud, Cochabamba, Bolivia, 4 Naval Medical Research Center Detachment, Lima, Peru, 5 Centro Nacional de Enfermedades Tropicales (CENETROP), Santa Cruz, Bolivia

Abstract

A small focus of hemorrhagic fever (HF) cases occurred near Cochabamba, Bolivia, in December 2003 and January 2004. Specimens were available from only one fatal case, which had a clinical course that included fever, headache, arthralgia, myalgia, and vomiting with subsequent deterioration and multiple hemorrhagic signs. A non-cytopathic virus was isolated from two of the patient serum samples, and identified as an arenavirus by IFA staining with a rabbit polyvalent antiserum raised against South American arenaviruses known to be associated with HF (Guanarito, Machupo, and Sabiá). RT-PCR analysis and subsequent analysis of the complete virus S and L RNA segment sequences identified the virus as a member of the New World Clade B arenaviruses, which includes all the pathogenic South American arenaviruses. The virus was shown to be most closely related to Sabiá virus, but with 26% and 30% nucleotide difference in the S and L segments, and 26%, 28%, 15% and 22% amino acid differences for the L, Z, N, and GP proteins, respectively, indicating the virus represents a newly discovered arenavirus, for which we propose the name Chapare virus. In conclusion, two different arenaviruses, Machupo and Chapare, can be associated with severe HF cases in Bolivia.

Citation: Delgado S, Erickson BR, Agudo R, Blair PJ, Vallejo E, et al. (2008) Chapare Virus, a Newly Discovered Arenavirus Isolated from a Fatal Hemorrhagic Fever Case in Bolivia. PLoS Pathog 4(4): e1000047. doi:10.1371/journal.ppat.1000047

Editor: Michael J. Buchmeier, University of California Irvine, United States of America

Received January 18, 2008; **Accepted** March 20, 2008; **Published** April 18, 2008

Funding: These investigations were funded by the Centers for Disease Control and Prevention and the United States Navy. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the funding agencies.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: snichol@cdc.gov

Introduction

The family *Arenaviridae* is composed of largely rodent-borne viruses which are divided into Old World and New World complexes [1,2]. Lassa and lymphocytic choriomeningitis (LCM) viruses are considered the most important of Old World arenaviruses due to their association with severe disease. The New World complex is divided into 3 major Clades (A, B and C), with Clade B containing all the hemorrhagic fever (HF) associated viruses [3,4,5,6]. These are Junín, Machupo, Guanarito and Sabiá viruses, the cause of Argentine, Bolivian, Venezuelan, and Brazilian HF, respectively [1]. Three of these viruses, Junín, Machupo, and Guanarito, can be associated with large HF outbreaks and untreated case fatalities can be in excess of 30%. The clinical picture is similar for each of these diseases. Onset of symptoms follows an incubation period of 1–2 weeks. Initial symptoms often include fever, malaise, myalgia and anorexia, followed approx. 3–4 days later by headache, back pain, dizziness, nausea, vomiting, and severe prostration. Hemorrhagic and neurologic symptoms, including petechiae and bleeding gums, tremors, and lethargy are common. About a third of untreated cases go on to develop more severe neurologic and/or hemorrhagic symptoms, with diffuse echymoses, and bleeding from mucous membranes or puncture sites, and/or delirium, coma and convulsions. Machupo virus, vectored by *Calomys callosus* rodents [7], is the only known pathogenic arenavirus found in Bolivia, although another arenavirus, Latino virus, has also been isolated from *Calomys callosus* in Bolivia [8]. Despite broad distribution of this rodent host, which is thought to include the lowlands of Bolivia, east-central Brazil, Paraguay and northern Argentina [9], Machupo virus-associated HF cases have originated only in the Beni department in northeastern Bolivia (Figure 1). We report here the investigation of a fatal HF case which occurred near Cochabamba, Cochabamba Department, Bolivia in December, 2003, and identify the associated arenavirus as a unique newly discovered virus, Chapare virus.

Results/Discussion

In late 2003 reports were received of a small cluster of HF cases in a rural area near the Chapare River, close to Cochabamba, Bolivia in the eastern foothills of the Andes (Figure 1). Exact details of the number of cases and verification of symptoms were difficult to obtain. However, a clinical description and blood specimens were available for one fatal case. This patient, a 22 year old male, had lived for the last 4 years in Samuzabeti, a small town located 35 km northeast of Villa Tunari. He was a tailor and also a farmer. Coca is the main crop in this area. He had no history of travel and no contact with any case with compatible illness for at least 4 weeks prior to his disease onset on January 3rd, 2004. In

Author Summary

Four rodent-borne arenaviruses are known to cause hemorrhagic fever (HF) in the New World. These include Junín, Machupo, Guanarito, and Sabiá viruses, which are found in rural areas of Argentina, Bolivia, Venezuela, and Brazil, respectively. In December 2003 and January 2004, a small number of HF cases were reported in rural Bolivia in an area outside the known Machupo HF endemic zone, and sera from one fatal case was available for laboratory testing. The man had symptoms similar to those seen with other arenaviral HF cases—acute febrile illness beginning with headache, joint and muscle pain, and vomiting—and rapidly progressed to shock, bleeding, and death at 14 days post onset of illness. Virus was isolated from two of the patient's serum samples and identified as an arenavirus by reaction of virus infected cells with arenavirus-specific antibodies and by genetic detection techniques (PCR). Subsequent complete genome analysis of the virus showed the virus to be a distinct newly discovered member of the arenavirus family, and the name Chapare virus was proposed. The virus is phylogenetically related to other arenaviruses that naturally cause hemorrhagic fever in South America, particularly Sabiá virus. Physicians should consider Chapare virus as a potential etiologic agent when encountering HF cases in the region.

addition, no members of case household or other close contacts were affected. His clinical course included fever, headache, arthralgia, myalgia and vomiting with subsequent deterioration and multiple hemorrhagic signs and death on January 17th, 2004 (14 days post onset). Based on these symptoms, the patient was initially suspected of having yellow fever or dengue HF. However, initial tests for these agents were negative. Initial IgM, IgG, antigen capture and RT-PCR testing for Machupo virus or related arenaviruses were also negative.

Patient specimens were sent to the biosafety level 4 (BSL4) containment laboratory at the Special Pathogens Branch in Atlanta where virus isolation attempts could be performed. These specimens consisted of 4 acute phase sera, collected on days 4, 7, 9 and 14 post onset of disease. Both the day 7 and day 9 sera yielded a non-cytopathic virus by growth in Vero E6 cells. These were identified by immunofluorescent antibody (IFA) staining with rabbit polyvalent hyperimmune serum raised against South American arenaviruses previously known to be associated with HF (Guanarito, Machupo, and Sabiá). RT-PCR analysis of the virus isolate RNAs amplified a 481 bp fragment which yielded nucleotide sequence related to known New World Clade B arenaviruses (which includes all the South American HF associated arenaviruses). Full length virus genome sequences were successfully determined for the virus isolated from the day 9 post onset bleed (designated strain 810419) by RT-PCR and sequence analysis followed by primer walking utilizing newly derived sequence information. The full length S segment was amplified by using the 19C primer designed based on the conserved RNA termini of New World Arenaviruses [10], whereas the L segment was amplified in multiple sections using a variety of primers (sequences available on request). Sequence analysis of the complete S and L segments confirmed that this virus, proposed name Chapare, was a unique member of the Clade B New World arenaviruses [3,4,5,10,11]. The virus was found to be most closely related to Sabiá virus, but with 26 and 30% nucleotide difference in the complete S and L segments, and 26, 28, 15 and 22% amino acid differences for the L, Z, N and GP proteins, respectively (Tables 1 and S1). The genetic differences between Chapare virus and other Clade B viruses range from 36–40% for the complete S segment and 39–40% for the complete L segment (data not shown). These nucleotide and amino acid sequence divergence levels are in excess of those seen among strains of the same species of New World arenavirus (Tables 2 and S1) [12,13,14]. For instance, the greatest difference seen between complete S segments of virus strains is 14% (within Allpahuayo virus strains) and 10% for the complete L segment (among Machupo virus strains) [15].

Chapare virus was found to be monophyletic with Sabiá virus on phylogenetic analysis of the nucleotide or encoded amino acid sequences of the complete S or L segment (Figure 2), or *NP*, *GP*, *L* or *Z* ORFs (data not shown). No evidence of reassortment or recombination between Chapare virus and other arenaviruses was found. There is no overall change in the structure of the trees except for the previously described [4,5] switch of the Clade A/Rec viruses from Clade A for the NP gene to Clade B for the GP gene (data not shown).

The pathogenicity of the New World Clade B viruses correlates with the efficient interaction of their GP1 surface glycoproteins with the human cellular receptor, transferring receptor 1 (TfR1) [16,17]. Presumably, Chapare virus will be found to have similar TfR1 binding properties, but this remains to be confirmed. Even assuming this to be true, the diversity of the GP1 amino acid sequences of Junín, Machupo, Guanarito, Sabiá and Chapare viruses is such that one cannot easily discern the GP1 domain involved in high efficiency binding to TfR1 solely on the basis of amino acid sequence alignments.

The relationship of Chapare virus from Bolivia to Sabiá virus from Brazil is intriguing. Both these virus clearly cause HF similar to that seen with Junín, Machupo and Guanarito viruses. The single HF case associated with a naturally acquired Sabiá virus infection was reported in the community of Sabiá, in Sao Paulo, Brazil in 1990 [18]. The exact site of exposure was unclear, as was the rodent reservoir. Yellow fever was the initial suspicion in the Sabiá case and that associated with the Chapare virus infection as both had associated extensive liver necrosis. More extensive liver involvement may be a feature shared between these viruses, as it is not commonly observed with HF associated with the other New World arenaviruses (although it is occasionally seen).

Due to the difficulties of working in this resource poor rural region, initial follow up efforts in the Chapare area, did not yield a more precise description of the reported cluster of cases with similar illness, and a limited ecological study did not reveal the rodent reservoir of this virus. It is hoped that more extensive studies in the area will reveal the extent to which Chapare virus poses a public health problem in this area, and shed light on the source of human infection. In summary, three arenaviruses are now known to be present in Bolivia, namely Machupo and Latino viruses (both hosted by *Calomys callosus*) and Chapare viruses (reservoir unknown). Furthermore, both Machupo and Chapare viruses are agents of fatal hemorrhagic fever in Bolivia.

Materials and Methods

Diagnostic Amplification & Identification

Initial virus genetic detection and analysis was conducted on total RNA extracted from infected Vero E6 cells, using Tripure Isolation Reagent (Roche Applied Science, Indianapolis, IN) in a ratio of 1:5 and incubated at room temperature for a minimum of 10 min. Total RNA was isolated by using the RNaid Kit following the manufacturer's recommendations (Qbiogene Inc., Carlsbad, CA), and the extracted RNA was reconstituted in 50 μL H_2O. Broadly reactive Arenavirus primers used for initial identification

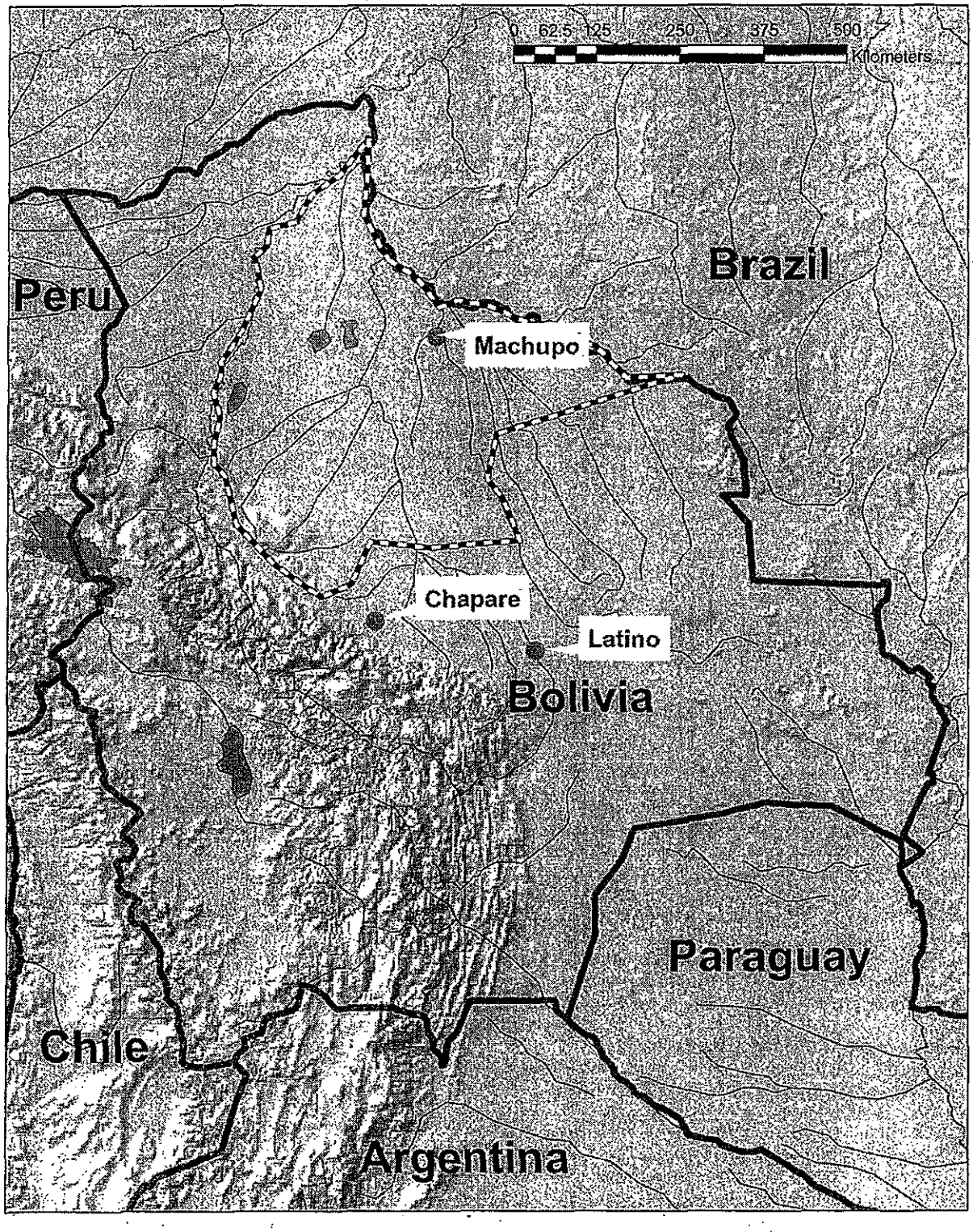


Figure 1. Map of Bolivia showing location of the Chapare virus-associated HF case relative to the Beni region where Machupo virus-associated HF cases originate. The Beni Department boundary is depicted by the checkered line. Multiple Machupo isolates have been recorded from the Beni Department. The single Latino and Chapare virus locations are labeled and represented as dots.
doi:10.1371/journal.ppat.1000047.g001

Table 1. Differences between Chapare virus and its closest relative, Sabiá, are similar to differences between other distinct species of arenavirus

Virus	Nucleotide[a]		Amino Acid[b]			
	S segment	L segment	GPC	NP	L	Z
Chapare to Sabiá	26	30	22	15	26	28
Machupo to Junín	25–27	31	25–27	11–14	25	18–20
Machupo to Tacaribe	31–32	33	32–33	19–20	27–28	21
Amapari to Guanarito	27	32	29	14	28	28–32
Paraná to Flexal	29	n/a[c]	17	21	n/a	n/a

[a]Complete nucleotide segments only
[b]Complete amino acid sequences only
[c]Complete segment or gene sequence is not available for one or both viruses
doi:10.1371/journal.ppat.1000047.t001

Table 2. Differences among strains of the same species of arenavirus

Virus	Nucleotide[a]		Amino Acid[b]			
	S segment	L segment	GPC	NP	L	Z
Allpahuayo	14	n/a[c]	2	2	n/a	n/a
Bear Canyon	3	3	2	1	2	0
Catarina	9	n/a	5	2	n/a	n/a
Flexal	0.1	n/a	0	0	n/a	n/a
Guanarito	2	n/a	1	0	n/a	11
Junín	7	3	2	4	2	1
Machupo	13	10	5	3	5	6
Pichindé	11	n/a	5	3	n/a	n/a
Whitewater Arroyo	0.4	n/a	0	1	n/a	n/a

[a]Complete nucleotide segments only
[b]Complete amino acid sequences only
[c]Complete segment or gene sequence is not available for more than one strain
doi:10.1371/journal.ppat.1000047.t002

were designed for the L polymerase gene on the L segment (L4160F, GCA GAR TTY AAA TCI AGA TT; L4393R, CCR TYI ASC CAR TCT ITI ACA TC; L4292F, GAT CAT TCI RTY GCI AAT GG; L4841R, CAI AII CCT ATA AAI CCW GAT G) [19] and the glycoprotein gene on the S segment (GP878+, GAC RTG CCW GGI GGI TAY TG; GP1126-, TAC CAA AAT TTG TGT ART TRC ART AIG G; GP1153+, CCT TAY TGY AAY TAC ACI AAA TTT TGG T; GP1396-, ATG TGY CTR TGI GTI GGI AW).

Reverse Transcription (RT) was done using 2.5 µL of RNA in a 25 µL total reaction volume and AMV RT (Promega Biosciences, San Luis Obispo, CA) at 42°C for 1 hr. Subsequent PCR amplification using FastStart Taq DNA Polymerase with GC-rich solution (Roche) was performed using 5 µL of the completed RT reaction in a 25 µL reaction volume with the following cycling conditions: 2 min at 95°C, (36 cycles of 1 min at 95°C, 1 min at 45°C, 2 min at 72°C), and a final elongation of 10 min at 72°C. Resulting DNA products were visualized and purified using a 1% agarose gel, and the Qiagen Gel Extraction Kit (Qiagen, Valencia, CA). PCR products were sequenced directly (without cloning) using the corresponding primers in a BigDye Terminator v3.1 reaction on a 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequence was further analyzed using Sequencher (Gene Codes Corporation, Ann Arbor, MI).

Complete Genome Amplification & Analysis

To obtain full length sequence for each segment, alignments of all New World arenavirus complete genomes were used to design primers for the conserved regions (available upon request). The full length S segment was generated following the Thermoscript RT-PCR system's directions (Invitrogen, Carlsbad, CA) and using the 19C primer [10]. Reverse transcription was conducted at 55°C, while the PCR profile was the same as stated above with an increased extension time of 4 minutes.

Different fractions of the full-length L RNA were amplified using 2-step or 1-step RT-PCR protocols and following the manufacturer recommendations. Briefly, cDNA was synthesized in the first approach using 10 µl of purified RNA, specific primers, dNTPs and Superscript III (Invitrogen) in 20 µl reactions. Amplification reactions were done using 5 µl of cDNA, specific primers, dNTPs and Platinum Taq DNA polymerase High Fidelity (Invitrogen) in 50 ul reactions. Alternatively, 1-step RT-PCR were performed using 5 µl of RNA, dNTPs and the enzyme blend provided by the SuperScript III One-Step RT-PCR System with Platinum Taq High Fidelity (Invitrogen) in a 50 µl reactions. Amplification reactions were analyzed in TBE/agarose gels and DNA bands purified using QIAquick Gel Extraction Kit (Qiagen). Sequencing reactions were done as described above.

Phylogenetic Analysis

All full length S and L segment sequences available in Genbank were used to compute pairwise uncorrected genetic distances using PAUP 4.0b10 (Sinauer Associates) for the following viruses: Allpahuayo, Amapari, Chapare, Flexal, Guanarito, Junín, Machupo, Paraná, Pichindé, Pirital, Sabiá, Tacaribe, Tamiami, and Whitewater Arroyo.

A representative sub-set of full length sequences (omitting multiple near identical variants of the same virus) were included in a Bayesian phylogenetic analysis. Sequence alignments were done with ClustalX [20] with manual adjustments and phylogenetic analysis was done with MrBayes3.1.2 [21] using the GTR+I+G model in 2 runs of 500,000 generations using the sequence of Pichindé virus as the outgroup.

Supporting Information

Table S1 Amino acid distances for complete L and Z genes and nucleocapsid and glycoprotein genes of the New World arenaviruses
Found at: doi:10.1371/journal.ppat.1000047.s001 (0.34 MB PDF)

Acknowledgments

The authors would like to thank Debbie L. Cannon and Jennifer B. Oliver for technical assistance, and Dr. Serena Reeder for her excellent assistance with the phylogenetic analysis presented in this work.

Author Contributions

Conceived and designed the experiments: CA JO SN. Performed the experiments: BE CA JC. Analyzed the data: BE RA PB EV CA JV JC PR TK JO SN. Contributed reagents/materials/analysis tools: SD RA PB EV

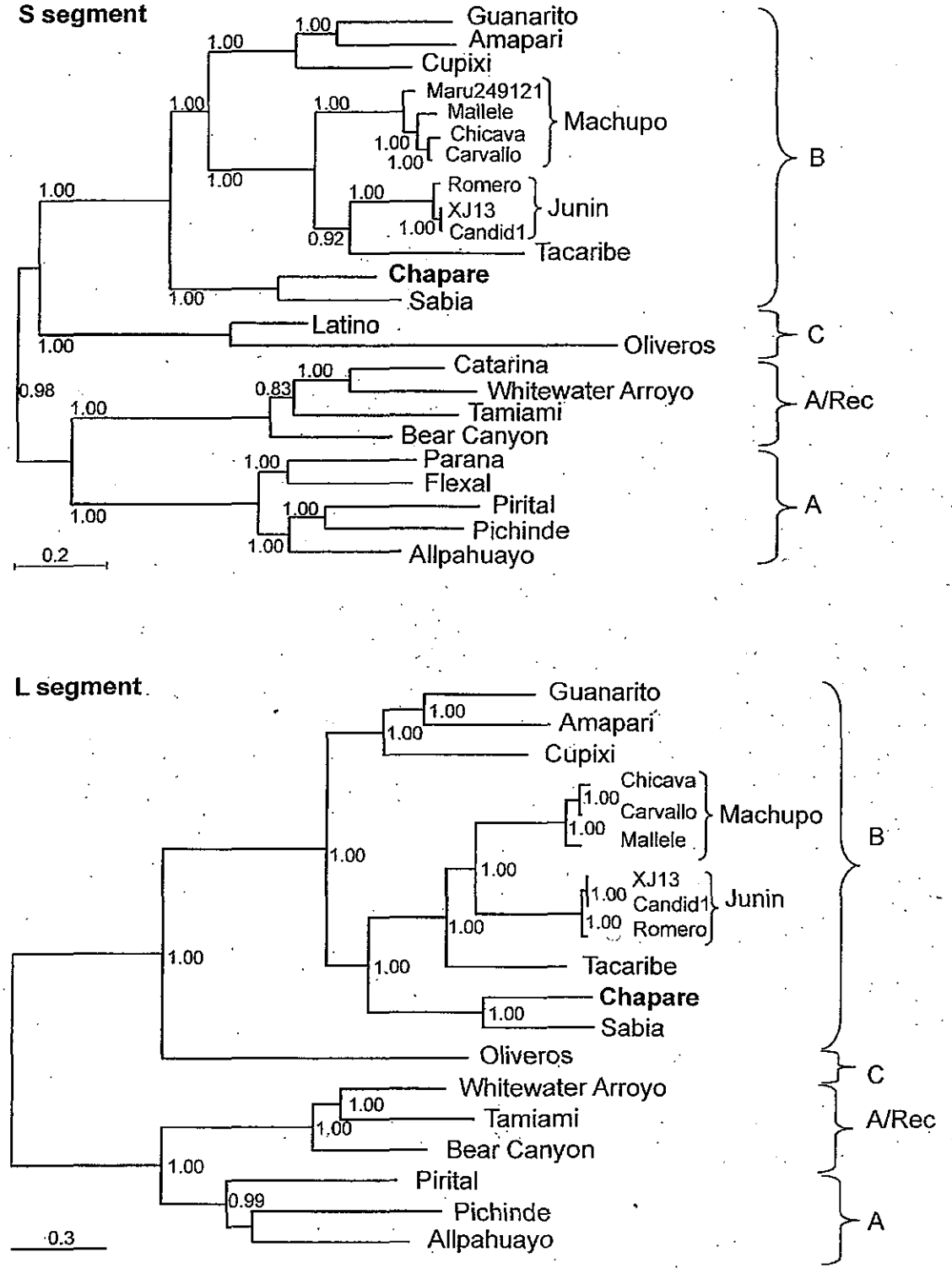


Figure 2. Phylogenetic analysis of the complete S and L RNA segments of New World arenaviruses. Complete S and L segments for New World arenaviruses were analyzed by Bayesian inference of phylogeny (MrBayes3.1.2) using the sequence of Pichindé virus as the outgroup. Multiple strains are grouped with small brackets and large brackets group the arenavirus Clades: A, A/Rec, B, and C. The Genbank accession numbers for the S segment analysis include: Allpahuayo (AY012686), Amapari (AF485256), Bear Canyon (AY924392), Catarina (DQ865245), Chapare (EU260463), Cupixi (AF512832), Flexal (AF485257), Guanarito (NC_005077), Junin (AY619641, NC_005081, AY746353), Latino (AF512830), Machupo (AY924208, AY619645, AY924202, NC_005078), Oliveros (U34248), Paraná (AF485261), Pichindé (NC_006447), Pirital (NC_005894), Sabiá (NC_006317), Tacaribe (NC_004293),

Tamiami (AF485263), and Whitewater Arroyo (AF485264). The Genbank accession numbers used for the L segment analysis include: Allpahuayo (NC_010249), Amapari (AY924389), Bear Canyon (AY924390), Chapare (EU260464), Cupixi (NC_010252), Guanarito (NC_005082), Junín (NC_005080, AY819707, AY619640), Machupo (AY624354, NC_005079, AY619644), Oliveros (NC_010250), Pichindé (NC_006439), Pirital (NC_005897), Sabiá (NC_006313), Tacaribe (NC_004292), Tamiami (AY924393), and Whitewater Arroyo (AY924395).
doi:10.1371/journal.ppat.1000047.g002

JV JC PR JO. Wrote the paper: BE SN. Initial clinician that reported the case, described the clinical course and provided specimens: SD. Clinicians involved in the case investigation: RA and EV. Participated in field investigation: PB. Participated in case investigation: JV. Edited the paper: TK. Led NMRCD investigation: JO.

References

1. Buchmeier MJ (2007) Arenaviridae. In: Knipe DMaH PM, ed. Fields Virology. 5th ed. Philadelphia: Lippincott, Williams and Wilkins. pp 1791–1828.
2. Gonzalez JP, Emonet S, de Lamballerie X, Charrel R (2007) Arenaviruses. Curr Top Microbiol Immunol 315: 253–288.
3. Bowen MD, Peters CJ, Nichol ST (1997) Phylogenetic analysis of the Arénaviridae: patterns of virus evolution and evidence for cospeciation between arenaviruses and their rodent hosts. Molecular Phylogenetics & Evolution 8: 301–316.
4. Archer AM, Rico-Hesse R (2002) High genetic divergence and recombination in Arenaviruses from the Americas. Virology 304: 274–281.
5. Charrel RN, Lemasson JJ, Garbutt M, Khelifa R, De Micco P, et al. (2003) New insights into the evolutionary relationships between arenaviruses provided by comparative analysis of small and large segment sequences. Virology 317: 191–196.
6. Salvato MS, Clegg JCS, Buchmeier MJ, Charrel RN, Gonzalez JP, Lukashevich IS, Peters CJ, Rico-Hesse R, Romanowski V (2005) Family *Arenaviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA, eds. Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses Elsevier Academic Press. pp 725–733.
7. Johnson KM, Kuns ML, Mackenzie RB, Webb PA, Yunker CE (1966) Isolation of Machupo virus from wild rodent Calomys callosus. Am J Trop Med Hyg 15: 103–106.
8. Murphy FA, Webb PA, Johnson KM, Whitfield SG, Chappell WA (1970) Arenoviruses in Vero cells: ultrastructural studies. J Virol 6: 507–518.
9. Salazar-Bravo J, Dragoo JW, Bowen MD, Peters CJ, Ksiazek TG, et al. (2002) Natural nidality in Bolivian hemorrhagic fever and the systematics of the reservoir species. Infection, Genetics & Evolution 1: 191–199.
10. Bowen MD, Peters CJ, Nichol ST (1996) The phylogeny of New World (Tacaribe complex) arenaviruses. Virology 219: 285–290.
11. Weaver SC, Salas RA, de Manzione N, Fulhorst CF, Duno G, et al. (2000) Guanarito virus (Arenaviridae) isolates from endemic and outlying localities in Venezuela: sequence comparisons among and within strains isolated from Venezuelan hemorrhagic fever patients and rodents. Virology 266: 189–195.
12. Cajimat MN, Fulhorst CF (2004) Phylogeny of the Venezuelan arenaviruses. Virus Research 102: 199–206.
13. Weaver SC, Salas RA, de Manzione N, Fulhorst CF, Travasos da Rosa AP, et al. (2001) Extreme genetic diversity among Pirital virus (Arenaviridae) isolates from western Venezuela. Virology 285: 110–118.
14. Cajimat MN, Milazzo ML, Hess BD, Rood MP, Fulhorst CF (2007) Principal host relationships and evolutionary history of the North American arenaviruses. Virology 367: 235–243.
15. Moncayo AC, Hice CL, Watts DM, Travassos de Rosa AP, Guzman H, et al. (2001) Allpahuayo virus: a newly recognized arenavirus (arenaviridae) from arboreal rice rats (oecomys bicolor and oecomys paricola) in northeastern peru. Virology 284: 277–286.
16. Flanagan ML, Oldenburg J, Reignier T, Holt N, Hamilton GA, et al. (2008) New world Clade B arenaviruses can use transferrin receptor 1 (TfR1)-dependent and -independent entry pathways, and glycoproteins from human pathogenic strains are associated with the use of TfR1. J Virol 82: 938–948.
17. Radoshitzky SR, Abraham J, Spiropoulou CF, Kuhn JH, Nguyen D, et al. (2007) Transferrin receptor 1 is a cellular receptor for New World haemorrhagic fever arenaviruses. Nature 446: 92–96.
18. Lisieux T, Coimbra M, Nassar ES, Burattini MN, de Souza LT, et al. (1994) New arenavirus isolated in Brazil. Lancet 343: 391–392.
19. Fischer SA, Graham MB, Kuehnert MJ, Kotton CN, Srinivasan A, et al. (2006) Transmission of lymphocytic choriomeningitis virus by organ transplantation. N Engl J Med 354: 2235–2249.
20. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25: 4876–4882.
21. Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574.

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	(製造承認書に記載なし)	研究報告の公表状況	Osselaer JC, Cazenave JP, Lambermont M, Garraud O, Hidajat M, Barbolla L, Tardivel R, Defoin L, Waller C, Mendel I, Raidot JP, Kandel G, De Meuter R, Fabrigli P, Dehenau D, Arroyo JL, Padrón F, Gouezec H, Corral M, Jacquet M, Sundin D, Lin L, Corash L. Vox Sang. 2008 May;94(4):315-23. Epub 2008 Jan 30.	公表国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社)			ベルギー

研究報告の概要

○アモトサレンと紫外線A波で光化学処理した血小板輸血7437件の安全性プロファイルを示す能動的血液安全監視プログラム
背景: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。輸血5106件の結果がすでに報告されている。我々はさらに7437件のPCT-PLTの結果を報告する。
方法:現行の血液安全監視プログラムの焦点は、PCT-PLT輸血に関連付けられるすべての有害事象を記録することである。有害事象データとして収集したデータは次の通り:輸血開始後の事象発現時間、臨床記述、バイタルサイン、放射線学的検査および細菌培養の結果、事象重症度(グレード0～4)、PCT-PLT輸血との因果関係。
結果:患者1400名(平均年齢60歳、範囲1～96歳)にPCT-PLTが輸血された。患者の大部分(53.4%)は、造血器腫瘍疾患を有し、従来の化学療法(44.8%)または幹細胞移植(8.6%)を要した。PCT-PLT 輸血68件が有害事象と関連付けられた。急性輸血反応(ATR)、PCT-PLT輸血との因果関係が「可能性あり」「可能性高」「確実」に分類された有害事象は発現頻度が低く(n=55, 55/7437=0.7%)、ほとんどがグレード1(即時／長期的な生命の危険なし)であった。患者39名(39/1400=2.8%)が何らかのATRを生じた。最も多く報告された自他覚症状は、悪寒、発熱、蕁麻疹、呼吸困難、悪心、嘔吐であった。5件の有害事象が重症(グレード2以上)であったが、PCT-PLT輸血との因果関係は認められなかった。PCT-PLTの複数回曝露は、ATR発現の確率を増加させなかった。輸血関連急性肺障害と死亡は報告されなかった。
結論:PCT-PLT輸血に関連したATRは発現頻度が低く、ほとんどが軽度であった。

報告企業の意見	今後の対応
アモトサレンと紫外線A波で光化学処理した血小板の輸血は、副作用は発現頻度が低く、ほとんどが軽度であったとの報告である。	日本赤十字社では8項目の安全対策の一つとして、不活化技術の導入について、各不活化技術の効果、血液成分への影響、製造作業への影響などについて評価検討を行っている。外国での不活化実施状況や効果、新たな技術、副作用等の情報収集も含め総合的に評価し、導入について関係機関と協議しているところである。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「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

Vox Sanguinis (2008) 94, 315–323

ORIGINAL PAPER

DOI: 10.1111/j.1423-0410.2007.01035.x

An active haemovigilance programme characterizing the safety profile of 7437 platelet transfusions prepared with amotosalen photochemical treatment

J. C. Osselaer,[1] J. P. Cazenave,[2] M. Lambermont,[3] O. Garraud,[4] M. Hidajat,[5] L. Barbolla,[6] R. Tardivel,[7] L. Defoin,[1] C. Waller,[2] I. Mendel,[2] J. P. Raidot,[2] G. Kandel,[2] R. De Meuter,[3] P. Fabrigli,[4] D. Dehenau,[5] J. L. Arroyo,[6] F. Padrón,[6] H. Gouezec,[7] M. Corral,[8] M. Jacquet,[9] D. Sundin,[9] L. Lin[9] & L. Corash[9]

[1]*Blood Transfusion Center, Cliniques Universitaires de Mont Godinne, Universite Catholique de Louvain, Yvoir, Belgium*
[2]*EFS Alsace, Strasbourg, France*
[3]*Service du Sang, Belgian Red Cross, Erasme University Hospital, Brussels, Belgium*
[4]*EFS Auvergne Loire, St. Etienne, France*
[5]*Blood Transfusion, AZ St Jan AV, Brugge, Belgium*
[6]*Centro de Hemoterapia y Hemodonacion de Castilla y Leon, Valladolid, Spain*
[7]*EFS Bretagne, Rennes, France*
[8]*Hospital Universitario de Salamanca, Salamanca, Spain*
[9]*Cerus Corporation, Concord, CA, USA*

Vox Sanguinis

Background An active haemovigilance programme was implemented to survey adverse events (AE) associated with transfusion of platelets photochemically treated with amotosalen and ultraviolet A (PCT-PLT). The results of 5106 transfusions have already been reported. Here we report the results of an additional 7437 PCT-PLT transfusions.

Methods The focus of this ongoing haemovigilance programme is to document all AEs associated with PCT-PLT transfusion. Data collected for AEs include: time of event after starting transfusion, clinical descriptions, vital signs, results from radiographs and bacterial cultures, event severity (Grade 0–4) and causal relationship to PCT-PLT transfusion.

Results One thousand four hundred patients (mean 60 years, range 1–96) received PCT-PLT transfusions. The majority of the patients (53·4%) had haematology–oncology diseases and required conventional chemotherapy (44·8%) or stem cell transplantation (8·6%). Sixty-eight PCT-PLT transfusions were associated with AE. Acute transfusion reactions (ATR), classified as an AE possibly related, probably related, or related to PCT-PLT transfusions were infrequent ($n = 55$, 55/7437 = 0·7%) and most were of Grade 1 severity. Thirty-nine patients (39/1400 = 2·8%) experienced one or more ATRs. The most frequently reported signs/symptoms were chills, fever, urticaria, dyspnoea, nausea and vomiting. Five AEs were considered severe (≥ Grade 2); however, no causal relationship to PCT-PLT transfusion was found. Repeated exposure to PCT-PLT did not increase the likelihood of an ATR. No cases of transfusion-related acute lung injury and no deaths due to PCT-PLT transfusions were reported.

Conclusions Routine transfusion of PCT-PLT is well-tolerated in a wide range of patients. ATRs related to PCT-PLT transfusion were infrequent and most were of mild severity.

Key words: PCT, platelets, haemovigilance, safety, INTERCEPT.

Received: 25 October 2007, revised 24 December 2007, accepted 24 December 2007, published online 30 January 2008

Correspondence: Lily Lin, Cerus Corporation, 2411 Stanwell Drive, Concord, CA 94520, USA
E-mail: llin@cerus.com

Introduction

INTERCEPT Blood System™ uses a photochemical treatment methodology [PCT: amotosalen plus ultraviolet A (UVA) light] to inactivate viruses, bacteria, protozoa, and leucocytes in platelet (PLT) and plasma components. The PLT system received CE Mark registration in Europe in 2002. Several centres in Belgium, Spain, Norway and Italy began routine production of PCT-PLT in 2003. An active haemovigilance programme was immediately implemented to prospectively collect information on PCT-PLT transfusions administered to patients in routine clinical settings. Prior to CE Mark registration, the safety data of PCT-PLT were primarily obtained from controlled clinical trials with a limited number of patients and predetermined clinical and safety end-points [1–3]. The postmarketing haemovigilance programme provided a means to extend the characterization of the safety profile of PCT-PLT in routine use and in a broad patient population. The results of the first 5106 PCT-PLT transfusions have already been reported [4]. With additional centres in Belgium, Spain and France starting with the routine production of PCT-PLT, the database of this haemovigilance programme has been expanded [5].

In March 2007, the Canadian Blood Services and Héma-Québec organized a consensus conference to provide recommendations and guide decision-making about new pathogen inactivation technologies [6]. The panel, consists of nine healthcare professionals and members of the public, stressed the importance of postmarketing surveillance studies in the introduction of new technologies for blood safety. The panel recommended that specific studies should be mandated by the regulatory authorities and supported by the manufacturers and/or the blood suppliers. Postmarketing surveillance for adverse reactions to pathogen inactivation products should be linked to the national haemovigilance systems if possible. Depending on the new pathogen inactivation technologies implemented, specific additional surveillance outcomes may be identified. The panel also suggested that chronically transfused patients might serve as an ideal surveillance population to identify long-term toxicities of pathogen-inactivated products.

The active haemovigilance programme described in this study is in concordance with these recommendations. Although this programme is not directly linked to a specific country haemovigilance system nor designed to replace any existing haemovigilance system, the format of data collection is modelled after the data collection format of the French haemovigilance system for documentation of transfusion incidents [7]. The focus of the current programme is on all adverse events (AE), serious or non-serious, occurring after the start of PCT-PLT transfusion. Following the recent report of 5106 PCT-PLT transfusions [4], here we report the results of an additional 7437 transfusions of PCT-PLT.

Materials and methods

General study design

This was a prospective observational active haemovigilance study. The objective of this study was to document the transfusion safety profile for approximately 7500 PCT-PLT components prepared with the INTERCEPT Blood System™ for platelets (Cerus Europe BV, Leusden, the Netherlands). These components were prepared in three centres in Belgium (CTS UCL Mont Godinne, CTS Brabant-Hainaut and AZ Sint Jan AV), three centres in France (EFS-Alsace, EFS-Auvergne-Loire and EFS-Bretagne), and one centre in Spain (CHEMCYL Valladolid) and administered to thrombocytopenic patients under standard clinical practice in hospitals. There were no randomization requirements, no inclusion criteria and no exclusion criteria of patients other than the need to receive a platelet transfusion. Baseline demographical information was collected on all study participants. Patients were assigned a centre-specific study number to preserve anonymity.

Patients who received transfusions of PCT-PLT were monitored for any AEs after the start of each platelet transfusion, which is consistent with European Haemovigilance Network recommendations for surveillance of AE to transfusion of labile blood components, and with those of national transfusion services [7,8]. However, in this study, reporting was obligatory for all PCT-PLT transfusions in each participating clinical site. A transfusion report was required for each PLT transfusion regardless of whether or not an AE occurred. In case of occurrence of an AE, additional clinical and biological information was collected to allow diagnosis and assessment of causality and severity. The data in the final database were anonymous and were reported on a per-transfusion basis as well as on a per-patient basis. Transfusions associated with serious AEs were reported in greater detail.

Study report forms

The report form used for this haemovigilance programme was developed on the basis of haemovigilance report forms already in use. Information was collected in several broad categories: patient demographic/diagnosis data, platelet component characteristics, transfusion events and documentation of all AEs following transfusion. An acute transfusion reaction (ATR) was defined as an AE possibly related, probably related, or related to a PCT-PLT transfusion.

AEs were graded for clinical severity within the following categories: Grade 0, isolated dysfunction without clinical or biological manifestation; Grade 1, absence of immediate or long-term life-threatening effects; Grade 2, long-term life-threatening effects; Grade 3, immediate life–threatening effects; and Grade 4, death. For each transfusion, the following

signs, symptoms and specific clinical syndromes were evaluated: fever, chills, cardiac arrhythmia, hypotension, itching, urticaria, skin rash, jaundice, pulmonary oedema, bronchospasm, dyspnoea, respiratory distress, nausea, vomiting, lower back pain, chest pain, abdominal pain, and shock. Any other findings could be entered as free text including refractoriness to platelet transfusion and transfusion-related acute lung injury. The following available clinical signs were recorded before and after each transfusion: temperature, blood pressure and heart rate. Abnormal clinical laboratory values, results of diagnostic procedures (chest X-ray) and bacterial cultures from patient and blood component sources were recorded when associated with an AE following a PCT-PLT transfusion.

Preparation of platelet components

Platelet components were collected by apheresis or from whole blood-derived buffy-coat procedures according to each centre's standard operating procedures. Volunteer donors were screened and tested for transfusion-transmitted pathogens according to each centre's standard operating procedures in compliance with respective national regulations. All components were leucocyte reduced, either by filtration (Sepacell PLS-5A, Asahi Biomedical, Tokyo, Japan) or process leucodepletion (Amicus Cell Separator, Fenwal, La Chatre, France; Haemonetics MCS+, Haemonetics, Braintree, MA, USA). Platelet components containing 2·5 to 6·0 × 10^{11} platelets were suspended in approximately 35% plasma and 65% InterSol™ (Fenwal) and prepared with amotosalen (nominal final concentration 150 μM) and a 3 J/cm^2 UVA light treatment (320–400 nm) according to the manufacturer's instructions for use (Cerus Europe BV). After treatment, PCT-PLTs were stored up to either 5 or 7 days under temperature-controlled conditions (22 ± 2 °C) before release for transfusion depending on country-specific regulations. PCT-PLTs were transfused before the expiration period of 5 days in France and Spain or 7 days in Belgium. PCT-PLTs were not cultured for bacterial contamination prior to release, and PCT was used in place of γ-irradiation for prevention of transfusion-associated graft-versus-host disease in all sites except EFS-Bretagne and EFS-Auvergne-Loire.

Platelet transfusion

PCT-PLT components for transfusion were ordered according to standard indications within each institution. The investigator was requested to report all AEs occurring after starting transfusion without time limitation. The severity of each AE (Grade 0 to 4) and the relationship of each AEs to the preceding platelet transfusion were assessed by the investigator. Serious adverse events were reported in greater detail with a narrative for each event.

Statistical analyses

All statistical analyses, summary tables and data listings were generated using SAS® version 8·2. The primary assessment of safety was the proportion of ATR for the transfusions reported. The safety profile of PCT-PLT transfusions included information on: the number of PCT-PLT transfusions by patient; the patient population profile; the characteristics of the PCT-PLT transfused, and the characteristics of the AE following platelet transfusion.

Data were analysed on a per-transfusion basis as well as on a per-patient basis. All PCT-PLT transfusions administered to a patient were included in the full analysis population, whether or not an AE was observed. Data were summarized for each parameter using descriptive statistics (mean, standard deviation, median, and range).

Statistical tests were performed for the exploration of risk factors only (multivariate logistic regression at 10% significant level). The variables included in the analysis are patient gender, age, previous transfusion history, type of platelet concentrate, γ-irradiation, antigen-matching and primary diagnosis. Variables with descriptive statistics were tested for *P* values and odds ratio. The number and proportion (%) of transfusions with one or more AEs were summarized overall, by seriousness and by relationship to platelet transfusion. Corresponding 95% confidence intervals (CIs) were calculated.

The non-survival analysis method is a univariate analysis of the number of transfusions received before the first occurrence of an AE. Only patients with at least one AE were considered in this analysis.

Results

Distribution of transfusions

A total of 7437 PCT-PLT transfusions were documented between May 2005 and January 2007 and constitute the full analysis population. The distribution of transfusion reports were: 3057 (41·1%) from CTS UCL Mont Godinne, 2048 (27·5%) from EFS-Alsace, 899 (12·1%) from CTS Brabant-Hainaut, 572 (7·7%) from EFS-Auvergne-Loire, 440 (5·9%) from AZ Sint Jan AV, 381 (5·1%) from CHEMCYL, and 40 (0·5%) from EFS-Bretagne.

Patient demographics

A total of 1400 patients underwent transfusion (Table 1). The majority of the patients were male (61·3%) and the mean age was 60 years (range < 1–96 years). Haematology–oncology diseases treated by chemotherapy (44·8%) and stem cell transplantation (8·6%) constituted 53·4% of the primary diagnoses and therapies among the transfused population. A significant number of patients receiving platelet transfusion (17·2%)

Table 1 Patient and transfusion demographics

	Patient characteristics (n = 1400)	Transfusion characteristics (n = 7437)
Gender (n, %)		
Male	858 (61·3%)	4354 (58·5%)
Female	542 (38·7%)	3082 (41·4%)
Unknown		1 (< 0·1%)
Age (years)		
Mean ± SD	60·0 ± 17·8	
Median	63	
(minimum–maximum)	(<1–96)	
Location of transfusion		
Intensive care unit		1145 (15·4%)
Outpatient		382 (5·1%)
Regular ward		5908 (79·4%)
Unknown		2 (< 0·1%)
Haematology–oncology patients	748 (53·4%)	5463 (73·5%)
Conventional chemotherapy	627 (44·8%)	4481 (60·3%)
Stem cell transplant	121 (8·6%)	982 (13·2%)
Surgery patients	241 (17·2%)	480 (6·5%)
Cardiovascular surgery	209 (14·9%)	349 (4·7%)
Solid organ transplantation	32 (2·3%)	131 (1·8%)
Other diagnoses	397 (28·4%)	859 (11·6%)
Missing diagnosis	14 (1·0%)	635 (8·5%)
History of a previous transfusion		
Yes	837 (59·8%)	5029 (67·6%)
No	398 (28·4%)	1927 (25·9%)
Unknown	165 (11·8%)	481 (6·5%)
If 'Yes' – did they experience a transfusion-related adverse event?[a]		
Yes	53 (6·3%)	382 (7·6%)
No	779 (93·0%)	4639 (92·2%)
Unknown	5 (0·6%)	8 (0·2%)

[a]For per-patient basis, the denominator is 837; for per-transfusion basis, the denominator is 5029.

were undergoing cardiovascular surgery or solid organ transplantation. Other diagnoses included haematology–oncology diseases not treated by chemotherapy and/or stem cell transplantation and surgery other than cardiovascular surgery and solid organ transplantation.

Of all patients, 837 patients (59·8%) had already received another blood product before the first PCT-PLT transfusion (Table 1). Among these patients, 53 patients (6·3% of 837) had a history of a transfusion reaction of some type in the past.

Platelet component demographics

Most of the PCT-PLT units were manufactured from apheresis platforms (4822, 64·8% vs. 2615, 35·2% for buffy-coat products). The majority of the PCT-PLTs (7357, 98·9%) were not treated with γ-irradiation [9]. Among the 7437 PCT-PLTs transfused, only 2·5% (189 units) of platelet units were human leucocyte antigen-matched products.

A large proportion of the PCT-PLT components (5908, 79·4%) were transfused in non-intensive care hospital wards (Table 1). Intensive care units and day-hospital units were the location for 15·4 and 5·1% of the PCT-PLT transfusions (1145 and 382 units, respectively). While most of the PCT-PLT components (5463, 73·5%) were administered to haematology–oncology patients, only 480 PCT-PLT components (6·5%) were administered to surgery patients.

The majority of the PCT-PLT components (5029, 67·6%) were administered to patients who had already received another blood component before the first PCT-PLT transfusion (Table 1). Among these transfusions, 382 (7·6% of 5029) PCT-PLT components were transfused to patients reported to have experienced at least one transfusion reaction in the past.

Number of transfusions per patient

The range of PCT-PLT transfusions per patient was 1 to 129, with an average of 5·3 ± 10·8 (median: 2) transfusions per patient. Of the 1400 patients who received PCT-PLT transfusions, 529 patients (37·8%) received only one PCT-PLT transfusion during this study period, 418 patients (29·9%) received two to three transfusions, and 453 patients (32·4%) received more than four PCT-PLT transfusions during the study. The majority of patients who received multiple transfusions had a primary diagnosis of haematology–oncology diseases treated by chemotherapy and/or stem cell transplantation.

Two patients from CTS UCL Mont Godinne received more than 100 transfusions analysed in this haemovigilance plan. One 56-year-old man (J01-636) who was treated by conventional chemotherapy for haematology–oncology disease received 129 PCT-PLT components within an 8-month period (from April 2006 to November 2006). One 72-year-old woman (J01-071) who was also treated by conventional chemotherapy for haematology–oncology disease received 107 PCT-PLT components within a 10-month period (from August 2005 to November 2006).

Adverse events following PCT-PLT transfusion

On a per-transfusion basis, 68 (0·9% of 7437 transfusions, 95% CI: 0·7–1·2%) transfusions were associated with an AE (Table 2). Of which, 55 (0·7% of 7437 transfusions, 95% CI: 0·6–1·0%) were classified as ATR possibly related, probably related, or related to PCT-PLT transfusion. Only five events were classified as serious AEs (0·07%, 95% CI: 0·0–0·2%), and were judged as probably unrelated to the PCT-PLT transfusion based on the observation of alternative causes for symptoms and no evidence of causal relationship to the platelet transfusion. No cases of transfusion-related acute lung injury and no deaths due to PCT-PLT transfusions were reported.

Table 2 Clinical characteristics of adverse events (AE)

	On a per-transfusion basis n (% = $n \times 100/7437$)				On a per-patient basis n (% = $n \times 100/1400$)			
	Any AEs	AE attributed to platelets (ATR)[b]	SAE[a]	SAE attributed to platelets[a,b]	Any AEs	AE attributed to platelets (ATR)[b]	SAEs[a]	SAE attributed to platelets[a,b]
Number with at least one event	68 (0·9%)	55 (0·7%)	5 (< 0·1%)	0 (0·0%)	45 (3·2%)	39 (2·8%)	4 (0·3%)	0 (0·0%)
Signs/Symptoms[c]								
Fever	8 (0·1%)	6 (< 0·1%)	0 (0%)	–	7 (0·5%)	5 (0·4%)	0 (0%)	–
Chills	45 (0·6%)	40 (0·5%)	2 (< 0·1%)	–	31 (2·2%)	28 (2·0%)	1 (< 0·1%)	–
Itching	2 (< 0·1%)	2 (< 0·1%)	0 (0%)	–	1 (< 0·1%)	1 (< 0·1%)	0 (0%)	–
Hypotension	1 (< 0·1%)	0 (0%)	1 (< 0·1%)	–	1 (< 0·1%)	0 (0%)	1 (< 0·1%)	–
Urticaria	14 (0·2%)	14 (0·2%)	0 (0%)	–	13 (0·9%)	13 (0·9%)	0 (0%)	–
Skin rash	5 (< 0·1%)	5 (< 0·1%)	0 (0%)	–	4 (0·3%)	4 (0·3%)	0 (0%)	–
Dyspnoea	8 (0·1%)	6 (< 0·1%)	1 (< 0·1%)	–	8 (0·6%)	6 (0·4%)	1 (< 0·1%)	–
Respiratory distress	1 (< 0·1%)	0 (0%)	1 (< 0·1%)	–	1 (< 0·1%)	0 (0%)	1 (< 0·1%)	–
Nausea/vomiting	8 (0·1%)	5 (< 0·1%)	3 (< 0·1%)	–	5 (0·4%)	3 (0·2%)	2 (0·1%)	–
Lower back pain	6 (< 0·1%)	1 (< 0·1%)	0 (0%)	–	2 (0·1%)	1 (< 0·1%)	0 (0%)	–
Chest/abdominal pain	1 (< 0·1%)	1 (< 0·1%)	0 (0%)	–	1 (< 0·1%)	1 (< 0·1%)	0 (0%)	–
Shock	4 (< 0·1%)	0 (0%)	4 (< 0·1%)	–	3 (0·2%)	0 (0%)	3 (0·2%)	–
Tachycardia	4 (< 0·1%)	3 (< 0·1%)	1 (< 0·1%)	–	3 (0·2%)	2 (0·1%)	1 (< 0·1%)	–
Other	14 (0·2%)	11 (0·1%)	3 (< 0·1%)	–	12 (0·9%)	10 (0·7%)	3 (0·2%)	–

[a]Serious adverse event (SAE): long-term life threatening, immediate life threatening or death.

[b]Causal relationship that was possibly related, probably related, or related to PCT-PLT transfusion.

[c]Number of signs/symptoms can exceed number of AE due to multiple observed signs/symptoms per AE.

On a per-patient basis, 45 patients (3·2% of 1400 patients) who received at least one transfusion of PCT-PLT experienced the 68 AEs following PCT-PLT transfusions (Table 2). Only 39 patients (2·8% of 1400 patients) experienced the 55 ATRs attributed to the PCT-PLT transfusion. Four patients experienced serious AEs following transfusion; however, no causal relationship to PCT-PLT transfusion could be established.

All AEs regardless of the relationship with the PCT-PLT transfusion occurred within 4 h after the start of the platelet transfusion (mean time: 0·3 ± 0·51 h, 0–3·3 h). The majority of AEs (64, or 94·1% of 68 AEs) occurred in patients who were not premedicated. The other four AEs occurred in patients who were premedicated with antipyretic or antihistaminic drugs, or corticosteroids.

Characteristics of clinical signs and symptoms associated with adverse event

On a per-transfusion basis, the most frequently observed symptoms/signs (≥ 0·1% of the total 7437 transfusions) were fever, chills, urticaria, dyspnoea, nausea and/or vomiting (Table 2). The individual incidence of each of the following signs/symptoms was < 0·1%: itching, hypotension, skin rash, respiratory distress, lower back pain, chest or abdominal pain, shock and tachycardia. All additional symptoms included in the category of other, such as refractoriness to platelet transfusion, hypertension, cephalea, pain in the leg, flush, malaise, cyanosis, oxygen desaturation and volume overload were also reported but with an individual incidence of less than 0·1%. Most of ATRs were described principally as Grade 1 chills and urticaria (Table 2).

On a per-patient basis, the most frequently observed symptoms/signs (≥ 0·5% of the total 1400 patients) were fever, chills, urticaria and dyspnoea (Table 2). Approximately 0·1–0·4% of the population (from 2 to 5/1400) experienced the following signs/symptoms: skin rash, nausea/vomiting, shock, lower back pain and tachycardia. Clinical refractoriness to transfusion, hypertension, headache and flushing were additional symptoms reported in the category of 'other'. Less than 0·1% of the study population (only 1/1400) experienced the following signs/symptoms such as hypotension, itching, respiratory distress and chest/abdominal pain. Symptoms such as pulse increase, leg pain, cyanosis, oxygen desaturation, malaise and/or volume overload were also reported in the category of 'other'. Most of the ATRs consisted of various combinations of fever (0·4%), chills (2·0%), urticaria (0·9%), skin rash (0·3%), dyspnoea (0·4%), nausea/vomiting (0·2%), tachycardia (0·1%) and others symptoms (0·7%) (Table 2).

Serious adverse events following platelet transfusion

During the course of this surveillance, five serious AEs were reported following transfusion of PCT-PLT (0·07%, 95% CI: 0·0–0·2). These serious AEs were assessed by the investigators as being 'unrelated or probably unrelated' to the PCT-PLT transfusions and were attributed to progression of underlying illness.

Patient B01-201 was admitted to hospital for a presumed pulmonary infection postchemotherapy. Additional co-morbidities at the time of admission were septic shock, acute renal insufficiency, neutropenia and thrombocytopenia. Intravenous (i.v.) antibiotic therapy was initiated and multiple transfusions of blood products (including PCT-PLT) were administered. One hour after administration of the second platelet unit, the patient complained of dyspnoea, respiratory distress was found to be hypotensive and tachycardic. Severe volume overload was determined to be the aetiology and treatment with oxygen, diuretics, and dialysis was initiated. The event was assessed by the investigator to be unrelated to the PCT-PLT transfusion.

Patient J01-382 experienced chills, nausea and sudden hypotension during transfusion with PCT-PLT. Prior to this, the patient had received at least four PCT-PLT transfusions with no AE. The transfusion was stopped and the patient was treated with i.v. fluids and recovered. Four days later, the patient experienced a second hypotensive episode after transfusion, which was spontaneously resolved. Subsequent to this, the patient received 19 additional PCT-PLT transfusions without any clinical sequelae. This patient did not receive any angiotensin-converting enzyme (ACE) inhibitors. Based on the patient's history and the lack of transfusion reaction with the subsequent transfusions, the investigator assessed both of these events as probably unrelated to PCT-PLT transfusion.

Patient J01-516 was admitted for ischaemic cardiomyopathy and underwent double vessel coronary artery bypass graft (CABG). The patient's postoperative recovery was complicated by a significant decrease in blood pressure, which occurred 10 min after start of transfusion of PCT-PLT. Despite vasopressor support and a 6-min period of circulatory arrest, the patient's condition continued to deteriorate and he died. Cause of death was attributed to an aortic dissection with major disseminated intravascular coagulopathy and mesenteric infarct and was assessed by the investigator as unrelated to the PCT-PLT transfusion.

Patient J01-780 experienced a hypotensive episode, cyanosis, oxygen desaturation and nausea approximately 30 min after receipt of PCT-PLT. The patient received oxygen therapy to treat the event and recovered. The patient had received two units of PCT-PLT before and one unit after this event with no adverse reactions. The patient had a history of hypotensive episodes, which occurred in the absence of transfusions. Based on the patient's history, the event was assessed by the investigator as probably unrelated to the PCT-PLT transfusion.

Risk factors associated with adverse event

The risk for AE was not correlated with the patient gender, age, or antigen-matching. The risk for AE for patients who already had been transfused before the first PCT-PLT transfusion appeared trending higher compared to patients who did not have any transfusion history; however, the difference did not reach statistical significance ($P = 0·0675$; odds ratio: 1·875; 95% CI: 0·956–3·648). Buffy-coat-derived platelets were associated with a lower risk for AE compared to apheresis products ($P = 0·0305$; odds ratio: 0·473; 95% CI: 0·240–0·932). Irradiated PCT-PLTs were of similar risk for AE compared to non-irradiated PCT-PLTs ($P = 0·0848$; odds ratio: 6·344; 95% CI: 0·776–51·862). No trending can be concluded because, of the total 7437 platelet transfusions, only 80 PCT-PLT components were γ-irradiated in EFS-Bretagne and EFS-Auvergne-Loire. Haematology-oncology patients treated with conventional chemotherapy were at a higher risk for AE compared to the other patients ($P \leq 0·0001$; odds ratio: 7·660; 95% CI: 3·014–19·467).

Number of transfusions prior to the first adverse event

Among the 45 patients who experienced at least one AE, repeated exposure to PCT-PLT did not appear to increase the likelihood of a transfusion reaction (Table 3). By using the non-survival analysis method (a subset analysis for patients with any AE only), the mean number of transfusions before first AE occurrence was 8·8 ± 10·1 (median = 4, minimum = 0 and maximum = 37).

Discussion

In accordance with the recommendations made by the panel of the Canadian Consensus Conference, an active haemovigilance programme has been implemented in Europe to document the occurrence of AE following transfusion of PCT-PLT [6]. To date, two reports have been prepared. The first report was on the transfusion of 5106 PCT-PLT components administered to patients in five European centres from October 2003 to December 2005 [4]. The second report as described here was on additional 7437 transfusions of PCT-PLT administered to patients in seven European centres between May 2005 and January 2007. This represents a total of 12 543 independent transfusions documented to date. There are no overlaps of PCT-PLT transfusions reported in this haemovigilance programme.

Overall, the incidence of ATR attributed to transfusion of PCT-PLT in both of the haemovigilance reporting periods was infrequent either on a per-transfusion basis (0·8% first period

Table 3 Number of PCT-PLT transfusions per patient prior to the first adverse event (AE)

Number of PCT-PLT transfusions per patient until first occurrence of AE	Full analysis population (n = 1400)
1	11 (0·79%)
2	6 (0·43%)
3	3 (0·21%)
4	3 (0·21%)
5	1 (0·07%)
6–10	9 (0·64%)
11–19	6 (0·43%)
≥ 20	6 (0·43%)
N (non survival analysis method)	45
Mean ± SD	8·8 ± 10·1
Median	4
Minimum–maximum	0–37

vs. 0·7% second period) or on a per-patient basis (4·9% first period vs. 2·8% second period). The slightly higher occurrence of ATR per patient in the first reporting period was not surprising, because the mean number of transfusions per patient (7·8 ± 16·2) [4] was greater than those observed in the second period (5·3 ± 10·8). All ATRs were mild in severity and of Grade 1 or lower. No serious AE from both study periods were attributed specifically to transfusion of PCT-PLT.

On a per-transfusion basis, the prevalence of ATR has been reported in the literature to range from 18 to 31%; however, these studies were conducted some years ago with variable methods of platelet preparation [10–13]. More recently, the incidence of moderate and severe ATR has been reported from the trial to reduce alloimmunization to platelets (TRAP) study, which examined 8769 platelet transfusions in 598 patients during induction therapy for acute leukaemia [14]. In the TRAP study, platelet components were prepared by four methods: unfiltered pooled whole blood-derived platelets in plasma; filtered pooled whole blood-derived platelets in plasma; unfiltered pooled whole blood-derived platelets in plasma treated with ultraviolet B illumination to reduce human leucocyte antigen sensitization; and filtered apheresis platelets in plasma. None of these components were prepared with additive solutions. The overall incidence of ATR was 2·2% of transfusions, and 22% of patients experienced at least one ATR. In comparison to the TRAP trial, the current study in which all grades of reactions were reported, both the proportion of transfusions associated with a reaction was lower (0·7%) as well as the proportion of patients (2·8%) experiencing at least one ATR. The use of 65% InterSol, a platelet additive solution, in the preparation of PCT-PLT may partially contribute to the reduction in the observed incidence of ATR [15].

The incidence of ATR in this study can be compared to data from the haemovigilance network in France [7]. In France, data were reported for transfusion reactions, with an incidence of four events per 1000 platelet components (0·4%), during 2 years in which the reporting system was first implemented. However, this may be an underestimate since each whole blood platelet concentrate in a pool was tabulated as an individual component transfusion. More recently, Kerkhoffs *et al.* [16] compared the incidence of transfusion reactions for leucoreduced pooled platelet components in plasma and plasma with additive solution in a study of 168 patients and 765 transfusions. They observed an incidence of 5·5% of transfusions with reactions for platelets in plasma vs. 2·4% of transfusions for platelets in a mixture of plasma and additive solution. On a per-patient basis, 9·5% of patients transfused with platelets in plasma plus additive solutions had reactions compared to 15·5% of patients supported with platelets suspended in plasma. These results further support the role of the platelet additive solution, InterSol, in the reduction of ATR observed in this study.

During the conduct of this study, an interim analysis of 2497 PCT-PLT transfusions administered to 606 patients in the three regions of France (EFS-Alsace, EFS-Auvergne-Loire and EFS-Bretagne) was performed [5]. Of the 606 patients, the predominant recipients of PCT-PLT were haematology–oncology patients (46·2%); 39·9% treated with chemotherapy and 6·3% treated with stem cell transplantation. These proportions were only slightly lower than those in the overall study population of 1400 patients, yet only four of the 606 patients (0·7%) reported an AE, including one serious AE of volume overload classified as unrelated to PCT-PLT transfusion. This low rate of AE observed in the French regions could contribute to the overall low incidence of ATR per patient in this study.

Premedication in patients did not play a role in the overall low incidence of ATR reported in this study. Information on premedication was only requested in case of AE occurrence. Of the 68 transfusions with occurrence of at least one AE, only two antipyretic, two antihistaminic and one corticosteroid were prescribed to patients. For the majority (64/68, or 94·1%) of these transfusions, patients were not premedicated.

The active haemovigilance programme described here is a prospective observational study, which was designed to assess the safety profile of PCT-PLT in routine clinical practice. The data from this programme represent the largest prospective experience to date for recording potential AE associated with platelet transfusions compared to prior studies of retrospective design and limited in size [10,16–18]. The present study was designed to be consistent with European haemovigilance practices in which reporting of all grades of transfusion-associated reactions has been emphasized [7,8]. In contrast to other haemovigilance studies, obligatory reporting for all platelet transfusions was required irrespective of whether or not an AE was observed. The current study focused on AE that could be linked to PCT-PLT transfusions after starting transfusion, but there were no specific limitations

on when adverse events could be reported following transfusion. Based on the patient population supported with platelet transfusion, the study was designed to capture repeated transfusions of PCT-PLT within patients to determine potential effects of repeated exposure to this new type of platelet component.

A limitation of the present study is the absence of a concurrent control group receiving conventional platelet components with which to determine a comparative baseline incidence of ATR. However, because reporting is obligatory, the expected outcomes of this active haemovigilance study are the increase in clinical experience with transfusion of PCT-PLT, the detection of unexpected AE following PCT-PLT transfusions in patient populations and for indications that were not studied previously in a formal clinical trial environment, and the establishment of a safety database for future reference.

In the current study, which was specifically designed to capture all grades of transfusion reactions, the prevalence of ATR per transfusion, was at the lower range of those reported in studies with conventional components. Prior exposure to PCT-PLT transfusions did not increase the likelihood of an ATR. The overall incidence of ATR was lower than that previously reported either on a per-transfusion or on a per-patient basis. Based on experience in a broad patient population, platelet components prepared with amotosalen photochemical treatment were well-tolerated in routine clinical practice.

Acknowledgements

The authors would like to thank the following individuals of Fenwal, formerly Baxter R&D Europe, who assisted in the implementation of the INTERCEPT Blood System at the blood centres, provided critical input into the scope and design of the haemovigilance programme, and training of site personnel on data entering: Jocelyne Flament, Jean-Marc Payrat, Nick Moerman, Valentine Franck, and Veronique Mayaudon. Quintiles provided data management and statistics service for this haemovigilance programme. This study was supported in part by Cerus Corporation. Three authors (D. Sundin, L. Lin and L. Corash) were affiliated with and held stock or stock options of Cerus Corporation during the conduct of this study. Three authors (J. C. Osselaer, J. P. Cazenave and O. Garraud) served on Cerus's European Scientific Advisory Board.

References

1 van Rhenen DJ, Gulliksson H, Cazenave JP, Pamphilon D, Ljungman P, Klüter H, Vermeij H, Kappers-Klunne M, de Greef G, Laforet M, Lioure B, Davis K, Marblie S, Mayaudon V, Flament J, Conlan M, Lin L, Metzel P, Buchholz D, Corash L: Transfusion of pooled buffy coat platelet components prepared with photochemical pathogen inactivation treatment: the euroSPRITE trial. *Blood* 2003; 101:2426–2433

2 McCullough J, Vesole DH, Benjamin RJ, Slichter SJ, Pineda A, Snyder E, Stadtmauer E, Lopez-Plaza I, Coutre S, Strauss RG, Goodnough LT, Fridey JL, Raife T, Cable R, Murphy S, Howard F, Davis K, Lin J-S, Metzel P, Corash L, Koutsoukos A, Lin L, Buchholz D, Conlan M: Therapeutic efficacy and safety of platelets treated with a photochemical process for pathogen inactivation: the SPRINT trial. *Blood* 2004; 104:1534–1541

3 Janetzko K, Cazenave JP, Kluter H, Kientz D, Michel M, Beris P, Lioure B, Hastka J, Marblie S, Mayaudon V, Lin L, Lin J-S, Conlan MG, Flament J: Therapeutic efficacy and safety of photochemically treated apheresis platelets processed with an optimized integrated set. *Transfusion* 2005; 45:1443–1452

4 Osselaer J, Messe N, Hervig T, Bueno JL, Castro E, Espinosa A, Lacone A, Junge K, Jacquet M, Flament J, Corash L: A prospective observational cohort safety study of 5106 platelet transfusions prepared with photochemical treatment. *Transfusion* 2008: doi: 10.1111/j.1537-2995.2008.01643.x

5 Garraud O, Fabrigli P, Tardivel R, Gouezec H, Waller C, Mendel I, Raidot JP, Kandel G, Isola H, Cazenave JP: Hemovigilance for transfusion of INTERCEPTTM platelets in routine therapeutic use: experience in Etablissement Francais du Sang (EFS) blood centers. *Vox Sang* 2007; 93(S1):32

6 Klein HG, Anderson D, Bernardi MJ, Cable R, Carey W, Hoch JS, Robitaille N, Sivilotti MLA, Smaill F: Pathogen inactivation: making decisions about new technologies. Report of a consensus conference. *Transfusion* 2007; 47:2338–2347

7 Andreu G, Morel P, Forestier F, Debeir J, Rebibo D, Janvier G, Hervé P: Hemovigilance network in France: organization and analysis of immediate transfusion incident reports from 1994–1998. *Transfusion* 2002; 42:1356–1364

8 Faber JC: Haemovigilance in Europe: now and tomorrow; in Rouger P, Hossenlopp C (eds): *Blood Transfusion in Europe*. Paris, Elsevier SAS, 2005:213–232

9 Corash L, Lin L: Novel processes for inactivation of leukocytes to prevent transfusion-associated graft-versus-host disease. *Bone Marrow Transplant* 2004; 33:1–7

10 Chambers LA, Kruskall MS, Pacini DG, Donovan LM: Febrile reactions after platelet transfusion: the effect of single versus multiple donors. *Transfusion* 1990; 30:219–221

11 Heddle NM, Kalma L, Singer J, Richards C, Fedak P, Walker I, Kelton JG: The role of the plasma from platelet concentrates in transfusion reactions. *N Engl J Med* 1994; 331:625–628

12 Mangano MM, Chambers LA, Kruskall MS: Limited efficacy of leukopoor platelets for prevention of febrile transfusion reactions. *Am J Clin Pathol* 1991; 95:733–738

13 Heddle NM, Klama LN, Griffith L, Roberts R, Shukla G, Kelton JG: A prospective study to identify the risk factors associated with acute reactions to platelet and red cell transfusions. *Transfusion* 1993; 33:794–797

14 Enright H: Factors influencing moderate to severe reactions to PLT transfusions: experience of the TRAP multicenter clinical trial. *Transfusion* 2003; 43:1545–1552

15 Isola H, Kientz D, Wiesel ML, Laforet M, Ohlmann P, Waller C, Mendel I, Raidot JP, Kandel G, Cazenave JP: Implementation of photochemical inactivation (INTERCEPT Blood System for platelets) in a regional blood center. *Vox Sang* 2007; 93(S1):165

16 Kerkhoffs JH, Eikenboom JC, Schipperus MS, van Wordragen-Vlaswinkel RJ, Brand R, Harvey MS, de Vries RR, Barge R, van Rhenen DJ, Brand A: A muticenter randomized study of the efficacy of transfusions with platelets stored in platelet additive solution II versus plasma. *Blood* 2006; 108:3210–3215

17 deWildt-Eggen J, Nauta S, Schrijver JG, van Marwijk Kooy M, Bins M, van Prooijen HC: Reactions and platelet increments after transfusion of platelet concentrates in plasma or an additive solution: a prospective, randomized study. *Transfusion* 2000; 40:398–403

18 Heddle NM, Blajchman MA, Meyer RM, Lipton JH, Walker IR, Sher GD, Constantini LA, Patterson B, Roberts RS, Thorpe KE, Levine MN: A randomized controlled trial comparing the frequency of acute reactions to plasma-removed platelets and prestorage WBC-reduced platelets. *Transfusion* 2002; 42:556–566

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 5 月 22 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	人ハプトグロビン		研究報告の公表状況	WHO Representative Office in China /19 May 2008	公表国 中国	
販売名（企業名）	ハプトグロビン注－ヨシトミ(ベネシス)					

研究報告の概要

2008 年 3 月後半、阜陽市（安徽省）で一人の病院臨床医が、重篤な肺炎と症状の悪化が急に進む未就学児症例が連続して 3 例発症したことによって、危険性を察知した。4 月中旬までに、15 人の小児が同様の重篤な疾患で死亡した。現地および国の専門家によって行われた疫学、臨床、検査及び病理のエビデンスに基づき、その疾患はエンテロウイルス 71(EV71)が引き起こす手足口病(HFMD)であることが 4 月 23 日に確認された。回顧的な症例調査により、小児が手、足及び口に皮膚発疹と水泡を示し、同じエリアで同じ時期に大流行していることが明らかになった。

阜陽市では、3 月 1 日から 5 月 9 日の間に 6,049 例の HFMD が報告され、353 例が重篤、そして 22 例が死亡した(致死率 0.4%)。回顧的な症例所見により、3 月 1 日から 4 月 22 日の間に 302 例の発生が確認され、第 1 症例は早くも 3 月 10 日に発生したことが明らかになった。発症日を基にした患者数は、4 月初めに増加し始め、4 月 28 日にピークに達した。阜陽市で報告された HFMD 症例数は、5 月 5 日以後減少した。阜陽市で報告された 6,049 症例中、性比は 1.9：1 であった。年齢範囲は生後 28 日から 18 歳であり、3 歳以下が 78%をしめた。阜陽市の全ての地区/郡は、HFMD 症例を報告し、3 つの地区（Yingzhou、Yingdong と Yingquan）に半分以上の症例が集中した。疫学的な調査において、22 の致死症例の間に接触はみられなかったが、症例の家庭の環境的調査でこれらの家庭内の低い衛生状態が明らかになった。

2008 年 1 月 1 日から 5 月 9 日までの 61,459 の HFMD 症例と 36 例の死亡は、中国本土の疾患報告制度を通して報告された。5 月 2 日に届出が必要になった後、報告症例数は急激に増加し、ほとんどすべての行政区から報告された。最も多く症例を報告した行政区は、広東（11,374）、安徽（9,235）、浙江（6,134）、山東（4,566）と河南（3,230）であった。

非ポリオエンテロウイルスは、ありふれたウイルスで世界中に存在する。感染は多くの場合症状を示さず、気づかれないが、これらウイルスは、時折通常より多くの患者に臨床的症状が現れ、時々死亡を伴う。1997 年以降、アジア太平洋地域で EV71 HFMD の多くの大流行があった。中国では、大流行が、1998 年に台湾省(100,000 以上の症例、78 例の死亡)で、2007 年に山東省(38,606 症例、14 例の死亡)であった。

報告企業の意見	今後の対応
中国の阜陽市（安徽省）でエンテロウイルス 71 による手足口病が大流行し、中国全土でも手足口病が流行しているとの報告である。 本剤の原料血漿は国内献血血漿のみであり、中国からは輸入していない。 また、万一原料血漿にエンテロウイルスが混入したとしても、EMC 及び CPV をモデルウイルスとしたウイルスバリデーション試験成績から、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、Cohn の低温エタノール分画で得た画分から人ハプトグロビンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及びろ過膜処理(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。

BENE2008-009

Report on the Hand, Foot and Mouth Disease Outbreak in Fuyang City, Anhui Province and the Prevention and Control in China

May 2008

Report prepared by
the Chinese Center for Disease Control and Prevention
the Office of the World Health Organization in China

List of Acronyms

CDC	Center for Disease Control and Prevention
EV71	Enterovirus 71
HFMD	Hand, foot and mouth disease
ICU	Intensive Care Unit
IHR(2005)	The International Health Regulations (2005)
MOH	Ministry of Health
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SARS	Severe Acute Respiratory Syndrome
WHO	World Health Organization

Contributors

Chinese CDC staff conducted the outbreak investigation, performed the data analysis and drafted the document. WHO staff helped reviewing the data and contributed to the revision of the manuscript.

Table of Contents

Executive Summary

In late March 2008, a hospital clinician was alarmed by the occurrence of 3 consecutive deaths of pre-school children presented with severe pneumonia and rapid deterioration in Fuyang City, Anhui Province. Up until mid-April, 15 children have died of similar severe illness.

Through an investigation conducted by local and national experts, the disease was confirmed on April 23 as hand, foot and mouth disease (HFMD) caused by enterovirus 71 (EV71) based on epidemiological, clinical, laboratory and pathological evidence. Retrospective case investigation revealed that a simultaneous outbreak had occurred in the same area with children presenting skin rashes and blisters over hand, foot, and mouth.

Anhui is an inland province located in the central part of Southeastern China. Fuyang City is located in the Northwest region of Anhui Province and has a total population of 9.76 million. The city covers a total area of 9,700 km^2 with one of the highest population densities in Mainland China (1,000 per square km). In Fuyang City, from March 1 to May 9, there have been 6,049 reported cases of HFMD of which 353 were severe and 22 were fatal (case fatality rate 0.4%). Retrospective case-finding identified 302 cases that occurred between March 1 and April 22, and revealed that the first case occurred as early as March 10. The number of cases according to date of onset began to increase in early April, and peaked on April 28. The number of reported HFMD cases in Fuyang City decreased after May 5.

Among the 6,049 reported cases in Fuyang City the gender ratio was 1.9:1. The age range varied between 28 days to 18 years of age, with 78% of the cases being 3 years of age or younger. All districts/counties in Fuyang City reported HFMD cases, with more than half the cases concentrated in 3 districts (Yingzhou, Yingdong and Yingquan). Epidemiological investigation revealed no contact between the 22 fatal cases, but environmental investigation of the cases' households revealed poor hygienic and sanitary conditions among these families.

The clinical symptoms of the mild HFMD cases are those typical for the disease: rashes on hands and feet, mouth and buttocks, fever and general malaise. All fatal cases presented an acute onset of fever and influenza-like-illness without catarrhal syndrome. The cases' condition rapidly deteriorated, developing tachypnea, cyanosis, and some presented seizures. All fatal cases died of serious complications such as neurogenic pulmonary oedema due to EV-71 infection.

Testing initial cases for a variety of diseases, including seasonal and avian influenza A/H5N1 and SARS did not reveal any conclusive results. Subsequently, additional testing by Chinese Center for Disease Control and Prevention (CDC) was performed and several

expert consultations were conducted. On April 23, a variety of specimens such as pharyngeal swabs, lung puncture fluid, lung tissues, and blood from 5 out of 12 fatal cases (42%), tested EV71 nucleic acid positive by RT-PCR. EV71 genotype C4 viruses were isolated from specimens of both mild and fatal cases. These sequences from mild and fatal cases exhibited high homogeneity. Isolated EV71 virus strain gene sequences were uploaded to GenBank on May 7.

From January 1 to May 9, 2008, 61,459 HFMD cases and 36 deaths were reported through the disease reporting system in Mainland China. The number of reported cases increased sharply after the disease became notifiable on May 2, and with cases being reported from nearly all provinces. The 5 provinces with the highest number of reported cases are Guangdong (11,374), Anhui (9,235), Zhejiang (6,134), Shandong (4,566) and Henan (3,230).

Because of the change in reporting policy and increased awareness of the general public about the disease, it is expected that there will be an increase in the number of reported HFMD cases in the upcoming weeks and months from Anhui Province and the rest of China.

Non-polio enteroviruses are common and exist worldwide. Although infection often has no symptoms and goes unnoticed, these viruses are also associated with occasional outbreaks in which a larger-than-usual number of patients develop clinical disease, sometimes with fatal consequences.

The initial high case fatality rate of the Fuyang City outbreak, 2.9% (18/610) from March 1 to April 23, was likely attributed to the following factors: rapid disease progression, late clinical presentation, and limited local medical capacities. Although there is no specific treatment for enterovirus infections and a vaccine is not currently available, once the aetiology of the disease was known and early treatment was provided to the severe patients, the case fatality rate decreased considerably to 0.07% (4/5439) from April 24 to May 9, due to enhanced surveillance and implementation of prevention and control measures.

There have been a number of outbreaks of EV71 HFMD in the Asia-Pacific region since 1997. In China, outbreaks have been reported in Taiwan Province in 1998 (>100,000 cases and 78 deaths) and in Shandong Province in 2007 (38,606 cases and 14 deaths).

The Government of China has shown its strong technical and political commitment to control the disease in Anhui and other provinces. In addition to enhanced surveillance, training was provided to clinicians and public health workers to improve the treatment success rate by increasing early identification and treatment of severe HFMD patients. Guidelines were written to enable early admission of severe cases to the hospital, and critical cases to the paediatric ICU. After that, the case fatality rate has decreased substantially. In addition, to prevent HFMD, the Ministry of Health started a nationwide health campaign, stressing the need for personal hygiene, in particular hand washing

practices.

In the future, China is devoted towards enhancing the above-mentioned measures for the prevention and control of HFMD in China. As part of the efforts to implement the International Health Regulations, IHR(2005), China will further strengthen the early warning system by immediate notification of clustering of clinically abnormal and severe cases, as well as increasing international collaboration and information exchange.

Section 1 - Investigation of HFMD Outbreak in Fuyang City, Anhui Province

I. Background

Anhui is an inland province located in the central part of Southeastern China, (29°41′~34°38′N, 114°54′~119°37′E). Two rivers, Yangtze and Huhe, run through the province, which covers an area of 139,600 km^2. Anhui Province is geographically composed of flatlands with a subtropical climate and has an average annual rainfall accumulation of about 900mm. Fuyang City is located in the Northwest region of Anhui Province and has jurisdiction over 8 counties (district, city) and 172 municipalities. The total population is 9.76 million, including approximately 1.5 million migrant workers that work outside of the city. The city covers a total area of 9,700 km^2 with one of the highest population densities in Mainland China.

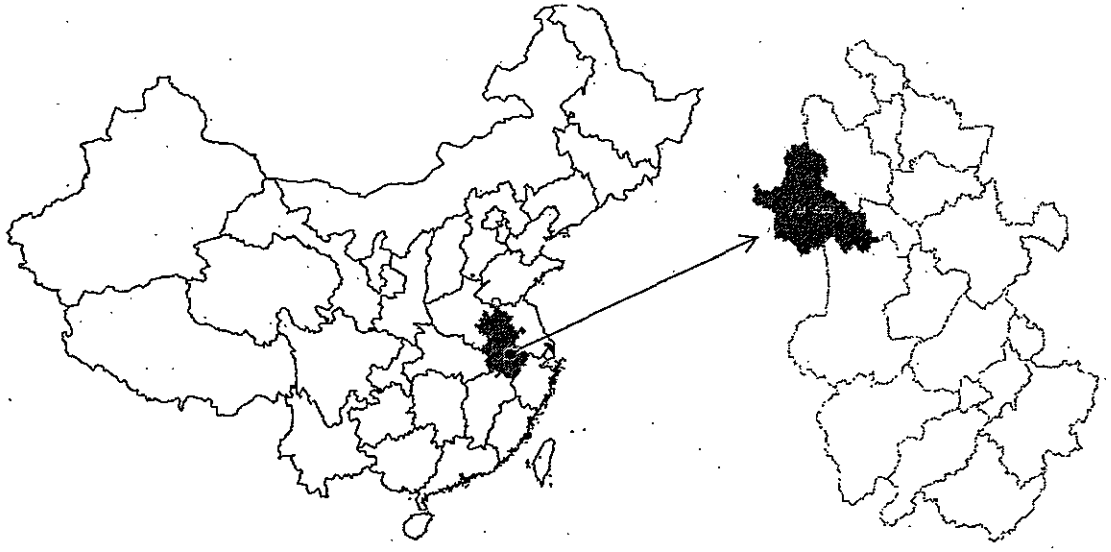


Figure 1. Geographic location of Fuyang City, Anhui Province in China.

II. Outbreak identification and investigation – chronology of events

1. Outbreak identification and reporting

From March 27 to 29 of 2008, 3 infants with severe pneumonia were admitted to the paediatric unit of Fuyang First People's Hospital. All 3 cases died despite medical treatment. These abnormal circumstances alarmed the health-care staff and were reported to the Fuyang Health Bureau.

2. Anhui provincial health department organized investigation

After receiving the report on March 31, Anhui Provincial Health Bureau subsequently dispatched 3 expert groups that included epidemiologists, clinicians and laboratory experts to conduct a field investigation. However, the etiology could not be confirmed at the time, and on April 15, the Anhui Health Bureau asked for assistance from the Ministry of Health (MOH).

3. MOH expert groups assist investigation and control

After receiving the report on April 15, MOH immediately dispatched an expert team comprised of epidemiologists, laboratory experts, clinicians and pathologists. The group arrived in Fuyang City on the morning of April 16 to assist with the epidemiological investigation and with the implementation of prevention and control measures. Afterwards, more experts were sent to Fuyang City to participate in the outbreak investigation and prevention and control.

4. Detection, reporting and treatment of severe cases

Since April 17, based on the main clinical manifestations of early fatal cases, a case definition for severe case screening was formulated and continuously revised in order to detect severe cases at an early stage and increase treatment effectiveness. All health-care facilities in the jurisdiction area were requested to evaluate the admitted suspect cases.

Severe cases are defined as having two of following clinical manifestations:

1. Continuous high fever.
2. Weakness, vomiting, irritability, etc.
3. Abnormal White Blood Cell count (WBC).
4. High blood glucose level.
5. Poor blood circulation of limbs.

All detected severe cases should be admitted for further monitoring and receive in-patient treatment. All the severe cases detected by these criteria were subjected to further investigation and analysis.

5. Laboratory testing and autopsy findings

From April 19 to 21, Chinese CDC performed Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) tests on samples from 12 fatal cases and 11 mild HFMD cases. Five of the fatal cases and 8 of the mild cases tested positive for EV71. Gene sequencing of samples from fatal and mild cases strongly exhibited homogeneity. Findings of the autopsies conducted on three fatal cases strongly suggest viral infection.

6. Etiology confirmation and outbreak information dissemination

On April 22, Chinese CDC organized an expert group composed of epidemiological, clinical, laboratory and pathology experts to review the existing evidence. The experts concluded that enterovirus 71 (EV71) was the main pathogen of the HFMD outbreak in Fuyang City, Anhui Province.

On April 23, MOH confirmed the experts' findings and recommendations. On the same day, Anhui Health Bureau disseminated the Fuyang City EV71 outbreak information on its website.

7. Surveillance and review of HFMD

Based on the results of the epidemiological investigation and etiology study, Fuyang City started HFMD case surveillance reporting on April 22, requesting all township and county or higher level healthcare facilities to report cases on a daily basis. Each day, surveillance output is reported through the submission of reporting forms. Since May 2, the web-based national disease surveillance and information management system also includes HFMD.

HFMD was not previously categorized as a notifiable infectious disease. In order to assess the local HFMD situation prior to the reclassification of HFMD, the MOH expert team conducted a HFMD retrospective review of data from all health care facilities in the jurisdiction area and were able to identify 302 unreported cases. The HFMD case inclusion criteria were as follows:

Any child under the age of 7 that sought medical care in Fuyang City between March 1 and April 21, 2008 which showed the following symptoms:

- Skin rash or blisters on hand, foot, or buttock, *and* fever, *in the absence of* measles, rubella, chicken pox and other febrile eruption diseases
- Skin rash or blisters on hand, foot, or buttock, *and* ulcers on the mouth or mucous membrane, *in the absence of* drug-related rash or allergy.

III. Investigation Results of Outbreak in Fuyang City

1. Characteristics of the outbreak

From March 1 to May 9, 2008, there were 6,049 HFMD cases reported in Fuyang City, of which 3,023 were hospitalized, 353 were severe and 22 were fatal. Hospitalized cases accounted for 50% of cases, and severe cases accounted for 6%. The incidence rate[1] was 69.6/100,000; while the case fatality rate was 0.4%. The 6,049 reported HFMD cases include 302 HFMD cases that occurred from March 1 to April 22 and were identified through retrospective investigation.

A. Descriptive analysis of reported cases

1) Epidemiological data

The gender ratio of the 6,049 reported cases in Fuyang City was 1.9:1 with 3,938 male cases and 2,111 female cases. The age range of the reported cases in Fuyang City varied between 28 days to 18 years of age; while the highest number of cases was found in children 3 years of age or younger (4,708 cases, 78% of all cases). (See Table 1).

Table 1. HFMD cases by age group and gender from March 1 to May 9, 2008 in Fuyang City

Age group (years)	Male		Female		Total	
	No. of cases	Incidence rate (‰)	No. of cases	Incidence rate (‰)	No. of cases	Incidence rate (‰)
0-	450	7.05	265	4.49	715	5.82
1-	1083	17.85	578	10.36	1661	14.27
2-	964	16.26	505	9.27	1469	12.91
3-	579	9.85	284	5.26	863	7.65
4-	366	6.20	166	3.06	532	4.69
5-	225	3.76	126	2.29	351	3.06
6-	102	1.78	67	1.66	169	1.73
7-	50	1.50	33	1.49	83	1.49
8-	42	0.88	18	0.57	60	0.76
9-	19	0.33	10	0.26	29	0.30
10-	15	0.04	22	0.07	37	0.05
11-15	40	0.07	33	0.07	73	0.07
16-20	1	0.00	2	0.01	3	0.00
20-	0.00	0.00	0.00	0.00	0.00	0.00
Unknown	2	-	2	-	4	-
Total	3938	0.91	2111	0.52	6049	0.70

All districts/counties of Fuyang City have reported cases with the highest number of cases in Yingzhou, Yingdong and Yingquan districts. The total number of cases reported by these

[1] HFMD cases reported by routine and enhanced surveillance system during the period, divided by the total population in Fuyang city

districts accounted for 56% (3,288 cases) of the total number of reported cases in Fuyang City. The incidence rates were also high in these three mentioned districts. (See Figure 2).

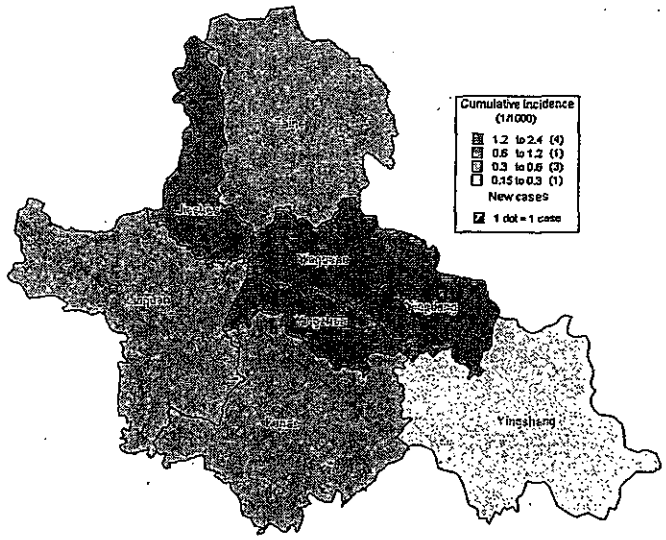


Figure 2.Incidence rate by districts/counties of Fuyang City, from March 1 and May 9, 2008

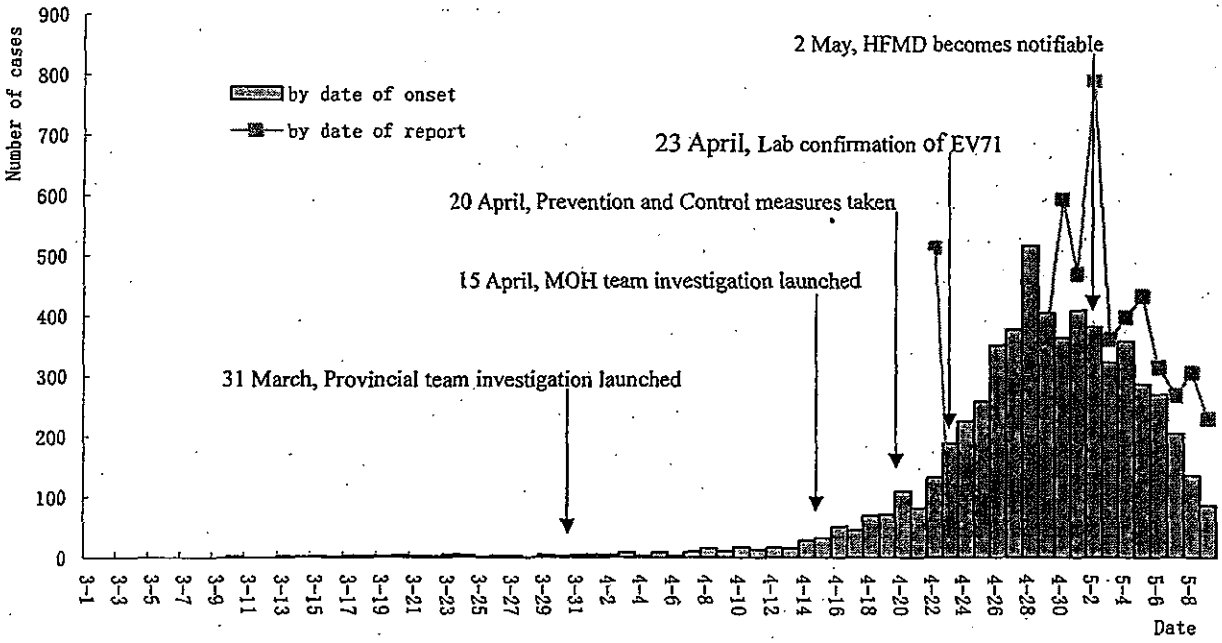


Figure3. The number of HFMD cases by date of onset and date of reporting from March 1to May 9, 2008 in Fuyang City

Figure 3 shows the distribution of the number of HFMD cases by date of onset and date of reporting between March 1 and May 9, 2008 in Fuyang City. Although the first case of HFMD occurred on March 10, the number of cases by date of onset only began to increase in early April with a rapid increase between April 16 and April 28. After April 28, the number of cases by date of onset decreased while the number of reported cases by date of reporting continued increasing until May 5 after which it decreased as well. Figure 4 shows

the total number of hospitalizations and discharges of HFMD cases per day between April 25 and May 7 in Fuyang City. The daily number of hospitalizations of HFMD cases, which had been increasing since April 29, has been decreasing since May 7.

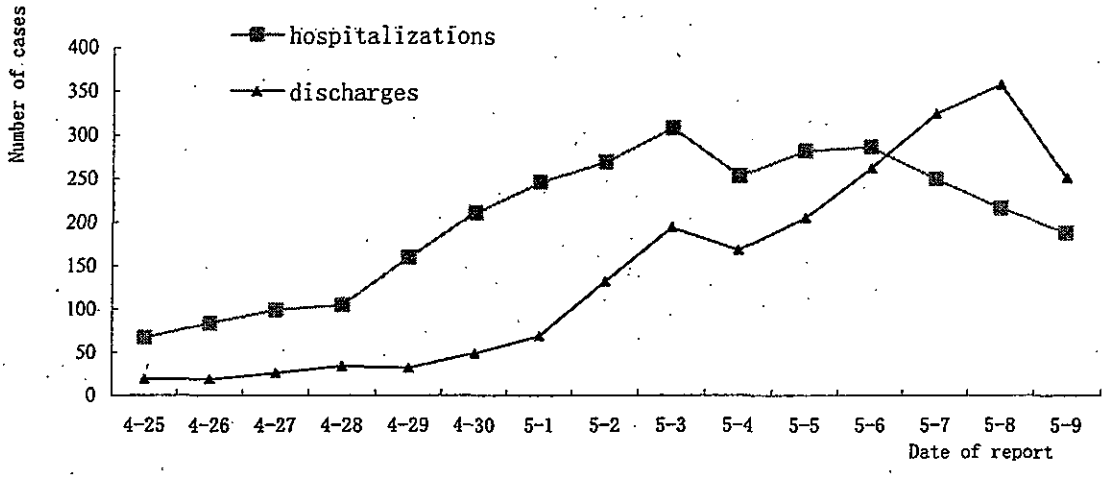


Figure 4. Daily number of hospitalizations and discharges of HFMD cases from April 25 to May 9 in Fuyang City.

2) Clinical symptoms

The clinical symptoms of 65 HFMD cases hospitalized in Fuyang No.2 Hospital were analyzed. The main clinical symptoms included rash, fever, general malaise, cough, and vomiting. Rashes (vesicular lesions/ulcers) were mostly localized on hands and feet (99%), mouth (80%) and buttocks (42%). Some cases had rashes around the anus, on their face or all over their bodies. (See Table 2). Routine blood tests were conducted on blood specimens collected from 39 of the 65 HFMD cases studied. The obvious abnormality reported was an elevated White Blood Cell count (WBC) in 15 (39%) of the samples tested.

Table 2. Clinical symptoms of hospitalized HFMD cases in Fuyang No.2 hospital (n=65).

Clinical symptom	Number of cases	Proportion (%)
Rash	65	100
Fever	46	70.8
General malaise	20	30.8
Coughing	12	18.5
Vomiting	8	12.3
Nasal discharge	6	9.2
Convulsion	4	6.2
Nasal occlusion	2	3.1
Diarrhea	2	3.1
Stiff neck	2	3.1
Pharyngalgia	1	1.5
Myoclonic twitching	1	1.5

Figure 5. Comparison of HFMD critical case prior (left) and after (right) medical treatment was provided at Fuyang No.2 hospital.

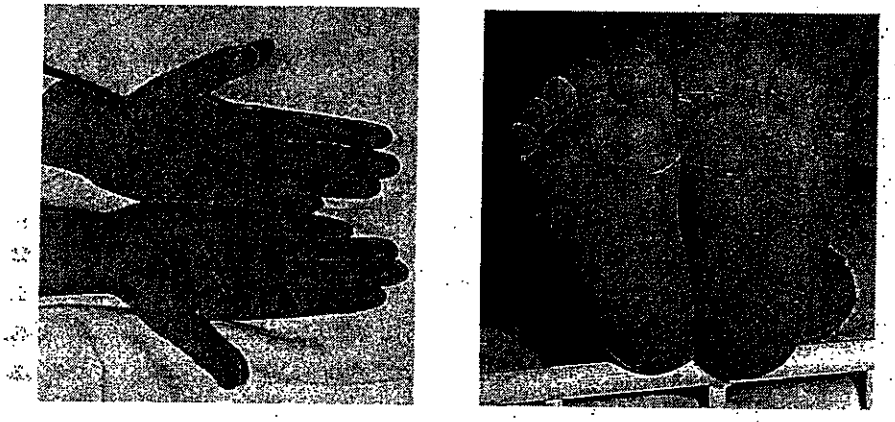


Figure 6. The rash of HFMD cases (on hand and foot)

B. Descriptive analysis of the initial fatal cases

1) Epidemiological data

As of May 9, there have been 8 consecutive days without any fatalities reported by Fuyang City (See Figure 7). For the 22 fatal cases reported to date in Fuyang City, the gender distribution was 1:1, and age ranged between 3 months and 3 years. The number of fatal cases in age groups 0-, 1-, 2-, 3- was 6, 8, 7, 1, which accounted for 28%, 36%, 31% and 5% of cases, respectively. Fatal cases were reported in 5 districts and counties: Yinzhou (9, 41%), Yingquan (6, 27%), Funan (4, 18%), Linquan (2, 9%) and Yindong (1, 5%). The majority of fatal cases occurred in rural areas. Figure 5 shows the distribution over time according to date of onset of the fatal cases reported in Fuyang City between March 23 to April 29.

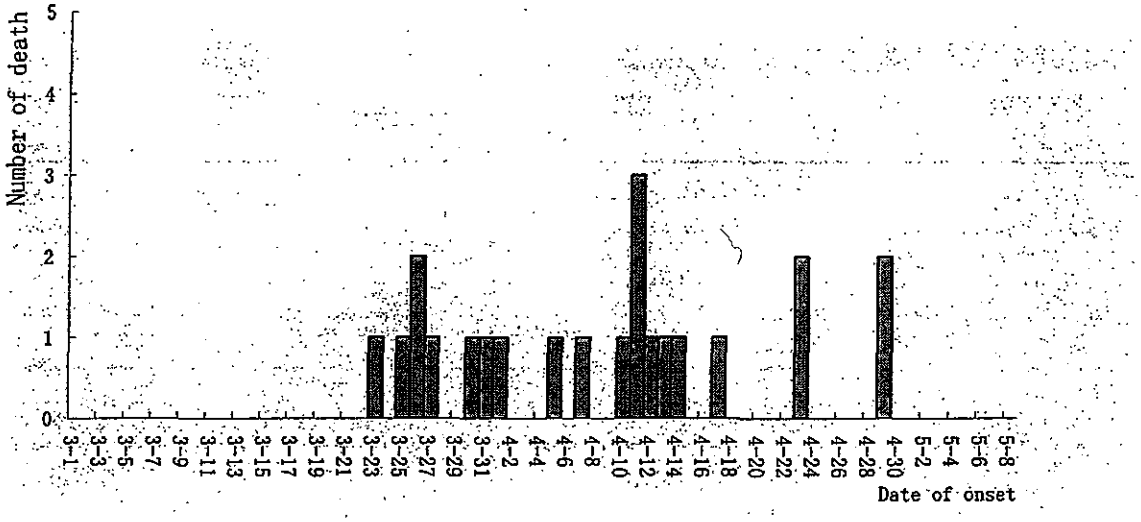


Figure 7. Fatal cases (n=22) by date of onset from March 23 to April 29 in Fuyang City.

2) Clinical symptoms

Data from 15 out of 22 fatal cases were analyzed. All cases were infants and had an acute onset of fever and influenza-like illness without catarrhal syndrome. Following general treatment guidelines for infectious respiratory diseases, local village or private doctors treated the patients with antibiotics without visible results. The condition of the cases deteriorated, developing tachypnea, cyanosis, and some presented seizures with foaming at the mouth of white or pink color. (See Table 3). Most hospitalized cases were preliminary diagnosed as severe pneumonia. Despite proactive medical procedures taken, the severe cases deteriorated rapidly and died. The mean duration between onset of symptoms and the hospitalization was 2 days; between hospitalization and time of death, 10 hours; and between onset of symptoms and time of death, 3 days. Further information on clinical conditions of the patients and their treatment will be discussed in an upcoming report.

Table 3. Clinical symptoms of fatal HFMD cases in Fuyang No.2 hospital (n=15).

Clinical symptom	Number of cases	proportion%
Fever	15	100
Tachypnea	14	93.3
Oral cyanosis	12	80.0
Pink foaming at the mouth	9	60.0
Coughing	7	46.7
Vomiting	8	53.3
Myoclonic twitching	3	20.0
Rash on palm, sole	6	40.0
Nasal discharge	2	13.3
Stiff neck	2	13.3

3) Exposure information

Epidemiological investigation revealed no contact between the 22 fatal cases. All cases were infants who had remained at home during the 2 weeks before the onset of symptoms. One case had been vaccinated within 1 month prior to onset of symptoms. Environmental investigation of the cases' households showed poor hygienic and sanitary conditions. All affected families had their own water well and none reported abnormalities after food consumption. In addition, no livestock die-off was reported by the affected families or in their village.

2. Laboratory testing and autopsy findings

A. Laboratory testing of fatal cases

Between March 31 and April 16, the Anhui CDC tested 53 specimens (including whole blood, blood serum, pharyngeal swab and tissue samples), collected from fatal HFMD cases, and tested negative for the presence of seasonal influenza, avian influenza A/H5N1, Severe Acute Respiratory Syndrome (SARS).

On April 18, the Institutes of Infectious Diseases and the Viral Diseases Prevention and Control of Chinese CDC received from Anhui CDC the previously collected specimens: pharyngeal swabs, lung puncture fluid, lung tissues, and blood. Laboratory testing for the presence of bacteria and virus was performed immediately on these samples. Specimens from 5 out of 12 fatal cases (42%) tested EV71 nucleic acid positive by RT-PCR. Of these 5 EV71 positive fatal cases, the lung tissue specimen tested EV71 positive for 1 case, and for another case, the intestinal lymph nodes, cerebrospinal fluid, spleen, thymus, kidney, brain, heart and lung specimens were EV71 positive. For the other 3 cases, the pharyngeal swabs were EV71 positive.

Viral isolation was conducted on 6 different types of specimens (including lung tissue, mouth efflux, brain tissue and pharyngeal swab) collected from 3 fatal cases. For all 6 specimens, the nucleotide sequencing and neutral antibody tests revealed the presence of EV71 virus.

B. Autopsy findings

The autopsy on 3 fatal cases performed by the Medical School of Beijing University, found mainly changes in the brain, lung, heart and lymphatic system. There were cases of severe brain oedema; lung congestion/pulmonary oedema, no significant lung inflammation, no significant bleeding; cardiac enlargement; hypertrophy of tonsils, thymus and lymph nodes; spleen enlargement; normal kidney, liver and gastrointestinal organs. The autopsy findings were overall consistent with the clinical manifestations and disease progression and strongly suggest viral infection.

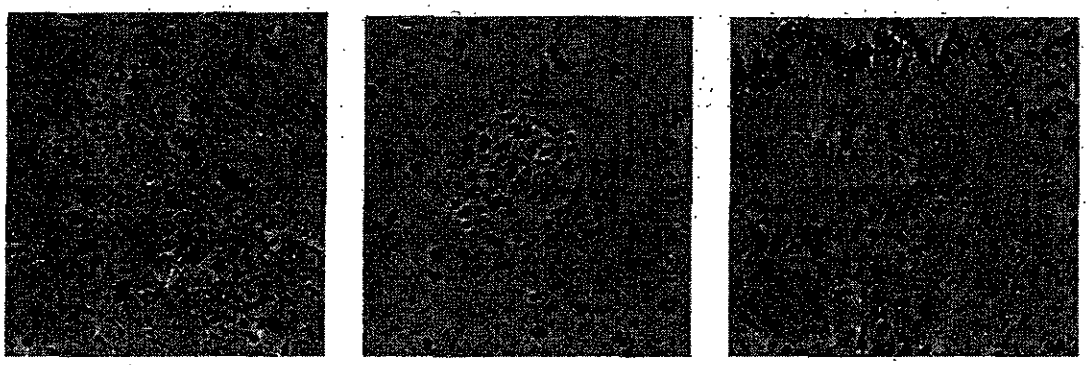


Figure 8. Left: brain tissue; Middle: spinal cord; Right: bronchus.

C. Etiology study of mild HFMD cases

RT-PCR testing for EV71 was performed on 122 different Fuyang city specimens, including pharyngeal and rectal swabs, of which 61 (50%) were EV71 nucleic acid positive. Gene homogeneity was 99.3%-99.97% between the virus strains from mild cases (2 strains were from 2 mild cases) and fatal cases (6 strains were from 3 fatal cases). No neuro-virulence site mutation of the EV71 virus was found among severe and mild cases through bioinformatics analysis. Chinese CDC submitted the gene sequences of 3 virus strains to GenBank on May 7.

Section 2 – Situation Analysis of HFMD in China

I. Current HFMD situation in China

1. HFMD surveillance

Before May 2, HFMD was not categorized as a notifiable disease and reporting of HFMD relied on voluntary reports submitted by clinicians. Since May 2, HFMD has been established as a class "C" notifiable disease, indicating that all clinical and laboratory diagnosed cases are reported through the web-based national disease surveillance and information management system. Standards for the clinical and laboratory diagnosis of HFMD cases can be viewed at the MOH website.[2]

2. Occurrence of HFMD in China

From January 1 to May 9, 2008, 61,459 HFMD cases were reported through the disease reporting system in Mainland China. The incidence rate was 4.5/100,000, and the number

[2] Guideline for HFMD prevention and control, 2008 edition
http://202.96.155.170/publicfiles/business/htmlfiles/mohjbyfkzj/s3577/200805/34775.htm

of deaths reached 38 (case fatality rate 0.06%).

1) Time distribution

The number of reported cases has been increasing since April 28. After categorizing HFMD as a class "C" notifiable disease, reported cases increased sharply. For HFMD time distribution and date of reporting see Figure 9.

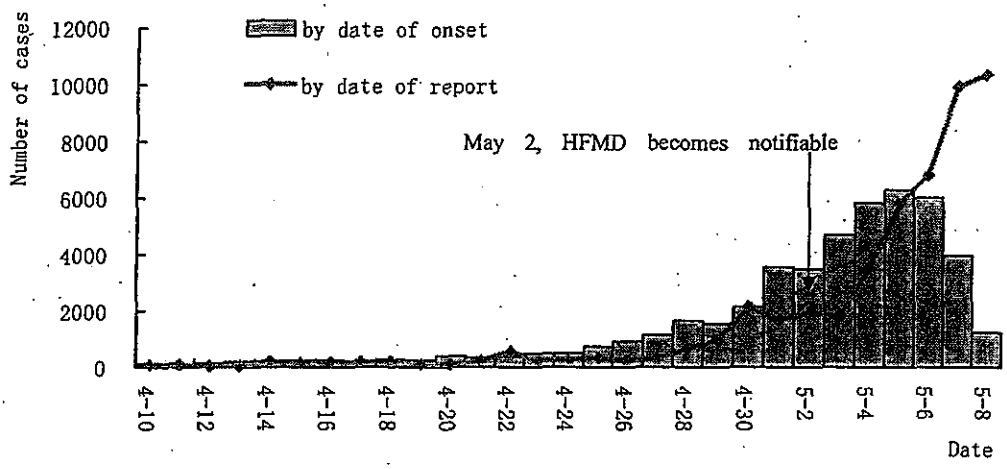


Figure 9. The number of HFMD cases by date of onset and date of reporting from April 10 to May 9, 2008 in China.

2) Geographical distribution

HFMD cases were reported in nearly all provinces in Mainland China. The 5 provinces with the highest number of reported cases are Guangdong (11,374), Anhui (9,235), Zhejiang (6,134), Shandong (4,566) and Henan (3,230).

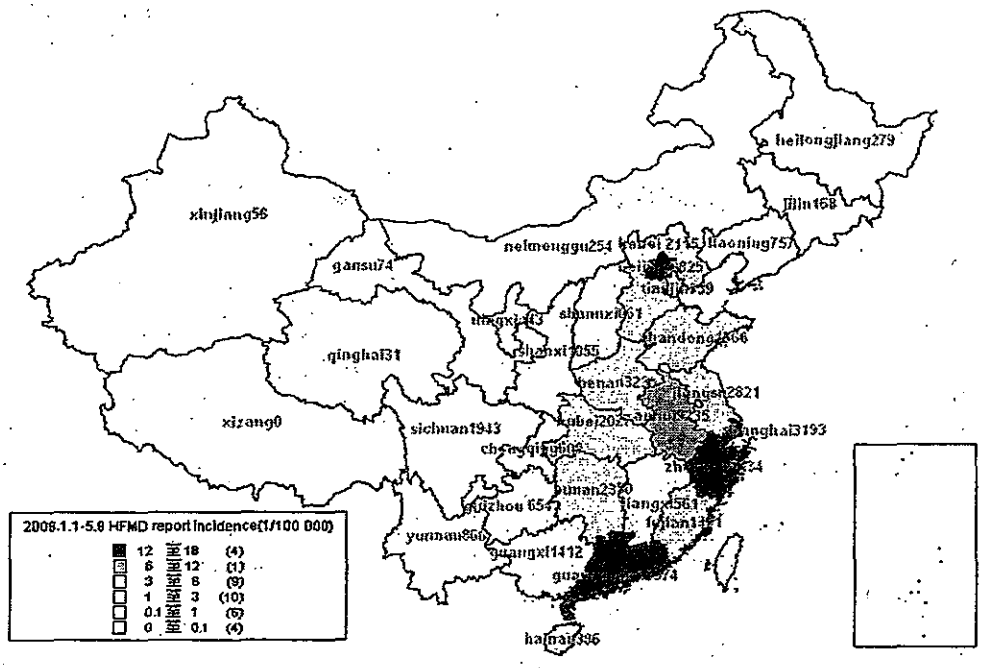


Figure 10 Incidence rate of HFMD cases by province in China from Jan 1 to May 9,2008

3) Age distribution

Children under 5 years old accounted for 92% of reported HFMD cases primarily affecting children ages 1 to 3 years old. See Figure 11 for the age distribution of HFMD in China.

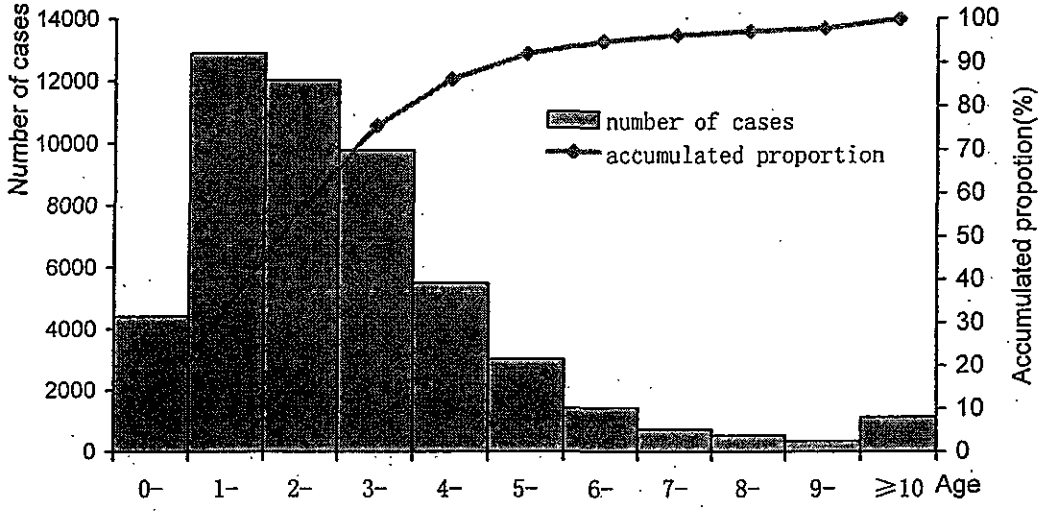


Figure 11. HFMD cases by age in China from January 1 to d May 9, 2008

3. Laboratory surveillance

After the identification of the HFMD outbreak in Fuyang City, Chinese CDC immediately began national-wide targeted laboratory testing on samples from HFMD cases. Up until May 9, 582 cases of samples from 23 provinces were tested positive for HFMD: EV71 accounted for 54.5%, Coxsackie A16 accounted for 17.4%, and other enteroviruses accounted for 28.2%.

II. HFMD and EV71 infection in China before 2008

The first reported case of HFMD in Mainland China occurred in Shanghai in 1981. Since then, cases have been reported in Beijing, Hebei, Tianjin, Fujian, Jilin, Shandong, Hubei, Qinghai and Guangdong. In 1995, the Wuhan Virus Institute isolated the EV71 virus from HFMD patients. In 1998, Shenzhen CDC also isolated the EV71 virus from HFMD patients. There was a HFMD and Herpangina outbreak in Taiwan in 1998 with two outbreak waves occurring in June and October. 129,106 cases were reported from sentinel sites with a total of 405 severe cases and 78 deaths. The majority of cases were children under 5 years of age, and complications included encephalitis, aseptic meningitis, pulmonary oedema/pneumorrahagia, acute flaccid paralysis and myocarditis. In 2007, an HFMD outbreak occurred in Linyi City of Shandong Province with a total of 39,606 cases reported, including 14 deaths. Laboratory testing found the main etiologic pathogen to be EV71

while other detected enteroviruses were Echo3 and/or Coxsackie A16.

In 2007, there were 83,344 HFMD cases identified in Mainland China. The incidence rate was reported as 6.34/100,000 with a total of 17 deaths and case fatality rate of 0.02%. The majority of cases occurred in pre-school children (41% of all cases) or children in childcare centres (52%). Prevalence of HFMD in children under 10 years of age was high, accounting for 97% of total reported cases. The HFMD peak season is from May to October with most cases occurring between June and July (see figure12). However, this may vary since HFMD was not a notifiable disease before 2008. Information on reported cases may be incomplete. Therefore, it is difficult to make an accurate estimation of past HFMD incidence in China.

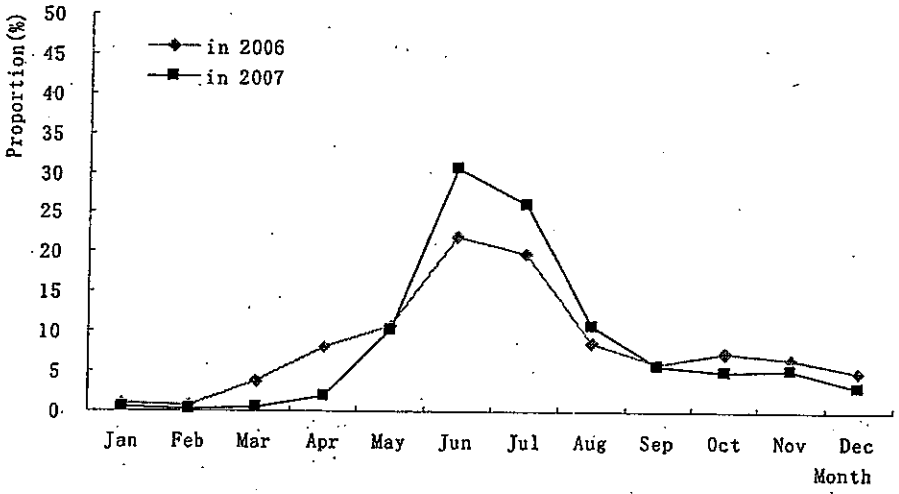


Figure 12 The distribution of HFMD cases by month in 2006 and 2007

Section 3 – Outbreak Response in Fuyang City, Anhui Province and China

I. The main response to the Fuyang City outbreak

1. Strengthening of disease surveillance

A case definition was formulated for the early detection of severe cases and for the reporting, monitoring and treatment of severe cases.
A HFMD reporting protocol was developed, and daily reporting of HFMD is performed at each level of health care facility.

2. Optimization of patient treatment and minimization of case fatalities

This includes the following:

- Designation of specific hospitals for the treatment of EV71-infected patients, who are allocated as follows: mild cases are sent to nearby health care facilities while designated hospitals focus on the treatment of severe cases.
- Establishment or expansion of paediatric Intensive Care Unit (ICU) facilities.
- Organization of training for high-level national and provincial ICU staff.
- Formation of a specialized medical team and 24 hour on-duty service.
- Enhancement of the monitoring and evaluation of severe cases based on clinical symptoms.
- Clinical monitoring for the early detection of severe cases and early provision of interventions to minimize fatalities.

3. Establishment of patient triage system and control of nosocomial infections

Consultation rooms were established for febrile rash cases within fever outpatient clinics or paediatric wards to prevent cross-transmission among other sick children. Medical equipment is required to be sterilized for each patient.

4. Strengthening technical guidance, development of technical training and improvement of health-care workers' professional skills

The national expert team has developed guidelines for the diagnosis and treatment of HFMD cases, a sampling plan and a HFMD prevention and control plan. The national expert team also assisted Anhui CDC in improving the quality of the provincial enterovirus laboratory. National and provincial experts have guided designated hospitals in Fuyang City in establishing paediatric ICU and have trained 350 health-care workers from 16 cities in Anhui Province on clinical diagnosis, ICU treatment, and epidemiological and sampling skills.

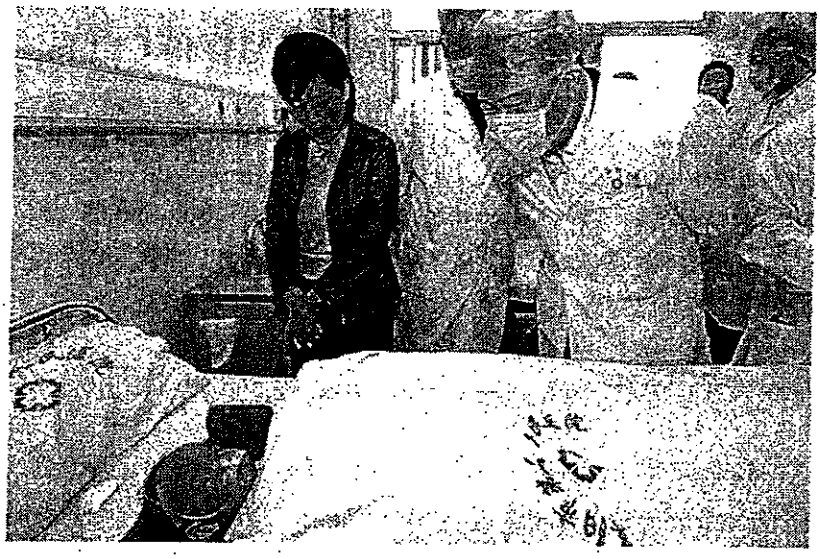


Figure13. Dr. Zhu Chen, Chinese Minister of MOH, visiting a HFMD patient at Fuyang No.2 People's Hospital on April 26, 2008.

5. **Establishment of full scale prevention and control measures with focus on childcare centres and schools.**

   a. Emphasis placed on promoting health education, disseminating information leaflets, and increasing public awareness.
   b. On a daily basis, the teacher in charge is expected to perform a clinical inspection of pupils in the morning, record absenteeism and reason for absence, and report daily to the local CDC. If children present with fever and rash, their parents should be informed immediately and should seek medical care. Subsequently, disinfection of the school building, tables, chairs and personal belongings should be conducted.
   c. Childcare centres are to disinfect toys daily, and tables should be disinfected before and after meals. Before and after class, the classrooms and school building should be ventilated by opening doors and windows for over 30 minutes.
   d. When 3 or more febrile/rash cases are identified per class, it should be reported to the local CDC immediately. The class should be divided or dismissed in order to avoid a possible outbreak situation.

6. **Establishment of HFMD medical fee assistance measure**

In order to ensure the prompt treatment of HFMD patients, especially severe cases, Fuyang City enacted the HFMD medical fee assistance measure to reimburse medical fees based on the new rural cooperative medical care regulation. This measure also provides free treatment to severe HFMD cases from low income families.

II. Current response measures for HFMD in China

HFMD is a common acute infectious disease that is widespread, and the peak season ranges from May to October. Following the death of many severe HFMD cases in Fuyang City and in order to strengthen HFMD surveillance, prevention and control and to protect the public's health, the Chinese Government enacted the following major outbreak response measures at the national level:

1. *Formation of a HFMD taskforce group*

On May 3, MOH formed a taskforce group for HFMD prevention and control with Minster Zhu Chen as team leader and deputy ministers Qiang Gao, Xiaowei Ma, Qian Liu as vice team leaders. There are 4 subgroups within this taskforce group: coordination, outbreak prevention and control, medical treatment and information dissemination.

2. Categorization of HFMD as a class "C" notifiable disease, prompt detection and treatment of severe cases and understanding the outbreak situation

Since May 2 2008, MOH categorized HFMD as a class "C" notifiable disease. All health care centres should report HFMD according to the "Law on notifiable infectious diseases prevention and control of the People's People's Republic of China" and "Infectious diseases report management regulation."

3. Strengthening implementation of HFMD prevention and control measures

a. On April 29, MOH issued the "Notice on the enhancement of HFMD and other enterovirus infectious diseases prevention and control measures." It requested all levels of health departments to emphasize prevention and control of HFMD and other infectious diseases caused by enteroviruses.
b. Since April 30, several guidelines have been published on the MOH website, including, "Guideline for HFMD medical treatment" and "Guideline for HFMD prevention and control (2008 edition)."

4. Increasing information exchange

Outbreak information was disseminated in a timely manner according to related national and international regulations. Information on the event was reported to WHO when EV71 was found as the causal agent of the outbreak by Chinese CDC on April 23. The gene sequence of isolated EV71 virus strain was submitted to GenBank[3] on May 7. Six outbreak

[3] The website is http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide. The serial numbers are: bankit1092219 EU703812; bankit1092307 EU703813; bankit1092309 EU703814.

information newsletters were also disseminated to Health Departments of Hong Kong, Macau and Taiwan since the beginning of the outbreak.

5. Improvement of health education

Prevention and control measures for HFMD are being promoted through various channels, and early reporting of symptomatic cases is encouraged.

Section 4 – Discussion

I. General background information on HFMD

HFMD is a common infectious disease caused by various enteroviruses, including EV71 and Coxsackie A. The predominant feature of HFMD is high morbidity in infants. HFMD is spread worldwide throughout the year and is transmitted predominantly by fecal-oral transmission, respiratory droplets, contact with case's blister fluid or general close contact with cases.

EV71, a non-polio enterovirus, was first ifentified in 1969. It is reported that the clinical manifestations of EV71 infection varies from classical HFMD symptoms to herpangaina, aseptic meningitis and encephalitis. Fifty to 80% of cases are asymptomatic or have mild flu-like symptoms. A few cases have severe nervous system damage that may result in death. The case fatality rate of severe cases is 10%-25%. No enterovirus vaccine is currently available. And because EV71 have a high asymptomatic infection rate, and can survive for long periods of time in the environment or sewage, it is a challenge to prevent and control.

II. HFMD outbreak in Fuyang City due to EV71 infection.

The HFMD outbreak in Fuyang City, Anhui Province that started in late March 2008 was caused by EV71 infection. Severe cases in Fuyang City are similar in terms of clinical manifestations and coincide with the population distribution, clinical manifestation and pathological findings of severe cases in the 1998 Taiwan outbreak and 2007 Shandong outbreak of EV71. Currently, testing of samples from Fuyang City cases reveal that the main etiologic pathogen is EV71. EV71 nucleic acid was identified in severe and mild cases in Fuyang City, and the viral nucleotide sequence was highly homogeneous.

III. Analysis of risk factors for high mortality rate in the initial phase of the outbreak

The case fatality rate for HFMD in Fuyang City, Anhui Province varied over time. Initially, from March 1 to April 23, it was 3% (18/610) and subsequently decreased to 0.07% (4/5439) from April 24 to May 9.

The initial high case fatality rate of the Fuyang City outbreak was likely attributable to the following factors:

a. According to the investigation of fatal cases, in the early clinical phase most cases had mild symptoms and were either treated as usual by rural doctors and private clinics or did not seek medical care. Most cases suddenly deteriorated in the first 2 or 3 days of clinical treatment with the situation worsening by the time of hospitalization, often already past the optimal time for treatment. The average time interval between hospitalization and time of death was only 10 hours.
b. Sixty percent of severe cases had no rash and therefore increased the difficulty for clinical doctors to diagnose enterovirus infection.
c. The proportion of severe cases caused by EV71 is higher and more likely to result in rapid disease progression and central nervous system damage with severe complications such as brainstem encephalitis, neurogenic pulmonary oedema, etc.
d. Mild cases normally did not seek medical care, and were therefore more difficult to detect and report. Underestimation of the number of mild cases based on hospital registration and disease reporting is possible.
e. The precarious socioeconomic status of some of the affected families may have resulted in a delay of presenting the patient to the hospital.

Based on the field investigation, no geographical clustering of HFMD cases was found around the severe cases in the initial stage of the outbreak while those severe cases and deaths occurred in Fuyang city. Further investigation of this is needed.

IV. Risk assessment

1. Risk of individual infection

Everyone is at risk of infection, but not everyone who is infected becomes ill. Young children under 5 years old are most susceptible. The clinical manifestation of most cases is mild. Since enteroviruses are omnipresent, it is likely for adults and older children to have immunity. The main transmission route for enterovirus 71 is via respiratory droplets, contact with fluid in the blisters or contact with infected faeces. The risk of transmission

can be minimized by avoiding contact with known infected individuals or activities that are of risk and by improving personal hygiene.

2. Risk of transmission

HFMD is a relatively common disease even outside of Fuyang City and other areas of China. There have been a number of outbreaks of EV71 HFMD in the Asia-Pacific region since 1997. Outbreaks have been reported in Bulgaria (1975), Malaysia (1997), Australia (1999) and Singapore (2000) among other areas in the region[4]. In China, an outbreak of HFMD due to EV71 was reported in Taiwan Province in 1998 with a total number of 129,106 cases of HFMD and Herpangina, of which 405 cases were severely ill and 78 cases were fatal[5]. Last year, Shandong Province experienced a HFMD outbreak with more than 40,000 cases and 14 deaths. This year, the number of EV71 cases has increased in Singapore[6] and Vietnam[7] while the disease has also been reported in Malaysia[8].

HFMD caused by EV71 is very common and not an emerging infectious disease. The public health impact of HFMD is not more serious than other common childhood diseases such as measles, Japanese encephalitis, epidemic meningitis, infectious diarrhea and pneumonia. According to the Chinese Ministry of Health it is not necessary to take public health measures regarding travel restrictions and quarantine in order to prevent the spread of the disease. WHO does not recommend restricting travel and trade to affected countries or regions but emphasizes improving personal hygiene for disease prevention.

V. Future work plan

1. Intensify the monitoring and control of EV71 infection

The Chinese government recognized the containment of HFMD as a high priority. The local governments are conducting a large-scale health education and public health promotion campaign to improve personal hygiene and ameliorate environmental sanitation. The Chinese MOH will continue to collect data on the clinical diagnosis and treatment of severe EV71 cases from Fuyang City and Anhui Province, evaluate existing control measures and strategies, update the technical guidelines and promptly organize training for paediatric doctors and public health workers in order to provide guidance on HFMD prevention and control in China.

Chinese CDC will further improve the technical capacity of its public health laboratory

---

[4] http://www.who.int/csr/don/2008_05_07/en/index.html

[5] http://www.cdc.gov/ncidod/EID/vol9no3/02-0285.htm

[6] http://www.sgdi.gov.sg/

[7] http://www.thanhniennews.com/healthy/?catid=8&newsid=38319

[8] http://thestar.com.my/news/story.asp?file=/2008/4/24/nation/21045923&sec=nation

network for enterovirus identification and monitoring in order to identify the enterovirus strains circulating in endemic areas and to analyze the molecular epidemiological characteristics of different strains and assess their clinical severity.

2. Improve and enhance the public health event surveillance and early warning system

MOH will reinforce the legislation for communicable disease surveillance and public health event reporting and improve the early warning and response for public health emergencies as part of an effort to implement the International Health Regulations, IHR (2005)[9]. The International Health Regulations (2005) are an international legal instrument which is legally binding for all WHO Member States. The purpose and scope of the IHR (2005) are to prevent, protect against, control and provide a public health response to the international spread of disease in ways that are commensurate with and restricted to public health risks, and which avoid unnecessary interference with international traffic and trade.

Recently, the Ministry of Health requested all doctors and public health workers to report deaths and clusters of severe cases of unknown cause immediately to the local health department. Subsequently, the local health department should promptly report these cases to MOH.

3. Strengthen international cooperation and information exchange

The Chinese government will share information on the HFMD outbreak and experience of containment and clinical treatment with WHO and other countries. Furthermore, the government plans to increase international cooperation to strengthen scientific research of enterovirus infection.

[9] http://www.who.int/topics/international_health_regulations/en/

別紙様式第2

別紙(3)-4

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4月7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	Blood 110 (11, Part 1): p853A NOV 16 2007 The risk of transfusion-transmitted babesiosis due to Babesia microti in Connecticut	公表国 米国	
販売名（企業名）	アンスロビンP-ベーリング（CSLベーリング株式会社）					

研究報告の概要

問題点（米国コネチカット州における Babesia microti によるバベシア症の輸血感染の危険性）

Babesia microti(Bm)はダニ媒介で感染して赤血球内に寄生し、米国のネブラスカ州と中東部以北では風土病である。

本来 Bm はダニ媒介の疾患だが、輸血により少なくとも 50 例の感染報告がある。症状は、発熱、溶血性貧血と血小板減少症で、輸血後 2-8 週で発症する。

コネチカット州での輸血による Bm 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試験した。慢性的に輸血を受けている人達から、輸血後 1,3,6,12 ヵ月後に採取され凍結された全血と血清と、関連するドナーの血清を検体とした。

全てのレシピエントの検体で、IFA を用いて Bm に対する抗体を測定した。

レシピエントの検体の IFA が陽性の場合、血液感染を特定するため対応するドナーの血清を Bm 抗体のスクリーニングを実施した。血清反応陽性のレシピエントの保存 DNA は Bm の real-time PCR でも評価された。筆者らは赤血球または血小板を輸血による Bm が 1 件の感染例を特定した。

鎌状赤血球貧血患者の検体 1 件を除いて全ての検体は Bm が陰性であった。その患者は 24 ヶ月で 45 回の赤血球輸血を受けていた。この検体は 2 施設のラボで血清反応陽性が再現された。血清反応陽性後 6 週と 11 週の血液検体は血清反応陰性であるが PCR は陽性であった。血清反応陽性の 5-21 か月前に採取された 11 検体は、全て血清反応陰性で、2 検体が PCR 陽性で、そのうち 1 検体は強陽性であった。対応するドナー3 人の検体は使用できなかった。

そのレシピエントはダニに暴露したことはなく、Bm が特有のコネチカット州のエリアに住んでいない。本研究に選ばれる前の 2 年間は 41 単位の赤血球を投与されていた。Bm に起因する臨床症状は現れていない。

血清反応陽性のドナーを特定できなかったが、輸血による Babesia microti 感染の可能性があるが、そのレシピエントがダニや以前の輸血から感染したかもしれない。

コネチカット州における Babesia microti 感染の危険性は 1920 回の赤血球輸血で 0 例または 1 例と計算できる。Gerber らの 1994 年の報告では、同州において 601 回の赤血球輸血で 1 例であり、結果が一致していた。

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
バベシア症は赤血球内にバベシア原虫が寄生するため発症するが、本剤は血漿を原材料にしているため感染はないと考えられる。	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。

Abstract# 2900 **Poster Board #-Session: 119-III**

The Risk of Transfusion-Transmitted Babesiosis Due to *Babesia microti* in Connecticut. Ritchard G. Cable,[1] Yan-Yun Wu*,[3] Stephanie Johnson*,[1] Kerri Dorsey*,[2] Russell Melmed*,[1] Jonathan Trouern-Trend*,[2] Shimian Zou*,[2] Laura Tonnetti*,[2] David Leiby*.[2] [1]*Blood Services, American Red Cross, Farmington, CT, USA;* [2]*Jerome H. Holland Laboratory, American Red Cross, Rockville, MD, USA;* [3]*Department of Laboratory Medicine, Yale University School of Medicine, New Haven, CT, USA.*

Babesia microti (Bm) is a tick-borne intra-erythrocytic parasite endemic in NE and the upper Midwest. Although primarily a tick-borne disease, Bm has been transmitted by transfusion in at least 50 documented cases. Symptoms include fever, hemolytic anemia, and thrombocytopenia, typically arising 2-8 weeks following transfusion. In order to assess the risk of Bm transmission by blood transfusion in Connecticut (CT), we tested a repository of donor and recipient samples collected in 2004-2007. *METHODS:* The repository consisted of frozen whole blood and serum samples collected generally 1, 3, 6, and 12 months after blood transfusions in a chronically transfused population, along with associated donor serum samples, collected at blood drives in CT. All recipient follow-up samples were screened for antibodies to *Babesia microti* by IFA, as were the initial samples of any seropositive recipient, using a 1:64 cut-off titer. If recipients tested IFA positive after being seronegative (seroconversion), corresponding donor sera were screened for Bm antibody to identify transfusion-transmission. Stored DNA from serial seroconverting recipient samples were also assessed by real-time PCR for Bm. We defined an evaluable transfusion for Bm as a platelet or RBC transfusion with at least one follow-up sample 14-180 days later. 107 recipients received evaluable transfusions. Altogether these recipients received 1920 evaluable RBC transfusions and 1634 evaluable platelet transfusions. *RESULTS:* All follow-up samples were seronegative for Bm except for a single follow-up sample in a recipient with sickle cell anemia transfused with 45 RBC over 24 months. This sample was reproducibly seropositive in 2 labs with a titer of 1:64 and was PCR negative. Blood samples 6 weeks before and 11 weeks after the seropositive sample were seronegative, but PCR +. To investigate, 11 earlier recipient samples taken 5-21 months before the seroconversion were tested and all were seronegative, although 2/11 were PCR + (one strongly positive). Donor serum samples from 18/21 RBC transfused prior to the strongly PCR + recipient sample were negative for Bm. Three donor samples were not available. The recipient reported no exposure to ticks and lived in a non-endemic area of Connecticut. The patient had received 41 units of red cells in the two years before enrollment in the study. There were no clinical symptoms attributable to Bm. *CONCLUSION:* This may be a case of transfusion-transmitted *Babesia microti,* despite our inability to identify a seropositive blood donor. However, the recipient may have acquired Bm from a tick bite or from earlier transfusions. The risk of *Babesia microti* transmission by transfusion in CT has thus been measured either as zero cases in 1920 RBC transfusions (95% CI 0.0 - 0.0016 per RBC) or as 1 case per 1920 RBC transfusions (0.005, CI 0.000013 - 0.0029 per RBC). A previous report (Gerber, et al. JID 1994; 170:231-234) directly measured the risk of transfusion transmission of *Babesia microti* in CT as 1 in 601 RBC (.0017). A recent risk estimate based on the prevalence of PCR positive CT donor samples is 1/1800 RBC (0.0006) (Cable RG, et al. Transfusion 2001: 41(suppl):12S-13S.) This current study of chronically transfused recipients is consistent with these earlier estimates.

Disclosure: No relevant conflicts of interest to declare.

別紙様式第2-1

No. 10

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	(製造承認書に記載なし)	研究報告の公表状況	Matsukura H, Shibata S, Tani Y, Shibata H, Furuta RA. Transfusion. 2008 May;48(5):1036-7.	公表国 日本
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社)			

研究報告の概要

○献血適格者におけるヒトパルボウイルスB19の持続感染

ヒトパルボウイルスB19持続感染の自然経過の特徴を明らかにするための長期的研究を実施した。日本では、全ての献血血液にRHA法によるB19抗原検査を行っている。この方法を用いて、1997年～1999年に大阪の献血者979,052人からB19感染102例を特定した。102名のうち、次の献血に訪れた20名(男性15名、女性5名;平均年齢34.3歳)から血漿検体を採取し、ウイルス力価及びB19 IgG・IgM抗体力価を測定することができた。B19 DNAについてはTaqMan PCR法、B19抗体については酵素免疫測定法を使用した。B19抗原陽性の血液は不適として廃棄されるため、初回献血時についてはこれらの検査を行っていない。平均フォローアップ期間は838日(範囲、101～1749日)だった。

血漿B19 DNAは、最初の6ヶ月間で急速に減少し、その後も減少は続いたが検出不能にはならなかった。B19抗体については、IgG、IgM両方が検出された9名ではIgMが検出不能となったが、他の9名ではIgGのみが検出され、IgMは2度目の献血の前に検出限界以下まで低くなったと考えられた。残り2名の献血者は調査期間の最後までIgMが検出可能だった(729日、743日)。B19抗体の当初の分析結果は異なるパターンを示したが、一度感染が成立すると、B19 IgGは20人の献血者全員で持続した。

これまでの研究結果と同様、本長期研究において献血者のB19持続感染が観察された。フォローアップ期間中、20名の献血者は高値のB19 IgGと低いウイルス力価を維持していたが、B19感染の症状を報告した者はいなかった。本研究のデータは、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を示された。ここで観察されたウイルス力価の動態は、B19 NATが実施できない状況において、より適切な献血者の選択に役立つだろう。これらの予備的な知見を実証するために、より大規模な研究が望まれる。

報告企業の意見	今後の対応
大阪のヒトパルボウイルスB19陽性献血者20名のB19 DNA、IgG・IgM抗体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との報告である。	今後も引き続き、ヒトパルボウイルスB19に関する新たな知見及び情報の収集に努める。日本赤十字社では、以前よりRHA法によるB19抗原検査を導入、ウイルス量の多い血液を排除している。また、2008年には感度向上のため検査法をCLEIA法に変更した。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」

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

9

LETTERS TO THE EDITOR

Persistent infection by human parvovirus B19 in qualified blood donors

Persistent parvovirus B19 infection with a low viral load has been reported in immunocompromised and in immunocompetent individuals (reviewed in Parsyan and Candotti[1]). Large cross-sectional studies using highly sensitive DNA amplification methods have also demonstrated persistent B19 infection.[2] Recently, Lefere and colleagues[3] conducted a longitudinal study of nonimmunodeficient patients who were multitransfused with red blood cells, demonstrating that asymptomatic chronic B19 infections may persist for a long period.[3] To characterize the natural course of persistent B19 infections, we conducted the following longitudinal study using an in-house TaqMan polymerase chain reaction method for B19 DNA and enzyme immunoassays to detect B19 immunoglobulin M (IgM) and immunoglobulin G (IgG; Denka Seiken, Tokyo, Japan). This study was approved by the ethical Committee of the Japanese Red Cross Osaka Blood Center. In Japan, all donated blood is tested for B19 infection with an in-house receptor-mediated hemagglutination method that detects B19 antigen as a marker of a high viremic stage of infection (cutoff, approx. 2.5×10^{10} IU/mL B19 DNA; data not shown). Using this method, we identified 102 cases of B19 infection among 979,052 blood donors in Osaka between 1997 and 1999. We were able to test the plasma samples of 20 of these 102 donors (15 male, 5 female; mean age, 34.3 years) when they returned for subsequent blood donations for viral load and B19-specific IgG and IgM. We did not examine the donors at their first visit because B19 antigen-positive blood was automatically disqualified and disposed. The mean duration of follow-up was 838 days (range, 101-1749 days). The results of sequential viral load testing for all donors are shown in Fig. 1A. In the first 6 months, we observed a rapid decline in plasma B19 DNA, which decreased continuously, but never became undetectable. Median plasma B19 viral loads for samples tested within every 6 months are shown in Fig. 1B. We analyzed the B19 antibody for all donors during the study period (Fig. 2A). For 9 donors (Donors 1-9) with both IgG and IgM, IgM became undetectable, while for 9 others (Donors 10-18), only B19 IgG was detected, presumably because B19 IgM had decreased to an undetectable level before the second visit. The remaining 2 donors (Donors 19 and 20) had B19 IgM–detectable until the last visit (at 729 and 743 days). Although the initial profile for B19 antibodies showed different patterns, once established, B19-specific IgG persisted in all 20 donors. Summaries for 3 representative cases corresponding to each of these patterns for IgM, IgG, and viral load are presented in Fig. 2B.

Consistent with previous studies that suggest that B19 DNA may persist for a long period in immunocompetent individuals,[3-5] we observed persistent B19 infection in healthy blood donors in the present longitudinal study. During the follow-up period, none of the 20 infected blood donors reported symptoms of B19 infection, although they retained high levels of B19 IgG and low viral load. Our data suggest that in healthy individuals, the B19 plasma viral load declines to below 10^4 IU per mL in approximately 1 year and to 10^3 IU per mL in approximately 2 years after an acute (high viremia) infection. The patterns of plasma B19 viral load observed in our study may be useful for identifying more suitable blood donors in circumstances where B19 NAT is unavailable. We encourage further studies with a larger sample size to validate these preliminary findings.

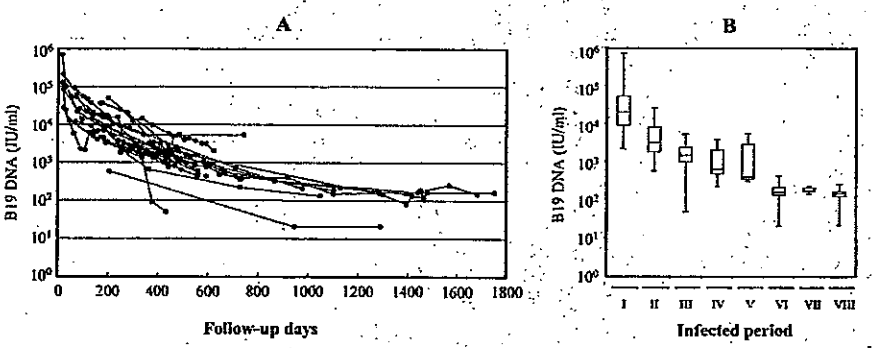


Fig. 1. (A) Changes in plasma B19 viral load in healthy blood donors after an acute B19 infection. Each line represents plasma B19 DNA of the same donor. Time 0 was defined as the first test visit when positive results were obtained for B19 antigen (high viremic phase). (B) Plasma B19 viral loads for all cases by 6-month intervals. Medians of the plasma B19 viral load with its 75th (top of the box) and 25th (bottom of the box) percentiles in each category were indicated. I = 0 to 0.5 years (0-182 days); II = 0.5 to 1.0 years (183-365 days); III = 1.0 to 1.5 years (366-549 days); IV = 1.5 to 2.0 years (550-730 days); V = 2.0 to 2.5 years (731-914 days); VI = 2.5-3.0 years (915-1096 days); VII = 3.0-3.5 years (1096-1279 days); VIII = at least 3.5 years (≥1280 days).

Harumichi Matsukura, BS
Sachiko Shibata, MT
Yoshihiko Tani, MD, PhD
Hirotoshi Shibata, MD, PhD
Rika A. Furuta, PhD
e-mail: furuta@osaka.bc.jrc.or.jp
Japanese Red Cross
Osaka Blood Center
Osaka, Japan

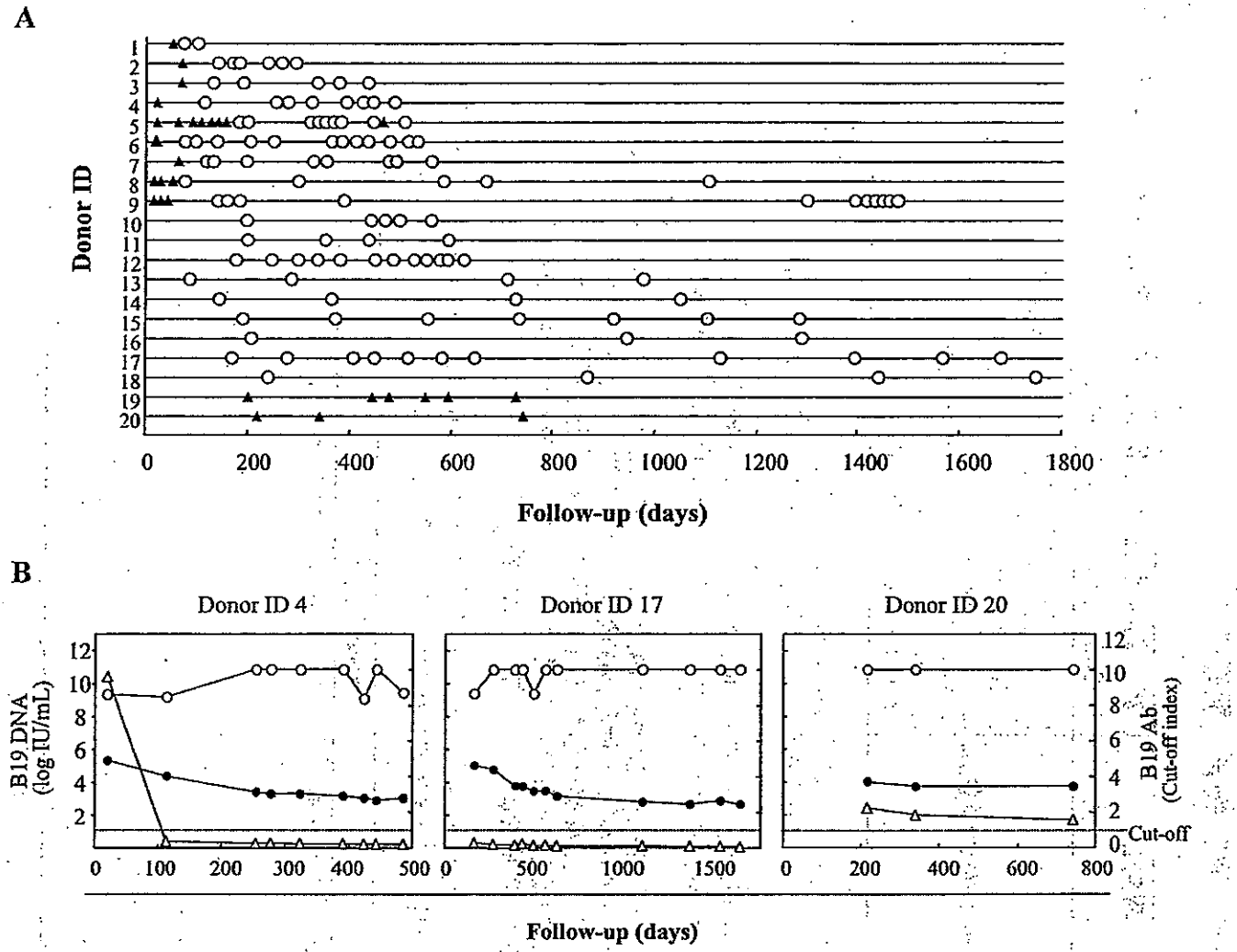


Fig. 2. (A) B19 IgM and IgG for individual donors at follow-up visits. (▲) Positive for both IgM and IgG; (○) positive for IgG. (B) Representative cases for three patterns of test results. Changes in viral load (●), IgM (△), and IgG (○). Donors correspond to those in A.

REFERENCES

1. Parsyan A, Candotti D. Human erythrovirus B19 and blood transfusion—an update. Transfus Med 2007;17:263-78.
2. Kleinman SH, Glynn SA, Lee TH, Tobler L, Montalvo L, Todd D, Kiss JE, Shyamala V, Busch MP; National Heart, Lung, Blood Institute Retrovirus Epidemiology Donor Study (REDS-II). Prevalence and quantitation of parvovirus B19 DNA levels in blood donors with a sensitive polymerase chain reaction screening assay. Transfusion 2007; 47:1756-64.
3. Lefrère JJ, Servant-Delmas A, Candotti D, Mariotti M, Thomas I, Brossard Y, Lefrère F, Girot R, Allain JP, Laperche S. Persistent B19 infection in immunocompetent individuals: implications for transfusion safety. Blood 2005; 106:2890-5.
4. Candotti D, Etiz N, Parsyan A, Allain JP. Identification and characterization of persistent human erythrovirus infection in blood donor samples. J Virol 2004;78:12169-78.
5. Norja P, Hokynar K, Aaltonen LM, Chen R, Ranki A, Partio EK, Kiviluoto O, Davidkin I, Leivo T, Eis-Hübinger AM, Schneider B, Fischer HP, Tolba R, Vapalahti O, Vaheri A, Söderlund-Venermo M, Hedman K. Bioportfolio: lifelong persistence of variant and prototypic erythrovirus DNA genomes in human tissue. Proc Natl Acad Sci U S A 2006; 103:7450-3.

New cell lines express HNA-1c, -4a, -4b, -5a, or -5b for identification of HNA antibodies

Antibodies to human leukocyte antigens (HLAs) or human neutrophil antigens (HNAs) are regarded to be the principal causes of nonhemolytic transfusion reactions, including transfusion-related acute lung injury. Although flow cytometric (FCM) analysis using panels of phenotyped neutrophils is widely used to detect and identify antibodies to HNAs, FCM is time-consuming and

別紙様式第 2-1

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-3

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2008 年 4 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			研究報告の公表状況	Inactivation of parvovirus B19 during STIM-4 vapor heat treatment of three coagulation factor concentrates Berting, A. et al, Transfusion, ahead of print	公表国 オーストリア	
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
ヒトパルボウイルス B19（B19V）の感染性アッセイが可能となる以前は，モデルウイルスと呼ばれる動物パルボウイルス（例：マウスパルボウイルス）が，血漿タンパクの製造工程におけるウイルス不活化の確認に使用されており，一般的にパルボウイルスは熱不活化に耐性があると考えられていた。しかし，最近の知見より，B19V は動物パルボウイルスよりも熱に弱いことが明らかになってきた。 本文献は，数種の血液凝固因子製剤において STIM-4 蒸気加熱処理装置を用いた不活化処理を行い，B19V とモデルウイルスとして用いられていたマウス微小ウイルス（MMV）間での不活化効果の比較を行っている。 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 1 型，2 型）はいずれも動物パルボウイルスと比較して，STIM-4 蒸気加熱処理工程によって効果的に不活化された（Log 減少ファクター，3.5～4.8）。これより，蒸気加熱処理による B19V の効果的な不活化が示唆され，B19V に対する STIM-4 蒸気過熱処理を行った血液凝固因子製剤の安全性が高まると考えられた。	BYL-2008-0320

報告企業の意見	今後の対応
加熱処理によるウイルス不活化の程度は各製剤によって左右されるため，製剤毎に確認する必要があると考える。 弊社のポリグロビン N の製造に使用されているプール血漿においては，B19V に対する NAT を実施し，10E5 IU/mL 以上が確認された場合は，そのプール血漿を製造工程から除去している。感染リスクを完全に排除することはできないが，伝播の可能性は非常に低いと考える。	今後とも利用可能な B19V の検出方法の改善に関する情報収集に努める。

(10)

Inactivation of parvovirus B19 during STIM-4 vapor heat treatment of three coagulation factor concentrates

Andreas Berting, Jens Modrof, Ulrike Unger, Matthias Gessner, Andreas Klotz, Gerhard Poelsler, and Thomas R. Kreil

BACKGROUND: To enhance the viral safety margins, nanofiltration has been widely integrated into the manufacturing process of plasma-derived medicinal products. Removal of smaller agents such as parvovirus B19 (B19V) by filtration, however, is typically less efficient. Because recent investigations have demonstrated that B19V may be more heat sensitive than animal parvoviruses, the potential B19V inactivation by a proprietary vapor heating procedure (STIM-4) as incorporated into the manufacturing processes of several nanofiltered coagulation factor concentrates was investigated.

STUDY DESIGN AND METHODS: An infectivity assay based on quantitative reverse transcription–polymerase chain reaction (TaqMan, Applied Biosystems) detection of B19V mRNA after inoculation of a permissive cell line (UT7 Epo S1 cells) was used to investigate the virus inactivation capacity of the STIM-4 vapor heat treatment as used during the manufacture of nanofiltered second-generation Factor VIII inhibitor-bypassing activity (FEIBA), F IX complex, and FVII products.

RESULTS: In contrast to animal parvoviruses, both B19V genotypes investigated, that is, 1 and 2, were shown to be surprisingly effectively inactivated by the STIM-4 vapor heat treatment process, with mean log reduction factors of 3.5 to 4.8, irrespective of the product intermediate tested.

CONCLUSION: The newly demonstrated effective inactivation of B19V by vapor heating, in contrast to the earlier used animal parvoviruses, results in significant B19V safety margins for STIM-4–treated coagulation factor concentrates.

To further enhance the safety margins of plasma-derived medicinal products against any residual virus safety concerns, manufacturers have continuously sought to implement dedicated virus reduction steps into the manufacturing processes of these products. Once robustly established and widely available, nanofiltration has frequently been considered an option for this purpose (for review see Burnouf and Radosevich[1]).

In selecting the appropriate pore sizes of these filters, commercially available between 15 and 75 nm, a delicate balance needs to be struck between maintaining an appropriate yield of the respective product intermediate while effectively removing viruses. Especially for larger-molecular-weight protein preparations the removal of smaller viruses has thus been difficult,[2] unless virus antibodies present in the intermediate increased the effective filtration size of a virus by formation of virus–antibody complexes,[3,4] or specific product formulations contributed to virus removal by inducing virus aggregation.[5] Particularly parvovirus B19 (B19V), currently the only known parvovirus associated with significant pathogenicity for humans, can thus often not be efficiently removed from larger-molecular-weight biologic entities of medicinal importance by these procedures.

B19V contaminates human blood or plasma donations, at reported frequencies of 1 in 800-5950[6,7] and levels

ABBREVIATIONS: B19V = parvovirus B19; FEIBA = Factor VIII inhibitor-bypassing activity; LRF(s) = log reduction factor(s); MMV = mice minute virus; NF/VH = nanofiltered and vapor heat treated.

From the Global Pathogen Safety and Molecular Biology, Baxter BioScience, Vienna, Austria.

Address reprint requests to: Thomas R. Kreil, PhD, Baxter BioScience, Benatzkygasse 2-6, A-1221 Vienna, Austria; e-mail: thomas_kreil@baxter.com.

Received for publication September 24, 2007; revision received November 19, 2007, and accepted November 24, 2007.

doi: 10.1111/j.1537-2995.2008.01662.x

TRANSFUSION ;**:**-**.

of viremia up to 10^{12} genome equivalents (geq) per mL.[6,8] Polymerase chain reaction (PCR) testing of the plasma supply has thus become state-of-the-art, and use of the technology has reduced the mean B19V load of plasma manufacturing pools by many orders of magnitude. Given the wide prevalence of B19V, however, supply considerations have prevented eliminating the virus from plasma by PCR testing, and thus virus reduction during the manufacturing process remains the critical safeguard of final product safety also in this instance.

Before an infectivity assay for B19V itself was available, animal parvoviruses, for example, porcine, murine, bovine, etc., parvoviruses, were used as so-called "model viruses"[9] in studies validating the virus reduction capacity of the manufacturing processes of plasma proteins. Based on the very high physicochemical resistance of these animal parvovirus models, virus inactivation procedures incorporated into these processes were considered less effective against parvoviruses.[10] More recently, however, initial data obtained with a novel infectivity assay for B19V itself indicated that the actual virus of concern for humans is much more heat sensitive than the animal parvoviruses used for earlier validation studies.[11-13]

Adding to the complexity, several more recently discovered human parvoviruses, for example, V9[14] and A6,[15] have now been reclassified to taxonomically represent B19V genotypes rather than distinct parvovirus species.[16]. Little is known, however, about the biologic properties of these newer B19Vs, for example, the sensitivity of these to inactivation.[17]

In this study the B19V reduction capacity of a proprietary and dedicated virus inactivation step was investigated, that is, the STIM-4 vapor heat treatment, in direct comparison to mice minute virus (MMV), an earlier used animal parvovirus model. The procedure was investigated with intermediates of several different coagulation factor concentrates that had been upgraded with respect to virus safety margins by implementation of nanofiltration during their manufacture, that is, Factor (F)VIII inhibitor-bypassing activity–nanofiltered and vapor heat treated (FEIBA NF/VH), F IX complex NF/VH, and FVII NF/VH.

MATERIALS AND METHODS

Viruses, cells, and infectivity assay

As a source of B19V, highly viremic plasma donations (990237, Genotype 1, 11.8 log geq/mL; IM81, Genotype 2, 11.4 log IU/mL) as identified by the routine plasma screening procedure of Baxter Bioscience were used. B19V were titrated on UT7 Epo S1 cells (provided by Dr Kevin E. Brown, Virus Reference Department, Center for Infections, Health Protection Agency, London, UK; with permission from Dr Kazuo Sugamura, Department of Microbiology and Immunology, Tohoku University, Graduate School of Medicine, Tohoku, Japan), essentially as earlier described.[18] Briefly, mRNA of infected cells was isolated and quantified by reverse transcription (RT)-PCR with the following procedure. Initially serial 10-fold dilutions of B19V samples of known PCR titer were incubated with UT-7 cells, and the B19V mRNA analyzed by RT-PCR. A regression line of the samples' known PCR titers versus the number of RT-PCR cycles required to obtain a positive signal for the same sample was then plotted to form a calibration curve. With this calibration curve, the PCR titer of any unknown sample was back-calculated from the mRNA RT-PCR titer obtained after incubation with susceptible cells. Typically, several 10-fold dilutions of unknown samples were analyzed by RT-PCR, to ensure that one or several of the results would lie on the linear part of the calibration curve. Whenever more than one result fitted onto the calibration curve, means were calculated for the PCR titer. The limit of detection was 3.7 log per mL,[18] and standard errors of means for multiple measurements were always not more than 0.5 log.

MMV, strain prototype (ATCC VR-1346, American Type Culture Collection, Rockville, MD) was propagated and titrated on A9 cells (ATCC CCL-1.4). Samples containing MMV were titrated by $TCID_{50}$ assay, that is, eightfold replicates of serial half-log sample dilutions were incubated with cells for 7 days before evaluation for a cytopathic effect. MMV concentrations were calculated according to the Poisson distribution and expressed as log $TCID_{50}$ per mL.

RT-PCR

For detection of B19V Genotype 1, primers sets for two mRNA splicing variants (splicing at nucleotide 1910 or 2030, Accession Number M13178[19]) were used (PA3 or PA4, respectively): PA3—primers PA3F (positions 365-386), PA3R (positions 1957-1978), and the fluorescent probe PA3P (5'-6-FAM-TTTGTGAGCTAACTAACAGATGCCCTCC ACCCAGAC-TAMRA-3'); and PA4—primers PA4F (positions 367-389), PA4R (2080-2102), and the fluorescent probe PA4P (5'-6-VIC-TGAGCTAACTAACAGGCGCCTGG AACA-TAMRA-3').

For detection of B19V Genotype 2 (Accession Number AY903437[17]), the primer set G2 was used, G2-F (positions 369-391), G2-R (positions 1962-1983), and the fluorescent probe G2-P (5'-6-FAM-TTTGCCTGCTAATTAACAGATGCC CTCCACCCAGAC-3').

Downscaled manufacturing processes for plasma derivatives

Downscaled versions of the manufacturing processes examined were established and the equivalence of critical product and process parameters to the respective manufacturing-scale processes established. Temperature is a critical process measure for virus inactivation and was

therefore monitored throughout all the processes investigated. Starting materials were process intermediates obtained from the manufacturing scale, which were spiked 1 in 10 with virus stock suspensions. Immediately after spiking, samples were drawn and titrated to confirm the amount of virus added. Further samples were collected and titrated at predetermined points throughout and at the end of the inactivation processes. MMV-spiked samples were directly titrated on A9 cells, whereas B19V-spiked samples were titrated on UT7 cells followed by mRNA isolation as described earlier.[18] Specific unspiked process intermediates were obtained from control procedures and tested for their potential cytotoxicity for the indicator cell line and for their potential interference with the detection of low virus titers. Virus reduction factors for the manufacturing processes examined were calculated in accordance with Committee for Proprietary Medicinal Products guidance.[9]

During their manufacture, the investigated products (all from Baxter BioScience, Zurich, Switzerland), that is, FEIBA NF, F IX complex NF (PPK NF), or together with FVII NF (prothrombin complex NF, PKT NF), are subjected to the STIM-4 vapor heating process. Specifically, a lyophilized intermediate of 7 to 8 percent residual moisture is heat treated for a minimum of 500 minutes at 60 ± 0.5°C, followed by heating to 80 ± 0.5°C, and then heating at 80 ± 0.5°C for 60 minutes. The downscaled versions of these processes were performed at the lower limits of these temperature and incubation time specifications or just below those specified for the manufacturing-scale process. To provide further assurance regarding the robustness of the virus inactivation by these processes, separate runs were performed at the upper and lower limits of the residual moisture content specified for manufacture; or runs were performed within these specifications.

Determination of the residual moisture was performed by the Karl Fischer method for non–virus-spiked control samples. The residual moisture content for all samples, including those containing virus, was confirmed by NIRVIS spectroscopy (System NIRVIS, Büchi Ltd, Flawil, Switzerland).

Product intermediates of 14.4 to 33 g per L protein concentration, 5 to 6 g per L salt concentration, and pH 7.0 to 8.0 were spiked with virus, lyophilized, and then heat-treated according to the procedure described above. Specific product measures, e.g., FEIBA (clotting assay), FII activity (clotting assay), FX activity (chromogenic assay), FVII activity (chromogenic assay), F IX activity (chromogenic assay), and protein concentration were determined for the downscale intermediate before and after the vapor heating process. The results were compared with the respective values for intermediates from the manufacturing scale to confirm equivalence of the different scale processes.

RESULTS

Vapor heating of FEIBA NF/VH

FEIBA intermediate was spiked with either B19V or MMV for downscaled vapor heating experiments. For B19V, two different primer sets (PA3, PA4) specifically designed to detect two different B19V VP1/VP2 splicing variants[20] were used for RT-PCR analysis (TaqMan, Applied Biosystems, Foster City, CA).

As can be seen in Table 1, significant inactivation of B19V was observed already after the 60°C heating phase of the process (experimentally conducted at 59.5 ± 0.5°C, i.e., worst case with respect to virus inactivation) with individual log reduction factors (LRFs) of 3.9 to 4.5. At completion of the 80°C heating phase of the process

TABLE 1. Inactivation kinetics of MMV and B19V during freeze-drying followed by vapor heating of FEIBA NF/VH process intermediates*

	MMV†		B19V‡					
Percent residual moisture content:	7	8	7-8					
			Titration 1		Titration 2		Titration 3	
Primer pairs:			PA3	PA4	PA3	PA4	PA3	PA4
Virus stock suspension	8.3	8.2	11.2	11.2	11.8	11.8	ND	ND
Spiked process intermediate§	7.2	7.2	10.3	10.4	10.6	10.4	10.6	10.4
Spiked and lyophilized intermediate	6.6	6.7	9.7	9.9	10.0	9.9	9.8	9.8
Heated at 59.5°C ± 0.5°C, 180 ± 5 min	ND	ND	7.8	7.7	7.7	7.6	ND	ND
Heated at 59.5°C ± 0.5°C, 505 ± 5 min	6.7	6.7	6.2	5.9	6.6	6.5	6.5	6.5
Reduction factor (after 60°C phase)	0.5	0.5	4.1	4.5	4.0	3.9	4.1	3.9
Heated at 79.5°C ± 0.5°C, 55 ± 5 min§	6.3	6.3	5.7	5.6	5.5	5.4	5.7	5.8
Reduction factor	0.9	0.9	4.6	4.8	5.1	5.0	4.9	4.6
Mean reduction factor	0.9		4.8					

* For the detection of spliced B19V mRNA two different primer sets, i.e., PA3 and PA4, were used.
† MMV titers are expressed as [log($TCID_{50}$/mL)].
‡ B19V titers are expressed as [log geq/mL].
§ Titers at this sampling stage were used to calculate the virus reduction factor after the entire heating phases.
ND = not determined.

(experimentally conducted at 79.5 ± 0.5°C), some residual B19V infectivity was still detectable, although LRFs of 4.6 to 5.1 were obtained, with a mean LRF of 4.8. Because use of primer sets PA3 and PA4 resulted in fully equivalent results, only one of the primer sets (PA3) was used for the detection of B19V Genotype 1 mRNA in further experiments.

In contrast to the effective inactivation of B19V by the vapor heating process, the inactivation observed for the animal parvovirus MMV was insignificant, with a mean LRF of 0.9.

Vapor heating of F IX complex NF/VH

To investigate the vapor heating process of F IX complex, the respective intermediate was spiked with either B19V or MMV. Because residual moisture during the vapor heating process might be considered a critical parameter for the effectiveness of virus inactivation, separate vapor heating runs were performed at the upper and lower limit of the specified residual moisture content of the manufacturing process, that is, 7 and 8 percent (wt/wt), to investigate the robustness of virus inactivation by the process. For B19V-spiked runs, some residual infectivity was still detected after the entire heating process, but the results obtained demonstrate comparable reduction factors for runs at either 7 or 8 percent residual moisture content with a mean LRF of 4.6 (Table 2). A substantial inactivation of B19V was observed already after the 60°C heating phase of the process (investigated at 59.5 ± 0.5°C) with individual LRFs of 3.7 to 4.2. As the reduction factors obtained between the individual titrations at 7 and 8 percent residual moisture content were comparable, consequently, the following vapor heating experiments were performed at between 7 and 8 percent residual moisture content, that is, within the specifications of the large-scale process.

MMV, again in sharp contrast to the effective inactivation of B19V by the vapor heating process, was not significantly inactivated even at the end of the entire heating process, with a mean LRF of 0.9. As seen with B19V before, there were again no differences between MMV inactivation results for individual heating runs conducted at 7 and 8 percent residual moisture.

Vapor heating of FVII NF/VH

F IX complex and FVII are separately produced and are individual products. Both components can, however, also be combined to the prothrombin complex total product. Because FVII is the second component of prothrombin complex total, the B19V and MMV inactivation by STIM-4 vapor heating was also investigated.

At the end of the entire heating phase, effective inactivation of B19V was observed, with a mean LRF of greater than 4.0 (Table 3). Also, substantial inactivation of B19V was found already after the 60°C heating phase (investigated at 59.5 ± 0.5°C) of the process (LRFs of 4.0 and 4.5), confirming earlier findings for the other prothrombin complex total compound. Again in sharp contrast to effective B19V inactivation, the parvovirus model MMV was inactivated only ineffectively, with a mean LRF of 1.7.

STIM-4 inactivation of B19V Genotype 1 versus Genotype 2: FEIBA, for example

To understand any potentially different thermosensitivity of the recently classified B19V Genotype 2, versus the earlier investigated B19V Genotype 1, FEIBA intermediate was now spiked with B19V Genotype 2 and treated as described earlier (see "Vapor heating of FEIBA NF/VH"). mRNAs isolated after culture with UT-7 cells were analyzed by TaqMan RT-PCR with either, as before, Genotype 1 primer sets (PA3), or now also specific Genotype 2 (G2) primer sets.

TABLE 2. Inactivation kinetics of MMV and B19V during freeze-drying followed by vapor heating of F IX complex NF/VH intermediate

	MMV*		B19V†			
Percent residual moisture content:	7	8	7		8	
			Titration 1	Titration 2	Titration 1	Titration 2
Virus stock suspension:	7.4	7.5	ND	ND	ND	ND
Spiked process intermediate‡	6.5	6.7	10.7	10.6	11.0	11.0
Spiked and lyophilized intermediate	6.5	6.4	10.8	10.5	10.1	10.4
Heated at 59.5°C ± 0.5°C, 495 ± 5 min	6.1	6.2	6.9	6.9	7.1	6.8
Reduction factor (after 60°C phase)	0.4	0.5	3.8	3.7	3.9	4.2
Heated at 79.5°C ± 0.5°C, 55 ± 5 min‡	5.8	5.6	6.4	6.6	5.8	6.3
Reduction factor	0.7	1.0	4.3	4.1	5.2	4.7
Mean reduction factor (log)	0.9		4.6			

* MMV titers are expressed as [log($TCID_{50}$/mL)].
† B19V titers are expressed as [log geq/mL].
‡ Titers at this sampling stage were used to calculate the virus reduction factor after the entire heating phases.
ND = not determined.

TABLE 3. Inactivation kinetics of MMV and B19V during freeze-drying followed by vapor heating of FVII NF/VH intermediate

	MMV*		B19V†			
Percent residual moisture content:	7	8	7-8			
			Run 1		Run 2	
			Titration 1	Titration 2	Titration 1	Titration 2
Virus stock suspension:	7.7	8.1	12.3	ND	ND	ND
Spiked process intermediate‡	6.5	7.2	11.0	10.6	10.8	10.8
Spiked and lyophilized intermediate	6.9	7.0	ND	9.9	9.9	10.1
Heated at 59.5°C ± 0.5°C, 525 ± 5 min	5.3	6.1	6.5	6.6	positive§	positive§
Reduction factor (after 60°C phase)	1.2	1.1	4.5	4.0	ND	ND
Heated at 79.5°C ± 0.5°C, 55 ± 5 min‡	5.2	5.2	<6.8	<6.8	<6.8	<6.8
Reduction factor	1.3	2.0	>4.1	>3.8	>4.0	>4.0
Mean reduction factor	1.7		> 4.0			

* MMV titers are expressed as [log($TCID_{50}$/mL)].
† B19V titers are expressed as [log genome equivalents/mL].
‡ Titers at this sampling stage were used to calculate the virus reduction factor.
§ These samples were tested positive; a titer could, however, not be calculated, because the PCR cycle numbers necessary to obtain a positive fluorescence signal were outside the range covered by the mean regression line.
ND = not determined.

TABLE 4. B19V Genotype 2 inactivation by vapor heat treatment during the manufacture of FEIBA NF/VH*

Percent residual moisture content:	B19V GT 2			
	7-8			
	Run 1	Run 2	Run 1	Run 2
	PA3	PA3	G2	G2
Reduction factor†	>3	4	>3	>4
Mean reduction factor	3.5			

* The TaqMan RT-PCR was performed with either Genotype 1 (PA3)- or Genotype 2 (G2)-specific primer sets.
† Reduction factor determined after the entire vapor heating procedure. The goodness-of-fit values of the standard regression lines were less than optimal resulting in a high standard deviation at lower virus titers. Therefore, reduction factors were determined by the difference in integer log sample dilutions between the spiked intermediate and the sample after completion of vapor heating.

As can be seen, use of both the two primer sets (PA3/PA4) designed for two Genotype 1 splicing variants revealed highly comparable results (Table 1). In addition insignificant differences between B19V Genotype 1 and Genotype 2 occurred, with both viruses effectively inactivated by the STIM-4 vapor heating process (Tables 1 and 4). Specifically, here for B19V Genotype 2, effective inactivation was observed at the end of the heating process, with calculated LRFs between greater than 3 and greater than 4, that is, a mean LRF of 3.5. Using the Genotype 1 (PA3)- or the Genotype 2 (G2)-specific primer sets, highly comparable inactivation results were obtained.

DISCUSSION

Since its discovery in 1975,[21] B19V has been associated with an ever-broadening panel of diseases. While initially only known as the causative agent of an erythematous childhood disease (fifth disease), more recently the virus is appreciated as the causative agent of more severe diseases such as, for example, hydrops fetalis,[22] arthritis,[23] hepatitis,[22] and possibly a significant number of myocarditis cases.[24]

The introduction of B19V PCR testing of plasma for fractionation, as initially defined under the Plasma Protein Therapeutics Association's voluntary standards,[25] has reduced plasma pool loads of the virus by several orders of magnitude[26] and correspondingly enhanced the B19V safety margins of plasma products. In support of the notion, while episodes of B19V transmissions have historically occurred,[27] such reports have not been received for the implicated products after the introduction of B19V PCR testing. There is, however, still a residual concern around the potential B19V contamination of plasma.

The final safeguard of product safety, that is, the virus reduction that occurs during the manufacturing process, has thus been of particular interest, also with respect to B19V. Unfortunately though, the lack of a widely available B19V infectivity assay has forced studies aimed at validating the B19V reduction capacity of manufacturing processes to be conducted with animal parvoviruses as "model viruses." Where these viruses are particularly resistant to physicochemical inactivation, the results obtained were often less reassuring.

There was consequently significant interest in a suitable B19V assay to investigate the virus of concern itself, and development efforts were lately rewarded. Initial use of the newly available approaches revealed that B19V itself was significantly more susceptible to inactivation by, for example, pasteurization,[11] low pH,[28] and dry heat,[13] than the earlier used animal parvovirus models. Also, however, research conducted with these assays has indicated that B19V has unique properties in terms of heat sensitivity

and its inactivation is particularly dependent on the composition of the matrices during (liquid) heating.[29]

The proprietary STIM-4 vapor heating process is a heat treatment step conducted at lyophilized product of 7 to 8 percent residual moisture that has been incorporated in the manufacturing process of several coagulation factor concentrates, in addition to a 35-nm nanofiltration step for FEIBA NF/VH, F IX complex NF/VH, and FVII NF/VH. Here we describe the efficient inactivation of B19V, in marked contrast to an animal parvovirus model, that is, MMV, by this vapor heat treatment.

The results of the two-phase vapor heat treatment demonstrate that B19V is effectively inactivated by this process step, whereas MMV is only marginally reduced. By use of both the two primer sets (PA3/PA4) designed to detect two Genotype 1 VP1/VP2 splicing variants highly comparable results were obtained (Table 1), indicating that the splicing variants in infected UT7-Epo S1 cells occur in rather similar concentrations.

After the entire heating process for B19V Genotype 1 mean log reduction factors of 4.8, 4.6, and more than 4.0 were obtained with highly comparable results for the panel of coagulation factor intermediates investigated, that is, FEIBA, F IX complex and FVII (see Tables 1-3). These findings support the robustness of the STIM-4 vapor heat treatment in inactivating B19V. Moreover, the significant inactivation of B19V already after the first heating phase at 60°C for the coagulation factors investigated (see Tables 1-3) provides further reassurance. In addition, varying the residual moisture content during the heat treatment, that is, to the lower (7%) and the upper limit (8%) specified for the manufacturing process, still resulted in highly comparable inactivation of B19V (Table 2).

The discovery of additional human erythrovirus genotypes, that is, Genotypes 1, 2, and 3,[16] and also novel parvoviruses,[30,31] has raised new questions about their biologic properties, their pathogenic potential and also their relevance to the viral safety of plasma-derived products. B19V Genotype 2 has been detected in human blood at high titers, and recently this genotype has also been found in a few lots of plasma-derived coagulation factor concentrates; all of them, however, were cocontaminated with Genotype 1 DNA.[32] These findings indicate that Genotype 2 has established a moderate prevalence in the population and therefore investigations regarding the inactivation of this B19V genotype may also be desirable. Recent studies that investigated the inactivation capacity of liquid heating and low pH[17] incubation for B19V Genotypes 1 and 2 in parallel suggested comparable inactivation capacity and kinetics and thus maybe also comparable physicochemical properties for the virus particles of both genotypes.[17]

In this report, the physicochemical stability of virus particles of Genotype 1 and 2 were compared during the vapor heat treatment process step described. The results demonstrate that both B19V genotypes are inactivated with comparable mean LRFs of 4.8 and 3.5 (Tables 1 and 4). These data indicate that B19V Genotype 1 and 2 particles have very similar physicochemical properties and thus data obtained in studies with Genotype 1 should also be indicative for Genotype 2 behavior. This argument is strengthened by very recent findings that anti-B19V–positive plasma samples or intravenous immune globulin product were able to neutralize B19V Genotype 1 and Genotype 2.[18] Furthermore, studies by Ekman and colleagues[33] suggest that all three B19V genotypes are similar variants of the same species and constitute a single serotype. Not surprising in this context, the pathogenic potential of different B19V genotypes also seems to be comparable.[14]

Altogether the results of this study demonstrate that the STIM-4 vapor heat treatment is a highly effective and robust virus inactivation step for the relevant parvovirus B19, both Genotype 1 and Genotype 2. Specifically, the STIM-4 vapor heat treatment process substantially contributes to the safety margins of the plasma-derived products FEIBA NF/VH, F IX complex NF/VH, and FVII NF/VH. As suggested by a recent article,[29] however, the inactivation capacity of heat treatment may significantly depend on the specific matrix investigated, and thus B19V inactivation needs to be confirmed for every specific product and process individually.

ACKNOWLEDGMENTS

Dr Donald A. Baker is acknowledged for unconditional and long-standing support of research into the safety of plasma products. We are grateful for all the contributions by the entire Baxter Bioscience Global Pathogen Safety team, most notably Angelika Hofmann and Gabriele Petutschnig, MSc (conduct of experiments); Alexandra Danzinger, Karin Berka, Bettina York, MSc, Claudia Schwarz, and Elisabeth Pinter (cell culture and virus propagation); Dr Geza Szabo and Johannes Geissler, MSc (data monitoring and compilation); and Dr Sandra Rieger and Dr Christina Forstner for the TaqMan PCR analysis.

REFERENCES

1. Burnouf T, Radosevich M. Nanofiltration of plasma-derived biopharmaceutical products. Haemophilia 2003;9: 24–37.
2. Burnouf-Radosevich M, Appourchaux P, Huart JJ, Burnouf T. Nanofiltration, a new specific virus elimination method applied to high-purity factor IX and factor XI concentrates. Vox Sang 1994;67:132–8.
3. Kreil TR, Wieser A, Berting A, Spruth M, Medek C, Pölsler G, Gaida T, Hämmerle T, Teschner W, Schwarz HP, Barrett PN. Removal of small nonenveloped viruses by antibody-

enhanced nanofiltration during the manufacture of plasma derivatives. Transfusion 2006;46:1143–51.
4. Omar A, Kempf C. Removal of neutralized model parvoviruses and enteroviruses in human IgG solutions by nanofiltration. Transfusion 2002;42:1005–10.
5. Yokoyama T, Murai K, Murozuka T, Wakisaka A, Tanifuji M, Fujii N, Tomono T. Removal of small non-enveloped viruses by nanofiltration. Vox Sang 2004;86:225–9.
6. Weimer T, Streichert S, Watson C, Groner A. High-titer screening PCR: a successful strategy for reducing the parvovirus B19 load in plasma pools for fractionation. Transfusion 2001;41:1500–4.
7. Aubin JT, Defer C, Vidaud M, Maniez MM, Flan B. Large-scale screening for human parvovirus B19 DNA by PCR: application to the quality control of plasma for fractionation. Vox Sang 2000;78:7–12.
8. Wong S, Brown KE. Development of an improved method of detection of infectious parvovirus B19. J Clin Virol 2006; 35:407–13.
9. CPMP. Note for guidance on virus validation studies: the design, contribution and interpretation of studies validating the inactivation and removal of viruses. CPMP/BWP/268/95/Rev. London: The European Agency for the Evaluation of Medicinal Products; 1996.
10. Roberts PL, Hart H. Comparison of the inactivation of canine and bovine parvovirus by freeze-drying and dry-heat treatment in two high purity factor VIII concentrates. Biologicals 2000;28:185–8.
11. Blumel J, Schmidt I, Willkommen H, Lower J. Inactivation of parvovirus B19 during pasteurization of human serum albumin. Transfusion 2002;42:1011–8.
12. 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.
13. Prikhod'ko GG, Vasilyeva I, Reyes H, Wong S, Brown KE, Jameson T, Busby TF. Evaluation of a new LightCycler reverse transcription-polymerase chain reaction infectivity assay for detection of human parvovirus B19 in dry-heat inactivation studies. Transfusion 2005;45:1011–9.
14. Nguyen QT, Sifer C, Schneider V, Allaume X, Servant A, Bernaudin F, Auguste V, Garbarg-Chenon A. Novel human erythrovirus associated with transient aplastic anemia. J Clin Microbiol 1999;37:2483–7.
15. Nguyen QT, Wong S, Heegaard ED, Brown KE. Identification and characterization of a second novel human erythrovirus variant, A6. Virology 2002;301:374–80.
16. Servant A, Laperche S, Lallemand F, Marinho V, De Saint MG, Meritet JF, Garbarg-Chenon A. Genetic diversity within human erythroviruses: identification of three genotypes. J Virol 2002;76:9124–34.
17. Blumel J, Eis-Hubinger AM, Stuhler A, Bonsch C, Gessner M, Lower J. Characterization of parvovirus B19 genotype 2 in KU812Ep6 cells. J Virol 2005;79:14197–206.
18. 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.
19. Shade RO, Blundell MC, Cotmore SF, Tattersall P, Astell CR. Nucleotide sequence and genome organization of human parvovirus B19 isolated from the serum of a child during aplastic crisis. J Virol 1986;58:921–36.
20. Ozawa K, Ayub J, Hao YS, Kurtzman G, Shimada T, Young N. Novel transcription map for the B19 (human) pathogenic parvovirus. J Virol 1987;61:2395–406.
21. Cossart YE, Field AM, Cant B, Widdows D. Parvovirus-like particles in human sera. Lancet 1975;1:72–3.
22. Young NS, Brown KE. Parvovirus B19. N Engl J Med 2004; 350:586–97.
23. Moore TL. Parvovirus-associated arthritis. Curr Opin Rheumatol 2000;12:289–94.
24. Pankuweit S, Ruppert V, Eckhardt H, Strache D, Maisch B. Pathophysiology and aetiological diagnosis of inflammatory myocardial diseases with a special focus on parvovirus B19. J Vet Med Infect Dis Vet Public Health 2005;52:344–7.
25. PPTA voluntary standard parvovirus B19. Report No. 017677. Annapolis (MD): Plasma Protein Therapeutics Association; 2001 Feb 8.
26. Groner A, Gurtler L. Parvovirus B19 infection and virus safety of plasma preparations. Biomed Prog 2000;13:46–50.
27. Blumel J, Schmidt I, Effenberger W, Seitz H, Willkommen H, Brackmann HH, Lower J, Eis-Hubinger AM. Parvovirus B19 transmission by heat-treated clotting factor concentrates. Transfusion 2002;42:1473–81.
28. Boschetti N, Niederhauser I, Kempf C, Stuhler A, Lower J, Blumel J. Different susceptibility of B19 virus and mice minute virus to low pH treatment. Transfusion 2004;44: 1079–86.
29. Hattori S, Yunoki M, Tsujikawa M, Urayama T, Tachibana Y, Yamamoto I, Yamamoto S, Ikuta K. Variability of parvovirus B19 to inactivation by liquid heating in plasma products. Vox Sang 2007;92:121–4.
30. Fryer JF, Kapoor A, Minor PD, Delwart E, Baylis SA. Novel parvovirus and related variant in human plasma. Emerg Infect Dis 2006;12:151–4.
31. Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Andersson B. Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proc Natl Acad Sci U S A 2005;102:12891–6.
32. Schneider B, Becker M, Brackmann HH, Eis-Hubinger AM. Contamination of coagulation factor concentrates with human parvovirus B19 genotype 1 and 2. Thromb Haemost 2004;92:838–45.
33. Ekman A, Hokynar K, Kakkola L, Kantola K, Hedman L, Bondén H, Gessner M, Aberham C, Norja P, Miettinen S, Hedman K, Söderlund-Venermo M. Biological and immunological relation of human parvovirus B19 genotypes 1-3. J Virol 2007;81:6927–35.

別紙様式第2-1

No. 12

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	(製造承認書に記載なし)	研究報告の公表状況		公表国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社)		Pettersson L, Boman J, Juto P, Evander M, Ahlm C. Emerg Infect Dis. 2008 May;14(5):808-10.	スウェーデン

研究報告の概要

○スウェーデンのPuumalaウイルス感染アウトブレイク
ハンタウイルスの一種Puumalaウイルスは、スウェーデン、フィンランド、ノルウェー、ロシア、中央ヨーロッパの一部に土着しており、自然宿主であるハタネズミの排泄物からヒトに感染する。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。

報告企業の意見	今後の対応
スウェーデンにおいてPuumalaウイルスの大規模アウトブレイクが発生し、2007年Västerbotten地方の流行性腎症患者の数は100,000人当り313人に至ったとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

(二)

Outbreak of Puumala Virus Infection, Sweden

Lisa Pettersson,* Jens Boman,*† Per Juto,* Magnus Evander,* and Clas Ahlm*

An unexpected and large outbreak of Puumala virus infection in Sweden resulted in 313 nephropathia epidemica patients/100,000 persons in Västerbotten County during 2007. An increase in the rodent population, milder weather, and less snow cover probably contributed to the outbreak.

Members of the genus *Hantavirus* (family *Bunyaviridae*) are rodent-borne pathogens, and virus is transmitted to humans by inhalation of infected rodent excreta (*1*). In Sweden, Finland, Norway, Russia, and parts of central Europe, Puumala virus (PUUV) is endemic in bank voles (*Myodes glareolus*). PUUV infection in humans cause nephropathia epidemica (NE), a mild form of hemorrhagic fever with renal syndrome (HFRS). In Sweden ≈90% of all NE cases are found in the 4 northernmost counties. Västerbotten County (Figure 1) has the highest incidence of human hantavirus infection in Sweden and probably one of the highest worldwide. Historically, the incidence rate is 20 per 100,000 persons per year (*2*), but the true incidence is considered to be 7–8 times higher (*3*).

There is a 3–4-year periodicity in the number of NE cases that is linked to the bank vole population dynamics in northern Sweden (*2*). After inhaling infectious aerosols originating from rodent saliva, urine, or feces, the patient has a 1–5-week incubation period before onset of disease symptoms. The most common NE symptoms are fever, headache, nausea, abdominal and back pain, vomiting, myalgia, and visual disturbance. One third of the patients have mostly mild hemorrhagic manifestations (*4,5*). Renal failure is typical with initial oliguria during the acute phase and polyuria in the convalescence phase. Dialysis is sometimes needed and <0.5% of NE cases are fatal. There is no effective treatment or available vaccine.

The Study

The local University Hospital of Umeå is the reference center for diagnosis of NE serving the 4 northernmost counties of Sweden, and many patients with NE are hospitalized here. In 2007, a sudden and large outbreak of hantavirus infections occurred in northern Sweden. The outbreak peaked in January 2007 (Figure 1) with many NE patients who had a considerable effect on public health services. The NE outbreak continued in the following months, but with fewer cases than in early 2007 (Figure 1).

For NE diagnosis, we used an immunofluorescence assay to detect PUUV-reactive immunoglobulin (Ig) M and IgG antibodies in serum of all patients with clinically suspected NE (*6*). A real-time reverse transcription–PCR (*6*) was used to obtain an amplification product from 1 patient sample. This product was sequenced and the S-segment sequence obtained (GenBank accession no. EU177630) was highly homologous to those of other rodent PUUV isolates from the area.

NE is a reportable disease under the Swedish Communicable Diseases Act. The outbreak peaked during the first 3 months of 2007; 972 cases were recorded in Sweden and 474 cases in Västerbotten County. NE patients mostly showed classic HFRS symptoms and mild to severe disease requiring hospitalization and occasionally intensive care. Accordingly, as many as 30% of the patients whose conditions had been diagnosed as NE were hospitalized, and 2 known deaths (case-fatality rate 0.25%) in the 2 northernmost counties in Sweden were recorded during the first 3 months of 2007. No patient had to continue dialysis after the acute phase of the disease.

We detected PUUV RNA in the milk of 2 breastfeeding women with a diagnosis of NE. Their children did not show any clinical symptoms of NE. However, we did not have access to samples to analyze whether the children had asymptomatic infections. Three pregnant women also had received a diagnosis of NE, but no clinical evidence of transmission from mother to child was reported. Analyses of the placentas did not detect any PUUV RNA. Only maternal IgG antibodies to PUUV were found in blood from umbilical cords. One woman miscarried after 12 weeks of pregnancy 3 weeks before showing symptoms of clinical NE, and death of the fetus may have been caused by viremia during the incubation period. During the peak of the outbreak (December 2006–March 2007), 488 cases occurred in Västerbotten County, and, as expected, more men (58%, 281/488) than women (42%, 207/488) had NE; most cases (72%) were among persons 35–74 years of age (Table).

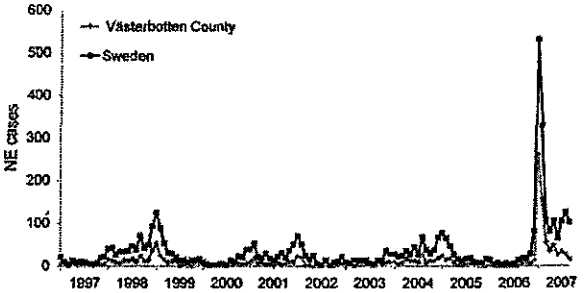


Figure 1. Monthly incidence of nephropathia epidemica (NE) in Sweden and Västerbotten County, Sweden, January 1997–September 2007.

*Umeå University, Umeå, Sweden; and †County Council of Västerbotten, Umeå, Sweden

The incidence of NE in Västerbotten County was 313 diagnosed cases/100,000 persons in 2007 compared with 73/100,000 in 1999, 38/100,000 in 2002, and 61/100,000 in 2005 (Figure 1). The number of NE cases usually depends on the size of the vole population, which peaks every third to fourth year (*2,7*). An increase in the bank vole population was reported in northern Sweden in the fall of 2006, with a trap index of 7.64. This index is similar to those of 2 NE peaks in the fall of 1998–1999 and 2004–2005 when trap indices were ≈8 (*8*). Trapping indices represents the number of voles captured per 100 trapping nights, a reflection of the relative population size on each sampling occasion (*9*). Thus, the bank vole population was high, but not more than in previous peak years and could not explain the high number of NE cases in 2007.

We considered other possible factors influencing hantavirus transmission to humans. One factor is increased exposure of humans to infected rodent excreta. We had received several reports from inhabitants in areas where bank voles normally live that more bank voles were found in traps inside houses than usual. When we investigated the weather conditions during this period, December 2006 was exceptional with respect to the mild weather with no or little snow and hard ice cover in the coastal area of northern Sweden. In Västerbotten County, the average temperature in December was 6.0°C–9.0°C warmer than normal (normally the average temperature in Västerbotten County varies by –4°C along the coast and –13°C in the mountains) The average temperature in Sweden was 4.5°C–9.5°C warmer than normal in December 2006 (Figure 2). The snow cover during winter is important for bank vole survival because bank voles have access to food below the snow and hide from predators and the cold (*10*). During 2 previous NE peak periods (2001–2002 and 2004–2005), the ground was already covered with snow in early winter (Figure 2). For these reasons, during December 2006, when the ground had no snow cover for 25 of 31 days (Figure 2), bank voles may have sought refuge in barns and houses and other buildings, thereby increasing the exposure for the human population at risk. A concurrent epizootic may have occurred among bank voles, which resulted in larger numbers of infectious animals, as shown in previous rodent studies (*11,12*). However, we did not have access to rodents during this period and this hypothesis needs to be studied.

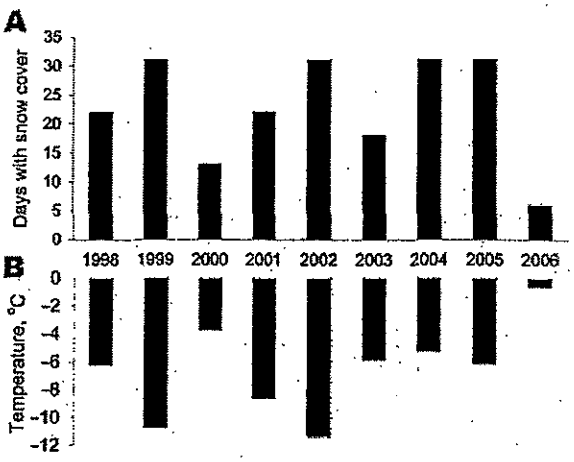


Figure 2. Climate conditions, December 1998–2006, in the nephropathia epidemica outbreak area of Västerbotten County, Sweden. A) Number of days with a snow cover. B) Average temperature. Snow cover was defined as a snow depth >0 cm. Measurements were made in locations ≈30 km from the coast. Data were obtained from the Swedish Meteorological and Hydrological Institute.

Table. Nephropathia epidemica cases in Västerbotten County, Sweden, December 1, 2006–March 31, 2007

Age group, y	No. (%) cases
<1–4	1 (0.20)
5–14	16 (3.3)
15–24	34 (7.0)
25–34	48 (9.8)
35–44	82 (17)
45–54	89 (18)
55–64	103 (21)
65–74	78 (16)
75–84	32 (6.6)
85–94	5 (1.0)
Total	488 (100)

Conclusions

This report shows how a zoonotic disease can suddenly result in an unexpected and large human outbreak. Presently, the numbers of NE cases in northern Sweden are still unusually high. Data indicate that the bank vole population during the fall of 2007 increased to an even higher level and a new outbreak is forecasted (*8*). However, the size of the rodent population is not the only factor that determines the size of a hantavirus epidemic. As shown in this report, climate factors may have contributed to the recent large outbreak in northern Sweden.

This study was supported by the county councils of northern Sweden and the medical faculty of Umeå University.

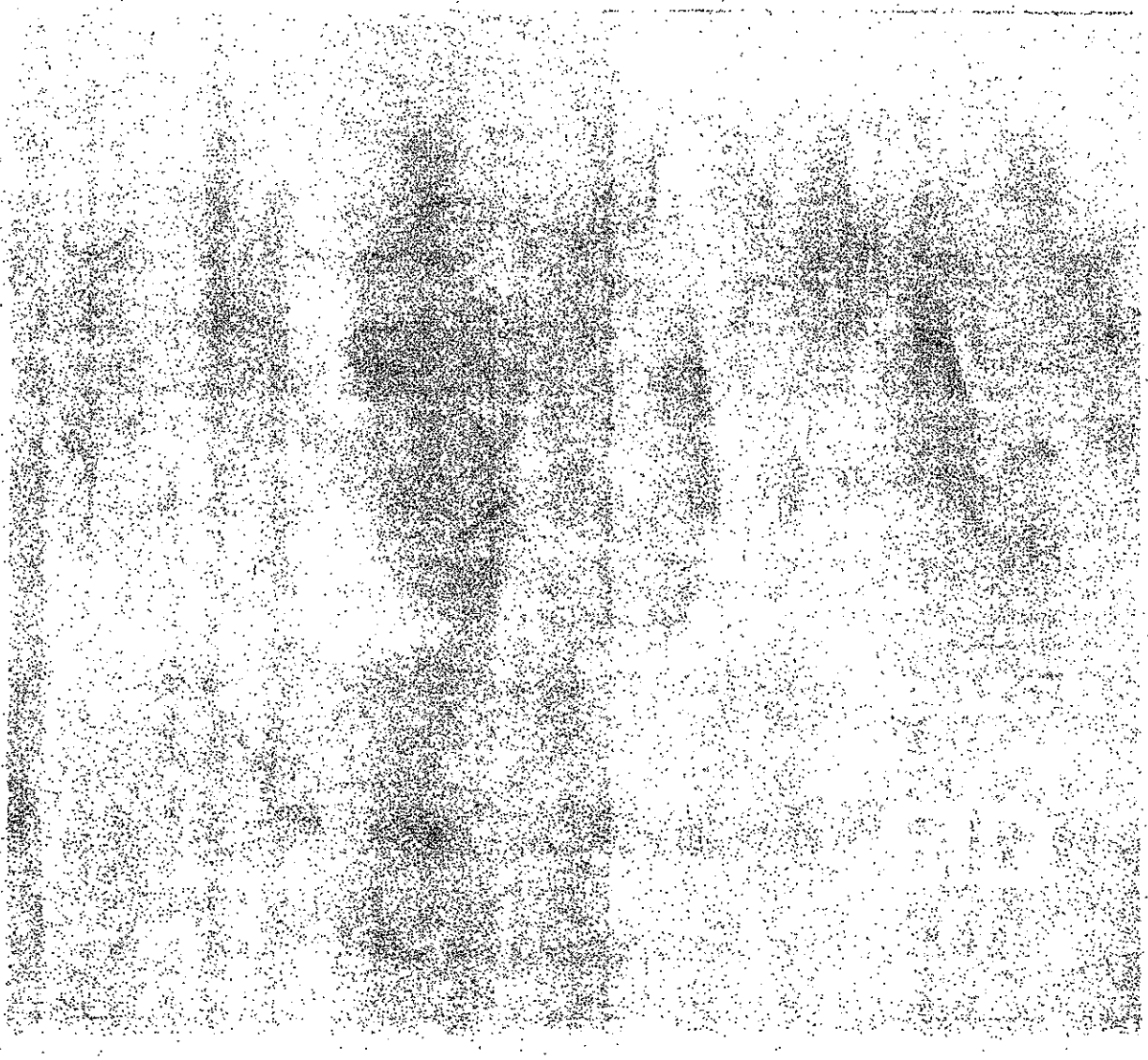
Dr. Pettersson is a clinical virologist working at the Umeå University Hospital. Her major research interest is the biology and epidemiology of hantaviruses.

References

1. Schmaljohn C, Hjelle B. Hantaviruses: a global disease problem. Emerg Infect Dis. 1997;3:95–104.
2. Olsson GE, Dalerum F, Hörnfeldt B, Elgh F, Palo TR, Juto P, et al. Human hantavirus infections, Sweden. Emerg Infect Dis. 2003;9:1395–401.

3. Ahlm C, Linderholm M, Juto P, Stegmayr B, Settergren B. Prevalence of serum IgG antibodies to Puumala virus (haemorrhagic fever with renal syndrome) in northern Sweden. Epidemiol Infect. 1994;113:129–36.
4. Settergren B, Juto P, Trollfors B, Wadell G, Norrby SR. Clinical characteristics of nephropathia epidemica in Sweden: prospective study of 74 cases. Rev Infect Dis. 1989;11:921–7.
5. Mustonen J, Brummer-Korvenkontio M, Hedman K, Pasternack A, Pietilä K, Vaheri A. Nephropathia epidemica in Finland: a retrospective study of 126 cases. Scand J Infect Dis. 1994;26:7–13.
6. Evander M, Eriksson I, Pettersson L, Juto P, Ahlm C, Olsson GE, et al. Puumala hantavirus viremia diagnosed by real-time reverse transcriptase PCR using samples from patients with hemorrhagic fever and renal syndrome. J Clin Microbiol. 2007;45:2491–7.
7. Brummer-Korvenkontio M, Vapalahti O, Henttonen H, Koskela P, Kuusisto P, Vaheri A. Epidemiological study of nephropathia epidemica in Finland 1989–96. Scand J Infect Dis. 1999;31:427–35.
8. Olsson GE, Hörnfeldt B, Hjertkvist M, Lundkvist Å. Nephropathia epidemica: high risk in Norrland during winter [in Swedish]. Lakartidningen. 2007;104:3450–3.
9. Olsson GE, White N, Ahlm C, Elgh F, Verlemyr AC, Juto P, et al. Demographic factors associated with hantavirus infection in bank voles (*Clethrionomys glareolus*). Emerg Infect Dis. 2002;8:924–9.
10. Hansson L, Hentonnen H. Gradients in density variations of small rodents: the importance of latitude and snow cover. Oecologia. 1985;67:394–402.
11. Ahlm C, Alexeyev OA, Elgh F, Aava B, Wadell G, Tärnvik A, et al. High prevalence of hantavirus antibodies in bank voles (*Clethrionomys glareolus*) captured in the vicinity of households afflicted with nephropathia epidemica. Am J Trop Med Hyg. 1997;56:674–8.
12. Escutenaire S, Chalon P, Verhagen R, Heyman P, Thomas I, Karelle-Bui L, et al. Spatial and temporal dynamics of Puumala hantavirus infection in red bank vole (*Clethrionomys glareolus*) populations in Belgium. Virus Res. 2000;67:91–107.

Address for correspondence: Magnus Evander, Department of Clinical Microbiology, Division of Virology, Umeå University, S-901 85 Umeå, Sweden; email: magnus.evander@clinmi.umu.se



別紙様式第２－１

医薬品　研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
一般的名称	－		研究報告の公表状況	http://www.boston.com/news/local/articles/2008/05/13/1_dies_1_ill_after_receiving_kidneys?mode=PF	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
ボストン病院で一人の臓器提供者から腎臓移植を受けた 70 歳の女性が死亡し、57 歳の男性が重態となった。 臓器提供者は回復不能な脳障害をこうむった 49 歳のホームレスの男性で、エイズウイルス、B 型肝炎、C 型肝炎などについては検査されていたが、2005 年にマサチューセッツとロードアイランドで 3 人の移植患者が死亡した際と同じ病原体である「リンパ球性脈絡髄膜炎ウイルス(LCMV)」に感染していた。 この LCMV は、一般的にげっ歯類によって伝播し、健康な人々ではインフルエンザ様の症状で特に問題はない。 臓器移植の際には、臓器の組織損傷を防ぐため、エイズウイルス、肝炎、ヘルペスのような容易に検査できる病原体は検査されるが、あまり一般的ではなく、時間のかかる LCMV のような病原体は検査されない。 移植を受けた女性は、家に帰ってすぐ具合が悪くになり(発熱、下痢などの、腎に特有でない症状)、外科手術のおよそ 2 週間後に再入院したが、さらに悪化し死亡した。 もう一例の男性は外科手術の 2.5 週間後に発熱で再入院しており、集中治療室で抗ウイルス薬を投与されている。 CDC において、死亡した臓器提供者と移植を受けた患者 2 人の検体が検査したところ、LCMV 陽性であることがわかり、臓器提供者からの伝播であることが確認された。	

報告企業の意見	今後の対応
リンパ球性脈絡髄膜炎ウイルスは、エンベロープを有するアレナウイルス科の RNA ウイルスで、直径 60～280nm の多形性であるので、ウイルス除去膜処理、加熱処理などにより、物理的除去又は不活化されると考えられる。	今後とも、リンパ球性脈絡髄膜炎ウイルスに関する情報に留意していく。

boston.com

1 dies, 1 ill after receiving kidneys

The Boston Globe

Donor infected with hard-to-find virus

By Stephen Smith, Globe Staff | May 13, 2008

A 70-year-old woman has died, and a 57-year-old man is critically ill in a Boston hospital after each received a kidney from a donor infected with a hard-to-detect virus, health authorities said yesterday.

The donor, a 49-year-old homeless man who suffered irreversible brain damage after cardiac arrest, carried a germ called lymphocytic choriomeningitis virus, or LCMV, the same infection that killed three transplant patients from Massachusetts and Rhode Island in 2005. The virus, most often transmitted by rodents, is usually unnoticed by healthy people who suffer no more than flulike symptoms.

Knowing that organs perish quickly, doctors test donors for what is easily analyzed, such as the AIDS virus, hepatitis, and a common herpes germ. But the lack of quick tests for less common conditions prevents screening for diseases such as the lymphocytic choriomeningitis virus.

Because the demand for organs always far exceeds the supply, recipients will accept organs even from high-risk donors such as the homeless. Waiting too long for a new kidney, liver, or heart can prove riskier.

"People are literally dying for organs," said Dr. Alfred DeMaria, top disease tracker at the Massachusetts Department of Public Health. "The list of potential things you can test for is enormous. But balancing that against the risk of not getting the organs, you have to make some decisions about what's feasible and what's not feasible to test for."

The homeless donor died in mid-March. After his family authorized the removal of viable organs, doctors took his kidneys. He had been tested for the AIDS virus, the liver diseases hepatitis B and C, and other diseases regularly checked by the New England Organ Bank, the region's organ procurement agency. There was no evidence of worrisome infections.

Still, his status as a man who had lived on the street, potentially exposed to a host of dangerous germs, led transplant surgeons to brand him as a high-risk donor.

Transplant surgeons at the hospitals with the two potential recipients - the woman was at Boston Medical Center, the man at Beth Israel Deaconess Medical Center - alerted the patients that the donor was regarded as high risk. The surgeons and patients decided to proceed.

"We all know that as much as we explain to the patients and inform them, they're relying on us and our medical judgment about whether this is a safe transplant," said Dr. Douglas W. Hanto, chief of the Division of Transplantation at Beth Israel Deaconess. "We feel a tremendous sense of responsibility to the patient and their family and feel terrible that this patient has had this infection and a bad outcome.

"But, on the other hand, we see patients who die every day on dialysis" awaiting a kidney transplant, he said.

The 57-year-old man transplanted at Hanto's hospital had lingered four years on the waiting list for a kidney. According to the United Network for Organ Sharing, an independent agency that sets organ procurement policies, 80,130 patients in the United States currently need a kidney.

It was the woman transplanted at Boston Medical who got sicker sooner after returning home. Like the donor and the other recipient, the woman was not identified by health authorities, who cited patient confidentiality laws.

The woman returned to Boston Medical about two weeks after her surgery, said Dr. Greg Grillone, the hospital's interim chief medical officer. She had a fever, diarrhea, "but oddly, symptoms not specific to the kidney," Grillone said.

Her condition kept deteriorating and, in mid-April, the woman died. Doctors at the hospital were stumped. There was no obvious cause of her precipitous demise.

But it turned out that one of the surgeons involved in the case, Dr. Amitabh Gautam, had been connected to

the 2005 Rhode Island and Massachusetts transplant cases.

He became suspicious that the Boston Medical patient had the same virus and alerted the federal Centers for Disease Control and Prevention. The virus has been known to have spread via transplant only two other times, in Wisconsin and Australia.

"Interestingly, what happened was this doctor had seen this before and thought, 'OK, this is a long shot, but I have seen it before and it can happen,' " Grillone said.

"If you take your car to the auto dealer with some very, very rare problem and you're lucky enough to get the mechanic who saw that same problem three years ago in the same make or model of the car, he might think: 'Oh, I saw this same problem three years ago. it might be the same problem," he said.

The man who had received his kidney at Beth Israel Deaconess returned with a fever 2 1/2 weeks after the surgery. On April 18, the doctors there got word that the Boston Medical patient had died. A transplant specialist at Beth Israel Deaconess also speculated that the virus might be at fault.

Samples from the deceased donor and the two patients were rushed to the CDC in Atlanta. All three tested positive for the virus, and investigators said all evidence points to the donor. The 57-year-old recipient remains in intensive care and is receiving the only drug known to possibly treat the virus.

"I don't believe this ever put the general public at risk," said Dr. Anita Barry, who leads the Boston Public Health Commission's investigation of the infections. "You have to be very, very unlucky to get LCMV from a transplant."

The virus is not transmitted casually from person-to-person; in addition to transplants, the only identified human transmissions have been from mother to fetus. Most people who are exposed catch it from the droppings of rodents, including wild animals and pets.

Because the virus causes few health problems in those who contract it, there has been little incentive to develop a rapid test.

The only tests currently available take time and are not widely available, said Dr. Eileen Farnon, a CDC medical epidemiologist.

"If you had a few days or a week for testing you could do that," Farnon said. "But in general that's not how the organ transplantation business works."

Stephen Smith can be reached at stsmith@globe.com. ■

医薬品　研究報告　調査報告書

<table>
<tr><td colspan="2">識別番号・報告回数</td><td></td><td>報告日</td><td>第一報入手日
2008年5月7日</td><td colspan="2">新医薬品等の区分
該当なし</td><td>総合機構処理欄</td></tr>
<tr><td colspan="2">一般的名称</td><td>別紙のとおり</td><td rowspan="2">研究報告の
公表状況</td><td rowspan="2">Infect Genet Evol. 2008 Mar 4. [Epub ahead of print]</td><td rowspan="2">公表国
コスタリカ</td><td rowspan="2"></td></tr>
<tr><td colspan="2">販売名（企業名）</td><td>別紙のとおり</td></tr>
<tr><td>研究報告の概要</td><td colspan="5">問題点：コスタリカにおいて、高病原性で新しい血清型に分類されるレプトスピラ株がヒトから分離された。

コスタリカにおいて、地域で流行しているレプトスピラの血清型を同定するため、通院している患者からレプトスピラを分離・解析した。レプトスピラ症の症状を呈して入院していた患者から分離された MAVJ401 株は、ウサギ抗血清パネルで Javanica 血清群型の血清型に対して著しく凝集価が上昇したが、標準的な Cross Agglutinin Absorption Test では血清学的にユニークであった。そのため MAVJ401 株は、Javanica 血清群型の新しい血清型（Arenal と命名）であると推測された。また、MAVJ401 株は、遺伝子学的解析によりラテンアメリカ諸国で多く発生している種である Leptospira santarosai に分類された。同じ地区の重症患者から分離された株も Arenal と同一の血清型であったことから、これが外来の血清型ではなく、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。この新しい血清型に分類されるレプトスピラ株は、地域の公衆衛生と家畜衛生に脅威をもたらすおそれがある。</td><td>使用上の注意記載状況・
その他参考事項等

記載なし</td></tr>
<tr><td colspan="3">報告企業の意見</td><td colspan="4">今後の対応</td></tr>
<tr><td colspan="3">別紙のとおり</td><td colspan="4">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。</td></tr>
</table>

MedDRA ver.11.0

(13)

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	レプトスピラ症は、病原性レプトスピラ感染に起因する人獣共通の細菌(スピロヘータ)感染症である。レプトスピラは通常、長さ 6～20μm、直径 0.1μm のらせん状の細菌で、病原性レプトスピラと非病原性レプトスピラに大別される。病原性レプトスピラは、げっ歯類をはじめ多くの野生動物や家畜(ウシ、ウマ、ブタ、ヒツジなど)、ペット(イヌ、ネコなど)の腎臓に保菌され、尿中に排出される。ヒトは、保菌動物の尿で汚染された水や土壌から経皮的あるいは経口的に感染する。レプトスピラ症は急性熱性疾患であり、感冒様症状のみで軽快する軽症型から、黄疸、出血、腎障害を伴う重症型(ワイル病)まで多彩な症状を示す。レプトスピラは現在、13 の遺伝種からなり、さらに免疫学的性状により 250 以上の血清型に分類されている。日本におけるレプトスピラ症の患者数は近年激減したが、南西諸島・本土南部地域では他の地域に比べて多く散発している。また世界的に見ると、特に東南アジアや中南米などの亜熱帯、熱帯地域で患者発生が多い。レプトスピラは感染初期にヒトの血液や尿から直接観察される場合があることから、本剤の原料への混入を完全に否定できないと考え、本報告を行った。 仮に、製造原料にレプトスピラが混入していたとしても、弊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、レプトスピラ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」が導入されており、これらの工程により除去されるものと考えられる。更に、これまでに当該製剤によるレプトスピラ感染の報告例は無い。 以上の点から、当該製剤はレプトスピラ感染に対する安全性を確保していると考える。しかし、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

Infection, Genetics and Evolution xxx (2008) xxx–xxx

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Arenal, a new *Leptospira* serovar of serogroup Javanica, isolated from a patient in Costa Rica

Ma. de los A. Valverde [a], J.M. Ramírez [b], L.G. Montes de Oca [c], Marga G.A. Goris [d], Niyaz Ahmed [e], Rudy A. Hartskeerl [d,*]

[a] *Centro Nacional de Referencia Leptospirosis, INCIENSA (Instituto Costarricense de Investigación y Enseñanza en Nutrición y Salud), Costa Rica*
[b] *C.H.F.A. (Caja Costarricense de Seguro Social), Costa Rica*
[c] *Hospital San Carlos (Caja Costarricense de Seguro Social), Costa Rica*
[d] *Royal Tropical Institute, Department of Biomedical Research, WHO/FAO/OIE Leptospirosis Reference Centre, Meibergdreef 39, 1105 AZ Amsterdam, The Netherlands*
[e] *Pathogen Evolution Laboratory, Centre for DNA Fingerprinting and Diagnostics, Hyderabad, India*

ARTICLE INFO

Article history:
Received 13 November 2007
Accepted 27 February 2008
Available online xxx

Keywords:
Leptospires
Leptospirosis
New serovar
Patient
Public health

ABSTRACT

Leptospirosis is a worldwide distributed zoonotic disease caused by pathogenic spirochetes of the genus *Leptospira*. The basic taxon of *Leptospira* is the serovar. Currently, nearly 300 serovars have been identified. Leptospirosis is particularly prevalent in warm and humid tropical regions where conditions for transmission and survival of pathogenic leptospires in the environment are optimal. Leptospirosis probably constitutes a serious veterinary and public health problem in Central America but solid figures are missing. To determine distribution of leptospirosis in Costa Rica and to identify locally circulating pathogenic serovars, we performed a sentinel-based study, isolating and characterizing leptospires from patients attending hospitals. Strain MAVJ 401 was isolated from a hospitalized patient in the Alajuela province. The isolate produced agglutination titers notably with reference rabbit antisera against serovars of serogroup Javanica but appeared serologically unique in the standard Cross Agglutinin Absorption Test. Therefore, MAVJ 401 was considered to represent a new serovar, designated Arenal, of the serogroup Javanica. Genotypic analysis revealed that strain MAVJ 401 belongs to *Leptospira santarosai*, a species that almost exclusively occurs in Latin America. This is not a unique finding of an exotic serovar. Recent isolates from severely ill patients in the same region appeared to be identical to Arenal.

We have identified a novel highly virulent serovar from a patient in Costa Rica that is common in this area, thus posing a threat for the local public and veterinary health.

1. Introduction

Leptospirosis is a worldwide zoonosis, transmitted to humans through contaminated water or direct exposure to the urine of infected animals. Human infection may be acquired through occupational, recreational, or avocational exposures. Direct contact with infected animals accounts for most infections in farmers, veterinarians, abattoir workers, meat inspectors, rodent control workers and other occupations which require contact with animals. Indirect contact is important for sewer workers, miners, soldiers, septic tank cleaners, fish farmers, gamekeepers, canal workers, rice field workers, taro farmers, banana farmers and sugar cane cutters (Levett, 2001).

The clinical spectrum of the disease ranges from mild influenza-like to severe forms such as the Weil's syndrome, characterized by hepato-renal dysfunctions and a bleeding tendency and Acute Respiratory Distress Syndrome (ARDS) with mortality rates exceeding 50% (Levett, 2001; McBride et al., 2005).

Development of a subclinical infection or clinical disease might depend on both host and causative agent related factors such as immunological competence, age, physical condition and virulence and size of the inoculum, respectively. Animals with subclinical infections as well as those that recover from the clinical disease become a potential source of infection for other susceptible hosts, because they continue to excrete leptospires for a prolonged period of time (Faine, 1982; Faine et al., 1999).

The causative agents of leptospirosis belong to the genus *Leptospira*, which contains both saprophytic and pathogenic species (Levett, 2001). The isolation and identification of an infecting *Leptospira* strain is cumbersome and time consuming. Isolation is difficult due to the slow growth rate, notably when combined with a concomitant contamination with faster growing microorganisms, and stringent and fastidious in vitro culture requirements of these bacteria (Faine, 1994; Faine et al., 1999). The

* Corresponding author. Tel.: +31 20 566 5437; fax: +31 20 697 1841.
E-mail address: r.hartskeerl@kit.nl (R.A. Hartskeerl).

1567-1348/$ – see front matter © 2008 Elsevier B.V.. All rights reserved.
doi:10.1016/j.meegid.2008.02.008

Please cite this article in press as: Valverde, M.A. et al., Arenal, a new *Leptospira* serovar of serogroup Javanica, isolated from a patient in Costa Rica, Infect. Genet. Evol. (2008), doi:10.1016/j.meegid.2008.02.008

initial identification of a *Leptospira* is morphological, by dark field microscopy observation. Definitive identification of the isolates requires the use of serological and molecular techniques (Dikken and Kmety, 1978; Brenner et al., 1999; Levett, 2003). In the conventional classification system, all pathogenic leptospires belong to the species *Leptospira interrogans* sensu lato (Dikken and Kmety, 1978; Faine and Stallman, 1982). Based on serological criteria, strains of *Leptospira* are differentiated into serovars, which represent the basic taxon (ICSB Sub-committee on the taxonomy of Leptospira, 1987; Kmety and Dikken, 1993). Serovars that are antigenically related are placed into serogroups. Serogroups do not have an official taxonomic status, but are of clinical and epidemiological importance (Levett, 2003). The list is updated periodically and more than 250 pathogenic serovars arranged in 26 serogroups are currently known. The recent genotypical classification system is based on DNA homology. In this system, leptospires are placed into 17 *Leptospira* species of a pathogenic, saprophytic and doubtful nature (Yasuda et al., 1987; Perolat et al., 1998; Brenner et al., 1999; Levett et al., 2006). There is a poor correlation between the serological and genotypic classification systems (Brenner et al., 1999; Yasuda et al., 1987).

The species *Leptospira santarosai* contains 61 serovars of multiple serogroups (Brenner et al., 1999). The type strain of *L. santarosai*, serovar Shermani strain 1342 K was isolated from a spiny rat (*Proechymis semispinousus*) in the Panama Canal Zone (Yasuda et al., 1987). Several additional reports confirmed that *L. santarosai* is pathogenic for humans and domestic animals (Brenner et al., 1999; Hsieh and Pan, 2004; Rossetti et al., 2005).

In this paper, we describe a new leptospiral serovar belonging to the species *L. santarosai* isolated from the blood of a severely ill leptospirosis patient.

2. Materials and methods

2.1. Case description

A 45-year-old man was hospitalized in Ciudad Quesada San Carlos Hospital, Costa Rica, with a 3–4 day history of fever, headache and myalgia. The patient is a biologist employed by a Costa Rican fish farm. At the day of admission his temperature was 39°. He had tachycardia and his blood pressure was 120/60 mmHg. Clinical examination showed a conscious man, with bilateral headache, sore throat, provoked myalgia of the legs, hepatalgia, hepatomegaly, and conjunctivitis. There were no signs of rash, meningeal irritation and cervical rigidity. Laboratory tests revealed increased SGOT:79.8 U/L (normal range (nr) 12.0–46.0), 76.2 U/L (nr 3–50), creatine phosphokinase: 915 U/L (nr 24–195), direct bilirubin: 0.53 mg/dL (nr 0.0–0.2), total bilirubin: 1.49 mg/dL (nr 0.0–1.0), associated with hyperglycemia: 143 mg/dL, alkaline phosphatase: 202 U/L (value is within normal range, nl), albumin: 3.3 g/dL (nl), and protein levels: 5.92 g/dL (nl), ureic nitrogen: 8.62 mg/dL (nl), creatinine: 1.26 mg/dL (nl). The leukocyte count was 8, $2 \times 10^3/\mu L$ with 80% polymorph nuclear forms. Thrombocytopenia: $145 \times 10^3/\mu L$ (last control: $99 \times 10^3/\mu L$) was also observed. Results of urinalysis were normal. Malaria blood smears, blood cultures and serology for dengue were negative.

The patient received a 7-day treatment with penicillin, 2 million units 4 times a day, which resulted in a resolution of symptoms. Oral treatment with penicillin was continued for 6 more days.

Leptospirosis was confirmed by seroconversion in the Microscopic Agglutination Test (MAT) with a titer of 1:100 with serovar Canicola in the second sample. Also the rapid screening test Lepto dipstick (Gussenhoven et al., 1997) gave a positive outcome (data not shown).

2.2. Bacterial culture

Culturing was performed in Ellinghausen and McCullough modified Johnson and Harris (EMJH) culture medium (DifcoTM). Aliquots of 0.1 and 0.01 mL of heparin anticoagulanted whole blood were inoculated into 6 mL EMJH culture medium. Incubation was at 30 °C and cultures were inspected by darkfield microscopy for growth of leptospires at regular intervals. Isolates were subcultured and maintained in EMJH medium and in Fletcher medium supplemented with 5 fluoro-uracil (200 μg/mL) as a selective inhibitor for contaminating microorganisms (Faine and Stallman, 1982; Faine et al., 1999; Hartskeerl et al., 2006).

2.3. Microscopic agglutination test

The microscopic agglutination test (MAT) was performed as per standard procedure (Comisión Científica Permanente sobre Leptospirosis de la AAVL, 1994) starting with a serum dilution of 1:20 up to 1:20480. The highest dilution of serum showing 50% reduction in free-moving leptospires under dark field microscope was considered the end-titre. Rabbit anti-*Leptospira* sera were prepared following the standard procedure (ICSB Sub-committee on the taxonomy of *Leptospira*, 1984).

2.4. Serological typing: MAT with group sera and monoclonal antibodies

To identify the isolate up to serogroup level, MAT was performed following standard procedure using a panel of 38 anti-*Leptospira* rabbit antibodies (Dikken and Kmety, 1978; Hartskeerl et al., 2006). Isolates were further typed at the serovar level by performing MAT with panels of monoclonal antibodies (mAbs) that characteristically agglutinate serovars from the serogroups Icterohaemorrhagiae and Sarmin (F12C3, F20C3, F20C4, F52C1, F52C2, F70C4, F70C7, F70C13, F70C14, F70C20, F70C24, F70C26, F82C1, F82C2, F82C7, F82C8, F89C3, and F89C12) as described by Korver et al. (1988) and from serogroup Javanica (F12C3, F20C3, F20C4, F70C20, F98C4, F98C5, F98C8, F98C12, F98C17, F98C19 and F98C20) with cross-agglutinations of serovars of the closely related serogroups Sarmin and Celledoni (Alex et al., 1993).

2.5. Cross Agglutinin Absorption Test

The Cross Agglutinin Absorption Test (CAAT), the standard assay for serological classification of *Leptospira* serovars was carried out by staff of INCIENSA as described elsewhere (Dikken and Kmety, 1978; Kmety and Dikken, 1993; Hartskeerl et al., 2006, ICSB Sub-committee on the taxonomy of *Leptospira*, 1984). Staff of the WHO/FAO/OIE Collaborating Centre for Reference and Research on Leptospirosis of the Royal Tropical Institute, The Netherlands confirmed the CAAT results.

2.6. Genetic characterization

Strains and isolates were grown at 30 °C in EMJH medium and harvested by centrifugation during the late logarithmic phase. DNA was isolated as described by Boom et al. (1990). PCR was performed on the DNA extracts using the primer set G1/G2 that specifically amplifies a 285 bp fragment of the *secY* gene from all pathogenic species except *L. kirschneri* (Gravekamp et al., 1993; Oliviera et al., 2003). PCR conditions and controls were as previously described (Gravekamp et al., 1993; Bal et al., 1994). PCR products were analyzed by electrophoresis in 1.5% agarose gels, stained with ethidium bromide using standard procedures and subsequently judged by eye under UV illumination.

For sequencing, DNA concentration of PCR products was adjusted in the range of 10–20 ng per reaction and applied to

Please cite this article in press as: Valverde, M.A. et al., Arenal, a new *Leptospira* serovar of serogroup Javanica, isolated from a patient in Costa Rica, Infect. Genet. Evol. (2008), doi:10.1016/j.meegid.2008.02.008

the sequence reaction using the BigDye® Terminator V1.1 Cycle Sequencing Kit (Applied Biosystems, United Kingdom) and subsequently analyzed on an ABI PRISM™ 310 Genetic Analyzer (Applied Biosystems, United Kingdom). DNA sequence clustal alignments were done using the LaserGene software package (DNASTAR). Species determination was done on basis of highest sequence identity of PCR products from *Leptospira* reference strains (Gravekamp et al., 1993; Oliviera et al., 2003; Rossetti et al., 2005; Priya et al., 2007).

3. Results

3.1. Isolation

The culture with 0.1 mL blood inoculation became positive after two weeks. The isolate was named strain MAVJ 401. Under the darkfield microscope, strain MAVJ 401 showed typical *Leptospira* motility and morphology. The strain grew well in EMJH and Fletcher medium at 30 °C.

3.2. Serological characterization

When testing the strain against a panel of 38 rabbit anti-*Leptospira* sera to determine potential serogroups, highest agglutination titers were found against serogroup Sarmin serovar Weaveri and serogroup Javanica serovar Poi. Low cross-agglutinating titers were also produced with members of the serogroups Icterohaemorrhagiae and Celledoni. No agglutinations were found with reference sera from intermediate and saprophytic reference strains, suggesting a pathogenic status of the isolate.

Subsequent testing with the panel of mAbs against serovars of the Icterohaemorrhagiae and Sarmin groups only revealed a titer 1:320 against one of the 18 mAbs in the panel. No match was found with the agglutination pattern of any of the serovars in these two serogroups (results not shown). The agglutination pattern obtained with the mAbs against serovars of the Javanica group was most similar with that of serovar Javanica, strain Veldrat Batavia 46 (Table 1). No match was found with serovars of the closely related serogroup Celledoni and, again, serogroup Sarmin.

Cross-agglutinations and CAAT were performed to confirm the presumptive results obtained via mAbs typing.

Cross-agglutination experiments were executed between strain MAVJ 401 and antiserum against all serovars from the groups Javanica, Sarmin and Celledoni and vice versa. No significant cross-agglutinations (>10% compared to the homologous agglutination) were observed with sera from the serogroups Celledoni and Sarmin and vice versa, serum against MAVJ 401, virtually excluding that MAVJ 401 belongs to these serogroups. A significant cross-agglutination titer in both cross-agglutination experiments was only found against serogroup Javanica serovar Fluminense strain Aa3. Surpisingly only low cross-agglutination titers were found against serovar Javanica strain Veldrat Batavia 46.

CAAT was performed in duplicate and independently by two persons to assure reproducibility. The following reference strains were included in the test, Javanica group: serovar Fluminense strain Aa3, serovar Sofia strain Sofia 874, serovar Coxi strain Cox, serovar Javanica strain Veldrat Batavia 46, serovar Sorexjalna strain Sorex Jalná, serovar Zhengkang strain L 82 and serogroup Sarmin; serovar Machiguenga strain MMD 3, serovar Rio strain Rr 5 and serovar Weaveri strain CZ 390.

According to the definition of the International Committee on Systematic Bacteriology, Subcommittee on the Taxonomy of *Leptospira* (1984, 1987), strain MAVJ 401 was not serologically identical to any of these strains (Table 2) and therefore MAVJ 401 represents a new serovar, designated Arenal. Based on the initial serological reactions it is proposed that this serovar is placed within the pathogenic serogroup Javanica.

Table 1
Comparison of agglutination titers of strain MAVJ 401 and the reference serovar Javanica, strain Veldrat Batavia 46 with mAbs against serogroup Javanica

mAb	Reciprocal titers against strain MAVJ 401	Reciprocal titers against strain Veldrat Batavia 46
F12C3	–	–
F20C3	–	–
F20C4	320	320
F70C20	–	–
F98C4	–	–
F98C5	–	–
F98C8	5120	5120
F98C12	20480	5120
F98C17	–	–
F98C19	10240	10240
F98C20	–	≤80

(–) No agglutination.
Up to a 4-fold titer difference is acceptable in mAbs typing.

Table 2
Cross-agglutinations and CAAT between MAVJ 401 and reference strains

Serum	Strain	Cross agglutination (%)[a]	CAAT, residual titer (%)[b]
Aa3	MAVJ 401	50	50
MAVJ 401	Aa3	12.5	100
Sofia 874	MAVJ 401	12.5	50
MAVJ 401	Sofia 874	0.2	ND
Cox	MAVJ 401	6.25	50
MAVJ 401	Cox	0.4	ND
Veldrat Batavia 46	MAVJ 401	1.5	100
MAVJ 401	Veldrat Batavia 46	0.2	ND
Sorex Jalná	MAVJ 401	100	100
MAVJ 401	Sorex Jalná	0.2	ND
L 82	MAVJ 401	12.5	100
MAVJ 401	L 82	0.8	ND
MMD 3	MAVJ 401	50	100
MAVJ 401	MMD 3	6.25	ND
Rr 5	MAVJ 401	25	50
MAVJ 401	Rr5	6.25	ND
CZ 390	MAVJ 401	25	100
MAVJ 401	CZ 390	1.5	ND

[a] (Heterologous titer: homologous titer) × 100%; >10% is significant.
[b] (Homologous titer after absorption: homologous titer before absorption) × 100%; <10% indicates similarity of the serovars.

3.3. Species determination

Consistent with its pathogenic status, DNA from MAVJ 401 was amplified by primer pair G1/G2 (Gravekamp et al., 1993). To determine the species of MAVJ 401, the sequence of its G1/G2 amplicon was compared with 65 other sequences (Oliviera et al., 2003; Rossetti et al., 2005; Priya et al., 2007). The sequence of the amplicon showed highest percentage identity with a number of strains from *L. santarosai*, i.e. 97.1% with serogroup Sejroe; serovar Caribe strain TRVL 61866 and serovar Gorgas strain 1413 U, serogroup Mini; serovar Georgia strain LT 117 and Tabaquite strain TRVL 3214, serogroup Pyrogenes; serovar Princestown strain TRVL 112499, serogroup Javanica; serovar Vargonis strain 24, serogroup Sarmin; serovar Weaveri strain CZ 390 and 96.7% identity with serogroup Pomona, serovar Tropica strain CZ 299.

Percentages sequence identity outside *L. santarosai* ranged from 71.3% (*L. meyeri*, serovar Semaranga strain Veldrat Semarang 173) to 94.7% (*L. weilii* serovar Mengrun strain A 102 and *L. weilii*, serovar Coxi strain Cox). Taking the highest percentage of identity with eight strains of *L. santarosai*, we believe that MAVJ 401 belongs to this species.

Please cite this article in press as: Valverde, M.A. et al., Arenal, a new *Leptospira* serovar of serogroup Javanica, isolated from a patient in Costa Rica, Infect. Genet. Evol. (2008), doi:10.1016/j.meegid.2008.02.008

4. Discussion

We describe the isolation and characterization of a novel *Leptospira* serovar isolated from a Costa Rican patient. The patient was admitted to the hospital with signs and symptoms compatible with leptospirosis and standard antibiotic treatment with penicillin was effective. Leptospirosis was serologically confirmed. It likely concerns here an occupational disease as the patient worked on a fish farm where he obviously acquired the infection via fish ponds contaminated with urine of carrier animals.

The morphology and motility of the bacterium under darkfield microscopy is consistent for the genus *Leptospira*. Serologically, the isolate showed titers notably against members of the serogroups Javanica and Sarmin. Cross-agglutination titers were also found in the serogroups Icterohaemorrhagiae and Celledoni. This likely represents intra-serogroup cross-agglutinations because serogroups Javanica and Celledoni on one hand and Javanica, Sarmin and Icterohaemorrhagiae on the other hand form 'serogroup complexes' comprising antigenic related serovars (Hartskeerl et al., 2006). Because of this overlapping antigenic relationship between these groups and the fact that highest agglutinating titers were produced with serovars of serogroup Javanica we suggest to place MAVJ 401 into this serogroup.

We found contrasting data by mAbs typing and the CAAT. mAbs typing generated a pattern that was highly similar to that of the reference serovar Javanica strain Veldrat Batavia 46 of the Javanica group. However, cross-agglutination and CAAT revealed only little similarity with this serovar. Moreover, CAAT, which is the standard method to determine the serovar as basic taxon, revealed that this isolate is unique. The serovar status is mainly, if not exclusively, based on the composition and structure of the highly antigenic LPS (Faine et al., 1999). A likely explanation of the discrepancy in typing with monoclonal and polyclonal sera is that panels of agglutinating mAbs are directed to a limited number of epitopes while polyclonal hyperimmune sera cover the full spectrum of epitopes. Apparently, it is possible that a set of mAbs recognizes a limited number of common epitopes on furthermore different LPS in distinct serovars within a serogroup. As shown in this study, incorrect mAbs-based identification can be avoided by determining cross agglutination with polyclonal hyperimmune serum against the presumably corresponding reference strain.

We designated the isolate serovar Arenal after the volcano in the Costa Rica near the residence of the patient in the province Alajuela.

DNA sequence analysis indicated that serovar Arenal most likely belongs to species *L. santarosai*, which is distributed almost exclusively in Latin America (Chappel et al., 1998).

Serovar Arenal likely is not an exotic serovar and might be common in and around the Alajuela province of Costa Rica. Recently, two out of 21 isolates obtained from Costa Rica were identified as serovar Arenal implying that 13.6% (3/22) of the isolates consisted of Arenal. The two additional Arenal isolates, preliminary coded as isolate 7 and 11, were cultured from severely ill patients living in the Puntarenas province that flanks Alajuela. Molecular analysis of MAVJ 401/isolate 7 by Multilocus Sequence Typing showed that it formed a distinct branch that was positioned closely to, but apart from the clade of *L. santarosai* (Ahmed et al., 2006). This supports the unique character of this novel serovar, also on genotypical grounds.

The infection source of isolate 11 is unknown. Infection with isolate 7 was very likely acquired via contact with cattle. The environment of the fish farm of MAVJ 401 makes it possible that the ponds have been contaminated with urine of infected cattle. It is therefore tempting to speculate that cattle form the infection reservoir of this novel serovar. However, further research on potential infection sources in the region will be needed to confirm or refute this.

L. santarosai, serovar Arenal, type strain MAVJ 401 has been deposited under this designation in the culture collections of the National Reference Center for Leptospirosis, Costa Rican Institute for Research in Nutrition and Health, Tres Ríos, Costa Rica and the WHO/FAO/OIE and National Collaborating Centre for Reference & Research on Leptospirosis, Royal Tropical Institute, Amsterdam, Netherlands. The novel serovar designation of strain MAVJ 401 has been ratified by the International Committee on Systematic Bacteriology, Subcommittee on the Taxonomy of *Leptospiraceae*.

Acknowledgments

The authors wish to thank Mr. J.I. Chaverri and Dr. M. Liem for their technical assistance. The work was supported by EU-INCO grant ICA4-CT-2001-10086. N.A. would like to thank CDFD for core grant support.

References

Ahmed, N., Devi, S.M., Valverde, M.A., Vijachari, P., Machang'u, R.S., Ellis, W.A., Harstkeerl, R.A., 2006. Multilocus sequence typing method for identification and genotypic classification of pathogenic *Leptospira* species. Ann. Clin. Microbiol. Antimicrob. 5, 28.

Alex, J.C., Korver, H., Ratnam, S., Venkatesan, R.A., 1993. *Leptospira* isolates of Madras City. Ind. J. Med. Microbiol. 17, 269–273.

Bal, A.E., Gravekamp, C., Hartskeerl, R.A., De Meza-Brewster, J., Korver, H., Terpstra, W.J., 1994. Detection of *Leptospira* in urine by PCR for early diagnosis of leptospirosis. J. Clin. Microbiol. 32, 1894–1898.

Brenner, D.J., Kaufmann, A., Sulzer, K.R., Steigerwalt, A.G., Rogers, F.C., Weyant, R.S., 1999. Further determination of DNA relatedness between serogroups and serovars in the family Leptospiraceae with a proposal for *Leptospira alexanderi* sp. nov. and four new *Leptospira* genomospecies. Int. J. Syst. Bacteriol. 49, 839–858.

Boom, R., Sol, C.J.A., Salisman, M.M.M., Jansen, C.L., Wertheim van Dillen, P.M.E., van der Noordaa, J., 1990. Rapid and simple method for purification of nucleic acids. J. Clin. Microbiol. 28, 495–503.

Chappel, R.J., Khalik, D.A., Adler, B., Bulach, D.M., Faine, S., Perolat, P., 1998. Serological titers to *Leptospira fainei* serovar Hurstbridge in human sera in Australia. Epidemiol. Infect. 121, 473–475.

Comisión Cientifica Permanente sobre Leptospirosis de la A.A.V.L.D., 1994. Manual de leptospirosis. Asociación Argentina de Veterinarios de Laboratorios de Diagnostico, Buenos Aires, Argentina.

Dikken, H., Kmety, E., 1978. Serological typing methods of leptospires. In: Bergan, T., Norris, J.R. (Eds.), Methods in Microbiology, vol. 11. Academic Press, London, U.K., pp. 259–307.

Faine, S., 1982. Guidelines for the Control of Leptospirosis. World Health Organization Geneva, Italy (WHO Offset Publication No. 67).

Faine, S., Stallman, N.D., 1982. Amended descriptions of the genus *Leptospira* Noguchi 1917 and the species *L. interrogans* (Stimson1907) Wenyon 1926 and *L. biflexa* (Wolbach and Binger 1914) Noguchi 1918. Int. J. Syst. Bacteriol. 88, 1493–1496.

Faine, S., 1994. *Leptospira* and Leptospirosis. CRC Press, Boca Raton, FL, USA.

Faine, S., Adler, B., Bolin, C., Perolat, P., 1999. *Leptospira* and Leptospirosis Armadale, second ed. Medi Sci Writing, Melbourne, Vic, Australia.

Gravekamp, C., van de Kemp, H., Franzen, M., Carrington, D., Schoone, G.J., van Eys, G.J., Everard, C.O., Hartskeerl, R.A., Terpstra, W.J., 1993. Detection of seven species of pathogenic leptospires by PCR using two sets of primers. J. Gen. Microbiol. 139, 1691–1700.

Gussenhoven, G.C., Hoorn, M.A.W.G., Goris, M.G.A., Terpstra, W.J., Hartskeerl, R.A., Mol, B.W., van Ingen, C.W., Smits, H.L., 1997. LEPTO dipstick, a dipstick assay for the detection of *Leptospira*-specific immunoglobulin M antibodies in human sera. *J. Clin. Microbiol.* 35, 92–97.

Hartskeerl, R.A., Smits, H.L., Korver, H., Goris, M.G.A., Terpstra, W.J., 2006. Manual International Course on Laboratory Methods for the Diagnosis of Leptospirosis. KIT, Amsterdam, The Netherlands.

Hsieh, W.J., Pan, M.J., 2004. Identification of *Leptospira* santarosai serovar shermani specific sequences by suppression subtractive hybridization. FEMS Microbiol. Lett. 235, 117–124.

International Committee on Systematic Bacteriology, Subcommittee on the Taxonomy of *Leptospira*, 1984. Minutes of the Meetings, August 6–10, 1982, Boston, MA, USA. Int. J. Syst. Bacteriol. 34, 258–259.

International Committee on Systematic Bacteriology, Subcommittee on the Taxonomy of *Leptospira*, 1987. Minutes of the Meetings, September 5–6 1986, Manchester, England. Int. J. Syst. Bacteriol. 37, 472–473.

Kmety, E., Dikken, H., 1993. Classification of the Species *Leptospira interrogans* and History of its Serovars. University Press, Groningen, The Netherlands.

Korver, H., Kolk, A.H.J., Vingerhoed, J., van Leeuwen, J., Terpstra, W.J., 1988. Classification of the serovars of Icterohaemorrhagiae serogroup by monoclonal antibodies. Isr. J. Vet. Med. 44, 15–18.

Levett, P.N., 2001. Leptospirosis. Clin. Microbiol. Rev. 14, 296–326.

Levett, P.N., 2003. *Leptospira* and leptonema. In: Murray, P.R., Baron, E.J., Jorgensen, J.H., Pfaller, M.A., Yolken, R.H. (Eds.), Manual of Clinical Microbiology, vol. 1. A.S.M. Press, Washington, DC, pp. 929–936.

Levett, P.N., Morey, R.E., Galloway, R.L., Steigerwalt, A.G., 2006. *Leptospira broomii* sp. Nov. isolated from humans with leptospirosis. Int. J. Syst. Evol. Microbiol. 56, 671–673.

McBride, A.J., Athanazio, D.A., Reis, M.G., Ko, A.I., 2005. Leptospirosis. Curr. Opin. Infect. Dis. 18, 376–386.

Oliviera, M.A.A., Baballero, O.L., Vago, A.R., Hartskeerl, R.A., Romanha, A.J., Pena, S.D.J., Simpson, A.J.G., Koury, M.C., 2003. Low-stringency single specific primer PCR for identification of *Leptospira*. J. Med. Microbiol. 52, 127–135.

Perolat, P., Chappel, R.J., Adler, B., Baranton, G., Bulach, D.M., Billinghurst, L.M., Letocat, M., Merien, M., Serrano, M.S., 1998. *Leptospira fainei* sp. nov., isolated from pigs in Australia. Int. J. Syst. Bacteriol. 48, 851–858.

Priya, C.G., Hoogendijk, K.T., Berg, M.V.D., Rathinam, S.R., Ahmed, A., Muthukkaruppan, V.R., Hartskeerl, R.A., 2007. Field rats form a major infection source of leptospirosis in and around Madurai, India. J. Postgrad. Med. 53, 236–240 (www.jpgmonline.com).

Rossetti, C.A., Liem, M., Samartino, L.E., Hartskeerl, R.A., 2005. Buenos Aires, a new *Leptospira* serovar of serogroup Djasiman, isolated from an aborted dog fetus in Argentina. Vet. Microbiol. 107, 241–248.

Yasuda, B.H., Steigerwalt, A.G., Sulzer, L.R., Kaufmann, A.F., Rogers, F., Brenner, D.J., 1987. Deoxyribonucleic acid relatedness between serogroups and serovars in the family Leptospiraceae with proposals for seven new *Leptaspira* species. Int. J. Syst. Bacteriol. 37, 407–415.

Please cite this article in press as: Valverde, M.A. et al., Arenal, a new *Leptospira* serovar of serogroup Javanica, isolated from a patient in Costa Rica, Infect. Genet. Evol. (2008), doi:10.1016/j.meegid.2008.02.008

別紙様式第 2-1

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-1

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 200 年 4 月 10 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	Portsmouth woman's death under investigation dailypress.com, April 11, 2008	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
異型クロイツフェルト・ヤコブ病(vCJD)に関連すると疑われる脳変性疾患を呈した米国の女性の症例が報告された。しかし，感染症，脳内酸素欠乏，肝不全，腎不全，毒物暴露，代謝疾患，脳腫瘍，頭蓋内圧の上昇，栄養不足など多数の原因が，本症例の脳疾患に関連していると考えられており，原因究明には更なる調査が必要である。MRI 又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，バージニア大学及び National Prion Disease Pathology Surveillance Center (NPDPSC) で更に調査されることになっているが，結果が出るまでには数ヶ月間を要すると考えられている。	BYL-2008-0316

報告企業の意見	今後の対応
弊社の血漿分画製剤は米国の血漿を使用しているが，現在までに報告されている米国での vCJD 3 例は，米国以外の国で暴露された患者に限定されている。また，弊社の血漿分画製剤の製造工程におけるプリオン除去能は 4 log を上回ることが確認されており，弊社製剤による vCJD 感染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。

(14)

dailypress.com/news/local/southofjames/dp-local_deathprobe_0411apr11,0,2417128.story

dailypress.com

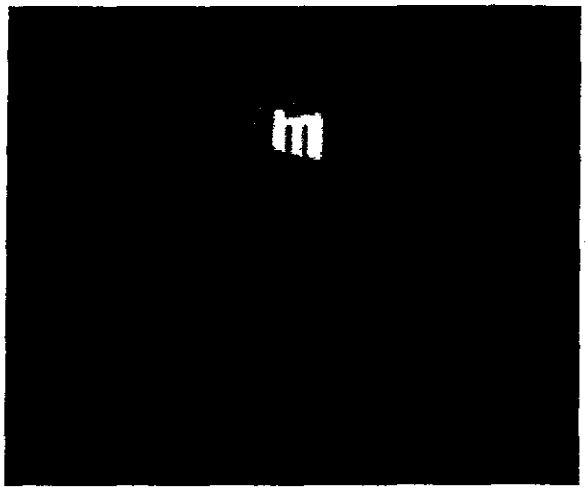
Portsmouth woman's death under investigation

By VERONICA GORLEY CHUFO

247-4741

April 11, 2008

RICHMOND



The illness and Wednesday death of a Portsmouth woman spurred a Virginia Department of Health investigation Thursday.

The woman suffered from encephalopathy, a degenerative brain disease. Her illness has been linked in news reports to variant Creutzfeldt-Jakob Disease — the human form of mad cow disease.

It's a very rare condition related to the consumption of beef infected with bovine spongiform encephalopathy. It's always fatal, the health department said in a news release.

The woman's name was not released by the health department but news reports have identified her as Aretha Vinson.

The illness could have been caused by a number of things, State Health Commissioner Karen Remley said in the release.

"Infections, lack of oxygen to the brain, liver failure, kidney failure, toxic exposures, metabolic diseases, brain tumors, increased intracranial pressure and poor nutrition are all related to encephalopathy," Remley said. "Further testing is the only way to know what caused this illness."

An MRI, or brain scan, was sent to the Centers for Disease Control and Prevention in Atlanta. Additional tests will be handled by the University of Virginia and the National Prion Disease Pathology Surveillance Center in Cleveland. Results are expected to take several months.

At least 200 cases of variant Creutzfeldt-Jakob Disease have been reported worldwide since 1996. Three cases have been reported in U.S. residents, and they were all exposed outside the country, Remley said. It's not spread casually from person to person.

For more information, visit cdc.gov, cjdfoundation.org or vdh.virginia.gov.

別紙様式第2-1

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙3-2

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2007 年 4 月 14 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	Prion Filter for Donated Blood Medgadget LLC, April 9, 2008	公表国 カナダ	
販売名（企業名）						

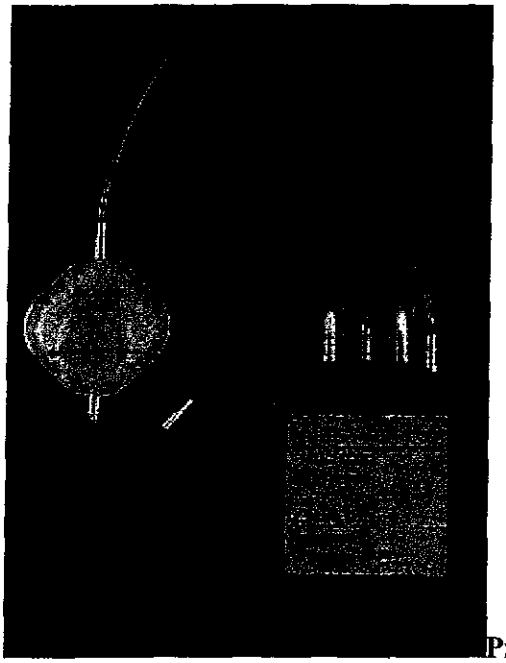
研究報告の概要	使用上の注意記載状況・その他参考事項等
カナダ，ケベック州の ProMetic Life Science 社が血液中の異型クロイツフェルト・ヤコブ病(vCJD)プリオンを除去するフィルターを開発した。 ProMetic Life Science 社の開発チームは，血液中のプリオンと親和性が高いペプチドをスクリーニングした後，市販のポリメタクリル酸樹脂にペプチドを固定し，これを膜状にしたものを幾層にも重ねて本フィルターを開発した。本使い捨てフィルターにより，1時間足らずで 1 ユニットの汚染血液からプリオンを除去することが可能であり，又，この工程によって血液自体の変性はおきない。さらに，プリオン感染ハムスターのフィルター処理した血液をプリオン非感染ハムスターに投与したところ，疾患は発現しなかった。	BYL-2008-0317

報告企業の意見	今後の対応
現在までに，血液中における vCJD 検査は可能となっていない。本フィルターの使用により，輸血による vCJD 感染に対する安全性は高まると考えられる。弊社の血漿分画製剤の製造工程におけるプリオン除去能は 4 log を上回ることが確認されている。	現時点で新たな安全対策上の措置を講じる必要はないと考える。

(57)

Print

Prion Filter for Donated Blood



ProMetic Life Sciences, a company out of Mont-Royal, Quebec, has developed a blood filter touted to remove prions responsible for variant Creutzfeldt-Jakob disease (vCJD). Considering that currently there is no available test for vCJD in donated blood, filtering may soothe the nerves of potential transfusion recipients.

> The team took five years to create the hand-sized filter, screening millions of small peptides to find one that had the strongest affinity for the prions found in contaminated blood. They stuck the best peptide onto commercial polymethacrylate resins, and then sandwiched these in alternating layers with a membrane
>
> In tests, the disposable filter can clean the prions out of a single pack of contaminated blood in less than an hour. No prions remain in the cleaned blood, which is otherwise unchanged by the process. Tests with prion-infected hamsters showed that their filtered blood could be injected into disease-free hamsters with no ill effects.
>
> The team hope that the UK's National Blood Service could be using the device by the end of this year. Peter Edwardson, ProMetic's vice-president of medical technologies, says that Ireland's clinical trial, aiming to confirm that the filtered red blood cells are just as effective as untreated blood when transfused into humans, should be complete in a few months.

More at the *Royal Society of Chemistry*...

ProMetic Life Sciences...

Flashbacks: Leukotrap® Affinity Prion Reduction Filter

Posted on April 9, 2008 09:14 AM

CLOSE

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. 4. 15	該当なし	

一般的名称	人赤血球濃厚液	研究報告の公表状況	Terpstra FG, van 't Wout AB, Schuitemaker H, van Engelenburg FA, Dekkers DW, Verhaar R, de Korte D, Verhoeven AJ. Transfusion. 2008 Feb;48(2):304-13. Epub 2007 Nov 19.	公表国
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)			オランダ

研究報告の概要

○血小板濃厚液の病原体を不活化するUVC照射の効果と限界

背景:輸血による疾患伝播を引き起こす血液製剤の病原体汚染は現在も懸念されている。本試験において、血小板濃厚液のUVC照射の病原体不活化能を検討した。複数のウイルス・細菌不活化の用量依存性を血小板の質への影響と比較した。

試験デザインおよび方法:さまざまな脂質エンベロープ(LE)、非脂質エンベロープ(NLE)ウイルスおよび細菌を用いてUVC照射の効力を調べた。

LEウイルスは、ウシ・ウイルス性下痢ウイルス(BVDV)、ヒト免疫不全ウイルス(HIV)、仮性狂犬病ウイルス(PRV)、伝播性胃腸炎ウイルス(TGEV)と水疱性口内炎ウイルス(VSV)とし、NLEウイルスは、イヌ・パルボウイルス(CPV)とシミアンウイルス40(SV40)、細菌は、表皮ブドウ球菌、黄色ブドウ球菌、大腸菌とセレウス菌とした。スパイクおよび照射後の検体について、残存する感染性と減少率(RF)を調べた。さらに、in vitroでUVC照射が血小板の品質に及ぼす影響を調べた。

結果:UVC量500J/㎡では、血小板の品質の変化は許容できるものであった(pH、乳酸産生、CD62P発現、ホスファチジルセリン曝露にて測定)。CPV、TGEV、VSV、表皮ブドウ球菌、黄色ブドウ球菌、大腸菌のRFは高く(>4log)、BVDV、PRV、セレウス菌のRFは中等度(約3log)であり、HIVおよびSV40のRFは低かった(約1log)。cell-freeウイルス、cell-associatedウイルス間で、ウイルスの軽減に差は認められなかった。

結論:UVC照射は、血小板の品質に影響を及ぼさずに、細菌および広範なウイルス(HIVを除く)を不活化することのできる、血小板濃厚液の有望な病原体低減技術である。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。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
UVC照射は、血小板の品質に影響を及ぼさずに、細菌および広範なウイルス(HIVを除く)を不活化することができるが、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との報告である。	日本赤十字社では8項目の安全対策の一つとして、不活化技術の導入について、各不活化技術の効果、血液成分への影響、製造作業への影響などについて評価検討を行っている。外国での不活化実施状況や効果、新たな技術、副作用等の情報収集も含め総合的に評価し、導入について関係機関と協議しているところである。

(18)

Potential and limitation of UVC irradiation for the inactivation of pathogens in platelet concentrates

Fokke G. Terpstra, Angélique B. van 't Wout, Hanneke Schuitemaker, Frank A.C. van Engelenburg, David W.C. Dekkers, Robin Verhaar, Dirk de Korte, and Arthur J. Verhoeven

BACKGROUND: Pathogen contamination, causing transfusion-transmitted diseases, is an ongoing concern in transfusion of cellular blood products. In this explorative study, the pathogen-inactivating capacity of UVC irradiation in platelet (PLT) concentrates was investigated. The dose dependencies of inactivation of several viruses and bacteria were compared with the effect on PLT quality.

STUDY DESIGN AND METHODS: The potential of UVC irradiation was studied with a range of lipid-enveloped (LE) and non–lipid-enveloped viruses (NLE) and bacteria. LE viruses were bovine viral diarrhea virus (BVDV), human immunodeficiency virus (HIV), pseudorabies virus (PRV), transmissible gastroenteritis virus (TGEV), and vesicular stomatitis virus (VSV). NLE viruses were canine parvovirus (CPV) and simian virus 40 (SV40). Bacteria were *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus cereus*. After spiking and irradiation, samples were tested for residual infectivity and reduction factors (RFs) were calculated. Furthermore, the effect of UVC irradiation on PLT quality was determined by measuring in vitro quality variables.

RESULTS: A UVC dose of 500 J per m^2 resulted in acceptable PLT quality (as measured by pH, lactate production, CD62P expression, and exposure of phosphatidylserine) and high RFs (>4 log) for CPV, TGEV, VSV, *S. epidermidis*, *S. aureus*, and *E. coli*. Intermediate RFs (approx. 3 log) were observed for BVDV, PRV, and *B. cereus*. Low RFs (approx. 1 log) were found for HIV and SV40. No differences in virus reduction were observed between cell-free and cell-associated virus.

CONCLUSION: UVC irradiation is a promising pathogen-reducing technique in PLT concentrates, inactivating bacteria, and a broad range of viruses (with the exception of HIV) under conditions that have limited effects on PLT quality. Further optimization of the UVC procedure, however, is necessary to deal with blood-borne viruses like HIV.

Next to careful selection of donors, safety of cellular blood products is primarily based on screening systems to detect markers for viral contamination (e.g., specific antibody testing and nucleic acid testing [NAT]) and bacterial contamination (e.g., BacT/ALERT culturing). In donors experiencing primary virus infections, however, antibodies are not yet detectable in the early phase of infection and NAT might also score negative (the so-called window phase). Moreover, in case of emerging infections, both safety measures will fail. Also, despite screening for bacteria, cases of bacterial transmission have been reported, due to the limited sensitivity of the system.[1] Hence, there is a strong need for in-process steps with broad pathogen-inactivating capacity. An additional requirement for such a step is that the quality of the appropriate blood products is not compromised.

For pathogen inactivation in platelet concentrates (PCs), a number of different techniques involving ultraviolet (UV) light have recently been described. One method utilizes the psoralen compound S-59 (amotosalen hydrochloride) in combination with UVA light. In this photochemical process S-59 intercalates into and binds to

ABBREVIATIONS: BVDV = bovine viral diarrhea virus; CA virus = cell-associated virus; CPV = canine parvovirus; LE virus = lipid-enveloped virus; NLE virus = non–lipid-enveloped virus; PC(s) = platelet concentrate(s); PRV = pseudorabies virus; PS = phosphatidylserine; RF(s) = reduction factor(s); SV40 = simian virus 40; TGEV = transmissible gastroenteritis virus; VSV = vesicular stomatitis virus.

From the Sanquin Research and Landsteiner Laboratory of the Academic Medical Center of the University of Amsterdam, Amsterdam, the Netherlands.

Address reprint requests to: Fokke G. Terpstra, Sanquin, Virus Safety Services, Plesmanlaan 125, 1006 AD Amsterdam, the Netherlands; e-mail: f.terpstra@sanquin.nl.

Received for publication March 29, 2007; revision received July 3, 2007, and accepted July 24, 2007.

doi: 10.1111/j.1537-2995.2007.01524.x

TRANSFUSION 2008;48:304-313.

nucleic strands. Upon irradiation with UVA light (320-400 nm), this binding becomes irreversible and the strands are cross-linked, resulting in inactivation of the pathogen.[2]

Another method takes advantage of the properties of a naturally occurring vitamin supplement, riboflavin. Riboflavin interacts between the bases of DNA or RNA and upon irradiation with broadband UV light (265-370 nm), riboflavin oxidizes guanine in nucleic acids, resulting in irreversible damage to the pathogen.[3]

A third method applies a two-step procedure of a photodynamic treatment with thionine and/or light to inactivate free viruses, followed by low-dose UVB treatment to inactivate white blood cells (WBCs) and bacteria.[4] Thionine has a high binding affinity to nucleic acids and specifically binds strongly in G-C–rich regions, but also formation of singlet oxygen is important.[5]

A disadvantage of most of the photochemical and photodynamic treatments developed to date is the need to add and to remove the sensitizer and/or its breakdown products. An alternative in this field is the use of UV light alone. The theoretically optimal wavelength for DNA damage without the need for a photosensitizer is 254 nm, that is, in the UVC range. A UVC technique applying light of 254 nm has, among others, been described by Caillet-Fauquet and colleagues[6] and shown to be effective in treating purified plasma-derived products. UVC mainly causes dimerization of adjacent pyrimidines[7] and the resultant intranucleotide cross-link abrogates subsequent pathogen replication. In addition, UVC also generates free radicals such as singlet oxygen.[8]

In this study, we explored the potential of UVC irradiation for pathogen inactivation in PCs. For a dose of 500 J per m^2, we found good pathogen reduction for bacteria and a broad range of viruses (with the exception of human immunodeficiency virus [HIV]) in a mixture of 10 percent plasma and additive solution (Composol-PS, Fresenius HemoCare, Emmer-Compascuum, the Netherlands), with a limited effect on several in vitro variables of platelet (PLT) quality.

MATERIALS AND METHODS

Preparation of PCs

PCs were prepared from whole blood–derived buffy coats by a modification of the standard protocol.[9,10] In short, 500 ± 50 mL of blood from nonremunerated, informed donors was collected in 70 mL of citrate-dextrose-phosphate in bottom-and-top blood collection systems (Fresenius HemoCare, Emmer-Compascuum, the Netherlands). After collection, the blood was stored for 12 to 16 hours at 20 ± 2°C.[11] After centrifugation (2780 × *g*, 8 min), the blood was separated in a plasma, buffy coat, and red cell (RBC) fraction with the aid of a semiautomated component preparation device (Compomat G4, Fresenius HemoCare).[12] Three buffy coats were mixed together and 240 mL of Composol-PS (consisting of 90 mmol/L NaCl, 5 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 27 mmol/L acetate, 23 mmol/L gluconate, 3.2 mmol/L citrate, pH 7.0; Fresenius HemoCare)[13] was added by means of sterile docking. Instead of a low-speed centrifugation step to prepare the PC, the pooled buffy coats were subjected to a high-speed centrifugation (2780 × *g*, 8 min), the supernatant was removed, and an additional 180 mL of Composol-PS was added to lower the plasma content of the final product. The buffy coats were then subjected to a low-speed centrifugation (5 min, 260 × *g*), and the PLT-rich supernatant was transferred across a leukoreduction filter (Compostop CS, Fresenius HemoCare) to an empty 1.3-L polyvinylchloride (PVC)-citrate storage bag (Compoflex, Fresenius HemoCare) with the use of a Compomat G4. This procedure had a yield of 60 to 65 percent and resulted in PCs with a residual plasma content of less than 15 percent, as determined by the protein content of the cell-free supernatant and a PLT count of 0.9×10^9 to 1.2×10^9 per mL. Residual WBCs and RBCs were less than 0.1×10^6 and 0.01×10^9 per mL, respectively, the detection limits of the hematologic counter used (Ac·T 10, Beckman Coulter, Mijdrecht, the Netherlands). After overnight storage at 22 ± 2°C (horizontally shaking with 1 cycle/second; PLT incubator, Model PF96, Helmer, Noblesville, IN), the PC was split into two equal volumes and the plasma concentration was adjusted to 10 percent (by addition of Composol-PS) or 30 percent (by addition of autologous plasma) with equal PLT counts. The final PLT counts were 0.6×10^9 to 0.8×10^9 per mL. The PLT was then subjected to UVC irradiation in portions of 5 mL, with or without pathogens added, as described below.

Bench-scale UVC irradiation

The UVC irradiation was performed as described previously.[6] Briefly, unless indicated otherwise, 5 mL of spiked material was irradiated from above in an open petri dish (84 mm in diameter) placed on an orbital shaker (50 r.p.m.). This resulted in a fluid layer with a thickness of approximately 1 mm. The irradiation device consisted of a UVC lamp with a low-pressure mercury arc (emission line at 254 nm, Germicidal 15T/8, General Electric, Fairfield, CT), a ventilator, filter, photoradiometer with UV sensor, and toothed rack.

In vitro measurements of PLT quality

After UVC irradiation, 5 mL of PC was transferred to 50-mL culture flasks with the addition of 10 mmol per L glucose, 12 mmol per L HCO_3^-, and 5 percent CO_2 in the gas phase and stored for 5 days at 22 ± 2°C (horizontal

shaking with 1 cycle/second). Control experiments had indicated that this down-scaled version of PLT storage results in similar loss of PLT quality as observed in whole PCs stored in PVC bags (data not shown). After the storage period, the PLTs were analyzed for pH (at 37°C), CD62P expression, exposure of phosphatidylserine (PS), and lactate production.

The expression of the activation antigen CD62P was measured essentially as described previously[14] with minor revisions. Briefly, PLTs were diluted to a concentration of 3×10^8 per mL with an electrolyte solution (Isoton II, Beckman Coulter, Mijdrecht, the Netherlands), and 5 µL of PLT suspension was then incubated with 45 µL of Isoton containing CD62P antibody (clone CLB-Thromb/6 conjugated with fluorescein isothiocyanate [FITC], Immunotech, Marseille, France) for 30 minutes at 22°C in the dark. Afterward, the PLTs were fixed by adding 0.5 mL of 0.5 percent (vol/vol) methanol-free formaldehyde (Polyscience, Inc., Warrington, PA; diluted in PBS) and analyzed via flow cytometry (FACScan, Becton Dickinson, Franklin Lakes, NJ). A parallel incubation with FITC-labeled murine immunoglobulin G (Sanquin Reagents, Amsterdam, the Netherlands) was used as negative control.

PS exposure after treatment and storage was determined by means of annexin V binding as follows. PLTs were diluted to a concentration of 1×10^8 per mL in HEPES medium (136 mmol/L NaCl, 3.2 mmol/L KCl, 2 mmol/L $MgSO_4$, 1.2 mmol/L K_2HPO_4, 10 mmol/L HEPES, pH 7.4), and 20 µL of PLT suspension was incubated with 180 µL of HEPES medium in the presence of annexin V-FITC (0.6 µg/mL, added from a stock solution of 250 µg/mL, VPS Diagnostics, Mijdrecht, the Netherlands) and 2.5 mmol per L $CaCl_2$. As negative control, all samples were also stained in the presence of 2.5 mmol per L ethylene glycol-bis(beta-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid.

Lactate was measured in cell-free supernatants (obtained by centrifugation of PLT samples for 5 min at $14,000 \times g$) by enzymatic conversion with lactoperoxidase (Trinity Biotech plc, Bray, Ireland). Rates of lactate production were calculated by comparison with cell-free supernatants obtained just before UVC irradiation.

Selection of viruses and cells

Viruses were selected for being blood-borne and pathogenic and/or to represent various genome types (RNA or DNA, single- or double-stranded) and sizes and with or without lipid envelopes[15,16] (Table 1). Five lipid-enveloped (LE) viruses were used: bovine viral diarrhea virus (BVDV; model for hepatitis C virus [HCV]), HIV (relevant blood-borne virus), pseudorabies virus (PRV; model virus for LE DNA viruses like hepatitis B virus [HBV]), transmissible gastroenteritis virus (TGEV; model for the corona virus causing severe acute respiratory syndrome), and vesicular stomatitis virus (VSV; model for LE RNA viruses). Two non–lipid-enveloped (NLE) viruses were used: canine parvovirus (CPV) and simian virus 40 (SV40), a specific model for parvovirus B19 and a general model for NLE DNA viruses, respectively.

BVDV, strain NADL (VR-534; ATCC, Rockville, MD), was cultured on MDBK cells (CCL-22; ATCC) and titrated on EBTr cells (ID-Lelystad, the Netherlands). HIV, strain HTLV-IIIB (National Cancer Institute, Bethesda, MD), was cultured on H9 cells (National Cancer Institute) and titrated on MT2 cells (Wellcome, Beckenham, UK). PRV, strain Aujeszki Bartha K61 (Duphar, Weesp, the Netherlands), was cultured and titrated on VERO cells (CCL-81; ATCC). TGEV, strain Purdue (VR-763; ATCC), was cultured and titrated on ST cells (CRL-1746; ATCC). VSV, strain Indiana (Sanquin Pharmaceutical Services, Amsterdam, the Netherlands), was cultured and titrated on BHK21 cells (CCL-10; ATCC). CPV, strain 780916 (Erasmus University Rotterdam, Rotterdam, the Netherlands), was cultured and titrated on A72 cells (Erasmus University Rotterdam). SV40, strain PML-2 (VR-821; ATCC), was cultured and titrated on BSC-1 cells (Organon, Oss, the Netherlands).

Test for cytotoxicity and interference

Before the actual spiking experiments, assays were performed to determine cytotoxic effects of the plasma-Composol mixture on the cell lines used for virus titrations and interference of the plasma-Composol mixture with the titration assays, as described previously.[17] Briefly, threefold serial dilutions of the plasma-Composol mixture

TABLE 1. Properties of the viruses used in UVC irradiation studies

Virus group	Virus	Size (nm)	Virus family	Genome	Size (kb)	Model virus for
LE	BVDV	37-50	Flavi	ss RNA	10-12	HCV
	HIV	100	Retro	2 ss RNA	2 × 10	Relevant virus
	PRV	100-200	Herpes	ds DNA	140	Large LE ds DNA viruses
	TGEV	100-120	Corona	ss RNA	27-32	SARS
	VSV	75 × 180	Rhabdo	ss RNA	11-15	Large LE ss RNA viruses
NLE	CPV	18-26	Parvo	ss DNA	5	Human parvovirus B19
	SV40	45	Polyoma	ds DNA	30	Human parvovirus B19

ss = single-stranded; ds = double-stranded; SARS = severe acute respiratory syndrome.

were prepared and tested on cells to determine cytotoxicity. Then, with the first dilution of the plasma-Composol mixture without cytotoxic effects, a known amount of virus was spiked and incubated. Subsequently, the infectivity of the virus inoculum and the spiked plasma-Composol mixture was measured to determine possible interference with the detection system. In none of the virus assays, interfering effects were observed for the plasma-Composol mixtures used.

Virus assays

Infectivity was measured in $TCID_{50}$ assays and bulk culture tests. For $TCID_{50}$ assays, 1-in-3 serial dilutions of samples were prepared in culture media and 50-µL (or 0.5 mL for HIV) volumes were tested in eight replicates. To detect small amounts of virus, up to 60 mL of prediluted sample was tested in duplicate bulk culture tests, with 25- and 175-cm^2 flasks. BVDV, CPV, PRV, SV40, TGEV, and VSV cultures were inspected microscopically for cytopathic effects at 6, 7, 5, 21, 3, and 4 days postinfection, respectively. HIV cultures were inspected microscopically twice a week for the formation of syncytia until 21 days postinfection. In all experiments, virus titers were calculated by the Spearman-Kärber method[18] with the exception of the factorial design experiment, in which the most probable number[19] method was used, and titers were expressed as $TCID_{50}$ per mL. If all cultures were negative, the titer ($TCID_{50}$/mL) was considered to be less than 1 ÷ total test volume (mL).

Reduction factors (RFs) were calculated by the formula

RF = log (total amount of virus spiked as derived from the reference sample ÷ total amount of virus recovered from the treated sample).

Bacteria assays

For these studies four different bacteria, representing potential contaminants in PCs as derived from the screening results in the Netherlands,[20] were selected: *Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli,* and *Bacillus cereus* (for each species, two different clinical isolates were used). Bacterial stocks were produced by overnight culture on regular blood agar plates, and colonies were picked and diluted in PBS to a concentration of approximately 3×10^7 colony-forming units (CFUs) per mL. After treatment, samples were collected and titrated in CFU assays: samples were serially diluted 1 in 10 with ice-cold saline. For *B. cereus*, after UVC irradiation an extra sample was taken and given an additional 10 minutes' incubation at 80°C to check for spores. Subsequently, 10-µL samples were tested on blood agar plates in duplicate and incubated at 35°C during 18 to 24 hours. All samples were tested in serial dilutions and for selected samples also a 200-µL sample was plated, in duplicate. Subsequently the number of bacteria was counted and CFU values were calculated.

RFs were calculated by the formula

RF = log (total amount of bacteria spiked as derived from the reference sample ÷ total amount of bacteria recovered from the treated sample).

RESULTS

In a first explorative study, efficacy of UVC irradiation for virus inactivation was tested by spiking BVDV in Composol containing varying amounts of residual plasma (5, 10, and 30%) in the absence of PLTs. Given the poor penetration of UVC light in plasma, an experimental setting with a thin plasma layer of approximately 1 mm was chosen, an orbital mixing speed of 50 r.p.m., UVC irradiation at a light intensity of 0.25 mW per cm^2, and doses ranging from 250 up to 1000 J per m^2 (1000 J/m^2 equals exposure for 400 sec). For both the 5 and the 10 percent plasma suspensions, reductions of approximately 6 log were observed at 1000 J per m^2, although the kinetics for the 10 percent plasma suspension was clearly slower than the 5 percent plasma suspension. In case of 30 percent plasma, the reduction was approximately 2.6 log at 1000 J per m^2 (Fig. 1). Although these data clearly indicated that the plasma concentration should be kept as low as possible, we used 10 and 30 percent plasma in subsequent experiments to avoid compromising PLT quality beyond acceptable limits.

The effect of UVC irradiation on PLT quality was evaluated in a similar experimental setup as above. Before analysis, the PLTs were stored for 5 days to allow detection of long-term effects of the treatment. In Fig. 2, pH, lactate production, CD62P expression, and PS exposure (as

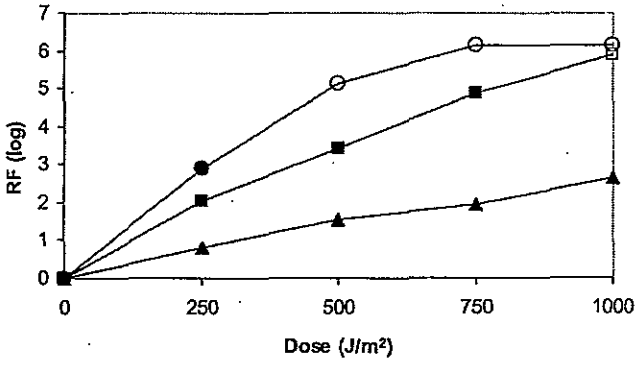


Fig. 1. Effect of UVC irradiation on BVDV inactivation in 5 (●), 10 (■), and 30 percent (▲) plasma. BVDV inactivation was tested with UVC irradiation in Composol containing 5, 10, or 30 percent plasma; RF values (log) are shown. Light intensity was 0.25 mW per cm^2, suspension depth 1 mm, and orbital mixing 50 r.p.m. Open symbols indicate maximal reduction.

measured by annexin V binding) are shown, as indicators of PLT quality. Increasing doses of UVC resulted in a clear deterioration in all of these variables, especially in the PCs suspended in 10 percent plasma. The pH had decreased from 7.23 to 6.52 with the highest UVC dose tested (Fig. 2A), concomitant with an increase in lactate production (Fig. 2B). CD62P expression increased up to 40 percent after a dose of 500 J per m^2 and leveled off at higher doses (Fig. 2C), probably due to shedding of the antigen. PS exposure also increased with increasing doses of UVC, but in 10 percent plasma and with 500 J per m^2 of UVC, the PS exposure remained below 30 percent (Fig. 2D). In 30 percent plasma, all PLT quality indicators remained within an acceptable range at all UVC doses tested, but, as noted above, under these circumstances virus inactivation may be limited.

To further optimize the UVC irradiation, three variables, light intensity, plasma percentage, and PLT concentration, were tested in a full factorial design (in duplicate) with inactivation of BVDV as readout, as described previously.[17] In this factorial design, light intensity was tested at 0.25 and 1.0 mW per cm^2, percentage plasma at 10 and 30 percent, and PLT concentration at 0 and 1.0×10^9 cells per mL. The irradiation dose was fixed at 500 J per m^2 based on the results obtained in the PLT quality experiments, and the depth was fixed at approximately 1 mm. Results for this series of experiments are shown in Table 2. Linear regression analysis of the complete data set showed that the percentage plasma was the most important determinant of UVC efficacy, with the highest virus reduction in the presence of 10 percent plasma. In addition, light intensity was also a significant parameter with highest virus reduction at 0.25 mW per cm^2. Finally, the presence or absence of PLTs did have a significant effect on the virus reduction obtained, but the contributing effect of the PLT concentration was slightly smaller than the other two variables. Given the relative low contribution of the PLT concentration, all subsequent virus studies were performed with the optimal setting as determined by the factorial design (i.e., 10% plasma, 0.25 mW/cm^2), but in the absence of PLTs.

To determine the generality of the results obtained with BVDV, we tested BVDV and six additional viruses with different genome type, size, and envelope status in a kinetic setting with irradiation values up to 1000 J per m^2. For CPV, TGEV, and VSV, high virus kill was observed, resulting in RF values of greater than 4 log already at 500 J per m^2 (sensitive viruses; Fig. 3A). For BVDV and PRV, the

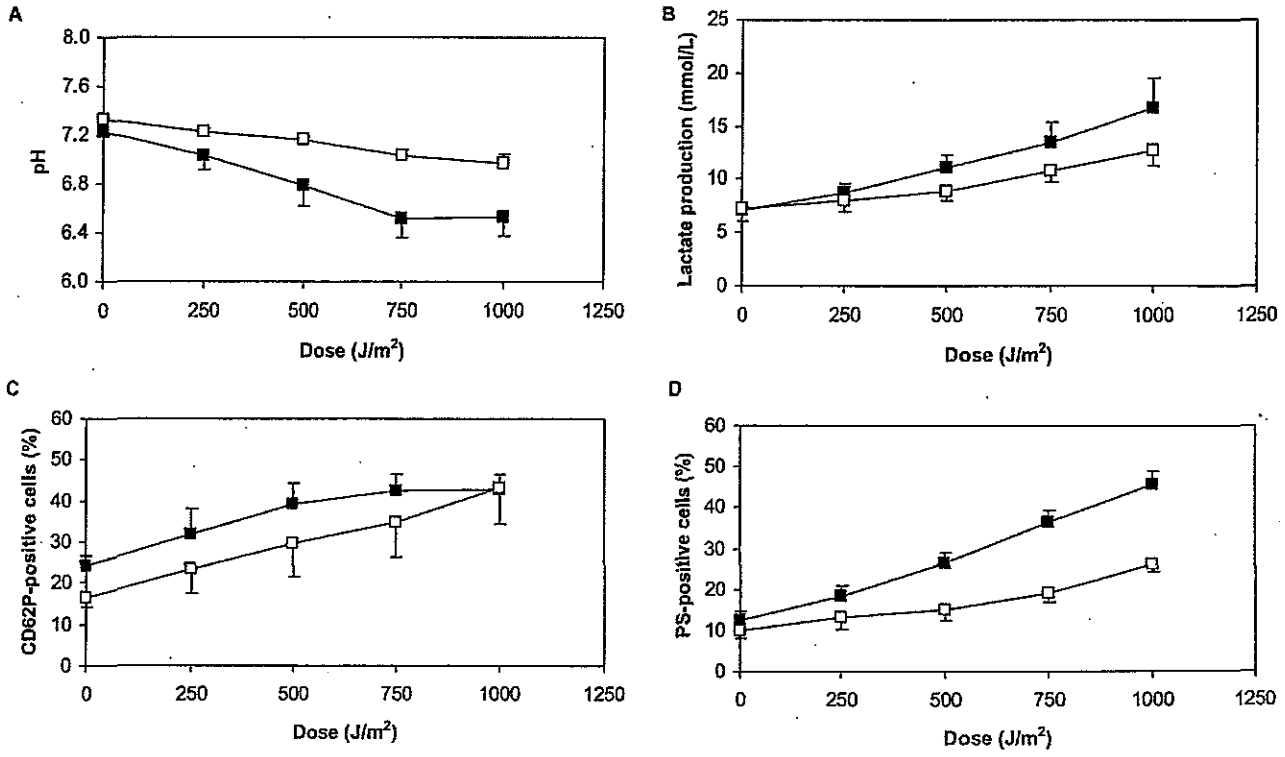


Fig. 2. Effect of UVC irradiation on PLT quality. PCs were subjected to UVC irradiation as described in the legend to Fig. 1. PLT quality variables after subsequent storage for 5 days were determined as described under Materials and Methods. (A) Medium pH; (B) lactate production; (C) CD62P expression; (D) PS-positive cells. (□) PCs in 30 percent plasma; (■) PCs in 10 percent plasma. Results shown are the mean ± SEM of 6 PCs.

TABLE 2. Factorial design for three variables (plasma, light intensity, and PLTs)*

Run	Plasma [%]	Light intensity (mW/cm²)	PLTs (cells/mL) × 10⁹	RF (log)
1	10	0.25	0	5.81
2	30	0.25	0	3.03
3	10	1.0	0	4.63
4	30	1.0	0	1.90
5	10	0.25	1.0 × 10⁹/mL	4.53
6	30	0.25	1.0 × 10⁹/mL	2.47
7	10	1.0	1.0 × 10⁹/mL	2.85
8	30	1.0	1.0 × 10⁹/mL	1.66
9	10	0.25	0	4.63
10	30	0.25	0	2.88
11	10	1.0	0	4.32
12	30	1.0	0	1.89
13	10	0.25	1.0 × 10⁹/mL	3.80
14	30	0.25	1.0 × 10⁹/mL	2.51
15	10	1.0	1.0 × 10⁹/mL	2.95
16	30	1.0	1.0 × 10⁹/mL	1.50

* Factorial design to determine the influence of three variables (percentage of plasma, light intensity, and absence or presence of PLTs) on the efficacy of UVC irradiation for the inactivation of BVDV in PCs. The percentage of plasma was tested at 10 and 30 percent, the light intensity at 0.25 and 1.0 mW per cm², and the PLT concentration at 0 and 1.0 (cells/mL) × 10⁹. The irradiation dose was fixed at 500 J per m², the depth was fixed at 1 mm, and orbital mixing was 50 r.p.m. The results for BVDV are shown as RF (log) calculated with the most probable number method.[19]

virus kill was somewhat lower, resulting in approximately 3 to 4 log at 500 J per m², but eventually more than 5 log at 1000 J per m² (medium-resistant viruses; Fig. 3B). For HIV and SV40, the virus kill was limited to approximately 1 log at 500 J per m² and approximately 2 log with doses up to 1000 J per m² (resistant viruses; Fig. 3C). Because cell-associated (CA) virus (intracellular and/or bound to the cellular membrane) may be more resistant to UVC damage, we also tested CA virus with HIV as a resistant virus and with VSV as a sensitive virus. In both cases, however, the CA-virus results mimicked those obtained with the corresponding cell-free viruses, that is, CA HIV was UVC-resistant and CA VSV was UVC-sensitive (Fig. 3D).

To further explore the resistance of HIV to UVC irradiation, we tested the effect of irradiation doses up to 4000 J per m². Although the virus kill slowly improved with increasing doses, infectious virus was still present even after irradiation at 4000 J per m² (RF = 3.5 log; data not shown).

Finally, we investigated the effect of UVC irradiation on the survival of bacteria. Experiments were performed with the same settings as for the virus studies. Irradiation at 250 J per m² resulted in greater than 4 log reduction for *S. epidermidis*, *S. aureus*, and *E. coli* and greater than 5 log at 500 J per m². In the case of *B. cereus*, the kill at 500 J per m² was limited to approximately 3 log (Fig. 4). An increase to 1000 J per m² resulted in a reduction of 3.7 log (data not shown). When after UVC irradiation, however, the *B. cereus* samples were incubated for 10 minutes at 80°C, all samples were below the detection limit (<0.5 log).

DISCUSSION

The risk of transmission of pathogens via cellular products, especially for PCs, is still a concern. Here we describe the potential of a UVC irradiation technique for pathogen inactivation in PCs.

Because of the high absorbance of UVC light by human plasma, we chose to study only PCs suspended in synthetic medium with 10 or 30 percent residual plasma. From the extinction coefficient per percentage of plasma (experimentally determined by us in a 1-cm cuvet as being close to 0.3), it can be calculated that, with a 1-mm light path as used in most of our experiments, 50 and 10 percent of the UVC light will reach the bottom of the suspension with, respectively, 10 or 30 percent plasma present. To avoid "dead" volumes not exposed to UVC, we chose the relative short light path of 1 mm, realizing that special containers of UV-permeable plastic of similar thickness would be required when the technique should be further developed into a blood bank procedure.

In our experimental setup, we then investigated whether varying a number of variables like percentage of plasma, irradiation dose, and light intensity would result in conditions with good pathogen inactivation in combination with good PLT quality. An acceptable compromise was found for 10 percent plasma in Composol, in combination with a depth of 1 mm, a light intensity of 0.25 mW per cm², and a total dose of 500 J per m². These conditions resulted in good (3-4 log) inactivation for the majority of pathogens tested with only limited effects on in vitro PLT quality. Evidently, this set of conditions can only be taken as a rough indication, because in this explorative study neither the irradiation nor the storage resembled blood bank conditions and the set of PLT quality variables was limited. The only quality variable for PCs mentioned in guidelines is the pH, which should be between 6.4 and 7.4 (at 22°C) according to European blood bank regulations.[21] Although this condition was met under all conditions tested, the results of the other in vitro quality variables indicated that in 10 percent plasma with doses higher than 500 J per m², PLT quality was seriously affected. An increase of 10 to 30 percent in CD62P-positive PLTs has been reported for standard PC at the end of their shelf life (5-7 days),[14,22,23] whereas with CD62P values higher than 50 percent the in vivo survival seems affected.[24] PS exposure during storage of standard PC usually remains below 20 percent at the end of the

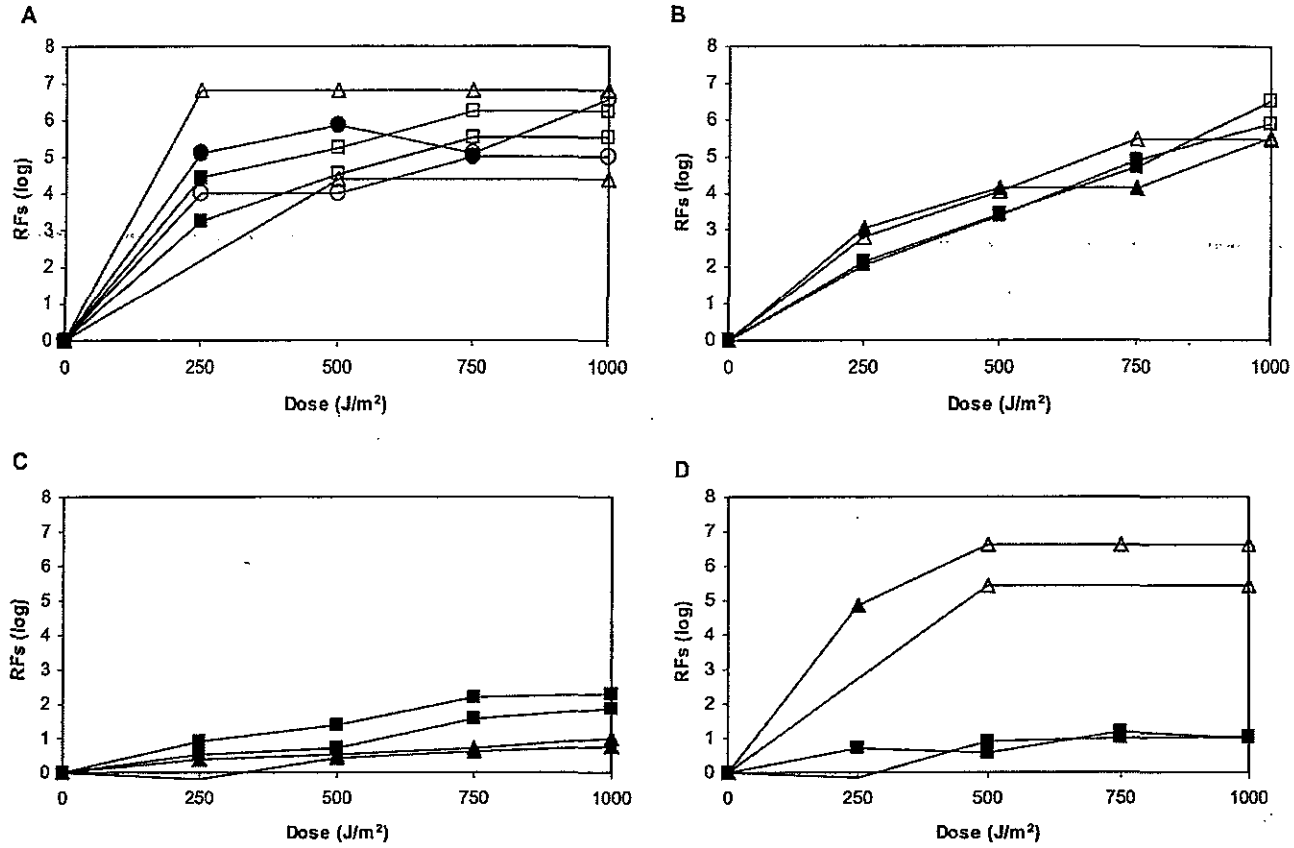


Fig. 3. Inactivation of different viruses by UVC irradiation. Virus inactivation by UVC irradiation was tested in Composol containing 10 percent plasma; RF values (log) are shown. Experimental conditions were as described in the legend to Fig. 1. (A) Sensitive viruses (■ = CPV; ▲ = TGEV; ● = VSV); (B) medium-resistant viruses (■ = BVDV; Δ = PRV); (C) resistant viruses (■ = HIV; ▲ = SV40); (D) CA viruses (■ = CA HIV; ▲ = CA VSV). Open symbols indicate maximal reduction. Results shown are representative for at least two experiments.

shelf life,[22,23] but so far there are no data about correlation of PS exposure and in vivo survival.

In a factorial design, it was shown that the percentage of residual plasma was the major variable affecting the outcome of the UVC irradiations. As expected, the percentage of plasma resulted in opposite effects on pathogen inactivation and PLT quality. To a lesser extent, this opposite effect was also found for the light intensity, whereas the presence or absence of PLTs had a relatively minor, although significant, effect on pathogen inactivation. The presence of PLTs, resulting in a decrease of approximately 1 log of pathogen inactivation, should be taken into account in interpreting much of our inactivation data. Furthermore, the necessity to lower the residual plasma concentration to guarantee sufficient pathogen inactivation will require adjustment of current procedures to produce suitable PCs, but it has been shown in earlier studies[25,26] that this may be achievable. Because UVC irradiation results in extra glucose consumption, additional measures should be taken to ensure provision of glucose.

It was anticipated that in case of bacteria, high inactivation values would be observed[6] and this was indeed the case for all bacteria tested, with the exception of *B. cereus*. The reason for the decreased sensitivity of *B. cereus* is not quite clear, but one might speculate that formation of spores plays a role. It has been shown that *Bacillus* spores are 10 to 20 times more resistant to UVC irradiation;[27] thus formation of spores can cause a suboptimal kill of *Bacillus*. Because freshly prepared bacteria cultures were used containing relatively low amounts of spores (as determined by specific staining of spores, data not shown), however, a higher resistance toward UVC irradiation of *B. cereus* itself compared to other bacteria is also a possible explanation.[28,29] Moreover, the UVC-surviving bacteria were killed upon incubation at 80°C for 10 minutes, a treatment that spores will survive, also indicating that *B. cereus* itself has a higher resistance toward UVC.

We found a broad spectrum of viral sensitivity to UVC irradiation. For CPV, TGEV, and VSV we found very high inactivation, whereas the inactivation for BVDV and PRV

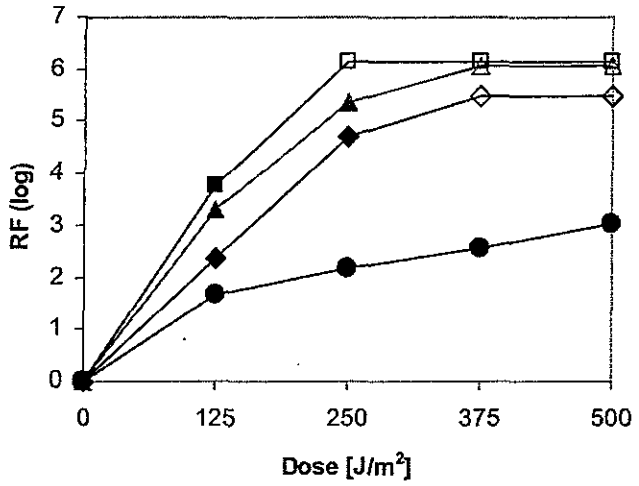


Fig. 4. Inactivation of different bacteria by UVC irradiation. (◆) *S. epidermidis*; (■) *S. aureus*; (▲) *E. coli*; and (●) *B. cereus*. Bacteria inactivation by UVC irradiation was tested in Composol containing 10 percent plasma; RF values (log) are shown. Experimental conditions were as described in the legend to Fig. 1. Open symbols indicate maximal reduction. Results shown are representative of two experiments.

was less and slower. In the case of HIV and SV40, the inactivation was very limited and only 1 to 2 log was observed. Increasing doses of UVC irradiation also induced increased damage to HIV. Even a dose of 4000 J per m^2, however, did not result in complete inactivation. Interestingly, the efficacy of UVC was not different for cell-free and CA virus because CA HIV and CA VSV showed susceptibilities very similar to the corresponding cell-free virus. This indicates that the presence of infected cells is not an impediment for virus inactivation with UVC irradiation, provided that the virus in question is sensitive to UVC irradiation.

Based on our results we would rank the viruses in the following order with respect to UVC sensitivity: TGEV > VSV > CPV > PRV > BVDV > HIV > SV40. This ranking is exactly in line with previous observations and theoretical considerations postulating that UVC is especially effective on viruses with large genomes (i.e., PRV)[30] and on viruses with single-stranded nucleic acid genomes (i.e., TGEV, VSV, and CPV).[31] Furthermore, it has been described that RNA is less severely damaged than DNA, because pyrimidine dimers and more specifically thymine are the most frequent lesions caused by UVC irradiation.[7] Our results are also in line with Caillet-Fauquet and coworkers[6] who determined a sensitivity of MVM > EMC > BHV and Li and colleagues[32] who showed a sensitivity of CPV > BVDV > HAV > PRV. Wang and coworkers,[33] however, reported SV40 to be highly sensitive, more or less comparable to parvovirus. In contrast, we found that SV40 is very resistant, similar to the resistance found for HIV, as was also predicted by Lytle and Sagripanti.[34] The reason for this discrepancy remains unclear, especially because the possible explanation of cell line–dependent repair can be ruled out, because both studies propagated the virus in the cell line BSC.

Considering the ranking of virus inactivation as observed in this study and as predicted by Lytle and Sagripanti,[34] it can be concluded that single-stranded DNA or RNA viruses are effectively inactivated by UVC irradiation. This confirms that UVC is distinct from several other techniques with respect to its capacity to inactivate the NLE viruses like parvovirus B19 and HAV. This effective elimination of NLE viruses, combined with B19 contamination in several blood and plasma products, renders this technique interesting for further consideration.

SV40 has been regarded to be a very resistant virus and was often used in the past as a general model for NLE viruses with a DNA genome (like parvovirus B19). At present, however, specific model viruses for parvovirus B19 are applied and/or parvovirus B19 itself. Given the fact that CPV is very effectively inactivated by UVC irradiation, the relevance of SV40 as model virus for parvovirus B19 can be questioned. We do recommend, however, continuing studies with SV40 as a general model virus as this virus may be representative for new currently unknown threats to the blood supply. Indeed, the inability of UVC irradiation to inactivate viruses with small double-stranded genomes like SV40 illustrates possible limitations of this treatment.

BVDV and PRV are effectively inactivated, although the kinetics are slower compared to the sensitive group of viruses. Therefore, it is expected that UVC is capable of inactivating problematic blood-borne viruses like HBV and HCV. The inability of UVC irradiation to sufficiently inactivate HIV, a very relevant virus, however, is a major disadvantage. There seem to be several reasons for the resistance of HIV to UVC irradiation. HIV is a retrovirus with a small RNA genome. It has a single-stranded genome, but each virion encapsulates two copies of the viral RNA that are tightly linked and might serve as each other's back-up in case of UVC-induced damage. Indeed, strand transfers during reverse transcription are an integral part of the HIV life cycle.[35]

The observation that UVC does not effectively inactivate HIV may be partially compensated by careful and efficient donor screening for HIV. Both specific antibody and NAT are routinely performed and the risk of HIV transmission via cellular products is estimated to be less than 1 in 1 million.[36,37] One should again keep in mind, however, that new viruses may emerge with similar characteristics as HIV that would not be affected by this treatment in its current state. Given the broad inactivation of bacteria and viruses, we believe, however, that UVC irradiation for PCs is a promising technology that warrants further investigation.

ACKNOWLEDGMENTS

The authors acknowledge the advice of Dr Ruth Laub and Mr Mario Di Giambattista (CAF/DCF, Brussels, Belgium) and Dr Jan Marcelis (Elisabeth Hospital, Tilburg, the Netherlands). The authors also acknowledge the technical assistance of Anita Boots, Linda Bos, Nicoline Brinkhuis, Edwin Gijsen, and Yvonne van Remmerden (Sanquin Research, Amsterdam, the Netherlands) and of Ms Jolanda Krabben-van Gils (Elisabeth Hospital, Tilburg, the Netherlands).

REFERENCES

1. te Boekhorst PA, Beckers EA, Vos MC, Vermeij H, van Rhenen DJ. Clinical significance of bacteriologic screening in platelet concentrates. Transfusion 2005;45:514-9.
2. Wollowitz S. Fundamentals of the psoralen-based Helinx technology for inactivation of infectious pathogens and leukocytes in platelets and plasma. Semin Hematol 2001; 38:4-11.
3. Ruane PH, Edrich R, Gampp D, Keil SD, Leonard RL, Goodrich RP. Photochemical inactivation of selected viruses and bacteria in platelet concentrates using riboflavin and light. Transfusion 2004;44:877-85.
4. Mohr H, Redecker-Klein A. Inactivation of pathogens in platelet concentrates by using a two-step procedure. Vox Sang 2003;84:96-104.
5. Floyd RA, Schneider JE Jr, Dittmer DP. Methylene blue photoinactivation of RNA viruses. Antiviral Res 2004;61: 141-51.
6. Caillet-Fauquet P, Di Giambattista M, Draps ML, Sandras F, Branckaert T, de Launoit Y, Laub R. Continuous-flow UVC irradiation: a new, effective, protein activity-preserving system for inactivating bacteria and viruses, including erythrovirus B19. J Virol Methods 2004;118: 131-9.
7. Douki T, Cadet J. Individual determination of the yield of the main UV-induced dimeric pyrimidine photoproducts in DNA suggests a high mutagenicity of CC photolesions. Biochemistry 2001;40:2495-501.
8. Wei H, Cai Q, Rahn R, Zhang X. Singlet oxygen involvement in ultraviolet (254 nm) radiation-induced formation of 8-hydroxy-deoxyguanosine in DNA. Free Radic Biol Med 1997;23:148-54.
9. *Pietersz RN, Loos JA, Reesink HW.* Platelet concentrates stored in plasma for 72 hours at 22 degrees C prepared from buffycoats of citrate-phosphate-dextrose blood collected in a quadruple-bag saline-adenine-glucose-mannitol system. Vox Sang 1985;49:81-5.
10. Pietersz RN, de Korte D, Reesink HW, van den Ende A, Dekker WJ, Roos D. Preparation of leukocyte-poor platelet concentrates from buffy coats. III. Effect of leukocyte contamination on storage conditions. Vox Sang 1988;55:14-20.
11. Pietersz RN, de Korte D, Reesink HW, Dekker WJ, van den Ende A, Loos JA. Storage of whole blood for up to 24 hours at ambient temperature prior to component preparation. Vox Sang 1989;56:145-50.
12. Loos H. Automation in blood component preparation. Prog Clin Biol Res 1986;211:333-41.
13. Fijnheer R, Veldman HA, van den Eertwegh AJ, Gouwerok CW, Homburg CH, Boomgaard MN, de Korte D, Roos D. In vitro evaluation of buffy-coat-derived platelet concentrates stored in a synthetic medium. Vox Sang 1991;60:16-22.
14. Fijnheer R, Modderman PW, Veldman H, Ouwehand WH, *Nieuwenhuis DR, De Korte D. Detection of platelet activation* with monoclonal antibodies and flow cytometry. Changes during platelet storage. Transfusion 1990;30:20-5.
15. Committee for Proprietary Medicinal Products (CPMP). Note for guidance on virus validation studies: the design, contribution and interpretation of studies validating the inactivation and removal of viruses (Internet). CPMP/BWP/268/95. London: The European agency for the evaluation of medicinal products; 1996. [cited 1996 February 14]. Available from: http://emea.eu/pdfs/human/bwp/026895en.pdf.
16. Committee for Proprietary Medicinal Products (CPMP). Note for guidance on plasma-derived medicinal products. CPMP/BWP/269/95, Rev. 3. London: The European agency for the evaluation of medicinal products; 2001. [cited 2001 January 25]. Available from: http://emea.eu/pdfs/human/bwp/026995en.pdf.
17. Terpstra FG, Parkkinen J, Tolo H, Koenderman AH, ter Hart HG, von Bonsdorff L, Torma E, Van Engelenburg FA. Viral safety of Nanogam(R), a new 15 nm-filtered liquid immunoglobulin product. Vox Sang 2006;90:21-32.
18. German Federal requirements 'Requirements of validation studies for demonstrating the virus safety of medicinal products derived from human blood plasma' (Bundesanzeiger Nr. 84, Mai 1994). 1994.
19. Cochran WG. Estimation of bacterial densities by means of the "most probable number." Biometrics 1950;6:105-16.
20. de Korte D, Curvers J, de Kort WL, Hoekstra T, van der Poel CL, Beckers EA, Marcelis JH. Effects of skin disinfection method, deviation bag, and bacterial screening on clinical safety of platelet transfusions in the Netherlands. Transfusion 2006;46:476-85.
21. Council of Europe. Guide to the preparation, use and quality assurance of blood components. 13th ed. Strasbourg: Council of Europe Press; 2007.
22. *Curvers J, van Pampus EC, Feijge MA, Rombout-*Sestrienkova E, Giesen PL, Heemskerk JW. Decreased responsiveness and development of activation markers of PLTs stored in plasma. Transfusion 2004;44:49-58.
23. Perrotta PL, Perrotta CL, Snyder EL. Apoptotic activity in stored human platelets. Transfusion 2003;43:526-35.
24. Goodrich RP, Li J, Pieters H, Crookes R, Roodt J, Heyns AP. Correlation of in vitro platelet quality measurements with in vivo platelet viability in human subjects. Vox Sang 2006; 90:279-85.
25. Boomgaard MN, Gouwerok CW, de Korte D, Loos JA.

Platelets stored for 6 days in a polyolefin container in a synthetic medium with minimal plasma carry-over. Vox Sang 1994;66:18-24.
26. Kostelijk EH, Gouwerok CW, Veldman HA, de Korte D. Comparison between a new PVC platelet storage container (UPX80) and a polyolefin container. Transfus Med 2000;10: 131-9.
27. Slieman TA, Nicholson WL. Artificial and solar UV radiation induces strand breaks and cyclobutane pyrimidine dimers in *Bacillus subtilis spore* DNA. Appl Environ Microbiol 2000;66:199-205.
28. Hanlin JH, Lombardi SJ, Slepecky RA. Heat and UV light resistance of vegetative cells and spores of Bacillus subtilis Rec-mutants. J Bacteriol 1985;163:774-7.
29. Romanovskaia VA, Rokitko PV, Malashenko I, Krishtab TP, Chernaia NA. [Sensitivity of soil bacteria isolated from the alienated zone around the Chernobyl Nuclear Power Plant to various stress factors]. Mikrobiologiia 1999;68: 534-9.
30. Kallenbach NR, Cornelius PA, Negus D, Montgomerie D, Englander S. Inactivation of viruses by ultraviolet light. Curr Stud Hematol Blood Transfus 1989, 70-82.
31. Kurth J, Waldmann R, Heiti J, Mausbach K, Burian R. Efficient inactivation of viruses and mycoplasma in animal sera using UVC irradiation. Dev Biol Stand 1999;99: 111-8.
32. Li Q, MacDonald S, Bienek C, Foster PR, Macleod AJ. Design of a UV-C irradiation process for the inactivation of viruses in protein solutions. Biologicals 2005;33:101-10.
33. Wang J, Mauser A, Chao SF, Remington K, Treckmann R, Kaiser K, Pifat D, Hotta J. Virus inactivation and protein recovery in a novel ultraviolet-C reactor. Vox Sang 2004;86: 230-8.
34. Lytle CD, Sagripanti JL. Predicted inactivation of viruses of relevance to biodefense by solar radiation. J Virol 2005;79: 14244-52.
35. Temin HM. Retrovirus variation and reverse transcription: abnormal strand transfers result in retrovirus genetic variation. Proc Natl Acad Sci U S A 1993;90:6900-3.
36. Chiavetta JA, Escobar M, Newman A, He Y, Driezen P, Deeks S, Hone DE, O'Brien SF, Sher G. Incidence and estimated rates of residual risk for HIV, hepatitis C, hepatitis B and human T-cell lymphotropic viruses in blood donors in Canada, 1990-2000. CMAJ 2003;169:767-73.
37. Pillonel J, Laperche S. [Trends in residual risk of transfusion-transmitted viral infections (HIV, HCV, HBV) in France between 1992 and 2002 and impact of nucleic acid testing]. Transfus Clin Biol 2004;11:81-6.

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. 4. 15	該当なし	

一般的名称	人赤血球濃厚液	研究報告の公表状況	Schlenke P, Hervig T, Isola H, Wiesel ML, Kientz D, Pinkoski L, Singh Y, Lin L, Corash L, Cazenave JP. Transfusion. 2008 Apr;48(4):697-705. Epub 2008 Jan 7.	公表国
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)			ドイツ

研究報告の概要

○アモトサレンおよび紫外線A波による光化学的プロセス:欧州の血液センター3施設での検証

背景:治療用血漿中の病原体および白血球(WBC)を不活化する光化学処理(PCT)プロセスが開発された。欧州の血液センター3施設において通常の稼動状況下でプロセスバリデーション試験を実施した。

試験デザインおよび方法:各センターで、アフェレーシス血漿30、PCT用全血由来血漿製剤30～36を用意した。全血由来血漿はいずれも、適合する供血血液2～3を混合したものであった。未処置対照検体(対照新鮮凍結血漿C-FFP)を除いてから、6 mmol/LのアモトサレンlSmLおよびUVA 3J/㎠で血漿546～635 mLを処理し、吸着装置を用いて残存アモトサレンを除去した。プロセス後、血漿検体(PCT-FFP)を採取し、採血後8時間まで-60℃で冷凍保存し、凝固因子および残存アモトサレンの測定を行った。

結果:合計186本の血漿にプロセスを実施した。C-FFPと比較してPCT-FFPの平均プロトロンビン時間(12.2±0.6秒)および活性化部分トロンボプラスチン時間(32.1±3.2秒)は若干延長した。フィブリノゲンおよび第VIII因子は、PCTへの感受性がもっとも高かった(平均減少率26%)。しかし、PCT処理-FFPを実施しても、治療用血漿に必要なフィブリノゲン(217±43mg/dL)と第VIII因子(97±29 IU/dL)は十分保持された。PCT-FFP中の第II、V、VII、IX、X、XI、XIII因子の平均値はC-FFP(活性保持81～97%)と同等であった。抗血栓性のタンパク質は、PCTによる有意な影響を受けず、83%～97%の範囲で保持された。アモトサレン平均残存量は、0.6±0.1 μmol/Lであった。

結論:欧州の3つのセンターにおけるプロセスバリデーション試験は、治療用血漿に関する欧州規制およびそれぞれの国内基準の範囲内で、PCT-FFP中の凝固因子の活性は保持されていた。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
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紫外線A波による不活化工程のプロセスバリデーション試験は、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を示したとの報告である。	日本赤十字社では8項目の安全対策の一つとして、不活化技術の導入について、各不活化技術の効果、血液成分への影響、製造作業への影響などについて評価検討を行っている。外国での不活化実施状況や効果、新たな技術、副作用等の情報収集も含め総合的に評価し、導入について関係機関と協議しているところである。

Photochemical treatment of plasma with amotosalen and UVA light: process validation in three European blood centers

Peter Schlenke, Tor Hervig,* Hervé Isola, Marie-Louise Wiesel, Daniel Kientz, Linda Pinkoski, Yasmin Singh, Lily Lin, Laurence Corash, and Jean-Pierre Cazenave**

BACKGROUND: A photochemical treatment (PCT) process has been developed to inactivate pathogens and white blood cells (WBCs) in therapeutic plasma. Process validation studies were performed in three European blood centers under routine operating conditions.

STUDY DESIGN AND METHODS: Each center prepared 30 apheresis and 30 to 36 whole blood–derived plasma units for PCT. Each whole blood–derived plasma unit contained a mixture of two to three matched donations. After removal of pretreatment control samples (control fresh-frozen plasma [C-FFP]), 546 to 635 mL of plasma was treated with 15 mL of 6 *mmol per L amotosalen*, 3 *J per cm*^{2} UVA treatment, and removal of residual amotosalen with a compound adsorption device. After processing, plasma samples (PCT-FFP) were withdrawn, frozen at –60°C within 8 hours of collection, and assayed for coagulation factors and residual amotosalen.

RESULTS: A total of 186 units of plasma were processed. The mean prothrombin time (12.2 ± 0.6 sec) and activated partial thromboplastin time (32.1 ± 3.2 sec) of PCT-FFP were slightly prolonged compared to C-FFP. Fibrinogen and Factor (F)VIII were most sensitive to PCT (26% mean reduction). PCT-FFP, however, retained sufficient levels of fibrinogen (217 ± 43 mg/dL) and FVIII (97 ± 29 IU/dL) for therapeutic plasma. Mean levels of FII, FV, FVII, F IX, FX, FXI, and FXIII in PCT-FFP were comparable to C-FFP (81%-97% retention of activity). Antithrombotic proteins were not significantly affected by PCT with retention ranging between 83 and 97 percent. Mean residual amotosalen levels were 0.6 ± 0.1 µmol per L.

CONCLUSION: Process validation studies in three European centers demonstrated retention of coagulation factors in PCT-FFP within the required European and respective national standards for therapeutic plasma.

The INTERCEPT Blood System for plasma (Cerus Europe B.V., Leusden, the Netherlands) received CE Mark registration based on extensive studies demonstrating pathogen inactivation,[1] preclinical safety,[2] and clinical efficacy.[3-5] This system uses a photochemical treatment (PCT) process with amotosalen and long-wavelength ultraviolet UVA light (320-400 nm). Amotosalen is a synthetic psoralen molecule that reversibly intercalates into the helical regions of DNA and RNA. Upon illumination with UVA light, amotosalen forms irreversible covalent bonds with pyrimidine bases of the nucleic acid.[6] The genomes of pathogens and white blood cells (WBCs) modified by amotosalen can no longer replicate.

ABBREVIATIONS: aPTT(s) = activated partial thromboplastin time(s); AP = α2-antiplasmin; AT = antithrombin; CAD = compound adsorption device; C-FFP = control fresh-frozen plasma; PC = protein C; PCT = photochemical treatment; PCT-FFP = photochemically treated plasma samples; PS = protein S; PT(s) = prothrombin time(s).

From the University of Lübeck, Institute of Immunology and Transfusion Medicine, Lübeck, Germany; Haukeland University Hospital, Bergen, Norway; Etablissement Français du Sang-Alsace, Strasbourg, France; and Cerus Corp., Concord, California.

Address reprint requests to: Lily Lin, PhD, Cerus Corp., 2411 Stanwell Drive, Concord, CA 94520; e-mail: lily_lin@cerus.com.

*Peter Schlenke, Tor Hervig, and Jean-Pierre Cazenave contributed equally to this study.

This study was supported in part by Cerus Corp. Four authors (LP, YS, LL, and LC) were affiliated with and held stock or stock options of Cerus Corp. during the conduct of this study. Three authors (PS, TH, and JPC) served on Cerus's European Scientific Advisory Board.

Received for publication July 18, 2007; revision received September 13, 2007, and accepted September 14, 2007.

doi: 10.1111/j.1537-2995.2007.01594.x

TRANSFUSION 2008;48:697-705.

There are two components in the PCT system intended for commercial use: an integrated disposable set and a UVA illuminator. The integrated disposable set is a closed system composed of sequentially connected container with amotosalen, illumination container, a flowthrough compound adsorption device (CAD), and three plastic storage containers (Fig. 1). The UVA illuminator is a microprocessor-controlled device capable of delivering the target UVA dose and illuminating two plasma units simultaneously. The PCT system is capable of treating plasma units within the volume range of 385 to 650 mL.

Plasma units for PCT processing can be obtained by apheresis collection or by mixing 2 to 3 matched units of whole blood–derived plasma, analogous to whole blood–derived platelets (PLTs). After sterile connection to the PCT disposable set, plasma is passed through the series of containers. The PCT steps include addition of amotosalen to plasma, illumination of the plasma mixture with UVA light, and the removal of residual amotosalen. Although this system has been evaluated for preparation of plasma in specialized centers during clinical trials, process validation studies were conducted in blood centers to verify the performance of the PCT system under different routine operating conditions. Blood centers in three European countries (France, Norway, Germany) participated in these studies. Each center collected 30 units of apheresis plasma and prepared 30 to 36 units of whole blood–derived plasma with a target volume ranging from 600 to 655 mL. Residual amotosalen concentrations in photochemically treated plasma samples (PCT-FFP) were measured to assess CAD performance. The performance of the PCT system was assessed based on in vitro coagulation function assays in treated plasma (PCT-FFP) compared to the plasma before treatment (C-FFP) as well as to European and national regulatory requirements for therapeutic plasma.

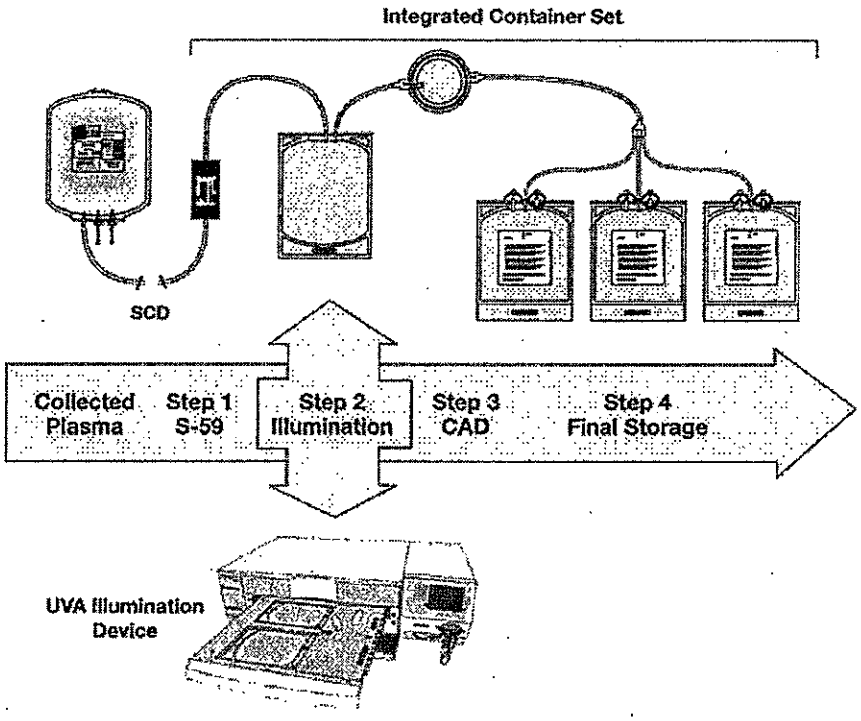


Fig. 1. The PCT system for plasma. The PCT system consists of a UVA illumination device and an integral disposable set. The UVA illuminator can illuminate 2 units of plasma per processing cycle. The disposable set provides a single-use, closed, integrated system for pathogen inactivation treatment of a plasma unit. The integrated disposable set is comprised of the following sterile components: a container with amotosalen (also known as S-59), a plastic illumination container, a CAD, and three plastic storage containers. The processing steps are as described under Materials and Methods section. SCD = sterile connection device.

MATERIALS AND METHODS

Plasma collection

Plasma collections were performed in three European blood centers and the collections methods varied slightly. In the Etablissement Français du Sang-Alsace in Strasbourg, France (Site S), apheresis plasma was collected on the Haemonetics PCS platform (Haemonetics Corp., Braintree, MA) in acid citrate dextrose anticoagulant. In blood centers of the University of Lübeck, Institute of Immunology and Transfusion Medicine, Lübeck, Germany (Site L), and Haukeland University Hospital, Bergen, Norway (Site B), apheresis plasma was collected on the Autopheresis-C platform (Baxter Healthcare Corp., Deerfield, IL) in citrate or in half-strength citrate-phosphate-dextrose (CPD) anticoagulant, respectively. Each site collected 30 units of apheresis plasma. Of the 90 apheresis units processed for this study, 27 were blood group A, 24 group AB, 22 group B, and 17 group O. The target volume of plasma was 600 to 655 mL. Fresh apheresis plasma units were kept at ambient temperature before and during the PCT process.

Whole blood was collected in CPD anticoagulant. Plasma was prepared by centrifugation with local standard operating procedures. A leukofiltration step was incorporated in the whole blood process only in Etablissement Français du Sang-Alsace in Strasbourg. Sites S and B each prepared 30 units of whole blood–derived plasma and Site L prepared 36 units. Of the 96 units, 44 were

blood group O, 42 group A, 7 group B, and 3 group AB. The target volume for the whole blood plasma unit before removing baseline samples was 600 to 655 mL and was obtained by mixing the appropriate volume of 2 to 3 ABO-matched whole blood–derived plasma units with a pooling set (FTC 0061, Baxter Healthcare Corp.). The plasma units were maintained at ambient temperature before and during the PCT process.

PCT disposable sets and UVA illumination device

The PCT disposable set for treatment of plasma (INT 3103 and INT 3104, Cerus Europe B.V.) consisted of the following sequentially integrated components: 15 mL of 6 mmol per L amotosalen HCl solution in saline packaged inside a plastic container (PL 2411, Baxter Healthcare Corp.) and protected from UVA light; a 1.3-L plastic container (PL 2410, Baxter Healthcare Corp.) for illumination of plasma; a flowthrough CAD consisting of an adsorbent disk composed of a copolymer of polystyrene and divinylbenzene particles fused with an ultrahigh-molecular-weight polyethylene plastic enclosed in an acrylic housing to reduce the concentration of amotosalen and its photoproducts; and three 400-mL plastic containers (PL 269, Baxter Healthcare Corp.) for storage of the treated plasma.

Illumination of plasma was performed in a UVA illumination device (Model INT100, Cerus Europe B.V.). The device was capable of illuminating 2 units of plasma per processing cycle. During illumination plasma units were reciprocally agitated at approximately 70 cycles per minute.

PCT process

For these studies, the entire process was completed to allow frozen storage of treated plasma within 8 hours of the start of plasma collection. A Luer adapter was sterile-connected to each plasma unit. Baseline coagulation factor samples (C-FFP) of approximately 20 mL were collected from each unit before PCT.

During the treatment process, plasma was passed through each component of the PCT set in a series of steps (Fig. 1). In Step 1, the plasma unit was sterile-connected to the amotosalen container, and the entire plasma volume was passed through the amotosalen container into the illumination container. In Step 2, the plasma containing amotosalen was illuminated with a 3 J per cm^2 UVA treatment. In Step 3, the illuminated plasma mixture was passed by gravity flow through the CAD into the storage containers.

After processing, plasma samples (PCT-FFP) were withdrawn and frozen in 1.5-mL aliquots in 2-mL polypropylene tubes at or below –60°C within 8 hours of the start of plasma or whole blood collection. The C-FFP and PCT-FFP samples were assayed for prothrombin time (PT), activated partial thromboplastin time (aPTT), Factor (F)I (fibrinogen), FII, FV, FVII, FVIII, F IX, FX, FXI, FXIII, protein C (PC), protein S (PS), antithrombin (AT), and α2-antiplasmin (AP). Samples were also withdrawn after addition of amotosalen before UVA illumination and after the entire PCT process (including CAD) for measurement of amotosalen concentrations. To minimize assay variability, samples collected from the three processing centers were shipped on dry ice to a single location for analysis. All assays were performed at Cerus Corp. with the exception of quantification of AP, which was performed by a reference laboratory (Esoterix Laboratories, Aurora, CO).

Measurement of the levels of amotosalen

The initial and residual amotosalen levels in each plasma unit were quantified. A 200-µL volume of plasma was diluted to 1 mL with 35 percent methanol in buffer. After centrifugation, the supernatant was filtered and 100 µL were analyzed on a C-18 (Zorbax) reverse-phase column (Agilent Technologies, Palo Alto, CA) with a gradient of increasing methanol in KH_2PO_4 buffer. Amotosalen was detected by optical absorption (300 nm).[7]

Measurement of in vitro coagulation function

Clottable fibrinogen (FI) was measured with a modified Clauss assay. Coagulation factors were assayed with one-stage PT-based clotting assays (FII, FV, FVII, FX) or one-stage aPTT-based clotting assays (FVIII, F IX, FXI). The clotting time of a mixture of diluted test plasma and plasma deficient in the factor being quantified was compared with a reference curve constructed from the clotting times of 5 dilutions, ranging from 1:5 to 1:320, of plasma with known activity mixed with deficient plasma. These coagulation assays, as well as the PT and aPTT, were performed on an automated coagulation analyzer (MLA Electra 1400C or 1600C, Instrumentation Laboratory Co., Lexington, MA). Reagents included brain thromboplastin (Hemoliance, Instrumentation Laboratory Co.), Platelin L (bioMérieux, Durham, NC), and congenitally depleted factor-deficient substrate (Helena Laboratories, Beaumont, TX; George King Bio-Medical, Overland Park, KS). The endpoint of all tests was the formation of a clot detected photooptically and measured in seconds. Factor Assay Control Plasma (FACT; George King Bio-Medical) was used as the reference standard for the procoagulation factor assays.

FXIII was measured with a commercially available FXIII kit (Berichrom, Dade Behring, Marburg, Germany). FXIII, activated by thrombin, releases an activation product that leads to a series of reactions resulting in a decrease in nicotinamide adenine dinucleotide, detected by monitoring absorbance at 340 nm. The assay was per-

formed on a Behring Clot Timer (BCT, Dade Behring), and standard human plasma (Dade Behring) was used as the reference standard.

PC and PS were measured with commercially available PC and PS kits (Staclot, both from Diagnostica Stago, Asnieres, France). PC and PS assays were based on prolongation of the aPTT resulting from inactivation of FV and FVIII by activated PC. The activator in the PC assay was an extract of *Agkistrodon contortrix* snake venom; the activator in the PS assay was activated PC. The tests were performed on the Behring Clot Timer. Unicalibrator (Diagnostica Stago) was used as the reference standard.

AT was measured with a commercially available ATIII kit (Stachrom, Diagnostica Stago). Plasma containing AT was incubated with a known excess of thrombin. A chromogenic substrate, imidolyzed by the remaining thrombin, was detected photooptically on a coagulation analyzer (MLA Electra 1400C or 1600C, Instrumentation Laboratory Co.). Factor assay control plasma was used as the reference standard.

α2-AP was quantified with reagents from Diagnostica Stago. In this chromogenic method, plasmin was added in excess to the test plasma, resulting in the formation of antiplasmin–plasmin complexes. The concentration of residual plasmin is measured by its amidolytic activity with a chromogenic substrate measured at 405 nm. α2-AP concentration is inversely proportional to the residual plasmin concentration and is determined by color intensity. This analysis was performed by Esoterix Laboratories (Aurora, CO) with an STA analyzer (Diagnostica Stago).

The mean and standard deviation (SD) were determined for each coagulation variable. All factor activities were expressed in IU per dL with the exception of fibrinogen, which is expressed in mg per dL. The activity of each coagulation variable remaining after PCT was also expressed as proportional (%) retention compared to the pretreatment (baseline) activity. Comparison of the PT and aPTT was based on the prolongation of the clotting time in seconds after PCT relative to baseline. Significant differences were determined by the t test at a p value of 0.05. Reference ranges for each assay were defined as the mean ± 2 SD for untreated plasma samples.[1]

RESULTS

Processing of plasma

A total of 186 units of plasma of approximately 600 to 655 mL was prepared in this study. After removal of control samples, the volume per plasma unit for PCT processing ranged from 546 to 635 mL. The mean pretreatment amotosalen concentration was 143 ± 8 µmol per L (Table 1). The illumination of a 3 J per cm^2 UVA treatment took 7 to 9 minutes. The mean time required for the plasma mixture to completely pass through the CAD was 21 ± 3 minutes (range, 15-30 min). After CAD, the mean residual amotosalen concentration was 0.6 ± 0.1 µmol per L (Table 1). All values were below the target performance value of less than 2.0 µmol per L. The PLT and RBC concentrations for both apheresis and whole blood–derived plasma before treatment and after treatment were less than 50×10^9 and less than 6×10^9 per L, respectively, and were within the European guidelines for therapeutic FFP.[8] The WBC concentrations for apheresis plasma were less than 0.1×10^9 per L before and after treatment. The WBC concentrations for nonleukofiltered whole blood plasma in Site L and Site B were higher before treatment ($6 \times 10^9 \pm 7 \times 10^9$ and $38 \times 10^9 \pm 22 \times 10^9$/L, respectively); however, after treatment, the WBC concentrations in PCT-FFP were within the European guidance limits.

TABLE 1. Amotosalen concentrations before illumination and after CAD treatment*

Measure	Amotosalen (µmol/L)	
	Before illumination	After CAD
Target range	110-225	<2
Apheresis plasma		
Site S† (n = 30)	139 ± 4	0.5 ± 0.1
Site B† (n = 30)	151 ± 6	0.5 ± 0.1
Site L† (n = 30)	142 ± 2	0.7 ± 0.1
Whole blood–derived plasma		
Site S (n = 30)	136 ± 8	0.6 ± 0.1
Site B (n = 30)	136 ± 4	0.5 ± 0.1
Site L (n = 36)	150 ± 3	0.6 ± 0.1
Overall (n = 186)	143 ± 8	0.6 ± 0.1

* Data are reported as mean ± SD.
† Site S = EFS Alsace, Strasbourg, France; Site B = Blood Bank of Haukeland University Hospital, Bergen, Norway; Site L = University of Lübeck, Lübeck, Germany.

The effect of PCT on global coagulation assays (PT and aPTT)

The mean C-FFP and PCT-FFP PT for both apheresis plasma and whole blood–derived plasma for all three processing sites were within reference ranges (Tables 2 and 3). PCT-FFP PTs were prolonged by a mean of 0.3 ± 0.2 seconds for apheresis plasma (n = 90) and 0.5 ± 0.1 seconds for whole blood–derived plasma (n = 96) compared to C-FFP measurements (Table 4).

Similarly, the mean C-FFP and PCT-FFP aPTT for both apheresis plasma and whole blood–derived plasma for all three processing sites were within reference ranges (Tables 2 and 3). PCT-FFP aPTTs were prolonged by a mean of 3.5 ± 1.3 seconds for apheresis plasma (n = 90) and 4.6 ± 0.9 seconds for whole blood–derived plasma (n = 96) compared to C-FFP measurements (Table 4). The overall (apheresis and whole blood combined for an n = 186) mean PCT-FFP PT and aPTT compared to C-FFP was prolonged by 0.4 ± 0.2 and 4.1 ± 1.2 seconds, respectively (Table 4).

TABLE 2. Apheresis plasma: clotting times and coagulation factor activity before and after PCT (mean ± SD)

Variable	Reference range†	Site S* (n = 30)			Site B* (n = 30)			Site L* (n = 30)		
		C-FFP	PCT-FFP	% Retention‡	C-FFP	PCT-FFP	% Retention‡	C-FFP	PCT-FFP	% Retention‡
PT (sec)	11.1-13.5	12.2 ± 0.6	12.7 ± 0.7	0.5 ± 0.2	12.3 ± 0.7	12.5 ± 0.7	0.2 ± 0.2	12.0 ± 0.4	12.4 ± 0.5	0.3 ± 0.2
aPTT (sec)	23-35	27.9 ± 2.5	32.4 ± 3.2	4.5 ± 1.1	26.1 ± 2.5	28.5 ± 2.7	2.3 ± 1.0	27.8 ± 2.5	31.5 ± 3.1	3.8 ± 0.8
FI (mg/dL)	200-390	295 ± 71	208 ± 55	70 ± 5	313 ± 48	261 ± 43	83 ± 3	287 ± 57	216 ± 43	75 ± 3
FII (IU/dL)	80-120	102 ± 17	89 ± 15	88 ± 7	117 ± 15	107 ± 16	91 ± 3	103 ± 14	91 ± 12	88 ± 3
FV (IU/dL)	95-170	120 ± 29	116 ± 26	97 ± 3	144 ± 23	142 ± 20	99 ± 5	125 ± 19	119 ± 17	95 ± 3
FVII (IU/dL)	70-175	116 ± 25	93 ± 21	80 ± 4	112 ± 27	92 ± 22	82 ± 3	111 ± 23	91 ± 19	81 ± 3
FVIII (IU/dL)	85-235	140 ± 34	99 ± 25	70 ± 3	161 ± 42	130 ± 35	81 ± 4	123 ± 39	91 ± 30	74 ± 3
F IX (IU/dL)	75-145	90 ± 13	75 ± 10	84 ± 4	101 ± 18	88 ± 14	87 ± 3	93 ± 12	78 ± 11	84 ± 4
FX (IU/dL)	75-130	108 ± 21	95 ± 19	88 ± 4	117 ± 20	106 ± 19	91 ± 3	112 ± 14	99 ± 12	88 ± 2
FXI (IU/dL)	60-150	93 ± 16	79 ± 13	85 ± 8	111 ± 19	101 ± 7	91 ± 6	103 ± 20	90 ± 18	87 ± 2
FXIII (IU/dL)	85-135	114 ± 22	110 ± 22	97 ± 9	126 ± 18	121 ± 18	96 ± 5	115 ± 21	108 ± 20	94 ± 3
PC (IU/dL)	80-140	122 ± 25	102 ± 24	84 ± 9	122 ± 20	107 ± 19	88 ± 4	119 ± 22	101 ± 20	85 ± 2
PS (IU/dL)	85-135	108 ± 25	107 ± 25	100 ± 9	108 ± 18	103 ± 17	95 ± 6	92 ± 20	88 ± 19	96 ± 3
AT (IU/dL)	85-105	102 ± 9	97 ± 9	95 ± 4	105 ± 9	101 ± 8	96 ± 4	95 ± 10	91 ± 10	95 ± 3
AP (IU/dL)	80-150	97 ± 11	81 ± 8	83 ± 10	108 ± 13	89 ± 8	83 ± 6	101 ± 12	82 ± 6	82 ± 8

* Site S = EFS Alsace, Strasbourg, France; Site B = Blood Bank of Haukeland University Hospital, Bergen, Norway; Site L = University of Lübeck, Lübeck, Germany.
† The reference range was calculated from the mean ± 2 SDs of untreated, conventional plasma. n = 80 for fibrinogen, FV, FVII, FVIII, F IX, and FXI; n = 50 for FII and FX; n = 25 for FXIII, AT, PC, and PS. The reference range for AT was established by Esoterix Laboratories.
‡ For PT and aPTT, the reported values = (PCT-FFP – C-FFP) in seconds.

TABLE 3. Whole blood–derived plasma: clotting times and coagulation factor activity before and after PCT (mean ± SD)

Variable†	Reference range†	Site S* (n = 30)			Site B* (n = 30)			Site L* (n = 30)		
		C-FFP	PCT-FFP	% Retention‡	C-FFP	PCT-FFP	% Retention‡	C-FFP	PCT-FFP	% Retention‡
PT (sec)	11.1-13.5	11.5 ± 0.3	12.0 ± 0.4	0.5 ± 0.1	11.5 ± 0.3	12.0 ± 0.3	0.4 ± 0.1	11.5 ± 0.4	12.0 ± 0.4	0.6 ± 0.1
aPTT (sec)	23-35	29.5 ± 1.8	34.2 ± 2.4	4.7 ± 0.7	28.6 ± 1.8	32.9 ± 2.4	4.3 ± 0.9	28.2 ± 1.4	33.1 ± 2.1	4.9 ± 0.8
FI (mg/dL)	200-390	290 ± 25	207 ± 23	71 ± 3	291 ± 30	222 ± 24	76 ± 3	272 ± 40	191 ± 30	70 ± 3
FII (IU/dL)	80-120	106 ± 8	95 ± 9	89 ± 3	100 ± 9	89 ± 8	89 ± 3	101 ± 7	89 ± 7	88 ± 3
FV (IU/dL)	95-170	123 ± 14	119 ± 13	97 ± 2	125 ± 15	121 ± 14	97 ± 3	119 ± 18	114 ± 17	96 ± 3
FVII (IU/dL)	70-175	113 ± 15	91 ± 12	80 ± 3	108 ± 14	86 ± 11	80 ± 3	114 ± 17	90 ± 14	79 ± 3
FVIII (IU/dL)	85-235	127 ± 24	91 ± 19	71 ± 3	118 ± 20	91 ± 17	77 ± 4	119 ± 23	84 ± 19	71 ± 4
F IX (IU/dL)	75-145	93 ± 10	78 ± 8	84 ± 3	92 ± 11	78 ± 10	85 ± 2	95 ± 9	78 ± 6	82 ± 4
FX (IU/dL)	75-130	108 ± 9	94 ± 8	87 ± 1	106 ± 12	92 ± 11	87 ± 3	105 ± 10	90 ± 9	85 ± 2
FXI (IU/dL)	60-150	83 ± 14	69 ± 12	83 ± 4	101 ± 11	87 ± 12	86 ± 6	94 ± 9	81 ± 9	85 ± 3
FXIII (IU/dL)	85-135	104 ± 12	101 ± 12	97 ± 2	121 ± 10	111 ± 10	92 ± 3	113 ± 14	106 ± 12	94 ± 3
PC (IU/dL)	80-140	115 ± 14	97 ± 12	85 ± 5	114 ± 19	102 ± 16	90 ± 5	116 ± 16	99 ± 14	85 ± 7
PS (IU/dL)	85-135	111 ± 15	107 ± 14	96 ± 3	117 ± 14	114 ± 14	97 ± 4	114 ± 16	109 ± 15	96 ± 5
AT (IU/dL)	85-105	101 ± 7	97 ± 7	96 ± 2	96 ± 4	93 ± 4	96 ± 2	91 ± 6	86 ± 6	95 ± 2
AP (IU/dL)	80-150	100 ± 8	85 ± 5	85 ± 5	95 ± 5	78 ± 4	82 ± 4	93 ± 6	76 ± 4	82 ± 3

* Site S: = EFS Alsace, Strasbourg, France; Site B = Blood Bank of Haukeland University Hospital, Bergen, Norway; Site L = University of Lübeck, Lübeck, Germany.
† The reference range was calculated from the mean ± 2 SDs of untreated, conventional plasma. n = 80 for fibrinogen, FV, FVII, FVIII, F IX, and FXI; n = 50 for FII and FX; n = 25 for FXIII, AT, PC, and PS. The reference range for AT was established by Esoterix Laboratories.
‡ For PT and aPTT, the reported values = (PCT-FFP – C-FFP) in seconds.

TABLE 4. Combined clotting times and coagulation factor activity before and after PCT (mean ± SD): comparison apheresis plasma and whole blood–derived plasma

Variable	Reference range*	Apheresis plasma (n = 90)			Whole blood plasma (n = 96)			Overall (n = 186)		
		C-FFP	PCT-FFP	% Retention†	C-FFP	PCT-FFP	% Retention†	C-FFP	PCT-FFP	% Retention†
PT (sec)	11.1-13.5	12.2 ± 0.6	12.5 ± 0.6	0.3 ± 0.2	11.5 ± 0.3	12.0 ± 0.4	0.5 ± 0.1	11.8 ± 0.6	12.2 ± 0.6	0.4 ± 0.2
aPTT (sec)	23-35	27.3 ± 2.6	30.8 ± 3.4	3.5 ± 1.3	28.7 ± 1.7	33.4 ± 2.3	4.6 ± 0.9	28.0 ± 2.3	32.1 ± 3.2	4.1 ± 1.2
FI (mg/dL)	200-390	299 ± 60	228 ± 52‡	76 ± 7‡	284 ± 34	206 ± 29‡	72 ± 4‡	291 ± 49	217 ± 43	74 ± 6
FII (IU/dL)	80-120	107 ± 17‡	95 ± 16	89 ± 5	102 ± 8‡	91 ± 8	89 ± 3	105 ± 13	93 ± 13	89 ± 4
FV (IU/dL)	95-170	130 ± 26‡	126 ± 24‡	97 ± 4	122 ± 16‡	118 ± 15‡	97 ± 3	126 ± 22	122 ± 20	97 ± 3
FVII (IU/dL)	70-175	113 ± 25	92 ± 21‡	81 ± 4‡	112 ± 15	89 ± 12‡	80 ± 3‡	112 ± 21	90 ± 17	81 ± 3
FVIII (IU/dL)	85-235	141 ± 41‡	107 ± 35‡	75 ± 6‡	121 ± 23‡	88 ± 18‡	73 ± 5‡	131 ± 34	97 ± 29	74 ± 5
F IX (IU/dL)	75-145	95 ± 15‡	80 ± 13‡	85 ± 4‡	94 ± 10‡	78 ± 8‡	84 ± 3‡	94 ± 13	79 ± 11	84 ± 4
FX (IU/dL)	75-130	112 ± 18‡	100 ± 17‡	89 ± 4‡	106 ± 10‡	92 ± 9‡	86 ± 2‡	109 ± 15	96 ± 14	88 ± 3
FXI (IU/dL)	60-150	102 ± 19‡	90 ± 18‡	88 ± 7‡	93 ± 13‡	79 ± 13‡	85 ± 5‡	97 ± 17	84 ± 17	86 ± 6
FXIII (IU/dL)	85-135	119 ± 21‡	113 ± 21‡	96 ± 6	113 ± 14‡	106 ± 12‡	94 ± 3	116 ± 18	110 ± 17	95 ± 5
PC (IU/dL)	80-140	121 ± 22	103 ± 21	85 ± 6	115 ± 16	99 ± 14	87 ± 6	118 ± 20	101 ± 18	86 ± 6
PS (IU/dL)	85-135	103 ± 22‡	99 ± 22‡	97 ± 7‡	114 ± 15‡	110 ± 15‡	96 ± 4‡	109 ± 20	105 ± 19	97 ± 5
AT (IU/dL)	85-105	100 ± 12‡	95 ± 12	95 ± 3	95 ± 7‡	91 ± 7	96 ± 2	98 ± 9	94 ± 9	96 ± 3
AP (IU/dL)	80-150	102 ± 12‡	83 ± 8‡	82 ± 7	96 ± 7‡	79 ± 6‡	83 ± 4	99 ± 11	82 ± 7	83 ± 6

* The reference range was calculated from the mean ± 2 SDs of untreated, conventional plasma. n = 80 for fibrinogen, FV, FVII, FVIII, F IX, and FXI; n = 50 for FII and FX; n = 25 for FXIII, AT, PC, and PS. The reference range for AT was established by Esoterix Laboratories.
† For PT and aPTT, the reported values = (PCT-FFP − C-FFP) in seconds.
‡ Significant differences between apheresis and whole blood plasma were detected at a p value of less than 0.05.

The effect of PCT on procoagulant factors

The mean procoagulant factor activities in apheresis C-FFP obtained in each processing site were within reference ranges (Table 2). Apheresis plasma processed with the PCT system retained mean factor activity ranging from 70 to 83 percent and 70 to 81 percent of C-FFP for fibrinogen (FI) and FVIII, respectively. Retentions of FII, FV, FVII, F IX, FX, FXI, and FXIII ranged from 80 to 99 percent (Table 2). After PCT, the mean procoagulant factor activities were also within the reference ranges. Mean FVIII activities were 99 ± 25, 130 ± 35, and 91 ± 30 IU per dL in Site S, Site B, and Site L, respectively, meeting national (70 IU/dL) and European Pharmacopoeia (50 IU/dL) requirements for therapeutic plasma.

Similarly, the mean activity levels of the procoagulant factors in whole blood–derived plasma were within reference ranges (Table 3). Whole blood plasma processed with the PCT system retained mean coagulation factor activities ranging from 70 to 76 and 71 to 77 percent of C-FFP for FI and FVIII, respectively. The mean retentions for FII, FV, FVII, F IX, FX, FXI, and FXIII ranged from 79 to 97 percent (Table 3). After PCT, the mean factor activities were also within the reference ranges with the exception of FI and FVIII in Site L. Mean FVIII activities were 91 ± 19, 91 ± 17, and 84 ± 19 IU per dL in Site S, Site B, and Site L, respectively, meeting national (70 IU/dL) and European Pharmacopoeia (50 IU/dL) requirements for therapeutic plasma.

To compare the processing characteristics between apheresis plasma (n = 90) and whole blood–derived plasma (n = 96), the results from all three sites were combined for analysis (Table 4). Whole blood–derived plasma generally exhibited statistically significantly lower factor activities compared to apheresis plasma (see Table 4). All mean values, however, fell within the reference ranges. The mean FVIII activity in apheresis PCT-FFP (107 ± 35 IU/dL, n = 90) and whole blood–derived plasma (88 ± 18 IU/dL, n = 96) met the national and European Pharmacopoeia requirements for therapeutic plasma.

The overall (n = 186) mean level of fibrinogen in PCT-FFP was 217 ± 43 mg per dL, which is 74 ± 6 percent of the C-FFP values. The overall (n = 186) mean activity of FVIII in PCT-FFP was 97 ± 29 IU per dL, which is 74 ± 5 percent of the C-FFP values. The retention of other factors in PCT-FFP was consistently higher (81%-97%; Table 4).

The effect of PCT on antithrombotic and fibrinolytic protein activity

All pretreatment activities of PC, PS, AT III, and α2-AP of apheresis plasma (Table 2) and whole blood–derived plasma (Table 3) were within reference ranges. After PCT, the mean activities of these antithrombotic proteins were still within the reference ranges with two exceptions: the

mean levels of AP activity in PCT-FFP from Site B (78 ± 4 IU/dL) and Site L (76 ± 4 IU/dL) were slightly outside the lower limit (80 IU/dL) of the reference range.

After PCT, the mean retention of antithrombotic proteins ranged from 84 to 90 percent for PC and 82 to 85 percent for AP (Tables 2 and 3). No differences were observed between apheresis and whole blood–derived plasma. Similarly, the mean retention in PCT-FFP ranged from 95 to 100 percent for PC and 95 to 96 percent for AT (Tables 2 and 3). The results were comparable between apheresis and whole blood–derived plasma.

To compare the processing characteristics between apheresis plasma (n = 90) and whole blood–derived plasma (n = 96), the results from all three sites were combined for analysis (Table 4). There were no significant differences in the activity of PC of either C-FFP or PCT-FFP between apheresis and whole blood–derived plasma. Whole blood–derived plasma contained significantly higher levels of PS before and after PCT than apheresis plasma (see Table 4). In contrast, the levels of AT and AP were generally lower in whole blood–derived plasma compared to apheresis plasma and reached significance (see Table 4).

The overall (n = 186) mean activities of the antithrombotic proteins in C-FFP and PCT-FFP were within the reference ranges. After PCT, mean retention of PC and AP was 86 and 83 percent, respectively, whereas mean retention of PS and AT was 97 and 96 percent (Table 4).

DISCUSSION

The PCT process for preparation of pathogen-inactivated FFP involves the addition of amotosalen to a nominal concentration of 150 µmol per L (range, 110-225 µmol/L), illumination of the plasma mixture with a 3 J per cm² UVA treatment, and removal of residual amotosalen to less than 2.0 µmol per L by a flow CAD. Three European centers participated in this study to validate the process under routine blood bank operation conditions. Each center processed 30 units of apheresis plasma and 30 to 36 units of whole blood–derived plasma with integral disposable sets that have received CE Mark approval and commercial UVA illuminator. The PCT process was completed within the time frame for FFP, allowing units to be frozen within 8 hours of collection.

The mean pretreatment amotosalen concentration from all three sites was 143 ± 8 µmol per L (n = 186), which is well within the target system performance range of 110 to 225 µmol per L. The use of the microprocessor-controlled UVA illuminator ensured delivery of the UVA treatment dose of 3 J per cm². All three centers demonstrated the addition of the correct amotosalen concentration, combined with a 3 J per cm² UVA treatment dose, thus ensuring robust pathogen inactivation. The mean amotosalen level after the CAD treatment for all centers was 0.6 ± 0.1 µmol per L (n = 186), which is significantly below the target performance value of 2.0 µmol per L. The mean residual amotosalen levels among the three sites ranged from 0.5 ± 0.1 to 0.7 ± 0.1 µmol per L with no units having residual amotosalen higher than 1.2 µmol per L, demonstrating the consistency and the efficacy of the CAD. These results demonstrate that the PCT process can be performed consistently under blood bank conditions.

The quality of PCT-FFP was assessed for activity of FVIII with respect to meeting national and European regulatory guidelines.[8] The consistency of the PCT process was assessed by the retention of all factor activities in PCT-FFP compared to levels in pretreatment plasma samples.

The factor most affected by PCT was FVIII with a mean of 26 percent reduction in activity. However, residual activity is within the current requirement for FFP as the level of FVIII is greater than 50 IU per dL in the European Pharmacopoeia standard for therapeutic FFP.[9] The mean FVIII activity after PCT was 107 ± 35 IU per dL (n = 90) for apheresis plasma and 88 ± 18 IU per dL (n = 96) for whole blood–derived plasma or an overall mean of 97 ± 29 IU per dL (n = 186). All units had FVIII activity greater than 50 IU per dL. In France, greater than 90 percent of quality control samples must have greater than 70 IU per dL in FVIII. Preliminary studies measuring the thrombin generation time for PCT-FFP have shown no difference from untreated plasma for peak thrombin levels, lag time to start of thrombin generation, or total thrombin produced.[10] These observations suggest that the reduction in FVIII levels are not critical to generation of thrombin and the ultimate conversion of fibrinogen to fibrin. These observations of normal thrombin generation are in contrast to those recently reported for plasma prepared with methylene blue and visible light.[11]

Fibrinogen was also affected by PCT with a mean of 26 percent reduction in the clottable fibrinogen levels. Although there is no required standard for the level of fibrinogen in FFP, the mean levels retained in PCT-FFP (217 ± 43 mg/dL, n = 186) were within the reference range. Prior clinical studies with PCT-FFP for support of liver transplant with massive transfusion have shown no increased requirement for plasma or cryoprecipitate, indicating that the levels of fibrinogen in PCT-FFP are sufficient.[4] These patients have a significant period of fibrinolytic activity after unclamping of the transplanted liver. The study examined the use of conventional FFP, cryoprecipitate, and PCT-FFP for support of these patients and observed no differences to indicate that the reduced levels of fibrinogen in PCT-FFP were clinically relevant. In addition, the levels of AP activity are reasonably conserved in PCT-FFP.[1] Although the levels of fibrinogen are reduced by the treatment, the levels appear adequate to support hemostasis in patients with active fibrinolysis.

Other coagulation factors (FII, FV, FVII, F IX, FX, FXI, FXIII) were less affected by PCT. The mean factor activities in PCT-FFP were within the reference ranges. Retention of activity after PCT ranged from 81 to 97 percent. Of specific importance were the levels of FVII, which is the factor with the shortest half-life and thus the most critical for transfusion support of acquired complex coagulopathy. In addition, levels of the anticoagulant PC and PS and AT were relatively unaffected by PCT and α2-AP was well conserved.

There was slight prolongation in PT and aPTT. PCT-FFP, however, retained PT and aPTT within the reference range. The slight changes in PT and aPTT after PCT were not associated with any adverse clinical observations in controlled clinical trial settings, and treatment of congenital coagulation defects has demonstrated consistent correction of both the PT and aPTT after transfusion with PCT-FFP.[3-5,12]

The results of this study demonstrate that there is good retention of relevant coagulation factor activities and antithrombotic protein function in PCT-FFP from either apheresis or whole blood and that these products meet the requirements for therapeutic plasma. In a separate study, the effect of storage on FI, FII, FV, FVII, FVIII, F IX, FX, and FXI has been evaluated.[13] The results show that therapeutic levels of these factors were conserved in PCT-FFP after 12 months of storage at –18°C and after 18 months of storage at –25°C. Similar results were obtained in storage studies conducted at one of the three centers (Site S, data not shown) with PCT-FFP prepared from apheresis plasma frozen up to 1 year. In addition, clinical trials with PCT-FFP have shown that this product is sufficient for therapeutic support of patients with each of the major clinical indications for plasma transfusion.

The effect of PCT on plasminogen and von Willebrand factor (VWF) has also been evaluated.[14,15] After treatment, plasminogen was within normal ranges and retained 94 percent. The von Willebrand antigen, VWF:ristocetin cofactor, components of the von Willebrand complex, including multimers and VWF:CP activity, remained within normal ranges and demonstrated greater than 98 percent retention. Because of the stability of these factors after treatment, they were not included in the current validation study.

When the results were compared between sites or between types of plasma, significant differences were observed, although the differences were small, not likely of clinical relevance, and did not appear to follow a specific pattern. The observed differences could simply be due to the geographic variation in the plasma characteristics and the slight variation in the processing techniques. Of particular interest is that the FVIII activities as well as the retention for apheresis PCT-FFP in Site B were significantly higher than the values obtained in the other two sites. This difference could not be completely explained by the different apheresis collection platforms. Site S used the Haemonetics platform, but the same Baxter platform was used in both Site B and Site L. The observed difference between Site B and Site L was most likely due to the variation in donor population and processing techniques. Different anticoagulants may introduce variability but were poorly defined and not well evaluated. Overall, PCT-FFP manufactured in the three different geographic locations were of comparable quality. All met the respective national and European standards for transfusable FFP.

Previous studies with cryoprecipitate prepared from photochemically treated plasma yielded approximately 95 and 88 percent retention of fibrinogen and FVIII, respectively, compared to cryoprecipitate prepared from untreated plasma.[16] Cryosupernatant prepared from photochemically treated plasma retained adequate levels of critical plasma proteins for plasma exchange therapy in acute thrombocytopenic purpura. These data indicate good preservation of hemostasis control proteins such as PS, α2-AP, and VWF-cleaving protease activity.[17]

In summary, the results of process validation studies from three European centers demonstrated the consistency of the PCT process for FFP. From a blood center perspective, scaleup manufacturing of PCT-FFP in routine is feasible by the ability to treat individual large-volume units of fresh apheresis plasma and small pools of whole blood–derived plasma. The mixture of whole blood plasma from two or three matched donations is similar to the procedure for whole blood–derived PLT components. Since adult patients will require 4 to 6 FFP units (200 mL each) for a therapeutic episode, donor exposures are consistent with current practice in which whole blood plasma units are processed individually. A similar PCT system utilizing amotosalen and UVA light for PLT components has been in routine use in some blood centers in European countries.[18] Both PLT and plasma components are treated with the same UVA illumination device thus simplifying the logistics of implementation of two pathogen inactivation systems in one blood center.

ACKNOWLEDGMENTS

The authors thank Silke Adresen for her technical assistance during the study in Site L; Katherine Ling and Tom Pangburn for performing the HPLC assay; Helen Lam, Simon Yeh, and Junous Bayat for analytical data review; Tracey Diaz and Theresa Sullivan for sample management; Treva Peterson and Melchor Dolor for technical assistance on coagulation assays and analysis; and William Greenman for critical review of the manuscript.

REFERENCES

1. Singh Y, Sawyer LS, Pinkoski LS, Dupuis KW, Hsu JC, Lin L, Corash L. Photochemical treatment of plasma with amo-

tosalen and long-wavelength ultraviolet light inactivates pathogens while retaining coagulation function. Transfusion 2006;46:1168-77.
2. Ciaravino V, McCullough T, Cimino G, Sullivan T. Preclinical safety profile of plasma prepared using the INTERCEPT Blood System. Vox Sang 2003;85:171-82.
3. de Alarcon P, Benjamin R, Dugdale M, Kessler C, Shopnick R, Smith P, Abshire T, Hambleton J, Matthew P, Ortiz I, Cohen A, Konkle BA, Streiff M, Lee M, Corash L, Wages D. Fresh frozen plasma prepared with amotosalen HCl (S-59) photochemical pathogen inactivation: transfusion of patients with congenital coagulation factor deficiencies. Transfusion 2005;45:1362-72.
4. Mintz PD, Bass NM, Petz LD, Steadman R, Streiff M, . McCullough J, Burks S, Wages D, Van Doren S, Corash L. Photochemically treated fresh frozen plasma for transfusion of patients with acquired coagulopathy of liver disease. Blood 2006;107:3753-60.
5. Mintz PD, Neff A, MacKenzie M, Goodnough LT, Hillyer C, Kessler C, McCrae K, Menitove J, Skiknee B, Damon L, Lopez-Plaza I, Rouault C, Crookston KP, Benjamin RJ, George J, Lin J-S, Corash L, Conlan MG. A randomized, controlled Phase III trial of therapeutic plasma exchange with fresh-frozen plasma (FFP) prepared with amotosalen and ultraviolet A light compared to untreated FFP in thrombotic thrombocytopenic purpura. Transfusion 2006; 46:1693-704.
6. Wollowitz S. Fundamentals of the psoralen-based Helinx technology for inactivation of infectious pathogens and leukocytes in platelets and plasma. Semin Hematol 2001; 38(4 Suppl 11):4-11.
7. Lin L, Conlan MG, Tessman J, Cimino G, Porter S. Amotosalen interactions with platelet and plasma components: absence of neoantigen formation after photochemical treatment. Transfusion 2005;45:1610-20.
8. Guide to the preparation, use and quality assurance of blood components. 9th ed. Strasbourg: Council of Europe Publishing; 2003.
9. European pharmacopoeia. 5th ed. Strasbourg: Council of Europe; 2005.
10. Cazenave JP, Netzer P, Schubler S, Ravanat C, Isola H, Kientz D, Gachet C, Wiesel ML. Retention of thrombin generation capacity of therapeutic apheresis plasma prepared with amotosalen and UVA light (INTERCEPT) pathogen inactivation. Blood 2007;110:75B.
11. Pock KH, Janisch S, Svae TE, Romisch J. Thrombin generation capacity is significantly impaired in methylene-blue treated plasma compared to normal levels in single-donor fresh-frozen plasma, a pharmaceutically licensed coagulation active plasma (Octaplas), and a development product (Uniplas) [abstract]. Proceeding of Pathogen Inactivation Consensus Conference, Toronto, Canada, March 29-30, 2007.
12. Hambleton J, Wages D, Radu-Radulescu L, Adams M, MacKenzie M, Shafer S, Lee M, Smyers J, Wiesehahn G, Corash L. Pharmacokinetic study of FFP photochemically treated with amotosalen (S-59) and UV light compared to FFP in healthy volunteers anticoagulated with warfarin. Transfusion 2002;42:1302-7.
13. Pinkoski L, Peterson T, Singh Y, Corash L. Coagulation factor activity in photochemically treated plasma after storage at –18C or –25C. Transfusion 2006;46(9S):115A.
14. Pinkoski L, Singh Y, Corash L. INTERCEPT plasma: further in vitro evidence of comparability to conventional FFP. Transfusion 2005;45(3S):109A.
15. Osselaer J, Debry C, Goffaux M, Pineau J, Calomme G, Dubuc E, Chatelain B, Vandendaele MC, Hsu J, Rheinschmidt M, Lin L. Coagulation function in fresh frozen plasma prepared with two photochemical treatment methods: methylene blue and amotosalen. Transfusion 2007 Sep 27 DOI: 10.1111/j.1537-2995.2007.01488.x.
16. Mintz PD, Avery NL, Moulaison SL, Flanagan HL, Boyd JC. Preparation of cryoprecipitate from photochemically treated fresh frozen plasma. Transfusion 2000;40 Suppl 1:63S.
17. Yarranton H, Lawrie AS, Mackie IJ, Pinkoski L, Corash L, Machin SJ. Coagulation factor levels in cryosupernatant prepared from plasma treated with amotosalen hydrochloride (S-59) and ultraviolet A light. Transfusion 2005;45: 1453-8.
18. Lin L, Hanson CV, Alter HJ, Jauvin V, Bernard KA, Murthy KK, Metzel P, Corash L. Inactivation of viruses in platelet concentrates by photochemical treatment with amotosalen and long-wavelength ultraviolet light. Transfusion 2005;45: 580-90.

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	(製造承認書に記載なし)	研究報告の公表状況	Blue DE, Cruz J, Limiac A, Spinola S, Davis TE, Waxman D, McCarthy L, Smith D. American Society for Microbiology 108th General Meeting; 2008 Jun 1-5; Boston.	公表国 米国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社)			

研究報告の概要

○輸血による*Babesia.microti*死亡例
輸血によって*Babesia microti*に感染し死亡する例は赤血球を含む輸血100万単位あたり1件未満と見積もられている。疾患は通常無症候性だが、無脾症、高齢、免疫抑制状態の患者では感染によって死に至ることがある。
症例:腎臓疾患で透析を必要としていた61歳の女性患者。入院45日前に赤血球2単位を輸血され、その後更に2単位追加輸血された。入院前日、吐き気と発熱を訴えたため、血液培養をオーダーし、抗生物質が投与された。リハビリ施設に戻る際に、体温は39.4℃を示し、低血圧で、昇圧剤を必要とし、敗血症の症状を呈した。血液塗抹標本では、赤血球の5~15%にトロフォゾイト(栄養体)があり、*Plasmodium falciparum*か*B. microti*と考えられた。静注キニジン及びクリンダマイシン投与が開始された。赤血球交換により寄生虫血症は1%まで低下した。投薬は適切だったが、播種性血管内凝固症候群(DIC)を発症し6日後に死亡した。外出や旅行はしていなかったため、唯一のリスクファクターは輸血と考えられた。
結果:*Babesia*はCDCで形態学的に確認された。患者の入院時の検体では6%の寄生虫血症と*B. microti* PCR陽性が認められた。輸血された製剤の供血者4名のうち1名がIFAで*B. microti*陽性となった。供血者はダニに噛まれた記憶はなく、流行地域に旅行したこともなかった。
結論:上の臨床症状と転帰は*Babesia*の輸血伝播による死亡例の中では珍しいものではないが、中西部で発生したという点が他と異なっている。ベクターIxodes scapularisが寄生する中西部のオジロジカの頭数増加に伴い、供血者における*Babesia microti*抗体陽性率を解明する為の研究を行うべきである。

報告企業の意見	今後の対応
輸血によると考えられる*Babesia microti*に感染し死亡した症例の報告である。	今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」

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

Abstract Title: Fatal Transfusion-Transmitted *Babesia microti*

Author Block: D. E. Blue[1,2], J. Cruz[3], A. Limiac[2], S. Spinola[1], **T. E. Davis**[1], D. Waxman[3], L. McCarthy[1], D. Smith[1,2];
[1]Indiana Univ. Sch. of Med., Indianapolis, IN, [2]Clarian Hlth. Partners (Methodist-IU-Riley Hosp.), Indianapolis, IN, [3]Indiana Blood Ctr., Indianapolis, IN.

Presentation Number: C-198

Poster Board Number: 0335

Keywords: Babesia,transfusion-transmitted

Background: Fatal transfusion-transmitted *B. microti* has an estimated incidence of <1:1,000,000 per unit of transfused red cell containing blood products. The disease is usually asymptomatic; however, fatal infections occur in asplenic, elderly or immunosuppressed individuals. **Case Report:** The 61-year-old female patient had renal disease requiring dialysis. Forty-five days prior to admission she received two units of packed red cells and then two more. One day prior to admission, the patient complained of nausea and fever. Blood cultures were ordered and antibiotics administered. Upon returning to the rehabilitation facility, she spiked temperatures to 103°F and was admitted to the hospital. She was hypotensive, requiring vasopressor support, and appeared to be septic. The blood smear revealed trophozoites in 5 to 15% of red cells, probable species: *Plasmodium falciparum* vs. *B. microti.* Treatment with intravenous quinidine and clindamycin was begun. A red cell exchange reduced parasitemia to 1%. Despite appropriate medication, the patient developed disseminated intravascular coagulation and expired 6 days later. Since she was confined indoors and did not travel, the only risk factor was transfusion. **Results:** *Babesia* was confirmed morphologically by CDC with 6% parasitemia and PCR positivity for *B. microti* from the patient's specimen at admission. The three donors available for testing were negative for *B. microti* and all samples were negative for *P. falciparum* by PCR. One blood donor and the patient were positive for *B. microti* by immunofluorescent antibody (IFA). The seropositive donor had no recollection of a tick bite and did not travel to endemic areas. **Conclusion:** The above clinical presentation and course is not atypical for rare fatal cases of transfusion-transmitted *Babesia*. This is an unusual case as it arose in the Midwest. With the expanding Midwest white-tailed deer populations harboring the vector, *Ixodes scapularis*, studies to determine the regional incidence of *Babesia microti* seropositive blood donors may be warranted.

別紙様式第2-1

No. 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. 4. 15	該当なし	

一般的名称	人赤血球濃厚液	研究報告の公表状況	飯岡大, 前追善智, 中村文彦, 林孝昌, 津田勝代. 第56回日本輸血・細胞治療学会総会; 2008 Apr 25-27; 福岡.	公表国
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)			日本

研究報告の概要

○血小板濃厚液の輸血後に、急性呼吸不全と*Bacillus cereus*(*B. cereus*)による髄膜炎を併発した症例

【緒言】輸血後細菌感染症は、診断・治療に難渋し、時に致命的な状態になることもある。我々は、*B. cereus*の輸血後感染症で急性呼吸不全および重症髄膜炎を併発した症例を経験し、その治療および診断経過が今後の対策につき有用と考えられここに報告する。

【症例】76歳女性。64歳に再生不良性貧血と診断、免疫抑制療法に不応で、71歳よりは赤血球および血小板輸血が定期的に必要となった。平成19年4月、血小板輸血を施行中、発熱・悪寒、その後急速な呼吸不全を認め、胸部X線・心エコー検査より、輸血関連肺障害(TRALI)と判断した。メチルプレドニゾロン500mg投与で呼吸状態は改善し発熱も消退した。しかし発症12時間後、嘔気・頭痛の出現と共に再び発熱を認めた。感染症を考え直ちに抗生剤を開始したが悪化し、発症16時間後には右方への眼球偏位と意識障害(昏睡)が出現した。髄液検査にて細胞数・蛋白の増加を認め、脳波でも異常波を認めたことから、細菌性髄膜炎および症候性てんかんと診断した。その後、抗生剤および抗てんかん薬が奏効し、発症第13日には意識清明となり、発症第25日には後遺障害なく退院できた。輸血関連感染の診断目的に当院で各種培養検査を施行したところ、血小板残液の鏡検・培養検査で*B. cereus*が検出された。髄液では、初回抗生剤投与後に採取した影響もあり鏡検・培養検査は陰性であったが、遺伝子検査PCR法にて、血小板製剤と同一菌株の*B. cereus*が検出され、今症例が輸血後感染症から髄膜炎に進展したと考えられた。一方で、凍結処理された供血者保存血漿では、培養検査・遺伝子検査共に陰性であった。

【考察】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。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

自発報告:2007年5月28日付1-07000033

報告企業の意見	今後の対応
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。 本症例について、日本赤十字社では抗白血球抗体、抗顆粒球抗体検査を実施し、臨床経過及び診断基準よりTRALIであると評価した。患者の血液培養が陰性で、当該血小板製剤と同一採血時の凍結血漿では無菌試験陰性であったことから、輸血による細菌感染があったかどうかは不明である。	日本赤十字社では、輸血による細菌感染予防対策として平成18年10月より血小板製剤について、また、平成19年3月より全血採血由来製剤について、初流血除去を導入した。また、全ての輸血用血液製剤について、平成19年1月より保存前白血球除去を実施している。さらに、輸血情報リーフレット等により細菌感染やウイルス感染について医療機関へ情報提供し注意喚起しているほか、細菌感染が疑われる場合の対応を周知している。今後も細菌やウイルスの検出や不活化する方策について情報の収集に努める。

WS-3-3 血小板濃厚液の輸血後に，急性呼吸不全と Bacillus cereus (B.cereus) による髄膜炎を併発した症例

天理よろづ相談所病院血液内科[1)]，天理よろづ相談所病院臨床病理部[2)]
飯岡　大[1)]，前迫善智[1)]，中村文彦[1)]，林　孝昌[1)]，津田勝代[2)]
TEL：0743-63-5611（223）E-mail：iioka@tenriyorozu-hp.or.jp

【緒言】輸血後細菌感染症は，診断・治療に難渋し，時に致命的な状態になることもある．我々は，*B.cereus* の輸血後感染症で急性呼吸不全および重症髄膜炎を併発した症例を経験し，その治療および診断経過が今後の対策につき有用と考えられここに報告する．【症例】76 歳女性．64 歳に再生不良性貧血と診断，免疫抑制療法に不応で，71 歳よりは赤血球および血小板輸血が定期的に必要となった．平成 19 年 4 月，血小板輸血を施行中，発熱・悪寒，その後急速な呼吸不全を認め，胸部 X 線・心エコー検査より，輸血関連肺障害（TRALI）と判断した．メチルプレドニゾロン 500mg 投与で呼吸状態は改善し発熱も消退した．しかし発症 12 時間後，嘔気・頭痛の出現と共に再び発熱を認めた．感染症を考え直ちに抗生剤（ビアペネム）を開始したが悪化し，発症 16 時間後には右方への眼球偏位と意識障害（昏睡）が出現した．髄液検査にて細胞数・蛋白の増加を認め，脳波でも異常波を認めたことから，細菌性髄膜炎および症候性てんかんと診断した．その後，抗生剤（バンコマイシンも併用）および抗てんかん薬が奏効し，発症第 13 日には意識清明となり，発症第 25 日には後遺障害なく退院できた．輸血関連感染の診断目的に当院で各種培養検査を施行したところ，血小板残液の鏡検・培養検査で *B.cereus* が検出された．髄液では，初回抗生剤投与後に採取した影響もあり鏡検・培養検査は陰性であったが，遺伝子検査 PCR 法にて，血小板製剤と同一菌株の *B.cereus* が検出され，今症例が輸血後感染症から髄膜炎に進展したと考えられた．一方で，凍結処理された供血者保存血漿では，培養検査・遺伝子検査共に陰性であった．【考察】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．

別紙様式第2-1

No. 9

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2008. 4. 9	該当なし	

一般的名称	人赤血球濃厚液	研究報告の公表状況		公表国
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)		SignOnSanDiego.com. 2008 Mar 26.	米国

研究報告の概要

○カリフォルニア州サンディエゴ郡の梅毒症例急激に増加

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。

全国的に、梅毒感染者の大部分は他のHIV陽性の男性と無防備な性交渉を行うHIV陽性の男性であり、サンディエゴ郡でも同様の傾向が見られる。昨年、当郡で梅毒と診断された人の84パーセントは男性と性交渉がある男性(MSM)であり、大半はHIV陽性だった。米国疾病対策予防センター(CDC)は、医師に対し、MSMの患者が年に1度梅毒のルーチンの検査を受けるよう奨励することを求めている。

連邦当局は、梅毒と他の性感染症(STD)の蔓延に対しても懸念を抱いている。先週、CDCは全国の梅毒症例数が7年連続で増加したと発表した。CDCは連邦の予算配分を変更してSTD教育と予防の取り組みに力を入れるよう要求している。AIDSや癌、心臓病と比較すると、米国のSTDの症例数は多くはない。しかし、連邦、州および地域の当局は、STD症例の増加が重要な公衆衛生問題であると認識している。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
カリフォルニア州サンディエゴ郡の梅毒症例数が、2000年以降急増しているとの報告である。	日本赤十字社では、輸血感染症対策として問診時に梅毒を含む性感染症の既往歴を確認し、梅毒の既往歴、治療歴のある人は献血不適としている。また、献血血液に対して梅毒血清学的検査を実施し、陽性血液を排除している。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。

SignOnSanDiego.com THE SAN DIEGO UNION-TRIBUNE

PRINT THI

More Health news

Syphilis cases up sharply in county

STD prevention, education efforts to be heightened

By Cheryl Clark
UNION-TRIBUNE STAFF WRITER

March 26, 2008

Alarmed by San Diego County's more than 1,100 percent rise in syphilis cases between 2000 and last year, state health officials are using five investigators to help the region stem the spread.

"San Diego's increase is a cause for concern because we're just not understanding why it's being transmitted in the frequency that we're seeing," said Dr. Douglas Hatch, chief of California's division of communicable disease control.

The search for causes and solutions includes debate about whether safe-sex education needs to be explicit and targeted at men who have sex with men. That group accounts for most of the syphilis infections in the county and nationwide.

San Diego County's annual syphilis caseload skyrocketed from 28 in 2000 – when the infection total hit a low – to 340 last year. It is a much sharper rise than in the state's other large urban counties, including San Francisco, Los Angeles, Orange and Alameda. It's also more than double the statewide percentage of increase and triple that of the nation.

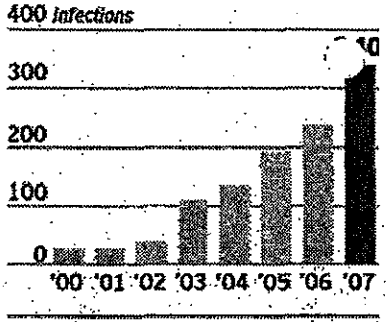
The team that the state dispatched to San Diego County consists of three investigators who recently started aiding county officials and two who were already working with them but have increased their level of assistance. They are contacting people who were diagnosed with syphilis, finding their sexual partners and urging those people to get tested so they can receive treatment and not transmit the bacteria.

Federal officials are also concerned by the spread of syphilis and other sexually transmitted diseases. Last week, the U.S. Centers for Disease Control and Prevention said the national syphilis case count had increased for the seventh consecutive year.

The agency wants to change federal funding formulas so states and counties can obtain more money for STD education and prevention efforts.

The United States' STD numbers aren't huge when compared to those for AIDS, cancer or heart disease.

REPORTED SYPHILIS INFECTIONS COUNTYWIDE



SOURCE: California Dept. of Public Health
MATT PERRY / Union-Tribune

REPORTED SYPHILIS INFECTIONS BY COUNTY

Sampling of counties with large populations

County	2000	2007	Change
San Diego	28	340	1,1 %
Los Angeles	152	827	444%
Orange	28	141	404%
San Francisco	54	202	274%
Alameda	14	39	179%
Statewide	330	2,002	507%

SOURCE: California Dept. of Public Health
MATT PERRY / Union-Tribune

But officials at the federal, state and local levels all have identified the growth of STDs as an important public health issue Public agencies and nonprofit groups spend hundreds of millions of dollars each year on STD prevention and testing.

Nationwide, an increasingly large portion of people infected with syphilis are HIV-positive men who have unprotected se with other HIV-positive men.

The trend is reflected in San Diego County. Last year, 84 percent of the people diagnosed with syphilis in San Diego County were men who had sex with men, and a big segment of them were HIV-positive, state health officials said.

Medical experts said the region's proximity to the border and its substantial tourist trade also could be contributing to

unsafe sex, which leads to higher numbers of syphilis cases and other STDs.

Syphilis likely has spread in San Diego County because at-risk men aren't telling health care providers about their sexual behavior and don't get tested, said Terry Cunningham, chief of the county's HIV, STD and Hepatitis branch.

He also blames health care providers who don't consistently address the issue with their patients.

The CDC is asking physicians to be more diligent in encouraging male patients who have sex with men to undergo routine screenings for syphilis, perhaps once a year.

Public health officials and various nonprofits diverge on a major point – how best to emphasize STD prevention. They ask:

▪How explicit must the anti-STD message be to draw enough attention?

▪Will public-service ads that are candid about sexual practices turn off or offend some people?

▪Should awareness campaigns aggressively focus on men who have sex with men, perhaps by using images of male couples?

San Diego County's approach is to address all individuals who face exposure to sexually transmitted diseases, said Holly Crawford, a spokeswoman for the county's Health and Human Services Agency.

"Targeting only specific populations . . . could lead to a false sense of security among the general population," she said. "STDs do not recognize gender, age, sexual orientation or location."

Greg Cox, chairman of the county Board of Supervisors, said the county is "addressing the issue of sexually transmitted diseases among all at-risk populations through bilingual education campaigns, alerts to doctors and STD education and testing at county clinics.

"I'm concerned about any disease or illness that affects the people of our region . . . but I am optimistic that our efforts wil succeed in bringing down the disturbing case numbers."

The county has allocated more than $1.5 million in state funds for this fiscal year to five nonprofit groups that work on STD prevention. They are the Family Health Centers of San Diego; San Diego Lesbian, Gay, Bisexual Transgender Community Center; San Diego Youth and Community Center; San Ysidro Health Center; and Vista Community Clinic.

The Vista clinic does outreach in neighborhoods where people who engage in high-risk sexual activities congregate, said director Barbara Mannino. The four other groups declined to discuss how they're using their grants.

The county also tries to reach high-risk populations in central San Diego by placing safe-sex ads in bus shelters and gay-themed publications.

Despite those efforts, some who work on STD prevention said more must be done to connect with specific risk groups.

"San Diego County's message is, 'We're all at risk.' That's true, but some people are much more at risk than others. What are they doing to target gay men and their sexual practices in bathhouses, for example?" said Oscar de la O, a founding member and president of Bienestar.

The nonprofit group tries to educate high-risk individuals in Southern California about syphilis and other STDs.

Bienestar outreach worker Abigail Madariaga is atransgendered woman, which means she was born a man but identifies and lives as a woman. On a recent evening, she and fellow STD educator Antonio Munoz talked to people watching a drag queen performance at Urban Mo's bar and nightclub in Hillcrest.

Madariaga said her orientation allows her to spread the safe-sex message more effectively to many of the estimated 1,500 other transgendered people in the region, as well as to gays, lesbians and bisexuals watching the drag show.

In San Francisco, public health workers spend time in Internet chat rooms, where gay men often make their connections. They began doing so after discovering how syphilis was spreading in many cases, said Dr. Jeffrey Klausner, director of STD prevention for the San Francisco Department of Public Health.

San Francisco has established medical clinics friendly to the gay community. Its health officials also conduct public awareness campaigns that show sex "as a positive, normal and healthy activity, rather than use fear and avoidance as a motivator," Klausner said.

One of those initiatives was the "Healthy Penis" program, which featured outreach workers dressed in costumes resembling penises and syphilis sores. That got people's attention, Klausner recalled.

The city has seen a drop in syphilis cases -- from 348 cases in 2004 to 202 last year.

Klausner blamed the spread of syphilis elsewhere on reduced funding for anti-STD efforts and said health leaders in each community need to target their messages to those most at risk. "Increasing rates of STDs are what you can expect when there are no resources," he said.

San Diego County's share of state money for STD education and prevention this fiscal year is down $280,000 from last year.

Beyond money, many safe-sex campaigns are thwarted by politicians' fear of candor, said Michael Weinstein, president of the AIDS Healthcare Foundation in Los Angeles.

"They must make it obvious they are talking about sex," he said. "The sooner we stop treating this as a dirty little secret, the better off we'll be."

■Cheryl Clark: (619) 542-4573; cheryl.clark@uniontrib.com

Find this article at:
http://www.signonsandiego.com/news/health/20080326-9999-1n26syphilis.html

☐ Check the box to include the list of links referenced in the article.

感染症定期報告の報告状況(2008/6/1～2008/8/31)

血対ID	受理日	報告者名	一般名	生物由来成分名	原材料名	原産国	含有区分	文献	症例	適正使用措置
80101	2008/06/06	ベネシス	ポリエチレングリコール処理抗破傷風人免疫グロブリン 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
80102	2008/06/10	バイエル薬品	pH4 処理酸性人免疫グロブリン	人免疫グロブリンG	ヒト血液	米国	有効成分	有	無	無
80103	2008/06/17	日本赤十字社	人赤血球濃厚液	人赤血球濃厚液	人血液	日本	有効成分	有	有	無
80104	2008/06/17	日本赤十字社	人全血液	人全血液	人血液	日本	有効成分	有	無	無
80105	2008/06/17	日本赤十字社	抗HBs人免疫グロブリン	抗HBs人免疫グロブリン	人血液	日本	有効成分	有	無	無
80106	2008/06/17	日本赤十字社	洗浄人赤血球浮遊液	洗浄人赤血球浮遊液	人血液	日本	有効成分	有	有	無
80107	2008/06/24	化学及血清療法研究所	乾燥スルホ化人免疫グロブリン	スルホ化人免疫グロブリンG	ヒト血液	①米国、②日本	有効成分	有	無	無
80108	2008/06/27	バクスター	乾燥イオン交換樹脂処理人免疫グロブリン	人免疫グロブリンG	人血漿	米国	有効成分	無	無	無
80109	2008/06/27	バクスター	乾燥イオン交換樹脂処理人免疫グロブリン	人血清アルブミン	人血漿	米国	添加物	無	無	無
80110	2008/07/03	ベネシス	人ハプトグロビン	人ハプトグロビン	人血液	日本	有効成分	有	無	無
80111	2008/07/15	化学及血清療法研究所	乾燥濃縮人血液凝固第Ⅷ因子	血液凝固第Ⅷ因子	ヒト血液	日本	有効成分	有	無	無
80112	2008/07/16	富士フィルムRIファーマ	テクネチウム大凝集人血清アルブミン(99mTc)	テクネチウム大凝集人血清アルブミン(99mTc)	ヒト血液	米国	有効成分	有	無	無
80113	2008/07/24	CSLベーリング	乾燥濃縮人アンチトロンビンⅢ	乾燥濃縮人アンチトロンビンⅢ	ヒト血液	米国、ドイツ、オーストリア	有効成分	有	有	無
80114	2008/07/25	ノボノルディスクファーマ	エプタコグ アルファ(活性型)(遺伝子組換え)	ウシ新生仔血清	ウシ血液	ニュージーランド	製造工程	無	無	無
80115	2008/07/25	ノボノルディスクファーマ	エプタコグ アルファ(活性型)(遺伝子組換え)	ウシ胎仔血清	ウシ血液	ニュージーランド、オーストラリア、米国及びカナダ	製造工程	無	無	無
80116	2008/07/25	ノボノルディスクファーマ	エプタコグ アルファ(活性型)(遺伝子組換え)	ブタ膵臓由来トリプシン	ブタ膵臓(抽出物)	不明	製造工程	無	無	無
80117	2008/07/25	ノボノルディスクファーマ	エプタコグ アルファ(活性型)(遺伝子組換え)	エプタコグ アルファ(活性型)(遺伝子組換え)	エプタコグ アルファ(活性型)(遺伝子組換え)	該当しない	有効成分	無	無	無
80118	2008/07/28	日本製薬	乾燥人血液凝固第Ⅸ因子複合体	血液凝固第Ⅸ因子複合体	人血液	日本	有効成分	有	無	無
80119	2008/07/29	日本メジフィジックス	放射性医薬品基準ガラクトシル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)注射液	ガラクトシル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
80120	2008/07/29	日本赤十字社		合成血	人血液	日本	有効成分	有	無	無
80121	2008/07/31	バクスター	ルリオクトコグ アルファ(遺伝子組換え)	培養補助剤(抗第Ⅷ因子モノクローナル抗体製造用-1)	ウシ血液	米国	製造工程	無	有	無
80122	2008/07/31	バクスター	ルリオクトコグ アルファ(遺伝子組換え)	ウシ血清アルブミン	ウシ血液	米国	製造工程	無	有	無

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
80123	2008/07/31	バクスター	ルリオクトコグ アルファ(遺伝子組換え)	ルリオクトコグ アルファ(遺伝子組換え)	遺伝子組換えチャイニーズハムスター卵巣細胞株	該当なし	有効成分	無	有	無
80124	2008/07/31	バクスター	加熱人血漿たん白	人血清アルブミン	人血漿	米国	有効成分	無	有	無
80125	2008/07/31	バクスター	ルリオクトコグ アルファ(遺伝子組換え)	インスリン(抗第Ⅷ因子モノクローナル抗体製造用)	ウシ膵臓	米国	製造工程	無	有	無
80126	2008/07/31	バクスター	ルリオクトコグ アルファ(遺伝子組換え)	人血清アルブミン	人血漿	米国	添加物	無	有	無
80127	2008/07/31	バクスター	ルリオクトコグ アルファ(遺伝子組換え)	アプロチニン	ウシ肺	ニュージーランド	製造工程	無	有	無
80128	2008/07/31	バクスター	ルリオクトコグ アルファ(遺伝子組換え)	ウシ胎児血清(抗第Ⅷ因子モノクローナル抗体製造用)	ウシ血液	オーストラリア	製造工程	無	有	無
80129	2008/07/31	バクスター	ルリオクトコグ アルファ(遺伝子組換え)	培養補助剤(抗第Ⅷ因子モノクローナル抗体製造用-2)	ウシ肝臓	米国又はカナダ	製造工程	無	有	無
80130	2008/08/14	富士フィルムRIファーマ	ヨウ化人血清アルブミン(131I)	ヨウ化人血清アルブミン(131I)	ヒト血液	日本	有効成分	有	無	無
80131	2008/08/25	日本製薬	①加熱人血漿たん白 ②人血清アルブミン(5%) ③人血清アルブミン(20%) ④人血清アルブミン(25%) ⑤乾燥ポリエチレングリコール処理人免疫グロブリン ⑥トロンビン ⑦乾燥濃縮人アンチトロンビンⅢ ⑧人免疫グロブリン ⑨乾燥人血液凝固第Ⅸ因子複合体	ヘパリン	ブタ腸粘膜	ブラジル	①～⑧製造工程、⑨添加物・製造工程	無	無	無
80132	2008/08/27	CSLベーリング	人血清アルブミン 破傷風抗毒素 フィブリノゲン加第XIII因子 乾燥濃縮人アンチトロンビンⅢ	ヘパリンナトリウム	ブタ腸粘膜	中国	製造工程	無	無	無
80133	2008/08/27	CSLベーリング	人C1-インアクチベーター	人C1-インアクチベーター	ヒト血液	米国、ドイツ、オーストリア	有効成分	有	無	有
80134	2008/08/28	化学及血清療法研究所	乾燥抗破傷風人免疫グロブリン	抗破傷風人免疫グロブリン	ヒト血液	米国	有効成分	有	無	有
80135	2008/08/28	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 乾燥濃縮人アンチトロンビンⅢ 人免疫グロブリン フィブリノゲン加第ⅩⅢ因子 乾燥濃縮人活性化プロテインC ヒスタミン加人免疫グロブリン製剤 トロンビン 乾燥スルホ化人免疫グロブリン 人血清アルブミン 乾燥ペプシン処理人免疫グロブリン	ヘパリンナトリウム	ブタ腸粘膜	中国、フランス、米国、カナダ	製造工程	無	無	無
80136	2008/08/28	バクスター	ルリオクトコグ アルファ(遺伝子組換え)	ルリオクトコグ アルファ(遺伝子組換え)	遺伝子組換えチャイニーズハムスター卵巣細胞株	該当なし	有効成分	無	無	無

別紙様式第4

感染症発生症例一覧

番号	感染症の種類		発現国	性別	年齢	発現時期	転帰	出典	区分	備考
	器官別大分類	基本語								
1	感染症および寄生虫症	菌血症	日本	男	51	2008/3/29	①回復	症例報告	当該製品	2008/4/15提出、識別番号1-08000029　未完了報告
2	感染症および寄生虫症	細菌感染	日本	女	64	2008/3/10	②軽快	症例報告	当該製品	2008/3/28提出、識別番号1-07000289　未完了報告
3	感染症および寄生虫症	細菌感染	日本	女	90	2008/3/5	⑤死亡	症例報告	当該製品	2008/3/21提出、識別番号1-07000278　未完了報告
4	感染症および寄生虫症	B型肝炎	日本	男	65	2008/2/19	③未回復	症例報告	当該製品	2008/3/11提出、識別番号1-07000249　未完了報告
5	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
6	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（5番と同一症例）
7	感染症および寄生虫症	細菌感染	日本	男	67	2008/2/18	①回復	症例報告	当該製品	2008/3/4提出、識別番号1-07000244　未完了報告
8	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
9	感染症および寄生虫症	敗血症性ショック	日本	女	82	2008/2/5	②軽快	症例報告	当該製品	2008/3/4提出、識別番号1-07000242　未完了報告
10	感染症および寄生虫症	敗血症	日本	女	82	2008/2/5	②軽快	症例報告	当該製品	2008/3/4提出、識別番号1-07000242　未完了報告（9番と同一症例）
11	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
12	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（12番と同一症例）
13	感染症および寄生虫症	C型肝炎	日本	女	75	2008/1/30	③未回復	症例報告	当該製品	2008/3/18提出、識別番号1-07000253　未完了報告
14	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
15	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
16	感染症および寄生虫症	C型肝炎	日本	男	35	2008/1/21	③未回復	症例報告	当該製品	2008/2/15提出、識別番号1-07000227　未完了報告
17	感染症および寄生虫症	C型肝炎	日本	男	57	2008/1/16	⑥不明	症例報告	当該製品	2008/2/14提出、識別番号1-07000222　未完了報告
18	感染症および寄生虫症	C型肝炎	日本	男	57	2008/1/16	⑥不明	症例報告	当該製品	2008/3/31提出、識別番号1-07000222　取り下げ（17番と同一症例）
19	感染症および寄生虫症	細菌感染	日本	男	74	2008/1/14	①回復	症例報告	当該製品	2008/2/5提出、識別番号1-07000200　未完了報告
20	感染症および寄生虫症	B型肝炎	日本	男	71	2008/1/9	⑥不明	症例報告	当該製品	2008/1/25提出、識別番号1-07000181　未完了報告
21	感染症および寄生虫症	B型肝炎	日本	男	66	2008/1/9	③未回復	症例報告	当該製品	2008/1/30提出、識別番号1-07000186　未完了報告
22	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
23	感染症および寄生虫症	C型肝炎	日本	女	76	2007/12/26	③未回復	症例報告	当該製品	2008/4/16提出、識別番号1-08000030　未完了報告
24	感染症および寄生虫症	サイトメガロウイルス感染	日本	女	2	2007/12/25	③未回復	症例報告	当該製品	2008/2/19提出、識別番号1-07000229　未完了報告
25	感染症および寄生虫症	C型肝炎	日本	女	42	2007/12/19	③未回復	症例報告	当該製品	2008/1/9提出、識別番号1-07000171　未完了報告
26	感染症および寄生虫症	敗血症	日本	男	74	2007/12/16	③未回復	症例報告	当該製品	2008/1/7提出、識別番号1-07000165　未完了報告
27	感染症および寄生虫症	C型肝炎	日本	女	97	2007/12/14	④回復したが後遺症あり	症例報告	当該製品	2008/1/7提出、識別番号1-07000166　未完了報告
28	感染症および寄生虫症	B型肝炎	日本	男	84	2007/12/14	⑥不明	症例報告	当該製品	2008/1/25提出、識別番号1-07000182　未完了報告
29	感染症および寄生虫症	C型肝炎	日本	女	80	2007/12/12	③未回復	症例報告	当該製品	2008/1/9提出、識別番号1-07000170　未完了報告
30	感染症および寄生虫症	C型肝炎	日本	男	62	2007/12/11	③未回復	症例報告	当該製品	2008/1/8提出、識別番号1-07000169　未完了報告
31	感染症および寄生虫症	C型肝炎	日本	男	53	2007/12/7	③未回復	症例報告	当該製品	2007/12/28提出、識別番号1-07000162　未完了報告
32	感染症および寄生虫症	C型肝炎	日本	女	84	2007/12/7	⑥不明	症例報告	当該製品	2008/3/6提出、識別番号1-07000247　未完了報告
33	感染症および寄生虫症	細菌感染	日本	女	83	2007/11/19	①回復	症例報告	当該製品	2007/12/4提出、識別番号1-07000135　未完了報告
34	感染症および寄生虫症	B型肝炎	日本	男	83	2007/11/16	③未回復	症例報告	当該製品	2007/12/5提出、識別番号1-07000139　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10回

35	感染症および寄生虫症	B型肝炎	日本	男	83	2007/11/16	⑥不明	症例報告	当該製品	2008/2/1提出、識別番号1-07000139　取り下げ（34番と同一症例）
36	感染症および寄生虫症	B型肝炎	日本	男	62	2007/11/15	③未回復	症例報告	当該製品	2007/12/14提出、識別番号1-07000145　未完了報告
37	感染症および寄生虫症	B型肝炎	日本	男	69	2007/11/14	③未回復	症例報告	当該製品	2007/12/19提出、識別番号1-07000149　未完了報告
38	感染症および寄生虫症	B型肝炎	日本	男	69	2007/11/14	③未回復	症例報告	当該製品	2007/12/28提出、識別番号1-07000149　未完了報告（37番と同一症例）
39	感染症および寄生虫症	伝染性紅斑	日本	女	55	2007/11/12	②軽快	症例報告	当該製品	2007/12/28提出、識別番号1-07000161　未完了報告
40	感染症および寄生虫症	細菌感染	日本	男	90	2007/11/9	②軽快	症例報告	当該製品	2007/11/27提出、識別番号1-07000129　未完了報告
41	感染症および寄生虫症	B型肝炎	日本	女	61	2007/11/9	③未回復	症例報告	当該製品	2007/11/27提出、識別番号1-07000130　未完了報告
42	感染症および寄生虫症	C型肝炎	日本	女	80	2007/11/7	②軽快	症例報告	当該製品	2007/11/28提出、識別番号1-07000132　未完了報告
43	感染症および寄生虫症	B型肝炎	日本	女	63	2007/11/6	⑥不明	症例報告	当該製品	2007/11/26提出、識別番号1-07000127　未完了報告
44	感染症および寄生虫症	B型肝炎	日本	女	26	2007/11/5	③未回復	症例報告	当該製品	2007/12/4提出、識別番号1-07000137　未完了報告
45	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
46	感染症および寄生虫症	細菌感染	日本	男	50	2007/10/24	②軽快	症例報告	当該製品	2007/11/8提出、識別番号1-07000114　未完了報告
47	感染症および寄生虫症	B型肝炎	日本	女	34	2007/10/19	③未回復	症例報告	当該製品	2007/11/8提出、識別番号1-07000113　未完了報告
48	感染症および寄生虫症	B型肝炎	日本	男	56	2007/10/19	①回復	症例報告	当該製品	2007/11/13提出、識別番号1-07000117　未完了報告
49	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
50	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（49番と同一症例）
51	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（49番、50番と同一症例）
52	感染症および寄生虫症	B型肝炎	日本	男	84	2007/10/12	③未回復	症例報告	当該製品	2007/10/31提出、識別番号1-07000107　未完了報告
53	感染症および寄生虫症	B型肝炎	日本	女	62	2007/10/12	③未回復	症例報告	当該製品	2007/11/19提出、識別番号1-07000124　未完了報告
54	感染症および寄生虫症	B型肝炎	日本	男	82	2007/10/1	⑥不明	症例報告	当該製品	2007/11/19提出、識別番号1-07000122　未完了報告
55	感染症および寄生虫症	B型肝炎	日本	男	82	2007/10/1	③未回復	症例報告	当該製品	2008/1/7提出、識別番号1-07000122　未完了報告（54番と同一症例）
56	感染症および寄生虫症	C型肝炎	日本	女	78	2007/9/22	②軽快	症例報告	当該製品	2007/11/5提出、識別番号1-07000108　未完了報告
57	感染症および寄生虫症	B型肝炎	日本	男	59	2007/9/10	③未回復	症例報告	当該製品	2007/11/26提出、識別番号1-07000125　未完了報告
58	感染症および寄生虫症	C型肝炎	日本	女	70	2007/9/3	⑤死亡	症例報告	当該製品	2007/12/4提出、識別番号1-07000095　取り下げ 第10回症例番号58は前回報告における第9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	67	2007/8/30	③未回復	症例報告	当該製品	2007/12/18提出、識別番号1-07000097　取り下げ 第10回症例番号59は前回報告における第9回症例番号6において報告したものの追加報告
60	感染症および寄生虫症	C型肝炎	日本	女	53	2007/8/28	②軽快	症例報告	当該製品	2007/11/20提出、識別番号1-07000104　未完了報告 第10回症例番号60は前回報告における第9回症例番号8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	B型肝炎	日本	男	73	2007/8/27	②軽快	症例報告	当該製品	2007/11/13提出、識別番号1-07000116　未完了報告
62	感染症および寄生虫症	B型肝炎	日本	男	73	2007/8/27	①回復	症例報告	当該製品	2008/2/1提出、識別番号1-07000116　取り下げ（61番と同一症例）
63	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
64	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（63番と同一症例）

MedDRA/J Ver. 11.0J

別紙様式第4

感染症発生症例一覧

155	65	感染症および寄生虫症	B型肝炎	日本	男	16	2007/7/23	③未回復	症例報告	当該製品	2007/10/31提出、識別番号1-07000106　未完了報告
	66	感染症および寄生虫症	B型肝炎	日本	男	16	2007/7/23	③未回復	症例報告	当該製品	2007/11/20提出、識別番号1-07000106　未完了報告（65番と同一症例）
	67	感染症および寄生虫症	B型肝炎	日本	男	16	2007/7/23	③未回復	症例報告	当該製品	2008/2/19提出、識別番号1-07000106　完了報告（65番、66番と同一症例）
	68	感染症および寄生虫症	B型肝炎	日本	女	52	2007/7/9	②軽快	症例報告	当該製品	2007/11/6提出、識別番号1-07000078　取り下げ 第10回症例番号68は前回報告における第9回症例番号18において報告したものの追加報告
	69	感染症および寄生虫症	B型肝炎	日本	男	62	2007/7/6	⑥不明	症例報告	当該製品	2007/11/6提出、識別番号1-07000064　取り下げ 第10回症例番号69は前回報告における第9回症例番号19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	B型肝炎	日本	男	58	2007/7/4	②軽快	症例報告	当該製品	2007/11/26提出、識別番号1-07000126　未完了報告
	71	感染症および寄生虫症	B型肝炎	日本	男	58	2007/7/4	③未回復	症例報告	当該製品	2008/2/6提出、識別番号1-07000126　未完了報告（70番と同一症例）
	72	感染症および寄生虫症	B型肝炎	日本	女	64	2007/6/13	③未回復	症例報告	当該製品	2007/11/6提出、識別番号1-07000055　取り下げ 第10回症例番号72は前回報告における第9回症例番号24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	C型肝炎	日本	男	84	2007/6/1	⑥不明	症例報告	当該製品	2007/12/18提出、識別番号1-07000053　取り下げ 第10回症例番号73は前回報告における第9回症例番号30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	B型肝炎	日本	男	73	2007/5/16	⑥不明	症例報告	当該製品	2007/12/4提出、識別番号1-07000029　取り下げ 第10回症例番号74は前回報告における第9回症例番号39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	女	82	2007/5/1	⑤死亡	症例報告	当該製品	2007/12/20提出、識別番号1-07000038　完了報告 第10回症例番号75は前回報告における第9回症例番号42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	B型肝炎	日本	女	71	2007/4/26	③未回復	症例報告	当該製品	2008/2/14提出、識別番号1-07000223　未完了報告
第9回	4	感染症および寄生虫症	C型肝炎	日本	女	70	2007/9/3	⑤死亡	症例報告	当該製品	2007/9/28 提出、識別番号 1-07000095 未完了報告
	6	感染症および寄生虫症	C型肝炎	日本	男	67	2007/8/30	③未回復	症例報告	当該製品	2007/10/9 提出、識別番号 1-07000097 未完了報告
	8	感染症および寄生虫症	C型肝炎	日本	女	53	2007/8/28	②軽快	症例報告	当該製品	2007/10/19 提出、識別番号 1-07000104 未完了報告
	18	感染症および寄生虫症	B型肝炎	日本	女	52	2007/7/9	③未回復	症例報告	当該製品	2007/8/16 提出、識別番号 1-07000078 未完了報告
	19	感染症および寄生虫症	B型肝炎	日本	男	62	2007/7/6	⑥不明	症例報告	当該製品	2007/7/25 提出、識別番号 1-07000064 未完了報告
	24	感染症および寄生虫症	B型肝炎	日本	女	64	2007/6/13	③未回復	症例報告	当該製品	2007/7/5 提出、識別番号 1-07000055 未完了報告
	30	感染症および寄生虫症	C型肝炎	日本	男	84	2007/6/1	③未回復	症例報告	当該製品	2007/7/5 提出、識別番号 1-07000053 未完了報告
	39	感染症および寄生虫症	B型肝炎	日本	男	73	2007/5/16	⑥不明	症例報告	当該製品	2007/5/30 提出、識別番号 1-07000029 未完了報告
	42	感染症および寄生虫症	B型肝炎	日本	女	82	2007/5/1	⑤死亡	症例報告	当該製品	2007/6/14 提出、識別番号 1-07000038 未完了報告

80103	2008/06/17	日本赤十字社	人赤血球濃厚液	人赤血球濃厚液

MedDRA/J Ver. 11.0J

別紙様式第4

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回	1	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

80106	2008/06/17	日本赤十字社	洗浄人赤血球浮遊液	洗浄人赤血球浮遊液

別紙様式第3

感染症の種類別発生状況

感染症の種類		前回調査期間終了時までの状況	当該調査期間	合計	外国製品による症例の内数	備考
器官別大分類	基本語					
感染症および寄生虫症	サイトメガロウイルス感染	1(0)	0(0)	1(0)	1	
感染症および寄生虫症	B型肝炎	2(0)	1(0)	3(0)	3	
感染症および寄生虫症	C型肝炎	18(0)	1(0)	19(0)	19	
感染症および寄生虫症	HIV感染	2(0)	0(0)	2(0)	2	
感染症および寄生虫症	ウイルス性肝炎	1(0)	0(0)	1(0)	1	
感染症および寄生虫症	伝染性紅斑	1(0)	0(0)	1(0)	1	
臨床検査	C型肝炎抗体陽性	3(0)	0(0)	3(0)	3	
臨床検査	C型肝炎RNA陽性	5(0)	0(0)	5(0)	5	
臨床検査	サイトメガロウイルス検査陽性	1(0)	0(0)	1(0)	1	
臨床検査	サイトメガロウイルス抗体陽性	2(0)	0(0)	2(0)	2	
臨床検査	B型肝炎表面抗原陽性	2(0)	0(0)	2(0)	2	
臨床検査	抗HBc抗体陽性	1(0)	0(0)	1(0)	1	
臨床検査	抗HBs抗体陽性	1(0)	0(0)	1(0)	1	
臨床検査	抗HBcIgG抗体陽性	1(0)	0(0)	1(0)	1	
臨床検査	抗HBe抗体陽性	1(0)	0(0)	1(0)	1	
臨床検査	C型肝炎陽性	3(0)	0(0)	3(0)	3	
臨床検査	ウイルス負荷	1(0)	0(0)	1(0)	1	

別紙様式第４　　感染症発生症例一覧　　別紙(5)

	番号	感染症の種類		発生国	性別	年齢	発現時期	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回	1	感染症および寄生虫症	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
	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
	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
	2	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
	2	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9回	報告なし										

MedDRA/J Ver.10.1

別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回	10-1	感染症および寄生虫症	C型肝炎	米国	男	43歳	1990	未回復	症例報告	当該製品	識別番号：07000020 報告日：2008年1月18日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎表面抗原陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎DNA測定値陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
第9回	該当なし										
第8回	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月9日 MedDRA: Version(9.1)
	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月27日 2007年4月9日に提出した症例番号8-1の追加報告 MedDRA: Version(9.1)
第7回	該当なし										

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６回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2006 年 2 月 6 日 第 6 回症例番号 5-1 は前回報告における第 5 回症例番号 5-1 において報告したものの取り下げ報告　MedDRA: Version(8.0)
	6-1	臨床検査	B 型肝炎抗原陽性	アメリカ	男	66 歳	2005/12/9	未回復	症例報告	当該製品	識別番号：05000495 報告日：2006 年 2 月 2 日 MedDRA: Version(8.1)
第５回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2005 年 8 月 18 日 MedDRA: Version(8.0)
第４回	該当なし										
第３回	3-1	臨床検査	C 型肝炎陽性	米国	男	14 歳	2001/11/30	不明	症例報告	当該製品	識別番号：04000072 報告日：2004 年 12 月 13 日 MedDRA: Version(7.1)
	3-2	臨床検査	C 型肝炎陽性	米国	男	10 歳	2002/9/11	不明	症例報告	当該製品	識別番号：04000073 報告日：2004 年 12 月 13 日 MedDRA: Version(7.1)
第２回	2-1	臨床検査	A 型肝炎抗体陽性	フランス	不明	50 歳	不明	不明	症例報告	当該製品	識別番号：03000021 報告日：2004 年 2 月 18 日 MedDRA: Version(6.1)

注）第１回は該当なし。

別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回	10-1	感染症および寄生虫症	C型肝炎	米国	男	43歳	1990	未回復	症例報告	当該製品	識別番号：07000020 報告日：2008年1月18日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎表面抗原陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎DNA測定値陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
第9回	該当なし										
第8回	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月9日 MedDRA: Version(9.1)
	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月27日 2007年4月9日に提出した症例番号8-1の追加報告 MedDRA: Version(9.1)
第7回	該当なし										

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６回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2006 年 2 月 6 日 第 6 回症例番号 5-1 は前回報告における第 5 回症例番号 5-1 において報告したものの取り下げ報告　MedDRA: Version (8.0)
	6-1	臨床検査	B 型肝炎抗原陽性	アメリカ	男	66 歳	2005/12/9	未回復	症例報告	当該製品	識別番号：05000495 報告日：2006 年 2 月 2 日 MedDRA: Version (8.1)
第５回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2005 年 8 月 18 日 MedDRA: Version (8.0)
第４回	該当なし										
第３回	3-1	臨床検査	C 型肝炎陽性	米国	男	14 歳	2001/11/30	不明	症例報告	当該製品	識別番号：04000072 報告日：2004 年 12 月 13 日 MedDRA: Version (7.1)
	3-2	臨床検査	C 型肝炎陽性	米国	男	10 歳	2002/9/11	不明	症例報告	当該製品	識別番号：04000073 報告日：2004 年 12 月 13 日 MedDRA: Version (7.1)
第２回	2-1	臨床検査	A 型肝炎抗体陽性	フランス	不明	50 歳	不明	不明	症例報告	当該製品	識別番号：03000021 報告日：2004 年 2 月 18 日 MedDRA: Version (6.1)

注）第１回は該当なし。

別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回	10-1	感染症および寄生虫症	C型肝炎	米国	男	43歳	1990	未回復	症例報告	当該製品	識別番号：07000020 報告日：2008年1月18日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎表面抗原陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎DNA測定値陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
第9回	該当なし										
第8回	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月9日 MedDRA: Version(9.1)
	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月27日 2007年4月9日に提出した症例番号8-1の追加報告 MedDRA: Version(9.1)
第7回	該当なし										

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６回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2006 年 2 月 6 日 第 6 回症例番号 5-1 は前回報告における第 5 回症例番号 5-1 において報告したものの取り下げ報告　MedDRA: Version (8.0)
	6-1	臨床検査	B 型肝炎抗原陽性	アメリカ	男	66 歳	2005/12/9	未回復	症例報告	当該製品	識別番号：05000495 報告日：2006 年 2 月 2 日 MedDRA: Version (8.1)
第５回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2005 年 8 月 18 日 MedDRA: Version (8.0)
第４回	該当なし										
第３回	3-1	臨床検査	C 型肝炎陽性	米国	男	14 歳	2001/11/30	不明	症例報告	当該製品	識別番号：04000072 報告日：2004 年 12 月 13 日 MedDRA: Version (7.1)
	3-2	臨床検査	C 型肝炎陽性	米国	男	10 歳	2002/9/11	不明	症例報告	当該製品	識別番号：04000073 報告日：2004 年 12 月 13 日 MedDRA: Version (7.1)
第２回	2-1	臨床検査	A 型肝炎抗体陽性	フランス	不明	50 歳	不明	不明	症例報告	当該製品	識別番号：03000021 報告日：2004 年 2 月 18 日 MedDRA: Version (6.1)

注）第１回は該当なし。

別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回	10-1	感染症および寄生虫症	C型肝炎	米国	男	43歳	1990	未回復	症例報告	当該製品	識別番号：07000020 報告日：2008年1月18日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎表面抗原陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎DNA測定値陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
第9回	該当なし										
第8回	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月9日 MedDRA: Version(9.1)
	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月27日 2007年4月9日に提出した症例番号8-1の追加報告 MedDRA: Version(9.1)
第7回	該当なし										

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６回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2006 年 2 月 6 日 第 6 回症例番号 5-1 は前回報告における第 5 回症例番号 5-1 において報告したものの取り下げ報告　MedDRA: Version (8.0)
	6-1	臨床検査	B 型肝炎抗原陽性	アメリカ	男	66 歳	2005/12/9	未回復	症例報告	当該製品	識別番号：05000495 報告日：2006 年 2 月 2 日 MedDRA: Version (8.1)
第５回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2005 年 8 月 18 日 MedDRA: Version (8.0)
第４回	該当なし										
第３回	3-1	臨床検査	C 型肝炎陽性	米国	男	14 歳	2001/11/30	不明	症例報告	当該製品	識別番号：04000072 報告日：2004 年 12 月 13 日 MedDRA: Version (7.1)
	3-2	臨床検査	C 型肝炎陽性	米国	男	10 歳	2002/9/11	不明	症例報告	当該製品	識別番号：04000073 報告日：2004 年 12 月 13 日 MedDRA: Version (7.1)
第２回	2-1	臨床検査	A 型肝炎抗体陽性	フランス	不明	50 歳	不明	不明	症例報告	当該製品	識別番号：03000021 報告日：2004 年 2 月 18 日 MedDRA: Version (6.1)

注）第１回は該当なし。

別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回	10-1	感染症および寄生虫症	C型肝炎	米国	男	43歳	1990	未回復	症例報告	当該製品	識別番号：07000020 報告日：2008年1月18日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎表面抗原陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎DNA測定値陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
第9回	該当なし										
第8回	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月9日 MedDRA: Version(9.1)
	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月27日 2007年4月9日に提出した症例番号8-1の追加報告 MedDRA: Version(9.1)
第7回	該当なし										

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６回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2006 年 2 月 6 日 第 6 回症例番号 5-1 は前回報告における第 5 回症例番号 5-1 において報告したものの取り下げ報告　MedDRA: Version(8.0)
	6-1	臨床検査	B 型肝炎抗原陽性	アメリカ	男	66 歳	2005/12/9	未回復	症例報告	当該製品	識別番号：05000495 報告日：2006 年 2 月 2 日 MedDRA: Version(8.1)
第５回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2005 年 8 月 18 日 MedDRA: Version(8.0)
第４回	該当なし										
第３回	3-1	臨床検査	C 型肝炎陽性	米国	男	14 歳	2001/11/30	不明	症例報告	当該製品	識別番号：04000072 報告日：2004 年 12 月 13 日 MedDRA: Version(7.1)
	3-2	臨床検査	C 型肝炎陽性	米国	男	10 歳	2002/9/11	不明	症例報告	当該製品	識別番号：04000073 報告日：2004 年 12 月 13 日 MedDRA: Version(7.1)
第２回	2-1	臨床検査	A 型肝炎抗体陽性	フランス	不明	50 歳	不明	不明	症例報告	当該製品	識別番号：03000021 報告日：2004 年 2 月 18 日 MedDRA: Version(6.1)

注）第１回は該当なし。

[redacted]

別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回	10-1	感染症および寄生虫症	C型肝炎	米国	男	43歳	1990	未回復	症例報告	当該製品	識別番号：07000020 報告日：2008年1月18日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎表面抗原陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎DNA測定値陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
第9回	該当なし										
第8回	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月9日 MedDRA: Version(9.1)
	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月27日 2007年4月9日に提出した症例番号8-1の追加報告 MedDRA: Version(9.1)
第7回	該当なし										

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６回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2006 年 2 月 6 日 第 6 回症例番号 5-1 は前回報告における第 5 回症例番号 5-1 において報告したものの取り下げ報告　MedDRA: Version(8.0)
	6-1	臨床検査	B 型肝炎抗原陽性	アメリカ	男	66 歳	2005/12/9	未回復	症例報告	当該製品	識別番号：05000495 報告日：2006 年 2 月 2 日 MedDRA: Version(8.1)
第５回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2005 年 8 月 18 日 MedDRA: Version(8.0)
第４回	該当なし										
第３回	3-1	臨床検査	C 型肝炎陽性	米国	男	14 歳	2001/11/30	不明	症例報告	当該製品	識別番号：04000072 報告日：2004 年 12 月 13 日 MedDRA: Version(7.1)
	3-2	臨床検査	C 型肝炎陽性	米国	男	10 歳	2002/9/11	不明	症例報告	当該製品	識別番号：04000073 報告日：2004 年 12 月 13 日 MedDRA: Version(7.1)
第２回	2-1	臨床検査	A 型肝炎抗体陽性	フランス	不明	50 歳	不明	不明	症例報告	当該製品	識別番号：03000021 報告日：2004 年 2 月 18 日 MedDRA: Version(6.1)

注）第１回は該当なし。

別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回	10-1	感染症および寄生虫症	C型肝炎	米国	男	43歳	1990	未回復	症例報告	当該製品	識別番号：07000020 報告日：2008年1月18日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎表面抗原陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎DNA測定値陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
第9回	該当なし										
第8回	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月9日 MedDRA: Version(9.1)
	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月27日 2007年4月9日に提出した症例番号8-1の追加報告 MedDRA: Version(9.1)
第7回	該当なし										

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６回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2006 年 2 月 6 日 第 6 回症例番号 5-1 は前回報告における第 5 回症例番号 5-1 において報告したものの取り下げ報告　MedDRA: Version (8.0)
	6-1	臨床検査	B 型肝炎抗原陽性	アメリカ	男	66 歳	2005/12/9	未回復	症例報告	当該製品	識別番号：05000495 報告日：2006 年 2 月 2 日 MedDRA: Version (8.1)
第５回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2005 年 8 月 18 日 MedDRA: Version (8.0)
第４回	該当なし										
第３回	3-1	臨床検査	C 型肝炎陽性	米国	男	14 歳	2001/11/30	不明	症例報告	当該製品	識別番号：04000072 報告日：2004 年 12 月 13 日 MedDRA: Version (7.1)
	3-2	臨床検査	C 型肝炎陽性	米国	男	10 歳	2002/9/11	不明	症例報告	当該製品	識別番号：04000073 報告日：2004 年 12 月 13 日 MedDRA: Version (7.1)
第２回	2-1	臨床検査	A 型肝炎抗体陽性	フランス	不明	50 歳	不明	不明	症例報告	当該製品	識別番号：03000021 報告日：2004 年 2 月 18 日 MedDRA: Version (6.1)

注）第 1 回は該当なし。

別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回	9-2	感染症および寄生虫症	Ｂ型肝炎	日本	女	26歳	2007/11/5	未回復	症例報告	当該製品	識別番号：07000143 報告日：2008年1月28日 第9回症例番号9-2において報告したもの（完了報告）の取り下げ報告 MedDRA: Version(10.1)
	10-1	感染症および寄生虫症	Ｃ型肝炎	日本	男	不明	1993	不明	症例報告	当該製品	識別番号：07000248 報告日：2008年3月10日 MedDRA: Version(10.1)
	10-1	感染症および寄生虫症	Ｃ型肝炎	日本	男	不明	1993	不明	症例報告	当該製品	識別番号：07000248 報告日：2008年3月21日 MedDRA: Version(10.1) 第10回　症例番号10-1（完了報告）の追加報告
第9回	9-1	感染症および寄生虫症	Ｃ型肝炎	日本	女	40歳代	不明	不明	症例報告	当該製品	識別番号：07000164 報告日：2007年12月28日 MedDRA: Version(10.1)
	9-2	感染症および寄生虫症	Ｂ型肝炎	日本	女	26歳	2007/11/5	未回復	症例報告	当該製品	識別番号：07000143 報告日：2007年12月13日 MedDRA: Version(10.1)
第8回	該当なし										
第7回	該当なし										

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６回	該当なし										
第５回	該当なし										
第４回	1-2	臨床検査	Ｃ型肝炎抗体陽性	日本	男	76 歳	2003/9/19	不明	症例報告	当該製品	登録番号：A03-120 報告日：2005 年 4 月 28 日 第 1 回症例番号 1-2 において報告したもの（未完了報告）の取り下げ報告 MedDRA: Version(7.1)
第３回	該当なし										
第２回	該当なし										
第１回	1-1	臨床検査	Ｂ型肝炎表面抗原陽性	日本	男性	72 歳	2003/7/18	不明	症例報告	当該製品	識別番号：A03-40 報告日：2003 年 9 月 5 日 MedDRA: Version(7.1)
	1-2	臨床検査	Ｃ型肝炎抗体陽性	日本	男性	76 歳	2003/9/19	不明	症例報告	当該製品	登録番号：A03-120 （未完了報告 報告日：2003 年 10 月 3 日） MedDRA: Version(7.1)

別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回	10-1	感染症および寄生虫症	C型肝炎	米国	男	43歳	1990	未回復	症例報告	当該製品	識別番号：07000020 報告日：2008年1月18日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎表面抗原陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
	10-2	臨床検査	B型肝炎DNA測定値陽性	韓国	男	不明	2008/3/8	未回復	症例報告	当該製品	識別番号：08000003 報告日：2008年4月21日 MedDRA: Version(10.1)
第9回	該当なし										
第8回	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月9日 MedDRA: Version(9.1)
	8-1	臨床検査	C型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	症例報告	当該製品	識別番号：07000005 報告日：2007年4月27日 2007年4月9日に提出した症例番号8-1の追加報告 MedDRA: Version(9.1)
第7回	該当なし										

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６回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2006 年 2 月 6 日 第 6 回症例番号 5-1 は前回報告における第 5 回症例番号 5-1 において報告したものの取り下げ報告　MedDRA: Version (8.0)
	6-1	臨床検査	B 型肝炎抗原陽性	アメリカ	男	66 歳	2005/12/9	未回復	症例報告	当該製品	識別番号：05000495 報告日：2006 年 2 月 2 日 MedDRA: Version (8.1)
第５回	5-1	臨床検査	HIV 検査陽性	韓国	男	5 歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2005 年 8 月 18 日 MedDRA: Version (8.0)
第４回	該当なし										
第３回	3-1	臨床検査	C 型肝炎陽性	米国	男	14 歳	2001/11/30	不明	症例報告	当該製品	識別番号：04000072 報告日：2004 年 12 月 13 日 MedDRA: Version (7.1)
	3-2	臨床検査	C 型肝炎陽性	米国	男	10 歳	2002/9/11	不明	症例報告	当該製品	識別番号：04000073 報告日：2004 年 12 月 13 日 MedDRA: Version (7.1)
第２回	2-1	臨床検査	A 型肝炎抗体陽性	フランス	不明	50 歳	不明	不明	症例報告	当該製品	識別番号：03000021 報告日：2004 年 2 月 18 日 MedDRA: Version (6.1)

注）第１回は該当なし。