

感染症定期報告に関する今後の対応について

平成16年度第5回
運営委員会確認事項
(平成16年9月17日)

1 基本的な方針

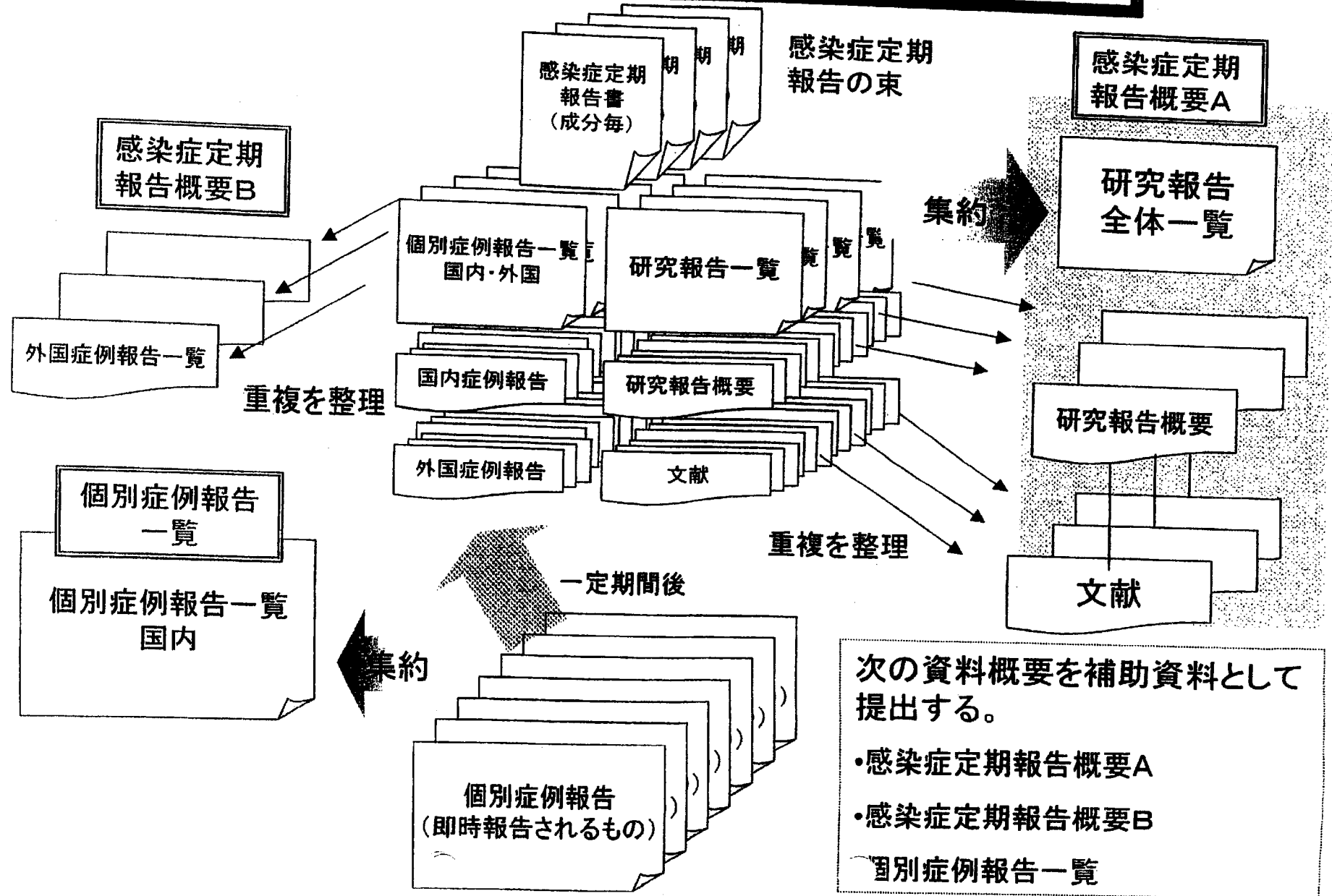
運営委員会に報告する資料においては、

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

2 具体的な方法

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



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抗体エスケープ変異株であるが、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感染と強く関連していた。	
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。	

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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、腎臓と脾臓の移植を受けた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切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。	

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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 α 2-6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関連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

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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。一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 Representative 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、チクングニヤウイルス(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、エンペロップ蛋白遺伝子の変異株(E1-A226V)が関係していた。この変異の、ネッタシマカおよびヒトシマカにおける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シマカがいない同地域でCHIKV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	バルボウイルス	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バルボウイルス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	バルボウイルス	Transfusion 2008; 48: 1036-1037	大阪における1997-1999年の献血者979052名中102名がヒトバルボウイルス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 10^4 IU/mL未満、約2年で 10^1 IU/mL未満まで下がること示された。	9
80105	2008/06/17	80248	バルボウイルス	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 ± 9 IU/mL(最小値11 IU/mL)のB19V IgG力価を含有し、これらの11 IU/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	バルボウイルス	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、ヒトバルボウイルス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	バルボウイルス	Vox Sanguinis 2007; 93: 341-347	過去30~35年間に製造された第VIII因子製剤中にヒトバ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シーケンスを含み、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 10^5 copies/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。	

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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 melitensis 2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	リンパ性脈絡髄膜炎	boston.com 2008年5月13日	2008年5月12日の保健当局発表によると、ボストンの病院で検出が難しいウイルスに感染したドナーから腎臓を移植された70歳女性が死亡し、57歳男性が危篤である。ドナーと患者2名の検体をCDCが検査したところ、全員、リンパ球性脈絡髄膜炎ウイルス(LCMV)陽性であり、ドナーからの伝播であったことが確認された。移植前にはエイズウイルス、肝炎ウイルスなどの検査は行ったが、LCMVの検査は行っていないかった。	12
80101	2008/06/06	80226	リンパ性脈絡髄膜炎	N Engl J Med 2008; 358: 991-998	オーストラリアで一人のドナーから臓器移植を受けた3例が移植後4-6週後に死亡した。他のいかなる方法でも原因不明であったが、2例のレシピエントの移植肝および腎から得られたRNAを偏りのない迅速シーケンシングで解析することにより、リンパ性脈絡髄膜炎に関係する新規のアレナウイルスが原因であることが明らかとなった。レシピエントの腎、肝、血液および脳脊髄液からこのウイルスが検出され、また免疫組織学的および血清学的に確認された。この方法は病原体発見の強力な手段である。	
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

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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で見られる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れないイムノプ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感染性が存在してい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感染性を有してい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は白血球を除きめつたに検出されない。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、スクレイビ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、スクレイビ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。	

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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、好氣的に22°C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

医薬品 研究報告 調査報告書

<p>識別番号・報告回数</p>			<p>報告日</p>	<p>第一報入手日 2008. 4. 15</p>	<p>新医薬品等の区分 該当なし</p>	<p>機構処理欄</p>
<p>一般的名称</p>	<p>人赤血球濃厚液</p>			<p>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.</p>	<p>公表国</p>	
<p>販売名(企業名)</p>	<p>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)</p>		<p>研究報告の公表状況</p>		<p>日本</p>	
<p>研究報告の概要</p>	<p>○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。</p>					<p>使用上の注意記載状況・ その他参考事項等</p>
	<p>赤血球濃厚液-LR「日赤」 照射赤血球濃厚液-LR「日赤」</p> <p>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>					
<p>報告企業の意見</p>			<p>今後の対応</p>			
<p>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。</p>			<p>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。</p>			



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

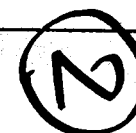
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【はじめに】日本赤十字社では、薬事法に基づき収集した副作用・感染症症例を独立行政法人医薬品医療機器総合機構へ報告している。2007年に全国の医療機関から報告された輸血関連感染症例(疑い例を含む)の現状とその解析結果について報告する。【対象と方法】2007年に医療機関から報告された症例を対象とし、ウイルス感染(疑)症例は当該献血者の保管検体の個別 NAT 等により、細菌感染(疑)症例は当該製剤(使用済みバッグ)又は同一製造番号の凍結血漿の無菌試験等により調査を行い評価した。【結果と考察】2007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養実施例は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、患者血培養陽性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 2008. 4. 15	新医薬品等の区分 該当なし	機構処理欄
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 日本	
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)				
研究報告の概要	<p>○HEV NAT陽性献血者におけるHEV感染のNatural course</p> <p>【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。</p> <p>【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。</p> <p>【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。</p> <p>【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。</p>				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			
<p>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。</p>		<p>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。</p>			



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

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【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 ヶ月間)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 抗体とも陰性のウィンドウ期の献血であり、献血時点から過去 6 ヶ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 ± 124 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

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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。				

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

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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 α 2–3-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 α 2–6-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 naive 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 α 2–3-linked SA, whereas human influenza viruses preferentially bind to α 2–6 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 α 2–6 SA-binding preference yet are thought to have originated from an avian virus possessing the α 2–3 SA-binding preference (15, 16). With few exceptions, avian H5N1 influenza viruses isolated from humans have maintained the classic α 2–3 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 α 2–6 SA glycans on upper respiratory tract epithelial cells and α 2–3 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 α 2–6 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-

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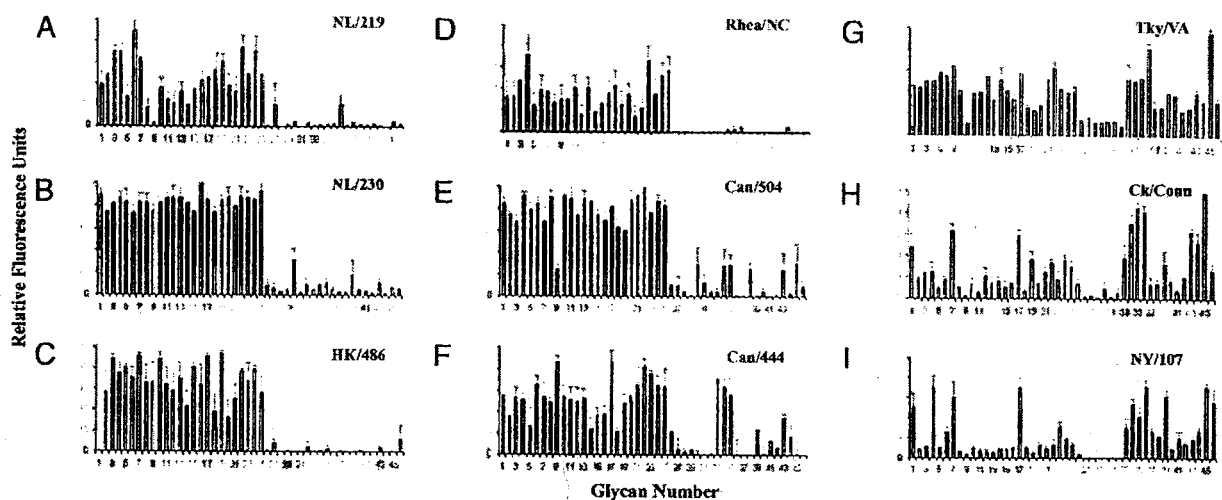


Fig. 1. Glycan microarray analysis of Eurasian and North American lineage H7 influenza viruses. Analysis was performed on the following viruses: NL/219 (A), NL/230 (B), HK/486 (H5N1) (C), Rhea/NC (D), Can/504 (E), Can/444 (F), Tky/VA (G), Ck/Conn (H), and NY/107 (I). The glycan microarray was performed by using whole virus with antisera raised against homologous or cross-reactive virus as a primary antibody. Colored bars highlight glycans that contain α 2-3 SA (yellow) and α 2-6 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 α 2-3 and α 2-6 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 α 2-3 SA (Fig. 1A and B). This pattern of binding closely resembles the strong α 2-3 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. 1C) (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 α 2-3- but not α 2-6-linked sialosides (Table 1). These findings suggest that HPAI H7N7 Eurasian lineage viruses, similar to HPAI H5N1 viruses, have maintained classic avian specificity for α 2-3 SA despite causing productive infections in humans.

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

Virus	Presence or absence of hemagglutination			
	TRBC	α 2-6 RBC	α 2-3 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 α 2-3 SA receptor-binding preference (Fig. 1D). 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 α 2-6 SA (Fig. 1G 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 α 2-6 SA binding and reduced binding to glycans with α 2-3 SA (Fig. 1I). 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. 1E and F), also revealed increased binding to α 2-6 SA compared with Eurasian lineage viruses (Fig. 1A–C). An assay using resialylated erythrocytes independently documented the dual α 2-6 and α 2-3 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 α 2-6 and α 2-3 glycans as summarized in Table 2. Although neither of the Eurasian viruses nor the Rhea/NC virus bound glycans with α 2-6 SA, all of the post-2002 North American viruses exhibited moderate to strong binding to the α 2-6 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 α 2-6 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 α 2-6 SA, they exhibited significant differences in their binding of glycans with α 2-3 SA. Tky/VA bound as well to glycans with α 2-3 SA as the Eurasian viruses. In contrast, Ck/Conn and NY/107 exhibited strong binding to only 4 of the 32 glycans with α 2-3 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 α 2-3 SA was significantly reduced, especially for NY/107. The reduced binding to glycans with α 2-3 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

Virus	α 2-3 sialosides						α 2-6 sialosides		
	Sulfated		Branched	Linear		Fucosylated	Branched	Linear	Internal
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 (-). \blacklozenge , NeuAc; \circ , Gal; \square , GalNAc; \bullet , Glc; \blacksquare , GlcNAc; \circ , Man; \blacktriangle , Fuc

Transmissibility of Eurasian H7 Influenza Viruses in Ferrets. To assess the impact of enhanced α 2-6 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 (EID₅₀), 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

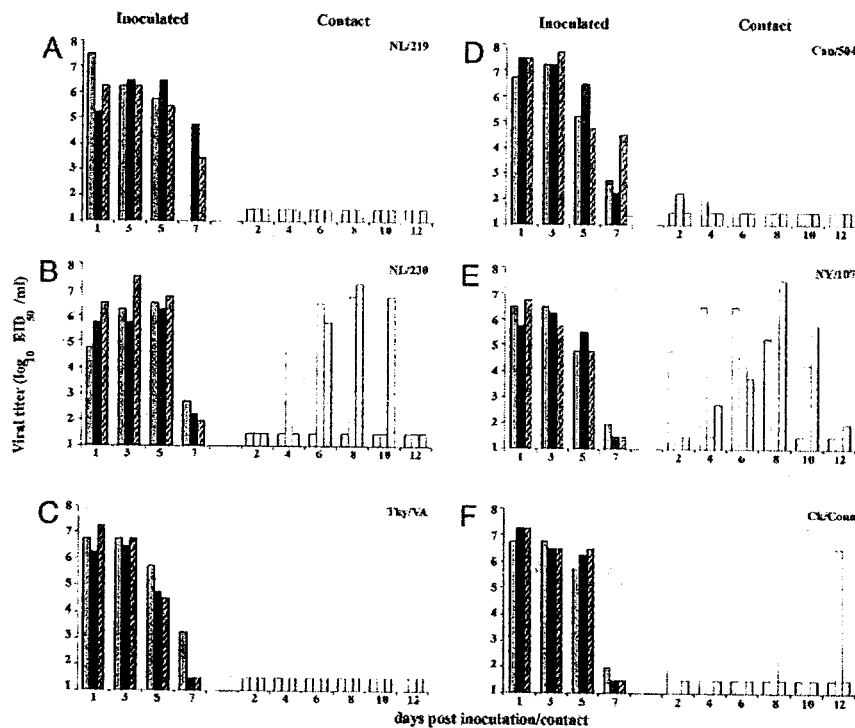


Fig. 2. Direct contact transmissibility of H7 influenza viruses. Three ferrets were inoculated with 10^7 EID₅₀ of NL/219 (A), NL/230 (B), Tky/VA (C), Can/504 (D), NY/107 (E), or Ck/Conn (F) virus, and nasal washes were collected from each ferret on the indicated days p.i. (dark bars). A naïve ferret was placed in the same cage with 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 $10^{1.5}$ EID₅₀/ml.

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

Virus	Subtype	No. of inoculated ferrets/total number				No. of contact ferrets/total number	
		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 $6.5 \pm 0.9 \log_{10}$ EID₅₀/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 $>10^{6.5}$ EID₅₀/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 resialylation 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 $6.75 \pm 0.5 \log_{10}$ EID₅₀/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 $7.4 \pm 0.3 \log_{10}$ EID₅₀/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 – $2.25 \log_{10}$ EID₅₀/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 $\alpha 2$ –6 SA binding with decreased binding to $\alpha 2$ –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 $6.3 \pm 0.5 \log_{10}$ EID₅₀/ml and $7.1 \pm 0.3 \log_{10}$ EID₅₀/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 $\geq 10^{5.25}$ EID₅₀/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 $10^{7.25}$ EID₅₀/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 $\alpha 2$ –3 SA receptors. Conversely, we observed an increase in $\alpha 2$ –6 binding among North American lineage H7 viruses isolated between 2002 and 2004. Several of these also showed reduced binding of $\alpha 2$ –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 α 2-6 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 α 2-6 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 α 2-6-binding preference (21, 22). Recent studies have highlighted increased complexity of the structural topology among α 2-3 and α 2-6 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 α 2-6 SA, corneal, conjunctival, and lacrimal duct epithelial cells of the human eye express predominantly α 2-3 SA (27, 44, 45). Additionally, the sialylated secretions (mucins) of both surfaces differ in their SA content; mucins in the airway epithelium contain α 2-3 SA, whereas ocular secreted mucins contain α 2-6 SA (46-48). The high α 2-3 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 transmissi-

bility, 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 α 2-6 SA binding along with decreased α 2-3 SA binding avidity. Strong α 2-6 SA binding appears to be an essential component of conferring transmissibility in human influenza viruses, because H1N1 variant viruses exhibiting the classic avian α 2-3-binding preference or dual α 2-3- and α 2-6-binding preference were unable to transmit efficiently (23). These results suggest that a decrease in α 2-3 SA binding may also be needed in addition to α 2-6 SA-binding avidity. However, despite similar α 2-3 and α 2-6 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 α 2-6 SA binding observed among these viruses. The classic avian specificity for α 2-3-linked SA observed with Rhea/NC could suggest a possible correlation between the acquisition of α 2-6 SA binding and the introduction of LPAI H7N2 viruses into the live bird markets of the northeastern U.S. The finding of enhanced α 2-6 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 embryonated hens' eggs as described (13). The 50% EID₅₀ 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/Rheal/NC/39482/93 (H7N1) (1:500) (for Rheal/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 stream 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 EID₅₀ 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.

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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。					使用上の注意記載状況・ その他参考事項等
	<p>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、ソウ、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。</p> <p>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。</p>					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		
<p>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。</p> <p>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。</p> <p>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。</p>				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。		



DISPATCHES

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 *Aphthovirus*, *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

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(LLC-MK2) cells. Cultures were incubated for 3 weeks at 37°C in 5% CO₂. 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,

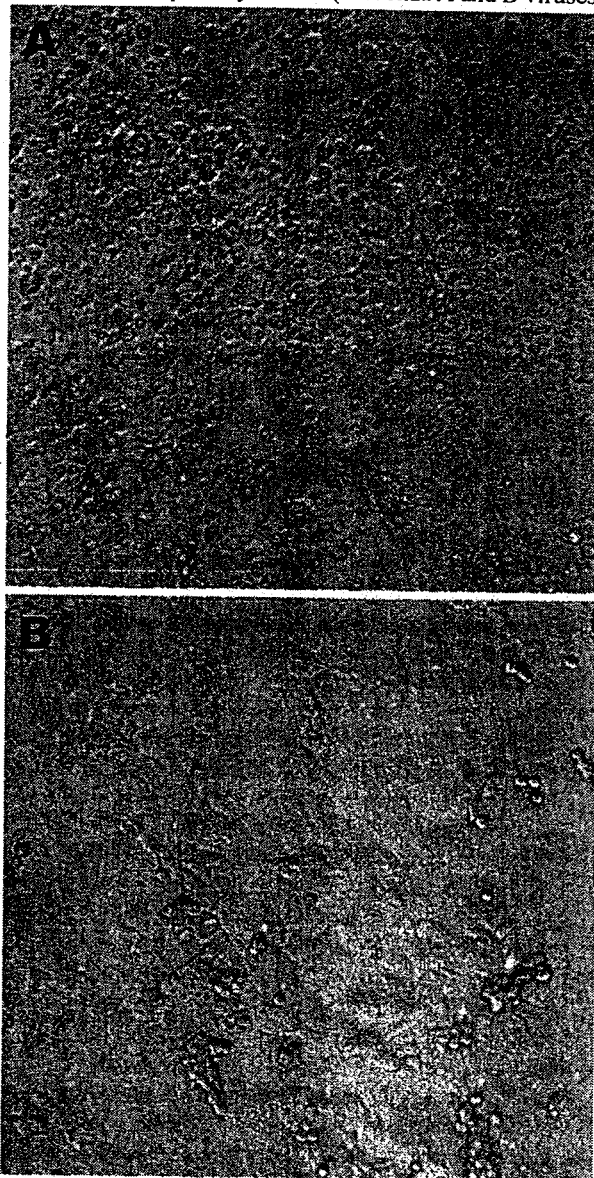


Figure 1. A) Cytopathic effects (round cells) observed 6 days after infection of rhesus monkey kidney (LLC-MK2) cells (second passage) with the Can112051-06 Saffold virus-like cardiovirus strain. B) Uninfected LLC-MK2 cells. Magnification $\times 10$.

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

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.

40 aa (residues 712–751 of the polyprotein), had 67.5% aa identity with the SAF-V counterpart (Figure 2, panel B).

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.

	EF Loop (I)	EF Loop (II)
SAF-V	PEPITSTSYVAHDSPPGDEPPVETETWVCSLRHSVDEKSCCTLRKPLALMKNQYR 59	
Can112051-06	PEFDTSRINATSEPKAVPTQMDTQHOSEKILQHSVDEKSCCTLRKPLALMKNQYR 59	
TMEV	PEFYTGKTKSGTNEPSSDFTMDTNRSPQSAITGVYVDMKNGE--FANNINGNW 59	
Theiler-like	PEFYTGKTFYGTTEBATFFFTKDTNQTQCNFVGVFRYDQRTGY--FALMKNQYR 59	
EMCV	PEYPT-----LDAPANDNRKGRDRALPAGTRIQTKKSP--FAMGKNQYR 59	
Mengovirus	PEYPT-----LDVFAKPKRASKDNLPNGIRIQTKKSP--FAMGKNQYR 59	

	CD Loop (I)	CD Loop (II)
SAF-V	LIPLPSTRLEKRRKFS----LDEQKRNLEFQSAFSSSEFVETKQD 40	
Can112051-06	LIPLPSTRLEKRRKFS----LDEQKRNLEFQSAFSSSEFVETKQD 40	
TMEV	LTPLPSS-YCPDSSSGPVHTKAPYQWHRVRSRGGANGNPIKMTKQD 44	
Theiler-like	LTPLPSS-YAPDSSSGPVTETQATFQNRHLRGTSGGDTTFPLMTKQD 44	
EMCV	LEPSPQDFAYDQLSP---QRLLTINRNGNEETSRVFLKSKQD 41	
Mengovirus	LEPSPQDFAYDQLSP---QRLLTINRNGNEETSRVFLKSKQD 41	

Figure 2. Comparison of amino acid sequences of the A) EF loop structure (part of the VP2 protein) and B) the CD loop structure (part of the VP1 protein) between Can112051-06 and other cardiioviruses 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 cardiioviruses 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 cardiiovirus-infected cells stained with an enterovirus antibody is uncertain because of the considerable difference between the capsid proteins of cardiioviruses 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 cardiioviruses 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.

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研究報告の概要	<p>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。</p>					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		
<p>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 のエンベロップを有する 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。</p>				<p>新規アレナウイルスに関する情報に注視する。</p>		

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

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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.

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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 Junin, 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.

In 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, transferrin 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 Junin, 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 Junin, 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 RNeasy Kit following the manufacturer's recommendations (Qiogene Inc., Carlsbad, CA), and the extracted RNA was reconstituted in 50 μ L H₂O. Broadly reactive Arenavirus primers used for initial identification

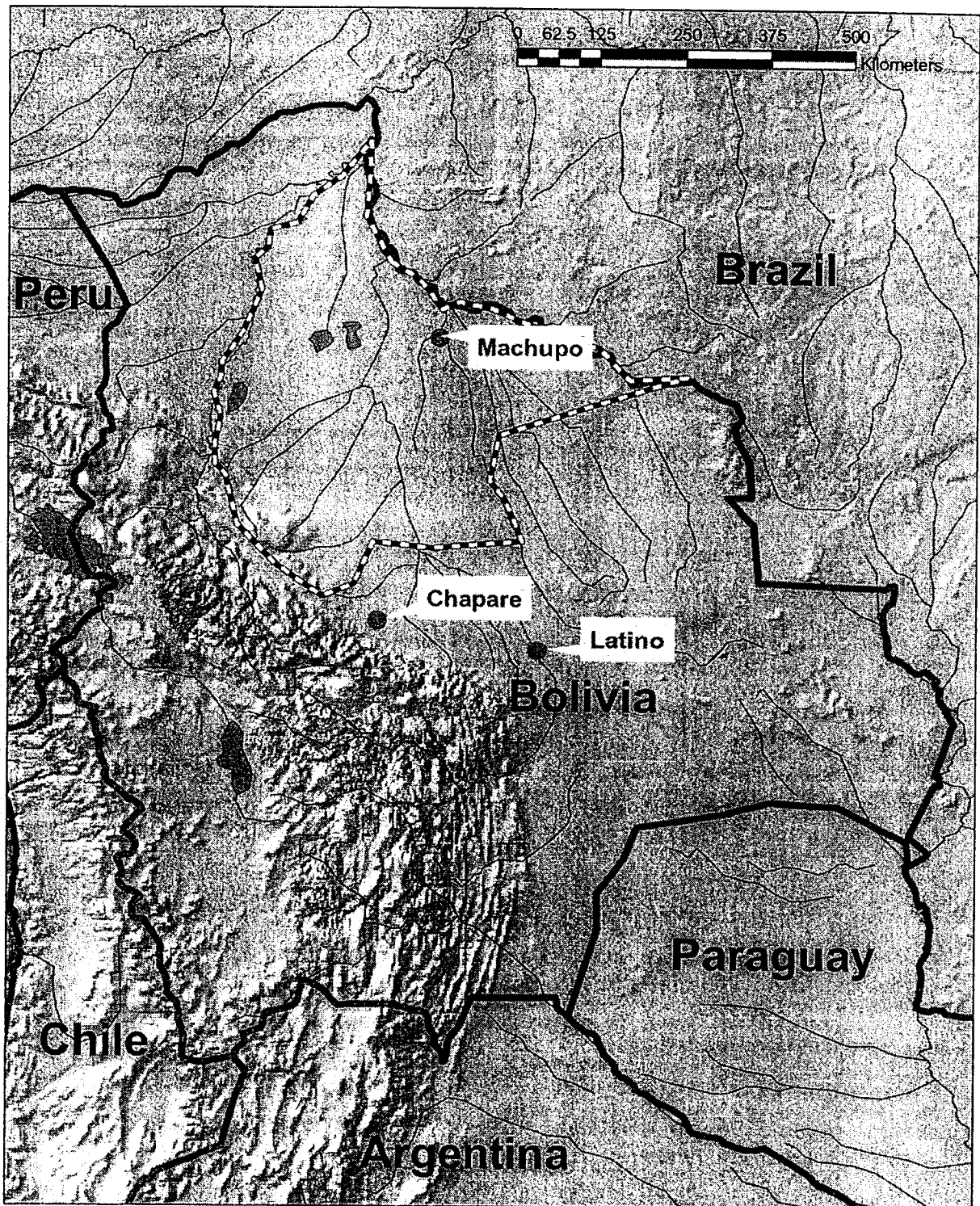


Figure 1. Map of Bolivia showing location of the Chapare virus-associated HF case relative to the Beni region where Machupo virus-associated HF cases originate. The Beni Department boundary is depicted by the checkered line. Multiple Machupo isolates have been recorded from the Beni Department. The single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

^aComplete nucleotide segments only

^bComplete amino acid sequences only

^cComplete segment or gene sequence is not available for one or both viruses
doi:10.1371/journal.ppat.1000047.t001

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 Qjagen Gel Extraction Kit (Qjagen, 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

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

^aComplete nucleotide segments only

^bComplete amino acid sequences only

^cComplete segment or gene sequence is not available for more than one strain
doi:10.1371/journal.ppat.1000047.t002

primers, dNTPs and Platinum Taq DNA polymerase High Fidelity (Invitrogen) in 50 µl 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 (Qjagen). 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)

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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

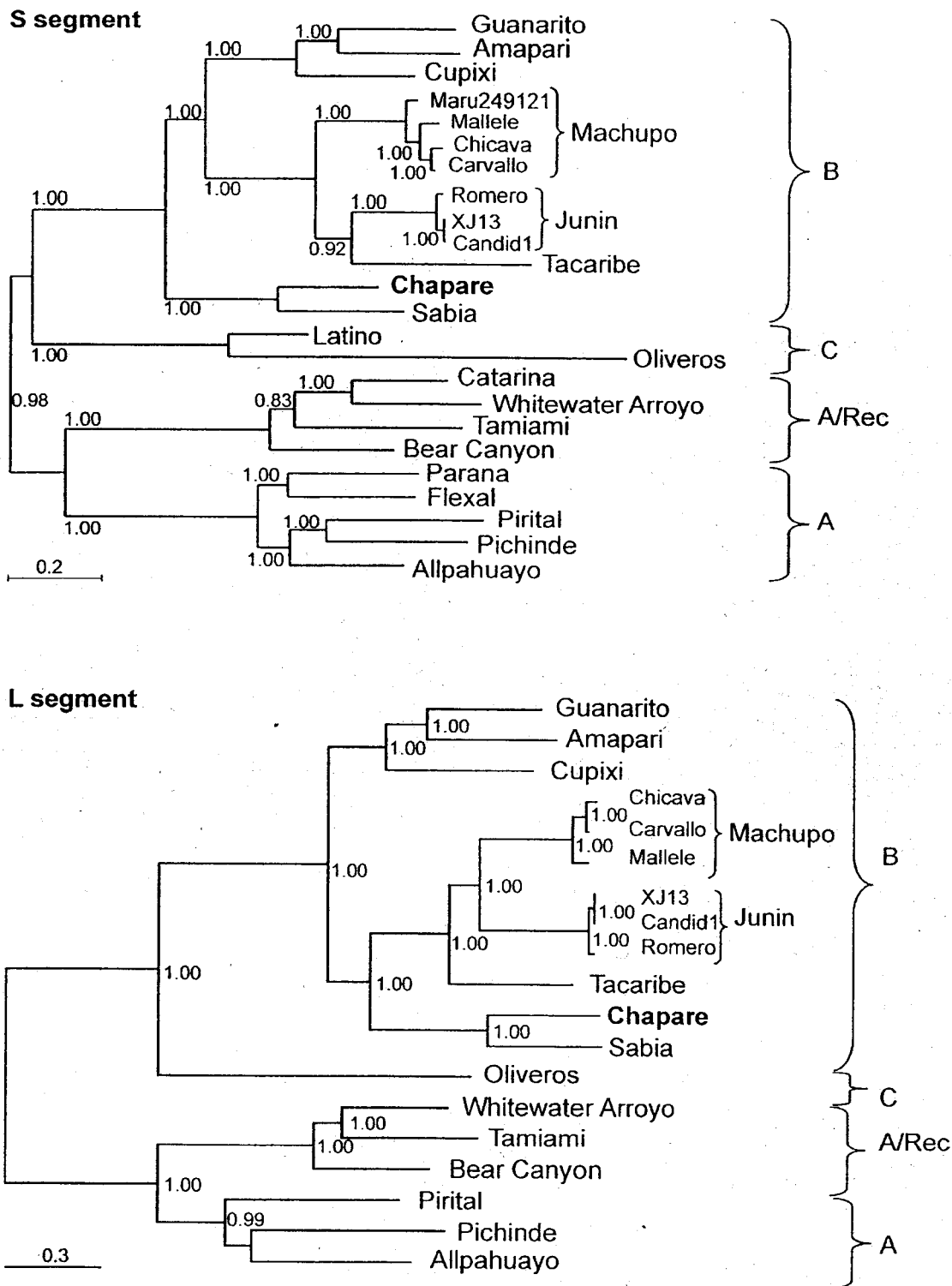


Figure 2. Phylogenetic analysis of the complete S and L RNA segments of New World arenaviruses. Complete S and L segments for New World arenaviruses were analyzed by Bayesian inference of phylogeny (MrBayes3.1.2) using the sequence of Pichindé virus as the outgroup. Multiple strains are grouped with 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), Piritál (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

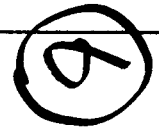
investigation: PB. Participated in case investigation: JV. Edited the paper: TK. Led NMRCD investigation: JO.

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医薬品 研究報告 調査報告書

<p>識別番号・報告回数</p>			<p>報告日</p>	<p>第一報入手日 2008. 5. 26</p>	<p>新医薬品等の区分 該当なし</p>	<p>機構処理欄</p>
<p>一般的名称</p>	<p>(製造承認書に記載なし)</p>			<p>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.</p>	<p>公表国</p>	
<p>販売名(企業名)</p>	<p>合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社)</p>		<p>研究報告の公表状況</p>		<p>ベルギー</p>	
<p>研究報告の概要</p>	<p>○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。</p>					<p>使用上の注意記載状況・ その他参考事項等</p> <p>合成血「日赤」 照射合成血「日赤」</p> <p>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>
<p>報告企業の意見</p>			<p>今後の対応</p>			
<p>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。</p>			<p>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。</p>			



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

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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 (\geq 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.

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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 $150 \mu\text{M}$) 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 ($22 \pm 2 \text{ }^\circ\text{C}$) 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 (<i>n</i> = 1400)	Transfusion characteristics (<i>n</i> = 7437)
Gender (<i>n</i> , %)		
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%)

^aFor 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	SAE ^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%)	-

^aSerious adverse event (SAE): long-term life threatening, immediate life threatening or death.

^bCausal relationship that was possibly related, probably related, or related to PCT-PLT transfusion.

^cNumber 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, cephalgia, 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 comorbidities 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.

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医薬品
医薬部外品 研究報告 調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 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	<p>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。</p> <p>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%を占めた。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。</p> <p>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。</p> <p>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、気づかれませんが、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。</p>					使用上の注意記載状況・その他参考事項等
	<p>報告企業の意見</p> <p>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。</p>					
<p>今後の対応</p> <p>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。</p>						

7

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.

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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 km² 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 km². 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 km² with one of the highest population densities in Mainland China.

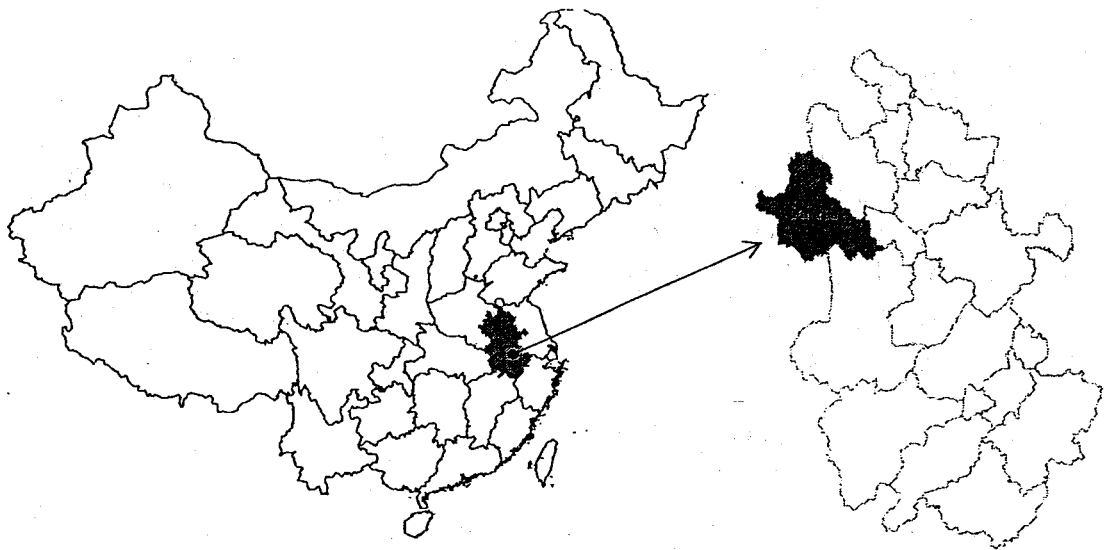


Figure 1. Geographic location of Fuyang City, Anhui Province in China.

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 rate¹ 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).

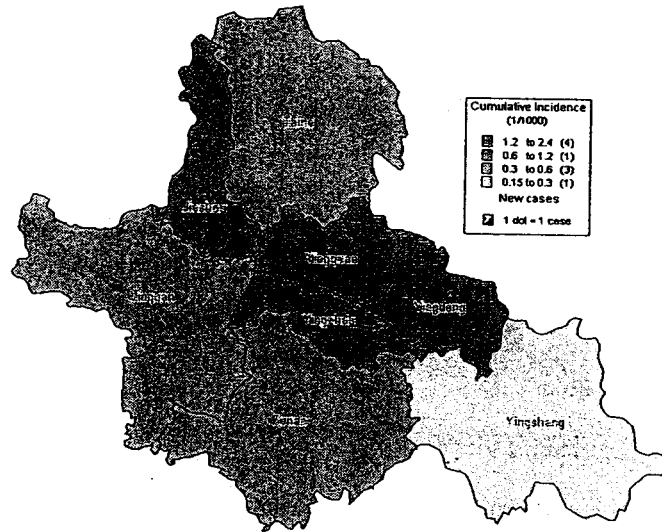


Figure 2. Incidence rate by districts/counties of Fuyang City, from March 1 and May 9, 2008

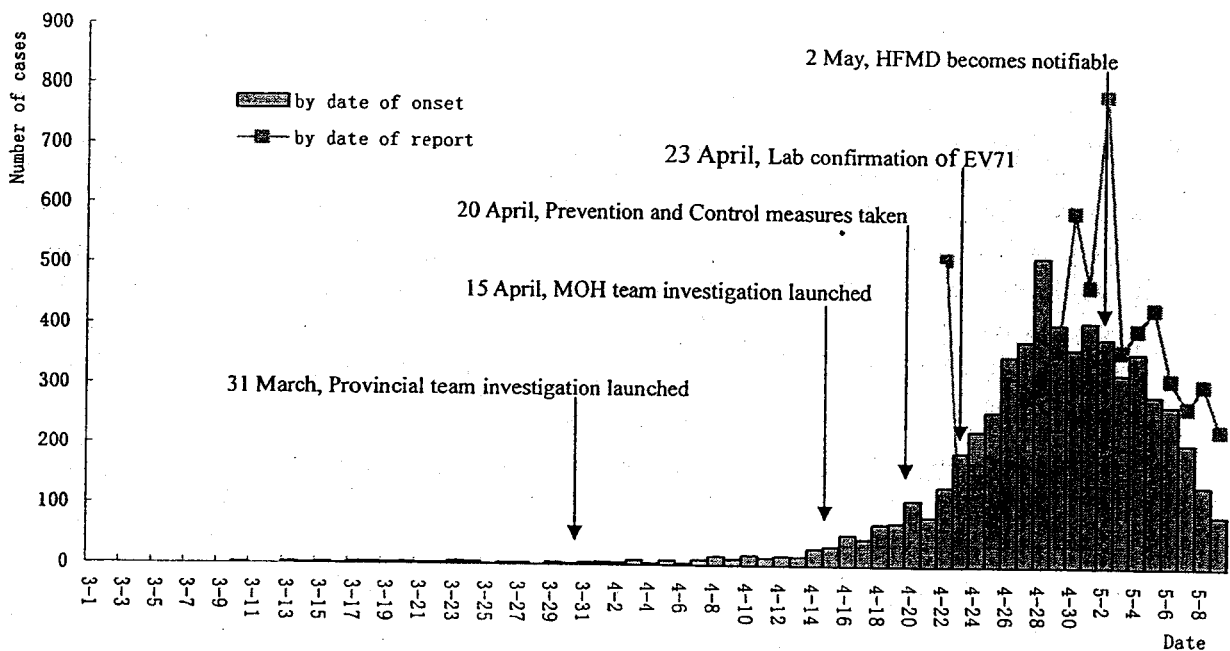


Figure 3. The number of HFMD cases by date of onset and date of reporting from March 1 to May 9, 2008 in Fuyang City

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.

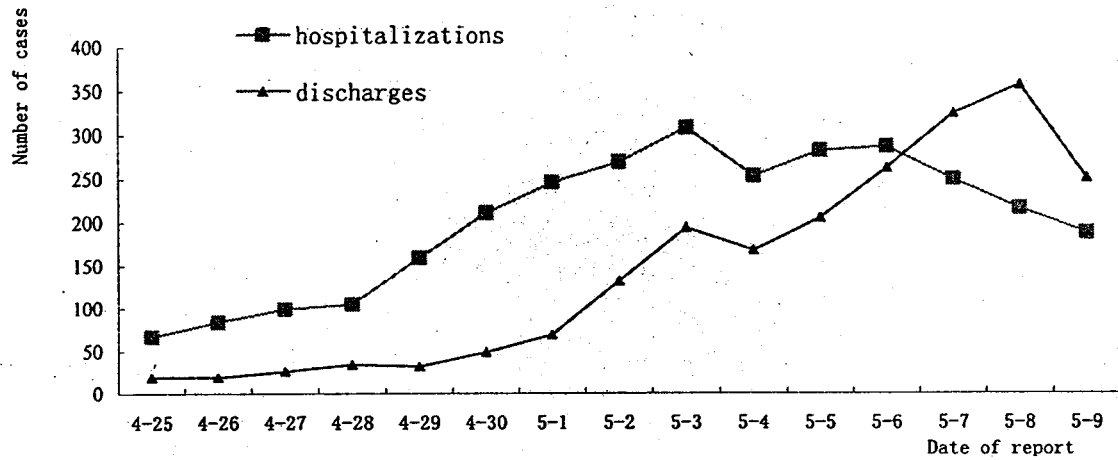


Figure 4. Daily number of hospitalizations and discharges of HFMD cases from April 25 to May 9 in Fuyang City.

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

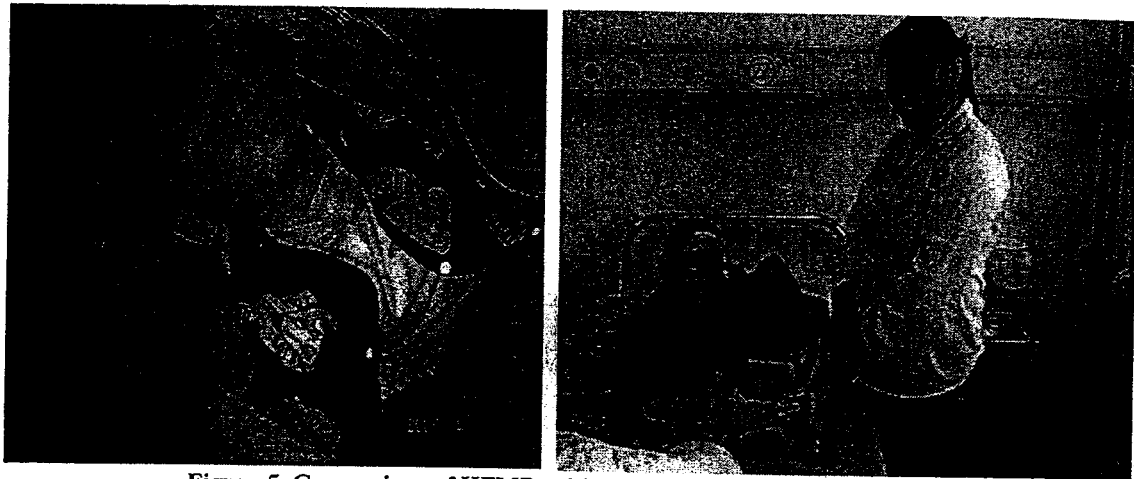


Figure 5. Comparison of HFMD critical case prior (left) and after (right) medical treatment was provided at Fuyang No.2 hospital.

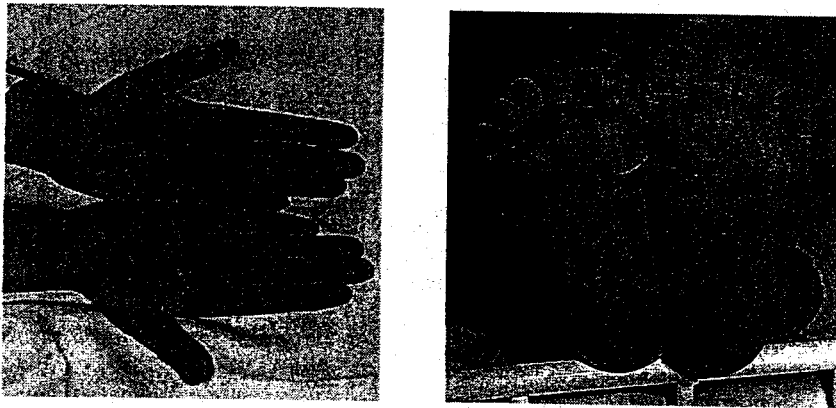


Figure 6. The rash of HFMD cases (on hand and foot)

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%), Linqun (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.

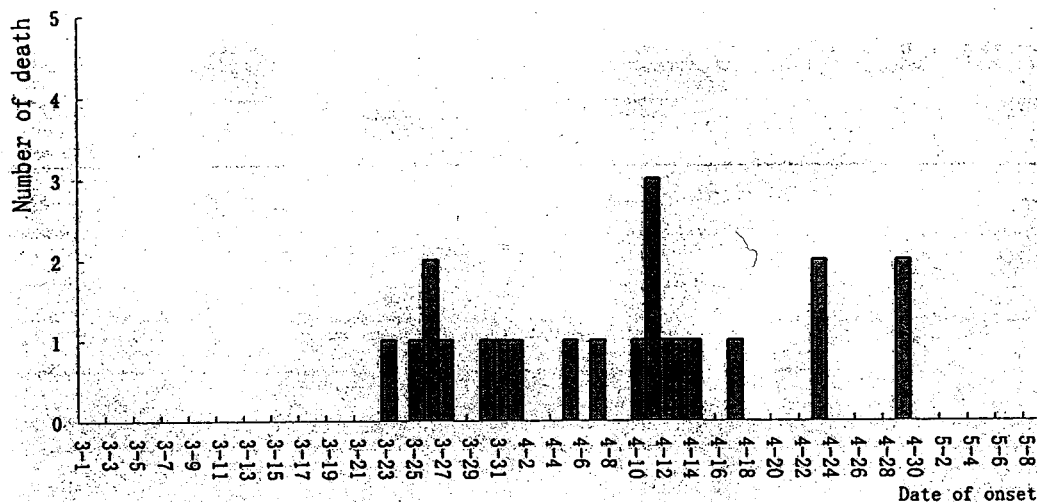


Figure 7. Fatal cases (n=22) by date of onset from March 23 to April 29 in Fuyang City.

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.

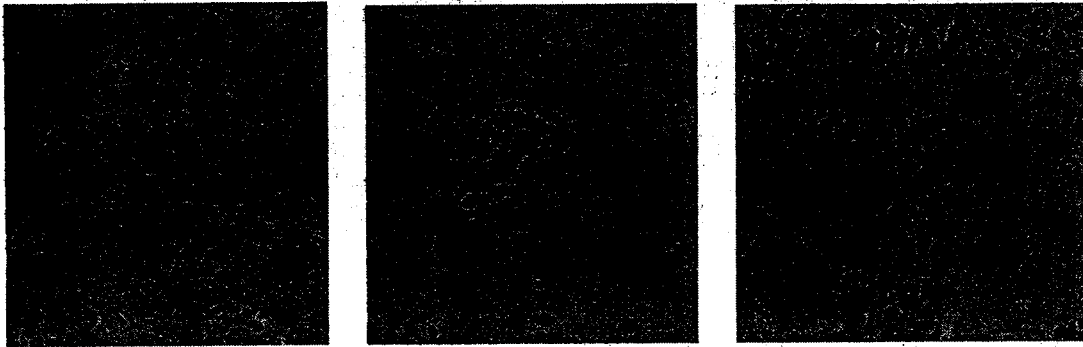


Figure 8. Left: brain tissue; Middle: spinal cord; Right: bronchus.

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.

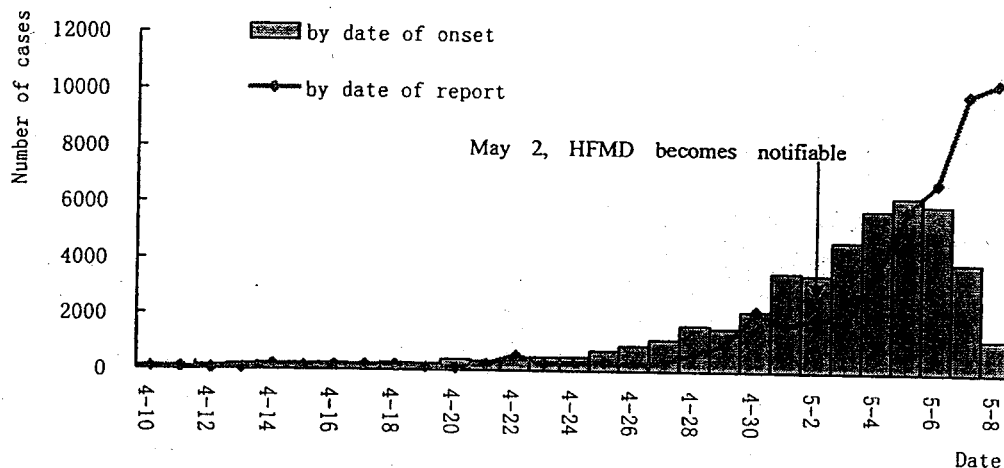


Figure 9. The number of HFMD cases by date of onset and date of reporting from April 10 to May 9, 2008 in China.

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).

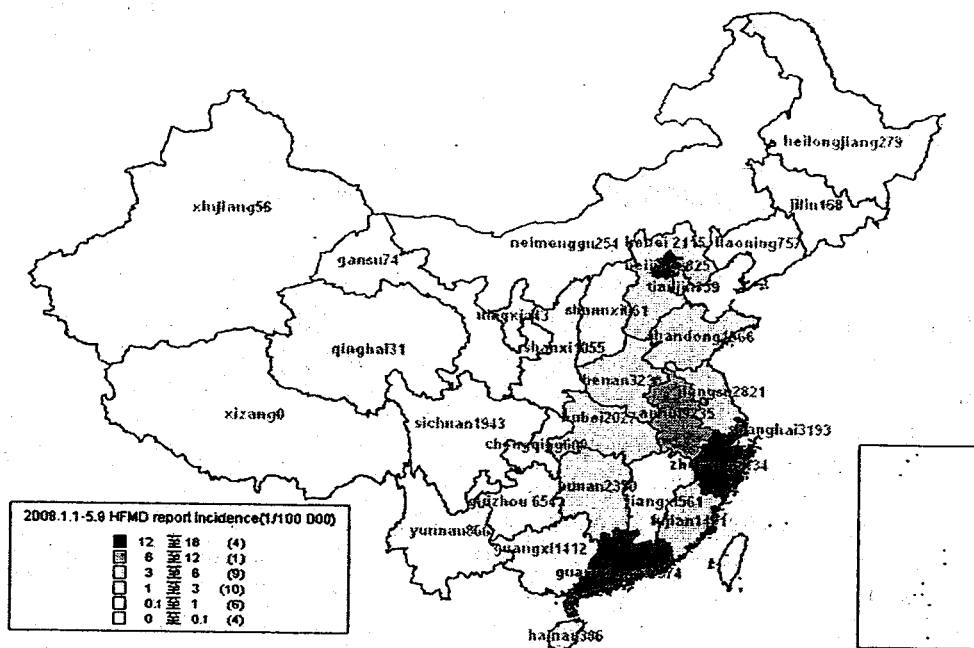


Figure 10 Incidence rate of HFMD cases by province in China from Jan 1 to May 9, 2008

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.

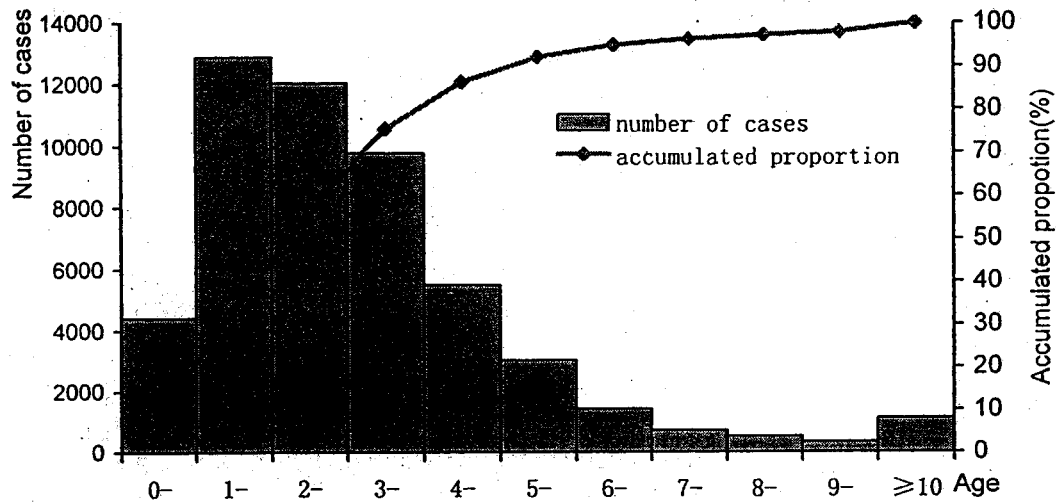


Figure 11. HFMD cases by age in China from January 1 to d May 9, 2008

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/pneumorrhagia, 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.

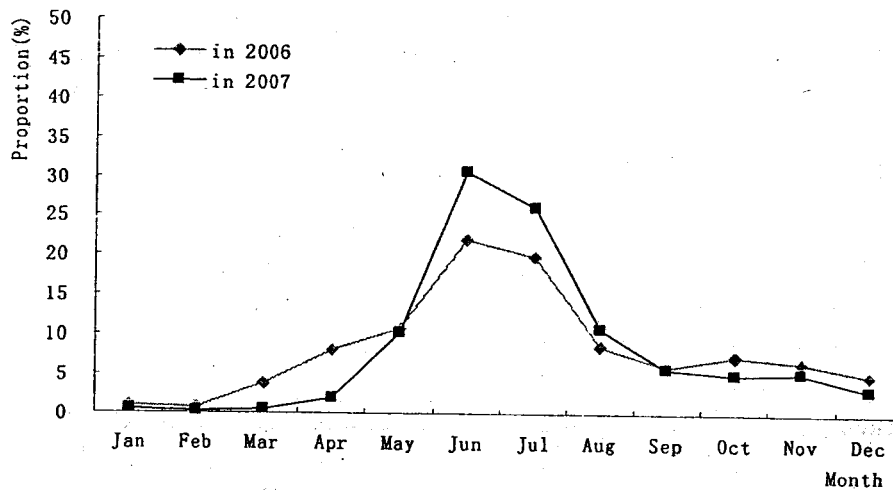


Figure 12 The distribution of HFMD cases by month in 2006 and 2007

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 Minister 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 GenBank³ 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 identified in 1969. It is reported that the clinical manifestations of EV71 infection varies from classical HFMD symptoms to herpangina, 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 region⁴. 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 fatal⁵. 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 Singapore⁶ and Vietnam⁷ while the disease has also been reported in Malaysia⁸.

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/

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			Blood 110 (11, Part 1): p853A NOV 16 2007	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)	研究報告の公表状況	The risk of transfusion-transmitted babesiosis due to Babesia microti in Connecticut			使用上の注意記載状況・ その他参考事項等
研究報告の概要	<p>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ビ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 ヶ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。全てのレシビエントの検体で、IFA を用いて Bm に対する抗体を測定した。 レシビエントの検体の IFA が陽性的の場合、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ビエントの保存 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 施設のラゴ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。 そのレシビ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 感染の可能性はあるが、そのレシビ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。</p>					
	報告企業の意見	今後の対応				
	<p>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。</p>					<p>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。</p>



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.

医薬品 研究報告 調査報告書

<p>識別番号・報告回数</p>			<p>報告日</p>	<p>第一報入手日 2008. 5. 26</p>	<p>新医薬品等の区分 該当なし</p>	<p>機構処理欄</p>
<p>一般的名称</p>	<p>(製造承認書に記載なし)</p>		<p>研究報告の公表状況</p>	<p>Matsukura H, Shibata S, Tani Y, Shibata H, Furuta RA. Transfusion. 2008 May;48(5):1036-7.</p>	<p>公表国</p>	
<p>販売名(企業名)</p>	<p>合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社)</p>				<p>日本</p>	
<p>研究報告の概要</p>	<p>○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10⁴IU/mL未満、約2年で10²IU/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裏証するために、より大規模な研究が望まれる。</p>					<p>使用上の注意記載状況・ その他参考事項等</p>
	<p>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</p>					
<p>報告企業の意見</p>			<p>今後の対応</p>			
<p>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10⁴IU/mL未満、約2年で10²IU/mL未満まで下がることを示されたとの報告である。</p>			<p>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。</p>			

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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 Candotti¹). Large cross-sectional studies using highly sensitive DNA amplification methods have also demonstrated persistent B19 infection.² Recently, Lefere and colleagues³ 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.

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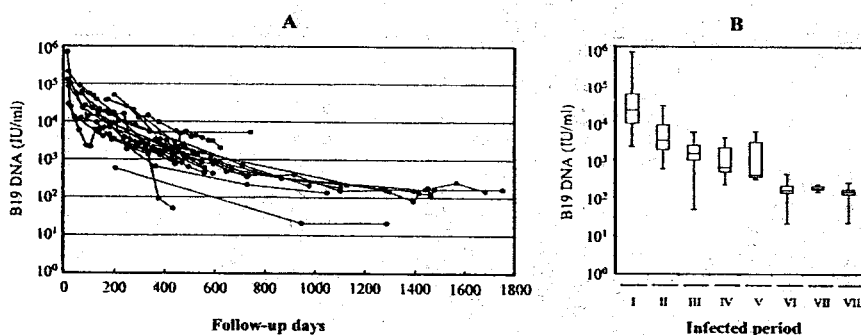


Fig. 1. (A) Changes in plasma B19 viral load in healthy blood donors after an acute B19 infection. Each line represents plasma B19 DNA of the same donor. Time 0 was defined as the first test visit when positive results were obtained for B19 antigen (high viremic phase). (B) Plasma B19 viral loads for 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).

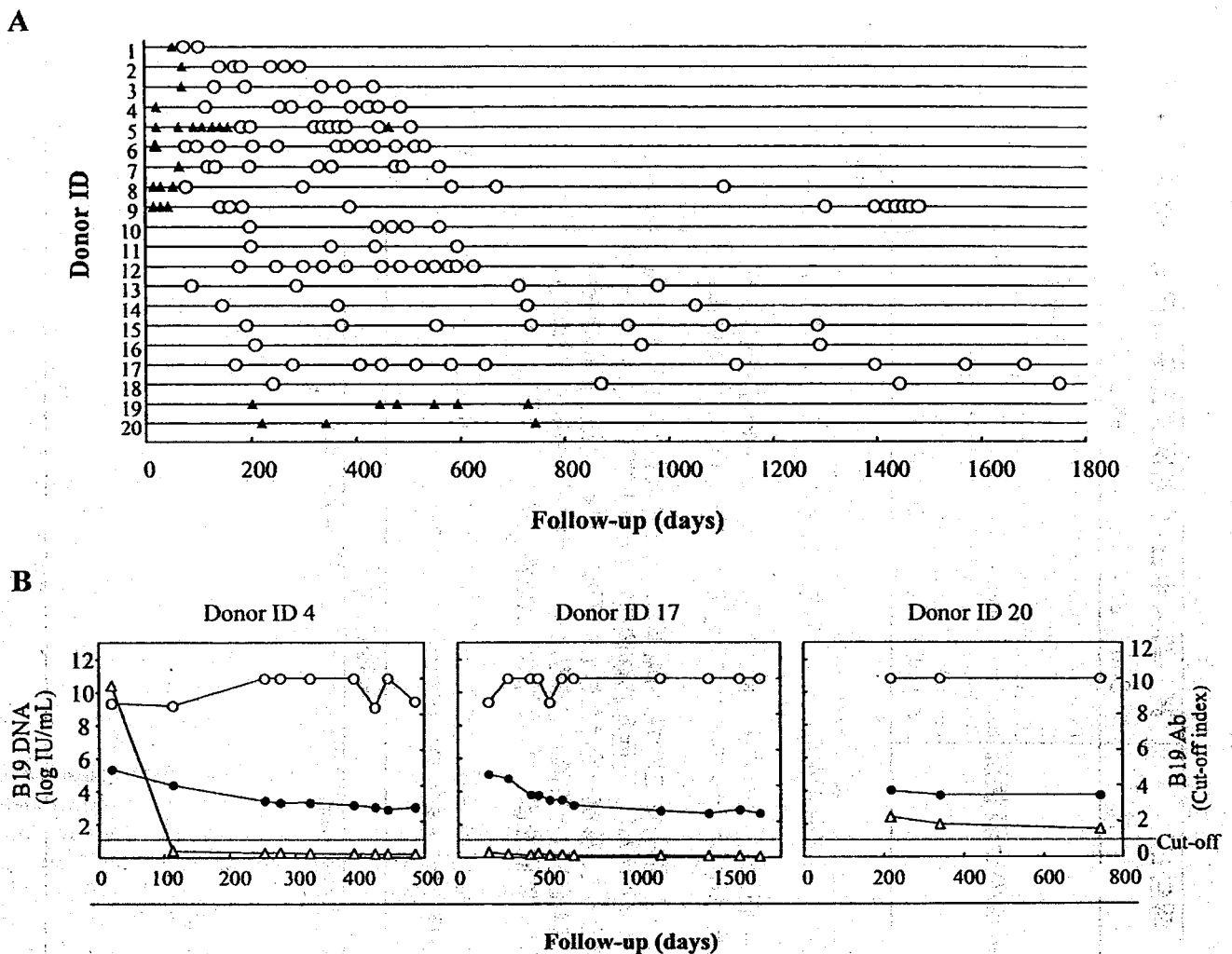


Fig. 2. (A) B19 IgM and IgG for individual donors at follow-up visits. (▲) Positive for both IgM and IgG; (○) positive for IgG. (B) Representative cases for three patterns of test results. Changes in viral load (●), IgM (Δ), and IgG (○). Donors correspond to those in A.

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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