# 薬事・食品衛生審議会
# 平成２０年度　第４回　血液事業部会運営委員会

## 議 事 次 第

日時：平成２１年２月１０日（火）
１５：００～１７：００
場所：財団法人日本教育会館（９Ｆ）
喜山倶楽部「平安の間」
東京都千代田区一ツ橋２－６－２

議題：

１．議事要旨の確認
２．感染症定期報告について
３．血液製剤に関する報告事項について
４．日本赤十字社からの報告事項について
５．その他

配付資料：

資　料　１　平成２０年度第３回血液事業部会運営委員会議事要旨（案）
資　料　２　感染症定期報告について
資料３－１　供血者からの遡及調査の進捗状況について
資料３－２　血液製剤に関する報告事項について
資料３－３　献血件数及びＨＩＶ抗体・核酸増幅検査陽性件数
資　料　４　血液凝固第Ⅷ因子製剤及び第Ⅸ因子製剤におけるインヒビターに係る対応について
資料５－１　輸血療法の実施に関する指針の一部改正（案）について
資料５－２　血液製剤の使用指針の一部改正（案）について
資料６－１　車検満了日を経過した移動採血車を運行した事例について（日本赤十字社提出資料）
資料６－２　献血者健康被害救済制度の運用状況について（日本赤十字社提出資料）
資料６－３　問診票改訂（案）について（日本赤十字社提出資料）

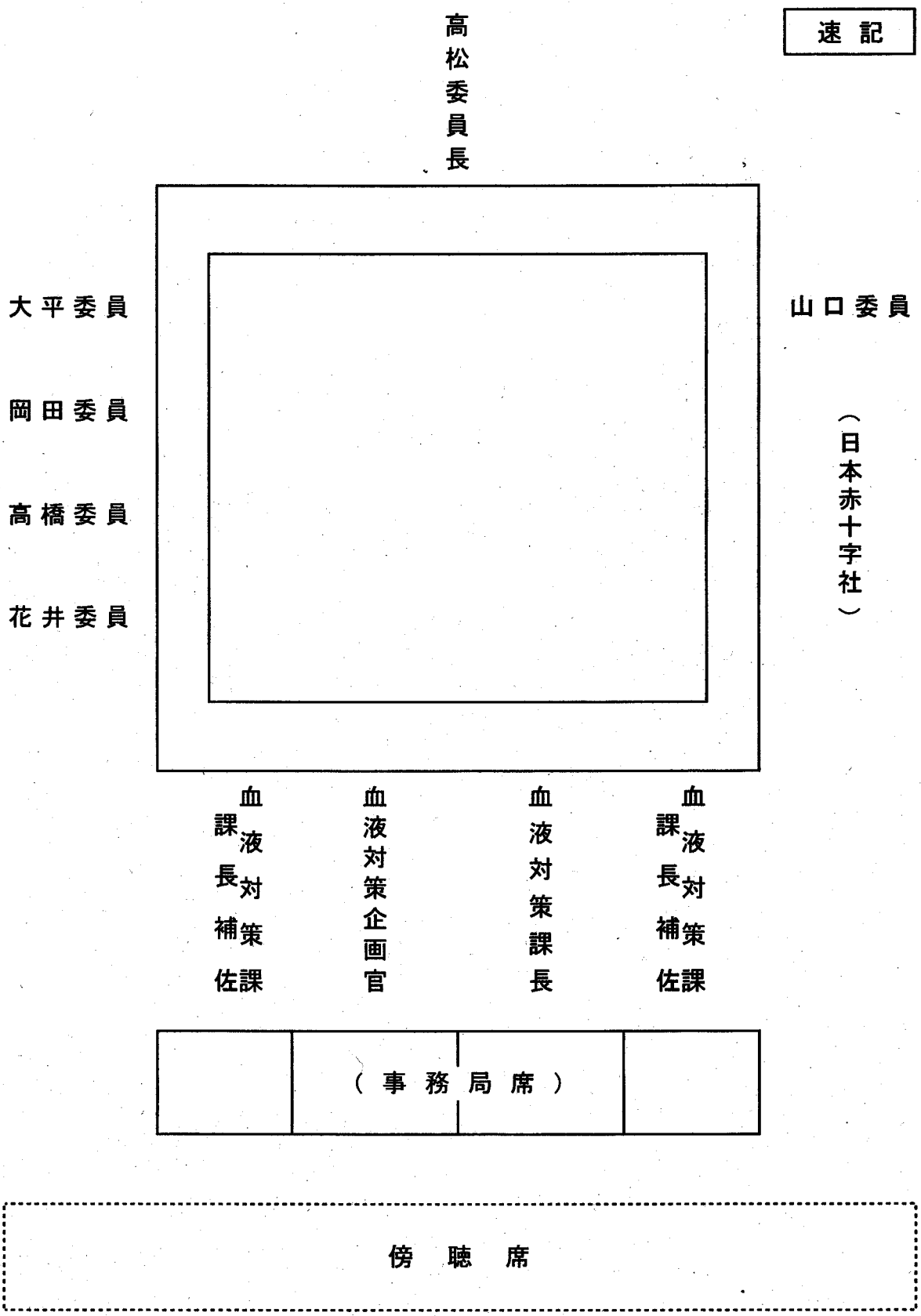
資料６－４　ＣＬＥＩＡ法の評価について（日本赤十字社提出資料）
資　料　７　注射用ノボセブン１．２ｍｇ及び同４．８ｍｇの不採算品再算定について
資料８－１　フィブリノゲン製剤納入先医療機関の追加調査について（平成２１年１月３０日公表）
資料８－２　フィブリノゲン製剤等に関する相談窓口について（平成２１年１月３０日公表）
資料８－３　Ｃ型肝炎訴訟の和解について（平成２１年２月５日公表）
資料８－４　田辺三菱製薬株式会社等における個人情報の開示請求への対応等について（平成２１年１月２８日公表）
資料８－５　田辺三菱製薬株式会社におけるフィブリノゲン製剤に係る４１８症例報告調査プロジェクトチームの活動状況等の報告について（平成２１年１月２７日公表）
資料８－６　国立病院訪問調査について（平成２０年１２月２６日公表）
資料８－７　血液凝固因子製剤の納入先医療機関の調査について（平成２０年１１月２８.日公表）

参考資料　「血液製剤等に係る遡及調査ガイドライン」（平成２０年１２月２６日付け一部改正）

# 平成20年度第4回
# 薬事・食品衛生審議会薬事分科会
# 血液事業部会運営委員会
# 座席表

平成21年2月10日(火)
財団法人日本教育会館(9F)
喜山倶楽部「平安の間」
15:00～17:00

速記



# 薬事・食品衛生審議会薬事分科会
# 血液事業部会運営委員会 委員名簿

1．大平 勝美 （おおひら　かつみ）
　　はばたき福祉事業団理事長

2．岡田 義昭 （おかだ　よしあき）
　　国立感染症研究所血液・安全性研究部第一室長

3．髙橋 孝喜 （たかはし　こうき）
　　日本輸血・細胞治療学会副理事長
　　（東京大学医学部附属病院輸血部教授）

4．高松 純樹 （たかまつ　じゅんき）
　　名古屋大学医学部附属病院輸血部教授

5．花井 十伍 （はない　じゅうご）
　　ネットワーク医療と人権理事

6．山口 照英 （やまぐち　てるひで）
　　国立医薬品食品衛生研究所生物薬品部長

（50音順、敬称略）

資料１

# 平成20年度第3回血液事業部会運営委員会議事要旨

日時: 平成20年10月29日(水) 10:00～12:15
場所: 財団法人日本教育会館(9F)喜山倶楽部「平安の間」
出席者:高松委員長、
大平、岡田、髙橋、花井、山口各委員
(事務局)
新村血液対策課長、林血液対策企画官、齋藤課長補佐、秋野課長補佐他
(採血事業者)
日本赤十字社血液事業本部 田所経営会議委員、日野副本部長

議 題: 1. 議事要旨の確認
2. 感染症定期報告について
3. 血液製剤に関する報告事項について
4. 日本赤十字社からの報告事項について
5. その他

(審議概要)

<u>議題1について</u>

議事要旨に関する意見等については、事務局まで連絡することとされた。

<u>議題2について</u>

感染症定期報告について、事務局から説明後、質疑応答がなされた。

<u>議題3について</u>

事務局及び日赤から、供血者からの遡及調査の進捗状況及び新しい感染症検査法の判定と陽性血液にかかる当面の取り扱い、血液製剤に関する報告事項、献血件数及びHIV抗体・核酸増幅検査陽性件数について説明後、下記のような意見が出された。

○ 新しい感染症試験法については、従来法と新法の十分な比較評価をせずにスタートしている印象を受ける。評価を行っているのであれば、きちんとデータを提示いただきたい。また、検査法の変更といった大がかりなシステムの変更は、全部のセンターで一遍に導入すると、何か問題が発生した場合に元に戻すのが大変なので段階的に導入すべき、という意見に対し、日本赤十字社より、できる範囲内で評価を

しており、データを提示することは可能である。またシステムの変更は一挙に導入したわけではなく、九州センターから段階的に行ってきた、との回答がなされた。

○ 大阪府において、献血者におけるHIV陽性率が減少してきたのは良い傾向である。

議題4について

日赤から、ヘモグロビン値が採血基準に満たない献血者から採血した事例について報告後、下記のような意見が出された。

○ 初歩的なミスである。危機管理意識が希薄であり、日本赤十字社内部での報告体制をきちんと整えることが重要である。

議題5について

事務局から、議題その他として血液凝固第Ⅷ因子製剤におけるインヒビターに係る対応について、白血球除去処理によると思われる血漿分画製剤の収量低下について、フィブリノゲン製剤及び血液凝固因子製剤に関する公表等についてそれぞれ説明後、下記のような意見が出された。

○ 第Ⅷ因子の添付文書の改訂に関連して、第Ⅸ因子についても同様の対応をすべきではないか。

以上

**資料2**

# 感染症定期報告に関する今後の対応について

平成１６年度第５回
運営委員会確認事項
（平成１６年９月１７日）

## 1 基本的な方針

運営委員会に報告する資料においては、

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

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

## 2 具体的な方法

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

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

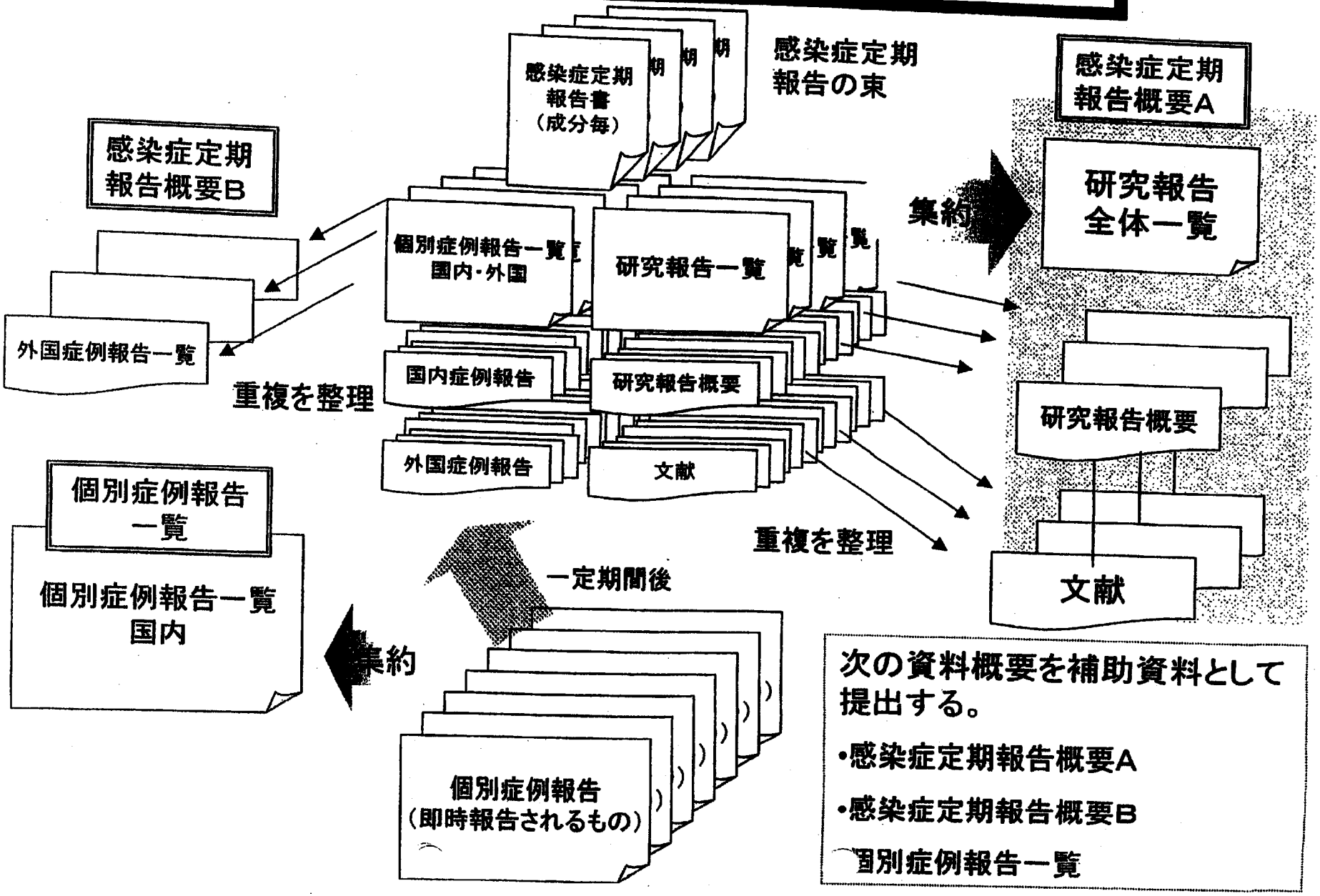
② 感染症発生症例報告のうち、発現国が「外国」の血漿分画製剤の使用による症例は、同一製品毎に報告期間を代表する感染症発生症例一覧（別紙様式第4）をまとめた**「資料概要B」**を事務局が作成し、送付する。

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

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

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

# 感染症定期報告・感染症個別症例報告の取り扱い




次の資料概要を補助資料として提出する。

- 感染症定期報告概要A
- 感染症定期報告概要B
- 個別症例報告一覧

# 感染症定期報告概要

（平成２１年２月１０日）

平成２０年９月１日受理分以降

Ａ　研究報告概要
Ｂ　個別症例報告概要

# Ａ　研究報告概要

○　一覧表（感染症種類毎）
○　感染症毎の主要研究報告概要
○　研究報告写

研究報告のまとめ方について

１　平成２０年９月１日以降に報告された感染症定期報告に含まれる研究報告（論文等）について、重複している分を除いた報告概要一覧表を作成した。

２　一覧表においては、前回の運営委員会において報告したもの以降の研究報告について、一覧表の後に当該感染症の主要研究報告の内容を添付した。

# 感染症定期報告の報告状況(2008/9/1～2008/11/30)

| 血対ID | 受理日 | 番号 | 感染症(PT) | 出典 | 概要 | 新出文献No. |
|---|---|---|---|---|---|---|
| 90013 | 2008/09/29 | 80516 | BSE | OIE／World animal health situation 2008年3月31日 | 1989年から2008年3月までに、英国以外の世界各国から国際獣疫事務局(OIE)に報告された畜牛におけるBSE症例数である。2006年は、スペイン68頭、アイルランド41頭、ポルトガル33頭、ドイツ16頭、日本およびポーランド10頭、フランス8頭、イタリア7頭、スイスおよびカナダ5頭、チェコ3頭、オーストリア、ベルギーおよびオランダ2頭、スロベニア、スウェーデンおよび米国1頭である。2008年には、これまでにカナダ1頭、アイルランド6頭が報告されている。 | |
| 90013 | 2008/09/29 | 80516 | BSE | OIE／World animal health situation 2008年4月17日 | 2008年3月までに、英国から国際獣疫事務局(OIE)に報告されたBSE症例数である。1987年以前は英国全体で446頭であったが、1992年には37280頭となった。その後、減少し、2007年には67頭となった。2008年は3月31までに10頭報告されている。 | |
| 90030 | 2008/10/23 | 80632 | B型肝炎 | Clin Infect Dis 2008; 47: e52-56 | 2000年1月から2004年12月に日本で新たにB型肝炎表面抗原陽性となった患者を調査したところ、552名中23名(4%)がHBV再活性化で、529名が急性B型肝炎であった。再活性化群は急性B型肝炎群に比べ、年齢およびHBV DNA値が有意に高く、ALTおよびアルブミンピーク値は低かった。また再活性化群の4分の1の患者が劇症肝不全となり、死亡した。肝臓関連死亡率は再活性化群の方が有意に高かった。 | 1 |
| 90013 | 2008/09/29 | 80516 | B型肝炎 | FDA/CBER 2008年5月 業界向けガイダンス(案) | FDAはB型肝炎コア抗原に対する抗体(抗HBc抗体)が陽性となったために供血延期となった供血者のリエントリー・アルゴリズムを提案するガイダンス案を発表した。これまで、抗HBc抗体が2回以上陽性となった供血者は無期限に供血延期とされていたが、本ガイダンスでは2回目に陽性となった後、8週間以上経ってからHBs抗原、抗HBc抗体および高感度HBV NATによってHBV感染が否定された場合は供血可能となる。 | 2 |
| 90060 | 2008/11/25 | 80719 | B型肝炎 | J Hepatol 2008; 48: 1022-1025 | スロヴェニアで、HBs抗原陰性で抗HBc抗体陽性、抗HBs抗体低力価陽性、HBV DNA陽性の濃厚赤血球と新鮮凍結血漿を輸血された59歳の患者が4ヶ月後に急性B型肝炎を発症した。また同じ供血血液由来のRCCの輸血を受けた71歳の患者も7ヶ月後にHBV感染を認めた。2例ともドナーと同じ配列を有するジェノタイプDが感染していた。潜在性B型肝炎ウイルス感染者の血液は抗HBs抗体が陽性にかかわらず、感染性を有した。 | 3 |
| 90007 | 2008/09/26 | 80498 | B型肝炎 | Transfusion 2008; 48: 286-294 | 最小感染量を求めるために、遺伝子型Aまたは遺伝子型CのHBVを含む急性期前の接種株をチンパンジーに接種したところ、最小50%チンパンジー感染量(CID50)は各々約10コピーと推定された。最低感染量を接種したチンパンジーにおけるHBV DNA ウィンドウ期は遺伝子型Aでは55-76日、遺伝子型Cでは35-50日、HBs Ag ウィンドウ期は遺伝子型Aでは69-97日、遺伝子型Cでは50-64日であった。またHBV DNAダブリングタイムは遺伝子型Cの方が遺伝子型Aに比べ有意に短かった。 | |

| 血対ID | 受理日 | 番号 | 感染症(PT) | 出典 | 概要 | 新出文献No. |
|---|---|---|---|---|---|---|
| 90013 | 2008/09/29 | 80516 | B型肝炎 | 第37回 日本肝臓学会西部会 2007年12月7-8日、肝臓 2007; 48(Suppl 3): A522 | 輸血によりHBs抗体エスケープ変異株に感染し、肝炎を発症した40歳代女性の症例報告である。献血者、受血者の塩基配列の解析を行って感染が証明された。核酸増幅検査を含む献血者のスクリーニングを行っているにもかかわらず、本邦では年間10-20例のHBV感染が報告されている。その原因の一つがHBs抗体エスケープミュータントであるが、本症例のように献血者、受血者ともに塩基配列の解析を行い感染が証明された例はきわめて稀である。 | |
| 90006 | 2008/09/26 | 80497 | B型肝炎、E型肝炎 | Veterinary Science in China 2007; 37: 921-925 | 中国の畜殺場から集めたブタの肝臓と血清からDNAを抽出し、PCRを使ってs遺伝子を増幅し配列決定を行った結果、ブタとヒトのHBVのS遺伝子の配列は98-100%の相同性を示した。また、RT-PCRによるHEV RNA検出を行った結果、HEV RNAがブタの肝臓に存在することが示された。 | 4 |
| 90013 | 2008/09/29 | 80516 | B型肝炎C型肝炎 | 第56回日本輸血・細胞治療学会総会 2008年4月25-27日 P-033 | 2007年に医療機関から日本赤十字社に報告された輸血関連感染症の報告数は124例(10月末現在)であり、一昨年及び昨年の同期間に比べ減少傾向にある。内訳はHBVが61例、HCV32例、細菌24例、その他のウイルスが7例であった。ウイルス感染(疑)症例の調査結果により病原体を確認した症例は、HBVの12例とHCVの1例であった。HCVの1例は20プールNAT開始後(2004年8月開始)初めての検出限界以下の献血血液による感染症例であった。 | |
| 90030 | 2008/10/23 | 80632 | C型肝炎 | Clin Infect Dis 2008; 47: 627-633 | フランスの大学病院の血液透析ユニットでのHCV伝播リスクにおける環境汚染および標準的注意の非遵守の役割を評価した。試験期間中にHCV陽性となった2名のうち1名は、同ユニットで治療中の慢性感染患者と同じウイルス株に感染していることが系統遺伝学的解析により明らかとなった。環境表面検体740例中82例がヘモグロビンを含み、その内6例がHCV RNAを含んでいた。手の衛生に関する遵守率は37%、患者ケアの直後に手袋をはずしていたのは33%であった。 | 5 |
| 90013 | 2008/09/29 | 80516 | E型肝炎 | Am J Trop Med Hyg 2008; 78: 1012-1015 | スペインでブタに曝露しているヒト101名と曝露していないヒト97名におけるHEV感染の有無を調べた。抗HEV IgG保有率は曝露群では18.8%、非曝露群では4.1%であった。ブタに接するヒトの抗HEV IgG保有リスクは5.4倍(P=0.03)であった。HEV感染は養豚作業員の職業病として扱うべきである。 | 6 |
| 90007 | 2008/09/26 | 80498 | E型肝炎 | N Engl J Med 2008; 358: 811-817 | 2004年1月1日～2006年12月31日に腎移植(241名)または肝移植(86名)を受けた患者の移植時の抗HEV IgG保有率は、各々14.5%または10.4%であった。この内、肝移植を受けた3名、腎移植を受けた9名、腎臓と膵臓の移植を受けた2名の計14名で急性HEV感染を同定したが、全員血清HEV RNA陽性であり、内8名が慢性肝炎となった。移植から診断までの時間は短く、慢性肝炎に進展した患者ではリンパ球数並びにCD2、CD3およびCD4 T細胞数が有意に低かった。 | |

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| 90060 | 2008/11/25 | 80719 | E型肝炎 | Transfusion 2008; 48: 1368-1375 | 2004年9月20日に39歳日本人男性から献血された血液はALT高値のため不適当とされ、HEV陽性であった。当該ドナーの遡及調査の結果、9月6日にも献血を行い、HEV RNAを含有する血小板が輸血されていた。当該ドナーと親戚は8月14日にブタの焼肉を食べており、父親は9月14日に急性肝炎を発症し、E型劇症肝炎で死亡した。他に7名がHEV陽性であった。レシピエントは輸血22日目にALTが上昇し、HEVが検出された。 | 7 |
| 90040 | 2008/10/24 | 80645 | E型肝炎 | Vox Sanguinis 2008; 95(Suppl.1): 282-283 | 2005年の中国の4都市(Beijing、Urmuchi、KunmingおよびGuangzhou)における供血検体のHEV感染率を調べた。その結果、ルーチン検査(抗HCV、抗HIV1/2、HBsAg、梅毒およびALT)陰性供血者の約1%は抗HEV IgMまたはHEV Ag陽性で、HEV感染の可能性があった。また、ALTスクリーニングは中国のHEV感染血排除に役立つ可能性があった。 | 8 |
| 90013 | 2008/09/29 | 80516 | E型肝炎 | 第56回日本輸血・細胞治療学会総会 2008年4月25-27日 O-026 | 北海道地区において現行プールNATスクリーニングの残量を用いてTaqMan RT-PCR法によるHEV NATスクリーニングを行った。陽性献血者85例について追跡調査および遡及調査などを行なった。陽性献血者の多くは動物内臓肉を食してHEVに感染したと考えられる新規感染者で、GenotypeはG3が多かった。多くは症状が現れないまま抗体が陽転化し、典型的な無症候性一過性感染の経過をたどった。 | |
| 90014 | 2008/09/29 | 80517 | HIV | ABC Newsletter 2008; No.26 2008年7月4日 | 米国医師会(AMA)は、男性同性愛行為を行った男性(MSM)の供血延期期間を生涯としている連邦の方針を5年間に変更することを支持するという声明を採択した。AMAはこの新方針をFDAに通告し、この方針を推し進めるグループと協力していく。FDAは1977年以降、MSMの供血を生涯延期することを血液事業者に要求しているが、アメリカ血液センターなどからは反対意見が出されている。 | 9 |
| 90013 | 2008/09/29 | 80516 | HIV | AIDS 2007; 21: 2351-2353 | フランスの新規HIV診断例におけるHIV-2およびHIV-1グループO型の感染率を調べた。2003年1月から2006年6月に10184例のHIV新規診断症例が報告されたが、HIV-2およびHIV-1グループO型感染の割合は、各々、1.8%および0.1%であった。これらの症例のほとんどは、異性との接触により感染した流行地域出身の患者であった。HIV-2感染のうち3例は男性と性的関係を持つ非アフリカ系男性であった。 | |
| 90014 | 2008/09/29 | 80517 | アメリカ・トリパノソーマ症 | Clin Infect Dis 2008; 46: e44-47 | 血液製剤の輸血によりシャーガス病に感染し、死亡したスペイン人患者の寄生虫学的、血清学的疾患経過、ならびに供血者の調査の報告である。患者は白血病の既往があり、176名以上の供血者由来の輸血を受けていた。臍帯血移植のための免疫抑制状態で、寄生虫が血液脳関門を通過して神経系に感染したことが確認された。特定された供血者は無症候であった。複数回輸血患者は、免疫抑制剤治療実施前に、抗Trypanosoma cruzi抗体のスクリーニングを受けるべきである。 | |

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| 90014 | 2008/09/29 | 80517 | アメリカ・トリパノソーマ症 | Vox Sanguinis 2008; 95(Suppl.1): 39 | 米国で全供血者を対象にしたTripanosoma Cruzi検査が導入された2007年1月30日以降、最初の10ヶ月間、供血者の調査を行った。適合供血のうちELISA法で反復陽性(RR)となったのは0.013%(90/651471)で、そのうちRIPA陽性は34%(28/82)で、陽性確認率は0.0043%であった。全供血のスクリーニングは費用対効果が低く、出生地と初回供血者に絞った対策の検討が示唆された。 | 10 |
| 90013 | 2008/09/29 | 80516 | インフルエンザ | AABB Weekly Report 2008年2月29日 | インフルエンザパンデミックと血液供給に関するAABBの作業部会は、パンデミック時に供血間隔の例外的な取り扱いを認めるよう2月14日にFDAに対し要望書を送付した。パンデミック時には適格な供血者数が制限されることが予想されるため、全血および赤血球採取の間隔を短くすることが最も有効であるとしている。 | |
| 90013 | 2008/09/29 | 80516 | インフルエンザ | Emerg Infect Dis 2007; 13: 1865-1870 | カナダの共同農場で生活していた7ヶ月齢の乳児から、A/Canada/1158/2006と名づけられたブタインフルエンザAウイルス(H3N2)が単離された。この農場のメンバー90名の内54名で同ウイルスに対する血清学的検査を行ったところ、54名中9名が陽性であった。また、ブタ10頭のうち1頭で血清陽性が明らかになった。ブタインフルエンザウイルス株は効率的にヒトからヒトへ伝染する形に適応または交雑することから、インフルエンザ流行への備えの一環として養豚者の定期的サーベイランスを検討すべきである。 | |
| 90013 | 2008/09/29 | 80516 | インフルエンザ | Vox Sanguinis 2008; 95(Suppl. 1): 40 | 米国におけるパンデミックインフルエンザの血液供給に対する影響をシミュレーションした。3ヶ月間の血液供血量が50%減少した場合、血液需要に制限がない場合は在庫のほとんどを使い尽くしたが、血液の使用を必要最低限に制限した場合は在庫がなくなることはなかった。 | 11 |
| 90018 | 2008/09/29 | 80523 | ウイルス感染 | AIDS Res Hum Retroviruses 2007; 23: 1330-1337 | Simian Foamy Virus (SFV)感染した男性7名を長期間追跡調査した。男性は非ヒト霊長類と接触する職業であった。男性の全ての末梢血単核球(PBMC)からプロウイルスDNAが検出され、口腔や尿生殖検体から検出されることもあった。長期間(中央値20年)の性的曝露にかかわらず妻たちは陰性であった。特異的な臨床症状は報告されなかった。限定的な追跡調査であるためSFV関連疾患やヒト-ヒト感染を特定できなかった。 | |
| 90007 | 2008/09/26 | 80498 | ウイルス感染 | Emerg Infect Dis 2008; 14: 834-836 | カナダにおいて、Saffoldウイルスに関連するカルジオウイルス分離株が呼吸器症状を有する3名の小児の鼻咽頭吸引物から検出された。Can112051-06分離株のポリプロテイン配列は、Saffoldウイルスと91.2%のアミノ酸同一性を有した。しかし、ウイルス表面のEF及びCDのループは、かなり異なっていた。 | |
| 90045 | 2008/10/29 | 80668 | ウイルス感染 | Lancet Infect Dis 2008; 8: 355 | ボリビア、ペルーおよび米国CDCのチームはボリビアの出血熱の致死症例から新規のアレナウイルスを発見し、Chapare virusと名付けられた。また、ウガンダでは赤オナガザルで新型ポックスウイルスの可能性があるウイルスが発見された。これら野生動物のウイルスがヒトへの感染能を獲得し、重篤な疾患を引き起こす可能性がある。 | 12 |

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| 90003 | 2008/09/26 | 80493 | ウイルス感染 | PLoS Pathogens 2008; 4: e1000047 | 出血熱症例の小さな流行が、2003年12月と2004年1月にボリビアのCochabamba付近で発生した。1死亡例から検体を入手し、患者血清検体から非細胞障害性ウイルスを単離し、アレナウイルスと同定した。RT-PCR分析、並びにS及びL RNAセグメント配列の解析の結果、このウイルスはサビアウイルスに最も近縁であるが、新規のウイルスであることが示された。我々はこのウイルスをChapareウイルスと命名することを提案する。 | |
| 90052 | 2008/11/14 | 80708 | ウイルス感染 | Proc Natl Acad Sci USA 2008; 105: 14124-14129 | インフルエンザ様疾患の小児の呼吸分泌物中から、汎ウイルスマイクロアレイ法を用いて、初めてヒトカルディオウイルスを同定した。系統遺伝学的分析から、このウイルスはTheilerのネズミ脳脊髄炎ウイルス亜型に属し、Saffoldウイルスと最も近縁であった。また、胃腸疾患患者群498名から得た751例の糞便検体中6検体からカルディオウイルスが検出された。 | 13 |
| 90013 | 2008/09/29 | 80516 | ウイルス感染 | ProMED-mail20080218.0645 | 2008年1月21日、Braziliaで32歳の男性が黄熱のため死亡した。これは、ブラジルにおける15人目の黄熱死亡患者である。Mato Grossoでも1名の感染と死亡が確認された。パラグアイ保健当局は首都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。 | |
| 90013 | 2008/09/29 | 80516 | ウイルス感染 | ProMED-mail20080720.2201 | オーストラリアBrisbaneの動物病院のスタッフが致死性のヘンドラウイルスに感染した。看護師1名と獣医1名が、感染したウマ数頭を治療後、感染した。前回のアウトブレイクは1994年で調教師1名とウマ14頭が死亡した。同ウイルスがヒト-ヒト感染するとのエビデンスはなく、拡大する危険性はない。 | 14 |
| 90007 | 2008/09/26 | 80498 | ウイルス感染 | Transfusion 2008; 48: 1180-1187 | 米国テキサス南東部の健康な成人ドナー100名の血液中のヒトヘルペスウイルス(HHV)陽性率とウイルスDNA量をRT-PCRにより調べた。その結果、HSV-1、HSV-2、VZV及びHHV-8 DNAはどの検体からも検出されなかった。一方、EBVは72%、HHV-7は65%、HHV-6は30%、CMVは1%に検出された。また、1名の血液から6.1×10^7geq/mlを超えるHHV-6 Type Bが検出されたが、健常者における異常な高値は活動性感染や免疫不全とは関連が無いと思われる。 | 15 |

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| 90054 | 2008/11/14 | 80710 | ウイルス感染 | WHO/EPR 2008年10月13日 | 南アフリカとザンビア出身者の最近の死亡例3例はアレナウイルス科のウイルスが原因あることが、NICDおよびCDCで行われた検査の結果明らかとなった。このウイルスに関する詳細な分析が継続されている。一方、南アフリカでは患者と密接に接触した看護師が感染し、入院中である。 | 16 |
| 90036 | 2008/10/24 | 80638 | ウイルス性脳炎 | ProMED-mail20080828.2697 | インド東部のウッタルプラデシ州で小児を死亡させている原因不明のウイルスは、インド保健省の専門家らにより急性脳炎症候群と診断された。同州の13の地区では、数週間におよそ800人の患者が発生し150人が死亡したと報告され、その数は増加すると見られている。血液検査で日本脳炎陽性となった患者は5%以下であった。日本脳炎とエンテロウイルスとの混合感染の可能性について調査中である。 | 17 |
| 90018 | 2008/09/29 | 80523 | ウエストナイルウイルス | J Med Virol 2008; 80: 557-563 | 中央ヨーロッパにおけるウエストナイルウイルス(WNV)の潜在的脅威を調べた。ドイツ人供血者14437名由来の検体中0.03%が抗WNV陽性であった。ドイツ人9976名由来の検体をWNV NAT法を用いてWNV-RNAの有無を調べた結果、全て陰性であった。米国由来血漿プールではWNV-RNAがしばしば検出されたが、ヨーロッパやアジア由来のプールからは検出されなかった。また、血漿製剤製造過程のウイルス不活化によりWNVに関する安全性は保証されることが明らかとなった。 | |
| 90013 | 2008/09/29 | 80516 | ウエストナイルウイルス | Rev Panam Salud Publica 2006; 19: 112-117 | 文献および未発表データから、ラテンアメリカやカリブ海地域のウエストナイルウイルス(WNV)感染の現状をまとめた。WNV感染は2001年にCayman諸島とFlorida Keysの住民で見られ、2002～2004年にジャマイカ、メキシコなど周辺地域で動物や鳥類での感染が確認されている。しかし、疾患報告数は少ない。この不可解な熱帯生態系でのウイルス減弱または他の可能性を検討するためには分離株が必要である。 | 18 |
| 90013 | 2008/09/29 | 80516 | コンゴ・クリミア出血熱 | ProMED-mail20080709.2092 | 2008年7月7日、トルコのBursa、CanakkaleおよびSamsunの病院でダニ媒介性疾患であるクリミア・コンゴ出血熱により3名が死亡し、この2ヶ月での死者数は37名となった。保健省はダニに注意するよう呼びかけ、咬まれた場合は決して手でつぶさずに、医師にピンセットで注意深く取り除いてもらい、ヨードで消毒することを推奨している。 | 19 |
| 90014 | 2008/09/29 | 80517 | サルモネラ | CDC 2008年7月8日 | CDCは関係機関と協力して複数の州で発生したサルモネラ血清型セントポールのアウトブレイクを調査している。生のトマトの摂食が原因と考えられている。2008年4月以降2008年7月7日までに、米国の41の州、ワシントンD.C.およびカナダで991名の患者が同じ遺伝子パターンのサルモネラ血清型セントポールに感染したことが確認された。 | 20 |

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| 90040 | 2008/10/24 | 80645 | チクングニヤウイルス感染 | Transfusion 2008; 48: 1333-1341 | 2005年から2007年に、チクングニヤウィルス(CHIKV)はレユニオン島で大流行し、供血は2006年1月に中断された。大流行中のウイルス血症血供血の平均リスクは、10万供血あたり132と推定された。2006年2月の最流行時におけるリスクは、10万供血あたり1500と最高であった。この期間中、757000人の住民のうち推定312500人が感染した。2006年1月から5月の平均推定リスク(0.7%)は、CHIKV NAT検査による血小板供血のリスク(0.4%)と同じオーダーであった。 | 21 |
| 90007 | 2008/09/26 | 80498 | 手足口病 | WHO Represetative Office in China 2008年5月19日 | 2008年3月下旬、中国Anhui省Fuyang市で未就学児3名が重症の肺炎と急激な悪化により死亡し、4月中旬までに15名の小児が同様の疾患で死亡した。調査の結果、エンテロウイルス71による手足口病と確定された。同市では、3月1日から5月9日の間に、6,049例報告され、353例が重篤で、22例が死亡した(致死率0.4%)。患者数は、4月の初めに増加し始めて、4月28日にピークに達し、5月5日以後減少した。 | |
| 90013 | 2008/09/29 | 80516 | デング熱 | Hong Kong Med J 2008; 14: 170-177 | 1998～2005年に香港の公立病院に入院したデング確定患者全員の医療記録をレトロスペクティブに検討した。126例中123例(98%)がデング熱、3例(2%)がデング出血熱であった。1例が輸血により感染したデング熱であった。116例が輸入症例、10例が地域症例であった。デングウイルス1型が最も多く、次に2型、3型、4型の順であった。死亡例はなかった。発熱、皮疹を呈し、血小板減少などを示す渡航歴のある患者には鑑別診断にデング熱を含めるべきである。 | 22 |
| 90020 | 2008/09/29 | 80525 | デング熱 | Transfusion 2008; 48: 1342-1347 | 高力価の培養デングウイルス セロタイプ2をアルブミンおよび免疫グロブリンの各種製造工程(低温エタノール分画、陽イオン交換クロマトグラフィー、低温殺菌、S/D処理およびウイルスろ過)前の検体に加え、各工程での同ウイルスのクリアランスをVero E6細胞培養におけるTCID50アッセイおよびRT-PCRで測定した。その結果、全ての工程が不活化・除去に有効であることが示された。 | 23 |
| 90040 | 2008/10/24 | 80645 | デング熱 | Transfusion 2008; 48: 1348-1354 | 2005年9月20日～12月4日のプエルトルコの米国赤十字におけるすべての供血16521検体中のデングウイルス(DENV) RNAをTMA(transcription-mediated amplification)法で測定したところ、12検体(0.07%)がTMA陽性であった。4検体は、RT-PCR(DENVセロタイプ2および3)陽性であった。RT-PCR陽性4検体中3検体でウイルスを培養することができた。TMA陽性12検体中1検体がIgM陽性であった。1:16に希釈した場合は5検体のみTMA陽性であった | 24 |

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| 90045 | 2008/10/29 | 80668 | 鳥インフルエンザ | N Engl J Med 2008; 358: 2573-2584 | 細胞培養で製造したH5N1鳥インフルエンザワクチンのPhase 1および2試験を行った。その結果、大多数の被験者においてアジュバントなしの7.5μgまたは15μgのヘマグルチニン抗原を含有するワクチンの2回接種が、様々なH5N1ウイルス株に対するする中和抗体の合成を誘発することが示された。注射部位での軽度の痛みと頭痛が主な有害反応であった。このワクチンが有用であることが示唆された。 | 25 |
| 90018 | 2008/09/29 | 80523 | 鳥インフルエンザ | Proc Natl Acad Sci USA 2008; 105: 7558-7563 | ユーラシアおよび北米系統のH7型トリインフルエンザウイルスの受容体結合能およびフェレットモデルにおける感染性を調べた。その結果、2004年にカナダで分離されたH7N3型、2002-2003年に米国北東部で分離されたH7N2型はα2-6結合シアル酸に対する親和性を高めたHAを保有していた。また2003年にニューヨークの男性から分離された低病原性H7N2型はフェレットの上気道で効率的に増殖し、直接接触で感染できることが確認された。 | |
| 90014 | 2008/09/29 | 80517 | バベシア症 | American Society for Microbiology 108th General Meeting 2008年6月1-5日、Boston | 米国中南部では稀な輸血によると考えられるBabesia microti感染症例の報告である。61歳の女性患者で、赤血球輸血後、吐き気と発熱を訴え、敗血症の症状を呈し、死亡した。血液塗抹標本で赤血球の5～15%にトロフォゾイト(栄養体)があった。患者血液検体中でBabesialは形態学的に確認され、PCRでB. microti陽性であった。輸血された製剤の供血者のうち1名がB. microti陽性であった。 | |
| 90018 | 2008/09/29 | 80523 | バベシア症 | Blood 2007; 110(11, Part 1): 853 | 米国コネチカット州での輸血によるBabesia microti感染の危険性を評価するため、2004-2007年に収集されたドナーとレシピエントの保存検体を検査した。その結果、45回の赤血球輸血を受けていた患者1例でBabesia microti感染が確認されたが、血清検体陽性の ドナーを特定することはできなかった。危険性は1920回の赤血球輸血で0例または1例と計算された。 | |
| 90045 | 2008/10/29 | 80668 | パルボウィルス | Biotechnol Prog 2008; 24: 554-560 | レジン上に3量体ペプチドを結合し、PBSまたは血漿溶液に添加したブタパルボウイルス(PPV)への選択的な結合能を有するペプチドをスクリーニングした。その結果、WRW(トリプトファン-アルギニン-トリプトファン)結合レジンは7.5%ヒト血漿中のPPVを検出限界以下に除去することができた。 | 26 |
| 90003 | 2008/09/26 | 80493 | パルボウィルス | FDA/CBER 2008年7月 業界向けガイダンス(案) | 血漿由来製品によるパルボウイルスB19伝播リスクを低減するための核酸増幅検査(NAT)についてのガイダンス案が示された。全ての血漿由来製剤について、製造プール中のパルボウイルスB19 DNAのウイルス負荷を確実に10000 IU/ml未満とするため、製造過程の品質管理検査としてNATを実施すべきである。ミニプール中でのNATの感度は少なくとも1000000 IU/mlとするべきである。これらの基準を超えるものは使用してはならない。 | 27 |

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| 90013 | 2008/09/29 | 80516 | パルボウィルス | Transfusion 2008; 48: 1036-1037 | 大阪における1997-1999年の献血者979052名中102名がヒトパルボウイルスB19感染者であった。B19感染者のうち20名のB19 DNA、IgGおよびIgMを長期間フォローアップしたところ、B19持続感染が観察されたが、B19感染の症状を報告した者はいなかった。B19急性感染後の血漿ウイルス力価は約1年で$10^4$IU/mL未満、約2年で$10^3$IU/mL未満まで下がることが示された。 | |
| 90045 | 2008/10/29 | 80668 | パルボウィルス | Transfusion in press | 3つの血液凝固因子製剤(第VIII因子インヒビターバイパス活性、第IX因子複合体および第VII因子)の製造工程においてSTIM-4蒸気加熱処理を用いた不活性化処理を行い、ヒトパルボウイルスB19(B19V)とマウス微小ウイルス(MMV)間で不活性化効果の比較を行った。その結果、血液凝固因子製剤の中間体の種類に関わらず、試験に用いたB19V(遺伝子型1型、2型)はいずれもMMVと比較して効果的に不活性化された。 | |
| 90013 | 2008/09/29 | 80516 | ハンタウイルス | Emerg Infect Dis 2008; 14: 808-810 | スウェーデンにおけるPuumalaウイルスの予期せぬ大規模アウトブレイクにより、2007年のVästerbotten地方の流行性腎症患者の数は100,000人当り313人に至った。齧歯類の増加の他、気候温暖化および地表を覆う積雪の減少により、ウイルスを媒介するハタネズミの活動が活発だったことが、当該アウトブレイクの一因であろうと考えられる。 | |
| 90007 | 2008/09/26 | 80498 | ヒトポリオーマウイルス感染 | Science 2008; 319: 1096-1100 | メルケル細胞癌(MCC)検体をdigital transcriptome subtraction法を用いて検査し、新種のポリオーマウイルスを同定し、メルケル細胞ポリオーマウイルス(MCVまたはMCPyV)と命名した。このウイルスはMCC腫瘍10検体中8例(80%)で検出されたが、対照組織検体では59例中5例(8%)、対照皮膚組織検体では25例中4例(16%)でしか検出されなかった。MCVがMCCの病原因子である可能性が示唆された。 | |
| 90014 | 2008/09/29 | 80517 | ブルセラ症 | Clin Infect Dis 2008; 46: e131-136 | 急性ブルセラ症患者39名の血液検体中のBrucella DNAの存在をRT PCR法により調べた。その結果、治療終了時では87%、治療完了後6ヶ月では77%、治療後2年を過ぎても70%の患者で、無症候性であるにもかかわらず、Brucella DNAが検出された。適切な治療を行い、回復したように見えても、Brucella DNAは存続する。ブルセラ菌は除去不可能な持続性の病原体である。 | 28 |
| 90014 | 2008/09/29 | 80517 | ペスト | Emerg Infect Dis 2007; 13: 1459-1462 | 2003年6月から7月にアルジェリアOran地区においてペストの集団感染が発生した。同国では、この疾患は50年以上報告されていなかった。腺ペスト症例18名が特定され、Yersinia pestisが6名から分離された。初発患者を除き、全員が回復した。標的予防的化学療法、衛生、ベクターコントロールが、感染制御上重要な役割を果たした。疫学的、分子生物学的な知見から、当該期間中、現地の保菌動物の存在が強く示唆されたが、その起源については特定できなかった。 | |

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| 90014 | 2008/09/29 | 80517 | リケッチア症 | Emerg Infect Dis 2008; 14: 1019-1023 | ネコノミが媒介するRickettsia felis感染症のヒト症例は世界中で報告されている。症状は発疹熱やデング熱などに類似しており、実際よりも少なく推定されている可能性が高い。ヒトの健康を脅かす感染症として今後調査が必要である。 | 29 |
| 90010 | 2008/09/26 | 80501 | リケッチア症 | ProMED-mail20080728.2306 | オランダ・ブラバント州の公衆衛生局が行った調査でQ熱の症例報告数が急激に増加し、2008年7月21日付けで491症例が報告されている。感染症管理センター長によると、実際の感染者数は報告された症例数の10倍であると思われる。2007年まではQ熱はオランダではほとんど存在しなかった。 | 30 |
| 90003 | 2008/09/26 | 80493 | リンパ性脈絡髄膜炎 | boston.com 2008年5月13日 | 2008年5月12日の保健当局発表によると、ボストンの病院で検出が難しいウイルスに感染したドナーから腎臓を移植された70歳女性が死亡し、57歳男性が危篤である。ドナーと患者2名の検体をCDCが検査したところ、全員、リンパ球性脈絡髄膜炎ウイルス(LCMV)陽性であり、ドナーからの伝播であったことが確認された。移植前にはエイズウイルス、肝炎ウイルスなどの検査は行ったが、LCMVの検査は行っていなかった。 | |
| 90003 | 2008/09/26 | 80493 | リンパ性脈絡髄膜炎 | N Engl J Med 2008; 358 10.1056/NEJMoa073785 | オーストラリアで一人のドナーから臓器移植を受けた3例が移植後4-6週後に死亡した。他のいかなる方法でも原因不明であったが、2例のレシピエントの移植肝および腎から得られたRNAを偏りのない迅速シークエンシングで解析することにより、リンパ性脈絡髄膜炎に関係する新規のアレナウイルスが原因であることが明らかとなった。レシピエントの腎、肝、血液および脳脊髄液からこのウイルスが検出され、また免疫組織学的および血清学的に確認された。この方法は病原体発見の強力な手段である。 | |
| 90007 | 2008/09/26 | 80498 | リンパ性脈絡髄膜炎 | N Engl J Med 2008; 358: 991-998 | オーストラリアで一人のドナーから臓器移植を受けた3例が移植後4-6週後に死亡した。他のいかなる方法でも原因不明であったが、2例のレシピエントの移植肝および腎から得られたRNAを偏りのない迅速シークエンシングで解析することにより、リンパ性脈絡髄膜炎に関係する新規のアレナウイルスが原因であることが明らかとなった。レシピエントの腎、肝、血液および脳脊髄液からこのウイルスが検出され、また免疫組織学的および血清学的に確認された。この方法は病原体発見の強力な手段である。 | |
| 90010 | 2008/09/26 | 80501 | レプトスピラ症 | Infect Genet Evol 2008, doi:10.1016 | コスタリカにおいて、レプトスピラ症の入院患者から分離されたレプトスピラは、Javanica血清群型に分類される新しい血清型で、Arenalと命名された。同じ地区の重症患者から分離された株も同じ血清型であったことから、この株は、この地域に流行する新規の高病原性の血清型であると考えられた。 | |

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| 90036 | 2008/10/24 | 80638 | レプトスピラ症 | Infect Genet Evol 2008; 8: 529-533 | コスタリカにおいて、レプトスピラ症の入院患者から分離されたレプトスピラは、Javanica血清群型に分類される新しい血清型で、Arenalと命名された。同じ地区の重症患者から分離された株も同じ血清型であったことから、この株は、この地域に流行する新規の高病原性の血清型であると考えられた。 | |
| 90029 | 2008/10/21 | 80614 | 異型クロイツフェルト・ヤコブ病 | American Society of Hematology/Press Releases 2008年8月28日 | Blood誌のprepublished onlineに掲載されたヒツジにおける研究によると、輸血によるBSE伝播のリスクは驚くほど高い。エジンバラ大学で行われた9年間の研究は、BSEまたはスクレイピーに感染したヒツジからの輸血による疾病伝播率を比較した。その結果、BSEおよびスクレイピーとも輸血によりヒツジに効率よく伝播された。症状を呈する前のドナーから採取された血液によっても伝播することが示された。 | 31 |
| 90007 | 2008/09/26 | 80498 | 異型クロイツフェルト・ヤコブ病 | Ann Neurol 2008; 63: 697-708 | 米国の国立プリオン病病因調査センターに2002年5月から2006年1月に紹介された患者11名(平均発症年齢62歳)を調べたところ、海綿状変性の型、PrP免疫染色パターンおよびマイクロプラークの存在が、既知のプリオン病とは異なり、通常の方法では典型的なプロテアーゼ抵抗性PrPは検出されなかった。我々はこれらをプロテアーゼ感受性プリオン病(PSPr)と名付けた。PSPrは、プリオン病の中では稀ではなく、我々のデータが示すよりもさらに多い可能性がある。 | 32 |
| 90055 | 2008/11/19 | 80714 | 異型クロイツフェルト・ヤコブ病 | Cell 2008; 134: 757-768 | マウスPrPScと混合させることによって折り畳み異常が起こったハムスターPrPCは、野生型ハムスターに対して感染性を起こす新規なプリオンを生成した。同様の結果は、反対方向でも得られた。PMCA増幅を繰り返すとin vitro産生プリオンの順応が起こる。このプロセスは、in vivoでの連続継代に観察される株の安定化を暗示させる。種の壁と株の生成がPrP折り畳み異常の伝播によって決定されることが示唆される。 | 33 |
| 90045 | 2008/10/29 | 80668 | 異型クロイツフェルト・ヤコブ病 | dailypress.com 2008年4月11日 | 米国Portsmouthで、脳変性疾患を呈し死亡した女性の死因を、vCJD疑いのため調査中である。MRIまたは脳スキャンの結果がアトランタの疾病対策センターに送付され、バージニア大学および国立プリオン病病因サーベイランスセンターで更に検査される。結果が出るまでには数ヶ月を要すると思われる。 | |
| 90030 | 2008/10/23 | 80632 | 異型クロイツフェルト・ヤコブ病 | Emerg Infect Dis 2008; 14: 1406-1412 | 263Kスクレイピーの臨床症状を呈するハムスター22匹の尿にTSE感染性があることが示された。これらの動物の腎臓と膀胱のホモジネートは20000倍以上希釈してもTSE感染性があった。組織学的、免疫組織化学的分析では、腎臓における疾患関連PrPの散発的な沈着以外、炎症や病変は見られなかった。尿中のTSE感染性が、自然のTSEの水平感染に何らかの役割を果たす可能性がある。 | 34 |

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| 90013 | 2008/09/29 | 80516 | 異型クロイツフェルト・ヤコブ病 | J Virol 2008; 82: 3697-3701 | 非典型的BSE株の1つであるBASE(またはBSE-L)の感染性およびヒトでの表現型を調べた。BASEウシ由来の脳ホモジネートを、ヒトプリオン蛋白を発現するトランスジェニック(Tg)マウスに接種したところ、60%が20-22ヶ月後に感染し、古典的BSEに関する報告より高い感染率であった。BASE感染ヒト化Tgマウス脳における病因性プリオンのアイソフォームは、元のウシBASEまたは孤発性ヒトプリオン病のものとは異なっていた。またBASEプリオンはリンパ向性であった。 | |
| 90045 | 2008/10/29 | 80668 | 異型クロイツフェルト・ヤコブ病 | Medgadget.com 2008年4月9日 | カナダQuebecのProMetic Life Science社は血液中のvCJDプリオンを除去する使い捨てフィルターを開発した。何百万ものペプチドをスクリーニングし、プリオンに最も親和性のあるものを探し、市販の樹脂に固定し、膜状にし、何層にも重ねた。本フィルターは汚染血液からのプリオン除去が可能であった。また、フィルターで処理したプリオン感染ハムスターの血液をプリオン非感染ハムスターに投与しても疾患は発現しなかった。 | |
| 90013 | 2008/09/29 | 80516 | 異型クロイツフェルト・ヤコブ病 | Microbiol Immunol 2007; 51: 1221-1231 | 感染動物モデルにおいても、血中のPrPresは白血球を除きめったに検出されない。新規の酸性SDS沈殿法と高感度化学発光法とを組み合わせることにより、プロテイナーゼK耐性3F4反応性タンパクが、スクレイピー感染ハムスターの血漿中からは検出されるが、疑似感染ハムスターでは検出されないことが示された。血漿中においてPrPresは他の血漿タンパクと糖鎖を通じて凝集しており、スクレイピー感染ハムスター血漿において検出可能となったことが示唆された。 | |
| 90030 | 2008/10/23 | 80632 | 異型クロイツフェルト・ヤコブ病 | PLoS ONE 2008; 3: e2878 | 野生型マウスおよびヒトPrPを発現しているトランスジェニックマウスに、輸血関連vCJD感染第1号症例由来の脳材料を接種し、輸血によるヒト-ヒト間の2次感染後のvCJD病原体の性質について調べた。その結果、潜伏期間、臨床症状、神経病理学的特徴およびPrP型について、vCJD(輸血)接種群はvCJD(BSE)接種群と類似していた。vCJD病原体は、ヒトにおける2次感染により、有意な変化が起こらないことが明らかとなった。 | 35 |
| 90055 | 2008/11/19 | 80714 | 異型クロイツフェルト・ヤコブ病 | PLoS ONE 2008; 3: e3017 | 非定型BSE(BASE)に感染した無症候のイタリアの乳牛の脳ホモジネートをカニクイザルに脳内接種した。BASE接種サルは生存期間が短く、古典的BSEまたはvCJD接種サルとは異なる臨床的展開、組織変化、PrPresパターンを示した。感染牛と同じ国の孤発性CJD患者でPrPが異常なウエスタンブロットを示す4例のうち3例のPrPresに同じ生化学的特徴を認めた。BASEの靈長類における高い病原性および見かけ上孤発性CJDである症例との関連の可能性が示唆された。 | 36 |

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| 90007 | 2008/09/26 | 80498 | 異型クロイツフェルト・ヤコブ病 | Transfusion 2008; 48: 609-619 | ヒツジのリコンビナントPrP(rPrP)のヒツジにおける血 液クリアランスならびにスクレイピー関連フィブリル(SAF)静注後のPrPresへの曝露について調べた。rPrPのARR変異型は、VRQ変異型よりもより早く除去された。また、PrPcのARR変異型のクリアランスがVRQ変異型のクリアランスよりも大きいことが示唆された。rPrPの血漿クリアランスは、両腎臓摘出後は52%減少し、rPrP除去に腎臓が重要であることが示された。PrPresはSAF静注後は緩やかに除去された。 | |
| 90007 | 2008/09/26 | 80498 | 感染 | 56th Annual Meeting of the American-Society-of-Tropical-Medicine-and-Hygiene 1044 | ヒト顆粒球アナプラズマ症(HGA)の発生率は、1999年以来2倍となった。原因病原体のAnaplasma phagocytophilumによる血液の安全リスクを調査するため、間接免疫蛍光法を用いてコネチカット州及びマサチューセッツ州の血液ドナーのA. phagocytophilumに対するヒトIgG抗体を測定した。その結果、2001年から2006年に採取された15,828ドナー血清中432例(2.7%)が抗体陽性であった。比較的高い陽性率が持続していることから、更なる調査が必要である。 | |
| 90045 | 2008/10/29 | 80668 | 感染 | Transfusion 2008; 48: 1739-1753 | 最近米国で承認された2つのフィブリノゲンおよびトロンビンについて、HIV、HCV、HBV、HAV、パルボウイルスB19およびvCJDに関する病原体感染リスクを評価した。これら血液製剤の製造過程では2つの異なった不活化および除去工程が使われている。全製剤とも1バイアル当たりの残存する病原体感染リスクは極めて低いことが示された。 | 37 |
| 90014 | 2008/09/29 | 80517 | 感染 | Transfusion 2008; 48: 304-313 | 血小板濃厚液におけるUVC照射の病原体不活化能を検討した。UVC照射は、血小板の品質に影響を及ぼさずに、細菌(表皮ブドウ球菌、黄色ブドウ球菌および大腸菌)ならびに伝播性胃腸炎ウイルスなど広範なウイルス(HIVおよびシミアンウイルス40を除く)を不活化することができた。しかし、HIVのような血液感染性ウイルスに対応するには、UVC法をさらに最適化することが必要である。 | |
| 90014 | 2008/09/29 | 80517 | 感染 | Transfusion 2008; 48: 697-705 | 欧州の3つの血液センターにおけるアモトサレンおよびUVAによるフォトケミカル処理(PCT)過程のプロセスバリデーション試験を行った。フィブリノーゲンおよび第VIII因子はPCTにより平均26%減少したが、治療用血漿として十分なレベルを保持していた。他の凝固因子は対照FFPのレベルの81-97%であった。PCT処理済FFP中の凝固因子が治療用血漿に関する欧州規制および国内基準の範囲内に保持されることが示された。 | |
| 90014 | 2008/09/29 | 80517 | 感染 | Vox Sanguinis 2008; 94: 315-323 | アモトサレンと紫外線A波で光化学処理した血小板(PCT-PLT)の輸血に関連する有害事象を調べるために能動的血液安全監視プログラムを実施した。患者1400名に7437件のPCT-PLTが輸血され、その内、68件が有害事象と関連付けられた。PCT-PLT輸血に関連した急性輸血反応は発現頻度が低く、ほとんどが軽度であった。 | |

| 血対ID | 受理日 | 番号 | 感染症(PT) | 出典 | 概要 | 新出文献No. |
|---|---|---|---|---|---|---|
| 90014 | 2008/09/29 | 80517 | 感染 | Vox Sanguinis 2008; 95(Suppl. 1), 2A-S01-02 | 化学的または光化学的遺伝子修飾に基づいた血液製剤中の病原体不活化(PI)は広範囲のスペクトルの予防的アプローチである。溶媒界面活性剤(SD)およびメチレンブルー法は欧州の多くの国で使われている。アモトサレン(Intercept)、リボフラビンを用いた新しい方法が導入されている。リボフラビン、UVおよび可視光線を用いる血小板(PC)、血漿および赤血球のためのPI法が開発中である。 | 38 |
| 90040 | 2008/10/24 | 80645 | 狂犬病 | ProMED-mail20080826.2660 | 1990年から2007年の中国における狂犬病発生傾向を調べた研究によると、最近8年間でヒト狂犬病症例数が急激に増加したことが明らかとなった。ヒト狂犬病は1990年から1996年の間は全国的な狂犬病ワクチン接種プログラムにより抑制され、わずか159症例が報告されただけであるが、2006年は3279症例と激増した。 | 39 |
| 90014 | 2008/09/29 | 80517 | 原虫感染 | Emerg Infect Dis 2008; 14: 1013-1018 | リーシュマニア症は生物媒介性疾患で、南ヨーロッパに定着しており、毎年700例近く、トルコを含めると3950例のヒトでの感染が報告されている。無症候症例は臨床症例の30～100倍とみられ、また飼い犬の血清陽性率は25%と推定される。薬剤耐性Leishmania infantumがイヌを介して拡大するおそれもある。全ヨーロッパレベルでの研究が必要である。 | 40 |
| 90013 | 2008/09/29 | 80516 | 細菌感染 | American Society for Microbiology 108th General Meeting 2008年6月1-5日 | マサチューセッツの医療センターで品質管理のため使用された廃棄製剤、使用期限切れロット、アフェレーシスの残りの人血清アルブミン製剤を入手し、クラミジアの有無を調べた。その結果、PCR及びウエスタンブロットにより、4社の20製剤全てにおいてクラミジアの存在が確認された。また、in vitro培養を行ったところ11検体(55%)でクラミジア生菌が生育した。 | 41 |
| 90030 | 2008/10/23 | 80632 | 細菌感染 | Transfusion 2008; 48: 1520-1521 | 骨髄異形成症候群と汎血球減少症の79歳男性が、血小板輸血と続いて赤血球1単位の輸血を受けた。 40分後に39.6℃の発熱、硬直、背部痛、低血圧および低酸素症を呈し、輸血は中止された。患者は抗菌剤による治療で回復した。患者の血液および赤血球バッグの残存物からStreptococcus pneumoniae血清型4が検出された。赤血球輸血によるS pneumoniae感染の初めての症例である。 | 42 |
| 90014 | 2008/09/29 | 80517 | 細菌感染 | Vox Sanguinis 2008; 94: 193-201 | ルックバック調査でPropionibacterium acnes汚染が推定される血小板濃縮製剤(PC)の保存から輸血までを追跡したところ、輸血後の有害事象は見られなかった。In vitro試験でプロピオン酸菌属の臨床分離菌をPCに接種し、好気的に22℃で10日間保存という条件下での生育を調べたところ、細菌の生育は緩慢か生育を認めなかった。プロピオン酸菌属はPC保存条件下では増殖しないため、検出されないか、輸血後に検出されると考えられた。 | |

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

別紙様式第2-1

No. 34

# 医薬品 研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 機構処理欄 |
|---|---|---|---|---|---|---|
| | | | | 2008. 8. 18 | 該当なし | |

| 一般的名称 | 新鮮凍結人血漿 | 研究報告の公表状況 | Umemura T, Tanaka E, Kiyosawa K, Kumada H; Japan de novo Hepatitis B Research Group. Clin Infect Dis. 2008 Sep 1;47(5):e52-6. | 公表国 |
|---|---|---|---|---|
| 販売名(企業名) | 新鮮凍結血漿「日赤」(日本赤十字社)<br>新鮮凍結血漿-LR「日赤」(日本赤十字社) | | | 日本 |

**研究報告の概要**

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

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

**使用上の注意記載状況・その他参考事項等**

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

BRIEF REPORT

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

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

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

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

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

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

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

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

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**Clinical Infectious Diseases 2008; 47:e52–6**



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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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## Acknowledgments

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

*Potential conflicts of interest.* All authors: no conflicts.

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別紙様式第2-1

No. 37

# 医薬品 研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 機構処理欄 |
|---|---|---|---|---|---|---|
| | | | | 2008. 6. 23 | 該当なし | |

| 一般的名称 | (製造販売承認書に記載なし) | 研究報告の公表状況 | FDA, CBER. 2008 May 20; Available from: URL: http://www.fda.gov/cber/gdlns/reentrybld.htm | 公表国 米国 |
|---|---|---|---|---|
| 販売名(企業名) | 合成血-LR「日赤」(日本赤十字社)<br>照射合成血-LR「日赤」(日本赤十字社) | | | |

**研究報告の概要**

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

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

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

**使用上の注意記載状況・その他参考事項等**

合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

| 報告企業の意見 | 今後の対応 |
|---|---|
| 米国食品医薬品局(FDA)は、B型肝炎コア抗体が陽性となったために供血延期となった供血者のリエントリー・アルゴリズムを提案するガイダンス案を発表したとの報告である。米国ではもともとHBV感染者が少なくワクチンも普及していることから、日本と状況は異なるが、偽陽性者のリエントリーの方法としては参考になると考えられる。 | 日本赤十字社では、HBs抗原検査及びHBc抗体検査を実施することに加えて、HBVについて20プールでスクリーニングNATを行い、陽性血液を排除している。HBV感染に関する新たな知見等について今後も情報の収集に努める。また、これまでの凝集法と比べて、より感度の高い化学発光酵素免疫測定法(CLEIA)及び精度を向上させた次世代NATを導入した。 |



(2)

# Guidance for Industry

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

### DRAFT GUIDANCE

**This guidance document is for comment purposes only.**

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

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

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

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

**Contains Nonbinding Recommendations**

*Draft –Not For Implementation*

# Table of Contents

**Contains Nonbinding Recommendations**

*Draft –Not For Implementation*

# Guidance for Industry

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

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

## I. INTRODUCTION

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

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

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

## II. BACKGROUND

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

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

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

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

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

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

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

### B. Rationale for the Requalification Method for Reentry

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

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

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

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

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

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

## III. RECOMMENDATIONS

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

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

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

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

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

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

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

and

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

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

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

## IV. IMPLEMENTATION

This guidance is being distributed for comment purposes only.

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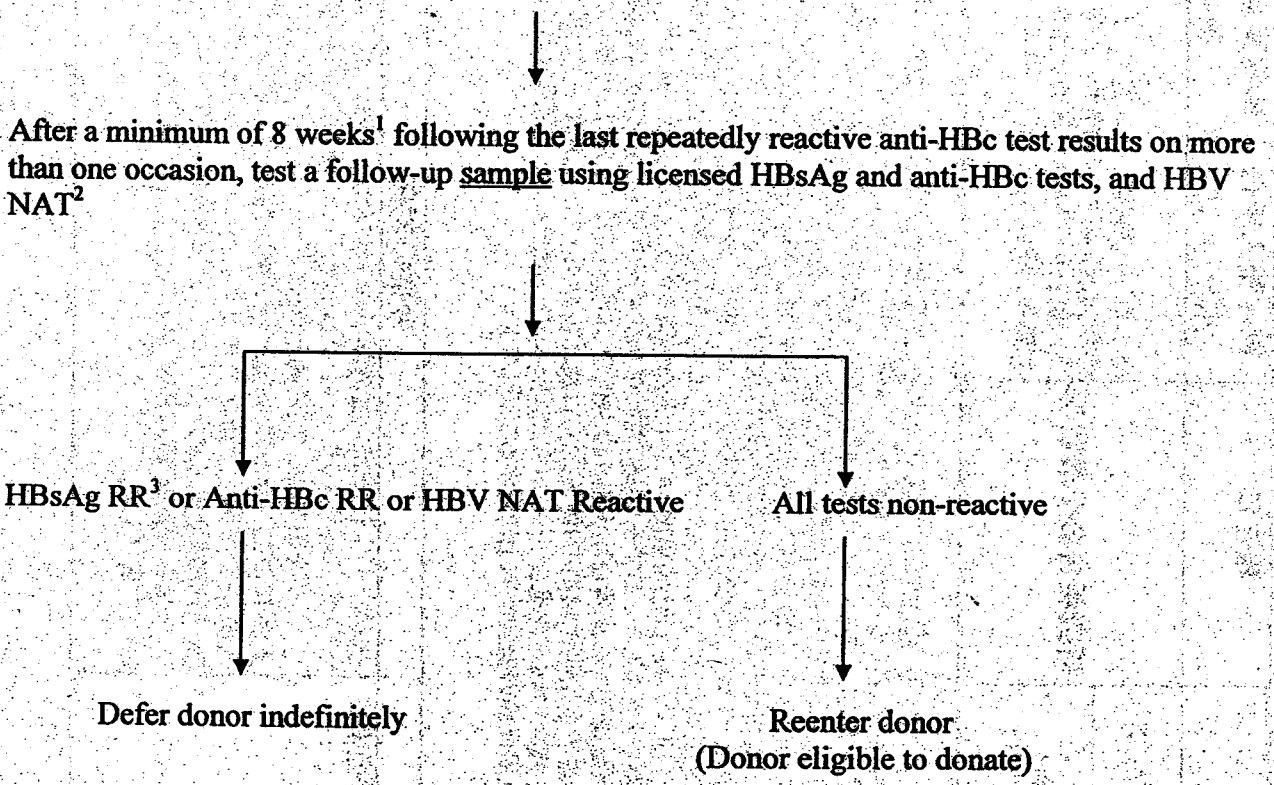
## APPENDIX

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




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

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

[3] Regardless of the neutralization test result.

別紙様式第２－１



# 医薬品　研究報告 調査報告書

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

**研究報告の概要**

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

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

**使用上の注意記載状況・その他参考事項等**

**重要な基本的注意**

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

ELSEVIER

Journal of Hepatology 48 (2008) 1022–1025

Journal of Hepatology

www.elsevier.com/locate/jhep

Case Report

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

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

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

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

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


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

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

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

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

## 1. Introduction

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

0168-8278/$34.00 
doi:10.1016/j.jhep.2008.02.016

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

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

## 2. Case report

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

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

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

### 2.1. Methods

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

## 3. Results and discussion

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

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

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

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

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

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

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

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

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

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

## Acknowledgements

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

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

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別紙様式第 2-1
番号 1

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<br>2008 年 3 月 13 日 | 新医薬品等の区分 | 厚生労働省処理欄 |
|---|---|---|---|---|---|---|

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

**研究報告の概要**

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

**使用上の注意記載状況・その他参考事項等**

代表としてノイアート（献血）の記載を示す。
2. 重要な基本的注意
(1) 本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-I 抗体陰性で、かつ ALT(GPT)値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分から人アンチトロンビン III を濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及びウイルス除去膜によるろ過処理を施しているが、投与に際しては、次の点に十分注意すること。

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



ヘパリン

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



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

# 屠宰猪肝和血清中乙型肝炎病毒及戊型肝炎病毒的检测

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

关键词:猪;乙型肝炎病毒;戊型肝炎病毒;电镜观察

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

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

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

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

收稿日期:2007-06-11;修回日期:2007-10-28
基金项目:国家自然科学基金项目(30771588)
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颗粒仅有很窄的宿主谱，一般可致持续病毒感染，而且有较明确的嗜肝性[1]。资料表明，畜禽中也存在一个嗜肝病毒群。自20世纪80年代以来，国内有关猪、鸡、牛、羊、犬等动物检出乙肝病毒表面抗原(HBsAg)的报道屡见不鲜，并有分离到类乙肝病毒的事实[2-9]。2006年余锐萍等[10-11]应用免疫组织化学方法对北京、河南等地肉联厂屠宰猪肝的人乙型肝炎病毒抗原进行检测，结果发现人乙型肝炎病毒抗原总检出率高达73%～100%。

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

## 1 材料与方法

### 1.1 样品

屠宰猪肝、血液采自北京市某肉联厂。

### 1.2 酶与试剂

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

### 1.3 SHBV的PCR检测

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

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

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

表1 用于检测猪体内HBV和HEV的引物

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

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

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

### 1.4 免疫电镜负染色样品的制备

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

### 1.5 HEV RNA的巢式PCR检测

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

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

## 2　结果

### 2.1　SHBV 检测

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

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

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

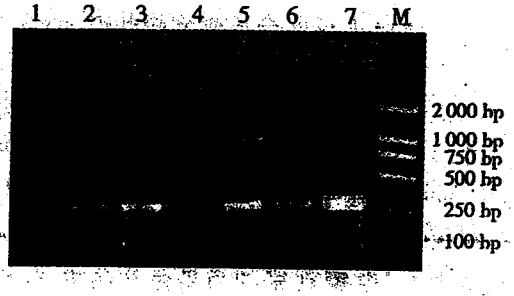


图 1　猪血清和肝中 HBV S 基因 PCR 产物的电泳结果

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

M：DNA 分子质量标准；1：阴性对照；2～4：血清样品；5～7：肝样品

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

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

图 2　扩增片段与 HBV X04615 株序列的同源性比较

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

### 2.2　HEV 的 RT-PCR 扩增

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

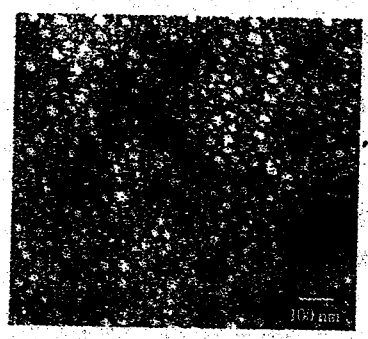

图 3　电镜下观察到的猪乙肝病毒颗粒

Fig. 3　Porcine HBV particles observed by TEM

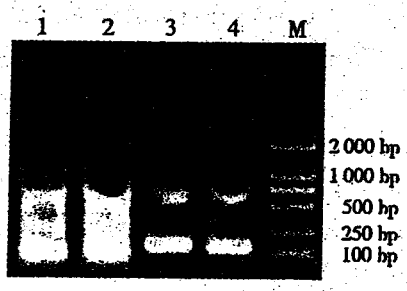


图 4　屠宰猪肝 HEV 的巢式 RT-PCR 电泳结果

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

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

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

## 3 讨论

### 3.1 关于猪乙肝病毒

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

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

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

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

### 3.2 关于戊肝病毒

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

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

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

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

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

## 1 材料および方法

### 1.1 サンプル

食肉処理したブタの肝臓および血液は、北京の某食肉生産連合にて採集を行った。

### 1.2 酵素および試薬

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

### 1.3 SHBV の PCR 検査

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

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

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

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

### 1.4 免疫電子顕微鏡を用いたネガティブ染色サンプルの調製

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

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

### 1.5 ネステッド PCR 法を用いた HEV RNA の検出

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

## 2 結果

### 2.1. SHBV 検査

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

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

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

### 2.2 HEV の RT-PCR による増幅

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

## 3 考察

### 3.1 ブタのB型肝炎ウイルスについて

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

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

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

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

### 3.2 E型肝炎について

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

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

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

別紙様式第2-1

No. 36

# 医薬品 研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 機構処理欄 |
|---|---|---|---|---|---|---|
| | | | | 2008. 8. 18 | 該当なし | |

| 一般的名称 | 新鮮凍結人血漿 | 研究報告の公表状況 | Girou E, Chevaliez S, Challine D, Thiessart M, Morice Y, Lesprit P, Tkoub-Scheirlinck L, Soing-Altrach S, Cizeau F, Cavin C, André M, Dahmanne D, Lang P, Pawlotsky JM. Clin Infect Dis. 2008 Sep 1;47(5):627-33. | 公表国 フランス |
|---|---|---|---|---|
| 販売名(企業名) | 新鮮凍結血漿「日赤」(日本赤十字社)<br>新鮮凍結血漿-LR「日赤」(日本赤十字社) | | | |

**研究報告の概要**

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

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

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

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

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

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

**使用上の注意記載状況・その他参考事項等**

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

MAJOR ARTICLE

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

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

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

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

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

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

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

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

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

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**Clinical Infectious Diseases 2008; 47:627–33**

1058-4838/2008/4705-0007$15.00
DOI: 10.1086/590564

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

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

## PATIENTS AND METHODS

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

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

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

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

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

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

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

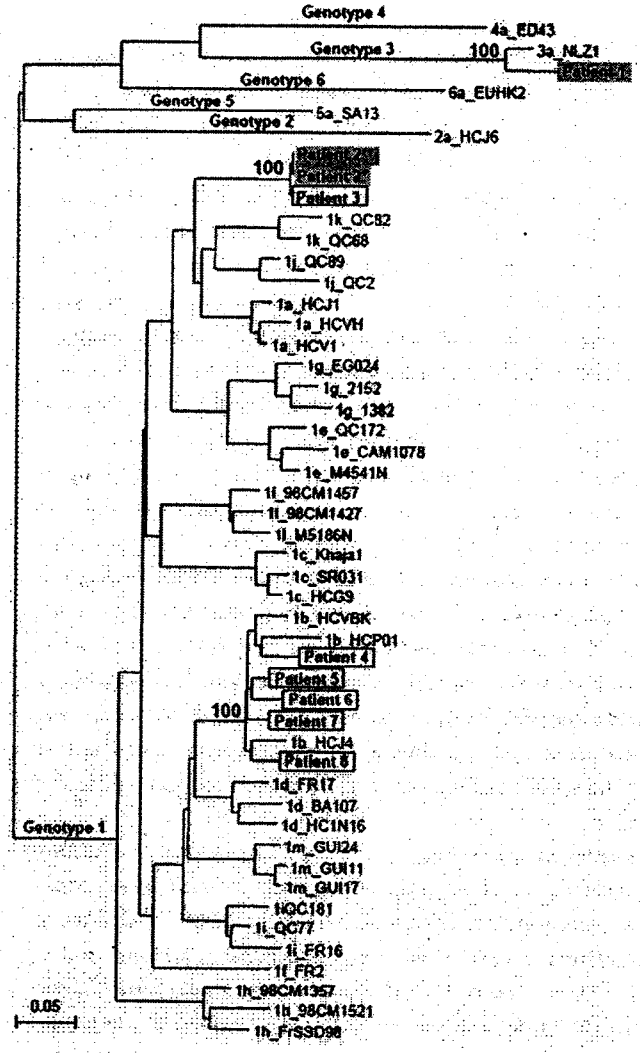


**Figure 1.** Phylogenetic tree plotted with nonstructural 5B sequences (nucleotide positions 8271–8597) from the 2 patients recently infected in our hemodialysis unit (patients 1 and 2; *shaded boxes*), the 6 patients known to be chronically infected with hepatitis C virus (HCV) and regularly treated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

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

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

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

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

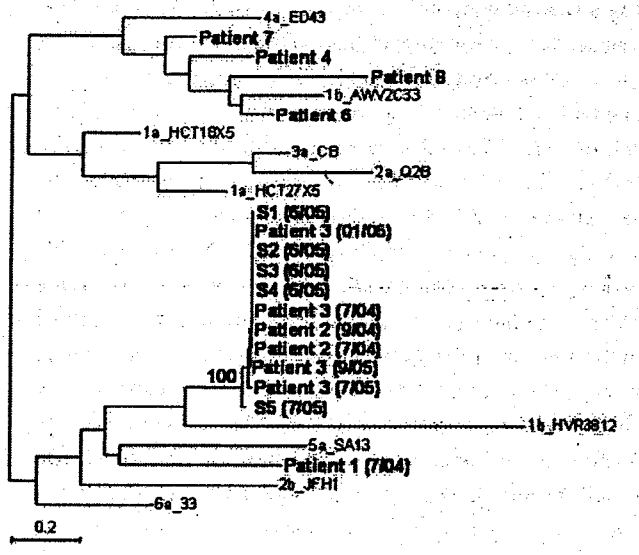


**Figure 2.** Phylogenetic tree plotted with hypervariable region (HVR) 1 sequences from the 6 patients known to be chronically infected with hepatitis C virus (HCV) and regularly treated in our hemodialysis unit (patients 3–8), including the patient who transmitted HCV to patient 2 (i.e., patient 3),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

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

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

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

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

## RESULTS

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

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

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

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

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

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

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

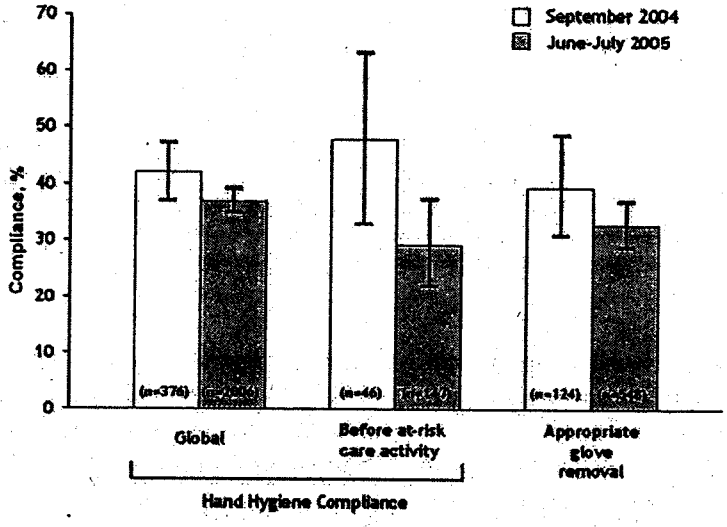


**Figure 3.** Compliance with guidelines for health care worker hand hygiene and appropriate glove use during dialysis. At-risk care activities consisted of handling dialysis catheters or fistulas. Whiskers, 95% CIs.

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

## DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

## Acknowledgments

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

***Potential conflicts of interest.*** All authors: no conflicts.

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別紙様式第2-1

No. 41

# 医薬品 研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 機構処理欄 |
|---|---|---|---|---|---|---|
| | | | | 2008. 6. 23 | 該当なし | |

| 一般的名称 | (製造販売承認書に記載なし) | 研究報告の公表状況 | | 公表国 |
|---|---|---|---|---|
| 販売名(企業名) | 合成血-LR「日赤」(日本赤十字社)<br>照射合成血-LR「日赤」(日本赤十字社) | | Galiana C, Fernández-Barredo S, García A, Gómez MT, Pérez-Gracia MT. Am J Trop Med Hyg. 2008 Jun;78(6):1012-5. | スペイン |

**研究報告の概要**

○養豚業従事者のE型肝炎ウイルス(HEV)への職業的曝露

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

| 報告企業の意見 | 今後の対応 |
|---|---|
| 職業上のブタ接触群と非接触群のHEVの陽性率とウイルス感染リスク因子を調べたところ、抗HEV IgG抗体陽性率はブタ接触群が有意に高く、陽性者は未処理水の摂取およびブタへの接触という2つのリスクファクターを示したとの報告である。HEV感染については血液の安全対策上だけではなく、公衆衛生及び食品衛生上の問題でもある。 | 日本赤十字社では、輸血による肝炎ウイルス感染防止のため、血液中のALT値61IU/L以上の血液を排除している。また、厚生労働科学研究「E型肝炎の感染経路・宿主域・遺伝的多様性・感染防止・診断・治療に関する研究班」と共同して、献血者におけるHEV感染の疫学調査を行っている。加えて、北海道における輸血後HEV感染報告を受け、試験的に北海道では研究的NATを行うなど安全対策を実施している。今後もHEV感染の実態に関する情報の収集及び安全対策に努める。 |

**使用上の注意記載状況・その他参考事項等**

合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク



6

*Am. J. Trop. Med. Hyg.*, 78(6), 2008, pp. 1012–1015


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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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別紙様式第２－１



# 医薬品　研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 総合機構処理欄 |
|---|---|---|---|---|---|---|
| 一般的名称 | 血小板 | | 研究報告の公表状況 | Transfusion (United States) Jul2008, 48 (7) p1368-75. | 公表国<br>米国 | |
| 販売名（企業名） | － | | | | | |

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

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

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

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

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

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

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

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

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

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

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

doi: 10.1111/j.1537-2995.2008.01722.x

TRANSFUSION 2008;48:1368-1375.

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

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

## MATERIALS AND METHODS

### Detection and quantitation of HEV RNA

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

### Phylogenetic analysis of HEV isolates

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

### Detection of serum anti-HEV

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

### Alanine aminotransferase testing

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

## RESULTS

### A lookback study of a causative blood donor

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

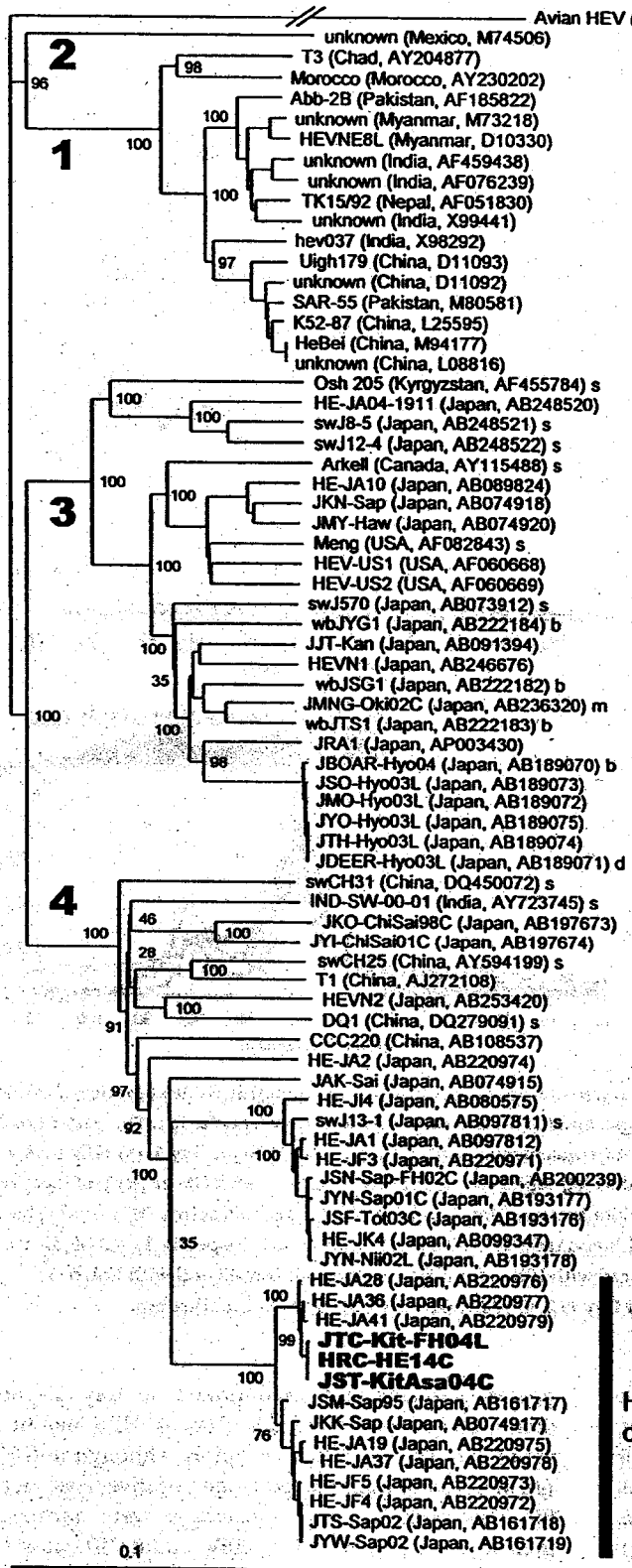


**Fig. 1. Phylogenetic tree constructed by the neighbor-joining method based on the entire or nearly entire sequences of HEV genome of 77 isolates using an avian HEV (AY535004) as an outgroup. After the isolate name, the name of the country where the strain was isolated and accession numbers are shown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

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

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

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

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

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

### Clinical course of the patient

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

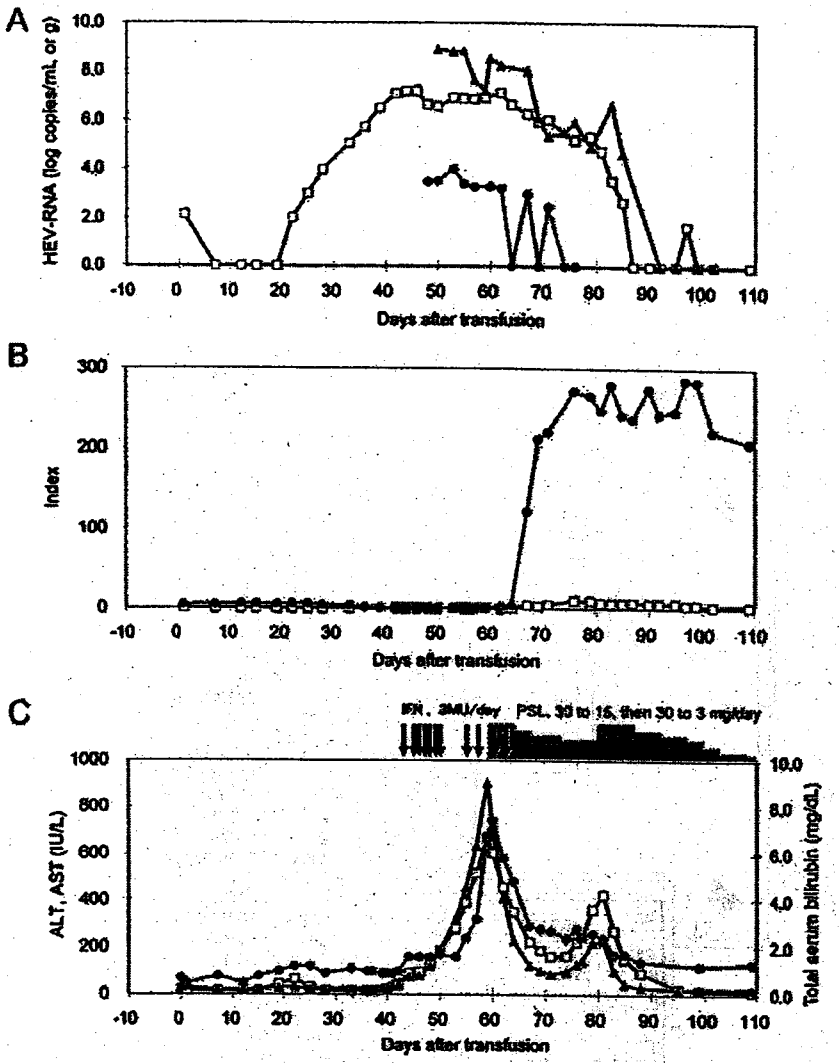


**Fig. 2. Clinical course of transfusion-transmitted hepatitis E with kinetics of (A) HEV RNA, (B) serologic, and (C) biochemical markers after transfusion. The patient had transfusion of PLT concentrates contaminated with HEV on Day 0. (A) HEV RNA load was represented as log copies per mL of serum (□)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

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

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

## DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

#### ACKNOWLEDGMENTS

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

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## APPENDIX 1

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

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

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

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|---|---|---|---|---|---|---|
| 一般的名称 | ①乾燥抗HBs人免疫グロブリン<br>②ポリエチレングリコール処理抗HBs人免疫グロブリン | | 研究報告の公表状況 | Vox Sanguinis 2008; 95(SUPPL. 1): 282-283 | 公表国<br>中国 | |
| 販売名（企業名） | ①ヘブスブリン（ベネシス）<br>②静注用ヘブスブリンーIH（ベネシス） | | | | | |

**研究報告の概要**

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

**使用上の注意記載状況・その他参考事項等**

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

| 報告企業の意見 | 今後の対応 |
|---|---|
| 中国における血液ドナーの約1%は、抗HEV IgM陽性又はHEV抗原陽性であり、HEV感染の可能性があるとの報告である。<br>静注用ヘブスブリン-IHについては、万一、原料血漿にHEVが混入したとしても、EMCおよびCPVをモデルウイルスとしたウイルスバリデーション試験成績から、本剤の製造工程において十分に不活化・除去されると考えている。<br>ヘブスブリンについては、EMCおよびCPVをモデルウイルスとしたウイルスバリデーション試験成績では本剤の製造工程において十分なLRVが得られないため、製造工程における不活化・除去が十分であるとは説明困難である。そのため、ヘブスブリン用の原料血漿については、弊社にてHEVについてのミニプールNATを試行的に導入した。 | 本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。 |

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

P-615

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

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In spite of the progress in the development of diagnostic, therapeutic and prophylactic methods, virus hepatitis still present a serious global health problem. The possibility of transmission of these infections through transfusion of blood and blood derivates implies obligatory control of the donated blood.
Aim: To show the prevalence of Hepatitis B (HBsAg) in volunteer blood donors for the period from 2001 till 2006.
Materials: The presence of virus markers was analyzed in the serum of 9166 blood donors who donated blood at the Department of transfuziology, General Hospital-Strumica, in the period from 2001 till 2006.
Methods: The samples were tested for the presence of viral markers (HBsAg), using tests for HBsAg (Abbott Auxyme Monoclonal EIA).
Results: The presence of markers for Hepatitis B (HBsAg) were found in 89 (0.97%) blood donors. In 2001 the presence of HBsAg was found in 12 blood donors, 2002 – in 20 blood donors, 2003 in 14 blood donors, 2004 in 17 blood donors, 2005 in 14 blood donors, 2006 in 12 blood donors. With A blood group were 42 (47.2%) blood donors, with 0 blood group were 28 (31.4%) blood donors, with B blood group were 10 (11.2%) blood donors and with AB blood group were nine (10.2%) blood donors.
Conclusion: The obligatory testing of the donors blood is of exceptional importance to prevent the transmission of diseases. Moreover, a significant ring in the chain for ensuring safe blood is the selection of a qualitative donor, that is a donor who donates blood volontarily, freely, anonymously and periodically.

P-616

## OCCULT HEPATITIS B VIRUS INFECTION IN BLOOD DONORS FROM CENTRAL PORTUGAL

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Background: The detection of HBV DNA in serum without HBsAg and with/without the presence of antibodies (anti-HBc/anti-HBs), defines the state of the occult hepatitis B virus infection. The prevalence in endemic areas varies from 7% to 19%, while in the west countries varies from 0% to 9%, being greater in people with anti-HBc and/or anti-HBs. Low serum HBV DNA titers, in the range of 100–1000 copies/mL, are typical in occult HBV infection. A high prevalence of occult HBV has been reported in hepatocellular carcinoma (HCC).
Aims: The appearance of the nucleic acid testing (NAT) with great sensibility allows us to identify a population with HBsAg negative but with low levels of HBV DNA in serum. In our Centre all donors are screened for HBV DNA, HIV RNA and HCV RNA.
Methods: In the screening of the hepatitis B serologic markers we have used ELISA and quimioluminiscence tests. In the screening of the HBV DNA we have used the Transcription Mediated Amplification (TMA) technology, in single testing, with predicted HBV detection rate of 50% and 95% of 3.1 and 7.4 IU/mL, respectively.In the screening of HBV viral load we have used PCR technology, with detection limit of 60 IU/mL.
Results: The Regional Blood Centre (Coimbra) started the screening of the HBV DNA to all donors in October 2006. Until November 2007 we have studied 28.881 donors. We found three cases of occult hepatitis B virus infection.
Conclusions: Some aspects need to be investigated, especially the relationship between the occult hepatitis B virus infection and the infectivity of the different blood components. The sensibility of the NAT is very important in the precocious detection of the HBV DNA in blood donors.

P-617

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

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

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

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

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

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

Table HEV Seroprevalence in blood donors

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

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

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

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

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

P-618

~~Abstract withdrawn.~~

P-619

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

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This abstract is read by title only.

P-620

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

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

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

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

Effect of I-P Adoption on Platelet and RBC Use

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

 

別紙様式第2-1

No. 30

# 医薬品 研究報告 調査報告書

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

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

**研究報告の概要**

○米国医師会がゲイ男性の供血5年延期を「容認できる」との考え
米国医師会(AMA)は、男性同性愛行為を行った供血者の供血延期期間を生涯から5年間に変更するとした連邦の方針を支持するという声明を採択した。この声明は2008年のAMA年次総会で採択され、「AMAは、現在の科学的エビデンスとリスク分析モデルに基づき、MSMに対する5年間の供血延期は容認できる(supportable)と認める」と述べている。AMAによると、「容認できる」という言葉は、基本的に、FDAに対して新しい方針を通知し「実施に協力する」ことを意味している。また、AMAは今回の変更に対して反対を主張しない。
FDAは1977年以降、採血事業者に対し、MSMの供血を生涯延期とすることを求めてきた。AMAの声明は、血液事業者団体が主張する1年間の供血延期により近いものとなっている。血液事業者は、供血延期は金銭や薬物と引き替えのセックスなどハイリスク行為に対して実施すべきであると主張してきた。また、最近ではゲイ・グループによる反対運動、政府機関や大学での議論も行われ、一部の大学では構内での移動採血を中止しようとする動きが出ていた。

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

**使用上の注意記載状況・その他参考事項等**

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# ABC NEWSLETTER

CURRENT EVENTS AND TRENDS IN BLOOD SERVICES

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2008 #26 July 4, 2008

INSIDE:



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

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

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

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

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

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

(continued on page 2)

AMA Statement (continued from page 1)

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

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

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

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

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

(continued on page 3)

The *ABC Newsletter* (ISSN #1092-9412) is published 46 times per year by America's Blood Centers and distributed by e-mail.

Contents and views expressed are not official statements of ABC or its Board of Directors.



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*ABC Newsletter* Co-Editors: Robert Kapler and Jane Starkey

Classified Advertising Manager: Deanna DeLuca

Annual Subscription Rate: $348
*(Residents, Fellows and SBB Students: $120)*

Publication Office:
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Tel: (202) 393-5725
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## Finnish Parliament Finds Red Cross MSM Policy Justified

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

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

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

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

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

AMA Statement (continued from page 2)

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

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

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

別紙様式第2-1

No. 13

# 医薬品 研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 機構処理欄 |
|---|---|---|---|---|---|---|
| | | | | 2008. 6. 23 | 該当なし | |

| 一般的名称 | (製造販売承認書に記載なし) | 研究報告の公表状況 | Custer S, Kamel HT, Tomasulo PA, Murphy EL, Busch MP. XXIIXth Congress of the International Society of Blood Transfusion; 2008 Jun 7-12; Macao. | 公表国 |
|---|---|---|---|---|
| 販売名(企業名) | 合成血-LR「日赤」(日本赤十字社)<br>照射合成血-LR「日赤」(日本赤十字社) | | | 米国 |

**研究報告の概要**

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

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

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

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

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

| 報告企業の意見 | 今後の対応 |
|---|---|
| 米国の供血者を対象にT. cruzi検査が導入された後10ヶ月間で、陽性確認率は0.0043%だった。症状に関連した質問は地理的なリスク要因の質問よりも有益ではないと考えられること、全供血のスクリーニングは費用対効果が低く、出生地と初回供血者に対象を絞ったスクリーニング戦略の検討が示唆されたとの報告である。 | 日本赤十字社は、輸血感染症対策として献血時に海外渡航歴の有無を確認し、シャーガス病の既往がある場合には献血不適としている。日本在住の中南米出身献血者については、厚生労働科学研究「献血血の安全性確保と安定供給のための新興感染症等に対する検査スクリーニング法等の開発と献血制限に関する研究」班と共同して検討する予定である。今後も引き続き情報の収集に努める。 |

**使用上の注意記載状況・その他参考事項等**

合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

# S19 - Emerging Infections

3C-S19-01

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

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

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

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

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

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

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

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

3C-S19-02

## EVALUATING THE EFFECTIVENESS OF MALARIA DEFERRALS THROUGH ANTIBODY TESTING

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

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

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

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

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

3C-S19-03

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

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

 

別紙様式第2-1

No. 33

# 医薬品 研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 機構処理欄 |
|---|---|---|---|---|---|---|
| | | | | 2008. 6. 23 | 該当なし | |

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

**研究報告の概要**

○パンデミックインフルエンザが米国の血液供給に与える影響のシミュレーション

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

| 報告企業の意見 | 今後の対応 |
|---|---|
| 米国におけるパンデミックインフルエンザのシミュレーションで、3ヶ月間の血液供血量が50%減少した場合、血液需要に制限がない場合は血液在庫のほとんどを使い尽くしたが、血液の使用を必要最低限に制限した場合は血液在庫がなくなることはなかったとの報告である。日本赤十字社では家禽に高病原性トリインフルエンザの流行が認められた場合、当該飼養農場の関係者や防疫作業従事者の献血制限を行っている。 | 日本においてもパンデミックインフルエンザの発生が予期されることから、安全な血液の安定供給を確保し血液事業を継続するための対応計画を検討する必要がある。今後も引き続き情報の収集に努める。 |

**使用上の注意記載状況・その他参考事項等**

合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

(二)

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

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

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

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

### 3C-S19-04

## SCREENING OF BLOOD DONORS FOR CHIKUNGUNYA VIRUS – DEVELOPMENT AND EVALUATION OF MINIPOOL-NAT AND ANTIBODY TESTS

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

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

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

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

### 3C-S19-05

## SIMULATING THE IMPACT OF PANDEMIC INFLUENZA ON THE US BLOOD SUPPLY

Walderhaug M, Menis M

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

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-6

| 識別番号・報告回数 | | 回 | 報告日<br>年　月　日 | 第一報入手日<br>2008 年 6 月 4 日 | 新医薬品等の区分<br>該当なし | 総合機構処理欄 |
|---|---|---|---|---|---|---|
| 一般的名称 | | | 研究報告の公表状況 | New arenavirus discovered in Bolivia<br>Lancet Infect Dis 2008; 8: 355 | 公表国<br>米国 | |
| 販売名（企業名） | | | | | | |

**研究報告の概要**

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

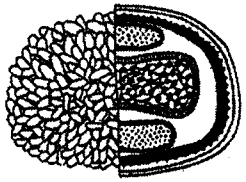
**使用上の注意記載状況・その他参考事項等**

BYL-2008-0336

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

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

Arenavirus



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

# Highlights from the 18th ECCMID

## First European Infection Day

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

## ECCMID research highlights

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

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

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

*Sally Hargreaves*

The printed journal includes an image merely for illustration

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

# New arenavirus discovered in Bolivia

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

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

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

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

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

*Cathel Kerr*

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

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

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

医薬品　研究報告　調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日<br>2008年9月16日 | 新医薬品等の区分<br>該当なし | 総合機構処理欄 |
|---|---|---|---|---|---|---|
| 一般的名称 | 別紙のとおり | | 研究報告の公表状況 | Proc Natl Acad Sci U S A.<br>2008;105:14124-14129 | 公表国<br>米国 | |
| 販売名（企業名） | 別紙のとおり | | | | | |

**研究報告の概要**

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

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

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

**使用上の注意記載状況・その他参考事項等**

記載なし

MedDRA/J ver.11.1



(3)

| | |
|---|---|
| 一般的名称 | ①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤乾燥ペプシン処理人免疫グロブリン、⑥乾燥スルホ化人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン*、⑧乾燥濃縮人活性化プロテインC、⑨乾燥濃縮人血液凝固第Ⅷ因子、⑩乾燥濃縮人血液凝固第Ⅸ因子、⑪乾燥抗破傷風人免疫グロブリン、⑫抗HBs人免疫グロブリン、⑬トロンビン、⑭フィブリノゲン加第ⅩⅢ因子、⑮乾燥濃縮人アンチトロンビンⅢ、⑯ヒスタミン加人免疫グロブリン製剤、⑰人血清アルブミン*、⑱人血清アルブミン*、⑲乾燥ペプシン処理人免役グロブリン*、⑳乾燥人血液凝固第Ⅸ因子複合体*、㉑乾燥濃縮人アンチトロンビンⅢ |
| 販売名(企業名) | ①献血アルブミン20“化血研”、②献血アルブミン25“化血研”、③人血清アルブミン“化血研”*、④“化血研”ガンマーグロブリン、⑤献血静注グロブリン“化血研”、⑥献血ベニロンーⅠ、⑦ベニロン*、⑧注射用アナクトC2,500単位、⑨コンファクトF、⑩ノバクトM、⑪テタノセーラ、⑫ヘパトセーラ、⑬トロンビン“化血研”、⑭ボルヒール、⑮アンスロビンP、⑯ヒスタグロビン、⑰アルブミン20%化血研*、⑱アルブミン5%化血研*、⑲静注グロブリン*、⑳ノバクトF*、㉑アンスロビンP1500注射用 |
| 報告企業の意見 | cardiovirusは、picornavirus科に分類される属名の一つである。cardiovirus属のウイルスはエンベロープを持たず、直径約30nmで正20面体のカプシッドを持ち、核酸は一本のプラス鎖RNAである。cardiovirus属には次の2つのサブグループがある；脳心筋炎ウイルス(encephalomyocarditis virus；EMCV)、タイラーのマウス脳脊髄炎ウイルス(Theiler's murine encephalomyelitis virus；TMEV)。これらのウイルスは、げっ歯類に感染し消化器官で増殖した後、糞便経口ルートで伝播する。ウイルスが腸管感染しても大抵は軽度か無症状であるが、腸管外に拡がると全身性の疾患を惹き起こす。EMCV系統のウイルスは脳炎及び心筋炎を惹き起こし、TMEV系統のウイルスは中枢神経系感染に関連している。ヒトから分離されたとされるcardiovirus属のウイルスも報告されているが、ヒトから直接クローニングされたことはなく、その罹患率、多様性、ヒトでの症状等についてはあまり知られていない。<br>本剤の製造工程には、冷エタノール分画工程、ウイルス除去膜ろ過工程あるいは加熱工程等の原理の異なるウイルス除去及び不活化工程が存在しているので、ウイルスクリアランスが期待される。各製造工程のウイルス除去・不活化効果は、「血漿分画製剤のウイルスに対する安全性確保に関するガイドライン(医薬発第1047号、平成11年8月30日)」に従い、ウシウイルス性下痢ウイルス(BVDV)、仮性狂犬病ウイルス(PRV)、ブタパルボウイルス(PPV)、A型肝炎ウイルス(HAV)または脳心筋炎ウイルス(EMCV)をモデルウイルスとして、ウイルスプロセスバリデーションを実施し、評価を行っている。今回報告したcardiovirus属には、モデルウイルスとして使用しているEMCVそのものが属しており、上記バリデーションの結果から、本剤の製造工程がEMCVの除去・不活化効果を有することを確認している。また、これまでに本剤によるcardiovirus感染の報告例は無い。<br>以上の点から、本剤はcardiovirusに対する安全性を確保していると考える。 |

*現在製造を行っていない

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

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

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

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

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

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

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

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

The authors declare no conflict of interest.

Freely available online through the PNAS open access option.

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





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

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

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

## Results

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

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

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

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

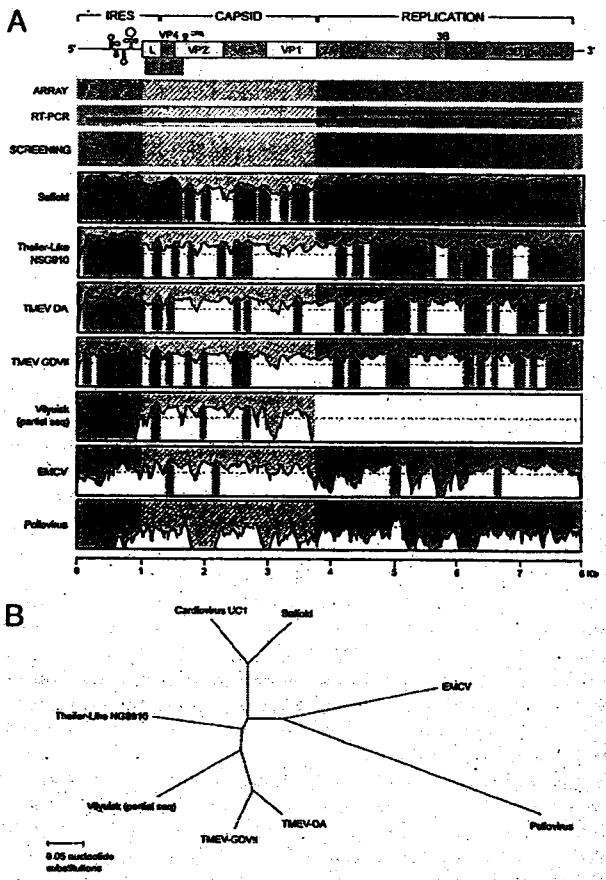


Fig. 1. Genome sequence of UC1. (*A*) Genome sequence similarity plots compare UC1 with Saffold virus, Theiler-Like NGS910 virus, TMEV-DA, TMEV-GDVII, Vilyuisk virus (partial sequence only), EMCV, and poliovirus. The *y* axis scale for each plot represents percentage of nucleotide identities from 0%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

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

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

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

**Table 1. Amino acid identity of predicted UC1 proteins**

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

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

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

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

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

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

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

**Table 2. Patients with stool positive for cardioviruses**

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

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

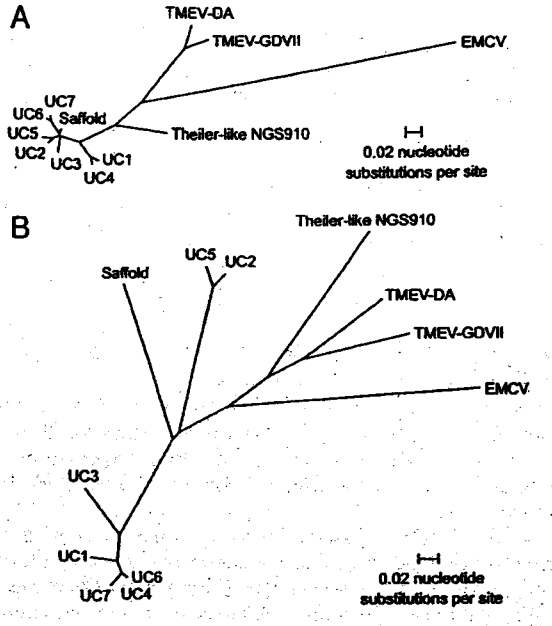


**Fig. 2.** Strain variation of human cardioviruses. (*A*) Radial tree of a 608-bp region within the 5′-UTR. (*B*) Radial tree of an 819-bp region corresponding to the VP1 gene. Strain designations UC2 to UC7 correspond to patients as listed in Table 2.

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

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

## Discussion

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

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

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

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

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

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

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

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

## Materials and Methods

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

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

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

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

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

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

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

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

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

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別紙様式第2-1

No. 23

# 医薬品 研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 機構処理欄 |
|---|---|---|---|---|---|---|
| | | | | 2008. 7. 23 | 該当なし | |

| 一般的名称 | (製造販売承認書に記載なし) | 研究報告の公表状況 | | 公表国 |
|---|---|---|---|---|
| 販売名(企業名) | 合成血-LR「日赤」(日本赤十字社)<br>照射合成血-LR「日赤」(日本赤十字社) | | ProMED 20080720.2201, 2008 Jul 20. 情報源:HeraldonSunday online, 2008 Jul 20. | オーストラリア |

**研究報告の概要**

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

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

**使用上の注意記載状況・その他参考事項等**

合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

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

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

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

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

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

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

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

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

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

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

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

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

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

[see also:
Hendra virus, human, equine - Australia (02): (QLD,NSW) 20080717.2168
Hendra virus, human, equine - Australia: (QLD) 20080715.2146
Hendra virus, equine - Australia: (Brisbane) 20080708.2076
2007
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Hendra virus, human, equine - Australia (QLD) (04): 2nd corr. 20070903.2902
Hendra virus, human, equine - Australia (QLD) (03): corr. 20070903.2896
Hendra virus, human, equine - Australia (QLD) (02): not 20070831.2871
Hendra virus, human, equine - Australia (QLD): RFI 20070830.2851
2006
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Hendra virus, equine - Australia (NSW): susp. 20061109.3222
2004
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Hendra virus - Australia (QLD) 20041214.3307
1999
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Hendra virus - Malaysia, Singapore: Fact sheet 19990319.0434
Hendra virus, horse - Australia (Queensland) 19990219.0218]

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別紙様式第 2-1
番号 11

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

| 識別番号・報告回数 | | | 報告日 | 第一報入手日<br>2008 年 7 月 16 日 | 新医薬品等の区分<br>該当なし | 厚生労働省処理欄 |
|---|---|---|---|---|---|---|
| 一般的名称 | 乾燥濃縮人血液凝固第Ⅷ因子 | | 研究報告の公表状況 | TRANSFUSION 2008; 48: 1180-1187 | 公表国<br>アメリカ | |
| 販売名（企業名） | コンコエイト-HT（ベネシス） | | | | | |

113

**研究報告の概要**

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

**使用上の注意記載状況・その他参考事項等**

2. 重要な基本的注意
(1) 本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-I 抗体陰性で、かつ ALT (GPT) 値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、人血液凝固第Ⅷ因子-vWF 複合体を濃縮・精製した製剤であり、ウイルス不活化を目的として、製造工程においてリン酸トリ-n-ブチル（TNBP）/ポリソルベート 80 処理、凍結乾燥の後、60℃、72 時間の加熱処理を施しているが、投与に際しては、次の点に十分注意すること。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 健常人血液ドナーから EBV、HHV-7、HHV-6 が高頻度に検出され、また HHV-6 については異常に高いレベルのウイルスが検出されたとの報告である。<br>万一、原料血漿にヘルペスウイルスが混入したとしても、BHVをモデルウイルスとしたウイルスバリデーション試験成績から、本剤の製造工程において十分に不活化・除去されると考えている。 | 本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。 |

BENE2008-012

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

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

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

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

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

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

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

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

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

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

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

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

doi: 10.1111/j.1537-2995.2008.01685.x

TRANSFUSION 2008;48:1180-1187.

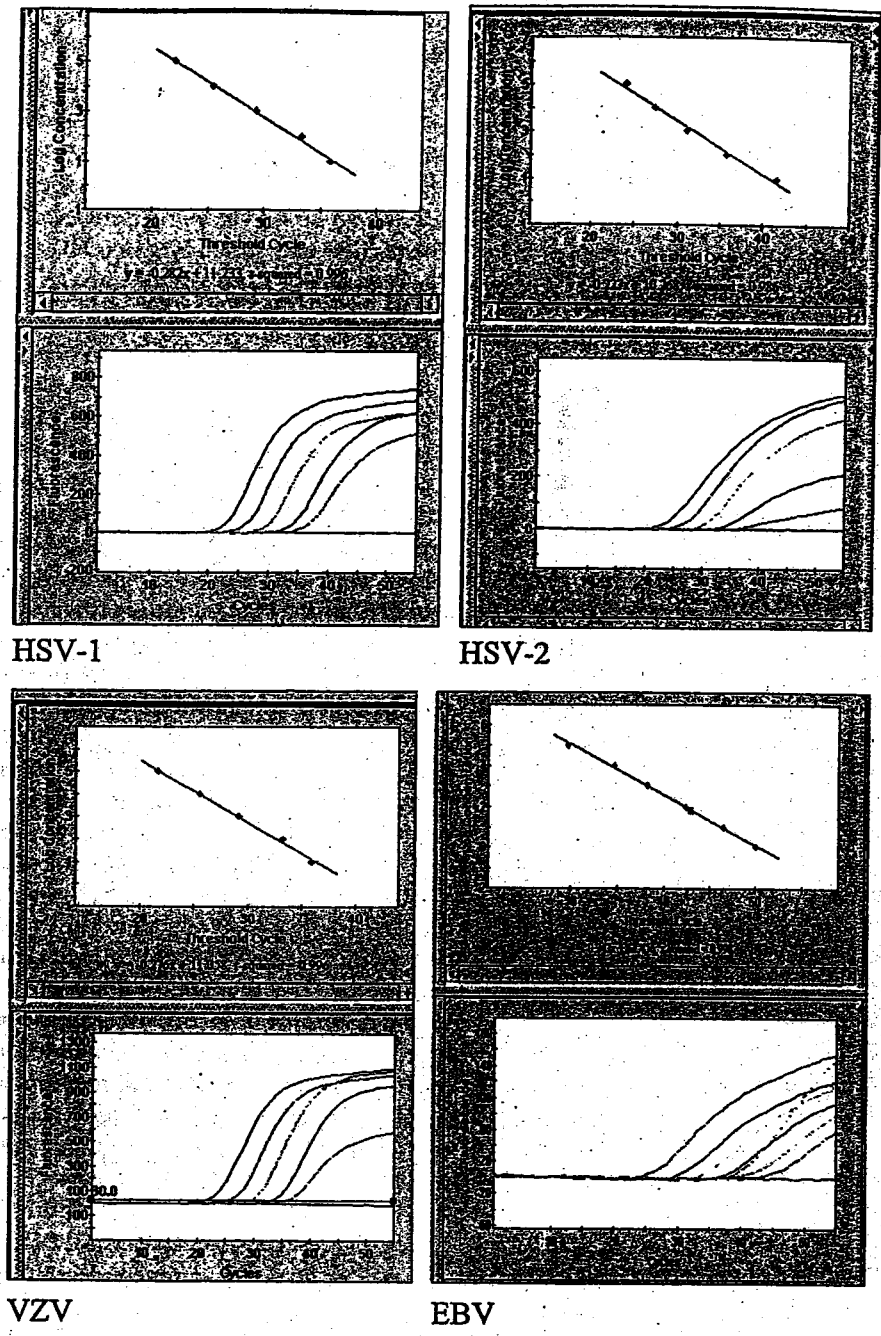


**Fig. 1. Real-time PCR standard curves. The top panel displays the linear relationship between log concentration of viral DNA and PCR cycle. The bottom panel demonstrates the relationship between fluorescence signal intensity and PCR cycle. The curves from left to right in the lower panel represent serial dilutions of viral DNA—$10^5$, $10^4$, $10^3$, $10^2$, and $10^1$ viral geq per PCR procedure (per µg). Results for $10^6$ geq are shown only for EBV, CMV, and HHV-8.**

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

## MATERIALS AND METHODS

### Real-time PCR

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

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

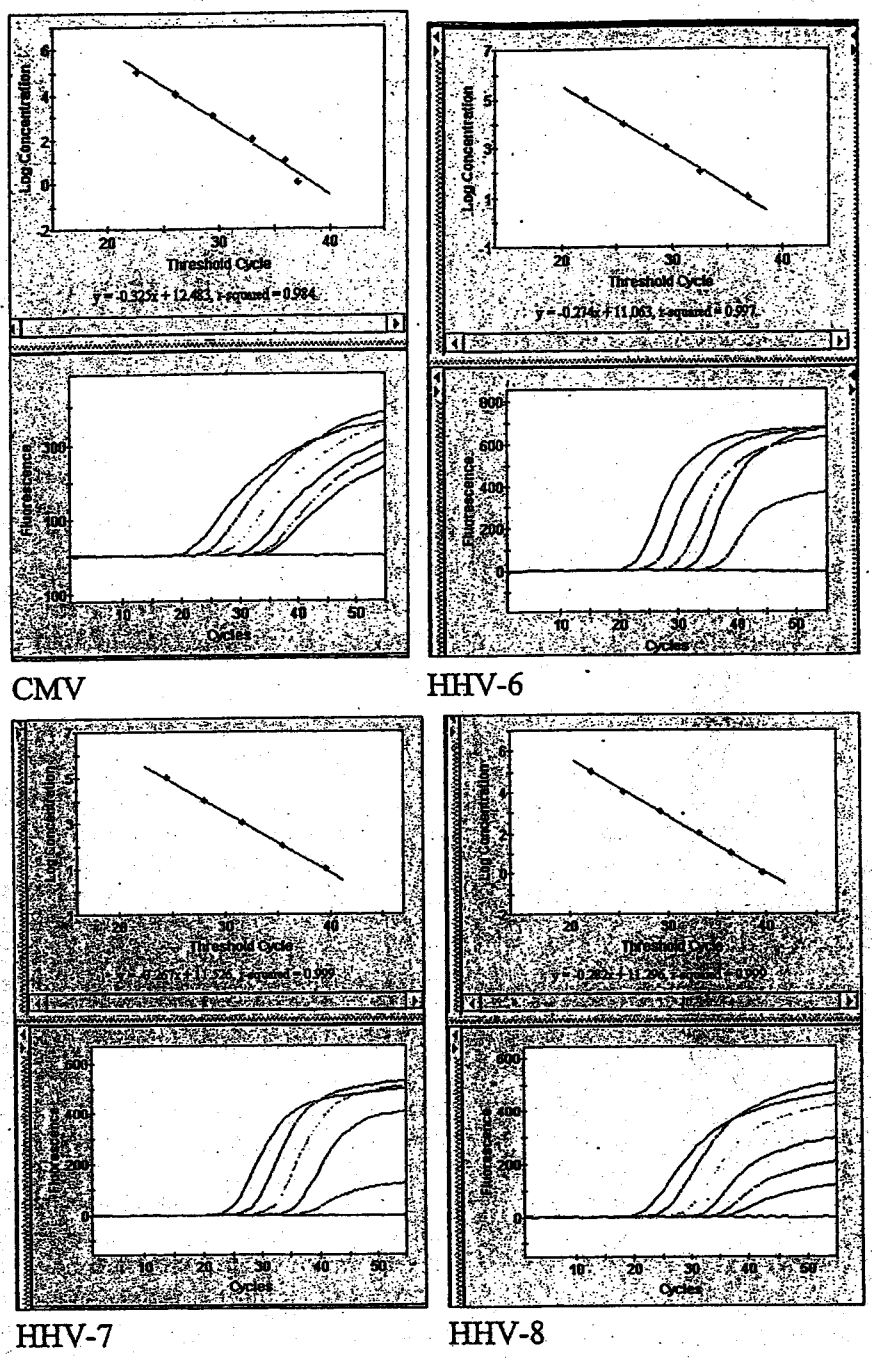


**Fig. 1.** *Continued*

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

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

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

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

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

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

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

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

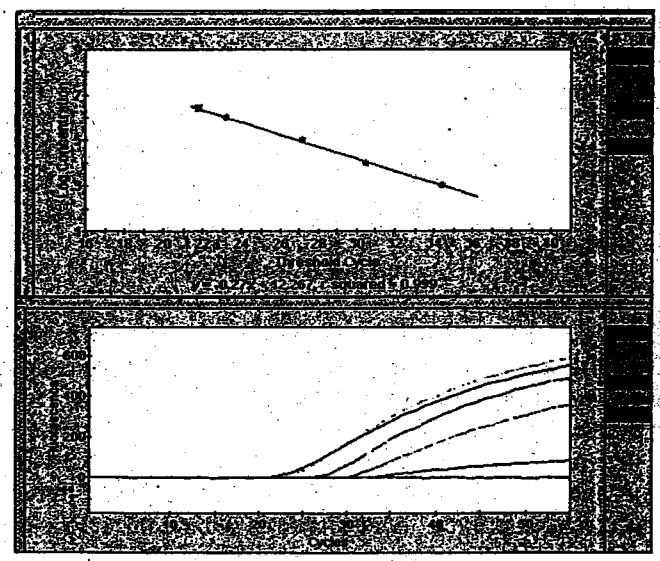

**Fig. 2. Real-time PCR standard curve for HHV-6 high-viral-load samples. PCR positivity of extremely high HHV-6 viral loads (>$10^6$ copies/reaction) was seen only after more than 20 cycles of single-step PCR and yields a highly linear log standard curve with a range of $10^3$ to $2.45 \times 10^6$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

### Virus load calculation

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

## RESULTS

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

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

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

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

## DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

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

### ACKNOWLEDGMENT

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

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## SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article:
**Appendix S1.** Real-time PCR reagents (Word document).

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

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番号：2

別紙様式第２－１

# 医薬品　研究報告　調査報告書

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

MedDRA/J Version(11.1)

World Health Organization

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

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

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

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

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

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<br>2008年9月1日 | 新医薬品等の区分<br>該当なし | 総合機構処理欄 |
|---|---|---|---|---|---|---|
| 一般的名称 | 別紙のとおり | | 研究報告の公表状況 | ProMED-mail, 20080828.2697 | 公表国<br>インド | |
| 販売名（企業名） | 別紙のとおり | | | | | |

**研究報告の概要**

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

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

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

使用上の注意記載状況・その他参考事項等

記載なし

MedDRA/J ver.11.0

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

*現在製造を行っていない

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

UNDIAGNOSED FATAL ILLNESS - INDIA (04): (UTTAR PRADESH)
**********

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[1]
Date: Tue 26 Aug 2008
Source: The Hindustan Times, online [edited]
<http://www.hindustantimes.com/StoryPage/StoryPage.aspx?sectionName=&id=3825990

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

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

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

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

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

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

[byline: Sanchita Sharm]

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communicated by:
ProMED-mail rapporteur Mary Marshall

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

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

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

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

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

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

[see also:
Undiagnosed fatal illness - India (03): (UP) RFI 20080826.2666
Undiagnosed fatal illness - India (02): (UP) RFI 20080811.2478
Undiagnosed fatal illness - India (Uttar Pradesh): RFI 20080331.1194
2007
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Japanese encephalitis - India (02) (Uttar Pradesh) 20071026.3486
Undiagnosed viral disease - India (02): (Uttar Pradesh) 20071026.3485
Undiagnosed viral disease - India: (Uttar Pradesh) 20071022.3440
Japanese encephalitis - India (Uttar Pradesh) 20070930.3233
2006
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Japanese encephalitis - India (Uttar Pradesh) (03): vaccine safety 20061222.3583
Leptospirosis - India (Gujarat): not hantavirus 20060831.2476
Leptospirosis - India (Maharashtra) 20060726.2058
Leptospirosis - India (Kerala) 20060609.1612
Leptospirosis - India (Karnataka) 20060123.0226
2005
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Undiagnosed deaths - India (Uttar Pradesh) (02) 20051115.3342
Undiagnosed deaths - India (Uttar Pradesh): RFI 20051113.3322
Leptospirosis - India (Maharashtra) 20050811.2348
2004
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Leptospirosis - India (South Gujarat)(02) 20040908.2509
Leptospirosis - India (South Gujarat) 20040902.2441
Leptospirosis - India (Tamil Nadu) 20040226.0602]

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# 医薬品 研究報告 調査報告書

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|---|---|---|---|---|
| 販売名(企業名) | 合成血-LR「日赤」(日本赤十字社)<br>照射合成血-LR「日赤」(日本赤十字社) | | Komar N, Clark GG. Rev Panam Salud Publica. 2006 Feb;19(2):112-7. | 米国 |

**研究報告の概要**

○ラテンアメリカおよびカリブ諸国のウエストナイルウイルスの活動性

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

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

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

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

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| 報告企業の意見 | 今後の対応 |
|---|---|
| ラテンアメリカおよびカリブ海地域の動物や鳥類において、ウエストナイルウイルスの抗体陽性率は高くなっているが疾患報告は少ない。この不可解な熱帯生態系での疾患の低減について、原因を検討するため分離株が必要であるとの報告である。 | 日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、ウエストナイルウイルス感染の発生に備え、緊急対応の準備を進めている。今後も引き続き情報の収集に努める。 |

Informe especial / Special report

# West Nile virus activity in Latin America and the Caribbean

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

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

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

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

## INTRODUCTION

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

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## METHODS

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

## RESULTS

### West Nile virus detected in 2001

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

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

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

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

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

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

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

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

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

## Spread of West Nile virus 2002–2004

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

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

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

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

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

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

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

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

## DISCUSSION

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

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

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

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

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

## CONCLUSION AND RECOMMENDATIONS

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

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

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

RESUMEN

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

***Objetivos.*** El virus del Nilo occidental (VNO, familia Flaviviridae, género *Flavivirus*) se ha propagado rápidamente por toda la cuenca del Caribe desde que se detectó por primera vez en 2001. En este informe se resumen nuestros conocimientos actuales acerca de la transmisión del VNO en zonas tropicales del continente americano.
***Métodos.*** Revisamos todo lo que se ha publicado sobre el tema y consultamos a autoridades de salud clave para obtener datos inéditos.
***Resultados.*** Las infecciones por el virus del Nilo occidental aparecieron por primera vez en seres humanos residentes de las Islas Caimán y de los Cayos de la Florida en 2001, y en pájaros de aspecto sano de los cuales se obtuvieron muestras a principios de 2002. En 2002 se encontraron pruebas serológicas de infección por el VNO en caballos, pollos y aves de corral no estabuladas oriundas de Guadalupe, la República Dominicana y la parte oriental de México. En 2003, el VNO se diseminó dentro de México y por la parte norte de Centroamérica y se encontraron pruebas serológicas en las Bahamas, Puerto Rico y Cuba. En 2004, las primeras pruebas serológicas de actividad vírica en ecosistemas sudamericanos se detectaron en septiembre y octubre en Colombia y Trinidad, donde se observaron anticuerpos neutralizantes contra el VNO en animales domésticos.
***Conclusiones.*** Estos informes esporádicos de enfermedad equina, humana y aviar en América Latina y el Caribe son desconcertantes. Es necesario aislar las cepas para determinar si la atenuación del virus u otro factor explica la carga de enfermedad reducida en ecosistemas tropicales.

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

別紙様式第2-1

No. 19

# 医薬品 研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 機構処理欄 |
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| | | | | 2008. 7. 11 | 該当なし | |

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

**研究報告の概要**

○クリミア・コンゴ出血熱—トルコ

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

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

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

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

**使用上の注意記載状況・その他参考事項等**

合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

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Back

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

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

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

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

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

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

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

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

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

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Communicated by:
ProMED-mail Rapporteur A-Lan Banks

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

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

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

[see also:
Crimean-Congo hem. fever - Turkey (10): treatment 20080704.2038
Crimean-Congo hem. fever - Turkey (09) 20080622.1935
Crimean-Congo hem. fever - Turkey (08) 20080620.1917
Crimean-Congo hem. fever - Turkey (07) 20080616.1892
Crimean-Congo hem. fever - Turkey (06) 20080615.1888
Crimean-Congo hemorrhagic fever - Turkey (05) 20080612.1866
Crimean-Congo hem. fever - Turkey (04) 20080611.1838
Crimean-Congo hem. fever - Turkey (03) 20080522.1686
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Crimean-Congo hemorrhagic fever - Turkey (04): WHO 20060809.2230
Crimean-Congo hemorrhagic fever - Turkey (03): comment on tick removal 20060728.2082
Crimean-Congo hemorrhagic fever - Turkey (02) 20060722.2013
Crimean-Congo hemorrhagic fever - Turkey 20060705.1844]
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別紙様式第2-1

No. 8

# 医薬品 研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 機構処理欄 |
|---|---|---|---|---|---|---|
| | | | | 2008. 7. 11 | 該当なし | |

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

**研究報告の概要**

○サルモネラ・セントポール流行の調査

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

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

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

| 報告企業の意見 | 今後の対応 |
|---|---|
| 2008年7月7日時点で、米国の41の州、ワシントンD.C.、カナダで991名の患者がサルモネラ・セントポール株に感染したことが確認されたとの報告である。 | 日本赤十字社では、輸血による細菌感染予防対策として問診時に献血者の健康状態を確認し、発熱を伴う食中毒様の激しい下痢症状がある場合は1ヶ月間献血不適としている。また、全ての輸血用血液製剤について、平成19年1月より保存前白血球除去を実施している。今後も細菌やウイルスの検出や不活化する方策について情報の収集に努める。 |

**使用上の注意記載状況・その他参考事項等**

合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

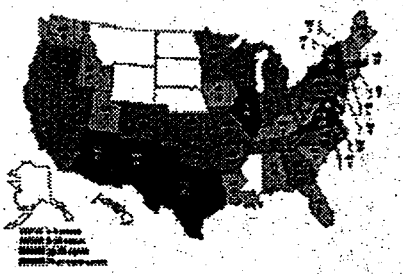


20

JRC2008T-046

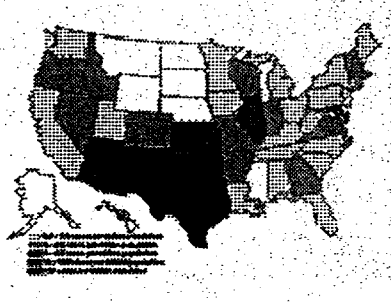
# Investigation of Outbreak of Infections Caused by *Salmonella* Saintpaul

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



Click map to view a larger image.

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



Click map to view a larger image.

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

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

**Click Here for Advice to Consumers**

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

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

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

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

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

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

## Clinical features of *Salmonella* Infection

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

## Advice to consumers

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

C sumers everywhere are advised to:

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

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

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

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

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

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

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

## Previous Updates on this Outbreak

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

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

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

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

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

別紙様式第2-1
番号11

医薬品
医薬部外品　研究報告　調査報告書
化粧品

| 識別番号・報告回数 | | | 報告日 | 第一報入手日<br>2008年8月1日 | 新医薬品等の区分<br>該当なし | 厚生労働省処理欄 |
|---|---|---|---|---|---|---|
| 一般的名称 | ①乾燥抗HBs人免疫グロブリン<br>②ポリエチレングリコール処理抗HBs人免疫グロブリン | | 研究報告の公表状況 | TRANSFUSION 2008; 48(7): 1333-1341 | 公表国<br>フランス | |
| 販売名<br>(企業名) | ①ヘブスブリン（ベネシス）<br>②静注用ヘブスブリンーIH（ベネシス） | | | | | |

**研究報告の概要**

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

| 報告企業の意見 | 今後の対応 |
|---|---|
| レユニオン島におけるチクングニヤウイルス（CHIKV）の流行時のCHIKV血症献血リスクに関する報告である。血漿分画製剤からのチクングニヤウイルス伝播の事例は報告されていない。また、万一原料血漿にチクングニヤウイルスが混入したとしても、BVDをモデルウイルスとしたウイルスバリデーション試験成績から、本剤の製造工程において十分に不活化・除去されると考えている。 | 本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。 |

**使用上の注意記載状況・その他参考事項等**

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

TRANSFUSION COMPLICATIONS

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

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

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

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

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

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

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

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

**ABBREVIATIONS:** CHIKV = Chikungunya virus; WNV = West Nile virus.

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Received for publication October 1, 2007; revision received November 13, 2007, and accepted November 13, 2007.

doi: 10.1111/j.1537-2995.2008.01646.x

TRANSFUSION 2008;48:1333-1341.

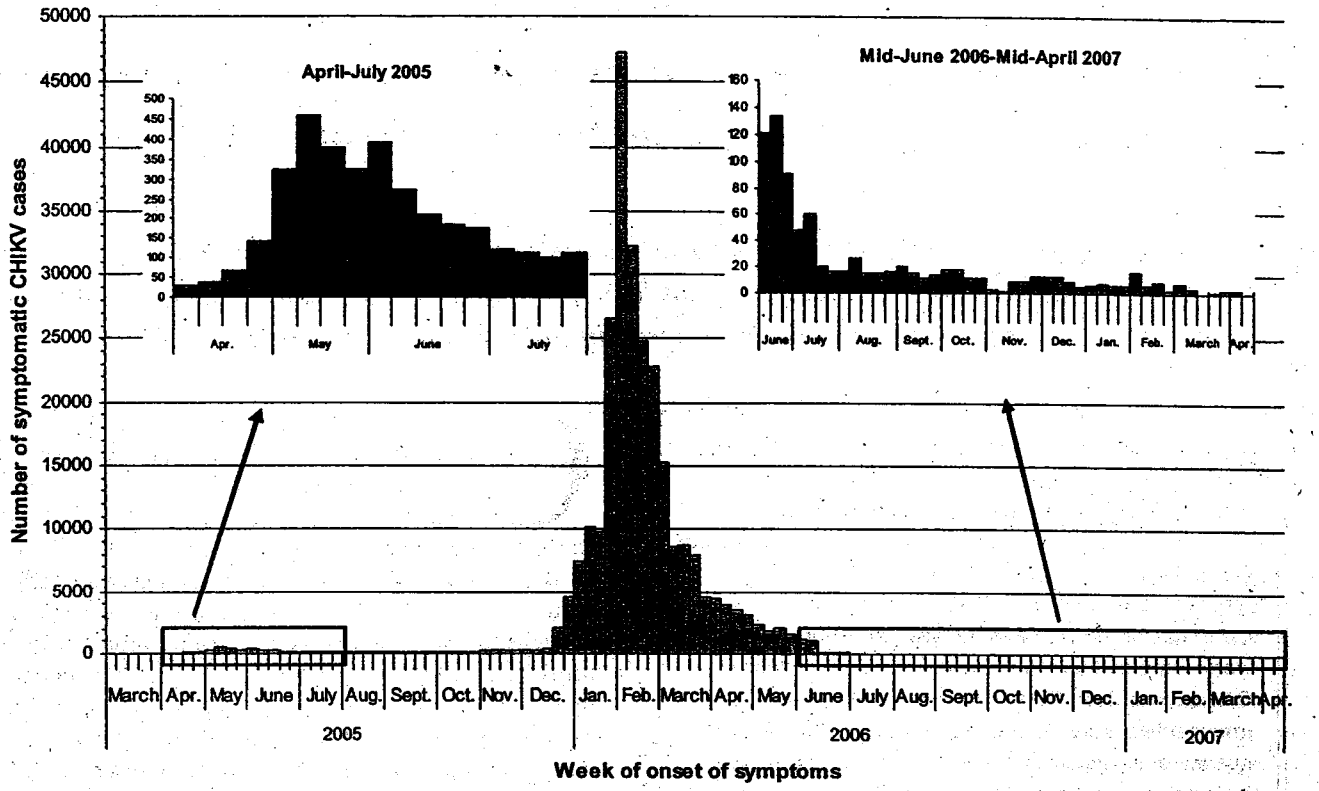


**Fig. 1. Distribution of symptomatic cases of CHIKV infection per week of onset of symptoms, Reunion Island, March 28, 2005, through April 15, 2007.**

Mayotte, Seychelles, Mauritius, and Madagascar Islands). On Reunion Island, the first cases were identified at the end of April 2005. After a first epidemic peak in May through June 2005 with a maximum of 450 cases during the second week of May, the number of cases decreased during the southern hemisphere winter season. At mid-December, an exponential increase in cases occurred, with almost 10,000 estimated cases at mid-January 2006 (Fig. 1). Because of concerns about the possible transmission of CHIKV by blood transfusion, the French Blood Services (EFS) interrupted blood donations on the island from January 23, 2006, except donations for platelets (PLTs) for which systematic screening for CHIKV genome by nucleic acid amplification testing (NAT) was set up.

At that moment, we estimated the risk of CHIKV viremic blood donation. Afterward, we updated these estimates since more accurate data were available on the incidence of infection and on the frequency of asymptomatic infections. We compared the estimated risk of viremic blood donation to the observed proportion of viremic PLT donations determined by CHIKV NAT screening.

## MATERIALS AND METHODS

The estimates were performed by the French Institute of Public Health Surveillance (InVS) in the setting of a work-group including the French Agency for the Safety of Health Products (Afssaps), the French Blood Services (EFS), and the National Institute for Blood Transfusion (INTS). In early 2005, this group initiated a project with the aim of obtaining a priori quantitative risk estimates of contamination of blood donations by infectious agents for various scenarios in terms of incidence and time-space distribution.[14]

### General approach

The first estimates performed in January 2006 ("preliminary estimates") concerned the two following periods: Period A, from the detection of the first cases in April 2005 to mid-December 2005 when a large increase of cases occurred (March 28-December 18, 2005; 266 days); and Period B, from mid-December until the interruption of blood collection (December 19, 2005-January 22, 2006; 35 days; Fig. 2).

These estimates were later refined with consolidated incidence data, corrected for delayed care-seeking and delayed reporting and more precise estimates of the proportion of asymptomatic infections obtained through a seroepidemiologic survey carried out at the final phase of the outbreak ("retrospective estimates"). We also estimated the risk of viremic blood donation for five different

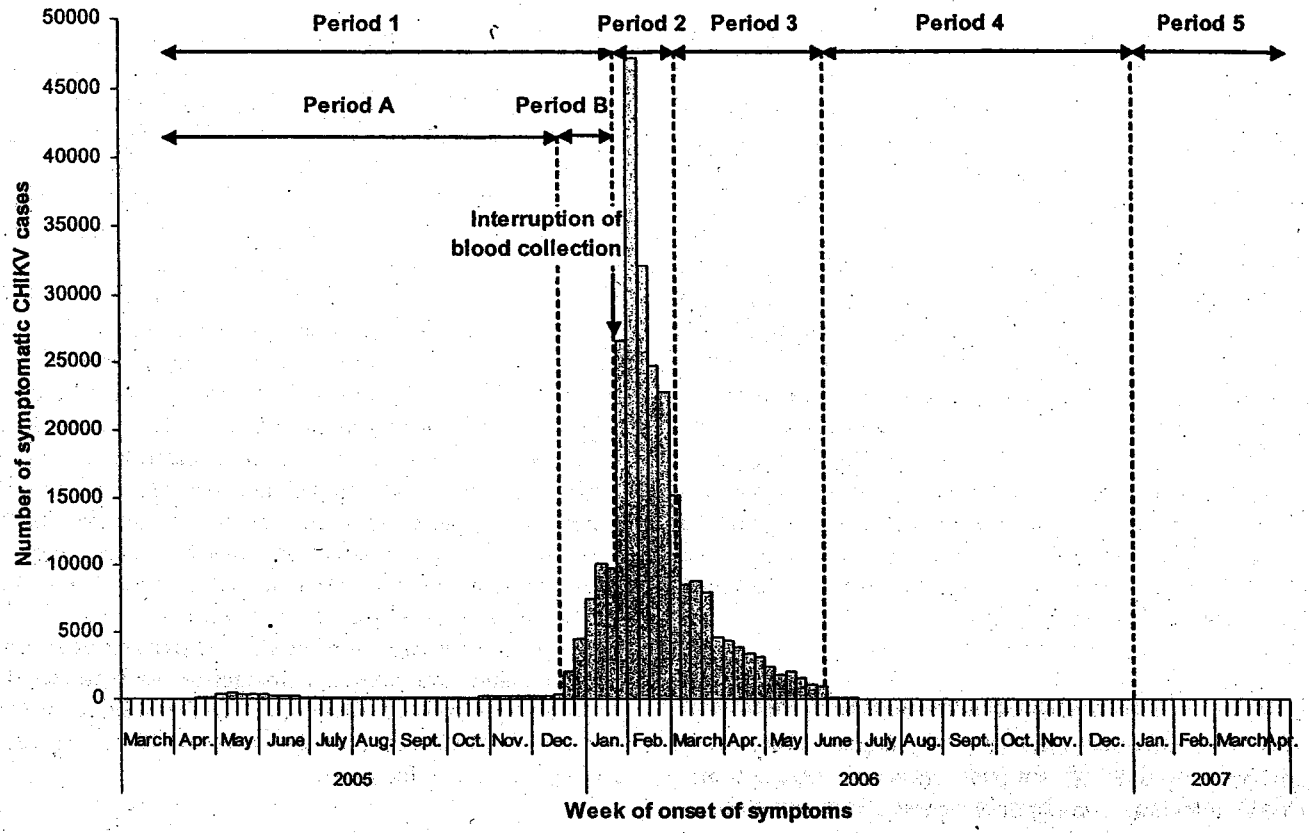


**Fig. 2. Periods for risk estimates and distribution of symptomatic cases of CHIKV infection per week of onset of symptoms, Reunion Island, March 28, 2005, through April 15, 2007.**

periods of the outbreak with these updated data (Fig. 2).

By use of the quarterly numbers of blood donations collected on Reunion Island in 2005 (unpublished data from EFS), we could then estimate the number of blood donations that would have been collected in 2006 if blood donations had not been interrupted.

To assess the validity of our risk estimates, we compared the estimated risk of viremic blood donation ("estimated risk") to the observed proportion of viremic PLT donations collected and screened for CHIKV genome ("observed risk") over the same period.

## Statistical approach

An approximating formula developed by Biggerstaff and Petersen[15] in 2002 for West Nile virus (WNV) was used to estimate the mean risk of viremic blood donation by CHIKV. This formula combines the proportion of asymptomatic (*Pa*) and symptomatic (*Ps*) infections with the duration of viremia among asymptomatic infected individuals (*Va*) and the duration between onset of viremia and onset of symptoms in symptomatic patients (*Vs*). This provides the mean time an infected individual is viremic and asymptomatic. Dividing this mean duration of viremia by the length of the outbreak period (*L*) then provides an estimate of the probability that an individual donates blood during viremia, assuming that a person with symptoms would self-defer or be excluded from donation by the predonation medical examination. Combined with the incidence (*I*) of the infection (including both symptomatic and asymptomatic infection), it gives an estimate of the mean risk of viremic blood donation:

$$\text{Mean risk} \approx \frac{(Pa \times Va) + (Ps \times Vs)}{L} \times I.$$

As suggested by Biggerstaff and Petersen,[15] risk confidence bounds were obtained by multiplying the confidence bounds of *I* by $[(Pa \times Va) + (Ps \times Vs)]/L$. Confidence intervals (CIs) of *I* were calculated with Fleiss quadratic method.[16]

## Data on duration of viremia

In January 2006, few data were available on the duration of CHIKV viremia. In 1964, Sarkar and coworkers[17] described, from virologic studies of hemorrhagic fever in Calcutta, that CHIKV was most frequently isolated from blood within 48 hours after the onset of symptoms, but that it had been isolated as late as 6 days after the onset of illness.

The duration of viremia has been more extensively documented for dengue viruses: 1 or 2 days before the onset of symptoms and between 4 and 6 days and as late as 12 days after the first symptoms.[18-20] We thus used the following parameters for CHIKV: 1.5 days for the mean duration between onset of viremia and onset of symptoms among symptomatic patients (*Vs*) and 1.5 + 6 = 7.5 days for the mean duration of viremia among asymptomatic infected individuals (*Va*) assuming that the whole duration of viremia is similar in symptomatic and asymptomatic infections.

The same estimates of duration of viremia were used for the retrospective estimates since consistent observations were reported during the outbreak on Reunion Island. Thus, during this epidemic, CHIKV has been isolated from blood mostly within 5 days and as late as 12 days after the onset of symptoms. In some cases, CHIKV viremia might have persisted over 12 days since viral loads at 12 days were high.[21]

### Data on the proportion of asymptomatic infections

For the preliminary estimates in January 2006, in the absence of data on the proportion of asymptomatic CHIKV infection, two hypotheses were formulated based on the proportion of asymptomatic infections reported during outbreaks of dengue:[22,23] a minimal proportion of asymptomatic infection of 30 percent and a maximal proportion of 70 percent.

Between August and October 2006, a seroprevalence study was conducted among the general population of Reunion Island.[24] This survey showed that 38 percent of the inhabitants of Reunion Island had been infected by CHIKV. The preliminary results indicated that 6 percent of the study population had a positive CHIKV serology without having reported CHIKV symptoms. This suggests that approximately 15 percent of infected individuals during this outbreak may have had an asymptomatic infection. Therefore, this proportion of 15 percent was used for *Pa* for the retrospective estimates.

### Incidence of CHIKV infection

We used the incidence data in the general population for the risk estimations assuming that potential blood donors had the same risk of CHIKV infection as the general population. The population of interest was the inhabitants of Reunion Island estimated at 756,745 by a population census conducted in 2004 by the National Institute for Statistics and Economics Studies (INSEE). CHIKV incidence data, by week of onset of symptoms, were obtained from the Reunion-Mayotte Interregional Epidemiology Unit, which had started surveillance for CHIKV infection as soon as the first cases were reported in April 2005. A suspect case of CHIKV infection was defined as a patient with an abrupt onset of fever over 38.5°C associated with incapacitating arthralgia in the absence of any other potential cause of infection. From April to December 2005, surveillance relied on vector control teams, which conducted active and retrospective case-finding around the cases reported by a sentinel physician network, medical laboratories, private practitioners, and patients themselves. The number of cases took into account the symptomatic patients responding to the case definition whether or not they had consulted a general practitioner. During this period, approximately 67 suspect CHIKV cases were identified by active case-finding for every suspect case identified by the sentinel network physicians. From mid-December onward, the number of cases exceeded the capacity of the active surveillance system, and surveillance was then entirely based on the sentinel network. To estimate the total number of cases from the sentinel network data, the multiplier of 67, derived during the phase of active case finding, was used.[25]

For the estimations of the risk of viremic donations, we calculated the estimated incidence of symptomatic and asymptomatic CHIKV infection by multiplying the estimated incidence of suspect cases by 100/(proportion of symptomatic infections).

## RESULTS

### Preliminary estimates

When the preliminary estimates were performed at the end of January 2006, the number of CHIKV suspect cases was 6500 for Period A and 25,000 for Period B. For Period A, the estimated mean risk of viremic blood donation was 15.2 per 100,000 donations, under the minimal hypothesis of 30 percent asymptomatic infections, and 61.3 per 100,000 donations, under the maximal hypothesis of 70 percent asymptomatic infections (Table 1). For Period B, the mean risk reached 445 per 100,000 donations, under the minimal hypothesis and 1,793 per 100,000 donations, under the maximal hypothesis.

### Retrospective estimates

The retrospective estimates used the results of the seroprevalence survey that estimated the proportion of asymptomatic CHIKV infections during this outbreak at 15 percent. The updated estimate of the number of symptomatic cases was 6,864 for Period A and 34,002 for Period B (Table 2). Risk of viremic blood donation was then estimated at 9.6 and 362.5 per 100,000 donations for Periods A and B, respectively. The risk estimates for the five periods of the outbreak are shown in Table 3. Between the identification of the first CHIKV cases and the interruption of blood donations (Period 1), 7 of 14,450 blood donations collected could have been viremic. During

**TABLE 1. Preliminary risk estimates of viremic blood donation, Reunion Island, March 28, 2005, through January 22, 2006**

| | Period A, Mar 28-Dec 18, 2005 | | Period B, Dec 19, 2005-Jan 22, 2006 | |
|---|---|---|---|---|
| | Minimal hypothesis | Maximal hypothesis | Minimal hypothesis | Maximal hypothesis |
| Estimated number of symptomatic cases | 6,500 | 6,500 | 25,000 | 25,000 |
| Proportion of asymptomatic infections (%) | 30 | 70 | 30 | 70 |
| Estimated number of infected cases | 9,286 | 21,667 | 35,714 | 83,333 |
| Period length (days) | 266 | 266 | 35 | 35 |
| Estimated incidence of CHIKV infection per 100,000 | 1,227 | 2,863 | 4,720 | 11,012 |
| Estimated risk of viremic blood donation | | | | |
| Per 100,000 blood donations (95% CI) | 15.2 (14.9-15.5) | 61.3 (60.6-62.2) | 445.0 (440.5-449.5) | 1,793.4 (1,781.9-1,804.9) |
| Per estimated number of blood donations (95% CI) | 2.0/12,800 (1.9-2.0) | 7.9/12,800 (7.8-8.0) | 7.1/1,600 (7.0-7.2) | 28.7/1,600 (28.5-28.9) |

**TABLE 2. Retrospective risk estimates of viremic blood donation, Reunion Island, March 28, 2005, through January 22, 2006**

| | Period A, Mar 28-Dec 18, 2005 | Period B, Dec 19, 2005-Jan 22, 2006 |
|---|---|---|
| Estimated number of symptomatic cases | 6,864 | 34,002 |
| Proportion of asymptomatic infections (%) | 15 | 15 |
| Estimated number of infected cases | 8,075 | 40,002 |
| Period length (days) | 266 | 35 |
| Estimated incidence of CHIKV infection per 100,000 | 1,067 | 5,286 |
| Estimated risk of viremic blood donation | | |
| Per 100,000 blood donations (95% CI) | 9.6 (9.4-9.8) | 362.5 (359.0-366.0) |
| Per estimated number of blood donations (95% CI) | 1.2/12,800 (1.2-1.3) | 5.8/1,600 (5.7-5.9) |

Period 2, at the height of the epidemic, the estimated risk of viremic blood donation was 1,500 per 100,000, that is, 29 potentially viremic donations if blood collection had continued. The estimated risk then decreased due to diminishing CHIKV transmission: 210 per 100,000 between March and June 2006 (Period 3), 1.4 per 100,000 for the second semester of 2006 (Period 4), and 0.27 per 100,000 for the first months of 2007 (Period 5), that is, 1 potentially viremic blood donation every 21 years on the basis of 17,500 blood donations collected each year. Finally, over the course of the outbreak, a total of 47 of 35,750 blood donations might have been viremic if blood collection had continued. Simultaneously, an estimated 312,500 of 757,000 inhabitants have been infected by mosquito-borne transmission.

### Comparison between estimated risk and observed risk

Between January 23 and May 7, 2006, 2 of the 500 PLT donations screened for CHIKV RNA were positive (0.4%). One donor developed CHIKV symptoms on the day after the blood donation, the other remained asymptomatic. The risk of viremic blood donation over this period was estimated at 720 per 100,000 blood donations, that is, 0.72 percent.

Although an estimated 7 viremic donors had donated blood before the collection was interrupted, no case of transfusion-transmitted CHIKV infection has been identified during this period.

## DISCUSSION

During this first and massive epidemic of CHIKV infection on Reunion Island, we computed estimates of the risk of CHIKV viremic blood donation, in real time during the ascending phase of the major epidemic peak, and afterward, we refined these estimates with newly available data. Although we underestimated the incidence of CHIKV infection in our preliminary calculations, we overestimated the proportion of asymptomatic infections. Consequently, the preliminary estimates were 1.2- to 6.4-fold greater than the retrospective calculations. The preliminary estimates, however, provided a right order of magnitude of the risk in real time in an emergency context. The retrospective calculations indicate a mean risk over the course of the outbreak, between April 2005 and April 2007, of 132 per 100,000 donations. The mean risk peaked at approximately 1,500 per 100,000 donations at the height of the outbreak in February 2006. In total, potentially 47 of 35,750 blood donations might have been viremic between April 2005 and April 2007 if blood collection had not been interrupted. We also estimated that 7 blood donations were viremic before the interruption of blood donations on the island. Therefore, this measure enabled the avoidance of 40 potentially viremic donations. By way of comparison, during the outbreak, the total number of individuals infected through mosquito-borne CHIKV transmission is estimated at 312,538 individuals.

This approach has several limitations. The estimates provided relate to a mean risk, which supposes that the risk is constant over the studied period and for the

TABLE 3. Retrospective risk estimates of viremic blood donation, Reunion Island, March 28, 2005, through April 15, 2007

| | Period 1, Mar 28, 2005-Jan 22, 2006 | Period 2, Jan 23, 2006-Mar 5, 2006 | Period 3, Mar 6, 2006-Jun 11, 2006 | Period 4, Jun 12, 2006-Dec 31, 2006 | Period 5, Jan 1, 2007-Apr 15, 2007 | Periods 1-5, Mar 28, 2005-Apr 15, 2007 |
|---|---|---|---|---|---|---|
| Estimated number of symptomatic cases | 40,866 | 169,008 | 54,936 | 772 | 75 | 265,657 |
| Period length (days) | 301 | 42 | 98 | 203 | 105 | 749 |
| Proportion of asymptomatic infections (%) | 15 | 15 | 15 | 15 | 15 | 15 |
| Estimated number of infected cases | 48,078 | 198,833 | 64,631 | 908 | 88 | 312,538 |
| Estimated incidence of CHIKV infection per 100,000 | 6,353 | 26,275 | 8,541 | 120 | 12 | 41,300 |
| Estimated risk of viremic blood donation | | | | | | |
| Per 100,000 blood donations (95% CI) | 50.7 (50.2-51.1) | 1,501.4 (1,495.8-1,507.1) | 209.2 (207.6-210.7) | 1.4 (1.3-1.5) | 0.27 (0.2-0.3) | 132.3 (132.0-132.7) |
| Per estimated number of blood donations (95% CI) | 7.3/14,450 (7.3-7.4) | 29.1/1,940 (29.0-29.2) | 9.9/4,710 (9.8-9.9) | 0.14/9,760 (0.13-0.15) | 0.01/4,890 (0.01-0.02) | 47.3/35,750 (47.2-47.4) |

geographic area. Although estimates were performed for several periods selected according to the level of incidence, the number of cases and consequently the risk might have been highly variable during the studied period. In addition, the risk of infection varied by geographic area as later demonstrated by the seroprevalence survey that showed that 29.6 percent of the inhabitants of the North have been infected whereas in the East, this proportion reached 48 percent.[24] Consequently, the mean risk underestimates the maximal risk, corresponding to the peak of the outbreak and to the area where CHIKV transmission was maximal. This maximum risk, however, is highly time and space limited.

To obtain a more dynamic sight of the risk over the course of the epidemic and estimates of the maximal risk, it would have been necessary to develop an approach similar to the one proposed by Biggerstaff and Petersen[15,26] for the WNV epidemic in 2002 in the United States. The latter is a statistical approach based on imputation and resampling techniques providing daily estimates of the risk of blood contamination in an epidemic setting. Conducting such an analysis in the context of this large and long-standing outbreak would have been computationally cumbersome. In our opinion, such a refinement was not essential in regard to the main objectives of the study, that is, providing a right order of magnitude of the risk as an aid for risk management. We considered that providing an approximation of the mean risk over five periods was a suitable alternative. To compute these mean risks, we therefore used the approximating formula proposed by Biggerstaff and Petersen.[15] In 2003, Biggerstaff and Petersen demonstrated for the WNV epidemic in 2002 in the United States that the approximating formula provides a reasonable approximation to the mean risk of transfusion.[15] The same work of comparison of the mean risks estimated by this method and by statistical resampling was carried out, in the setting of our workgroup, for an outbreak of acute hepatitis A in France that occurred in 1996 through 1997.[14,27] It also concluded to a good concordance of the results of both methods. Note that the CIs presented with our mean risk estimates do not take into account the uncertainty on the duration of viremia, the proportion of asymptomatic infections, nor the coefficient of 67, used to estimate incidence of symptomatic infections from the sentinel network data. Even though this limitation led to artificially narrow CIs, point estimates of mean risk should not be affected.

Our incidence data were derived from a sentinel surveillance system. Because a clinical case definition was used, it is possible that other febrile illnesses, not due to CHIKV, were included in the case count. The positive predictive value of a clinical case definition, however, greatly improves if incidence is high. Therefore, the inclusion of noncases in the case count, leading to

overestimation of the incidence and hence the risk of viremic donation, is more likely to occur outside an outbreak setting. The validity of the extrapolated data derived from a sentinel surveillance system estimating the total number of cases in the community should also be questioned. The serosurvey estimated that 38 percent of the inhabitants had been infected and that 32 percent had suffered from symptomatic infections. These data are consistent with the 35 percent of the inhabitants having suffered from symptomatic illness, estimated by the sentinel surveillance system and corroborate our incidence estimates.

We assumed that potential blood donors had the same risk of CHIKV infection as the general population. This assumption was supported by the findings of the serosurvey that showed similar antibody prevalences among adults of both sexes.[24] In addition, when we applied age-specific CHIKV antibody prevalence rates of the serosurvey to the donor population of Reunion Island, the overall seroprevalence among donors was estimated at 37.2 percent, similar to the overall antibody prevalence in the general population (38%).

One major limitation of the validity of our estimates relates to lack of a precise knowledge on the distribution of the duration of asymptomatic viremia in individuals with apparent and inapparent infection. To refine the estimates, further studies are necessary to document the kinetics of CHIKV viremia. This approach also hypothesizes that symptomatic individuals would self-defer or be excluded by the predonation examination. In real life, this may not always be the case. In the United States, among the first 14 identified donors associated with transfusion-related WNV transmission to recipients, 3 were shown to have been symptomatic at the moment of the donation.[15] Nevertheless, for CHIKV infection which is characterized by sudden onset of symptoms, this assumption is more plausible than for WNV which frequently causes paucisymptomatic infection.

Lack of data on the frequency of asymptomatic infection was the most important limiting factor for the preliminary estimates. This variable has a preponderant role in the risk estimate since it contributes both in the computation of the weighted mean of the duration of asymptomatic viremia and in the estimate of the incidence of infection. Valid data were available, however, for the retrospective calculations from the seroprevalence survey. This survey provided an estimate of the proportion of asymptomatic infections obtained directly among the studied population and for the epidemic CHIKV strain circulating.

In spite of the above limitations, the retrospective estimates are likely to give a good approximation of the real risk, as suggested by the observed risk of viremic PLT donations. From January to May 2006, this observed risk was 400 per 100,000 donations, of the same order of magnitude as the risk of 720 per 100,000 donations estimated over the same period.

Up to date, CHIKV infections from transfusion of blood or blood components have not been reported in the literature. On Reunion Island, no case of transfusion-transmitted CHIKV infection has been identified in spite of the estimated seven viremic donations collected before donations were interrupted. Despite the lack of data about transfusion-transmitted CHIKV infection, the high viral load during the acute phase of the infection,[21,28] the fact that several cases of CHIKV transmission have occurred among laboratory personnel handling infected blood,[29] and the fact that CHIKV has been transmitted to a health care worker drawing blood from an infected patient[28] provide evidence that transfusion-related transmission of CHIKV is highly plausible. It is possible that transfusion-related infections have not been recognized or have not been distinguished from infection from mosquito vectors. Also, the true transmission rate from viremic donors to recipients is not known. Several issues may influence the possibility of transmission of CHIKV through transfusion, such as the stability of the virus during storage of blood and the efficiency of virus elimination of blood processing methods, as viral inactivation. Also, the presence of IgM or IgG antibodies in donor blood may neutralize infectivity, as demonstrated for other viruses such as parvovirus[30] and suggested for WNV.[31] In addition, the assessment of the risk of CHIKV transmission from a viremic donor to a recipient would need to take into account the recipient's immune status.

In conclusion, despite the absence of documented cases, blood transfusion–related CHIKV transmission is plausible and the risk of viremic donation can be substantial in an outbreak setting. During this large outbreak, the estimated risk of viremic blood donation was high, but low compared to the risk of mosquito-borne CHIKV transmission. Despite its limitations, this work provided a right order of magnitude of the risk of viremic blood donation in real time during the ascending phase of the epidemic peak. At this moment, the decision of interrupting blood collection relied on the precautionary principle. The low risk estimated for early 2007 was, however, useful to contribute to the decision making process to start again the collection of blood donations on the island from June 14, 2007. This illustrates how this approach may contribute to guiding prevention measures.

## ACKNOWLEDGMENTS

The authors thank Reunion-Mayotte Interregional Epidemiology Unit (Reunion-Mayotte Cire) and the Reunion Regional Observatory of Health plus sentinel general practitioners for providing the case onset data and for their important work during this massive and long outbreak.

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# 医薬品 研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 機構処理欄 |
|---|---|---|---|---|---|---|
| | | | | 2008. 6. 23 | 該当なし | |

| 一般的名称 | (製造販売承認書に記載なし) | 研究報告の公表状況 | Chuang VW, Wong TY, Leung YH, Ma ES, Law YL, Tsang OT, Chan KM, Tsang IH, Que TL, Yung RW, Liu SH. Hong Kong Med J. 2008 Jun;14(3):170-7. | 公表国 中国 |
|---|---|---|---|---|
| 販売名(企業名) | 合成血-LR「日赤」(日本赤十字社)<br>照射合成血-LR「日赤」(日本赤十字社) | | | |

**研究報告の概要**

○1998年～2005年の香港におけるデング熱症例の検討
目的:デング患者の疫学的、臨床的知見、臨床検査知見、並びに転帰の検討。
患者:1998年～2005年に香港の公立病院に入院したデング患者(臨床検査による確定例)全員の医療記録を後方視的に検討した。
結果:合計126名の患者を特定した[デング熱123名(98%)、デング出血熱3名(2%)]。輸血によりデング熱が伝播した患者1名が明らかとなった。合計116名(92%)は「輸入感染」で、10名(8%)は「地域内感染」であった。RT-PCRで確定したデング症例56名のうち、もっとも多かったのはデングウイルス1型(48%)であり、ついで2型(23%)3型(16%)、4型(13%)であった。地域内感染は1、2型のみであった。患者の年齢の中央値は38歳で、入院期間の平均は6日間であった。死亡例はなく、ほぼ全員(98%)が発熱を呈した。入院時のその他の症状は次の通り:筋肉痛(83%)、頭痛(65%)、倦怠感(59%)、皮疹(60%)。3分の1以上の患者が胃腸および上気道の合併症を発現した。もっとも多く認められた身体的所見は斑丘疹状皮疹であった。血小板減少、好中球減少、リンパ球減少は、それぞれ86%、78%、69%の患者に発現した。人口統計学的・臨床的知見、臨床検査知見、ならびに転帰は、4つのデング血清型間で差はなかったが、リンパ球数は、他の型と比べて3型がもっとも低かった(P=0.004)。
結論:発熱、皮疹を呈し、合致する血液学的知見を持ち、流行地への渡航歴のある患者に遭遇した場合には鑑別診断にデング熱を含めるべきである。

**使用上の注意記載状況・その他参考事項等**

合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

| 報告企業の意見 | 今後の対応 |
|---|---|
| 1998年～2005年に香港の公立病院に入院したデング患者は合計126名で、うち10名(8%)は「地域内感染」であり1名は輸血による感染だった。ウイルス型は1型が最も多く、地域内感染は1、2型のみであったとの報告である。デングウイルスは東南アジアに定着しており、中国や台湾など日本に近い地域での流行状況を注視していく必要がある。 | 日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。問診でデング熱の既往があった場合には、治癒後1ヶ月間献血不適としている。また、厚生労働科学研究「献血血の安全性確保と安定供給のための新興感染症等に対する検査スクリーニング法等の開発と献血制限に関する研究」班に協力する予定である。今後も引き続き情報の収集に努める。 |

ORIGINAL ARTICLE

# Review of dengue fever cases in Hong Kong during 1998 to 2005

CME

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**Objective** To describe the epidemiology, clinical and laboratory findings, and outcomes of patients presenting locally with dengue.

**Design** Retrospective review of case records.

**Setting** Public hospitals, Hong Kong.

**Patients** Medical records of all laboratory-confirmed dengue patients admitted to public hospitals during 1998 to 2005 were reviewed retrospectively.

**Results** A total of 126 cases were identified, 123 (98%) being dengue fever and three (2%) dengue haemorrhagic fever. One patient who had blood transfusion–acquired dengue fever was highlighted. A total of 116 (92%) cases were 'imported', while 10 (8%) were local. Among the 56 dengue cases confirmed by reverse transcription–polymerase chain reaction, dengue virus type 1 was the most common accounting for 48% of them, followed by type 2, type 3, and type 4 responsible for 23%, 16%, and 13%, respectively. Only type 1 and type 2 were present in locally acquired infections. The median age of the patients was 38 years and the mean duration of hospitalisation was 6 days. There was no mortality, and nearly all patients (98%) presented with fever. Other symptoms at presentation included: myalgia (83%), headache (65%), fatigue (59%), and skin rash (60%). More than one third of patients had gastro-intestinal and upper respiratory complaints. Maculopapular skin rash was the most common physical finding. Thrombocytopenia, neutropenia, and lymphopenia were present in 86%, 78%, and 69% of the patients, respectively. In only 29% of the patients was dengue fever included in the initial differential diagnosis. The demographic, clinical, and laboratory findings as well as outcomes did not differ significantly among the four dengue serotypes, but the lowest lymphocyte counts of type 3 was lower than the other serotypes (P=0.004).

**Conclusion** When physicians encounter patients with a relevant travel history, presenting with fever and skin rash, and having compatible haematological findings, dengue fever should be included in the differential diagnosis.

Key words
Dengue; Dengue hemorrhagic fever; Serotyping

*Hong Kong Med J 2008;14:170-7*

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## Introduction

Dengue is the most common and widespread arthropod-borne viral infection in the world today. It is recognised in over 100 countries throughout the tropics and subtropical areas and threatens the health of approximately 40% of the world's population, of nearly 2.5 billion people.[1] The highest burden of disease occurs in South-East Asia and the Western Pacific, where it is one of the 10 leading causes of hospitalisation and childhood mortality.[2]

In Hong Kong, dengue fever was made notifiable since March 1994 and all infections reported to the Department of Health (DH) are investigated to establish their source. The number of cases reported is showing an increasing trend in recent years; the vast majority being imported from other countries. Hong Kong experienced its first local dengue case in September 2002.[3] Thereafter, several others were encountered in Ma Wan and local cases were subsequently identified sporadically in 2002 and 2003.

The epidemiology, clinical manifestations, and laboratory findings of dengue fever infections and its complications have been extensively described in the medical literature,[4,5] but comprehensive review is lacking for our local patients.

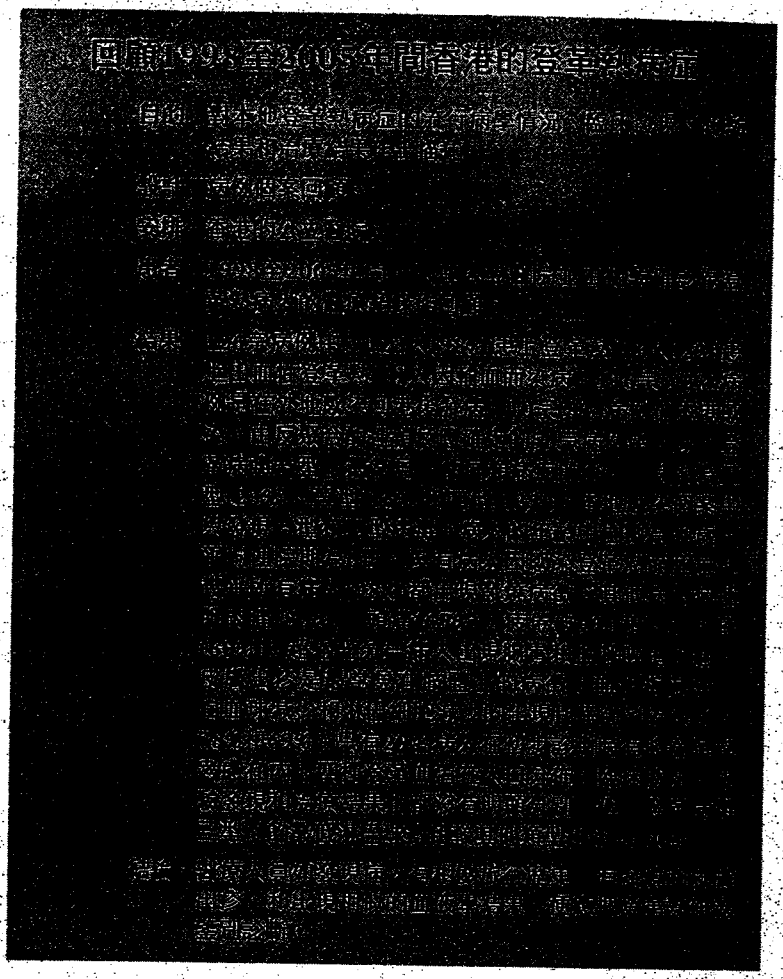
The objective of this review was to describe the epidemiology and explore the clinical characteristics and laboratory findings of dengue fever and dengue haemorrhagic fever (DHF) cases admitted to Hong Kong public hospitals during the period 1998 to 2005. We also compared the clinical and laboratory features of the four dengue serotypes identified by the polymerase chain reaction (PCR) technique.

## Methods

We included patients admitted to public hospitals during 1998 to 2005 by using selective criteria "any diagnosis ICD9CM code" starting with "061 dengue" through the Clinical Data Analysis and Reporting System. A patient list was retrieved and matched with the laboratory-confirmed dengue cases notified to the DH. A case was defined as confirmed by detection of viral genomic sequences in autopsy tissue, serum or cerebrospinal fluid samples by PCR; a four-fold or more rise in immunoglobulin G (IgG) or IgM antibody titres to one or more dengue virus antigens in paired serum samples; or a positive IgM antibody titre in late acute or convalescent phase serum specimens (obtained between September 2003 and July 2004). The epidemiological data and virological results were provided by the Surveillance and Epidemiology Branch, Centre for Health Protection, DH. The clinical presentations, laboratory findings, and outcomes of all the confirmed cases were retrospectively reviewed through medical records.

The dengue cases were categorised into dengue fever, DHF, and dengue shock syndrome. In this paper, the definition of DHF was based on the World Health Organization's criteria and defined as: fever lasting 2 to 7 days, haemorrhagic tendencies (a positive tourniquet test; petechiae, ecchymoses or purpura; bleeding from the mucosa, gastro-intestinal tract, injection sites or other locations; haematemesis or melaena), thrombocytopenia (with platelet counts $\leq 100 \times 10^9$ /L) and evidence of plasma leakage due to increased vascular permeability (a rise in haematocrit ≥20% above average for age, sex in the population, a drop in the haematocrit following volume-replacement treatment of ≥20% from baseline, and features consistent with plasma leakage such as pleural effusion, ascites, and hypoproteinaemia). Dengue shock syndrome was defined as DHF together with direct evidence of circulatory failure or indirect evidence manifested as a rapid and weak pulse, narrow pulse pressure (20 mm Hg or hypotension for age) or cold, clammy skin and altered mental status.

Statistical analysis was carried out to compare the epidemiological, clinical, and laboratory findings among the four dengue serotypes. The categorical variables were compared by the Chi squared and Fisher's exact tests. Normally distributed data were compared by analysis of variance and data with skewed distributions by the Kruskal-Wallis test.



## Results

### Disease trend

In all, 126 patients with laboratory-confirmed dengue fever were admitted to public hospitals from 1998 to 2005. Only three (2%) patients suffered from DHF, while the remaining 123 (98%) had dengue fever; no dengue shock syndrome was reported. The number of patients encountered showed an upward trend from 1998 (2 cases) to 2003 (35 cases), and subsequently remained more or less constant in 2004 (20 cases) and 2005 (24 cases). A total of 116 (92%) were imported, while in 10 (8%) the infection was locally acquired (Fig 1).

No locally acquired disease was reported until in 2002, when nine patients were identified. Among them, six cases were confirmed to be epidemiologically related to the Ma Wan outbreak. Another patient acquired the infection through blood transfusion from one of the Ma Wan cases. The remaining two locally acquired cases in 2002 and one

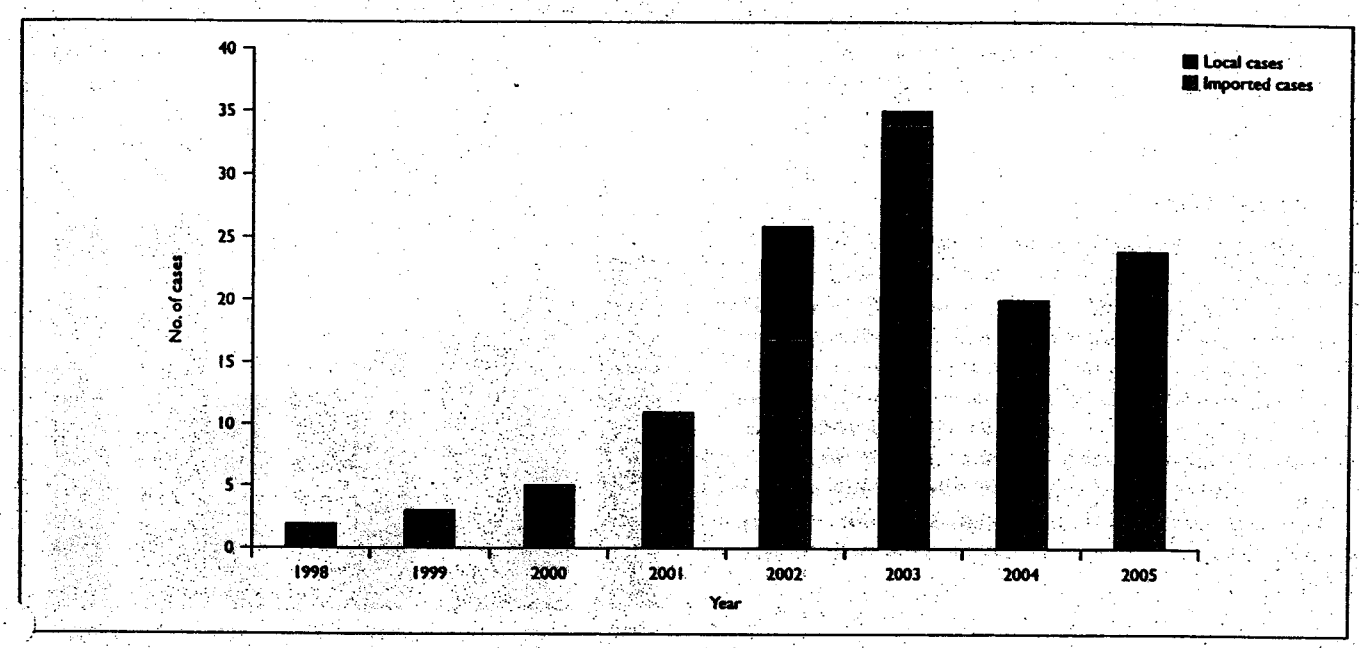



FIG 1. Numbers of dengue fever cases admitted to public hospitals in Hong Kong, 1998-2005

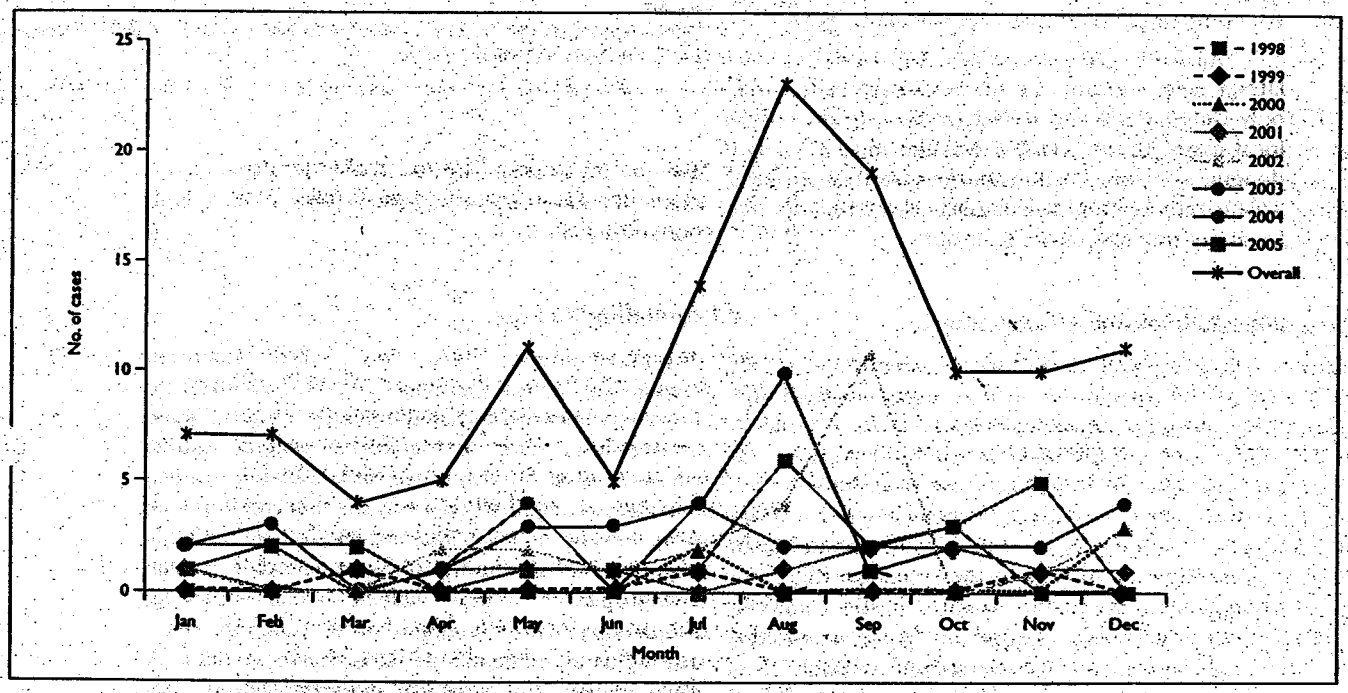



FIG 2. Seasonal variation of dengue fever cases admitted to public hospitals in Hong Kong

in 2003 were sporadic.

## Seasonality

In Hong Kong, dengue cases were reported all year round. Figure 2 demonstrates the seasonal variation of cases, with a peak from July to September.

## Country of origin for infection

Among the 116 imported cases, 106 (91%) were acquired in South-East Asian countries (Indonesia, Thailand, the Philippines, Vietnam, Singapore, Malaysia, Cambodia, Macau, and the Pacific Islands), eight (7%) originated from South Asia (India, Pakistan, Bangladesh, Sri-Lanka, and Nepal), and one (1%)

from Pitcairn island. Data for one case could not be determined as the patient had recently travelled to more than one country where the infection was endemic.

### Patient demographics

The median age of the patients was 38 (range, 5-72) years and the female-to-male ratio was 1:1.2; five (4%) were paediatric patients (aged under 16 years); 114 (90%) were Hong Kong residents. A small proportion of the patients were migrant workers or tourists (4% and 5%, respectively). Among the Hong Kong residents, 86 (75%) were Chinese, 11 (10%) were from other Asian nations (Indonesia, the Philippines, Myanmar, Thailand), three (3%) were White and two (2%) belonged to the Pakistani/Nepalese group. Data on the origin of the remaining 12 patients were missing.

### Serotype prevalence

Laboratory data on reverse-transcription PCR serotyping were available since 2002 and the serotypes of the corresponding 56 cases are shown in Figure 3.

All four serotypes, DEN-1, DEN-2, DEN-3 and DEN-4 were present among imported cases; while only DEN-1 (n=6) and DEN-2 (n=1) were present in local cases. Overall, DEN-1 was the most prevalent dengue serotype, responsible for nearly half (48%, 27/56) of all cases, followed by DEN-2 which accounted for about one quarter (23%, 13/56).

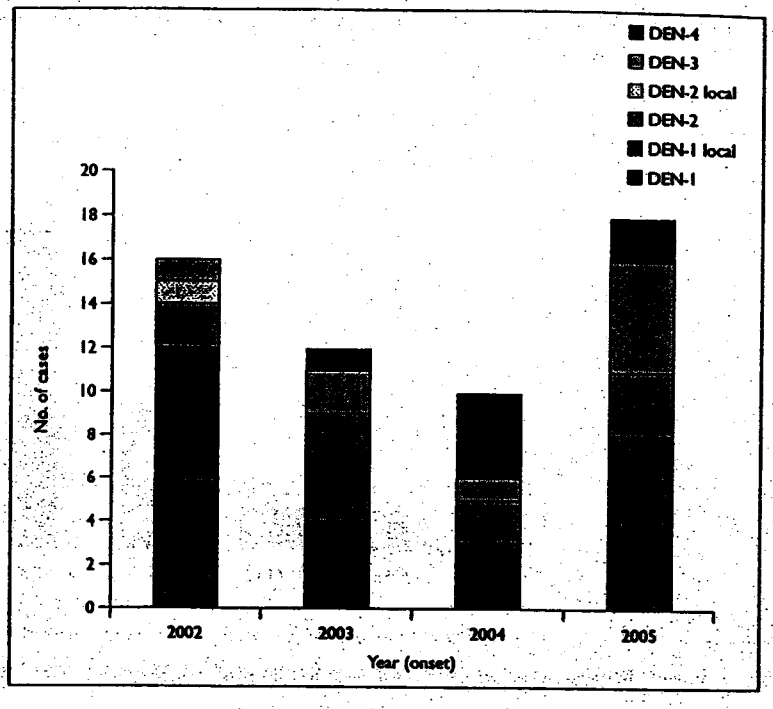



FIG 3. Distribution of serotypes among the dengue fever cases identified from 2002 to 2005
DEN-1 denotes dengue virus type 1, DEN-2 dengue virus type 2, DEN-3 dengue virus type 3, and DEN-4 dengue virus type 4

### Clinical presentations and outcome

Approximately 98% (122/124) of patients presented with fever; the mean value for the highest temperature being 38.2°C (standard deviation, 1.0°C) [Table 1]. The second commonest presenting symptom was myalgia, 83% (75/90). Two thirds of patients had headache, fatigue, and skin rashes. One third of the patients (24/71) complained of retro-orbital pain. The chief presenting complaints in more than one third of the patients were gastro-intestinal (nausea, vomiting and/or diarrhoea) or upper respiratory tract (dry cough and/or sore throat) or both. Over one quarter of patients (28/108, 26%) complained of abdominal pain, and one complained of blurred vision. Except for petechiae which were present in 45% (47/105) of the patients, other spontaneous bleeding was uncommon. Maculopapular skin rash was the commonest physical finding; in 70% of those with a rash it occurred predominately on the trunk. Lymphadenopathy was uncommon, which was only elicited in 16% of the patients. No patient demonstrated biphasic fever. Only one patient had clinical and radiological features of plasma leakage (pleural effusion), and was confirmed to be due to DHF as the final diagnosis. The mean duration of hospitalisation for these patients was 6 days, and there was no mortality.

### Laboratory findings

Thrombocytopenia was the most common haematological finding, which affected 107 (86%) of the 124 patients with available platelet counts (Table 1). The mean value of the lowest platelet counts was 64 x $10^9$/L. Among those with available results, neutropenia, atypical lymphocytes, and lymphopenia were present in 78%, 75%, and 69% of the patients respectively; half had prolonged activated partial thromboplastin time values. Corresponding proportions with deranged liver function tests and hypoalbuminaemia are also shown in Table 1. Mean values for aspartate aminotransferase and alanine aminotransferase were 212 IU/L and 169 IU/L, respectively.

### Clinical differential diagnosis

Dengue infection was included as an initial clinical differential diagnosis in only 29% of the patients. Other differential diagnoses included: viral infection, upper respiratory tract infection, gastroenteritis, typhoid fever, chest infection, malaria, scarlet fever, scrub typhus, influenza, and fever for investigation.

TABLE 1. Recorded clinical symptoms, physical and laboratory findings of dengue cases

| Symptoms/findings | No. of patients (%) | Remarks (reference range for laboratory tests) |
|---|---|---|
| Clinical symptoms | | |
| Fever | 122/124 (98) | |
| Myalgia | 75/90 (83) | |
| Headache | 68/105 (65) | |
| Skin rash | 72/121 (60) | |
| Fatigue | 50/85 (59) | |
| Dizziness | 20/44 (45) | |
| Retro-orbital pain | 24/71 (34) | |
| Gastro-intestinal tract (nausea, vomiting, and/or diarrhoea) | 39/112 (35) | |
| Upper respiratory tract (non-productive cough, sore throat) | 32/112 (29) | |
| Bleeding manifestations | | |
| Epistaxis | 7/67 (10) | |
| Gum bleeding | 8/66 (12) | |
| Haematemesis | 1/65 (2) | Dengue haemorrhagic fever |
| Tarry stool | 1/69 (1) | Dengue haemorrhagic fever |
| Petechiae | 47/105 (45) | |
| Clinical signs | | |
| Skin rash | 86/124 (69) | |
| Lymphadenopathy | 19/116 (16) | |
| Laboratory findings | | |
| Thrombocytopenia | 107/124 (86) | Platelets: 145-370 x $10^9$/L |
| Lymphopenia | 79/114 (69) | Lymphocytes: 1.0-3.1 x $10^9$/L |
| Neutropenia | 89/114 (78) | Neutrophils: 1.7-5.8 x $10^9$/L |
| Atypical lymphocytes | 92/123 (75) | |
| Prolonged activated partial thromboplastin time | 49/97 (51) | Activated partial thromboplastin time: 27.6-40.5 sec |
| Elevated aspartate aminotransferase | 29/32 (91) | Aspartate aminotransferase: <38 IU/L |
| Elevated alanine aminotransferase | 98/123 (80) | Alanine aminotransferase: 3-36 IU/L |
| Hypoalbuminaemia | 34/123 (28) | Albumin: 35-52 g/L |

### Comparison of epidemiological, clinical, and laboratory findings among the four dengue virus serotypes

There were no statistically significant differences in terms of disease severity between the four virus types, patient gender, age and duration of hospitalisation, headache, myalgia, arthralgia, retro-orbital pain, skin rash, fatigue, gastro-intestinal and respiratory symptoms (Table 2). The percentages of patients with bleeding tendencies were 50%, 67%, 63%, and 33% for DEN-1, DEN-2, DEN-3, and DEN-4 virus type infections, respectively. Further analysis of the haemorrhagic manifestations was conducted by categorisation into epistaxis, gum bleeding, haematuria, and petechiae; 75% of these patients exhibited petechiae only, with no statistically significant difference between virus types (P=0.58). Nor was there any statistically significant difference between patients having different virus subtype infections for laboratory variables, except that the lowest lymphocyte counts of patients infected by serotype 3 was lower than the other serotypes (P=0.004).

### Dengue haemorrhagic fever

Of the 126 patients under study, three (2%) were classified as DHF; all were imported from South-East Asian countries, and none could recall a previous history of dengue infection. Their demographic, clinical, and laboratory findings are shown in Table 3. They all received intravenous fluid replacement and platelet transfusions, recovered uneventfully without progression to dengue shock syndrome, and were discharged on day 6 or day 7 after hospital admission. Although these three patients did not recall prior infection, in one it was likely, as evidenced by respective acute and convalescence antibody titres.

## Discussion

This is a comprehensive review of dengue fever patients admitted to Hong Kong public hospitals over the past 8 years. Epidemiological data showed that more than 70% of the patients were local Chinese residents with a travel history to neighbouring South-East Asian countries, where dengue fever is more endemic.[6] The most prevalent serotype was DEN-1, followed by DEN-2, DEN-3, and DEN-4, which was consistent with the serotype patterns in the countries from which such infections were imported.[7,8] The outbreak in Ma Wan was the first local one in Hong Kong; only DEN-1 and DEN-2 virus subtypes were encountered in local patients during 2002 and 2003.

Seasonal variations in dengue infections should be interpreted with cautions. Dengue fever is a travel-related arthropod-borne viral disease in Hong Kong; disease activity is closely related to and depends on the seasonal and weather conditions of countries from which the virus is imported. It is difficult to determine the seasonal patterns of dengue fever acquired locally based on the few reported cases. Monthly ovitrap surveillance in Hong Kong showed that the density of *Aedes albopictus* increases from April and peaks in June.[9] It is important to alert the public to keep vigilance against this mosquito-borne viral disease during this peak period.

We report here the first blood transfusion–transmitted dengue in the literature. The patient was a 76-year-old woman, with a history of hypertension and bronchiectasis. She was admitted in 2002 because of progressive malaise. Blood tests revealed

TABLE 2. Comparison of demographic, clinical, and laboratory findings in patients infected with the four dengue serotypes*

| | PCR† type 1 (n=27) | PCR type 2 (n=13) | PCR type 3 (n=9) | PCR type 4 (n=7) | Overall (n=56) | P value |
|---|---|---|---|---|---|---|
| Gender (M:F) | 13:14 | 6:7 | 5:4 | 3:4 | 27:29 | 1.0000 |
| Age, median (IQR) | 36.0 (24.0-52.0) | 54.0 (33.0-66.0) | 28.0 (23.5-61.0) | 35.0 (21.0-63.0) | 36.0 (26.3-57.8) | 0.3559 |
| Duration of hospitalisation, median (IQR) (days) | 5.0 (4.0-7.0) | 6.0 (3.0-7.5) | 7.0 (4.5-8.5) | 5.0 (4.0-6.0) | 5.0 (4.0-7.0) | 0.4589 |
| Retro-orbital pain | 9/18 (50) | 3/10 (30) | 0/4 (0) | 1/6 (17) | 13/38 (34) | 0.2297 |
| Rash—symptom | 15/25 (60) | 8/13 (62) | 2/9 (22) | 2/7 (29) | 27/54 (50) | 0.1332 |
| Rash—sign | 17/26 (65) | 10/13 (77) | 6/9 (67) | 1/7 (14) | 34/55 (62) | 0.0509 |
| Abdominal pain | 3/25 (12) | 2/12 (17) | 1/8 (13) | 1/7 (14) | 7/52 (13) | 1.0000 |
| Diarrhoea | 11/25 (44) | 5/12 (42) | 2/8 (25) | 1/6 (17) | 19/51 (37) | 0.5956 |
| Bleeding manifestation (epistaxis, gum bleeding, petechiae, haematuria) | 13/26 (50) | 8/12 (67) | 5/8 (63) | 2/6 (33) | 28/52 (54) | 0.5775 |
| Hepatomegaly | 2/26 (8) | 2/13 (15) | 0/9 (0) | 1/7 (14) | 5/55 (9) | 0.5883 |
| Leukopenia | 25/26 (96) | 10/13 (77) | 9/9 (100) | 5/7 (71) | 49/55 (89) | 0.0529 |
| Lymphopenia | 20/22 (91) | 9/13 (69) | 8/9 (89) | 4/6 (67) | 41/50 (82) | 0.2550 |
| Atypical lymphocyte | 18/26 (69) | 10/13 (77) | 7/9 (78) | 6/7 (86) | 41/55 (75) | 0.8848 |
| Thrombocytopenia | 26/26 (100) | 11/13 (85) | 8/9 (89) | 6/7 (86) | 51/55 (93) | 0.0931 |
| Elevated aspartate aminotransferase | 8/9 (89) | 3/4 (75) | 4/4 (100) | 2/2 (100) | 17/19 (89) | 1.0000 |
| Elevated alanine aminotransferase | 23/26 (88) | 11/13 (85) | 7/9 (78) | 6/7 (86) | 47/55 (85) | 0.8954 |
| Hypoalbuminaemia | 10/26 (38) | 5/13 (38) | 5/9 (56) | 4/7 (57) | 24/55 (44) | 0.6658 |
| Highest temperature, mean (SD) | 38.6 (1.0) | 38.2 (1.1) | 38.6 (1.3) | 38.7 (0.6) | 38.5 (1.0) | 0.6893 |
| Transfusion | 4/23 (17) | 2/12 (17) | 1/8 (13) | 2/6 (33) | 9/49 (18) | 1.0000 |

* Data are shown in No. (%), except otherwise stated
† PCR denotes polymerase chain reaction

TABLE 3. Demographic, clinical, and laboratory findings in patients with dengue haemorrhagic fever

| Sex/age (years) | Ethnicity | Fever | Haemorrhagic manifestations | Lowest platelet count (x $10^9$/L) | Plasma leakage | Laboratory findings | |
|---|---|---|---|---|---|---|---|
| | | | | | | Serotype | Serology titer |
| M/38 | Thai | 37.2°C | Petechiae, bloody diarrhoea | 9 | Pleural effusion | Not done | Immunoglobulin M +ve |
| M/46 | Chinese | 38.4°C | Petechiae, bruises | 9 | Ascites | DEN 2 | Immunoglobulin M +ve |
| F/49 | Thai | 38°C | Coffee ground vomitus, petechiae | 8 | Hypoalbuminaemia, haemoconcentration | DEN 1 | 4-fold increase* |

* 1st titre: 640 (DEN-1), 5120 (DEN-2), 1280 (DEN-3), 1280 (DEN-4); 2nd titre: 5120 (DEN-1), 10 240 (DEN-2), 10 240 (DEN-3), 10 240 (DEN-4)

macrocytic anaemia and pancytopenia. She was diagnosed to have vitamin B12 deficiency anaemia, which was treated by vitamin B12 replacement and received a blood transfusion on 24 August 2002. On day 2 post-transfusion, she developed low-grade fever, but no skin rash, headache, myalgia, arthralgia, or retro-orbital pain. The patient was treated with antibiotics as for a urinary tract infection, based on the microbiological findings. The fever subsided 3 days later and the patient recovered uneventfully. The blood product she received was donated by a 17-year-old asymptomatic patient living in Ma Wan, during his viremic phase on 17 July 2002. On 24 July 2002, he developed generalised skin rash and attended the Accident and Emergency Department of Yan Chai Hospital. In October, he was subsequently picked up as one of the dengue cases based on serology results during the active case finding exercise in Ma Wan. Molecular testing performed on the donated blood product was positive for dengue virus type 1. The woman who had received the blood transfusion was recalled for blood testing on 7 October 2002, and was found to be positive for corresponding IgM antibodies and had a haemagglutination-inhibition titre of 1:2560. This incident was the first documented cases of such transmission in the literature, and since October

2002, the Hong Kong Red Cross Blood Transfusion Service (BTS) has intensified its donor deferral systems to counter this possibility. Specifically, it now asks about symptoms of dengue fever in the Blood Donor Registration Form (Supplement) by reminding all prospective donors to inform the BTS staff of all instances for flu, fever, headache, eye pain, muscle/joint pain, vomiting, and skin rash experienced 2 weeks before or after blood donation.

In our study, dengue fever was far more common than DHF and dengue shock syndrome, which were rare events. Our patients only manifested mild bleeding with good clinical outcomes and no fatalities. The clinical presentations of dengue fever, such as fever, myalgia, headache, and arthralgia, were comparable to findings reported in other studies.[10-12] Our patients (35%) presented with fewer gastroenteritis symptoms compared to those of others (50-98%).[11,12] Lymphadenopathy was documented in only 16% of our patients, which is much lower than the figure of 50% reported elsewhere.[13] This difference may be accounted for by less-than-adequate physical examination. Gum bleeding and epistaxis were reported in 12% and 10% of our patients respectively, which was also much lower than that reported previously.[11,12] Such differences could be due to the populations studied; patients recruited in endemic countries were mainly encountered during outbreaks in which both dengue fever and DHF were common. Previous studies showed dengue disease severity correlated with high viremia titres, secondary infection, and DEN-2 serotype infection.[14,15] Our findings showed that the haemorrhagic tendencies and duration of hospitalisation were not related to specific serotypes. Although some of our patients did receive platelet transfusions, the efficacy of such treatment in speeding recovery remains controversial. According to Thai experts, platelets are almost immediately destroyed by immune lysis after administration.[16]

Our study had several limitations. First, the target patients were limited to those with laboratory-confirmed dengue admitted to public hospitals. During 1998 to 2005, DH received notification of 203 dengue cases, including 77 who were admitted to private hospitals or consulted general practitioners only. The disease burden might also be underestimated, because some patients might have recovered, without seeking medical attention, while others might not have undergone serological testing. Second, statistical analysis could not be carried out to compare clinical and laboratory parameters in patients with dengue fever and DHF, as there were too few of the latter. Third, laboratory results before 2002 were not available in the Public Health Laboratory Information System. Fourth, not all clinical symptoms and signs listed in Table 2 could be retrieved from the medical records, as some may not have been specifically asked for or looked for.

In conclusion, dengue fever should be considered in the differential diagnosis of febrile patients with or without a travel history. Health care providers should therefore have an understanding of the infection, the spectrum of its clinical features, and methods of diagnosis and appropriate treatment. Until the *Aedes* mosquito can be effectively controlled or a cost-effective vaccine is developed, dengue fever will remain a public health concern, especially in South-East Asia. Control at source is one of the keys to combating dengue fever and requires active participation from all sectors of the community.

## Acknowledgements

The members of Dengue Review Working Group gratefully acknowledge the Central Committee on Infectious Disease, Hospital Authority for the provision of patient medical records and Surveillance and Epidemiology Branch, Centre for Health Protection for the epidemiological and laboratory input. Without their support, our review would not have been achieved.

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別紙様式第2

別紙(3)-3

# 医薬品　研究報告　調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日<br>2008年8月11日 | 新医薬品等の区分<br>該当なし | 厚生労働省処理欄 |
|---|---|---|---|---|---|---|
| 一般的名称 | フィブリノゲン加第 XIII 因子 | | 研究報告の公表状況 | Clearance of dengue virus in the plasma-derived therapeutic proteins. Transfusion. 2008 Jul;48(7):1342-7. 2008 Feb 22. | 公表国<br>中国 | |
| 販売名（企業名） | ベリプラスト P コンビセット<br>（CSL ベーリング株式会社） | | | | | |

**研究報告の概要**

問題点（血漿分画製剤でのデングウイルスの不活化・除去）

デングウイルスは年間に世界で 5 千万から 1 億人が感染し、感染者の数十万人がより重篤で生命を脅かすデング出血熱やデングショック症候群に進展する。

デングウイルスはフラビウイルス科に属し、直径 50nm のエンベロープを有する RNA ウイルスである。一般に血液などの高蛋白な体液で長期間生存するので、輸血により感染する可能性がある。針刺し事故や骨髄移植、分娩での血液に関連するデングウイルス感染が報告されている。

本研究は血漿分画製剤でのデングウイルス伝播の危険性が、特定のウイルス除去・不活化工程で除去されることを初めて証明するため実施された。

低温エタノール分画、陰イオン交換クロマトグラフィー、パスツリゼーション、S/D 処理とウイルスろ過を含むアルブミンやグロブリンの各製造工程前に、高力価の培養デングウイルスセロタイプ 2 を正常人血漿にスパイクし、各製造工程でのデングウイルスのクリアランスを $TCID_{50}$ アッセイ、RT-PCR で測定した。

デングウイルスの不活化・除去に対して、各製造工程前は全てで有効であった。

また、アルブミンの全製造工程（低温エタノール分画、パスツリゼーション）で少なくとも 10.12 log 減少すること、グロブリンの全製造工程（低温エタノール分画、ウイルスろ過、S/D 処理、クロマトグラフィー）では少なくとも 14.24 log 減少することが証明された。

現在実施されている血漿分画製剤の製造方法は、デングウイルス伝播に関して安全である。

使用上の注意記載状況・その他参考事項等

| 報告企業の意見 | 今後の対応 |
|---|---|
| 本剤の製造工程（低温エタノール分画、パスツリゼーション、イオン交換樹脂等）でデングウイルスが不活化・除去できると考えられる。 | 今後とも新しい感染症に関する情報収集に努める所存である。 |



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# Clearance of dengue virus in the plasma-derived therapeutic proteins

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**BACKGROUND:** Viral safety is of paramount importance for human plasma–derived therapeutic proteins. Recent reports of blood-associated transmission and continuous regional outbreaks of dengue fever have prompted a validation of clearance of dengue virus in the manufacture processes of the plasma-derived products.

**STUDY DESIGN AND METHODS:** A high titer of cultured dengue virus serotype 2 was spiked into process samples before individual steps of albumin and immunoglobulin manufacture processes, including cold ethanol precipitation, cation-exchange chromatography, pasteurization, solvent/detergent treatment, and virus filtration. Clearance of dengue virus was quantified with $TCID_{50}$ assays in the culture of Vero E6 cells and, when appropriate, real-time polymerase chain reaction (RT-PCR) assays.

**RESULTS:** The individual process steps were all effective in the inactivation and/or removal of dengue virus, and the data obtained clearly demonstrate that the risk of dengue virus transmission was reduced cumulatively by at least 10.12 and at least 14.24 log in the albumin and immunoglobulin manufacture processes, respectively.

**CONCLUSION:** The dedicated viral inactivation and/or removal approaches currently implemented in the manufacture of plasma-derived products provide a good safety margin with regard to the transmission of dengue virus.

Dengue virus infects 50 to 100 million people worldwide a year; of those infected, several hundred thousand develop the more severe and life-threatening diseases, dengue hemorrhagic fever and dengue shock syndrome. Dengue virus belongs to the family Flaviviridae, which in general is known to survive over long periods in fluids with high protein contents, for example, blood. Therefore, dengue viruses may be transmitted via transfusion of blood or blood components. Albeit rare, it has indeed been documented that blood-associated transmission of dengue virus occurs via routes including needle-stick injuries,[1] marrow transplantation,[2] intrapartum and vertical transmission,[2] and mucocutaneous transmission.[3] This can be a serious public health problem without proper control measures.

Dengue virus is a lipid-enveloped RNA virus, with a diameter of approximately 50 nm.[4] Reportedly, dengue virus has been effectively inactivated by photosensitizers[5,6] and is sensitive to high temperatures and acidic pH.[7] This study aims to demonstrate for the first time that the

**ABBREVIATION:** BVDV = bovine viral diarrhea virus.

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The authors are thankful for partial funding support from the Innovation and Technology Fund (S/P 583/03), the Government of the Hong Kong Special Administrative Region. Some results of the present study are used in the U.S. patent application number 11/294,432 and Chinese patent application number 200610150052.2.

Received for publication March 27, 2007; revision received November 12, 2007, and accepted November 19, 2007.

doi: 10.1111/j.1537-2995.2008.01647.x

**TRANSFUSION** 2008;48:1342-1347.

risk of dengue virus transmission in plasma derivatives is eliminated by specific virus removal and inactivation procedures. Log reduction of dengue virus is investigated at individual steps of the manufacture processes of plasma-derived albumin and immunoglobulins, which include cold ethanol precipitation, cation-exchange chromatography, pasteurization, solvent/detergent (S/D) treatment, and virus filtration. The evaluation of the manufacture processes provides a measure of confidence for eliminating dengue virus.

## MATERIALS AND METHODS

### Raw materials

Normal human plasma was obtained from the plasma fractionator Shenzhen Weiwu Guangming Biological Products Co. (Shenzhen, China). All chemicals used in this study were of either pharmaceutical grade or analytical grade. Virus filters (Planova 35N, 10 $cm^2$) were a gift from Asahi Kasei (Tokyo, Japan).

### Virus culture and quantification

Dengue virus serotype 2 (S047/00 from Environmental Health Institute, Singapore) was propagated in C6/36 cells (CDC Guangdong, China) in minimal essential medium with 1 percent fetal bovine serum (Gibco, Grand Island, NY). Dengue virus was quantified with $TCID_{50}$ assays in the culture of Vero E6 cells (ATCC, Manassas, VA). Vero E6 cells ($2.5 \times 10^5$ cells/mL) were seeded in 96-well plates in a volume of 100 µL per well. After 1 day of incubation, 50 µL of medium was added to each well. Each dilution of sample was added at 50 µL per well, and further incubation was carried out at 36 ± 2°C with 5 percent $CO_2$. Plates were assessed for $TCID_{50}$ endpoint as cytopathic effects developed on the fifth day. The $TCID_{50}$ endpoint was calculated according to the Spearman-Kärber method, and the Poisson distribution was used when no virus was detected in samples. Quantitative real-time polymerase chain reaction (RT-PCR) was used to determine virus titer in the chromatography and cold ethanol precipitation steps. RNA of dengue virus was extracted in duplicate from samples with a viral RNA mini kit (QIAamp, Qiagen, Hilden, Germany) according to the procedure provided by the manufacturer. Dengue virus cDNA was reverse transcribed with random hexamers with reverse transcriptase (Supercript III, Invitrogen, Carlsbad, CA). Quantitative RT-PCR utilizing TaqMan technology (Applied Biosystems, Foster City, CA) was performed on samples and proper controls with specific primers (GTCAACATAGAAGCAGAACCTCCA and CTCTATGATGATGTAGCTGTCTCCG) and SYBR Green fluorescent probes with conditions optimized to detect 4.67 copies of viral RNA for dengue virus. Duplicate PCR procedures were performed for each sample with a sequence detection system (ABI 7900 HT, Applied Biosystems), and the cycling conditions were 50°C for 2 minutes, 95°C for 10 minutes followed by 40 cycles of 95°C for 15 seconds, and 60°C for 1 minute, as well as a dissociation stage of 95°C for 15 minutes, 60°C for 15 minutes, and 95°C for 15 minutes.

### Fraction IV precipitation

The supernatant II + III was prepared from frozen human plasma through two consecutive steps of cold ethanol precipitation with 8 percent ethanol at pH 7.1 followed by 19 percent ethanol at pH 5.85.[8] Duplicates of 20 mL of supernatant II + III were spiked with 7.00 log per mL each of dengue virus at a ratio (vol/vol) of 1:10. Ethanol (95%) was added drop by drop into the supernatant II + III to a final ethanol concentration of 40 percent, which was further mixed at –5 to 5.5°C for 1 hour, before being centrifuged at 2300 × *g* to separate the fraction (F)IV from the supernatant IV. The supernatant II+III, the FIV, and the supernatant IV were titrated for quantity of viruses by $TCID_{50}$ assay or RT-PCR assay.

### Pasteurization

The purified albumin solution was diafiltrated with 8 volumes of water and then concentrated to a concentration of 22 percent with a 30-kDa cutoff cassette (Millipore, Bedford, MA). Sodium caprylate was added to the concentrated albumin solution to a final concentration of 32 mmol per L, before adjustment of pH to 6.8 to make 20 percent albumin bulk. Two-hundred milliliters of albumin bulk and duplicates of the sterile-filtered 20 percent albumin in a 50-mL bottle was heated to 59°C in a water bath, followed by spiking with dengue virus (6.67, 7.50, or 7.67 log/mL) at a ratio (vol/vol) of 1:20 and 1:25, respectively. Gentle mixing with a mechanical stirrer (stainless steel) was applied to the bulk pasteurization. Samples were taken out for virus titration during the time course of a 10-hour treatment at 59 to 60°C.

### FIII precipitation

The FII + III separated from the supernatant II + III above was redissolved, and NaAc-HAc buffer (0.8 mol/L-4 mol/L, pH 3.9) was added dropwise to adjust pH to 5.1. Dengue virus (7.17 or 7.67 log/mL) was spiked at a ratio (vol/vol) of 1:10 into duplicates of 20 mL of the pH-adjusted FII + III. Ethanol (95%) was added drop by drop into the FII + III to a final ethanol concentration of 15 percent, which was further mixed at –5 to 5.5°C for 1.5 hour, before being centrifuged at 2300 × *g* to separate the FIII from the supernatant III. The FII + III, the supernatant III, and the FIII were titrated for quantity of viruses by $TCID_{50}$ assay.

### Virus filtration

A quantity of 196 mL of partially purified immunoglobulin was spiked with 7.67 log per mL dengue virus at a ratio (vol/vol) of 1:49, followed by filtration with a 0.22-μm filter (Steritop, Millipore) to remove viral aggregates. The filtered immunoglobulin was subject to virus filtration with the 35N filter in a normal-flow manner, under constant pressure of 80 kPa. Samples were titrated for quantity of viruses by $TCID_{50}$ assay or RT-PCR assay.

### S/D treatment

Duplicates of 27 mL of the immunoglobulin purified through virus filtration were heated to 28°C in a water bath, followed by spiking with 7.16 or 7.83 log per mL dengue virus at a ratio (vol/vol) of 1:9. Triton X-100 and tri-*n*-butyl phosphate were added drop by drop into the immunoglobulin to a final concentration of 1 and 0.3 percent, respectively. Gentle mixing was achieved with a mechanical stirrer (stainless steel) for the time course of 16-hour treatment at 28 to 30°C, during which samples were removed for virus titration by $TCID_{50}$ assay.

### Cation-exchange chromatography

A chromatography column of 10-mm diameter was packed to a bed height of 11 cm with either new CM Sepharose Fast Flow resin (Pharmacia Biotech, Uppsala, Sweden) or the used resin that had previously been recycled 476 times with the immunoglobulin purification process. The column was equilibrated with 20 mmol per L NaAc buffer, pH 4.0. Adjusted to a pH of 4.0 with 1 M HCl and an ionic strength of 1.4 mS per cm with purified water, duplicates of 75 mL of the S/D-treated immunoglobulin solution were spiked with 7.67 or 7.83 dengue virus at a ratio (vol/vol) of 1:20. The virus-spiked immunoglobulin solution was applied to the column at a linear flow rate of 40 cm/hr at ambient temperature. After washing of the column with 10 column volumes of 10 mmol per L glycine, pH 7.0, immunoglobulins were eluted with 100 mmol per L glycine together with 150 mmol per L sodium chloride, pH 9.0. The column load, the flow-through fraction, and the eluate fraction containing immunoglobulins were titrated for quantity of viruses by $TCID_{50}$ assay or RT-PCR assay.

## RESULTS

### FIV precipitation

After the 40 percent ethanol precipitation of the supernatant II + III, no dengue virus was detected with the $TCID_{50}$ assay in both the supernatant IV and the FIV (Table 1). Despite its direct cytotoxicity to the virus detector Vero E6 cells, when diluted 500-fold, 40 percent ethanol did not affect the determination of virus titer. Results of quantitative RT-PCR clearly showed that genetic materials of dengue virus were concentrated in the FIV (Table 1), which is discarded during the albumin manufacture. Because chemical inactivation by high concentrations of ethanol is mechanistically different from the physical partitioning effects between fractions, this FIV precipitation step provides an extra safety margin in the effective clearance of dengue viruses.

### Pasteurization

The kinetics of inactivation of dengue virus in the 20 percent albumin during the 10-hour pasteurization at 59 to 60°C are shown in Fig. 1. The pasteurization was carried out at 0.5°C below what is normally used in the manufacture, representing a worst-case scenario. Dengue

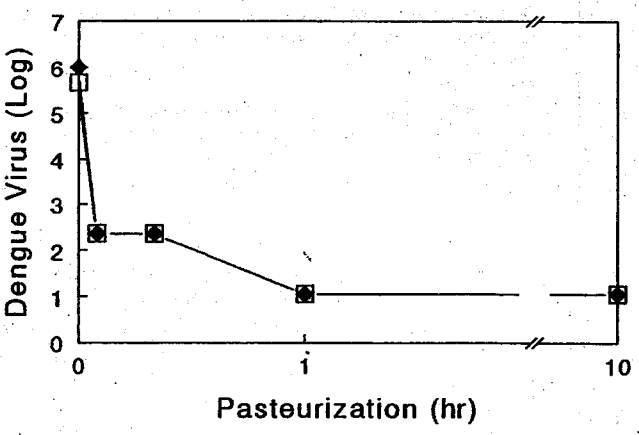


**Fig. 1. Inactivation of dengue virus in albumin by pasteurization over time. (□) Bulk pasteurization; (◆) terminal pasteurization. Stock dengue viruses (6.67 or 7.50 log/mL) were spiked at a ratio (vol/vol) of 1:20 and 1:25, respectively, in the bulk pasteurization and terminal pasteurization.**

**TABLE 1. Clearance of dengue virus in the precipitation of FIV***

| Assay (log) | Supernate II + III | FIV | Supernate IV | Log reduction, II + III → supernate IV |
|---|---|---|---|---|
| $TCID_{50}$ | 6.83/7.00 | 2.06†/2.06† | 1.65†/1.65† | ≥5.18/≥5.35 |
| Quantitative RT-PCR | 7.15/8.33 | 7.40/7.56 | 3.30/4.98 | 3.85/3.35 |

* Data shown are total viral titers (log number multiplied by volume) from duplicate experiments, where stock virus had a titer each of 7.00 log per mL spiked at a ratio of 1:10.
† No dengue virus was detected, and the number was calculated according to the Poisson distribution.

virus was quickly inactivated by the heat treatment, and infectious virus became undetectable within 5 minutes (Fig. 1). Total viral reduction for both the bulk pasteurization and the terminal pasteurization is shown in Table 2.

### FIII precipitation

After the 15 percent ethanol precipitation, dengue virus was detectable with the $TCID_{50}$ assay in both the supernatant III and the FIII, with a majority of infectious virus in the FIII. Viral reduction from the FII + III to the supernatant III was calculated and is shown in Table 3.

### Virus filtration

The immunoglobulin spiked with dengue virus was processed at 24 to 25°C through the 35N virus filter within 7 hours. No infectious virus was detectable by the $TCID_{50}$ assay in the immunoglobulin filtrate, the sample obtained when the virus filter was reversely flushed with purified water, or the virus-spiked immunoglobulin control standing along the whole virus filtration process. To differentiate physical separation from the chemical inactivation by the low pH, the samples were further quantified for dengue virus with the quantitative RT-PCR assay. The RT-PCR data show that dengue virus was much more concentrated in the back-flush fraction than in the immunoglobulin filtrate. Viral reduction by the virus filtration was calculated and shown in Tables 3 and 4. These results indicate that dengue virus is effectively removed by the 35N virus filtration.

### S/D treatment

The presence of S/D was cytotoxic to the virus detector Vero E6 cells; when diluted 1000-fold, S/D did not affect the determination of virus titer. The kinetics of inactivation of dengue virus in the immunoglobulin during the 16-hour S/D treatment at 28 to 30°C is shown in Fig. 2. Dengue virus was quickly inactivated by the S/D treatment, and infectious virus became undetectable within 1 minute. Total viral reduction for the S/D treatment was shown in Table 3.

**TABLE 2. Clearance of dengue virus in the albumin process***

| Process step | Reduction of virus (log) |
|---|---|
| FIV precipitation | ≥5.18†/≥5.35† |
| Bulk pasteurization | ≥4.61 |
| Terminal pasteurization | ≥4.94/≥5.44 |
| Cumulative | ≥10.12‡/≥10.79‡ |

* Data are shown from duplicate experiments, except bulk pasteurization, which was conducted once.
† The data from the $TCID_{50}$ assay, but not the RT-PCR assay, are included.
‡ "Bulk pasteurization" is not included in the "cumulative," because it is similar mechanistically to "terminal pasteurization."

**TABLE 3. Clearance of dengue virus in the immunoglobulin process***

| Process step | Reduction of virus (log) |
|---|---|
| FIII precipitation | 2.16/2.65 |
| Virus filtration | 3.37† |
| S/D treatment | ≥5.05/≥5.38 |
| Chromatography | 3.66‡/4.18‡ |
| Cumulative | ≥14.24/≥15.58 |

* Data of single virus filtration experiment and duplicate experiments of other processing steps are shown.
† Only RT-PCR data are included.
‡ Virus reduction caused by the presence of S/D is not included.

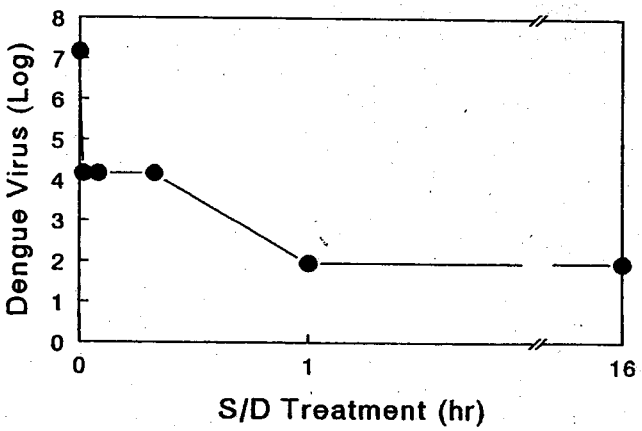


**Fig. 2. Inactivation of dengue virus in immunoglobulins by S/D treatment over time. Stock dengue viruses (7.16 log/mL) was spiked at a ratio (vol/vol) of 1:9 in the S/D treatment step; when it was spiked to the immunoglobulin sample neutralized to pH 7.0 a viral titer of 7.00 log was obtained.**

**TABLE 4. Clearance of dengue virus in the virus filtration***

| Assay | Load | Immunoglobulin filtrate | Back-flush | Log reduction, load → filtrate |
|---|---|---|---|---|
| $TCID_{50}$ | 8.37 | ≤2.58† | ≤2.51† | ≥5.79 |
| Quantitative RT-PCR | 8.47 | 5.10 | 7.08 | 3.37 |

* The numbers shown are total viral titers (log number multiplied by volume), and the stock virus spiked at a ratio of 1:49 had a titer of 7.67 log per mL.
† No dengue virus was detected, and the number was calculated according to the Poisson distribution.

**TABLE 5. Clearance of dengue virus in the chromatography***

| Assay | Resin | Load | Flow through | Eluate | Log reduction, load → eluate |
|---|---|---|---|---|---|
| $TCID_{50}$ | New | 8.20 | ≤6.19† | ≤1.16† | ≥7.04 |
| | Used | 8.04 | ≤6.19† | ≤1.11† | ≥6.93 |
| Quantitative RT-PCR | New | 9.24 | 8.31 | 5.58 | 3.66 |
| | Used | 8.99 | 8.14 | 4.81 | 4.18 |

* The numbers shown are total viral titers (log number multiplied by volume), and the stock virus spiked at a ratio of 1:20 had a titer of 7.67 and 7.83 log per mL for the new and used resins, respectively.
† No dengue virus was detected, and the number was calculated according to the Poisson distribution.

### Cation-exchange chromatography

After the chromatography step of the immunoglobulin process, no infectious virus was detectable by the $TCID_{50}$ assay in the column load, the flow-through fraction, the eluate fraction, or the virus-spiked load control standing along the whole chromatography process. Total viral reduction from the column load to the eluate fraction was at least 7.04 and at least 6.93 log for the new resin and the 476-cycled used resin, respectively (Table 5). Because S/D was present in the starting material, the elimination of dengue virus could be a result of inactivation by the chemicals. To differentiate physical removal from chemical inactivation, the samples were further quantified for dengue virus with the quantitative RT-PCR assay. The RT-PCR data show that a majority of dengue virus was observed in the flow-through fraction. Total viral removal by the chromatography process was calculated to be 3.66 and 4.18 log for the new resin and the 476-cycled used resin, respectively (Tables 3 and 5).

## DISCUSSION

Viral safety is of paramount importance for human plasma–derived therapeutic proteins such as albumin, α1-proteinase inhibitor, clotting factors, and immunoglobulins. Recent documentation of blood-associated transmission[1-3] and continuous regional outbreaks of dengue fever have prompted a validation of clearance of dengue virus in the manufacture processes of the plasma-derived products. It was the intention of this study to investigate clearance of dengue virus in individual steps of manufacture processes of plasma-derived albumin and immunoglobulins. The results shown in Tables 2 and 3 clearly demonstrate for the first time that specific virus removal and inactivation procedures reduce the risk of dengue virus transmission by more than 10 log cumulatively in plasma-derived albumin and immunoglobulins.

In this study, cold ethanol precipitation is very effective in inactivating dengue virus in the albumin process, but mildly effective in removing dengue virus in the immunoglobulin process. This difference in effectiveness is probably due to the fact that higher concentrations of ethanol were used in the albumin process. It is fairly reasonable to speculate that other therapeutic proteins prepared from plasma by similarly high concentrations of ethanol, for instance, α1-proteinase inhibitor and transferrin purified from the Cohn FIV, would have a good safety margin with regard to transmission of dengue virus.

Pasteurization inactivated dengue virus very quickly and effectively in the albumin process. The presence of a high concentration of albumin or the albumin stabilizing agent sodium caprylate did not seem to protect dengue virus from the heat inactivation. Caprylate has been shown to be an effective virus-inactivating agent at millimolar concentrations under acidic conditions;[9-12] however, caprylate appears unlikely to contribute much to the viral inactivation capacity of the pasteurization step as in the albumin formulation it is used under neutral pH, which do not favor the formation of the active component—the nonionized form of caprylate. As shown by albumin's long history of viral safety in clinical applications, the dedicated viral inactivation step in albumin manufacture processes has been very robust in the inactivation of many different viruses including West Nile virus and bovine viral diarrhea virus (BVDV), both from the same Flaviviridae family as dengue virus.[13,14]

Virus filtration was very effective in separating dengue virus from the immunoglobulin filtrate (Table 4). The data suggest that chemical inactivation by the low pH condition can probably contribute to the viral clearance capacity of this process step. In a separate study with BVDV, which is of similar size but not sensitive to low pH treatment, a majority of the spiked BVDV was trapped in the Planova 35N filter, which was recovered in the backflush sample (unpublished observation).

Like pasteurization, S/D treatment very quickly and effectively inactivated dengue virus in the immunoglobulin process. This dedicated viral inactivation step in the immunoglobulin manufacture processes has been very robust in the inactivation of many different viruses including West Nile virus and BVDV.[13,14]

The cation-exchange chromatography was originally intended to remove S/D from the immunoglobulin process; however, it was also observed in this study to effectively remove dengue virus by affinity adsorption. In addition, this purification step was mildly effective in the physical removal of BVDV (unpublished observation). Although the chromatographic process may not be a

robust viral removal step in general, it is indeed effective in the clearance of dengue virus.

In summary, this study has shown that effective clearance of dengue virus is achieved in the manufacture processes of albumin and immunoglobulins, providing additional evidence supporting the viral safety of plasma-derived products.

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