薬事・食品衛生審議会

平成２４年度　第３回　血液事業部会運営委員会

議 事 次 第

日時：平成２４年１２月１９日（水）
１６：００～１８：００
場所：弘済会館　４階 萩の間

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

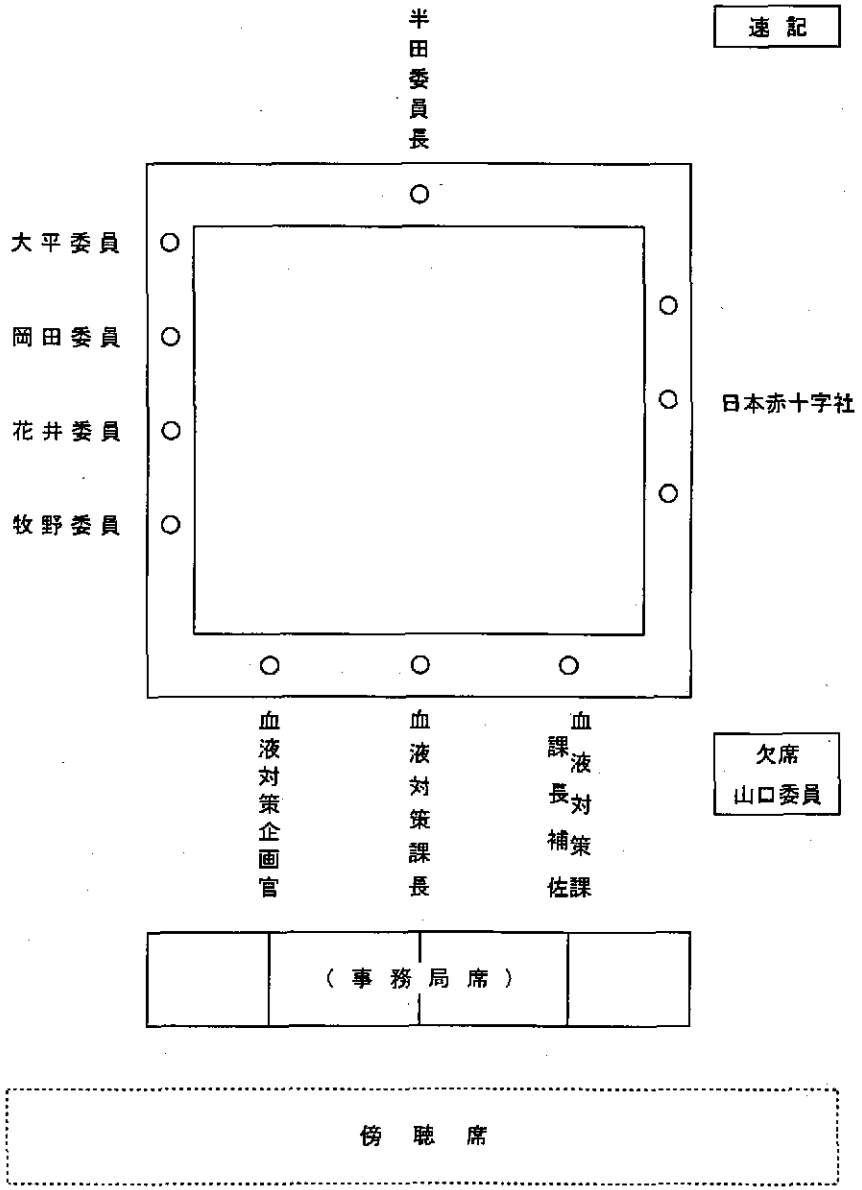
配付資料：

座席表
委員名簿
設置要綱
資　料　１　平成２４年度第２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　血液製剤に関する医療機関からの感染症報告事例等について
資料３－３　献血件数及びＨＩＶ抗体・核酸増幅検査陽性件数について
資　料　４　血小板製剤に対する感染性因子低減化（不活化）技術導入準備の進捗状況について（日本赤十字社提出資料）
資　料　５　「血液製剤の安全性の向上及び安定供給の確保に関する基本的な方針」の改訂案について
資　料　６　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
資　料　７　株式会社ベネシスに対する改善命令について（日本血液製剤機構提出資料）

参考資料１　「献血血液の研究開発等での使用に関する指針」に基づく公募について（平成 24 年 11 月 27 日薬食血発 1127 第１号）
参考資料２　「献血血液の研究開発等での使用に関する指針」に基づく事前評価等に関する指針について（平成 24 年 11 月 27 日薬食血発 1127 第２号）
参考資料３　「献血血液の研究開発等での使用に関する指針」について（平成 24 年８月１日薬食発 0801 第１号）

平成２４年度第３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24年12月19日(水)
弘済会館 4階 萩の間
16:00～18:00



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．花井 十伍 （はない　じゅうご）
　　ネットワーク医療と人権理事

4．半田 誠 （はんだ　まこと）
　　慶應義塾大学医学部輸血・細胞療法部教授

5．牧野 茂義 （まきの　しげよし）
　　国家公務員共済組合連合会虎の門病院輸血部長

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研究員

（50音順、敬称略）

薬事分科会血液事業部会運営委員会規程

（目的）

第１条　この規程は薬事分科会規程（以下「規程」という。）第２条第１項に基づき設置される血液事業部会（以下「部会」という。）に置かれる運営委員会（以下「委員会」という。）の運営に関し必要な事項を定め、審議の円滑な実施を図ることを目的とする。

（所掌）

第２条　委員会は、規程第３条第５項に規定する部会が調査審議すべき血液製剤（血液製剤代替医薬品を含む。以下同じ。）に係る事項（以下「部会で調査審議すべき事項」という。）を検討するとともに、以下に掲げる事項を確認し、部会に報告するものとする。

一　安全な血液製剤の安定供給の確保等に関する法律（以下「血液法」という。）第２６条第１項に規定する血液製剤の製造又は輸入の実績に係る報告

二　血液法第２９条に規定する薬事法第６８条の８第１項に規定する生物由来製品（血液製剤に限る。）の評価に係る報告

三　規程第４条第１項の規定に基づき部会に置かれる調査会における調査審議の状況

四　その他部会で調査審議する事項のうち特別の事項についての状況

（委員会への所属）

第３条　委員会に所属すべき委員は、部会に所属する委員、臨時委員及び専門委員（以下「委員等」という。）の中から、部会長が指名する。

２　部会長は、前項の規定により委員会に属すべき委員等を指名する場合は、血液製剤を使用する患者の代表、医療関係者、血液事業の専門家を含め、数名を指名する。

３　部会長は、第一項の規定により委員会に属すべき委員等を指名した場合は、部会においてその旨を報告しなければならない。

（委員長の選任）

第４条　委員会に委員長を置き、委員会に属する委員等の互選により選任する。

２　委員長は、委員会の事務を掌理する。

３　委員長に事故があるときは、委員会に属する委員等のうちから委員長があらかじめ指名する者が、その職務を代理する。

（委員会の開催）

第５条　委員会は、四半期（１月から３月まで、４月から６月まで、７月から９月まで及び１０月から１２月までの各期間をいう。）ごとに開催する。

２　前項に規定する場合のほか、委員等が必要と認めるときは委員会を開催することができる。

（議決）

第６条　部会への報告の要否等、議決を行う必要がある委員会の議事は、委員会に属する委員等で会議に出席したものの過半数で決し、可否同数のときは、委員長の決するところによる。

（議事の公開）

第７条　委員会は原則として公開する。ただし、公開することにより、委員の自由な発言が制限され公正かつ中立な審議に著しい支障をおよぼすおそれがある場合、又は、個人の秘密、企業の知的財産等が開示され特定の者に不当な利益又は不利益をもたらすおそれがある場合については、委員長は、これを非公開とすることができる。

（雑則）

第８条　この規程に定めるもののほか、委員会の運営に関し必要な事項は、部会長が部会に諮り決定するものとする。

附則

この規程は、平成１５年７月３０日から施行する。

資料１

平成24年度第2回　血液事業部会運営委員会議事要旨（案）

日時：　平成24年9月28日（金）　10:00～12:00
場所：　厚生労働省17階（専用第21会議室）
出席者：
（委員）
半田委員長、大平委員、岡田委員、花井委員、牧野委員、山口委員
（日本赤十字社）
田所経営会議委員、中西総括副本部長、日野副本部長、井上献血推進課長
（事務局）
加藤課長、丈達企画官、岡村補佐、笠松補佐

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）

議題1について

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。

議題2について

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。

議題3について

事務局より、供血者からの遡及調査の進捗状況、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がなされた。

議題4について

（シャーガス病に対する献血血液の安全対策）

事務局及び日本赤十字社より、平成24年度第1回安全技術調査会でのシャーガス病に関する審議結果が報告された。シャーガス病に対する献血血液の安全対策として、中南米出身者又は中南米に4週間以上滞在した方等からの献血血液を原料血漿にのみ使用する対応が、平成24年10月15日より開始されることが報告された。

（平成23年度日本赤十字社血液事業本部の取り組み）

日本赤十字社より、平成23年度の血液事業本部の取り組みに関する報告がなされた。

（献血の同意説明書）

日本赤十字社より、「献血血液の研究開発等での使用に関する指針」を受けて、新たに作成された「献血の同意説明書」の案が提示された。委員より、一部誤解を招く記載があることから、より分かりやすい表現に改める必要があるとの意見が出された。

議題5について

（基本方針の改訂案）

事務局より、「血液製剤の安全性の向上及び安定供給の確保に関する基本的な方針」の改訂案の提示及びその説明がなされた。改訂案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。

（フィブリノゲン製剤に係る調査）

事務局より、フィブリノゲン製剤の調査に係る公表事項の報告がなされた。

（厚生労働省行政事業レビュー）

事務局より、平成24年度厚生労働省行政レビューの結果、日本赤十字社への補助事業が「廃止」となったことが報告された。

（その他）

献血後に交通事故で死亡する事例が発生したことを受け、事務局より、水分補給や休憩の必要性等について、改めて現場に周知徹底する対応をとることが報告された。

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。

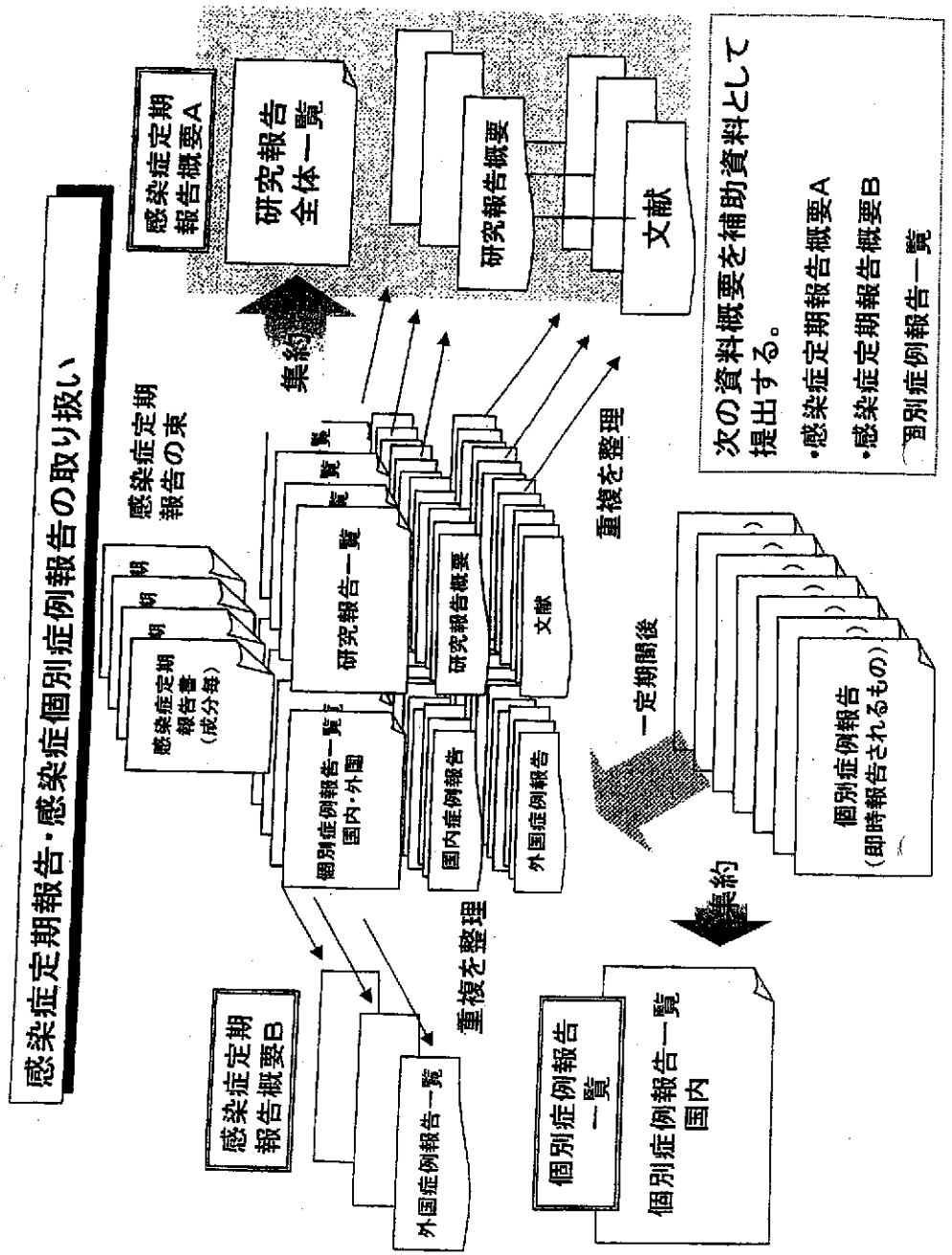
① 研究報告は、同一文献による重複を廃した別紙のような形式の一覧表を作成し、当該一覧表に代表的なものの報告様式（別紙様式第2）及び該当文献を添付した「資料概要A」を事務局が作成し、送付する。

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。



感染症定期報告概要

（平成２４年１２月１９日）

平成２４年８月１日～１０月３１日受理分

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２４年８月１日～１０月３１日まで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(2012/8/1～2012/10/31)

血対課ID	受理日	感染症(PT)	出典	概要	新出文献番号
120094	27-Sep-12	B型肝炎	Transfusion. 52(2012)880-892	南アフリカにおける個別核酸増幅検査(ID-NAT)済み血液輸血によるB型肝炎ウイルス(HBV)感染に関する報告。2005年以来4年間で2,921,561の供血血液が、南アフリカにてHBV ID-NATスクリーニングを受け、149件のHBs抗原陰性のHBV NAT陽性供血が同定された。遡及調査によって1件のHBV感染疑い症例が同定された。供血者及び受血者から分離したHBVのゲノム配列決定、増幅、系統発生解析を行ったところ、149件のHBV NAT陽性検体中114件は抗HBc抗体が検出される前のウインドウピリオド(WP)で、35件は抗HBc抗体WP後であると分類された。HBV DNA陰性の状態での抗HBc抗体WP前、後における急性感染リスクはそれぞれ1:40,000と1:480,000であると推算された。報告されたHBV感染症例は、抗HBc抗体検出前のWPにおけるID-NAT陰性の輸血を受けた患者であった。	1
120094	28-Sep-12	B型肝炎	J Hepatol. Jun 2, 2012	台湾におけるワクチン接種プログラムによるB型肝炎ウイルス(HBV)感染の低減に関する報告。台湾で1984年に開始された乳幼児へのユニバーサルワクチン接種プログラムの評価として、25年後の血清疫学調査が行われた。30歳未満の各年齢集団から約100人ずつ、3,332人の被験者を登録し、HBs抗原、HBs抗体、HBc抗体の陽性率を比較したところ、2009年におけるプログラム開始後に出生した被験者と、1984年のベースライングループとの間で大きく異なっていた。また、前回(プログラム開始20年後)調査よりも、HBs抗原保有率がワクチン接種群でさらに減少していた。ワクチン無効者のうち86%は、母親がHBs抗原陽性であった。この結果より、若年者へのワクチンの有効性が明確であることが示された。	2
120095	03-Oct-12	B型肝炎	Virology Journal 2012, 9: 2	ニワトリの血清及び肝中に含まれるB型肝炎ウイルス(HBV)に関する報告。ブロイラーの血液検体129例及び肝臓検体193例を用いて、HBV関連検査を行ったを行ったところ、HBsAg、HBsAb、HBcAbの全陽性率はそれぞれ26.8%、53.49%、17.05%であり、一方HBeAg及びHBeAbが検出されたのは僅かであった。透過型電子顕微鏡(TEM)により血液検体を分析した結果、HBVと類似した粒子が確認された。また、2例の肝臓検体において確認されたウイルスDNA配列は、既知のHBV株と97.9%の相同性があることが示された。今後ニワトリで確認されたHBVがヒトHBVと同一であると示されるならば、ニワトリのHBV感染は重要な問題となり得るであろう。	3
120121	27-Oct-12	B型肝炎	J Infect Dis. 206(2012)478-485	B型肝炎ウイルス(HBV)感染症患者の体液を介した実験的HBV感染に関する報告。慢性HBV感染症の小児39例及び成人8例における尿、唾液、涙液及び汗中のHBV DNAをリアルタイムPCRを用いて調査したところ、尿検体の73.7%、唾液検体の86.8%、涙液検体の100%、汗検体の100%にHBV DNAが検出された。血清検体と、唾液及び涙液のHBV DNAレベル間には有意な相関が見られた。また、小児1例の涙液検体をヒト肝細胞移植キメラマウス2匹に静注したところ、接種1週間後にキメラマウスの血清はいずれもHBV DNA陽性となった。マウスにおいて涙液の感染性が確認されたことから、高レベルのウイルス血症を有するHBV保有者の体液に直接接触することを防ぐ対策が必要である。	4
120121	28-Oct-12	C型肝炎	J Infect Dis. 206(2012)654-661	C型肝炎ウイルス(HCV)感染者の経過と伝播様式に関する報告。738例のHCV抗体陽性供血者について感染のリスク要因と経過に関して評価された。まず偽陽性排除のために第3世代リコンビナント免疫ブロット法(RIBA)を用いてHCV抗体を検査したところ、469例(64%)がRIBA陽性、217例(29%)が陰性、52例(7%)が不確定であった。主な独立リスク因子は静注薬物使用、輸血や経鼻コカイン使用であった。計384例(82%)のRIBA陽性供血者がHCV RNA陽性であり、うち185例(48%)からの肝生検検体において33%に線維化は見られず、52%に軽度の線維化、12%に架橋線維化が見られた。感染後平均25年で2%に肝硬変が確認された。反復生検を行った63例の解析の結果、8%が5年以上でIshakステージ2以上に進行したことを示した。	5
120094	29-Sep-12	E型肝炎	Emerging Infectious Diseases. 18(2012)869-872	オランダにおける臓器移植レシピエントのE型肝炎ウイルス(HEV)感染に関する報告。HEV感染は免疫抑制状態にある患者にとって生命を脅かす可能性がある。2000～2011年、オランダにおいて臓器移植を受けた1200例の生存レシピエントに対しHEV RNA検査を行ったところ、12例のHEV感染が判明し、11例は慢性感染症であった。患者の年齢中央値は56.9歳、9例(75%)が男性であった。慢性HEV感染症の全患者で肝酵素レベルが上昇しており、HEV RNA検出はALTレベルの上昇と同時かまたはその後に続いた。HEV RNA陽性時からIgMが検出されるまでの期間の中央値は32日、IgGが検出されるまでの期間は平均124日であった。11例のHEV感染患者のサンプルから分離したウイルスは全てジェノタイプ3であった。HEV感染の原因が市中感染か院内感染かは不明であった。慢性HEV感染患者ではRNAが検出されてからIgM及びIgGの検出までに期間があるため、高い肝酵素値を示す臓器移植患者におけるHEV感染の診断にRNAの検出を行うべきである。	6
120094	30-Sep-12	E型肝炎	Vox Sang. 103(2012)89-90	スウェーデン、ドイツ及び米国からの血漿供血におけるE型肝炎ウイルス(HEV)陽性率に関する報告。各国由来の血漿供血において、96供血以下ミニプール血漿中のHEV RNAについて調査した。その結果、スウェーデン由来供血の95,835本中12本、ドイツ由来の18,100本中4本がHEV RNA陽性であった。ミニプールを考慮すると、HEV陽性供血の割合はスウェーデンでは1:7,986、ドイツでは1:4,525であった。一方、米国由来の51,075供血においてHEV陽性は検出されなかった。分子学的及び血清学的分析が行われた12本の供血について、HEV株は全てジェノタイプ3であり、ウイルス量は3.2-5.7 log10IU/mL HEV RNAと日本人献血者での報告と同様の範囲であった。IgGおよびIgMの測定を行ったところ、大部分のサンプルはウインドウ期供血であった。また、検査された12本のウイルス血症供血のうちALTレベルの上昇がみられたのは3本のみであった。これはALTによるスクリーニングがHEVの除外の方法として信頼できないことを示している。	7
120094	02-Oct-12	パルボウイルス	Emerging Infectious Diseases. 18(2012)680-683	カメルーンにおけるヒトパルボウイルス4(PARV4)感染の報告。2009年に採取されたカメルーン人の血清サンプル451例を用いてPARV4抗体陽性率の調査を行った結果、79例(17.5%)がPARV4抗体を有していた。PARV4抗体陽性はマラリア予防薬の静脈注射、結核の非経口治療、避妊薬の筋肉注射に関連があった。また、高齢者よりも60－64歳の初老の人に陽性者が多かった。これはウイルス曝露の経年的変化や、PARV4抗体価が次第に弱まっていき、最終的に偽陰性となること等が考えられる。今回の調査結果より、ある程度のPARV4非経口感染があることが示唆された。	8
120094	03-Oct-12	パルボウイルス	Transfusion. 52(2012)1482-1489	血友病患者におけるヒトパルボウイルス4(PARV4)の感染に関する報告。血漿由来ウイルス不活化凝固因子製剤の治療を受けている血友病患者に対するPARV4の潜在的伝播を調査するため、血友病患者集団194例におけるPARV4抗体の陽転化についてスクリーニングを行った。その結果、検査開始時のPARV4抗体陽性率は44%であり、観察期間中9例の被験者(うち7例はHIV陽性)において抗体が陽転化した(発生率、1.7%/年)。感染した被験者は比較的長期のウイルス血症期間を示し、感染急性期に弱い一過性のIgM応答を示した。この研究により、PARV4はウイルス不活化処理に抵抗性を持つ輸血感染性病原体であることが確認された。血漿由来血液製剤を使用する人々において、定期的に感染が発生する可能性が懸念される。	9
120137	31-Oct-12	インフルエンザ	http://www.cdc.gov/flu/spotlights/h3n2v-more-cases.htm	米国におけるインフルエンザA(H3N2)v感染の報告。米国疾病予防管理センター(CDC)は、米国において新たに12例のH3N2vインフルエンザ感染が発生し、オハイオ州で初めての死亡例が報告されたことを発表した。死亡した患者は複数基礎疾患を持つ高齢者で、祭りでブタに直接接触していた。本ウイルスのヒト-ヒト感染は限られており、散発的に発生しているが、集団での持続的な発生は認められていない。	10

血対課ID	受理日	感染症(PT)	出典	概要	新出文献番号
120141	01-Nov-12	鳥インフルエンザ	MMWR. 61(2012)726-727	メキシコにおける高病原性鳥インフルエンザA(H7N3)ウイルス感染の発生報告。メキシコハリスコ州の農場一帯で報告されている家きんでの高病原性鳥インフルエンザA(H7N3)の集団発生に関連して、同ウイルスへのヒト感染事例が2例報告された。1例はインフルエンザA(H7N3)が検出された飼育場の従事者の32歳女性であり、2012年7月に結膜炎と診断された。両眼のスワブの検査により、インフルエンザA(H7)ウイルスが陽性であった。もう1例は1例目の患者の親族であり、同じく2012年7月に結膜炎を発症し、インフルエンザA(H7)ウイルス感染が判明した。両症例とも重篤な症状に至ることなく回復している。	11
120132	06-Nov-12	ウエストナイルウイルス	http://www.cdc.gov/ncidod/dvbid/westnile/index.htm/2012/08/17	米国におけるウエストナイルウイルス(WNV)感染の発生状況に関する報告。2012年8月第2週時点で、米国43州において26例の死亡を含むWNVヒト感染例693例が報告されている。これは1999年に米国で初めて検出されて以降最多のペースである。693例のうち406例(59%)が神経侵襲性疾患、287例(41%)が非神経侵襲性疾患であった。	12
120121	06-Nov-12	デング熱	Transfusion. 52(2012)1657-1666	プエルトリコにおける輸血によるデング熱感染に関する報告。2007年、プエルトリコにおいて計10,508例のデング熱疑い症例が報告された。これを受けて、供血がデングウイルス(DENV)RNAについて検査され、RNA陽性供血の受血者は輸血感染の評価のために追跡された。検査された検体15,350例のうちTMA法によるDENV RNA検査に対する繰り返し反応(RR)が確認されたのは29例であり、1/529の割合であった(0.19%)。蚊での培養により感染性を示した12例において、RT-PCRによりウイルス力価105～109copies/mLでDENV 1型、2型及び3型が検出された。TMA-RR供血の受血者29例のうち3例が検査されたところ、108copies/mLのDENV-2を含む赤血球を輸血されたプエルトリコの受血者1例が輸血3日後に発熱し、デング出血熱に進行した。受血者もDENV-2陽性であり、供血者と受血者の両方が同一のエンベロープ配列を有していた。	13
120094	07-Oct-12	HHV-8感染	J Med Virol. 84(2012)792-797	サウジアラビアの血液透析患者の口腔及び血液中のヒトヘルペスウイルス8(HHV-8)に関する報告。サウジアラビアにおいて、血液透析患者72例の血漿中抗HHV-8抗体及びCD45(+)末梢血液細胞におけるHHV-8 DNA感染率を、供血者178例、妊婦60例と比較した。その結果、血液透析患者と健康な被験者間での抗体検出率は16.7%対0.4%、DNA検出率は4.2%対0.4%であった。また、透析患者の口腔内におけるHHV-8を調査したところ、HHV-8 DNAが口腔内から検出された患者5例における唾液中に排出されたウイルス量は、8,600～119,562,500GE/mlにまで分布した。さらに、サブゲノムシークエンスを実施したところ、口腔内のHHV-8は、4例がジェノタイプC2、1例がジェノタイプA1及びC2に属していたことが示された。血液透析患者の口腔内のHHV-8は高ウイルス量で、多様性があったことから、血液とともに唾液はHHV-8感染を媒介し、血液透析患者における高いHHV-8感染リスクとなり、腎移植後のカポシ肉腫の原因となることが推測された。	14
120094	05-Oct-12	ハンタウイルス	Emerging Infectious Diseases. 18(2012)750-757	ボリビアにおける新規アンデスウイルスに関する報告。南アメリカにおけるハンタウイルスの遺伝的多様性を調べるため、2008年～2009年にボリビア中央部のチャパレで発熱患者の血液検査を行ったところ、ハンタウイルス属RNAは死亡した1例を含む3例の患者に認められた。ウイルスのS及びMセグメントの部分的RNA配列はアンデスウイルス系統に最も密接に関連していたが、既報告株とは異なっていた。チャパレ住民間での抗ハンタウイルスIgG抗体調査は人口の12.2%が過去にハンタウイルスへ曝露していたことを示し、農業従事者間で最も検出率が高かった。ハンタウイルス株へ曝露する人が多いことと、結果的に生じる疾病が重大であることから、この新しいハンタウイルスの宿主、浸淫地域、及び公衆衛生への影響を決定するための更なる研究が必要とされる。	15

血対課ID	受理日	感染症(PT)	出典	概要	新出文献番号
120094	06-Oct-12	ハンタウイルス	Euro Surveill. 2012;17(21):pii=20180	ドイツにおけるヒトハンタウイルス感染症報告増加の報告。2011年10月から2012年8月、852件のヒトハンタウイルス感染症がドイツで報告され、そのうち68%がバーデンーヴュルテンベルク州で発生していた。ヒトハンタウイルスは保有宿主であるハタネズミの排泄物への曝露によりヒトに伝播し、2～4週間の潜伏期後に流行性腎症を引き起こす。2012年第17週の最新報告数は87件であり、歴史的な最多報告数である2007年第22週の96件にほぼ到達している。症例数急増の原因は不明であるが、気候要因と、恐らく2011年のブナの繁茂によるハタネズミの増加に関連すると推定されている。2012年夏季期間にさらなる症例数増加が見込まれるため、予防対策のためのさらなる情報が必要とされる。	16
120094	30-Sep-12	ウイルス感染	ProMED-mail 20120713.1200936	カンボジアの幼児における手足口病(HFMD)に関する報告。カンボジアの幼児において原因不明の致死性疾患が報告されたことを受け、31例の患者サンプルについてパスツール研究所で検査を行ったところ、大部分の症例の原因はHFMDの重症型であるという結論に至った。サンプルの多くがHFMDの原因であるエンテロウイルス71(EV-71)陽性であった。確認された計78症例のうち61例について調査を行った結果、大部分の患者は3歳以下で、異なる14州から報告があり、数例は慢性状態であることが分かった。WHOや関連機関の援助を受け、保健省は調査を継続するとともに、全てのHFMD患者を報告するよう指示し、サーベイランスを強化した。	17
120095	02-Oct-12	ウイルス感染	WHO/GAR/Disease Outbreak News. Jul 9, 2012	原因不明の疾患で死亡したカンボジアの小児におけるウイルス検出の報告。カンボジアにおいて、2012年4月から7月5日までの間に原因不明の疾患にかかった小児59例が報告され、うち52例が死亡した。患者は大多数が3歳未満であり、男女比は1.3:1であった。最新の検査結果によると、患者検体の多くで手足口病(HFMD)を引き起こすエンテロウイルス71(EV-71)に対して陽性反応を示した。他にも、デング熱とブタ連鎖球菌等の病原体について検出されたが、インフルエンザウイルスやSARS、ニパウイルスには陰性であった。更なる検査が現在進行中であり、数日以内に結論が出される予定である。	18
120095	04-Oct-12	ウイルス感染	Chinese journal of Zoonoses. 28(2012)442-448	中国におけるヒトへの山羊痘感染発生の報告。2010年、中国重慶市において山羊痘感染症例が34例報告された。そのうち5例から小嚢液、眼脂又はかさぶたを採取し、4例の山羊痘感染ヤギの検体と共にウイルスDNAの検出を行ったところ、全ての検体が山羊痘ウイルスA29L遺伝子に陽性であった。また、ヒト症例5例中4例について山羊痘ウイルスP32遺伝子に対しても陽性であった。ヒト2例及びヤギ1例から得られたP32遺伝子の配列を比較すると100%の相同性が得られ、双方のウイルスが同一であることが示された。今回の感染者には全て罹患ヤギとの接触歴があったため、直接ヤギと接触したことが、山羊痘感染の主な原因であったと考えられた。	19
120098	26-Sep-12	ウイルス感染	Arch Virol. 157(2012)521-524	中国のブタにおけるブタ・サイトメガロウイルス(PCMV)及びサポウイルス(SaV)の抗体陽性率に関する報告。2005年5月から2010年10月にかけて、中国湖南省でブタの血清検体500例中のPCMV及びSaVに対する抗体をELISAにより評価した。対象のブタは省内の10の地域に分布する農場から集められた。その結果、全陽性率はPCMVについて96.40%、SaVについて63.40%であり、繁殖期の雌ブタで陽性率が最も高かった。調査結果はPCMV及びSaV感染の両方が湖南省のブタで広く流行していることを示している。	20

血対課ID	受理日	感染症(PT)	出典	概要	新出文献番号
120121	18-Nov-12	ウイルス感染	Hepatol Res. 41(2011)971-981	高いALTレベルの献血者から分離された新規DNA配列に関する報告。ALT値が上昇した500例の献血者の血清検体についてPCRによりスクリーニングされ、得られた配列のウイルス特性について調査された。その結果、4例の血液サンプルに9496 bpの新しいDNA配列が含有されていることが判明し、これをKIs-Vと命名した。KIs-Vは制限酵素SalI及びBstXIに反応した。KIs-Vはヒト白血球DNAから検出されなかった。連続濾過により、KIs-Vは30-50nmの粒子であることが示唆された。in silico分析より、KIs-Vは13のORFを含むことが分かり、既報告のいかなるウイルスタンパク質とも相同性を示さなかった。1つの遺伝子は、DNAポリメラーゼ領域に類似性を示した。転写開始及びCpGアイランドの強いシグナルが確認された。KIs-Vのヌクレオチド構成は複製開始点と終点を含む環状DNAゲノムの特徴を示した。予備研究において、KIs-Vは高いALT値を示すE型肝炎ウイルス抗体陽性者において度々検出された。これらの結果より、KIs-Vはエンベロープを有する新しい分類の二本鎖環状DNAゲノムであることが示された。	21
120128	31-Oct-12	ウイルス感染	Euro Surveill. 2012;17(21):pii=19935	ドイツにおける初めてのウスツウイルス感染の報告。2012年8月、Bernhard Nocht熱帯医学研究所(BNI)はドイツにおいて初めてのウスツウイルス感染者が検出されたことを発表した。4200例の血液検体に対して検査を行った結果、1例の陽性が検出された。感染が確認された男性に感染症状はなかった。2011年夏期に、南ドイツでウスツウイルス感染により多数のクロウタドリが死亡し、2012年においても既に多数の鳥の死亡が報告されていた。	22
120128	01-Nov-12	ウイルス感染	ProMED-mail 20120821.1255556	ドイツにおける初めてのウスツウイルス感染の報告。2012年8月、Bernhard Nocht熱帯医学研究所(BNI)はドイツにおいて初めてのウスツウイルス感染者が検出されたことを発表した。4200例の血液検体に対して検査を行った結果、1例の陽性が検出された。感染が確認された男性に感染症状はなかった。2011年夏期に、南ドイツでウスツウイルス感染により多数のクロウタドリが死亡し、2012年においても既に多数の鳥の死亡が報告されていた。	23
120142	02-Nov-12	ウイルス感染	WHO/GAR/*Disease* Outbreak News. Sep 23, 2012	サウジアラビアにおける新規コロナウイルス感染者の報告。49歳のカタール国籍の男性がサウジアラビアへの旅行から帰国後、急性の呼吸器症候群を発症した。患者は2012年9月にカタールのICUへ入院した後に英国へ緊急搬送され、英国健康保護局(HPA)での検査により新規のコロナウイルスへの感染が判明した。遺伝子配列の解析の結果、2012年始めに死亡した60歳のサウジアラビア人の肺組織から得られたウイルスと99.5%の相同性が示された。WHOは更なる情報収集を行っている。	24
120121	16-Nov-12	細菌感染	Vox Sang. 103(2012)93-98	皮膚疾患患者と対照群での皮膚細菌の比較に関する報告。供血を延期された皮膚疾患の供血者55例について、各症例に3例のコントロールを対応させ、静脈穿刺前腕部の皮膚から細菌培養サンプルを採取して検査を行った。その結果、コロニーを形成した皮膚細菌の全数の中央値は、コントロール群(105 CFUs/例)に比べ症例群(224 CFUs/例)で有意に高かった。黄色ブドウ球菌は、コントロール群(7%)と比較して症例群(49%)で有意により多く存在した。他の細菌属に関しては症例群とコントロール群の間に違いは見られなかった。この研究は、皮膚疾患を有する供血者の現行供血延期ガイドラインが、皮膚に細菌を多く有する者や黄色ブドウ球菌保有者を効果的に識別することを示している。	25
120128	30-Oct-12	細菌感染	Euro Surveill. 2012;17(21):pii=20186	英国における、ヒトからヒトへの感染が疑われたオウム病症例の報告。2012年2月、テイサイド州において肺炎患者5例が報告された。患者は親族の4例と医療従事者1例であり、この医療従事者は最初に症状が認められた患者の世話をしていた。患者検体より、Chlamydophila psittaciがPCR法により確認された。感染源の推定は不可能であったが、症例発現の時間範囲が1～22日間であったことから、ヒトからヒトへの感染が示唆された。オウム病は一般的に動物(主に鳥類)からヒトへの感染症と考えられていたが、ヒト間で感染する可能性があることが報告された。	26

血対課ID	受理日	感染症(PT)	出典	概要	新出文献番号
120121	17-Nov-12	赤痢	Jpn J Infect Dis. 65(2012)277-278	東京における男性と性交渉のある男性(MSM)間での赤痢アウトブレイクの報告。2011年9月から11月に5例の細菌性赤痢患者が東大医科学研究所に入院した。患者は全てHIVに感染したMSMであり、CD4 T細胞数は168－415 cell/μLで、3例は既にART治療を受けていた。患者は腹痛、水様下痢、発熱などを呈した。全員の糞便培養からShigella sonneiが検出され、レボフロキサシンによる治療を受けた。患者の平均発症期間は10日と、通常(2-3日)より少し長かった。問診では5例の患者間における直接性的接触のような密接な関係は認められなかった。全患者の分離株の分析の結果、類似のパターンを示すことが明らかとなり、単一のS.sonnei株がMSM間に広まったことが示唆された。日本でのMSMにおける初めての赤痢菌アウトブレイクの報告は、MSMに対して赤痢菌を含む性感染性病原体に対する予防行為の重要性をより強調するものとなる。	27
120095	01-Oct-12	マラリア	http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/ucm077061.htm	輸血関連マラリア感染のリスク低減のための輸血管理に関するガイドラインのドラフトが発表された。主に以下の供血延期措置をとるよう勧告されている。 ・マラリア感染既往がある者は、治療成功証明書がない限り無期限の供血延期とする。 ・マラリア流行国での居住後3年間は供血延期とする。 ・マラリア流行地域への渡航歴及び通過歴のある者は出国後1年間の供血延期とする。ただし、メキシコの中でもマラリア感染率が低い特定の州に対する渡航は供血延期に該当しない。	28
120121	07-Nov-12	バベシア症	Transfusion. 52(2012)1509-0516	米国におけるBabesia microti(B.microti)抗体陽性供血者の遡及調査に関する報告。コネチカット州において、1999年から2005年の間に、B.microti検査が陽性であり遡及調査対象となった474供血、656製剤から、合計208例の抗体陽性供血者が同定された。63例の受血者がB.microti検査を受け、8例(12.7%)が免疫蛍光アッセイ(IFA)やPCRで陽性であった。抗体陽性供血者の供血延期実施後の2001年(3/48人、6.3%)に比べて、1999年から2000年(5/15人、33.3%)においてB.microti陽性受血者の割合が有意に高いことが判明した。有意差は、IFA陽性となった供血と前回供血及び寄生虫血症供血者と非寄生虫血症供血者からの製剤受血者の陽性率を比較した時にも見られた。遡及調査を通してB.microti感染が検出されたこの報告は、米国の血液受血者におけるB.microti感染を減少させるための介入が必要であることを示している。	29
120121	08-Nov-12	バベシア症	Emerging Infectious Diseases. 18(2012)1318-1321	米国におけるバベシア症の垂直感染に関する報告。2002年9月16日、生後6週目の女児が発熱、不穏、食欲不振から入院した。母親は妊娠中、出産後とも無症候であり、妊娠中にダニに咬まれた覚えはなかった。乳児のダニ曝露は確認されておらず、母子ともに輸血歴はなかった。児の末梢血スメアは赤血球の4%にB.microtiを示し、血液検体はB.microti DNA陽性であった。また、総B.microti抗体価は256倍以上であった。生後3日目に採取した血液が検査されたところ、B.microti DNAは陰性であり、IgM抗体陰性であるが、総抗体は陽性(128倍以上)であることが分かった。パラフィン包埋胎盤組織の検査によりBabesia DNAが検出された。児の罹患時、母親はPCRとスメアではBabesia陰性であったが、総抗体価は陽性であった(256倍以上)ことが判明した。以上より本症例は、母親の分娩前感染症が原因の先天性バベシア症であると診断された。患児の生後3日目の血液サンプルの分析で検出されたBabesia抗体は、恐らく母親のIgG抗体が移行したことを意味する。バベシア症の流行地域における乳児の発熱及び溶血性貧血の鑑別診断において、この診断は考慮されなくてはならない。	30

血対課ID	受理日	感染症(PT)	出典	概要	新出文献番号
120121	02-Nov-12	アメリカ・トリパノソーマ症	Transfusion ; published online. Mar 8, 2012	米国の供血者におけるシャーガス病原因原虫の昆虫媒介性感染に関する報告。米国内の昆虫媒介性感染負荷を評価し、推定されるリスク要因を明らかにするため、約2900万供血のスクリーニングから確認された1084例のTrypanosoma cruzi(T.cruzi)陽性者のうち調査参加資格を満たす供血者37例について調査が行われた。15例(41%)が血清学検査結果が4回もしくは5回陽性であり、T.cruzi感染陽性とみなされ、うち1例は血液培養検査陽性だった。15例中3例が流行国の農村地域を訪れたことがあったが、2週間以上滞在した者はいなかった。全例がT.cruzi媒介昆虫や感染したほ乳類の生息地に居住した経験があり、13例が野外でレジャーや仕事をしたと報告し、11例が私有地で宿主動物を見たと報告した。この研究に基づく土着性感染の推定割合は供血者354,000人につき1人である。米国での昆虫媒介性感染の発生源を特定することが、感染リスクのさらなる評価のために必要である。	31
120121	03-Nov-12	アメリカ・トリパノソーマ症	32nd International congress of the ISBT. 5D-S43-03	米国の供血者におけるTrypanosoma cruzi(T. cruzi)新規感染発生の検証に関する報告。米国において、供血者の新規T.cruzi感染はないという予備データに基づき、一度T.cruzi抗体検査が陰性であれば、将来の全ての供血を適格とする選択的抗体検査について検証された。現在、T.cruziがハイリスクである4つの地域で全数検査が維持される一方で、残りは選択的検査が実施されている。4年間の研究において、422万人の複数回供血者が1,435年の平均供血間隔で追跡されたところ、抗体が陽転した供血者はいなかった。調査期間中、前回の供血がELISAで陰性であったRIPA陽性供血者が22例確認されたが、さらなるサンプリングにおける抗体陽性は断続的で、40日以上4年間の追跡調査中に完全に抗体陽転化することはなかった。また、PCRや培養により寄生虫血症となった供血者はいなかった。よってこれら22例は偽陽性または遠い過去での初感染であったと思われる。今回の調査結果より、観察された新規感染率がゼロであることに基づき、米国において初回陰性結果に基づく選択的検査は、全数検査に匹敵する安全性を提供していると示された。	32
120095	30-Sep-12	クロイツフェルト・ヤコブ病	Transfusion . 52(2012)1290-1295	フランスの供血者における無症候の孤発性クロイルフェルト・ヤコブ病(sCJD)患者に関する疫学的研究の報告。フランスにおける1999～2008年のデータを用いて、一般でのsCJD症例及び献血者集団の人口統計学的特性から、臨床症状を呈する前のsCJD供血者の年間推定患者数が推定された。その結果、供血時から1年以内にsCJDを発症するドナーは毎年平均1.1例であり、5年以内が6.9例、10年以内が18.0例、そして15年以内の発症が33.4例であると推定された。供血時にほとんどのsCJD感染者が後期前臨床段階でないことが予想された。今回の結果及び長年に渡ってsCJDの増加が世界的に確認されていないことは、輸血によるsCJD感染のリスクが非常に低いことを示している。	33
120090	25-Sep-12	異型クロイツフェルト・ヤコブ病	Emerging Infectious Diseases. 18(2012)901-907	医原性クロイツフェルト・ヤコブ病(CJD)の最終評価に関する報告。医原性CJDの発現はほぼ収束を迎えたが、例外的に長い潜伏期間を伴う発現症例が現在もみられている。主因はCJD感染死体に由来する成長ホルモンや硬膜移植片であり、他には少数例として脳神経外科器具の汚染、角膜移植片、性腺刺激ホルモンを介したものや、輸血による変異型CJDの二次感染が挙げられる。医原性感染を防止する最良の方法は一次感染の防止であるが、無症状の感染者を特定する検査がない限り、リスクを完全に除くことはできない。従って、現段階では、①CJD発症リスクが高い人間の識別及び臓器提供の延期、②医療器具の殺菌時や組織及び体液の処理へのプリオン低減工程の組み込み、という方法をとらざるを得ず、この組み合わせがリスクを最小化することに繋がっている。	34
120094	08-Oct-12	異型クロイツフェルト・ヤコブ病	Prion 2012, PO-251	赤血球輸血経由vCJD感染のリスク評価モデルバリデーションに関する報告。英国とフランスにおける輸血感染vCJD(TTvCJD)のリスク推定モデルが開発された。入力値として両国の潜在的vCJD有病率、供血者数と赤血球輸血数、疾病の感染性、受血者の感受性等が使用された。英国の有病率は疫学的モデリング研究から算出された低い推定値と、組織サーベイランス研究による高い推定値に層別化され、フランスの有病率は英国のデータを元にそれぞれ算出された。モデルの評価のため、1980年以降の症例数予測を観察症例数と比較したところ、TTvCJDリスク推定はモデルに使用された推定有病率に大きく依存していたが、低い推定有病率を用いたモデルは臨床TTvCJD報告数とほぼ一致していた。また、高い推定値を用いると、推定無症候性感染数は推定臨床症例数の10倍以上多いと予測された。これは感染した受血者の約90%が明確なvCJD兆候を示す前に他の要因で亡くなった可能性を示している。将来、このモデルは米国におけるTTvCJDリスク及び現在の安全性介入の有効性の推定に適用されることが予測される。	35
120095	29-Sep-12	異型クロイツフェルト・ヤコブ病	http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/UCM307137.pdf	米国FDAによる、血液製剤を介した変異型クロイツフェルト・ヤコブ病(vCJD)の伝播リスク減少のための措置に関するガイダンス案。動物実験およびFDAのリスク・アセスメントの結果、血漿分画製剤によりvCJDを発症する可能性は極めて低いが、完全には排除できないと結論付けられた。これを受け、血漿分画製剤の添付文書において新たにvCJDに言及し、その感染リスクを明記するよう勧告する。同様に、血漿由来のアルブミン及び血漿由来アルブミンを使用した製品についても、改訂を勧告する。本ドラフトが最終版となる際には、2010年のCJD/vCJDガイダンスのセクションVII.Bに置き換わるものである。	36
120135	31-Oct-12	異型クロイツフェルト・ヤコブ病	Health Protection Report. 6(2012)4-5	英国における異常プリオンの保有率に関する報告。英国海綿状脳症諮問委員会が、変異型クロイツフェルト・ヤコブ病の有病率を調査するため、2000年～2012年に英国の41病院から収集された虫垂検体32441例を免疫組織化学的に検査したところ、異常プリオンが16例において検出された。これらの陽性検体は既知の英国vCJD症例176例のものではなかった。全体の有病率の推定値は百万分の493(95%信頼区間:282～801)で、1995年～1999年に実施された前回調査結果の百万分の237(95%信頼区間:49～692)と統計的に一致していた。今回の調査では、前回よりも広い出生集団においてプリオンが存在していることが示された。	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	総合機構処理欄
				2012. 4. 21	該当なし	
一般的名称	人血清アルブミン		研究報告の公表状況	Vermeulen M, Dickens C, Lelie N, Walker E, Coleman C, Keyter M, Reddy R, Crookes R, Kramvis A. Transfusion. 2012 Apr;52(4):880-92. doi: 10.1111/j.1537-2995.2011.03355.x. Epub 2011 Oct 7.	公表国 南アフリカ	
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン5%静注12.5g/250mL(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)					

研究報告の概要

○南アフリカでの4年間の個別核酸増幅検査(ID-NAT)済み血液輸血によるB型肝炎感染

背景: 2005年以来、2,921,561の供血血液が、南アフリカ国立血液サービスの実施するB型肝炎ウイルス(HBV)ID-NATスクリーニングを受けた。4年間で149件のHBs抗原陰性の急性期HBV NAT陽性供血が同定された(1:19,608)。遡及調査によって1件のHBV感染疑い症例が同定された。

研究方法: 供血者及び受血者から分離したHBVのゲノム配列決定、増幅、系統発生解析を行った。HBVウィンドウピリオド(WP)感染リスクは、最小感染ウイルス量を3.7 HBVビリオン、HBV DNAが一時的に検出可能な感染発生率補正係数を1.34と想定して推算された。

結果: 149件の急性期HBV NAT陽性結果のうち、114件(1:25,627)は抗HBc抗体が検出される前のWPで、35件(1:83,473)は抗HBc抗体WP後であると分類された。HBV DNA陰性 抗HBc抗体WP前、後(それぞれ15.3日、1.3日)における急性感染リスクはそれぞれ1:40,000と1:480,000であると推算された。1件のHBV感染(1:2,900,000)は抗HBc抗体検出前のWPにおけるID-NAT陰性の輸血を受けた患者に認められた。配列解析によって供血者と受血者間で99.7%の核酸相同性を持つHBVサブジェノタイプA1の感染であることが確認された。感染性赤血球製剤のウイルス量は、20 mLの血漿中に32(22-43)HBV DNAコピーであったと推定された。

結論: ID-NATを使用した血液スクリーニングをすり抜けた血液による、南アフリカにおける初めての輸血関連HBV感染症例であり、0.34/100万供血のHBV感染率である。ID-NATスクリーニングを受けた供血者人口における急性期前の感染リスクの推算値は、観察されたWP感染率より73倍高かった。南アフリカにおける血液供給の安全性はID-NATの導入によって非常に強化されたが、感染リスクが完全に排除されたわけではないことを今回の研究結果は証明している。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン5%静注12.5g/250mL
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
南アフリカで行われた4年間にわたる供血血液へのHBV ID-NATスクリーニングにおいて、2,921,561供血から149件のHBs抗原陰性の急性期NAT陽性供血が同定され、遡及調査によって1件のID-NAT陰性の輸血関連感染疑い症例が確認されたとの報告である。 これまで、本剤によるHBV感染の報告はない。また本剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれている。さらに最終製品についてHBV-NAT陰性であることを確認しており、安全性は確保されていると考える。	これまでの使用実績やバリデーション成績に鑑み、特別の対応を必要としないが、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。

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DONOR INFECTIOUS DISEASE TESTING

Hepatitis B virus transmission by blood transfusion during 4 years of individual-donation nucleic acid testing in South Africa: estimated and observed window period risk

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BACKGROUND: Since October 2005, a total of 2,921,561 blood donations have been screened by the South African National Blood Service for hepatitis B virus (HBV) by individual-donation nucleic acid testing (ID-NAT). Over 4 years, 149 hepatitis B surface antigen–negative acute-phase HBV NAT–positive donations were identified (1:19,608). The lookback program identified one probable HBV transmission.

STUDY DESIGN AND METHODS: The complete genomes of HBV isolated from the donor and recipient were sequenced, cloned, and analyzed phylogenetically. The HBV window period (WP) transmission risk was estimated assuming a minimum infectious dose of 3.7 HBV virions and an incidence rate correction factor of 1.34 for transient detectability of HBV DNA.

RESULTS: Of 149 acute-phase HBV NAT yields, 114 (1:25,627) were classified as pre–antibody to hepatitis B core antigen (anti-HBc) WP and 35 (1:83,473) as post–anti-HBc WP. The acute-phase transmission risk in the HBV DNA–negative pre- and post–anti-HBc WPs (of 15.3 and 1.3 days, respectively) was estimated at 1:40,000 and 1:480,000, respectively. One HBV transmission (1:2,900,000) was identified in a patient who received a transfusion from an ID-NAT–nonreactive donor in the pre–anti-HBc WP. Sequence analysis confirmed transmission of HBV Subgenotype A1 with 99.7% nucleotide homology between donor and recipient strains. The viral burden in the infectious red blood cell unit was estimated at 32 (22-43) HBV DNA copies/20 mL of plasma.

CONCLUSION: We report the first known case of transfusion-transmitted HBV infection by blood screened using ID-NAT giving an observed HBV transmission rate of 0.34 per million. The estimated pre–acute-phase transmission risk in the ID-NAT screened donor population was 73-fold higher than the observed WP transmission rate.

In October 2005, the South African National Blood Service (SANBS) implemented individual-donation nucleic acid testing (ID-NAT) to screen all blood donations for human immunodeficiency virus (HIV), hepatitis C virus (HCV), and hepatitis B virus (HBV).[1] Although the development of systems to screen blood donations using NAT has significantly reduced the residual risk of transfusion-transmissible infections (TTIs),[1-5] it has not eliminated it completely.[4,6-12] Factors that contribute to the transmissibility of infections by blood transfusion during the window period (WP) include the level of viremia, the sensitivity of the screening assay, and the minimal infectious dose.[13,14] The length of the WP is determined by the doubling time of viremia and the analytical sensitivity of the testing system used for screening.[13,15,16] ID-NAT is generally more sensitive and detects

ABBREVIATIONS: ID = individual donation; ID_{50} = 50% minimum infectious dose; LDD = limit of detection; MP = minipool; OBI = occult hepatitis B infection; SANBS = South African National Blood Service; TTI(s) = transfusion-transmissible infection(s); WP(s) = window period(s).

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lower levels of nucleic acid compared to minipool (MP) NAT, although for HBV, three head-to-head comparison studies showed no significant difference in analytical and clinical sensitivity between the Ultrio assay (Novartis Diagnostics, Emeryville, CA) in ID-NAT format and the TaqScreen assay (Roche Molecular Systems, Pleasanton, CA) utilized in MP6 configuration.[17-19]

A review of the literature revealed that to date there has been no documented case of transfusion-transmitted HBV where these latter systems have been used to screen donated blood. However, there have been a number of cases of HBV transmission that have been reported in blood screened by NAT using larger pool sizes.[7,20-22] Interestingly, when some of these donations were tested by ID-NAT they were also not reactive, but the diagnostic polymerase chain reaction (PCR) methods used in these cases might have been less sensitive than the current triplex NAT blood screening assays.

The benefit of implementing a more sensitive ID-NAT system (as opposed to an MP system) is expected to be greater in regions of high HBV incidence and prevalence, where the rate of donations in the WP and in cases of occult hepatitis B infection (OBI) is high.[23]

Before the implementation of hepatitis B vaccination in the immunization program of infants in 1995, it was reported that more than 70% of the South African population had been exposed to HBV, with an estimated 10% being hepatitis B surface antigen (HBsAg) carriers.[24] Interestingly, when the background prevalence of HBV was examined in an adult population 5 years after introduction of infant immunization, the HBsAg- and anti-HBc–positive rate was reported to be lower (3.3 and 36.7%, respectively), which indicated that beneficial factors other than HBV vaccination play a role in reducing HBV transmission in a community.[24] The HBsAg and anti-HBc prevalences in a South African blood donor population were reported to be 0.54 and 6.0%,[1,25] six times lower than in the general population.[24] Even though the potential transmission of HBV infection by blood transfusion in South Africa has been significantly reduced by ID-NAT, the residual risk of HBV transmission by donations in the window phase remains relatively high.[1] SANBS operates a comprehensive lookback program to identify possible cases of transfusion-transmitted HIV, HCV, and HBV infections. Donor-triggered lookback investigations are undertaken on all HBV DNA– and/or HBsAg-positive donations, including OBI, for donations procured within 1 year of the positive (index) donation. SANBS does not routinely screen for anti-HBc; however, should recent seroconversion to anti-HBc be identified, a lookback investigation will be conducted. Recipient triggered lookback investigations are carried out whenever there is a reported case of a possible transfusion-transmitted infection.

In this study, we report the first case of transmission of HBV by a blood donation in the HBV DNA–negative WP, which occurred during the fourth year of ID-NAT screening and which was confirmed by comprehensive nucleic acid sequencing and phylogenetic analyses. Moreover, we compare the observed HBV WP transmission rate with the estimated transmission risk caused by donors with acute HBV infection undetectable by ID-NAT.

MATERIALS AND METHODS

Screening and confirmation of HBV infection

All blood donations in South Africa are donated by voluntary, nonremunerated donors and are routinely screened for HIV, HBV, and HCV by both serologic testing and ID-NAT. Screening for HBsAg was performed on a chemiluminescent immunoassay system (Abbott PRISM ChLIA system, Abbott, Delkenheim, Germany) and, for HBV DNA, on a multiplex NAT assay system (Procleix Ultrio multiplex system on TIGRIS, Novartis Diagnostics). Serology and ID-NATs were performed concurrently using two donor samples, a citrated plasma sample for HBsAg and an ethylenediaminetetraacetic acid gel-separated plasma sample for NAT. Initial reactive donations in the Ultrio assay are tested in duplicate on the primary test tube as well as in the discriminatory probe assays. Donors that are concordantly repeat reactive in Ultrio or dHBV assays and the HBsAg assay are considered HBV infected. HBsAg-negative donations that are Ultrio repeat reactive or reactive in the dHBV assay are tested for viral load by quantitative PCR (on the Cobas TaqMan, Roche Molecular Diagnostics, Pleasanton, CA) assay as well as by triplicate or fivefold Ultrio and dHBV assays on samples taken from the frozen plasma unit. These potential HBV NAT–yield samples are also tested for immunoglobulin (Ig)M anti-HBc, total anti-HBc, and anti-HBs titer (Elecsys, Roche Molecular Systems, Pleasanton, CA).

Potential HBV NAT–yield donors, as well as HBsAg neutralization–positive donors with Ultrio-nonreactive or nonrepeatable reactive results, are recalled to confirm HBV infection markers in a follow-up sample. On the basis of the pattern of results in index and follow-up samples, HBV NAT–yield infections in first-time, lapsed, and repeat donors are categorized into acute and chronic NAT-yield cases. The first group is further classified as pre-HBsAg or pre–anti-HBc WP if all serum markers in the index donation are nonreactive or as post HBsAg or post–anti-HBc WP (or early recovery phase) when IgM anti-HBc is positive (Elecsys, Roche Molecular Systems). If two sequential samples were HBV DNA reactive followed by seroconversion to anti-HBc (and anti-HBs) in a later follow-up sample, we classified the infection as primary OBI (Fig. 1). In some of these cases, it is unlikely that HBsAg would have been detectable at some point in time because of the length of the sampling intervals. A second subcategory of WP infections could be classified as either abortive HBV infection or, more likely, vaccine breakthrough infections,

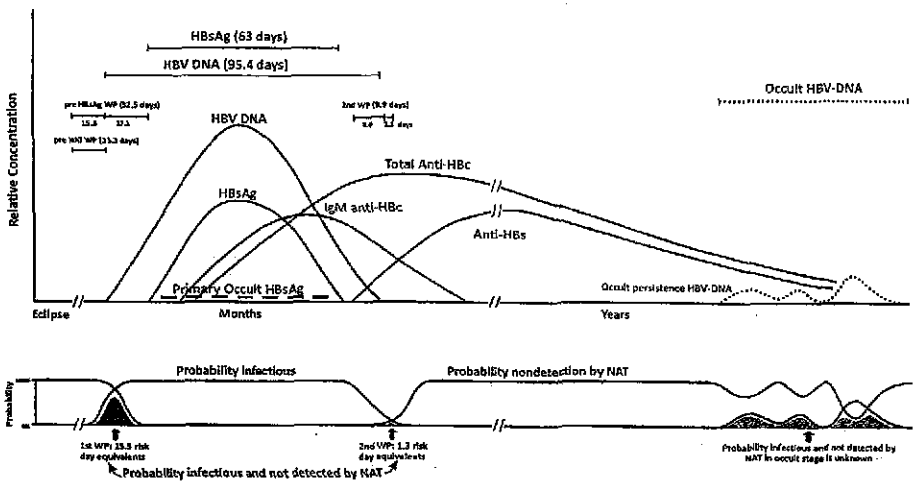


Fig. 1. Course of serum markers in acute resolving hepatitis B infection. The curves in the upper part of the diagram show the relative concentration of the markers in a typical infection. The lines above the curves show the mean lengths of the detection periods of HBV DNA and HBsAg as estimated from the numbers of HBV NAT yields with and without detectable HBsAg. The lengths of the pre- and post-HBsAg WPs and pre- and post-NAT (Ultrio) WPs were calculated for RBCs (20 mL of plasma) from the 50% LODs of 9.5 and 1000 copies/mL for HBV DNA and HBsAg by the transmission risk model of Weusten and coworkers,[16] assuming a 50% minimum infectious dose of 3.7 copies in the ramp-up viremia phase and 370 copies in the declining viremia phase. The resulting probability curves that RBCs are infectious and the probability of nondetection by Ultrio based on 95 and 50% LODs of 98.5 and 9.5 copies/mL determined by comparison against the Eurohep standard are shown at the bottom part of the diagram. The shaded area shows the probability that RBCs are infectious but not detected by NAT and give the number of risk-day equivalents in the first and second WPs, which were estimated to be 15.3 and 1.3 days, respectively, for Ultrio and 12.6 and 0.74 days, respectively, when the more sensitive Ultrio Plus assay with 95 and 50% LODs of 46.9 and 4.5 copies/mL would be used. In a later stage of OBI when anti-HBs titers have declined to below 10 to 100 mIU/mL occult persisting HBV DNA in the liver can reappear in plasma and the blood can become potentially infectious again. The probability that blood from occult HBV carriers is infectious but not detected by NAT is unknown and not addressed in this article.

when HBV DNA was detectable in the presence of anti-HBs as the sole detectable serum marker, followed by an increase in anti-HBs titer and delayed anti-HBc seroconversion. If, however, no follow-up sample was available or no increase in anti-HBs titer was observed, it cannot be excluded that the donor was a chronic occult HBV carrier with anti-HBs as the sole detectable marker. Since there were only a few of these, we classified them as acute NAT yields.

Analyses of the HBV transmission case

A 47-year-old regular male blood donor tested positive for HBsAg and HBV DNA on his 53rd whole blood donation in January 2009. The donor had given a whole blood donation 60 days before the positive index donation (X) and this donation had tested negative for all markers of TTI. The previous whole blood donation (X – 1) had been processed into a red blood cell unit (RBC), a random-donor platelet (PLT) unit, and a fresh-frozen plasma (FFP) unit.

Once it was confirmed that the X donation was positive for HBsAg and HBV-DNA, a donor- triggered lookback investigation was initiated. This investigation established that the RBC unit and PLT unit of the X-1 donation had been transfused and that the fresh frozen plasma (FFP) unit had been forwarded to the South African National BioProducts Institute (Pinetown SA), a plasma fractionation facility.

To confirm transmission of HBV by the X-1 donation, follow-up blood samples were taken from the recipient and from the donor at 110 days and 120 days post-transfusion, respectively. The follow-up samples were

sequenced and genotyped (see below) and tested for HBsAg, anti-HBc (IgM), anti-HBc (total), and anti-HBs titer. Liver function tests—alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH) and total bilirubin—were also performed on donor and recipient samples.

Written informed consent to perform additional laboratory tests, including tests to characterize the virus, was obtained from both the donor and the recipient. Approval to molecularly characterize the HBV isolates was obtained from the Human Ethics Committee of the University of the Witwatersrand.

Viral load estimation

The FFP unit from the X – 1 donation was recovered from the National Blood Fractionation Centre and aliquoted into 3.5 mL samples and frozen at –80°C. Probit analysis can be used to estimate the HBV concentration and the number of viral particles in the units transfused, which are below the detection limit of the Ultrio assay. This was undertaken by testing the donor's FFP unit in 30 replicate tests on the Ultrio and the new generation Ultrio Plus assay and comparing the proportions of positive results with those found on the Eurohep HBV DNA Subgenotype A2 standard dilutions calibrated in copies/mL,[25] comparable to the copies/mL quantified by the Versant bDNA 3.0 viral load assay (Siemens, Tarrytown, NY).[16] The Eurohep standard has subsequently been used for preparation of batches of the WHO standard and it has been estimated that one IU of the lyophilized material in the international standard is equivalent to approximately 5 HBV DNA copies or virions.[26,27] An aliquot of plasma from the FFP unit was also provided to Gen-Probe for testing 30 replicates on the Procleix Ultrio Plus assay. Another aliquot was sent to the Paul Ehrlich Institute for testing 12 replicates in the Ultrio and TaqScreen assays (Roche Molecular Systems) in ID format.

HBV amplification

For both the donor and the recipient, total DNA was extracted from the plasma samples using a DNA mini kit (QIAamp, QIAGEN GmbH, Hilden, Germany), according to the manufacturer's instructions. The surface and basic core promoter regions of both the donor and the recipient HBV isolates were amplified directly from DNA extracted from serum. A nested PCR procedure was carried out to amplify the complete S open reading frame: primers S1F 5′-TCAATCGCCGCGTCGCAGAAGAT CTCAATC-3′ (2410-2439 from *EcoRI* site) and S1R 5′-TCCAGACCXGCTGCGAGCAAAACA-3′ (1314-1291 from *EcoRI* site) were used for the first round (denaturation 94°C for 60 sec, annealing 66°C for 3 min, extension 72°C for 3 min, 40 cycles) and S2F 5′-AATGTTAGTATTCCTT GGACTCATAAGGTGGG-3′ (2451-2482 from *EcoRI* site) and S2R 5′-AGTTCCGCAGTATGGATCGGCAGAGGA-3′ (1280-1254 from *Eco*RI site) for the second round (denaturation 94°C for 60 sec, annealing 68°C for 3 min, extension 72°C for 3 min, 40 cycles).

The basic core promoter/precore region was amplified using a slight modification of a previously published nested PCR:[28] primers BCP1F 5′-GCATGGAGAC CACCGTGAAC-3′ (1606-1625 from *Eco*RI site) and BCP1R 5′-GGAAAGAAGTCCGAGGGCAA-3′ (1974-1955 from *Eco*RI site) were used for the first round (denaturation 94°C for 60 sec, annealing 55°C for 1 min, extension 72°C for 2 min, 40 cycles) and BCP2F 5′-CATAAGAGGA CTCTTGGACT-3′ (1653-1672 from *Eco*RI site) and BCP2R 5′-GGCAAAAAACAGAGTAACTC-3′ (1959-1940 from *Eco*RI site) for the second round, with the identical cycling conditions as the first-round PCR.

The complete HBV genome was amplified using a single amplification,[29] with modified primers P1 5′-CTTTT TCACCTCTGCCTAATCA-3′ (1820-1841 from *Eco*RI site) and P2RM 5′-AAAAAGTTGCATGRTGMTGG-3′ (1825-1806 from *Eco*RI site).

Cloning

The complete genome amplicons were gel purified using a gel DNA recovery kit (Zymoclean, Zymo Research, Irvine, CA) and cloned individually into a cDNA vector (pSMART, Lucigen, Middleton, WI) according to the protocol provided by the manufacturer and the clones sequenced directly.[30]

Sequencing

The amplicons and clones were prepared for direct sequencing using a cycle sequencing ready reaction kit (BigDye Terminator v3.1, Applied Biosystems) and sequenced on a genetic analyzer with 16 capillaries (ABI3130xl, Applied Biosystems). In addition to the primers used for amplification, vector-specific primers as well as HBV-specific primers (1959F 5′-CTTCTGACTT CTTTCCTTC-3′ [1959-1977 from *Eco*RI site]; 2837R 5′-CCAAGAATATGGTGACC-3′ [2837-2821 from *Eco*RI site]; 2454F 5′-GTTAGTATTCCTTGGACT-3′ [2454-2471 from *Eco*RI site]; 185R 5′-GTCCTAGGAATCCTGATG-3′ [185-168 from *Eco*RI site]; 60F 5′-CTGGTGGCTCC AGTTC-3′ [60-75 from *Eco*RI site]; 734R 5′-CTGAAAG CCAAACAGT-3′ [734-719 from *Eco*RI site]; 595F 5′-CACCTGTATTCCCATC-3′ [595-610 from *Eco*RI site]; 1431R 5′-GACGTAAACAAAGGACG-3′ [1431-1415 from *Eco*RI site]; 1258F 5′-CTGCCGATCCATACTG-3′ [1258-1273 from *Eco*RI site]; and 1769R 5′-CAATTTATGCCTACAGCCTC-3′ [1769-1777 from *Eco*RI site]) were used for sequencing. All sequences were analyzed in both the forward and the reverse directions. The accession numbers of HBV isolates sequenced in this study have been deposited in GenBank/EMBL/DDBJ: JN182318-JN182334.

Phylogenetic analyses

Complete and subgenomic HBV sequences were compared with corresponding sequences belonging to the eight genotypes of HBV (A-H) from GenBank. Multiple sequence alignments and nucleotide divergence calculations were carried out using Dambe.[31] The alignments were edited manually in GeneDoc[32] and fed into PHYLIP (Phylogeny inference package) version 3.69.[33] DNAML (maximum likelihood) alone and DNADIST consecutively with NEIGHBOR (neighbor-joining) were used to generate dendograms. SEQBOOT, DNADIST, and NEIGBOR were used for bootstrapping of 1000 data sets. CONSENSE was used to compute a consensus tree. Trees were visualized using TreeView Win 32 Version 1.6.6.[34]

WP transmission risk analysis

We used the recently refined transmission risk model of Weusten and colleagues[16] to estimate the HBV residual transmission risk posed by RBC transfusions from repeat donors. Using a preformatted calculation spreadsheet the relevant variables were entered, that is, the MP size, the amount of plasma in a RBC unit (estimated at 20 mL), the total number of repeat donations over 4 years of testing, the number of acute HBV infections or HBV DNA and/or HBsAg seroconversions in the repeat donors, the mean preseroconversion interdonation interval, the mean viral doubling time of 2.56 days for the ramp-up phase, and the mean half-life of HBV (1.6 days) in the recovery phase.[35] For the Ultrio assay the recently established 95% and 50% detection limits on the Eurohep genotype A2 standard in a SANBS validation study were used for the calculations (98.5 and 9.5 copies/mL, respectively). The 50% minimum infectious dose (ID_{50}) estimated in the pre-ID-NAT WP was estimated at 3.7[15] but in the second HBV DNA–negative WP the ID_{50} was estimated to be approximately 100-fold higher (and set at 370 copies) as could be deduced from infectivity studies in human liver chimera mice.[36]

The formula below describes the WP transmission risk for HBV in repeat donors:

$$\text{risk} = \frac{r_{\text{days}}}{t_{\text{between}}} \frac{D_{\text{conv}}}{D_{\text{total}}} \times \text{incidence rate adjustment factor},$$

where the total number of risk-day equivalents in the acute phase is the sum of that in the first and second WP, t_{between} is the mean preseroconversion interdonation interval in repeat donors with interdonation intervals less than 12 months, D_{conv} is the number of seroconverting repeat donors, and D_{total} is the total amount of repeat donations. *Because of the transient detectability of HBsAg and HBV* DNA in the acute phase, the observed incidence rate is an underestimation since seroconversion to anti-HBc and anti-HBs, without detectable HBsAg and HBV DNA, remains unrecognized. Korelitz and colleagues[37] estimated a mean HBsAg detection period of 63 days on the basis of previous follow-up studies. The length of the HBV DNA detection period in acute infection can be estimated from the number of concordant HBsAg and HBV DNA–reactive donations in repeat donors and the additional number of acute NAT-yield infections in this study. The NAT detection period can then be estimated by dividing the total number of viremic acute infections by the number of acute HBsAg-positive infections and multiplying the estimated HBsAg detection period with this factor.[35]

The incidence rate adjustment factor can then be deduced by dividing the mean preseroconversion interdonation interval by the estimated HBV DNA detection period. To estimate the risk of HBV infection in first-time, lapsed, and all donations, the acute-phase NAT-yield rate in repeat donors was compared with those in first-time, lapsed, and all donors. The HBV transmission risk in first-time, lapsed, and all donors was then determined by multiplying the residual risk in repeat donors with the NAT-yield rate ratios in the respective donation categories.

RESULTS

Classification of acute HBV infections

To estimate the risk of HBV transmission by WP donations, it is important to classify the HBV NAT–yield donations as acute or chronic infections. Table 1 shows the number of acute and chronic HBV infections in first-time, lapsed, and repeat donations as detected by HBV DNA and HBsAg screening during 4 years of ID-NAT screening. Over 4 years, 170 repeat donors seroconverted to both HBsAg and HBV DNA, while another 84 seroconverted to HBV DNA without detectable HBsAg. The mean preseroconversion interdonation interval (and range) in the total of 254 acutely infected repeat donors was 127 (35-364) days.

Of the 149 acute-phase NAT-yield cases found in first-time, lapsed, and repeat donors, 114 were classified as early WP donations in the pre-HBsAg or pre–anti-HBc seroconversion phase. Seventy-nine (69%) of these WP infections were confirmed by the presence of HBsAg, HBV DNA, and/or anti-HBc in a follow-up sample, whereas in 35 (31%) no follow-up sample was available. These latter seronegative donations were classified as being in the WP because the TaqMan viral load assay was positive and/or HBV DNA was reactive in multiple replicate Ultrio and dHBV assays on aliquots taken from the frozen plasma unit. Six infections were classified as probable primary OBIs because HBV DNA was reactive in at least two follow-up samples several weeks apart without seroconversion to HBsAg, followed by seroconversion to anti-HBc and anti-HBs in a later sample. Thirteen infections were classified as possible abortive or vaccine breakthrough infections because of presence of HBV DNA in anti-HBs–

TABLE 1. Acute and chronic HBV infection rates as detected by HBV-DNA and HBsAg screening

HBV infection classification	Number HBV infections in donations			
	First time	Lapsed	Repeat	All
Number donations	315,488	302,970	2,303,103	2,921,561
HBsAg+, DNA+	2,302	113	170	2585
(rate)	(1:137)	(1:2,681)	(1:13,548)	(1:1,130)
Acute NAT yield	46	19	84	149*
(rate)	(1:6,858)	(1:15,946)	(1:27,418)	(1:19,608)
Chronic OBI NAT yield	89	27	61	177†
(rate)	(1:3,545)	(1:11,221)	(1:34,375)	(1:16,506)
HBsAg+/DNA–	86	0	0	86
(rate)	(1:3,668)			(1:33,972)
Unclassified	3	1	6	10
All HBV infections	2,523	159	315	3,007
(rate)	(1:125)	(1:1,905)	(1:7311)	(1:972)

* Of 149 acute-phase NAT yields, 95 donors were in the pre–anti-HBc WP, six were likely to be primary occult infections, 13 were possible vaccine breakthrough infections, and 35 were in the IgM anti-HBc+ early recovery phase.
† Of 177 donors with chronic OBI, 75 were potentially infectious (anti-HBc+, anti-HBs–) and 102 were not infectious (anti-HBc+, anti-HBs+). Another five donors could have been classified as OBI with anti-HBs as the sole marker, but we chose to classify these as acute phase NAT yields.

positive (or negative) donations, followed by a significant increase in anti-HBs titer in the second sample (and in four cases followed by delayed anti-HBc seroconversion detected in a third or fourth sample obtained from the donor). In four of the possible vaccine breakthrough infections no follow-up sample was available. Theoretically, these donations could also be classified as occult carriers with anti-HBs as the sole detectable serum marker, but for the purposes of this study they were assumed to be acute infections. One of these four infections was a repeat donor. Misclassification of this case as an acute phase NAT yield would negligibly affect the incidence rate of 254 in 2.3 million repeat donations.

Another group of acute NAT yields, most of them with very low viral loads (below the detection limit of the viral load assay), were the IgM anti-HBc–positive donations in the early recovery phase. In 33 of the 35 early recovery NAT-yield donations, the anti-HBs titer was determined. In 23 of 33 (70%), anti-HBs was reactive and in 19 (58%) anti-HBs titers were above 100 mIU/mL. Ten of the IgM anti-HBc–reactive early recovery phase donations (30%) were anti-HBs negative (<10 mIU/mL) and potentially infectious. A total of 177 (54%) of the HBV NAT yields were OBIs, of which 75 (42%) had no detectable anti-HBs and could potentially be infectious.

HBV transmission case

The blood donor was confirmed positive for HBsAg and HBV DNA on his 53rd whole blood donation. It was determined that the RBC component had been transfused to a 28-year-old male undergoing surgery after a motor vehicle accident. This recipient developed clinical signs and symptoms of acute hepatitis B, 84 days after transfusion, with markedly abnormal liver function test results: ALT 866 IU/L (normal range <50 IU/L), AST 470 IU/L (normal range <38 IU/L), ALP 254 µmol/L (normal range 40-130 µmol/L), GGT 222 IU/L (normal range <60 IU/L), LDH 322 IU/L (normal range 100-250 IU/L), and total bilirubin of 60 µmol/L (normal range 5-21 µmol/L). A further sample taken 110 days after transfusion showed that the recipient was positive for HBV DNA, HBsAg, and IgM anti-HBc. Six months after transfusion the recipient was clinically asymptomatic but did not avail himself for repeat serologic and liver function testing. The PLT unit was issued to a patient with an unreported diagnosis, who, despite concerted efforts, remains untraceable.

The donor remained clinically asymptomatic throughout the follow-up period and upon questioning did not reveal any risk behavior for HBV. He was retested at 2 and 5 months after the X (positive) donation (Table 2). Two months after the X donation, although he was HBsAg negative, HBV DNA was still detectable by ID-NAT. This sample was anti-HBc IgM positive and anti-HBs negative. Five months after the X donation, the donor was anti-HBc and anti-HBs positive and HBV DNA negative.

Viral load estimation

Table 3 shows the proportion of reactive results in replicate Ultrio, Ultrio Plus, and TaqScreen assays in the HBV transmission sample as found by three laboratories. Three of 30 (10%) replicate Ultrio and 10 of 30 (33%) replicate Ultrio Plus assays performed on the recovered FFP unit tested positive for HBV DNA by SANBS. The sample was also tested in Ultrio Plus assays by Gen-Probe and was found reactive in 7 of 30 (23%) replicates. In the Paul Ehrlich Institute the infectious HBV WP sample tested reactive in 2 of 12 (17%) replicates in both the TaqScreen and the Ultrio assay. Using probit analysis, the 95 and 50% detection limit on the Eurohep standards were 98.5 (59-189) and 9.5 (6.5-13.9) copies/mL for the Ultrio and 46.9 (28.4-89) and 4.5 (3.0-6.7) copies/mL for the Ultrio Plus assays, respectively, showing a 2.1 (1.2-4.0)-fold enhancement in analytical sensitivity of the Ultrio Plus assay. From the probit curves the HBV DNA concentration in the plasma unit was estimated to be 1.5 (1.2-1.8) and 1.6 (1.1-2.1) copies or virions/mL in Ultrio and Ultrio Plus, respectively. From this estimation of the low viral load the RBC unit would have contained 32 (22-43) HBV virions in 20 mL of plasma. According to the infectivity risk formula

TABLE 2. HBV markers in samples from both donor and recipient post transfusion

Marker	Donor (47-year-old regular blood donor)			Recipient (110 days after transfusion)
	Index donation	(X + 2 months)	(X + 5 months)	
HBsAg (S/CO ratio)	Positive (352)	Negative (0.27)	Negative	Positive (743.45)
HBV DNA (S/CO ratio)	Positive*	Positive†	Negative	Positive (14.3)
dHBV (S/CO ratio)	Positive (23.1)		Negative	Positive (25.2)
Anti-HBc (IgM)		Positive	Negative	Positive
Anti-HBc (total)		Positive	Positive	Positive
Anti-HBs		Negative	Positive (310 U/L)	Negative

* Positive on three replicates (S/CO ratios of 15.3, 15.6, and 14.9).
† Positive on two replicates (S/CO ratios of 13.3 and 12.8) and negative on one replicate (S/CO ratio 0.12).
S/CO = signal to cutoff.

TABLE 3. Proportion of reactive test results on WP sample in different laboratories*

Laboratory	cobas TaqScreen MPX test	Ultrio assay	Ultrio Plus assay
Paul Ehrlich Institute	2/12 (17)	2/12 (17)	
SANBS		3/30 (10)†	10/30 (33)
Gen-Probe			7/30 (23)†

* Data are reported as number (%) reactive.
† Estimated concentration in probit analysis against the Eurohep Genotype A2 standard was 1.5 (1.2-1.8) and 1.6 (1.1-2.1) copies/mL in Ultrio and Ultrio Plus, respectively.

of Weusten and colleagues,[16] the transmission risk of the amount of HBV virions in the RBC unit would have been 99.7% (98.4%-100%).

Sequence analysis of HBV in donor and recipient

The complete genome of HBV isolated from both the recipient and the donor was amplified and cloned. The full 3221 nucleotides of the HBV genome were successfully sequenced from five clones from the donor and three clones from the recipient. In addition, less than genome length sequences were obtained for four clones from the donor and five clones from the recipient because nucleotides were lost from the 5′ and 3′ ends during the cloning process. These sequences were compared phylogenetically to reference sequences from public databases. Neighbor joining trees for both the complete HBV genome (Fig. 2) and a 3141-nucleotide subgenomic fragment (Nucleotides 1855-1775; Fig. 2 inset) show the isolates from both the donor and the recipient cluster together and belong to Subgenotype A1.

The intragroup divergence of the recipient and donor clones did not differ significantly from each other or from the donor-recipient clone intergroup divergence. On the other hand, the mean intragroup nucleotide divergence for the donor-recipient HBV clones (0.31 ± 0.0006%, mean ± standard deviation [SD]) was significantly lower than intragroup divergences for the South African (2.1% ± 0.006, mean ± SD), African (2.33% ± 0.006, mean ± SD), and global (2.81% ± 0.85, mean ± SD) Subgenotype A1 sequences, respectively ($p < 0.05$).

The consensus sequence of the donor HBV clones was identical to that of the recipient HBV clones but differed at 28 nucleotide positions from the consensus generated from 33 Subgenotype A1 sequences. Fifteen of these mutations were synonymous and 13 were nonsynonymous (Fig. 3).

HBV WP transmission risk

ID-NAT screening interdicted an additional 84 of 254 (33%) acute viremic infections in repeat donors. From the relative proportions of detection of HBsAg and HBV DNA it was estimated that the mean HBsAg detection period of 63 days[37] was extended to 95.4 days (Fig. 1) by multiplying the 63-day period by a factor of 254 in 170 or 1.49. Assuming a 50% HBsAg seroconversion point at 1000 copies/mL[18] in HBsAg PRISM and a ID_{50} of 3.7 copies[15] the infectious pre-HBsAg WP is estimated at 32.5 days, which can be reduced to 15.3 days by Ultrio and 12.6 days with the Ultrio Plus assay in ID-NAT format, when modeled on the detection limits found on the Eurohep standard. The WP reduction times by introduction of ID-NAT are then calculated to be 17.1 days for Ultrio and 19.9 days for Ultrio Plus. Using the same seroconversion risk model of Weusten and colleagues[16] in a reversed manner in the declining HBsAg and HBV DNA clearing phase it can be estimated that with a half-life of 1.6 days[35] and a 100-fold reduced infectivity of HBV because of immune neutralization,[36] still 1.29 and 0.74 risk-day equivalents remain in the second WP for Ultrio or Ultrio Plus, respectively (Fig. 1). If the relative sensitivity of HBsAg and HBV DNA assays were to be the same in the pre-HBsAg ramp-up phase as in the HBsAg declining phase, the reversed Weusten model estimates a post-HBsAg infectious WP of 9.9 days. This means that Ultrio has shortened the second post-HBsAg WP by 8.6 days. However, when the risk-day equivalents are estimated on the basis of the proportion of IgM anti-HBc–positive NAT yields we can calculate a shorter reduction period as follows: In 14 repeat donors HBV DNA was detectable

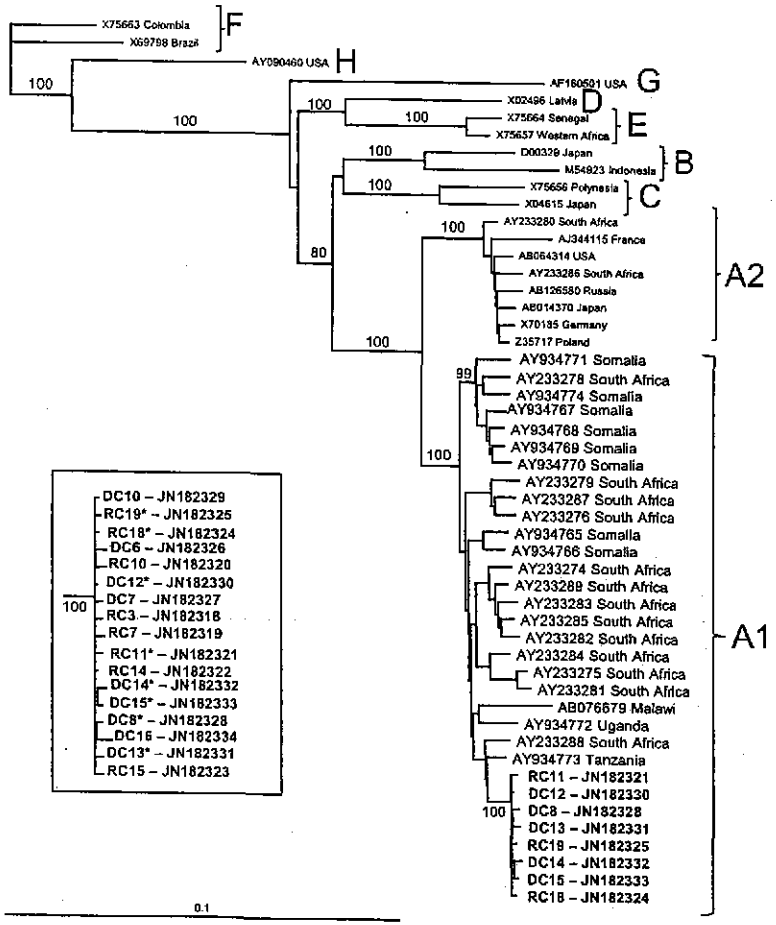
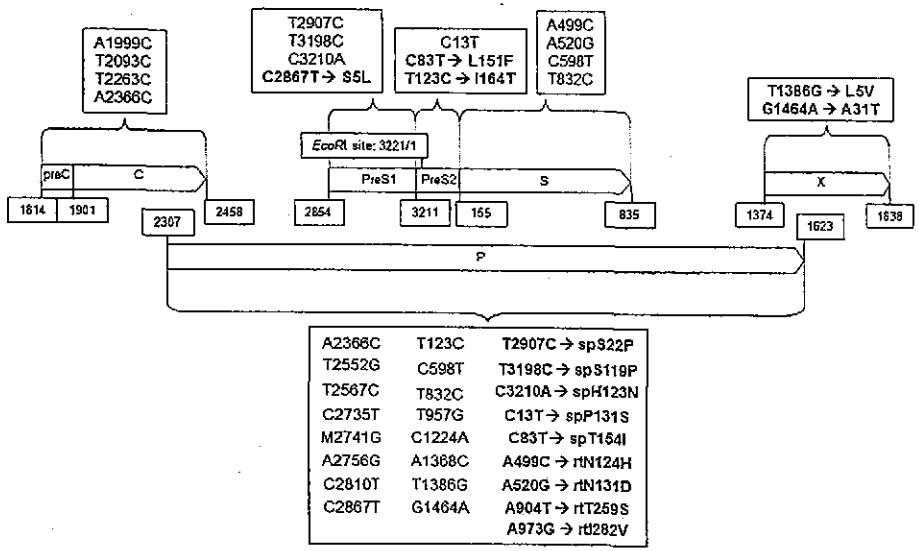


Fig. 2. Phylogenetic relationship of eight HBV clones, shown in bold (five from the donor and three from the recipient) to full-length sequences of other HBV isolates obtained from GenBank, established using neighbor-joining methods. Bootstrap statistical analysis was performed using 1000 data sets with the numbers on the nodes indicating the percentage of occurrences. Clones are designated DC (donor clone) or RC (recipient clone) followed by their GenBank accession number. Other sequences are designated by their GenBank accession number followed by the country of origin. (Inset) Phylogenetic relationship of a 3141-nucleotide subgenomic fragment (minus nucleotides 1775-1855) from an additional nine shorter than full-length HBV clones (four from the donor and five from the recipient) relative to the full-length clones (*), established using neighbor-joining methods.



A2366C	T123C	T2907C → spS22P
T2552G	C598T	T3198C → spS119P
T2567C	T832C	C3210A → spH123N
C2735T	T957G	C13T → spP131S
M2741G	C1224A	C83T → spT154I
A2756G	A1368C	A499C → rtN124H
C2810T	T1386G	A520G → rtN131D
C2867T	G1464A	A904T → rtT259S
		A973G → rtI282V

Fig. 3. Comparison of the genomic organization of the donor-recipient consensus sequence and Subgenotype A1 consensus sequence showing the differences found in each of the four open reading frames. Both the synonymous and the nonsynonymous (indicated by bold type) mutations are shown, with the corresponding amino acid changes for the nonsynonymous mutations included. The start and end sites of the four open reading frames are indicated: preC = precore; C = core; P = polymerase gene; S = surface gene; X = X gene. The first T of the *Eco*RI cleavage site (5′-GAATTC-3′) is Position 1.

longer than HBsAg in the IgM anti-HBc–positive early recovery phase. Since we found 70% of IgM anti-HBc–positive donors to be anti-HBs positive (>10 mIU/mL), 30% of the 14 repeat donors (equivalent to 4.2 cases) could be potentially infectious. Hence the proportion of HBsAg-negative donors in the anti-HBs–negative, but HBV DNA– and IgM anti-HBc–positive detection phase, can be estimated to be 4.2 in 254 (1.6%). Since the HBV DNA detection period by Ultrio in ID-NAT was estimated at 95.4 days the length of the HBV DNA–positive but HBsAg- and anti-HBs–negative second WP would be 0.016 × 95.4 days or 1.5 risk-day equivalents. This is less than the 8.6 risk-day equivalents determined by the reversed modeling according to Weusten and coworkers,[16] indicating that worst-case risk estimates for the second WP were calculated in this report.

Since the mean preseroconversion interval in repeat donors was 127 days and the length of the HBV DNA detection period was estimated at 94.5 days, one-third of the acute HBV infections would remain unrecognized. Therefore, the incidence rate in our study needs to be multiplied with an adjustment factor of 127 per 94.5 or 1.34. Using the refined risk analysis[15] for Ultrio in ID-NAT based on 15.3 and 1.3 risk-day equivalents for the first and second WP (total 16.6 risk-day equivalents) a residual HBV transmission risk of 17.9 per million donations in the first WP and 1.5 per million in the second WP was calculated (for a total residual risk of 19.4 HBV transmissions per million repeat donations). Since the HBV WP NAT-yield rate (Table 1) was found to be 4.00-, 1.72-, and 1.40-fold higher in first-time, lapsed, and all donations, respectively, we estimated the residual risk in these donations to be accordingly higher than in repeat donations, that is, 77.6, 33.4, and 27.1 per million, respectively. In other words, the residual HBV transmission risk from acute HBV infections was estimated at 1:12,880, 1:29,966, 1:46,164, and 1:36,800 donations in first-time, lapsed, repeat, or all donors, respectively. Note that these risk estimates do not take into account the unknown risk caused by ID-NAT–negative donors with OBI (see Fig. 1). During 4 years of observation the SANBS lookback program has revealed one clinical posttransfusion hepatitis B event in 2.9 million ID-NAT-screened donations, an observed HBV transmission rate of 0.34 per million.

DISCUSSION

This communication reports the first transfusion-transmitted HBV infection by blood that had been screened by ID-NAT. Definitive proof of a TTI can only be obtained by genomic analysis of both the donor and the recipient viral strains.[38] In this case, the clones of the HBV strains derived from the donor and the recipient were 99.7% homologous but had a number of nucleotide positions, which distinguished this strain from the consensus sequence of Subgenotype A1, the predominant subgenotype circulating in South Africa[38] (Fig. 3). Of the mutations depicted in Fig. 3, as far as we can ascertain, only the synonymous C1224A mutation in the polymerase open reading frame could possibly have any functional or regulatory significance. The C1224A mutation is within the NF-1 binding site of the HBV enhancer I[40] and may therefore affect transcription of the pregenomic and HBx mRNAs.[41] Further functional characterization would be required to confirm this. The high homology of the consensus sequences of the HBV strains from both the donor and the recipient excludes any possibility of a hospital-acquired infection or viral reactivation in the recipient. Furthermore, these strains form a unique branch within the Subgenotype A1 clade with 100% bootstrap support (Fig. 2).

The viral load in the pre–ID-NAT WP donation was estimated by probit analysis to be 1.6 (1.1-2.1) copies/mL and was only detectable in 10% of replicate Ultrio assays. This translates to approximately 32 (22-43) HBV virions in the estimated volume of 20 mL of plasma in the transfused RBC unit. The viral load in the WP donation was also below the detection limit of the more sensitive Ultrio Plus and TaqScreen assays, which detected the sample 33 and 17% of the time in replicate assays, respectively.

The possibility that a unit of blood will transmit virus to a blood recipient is determined by the infectious WP. For HBsAg, using the Abbott PRISM ChLIA system, the infectious WP has been estimated to be 35.5 days if one viral particle or DNA copy in a RBC transfusion is infectious.[18] The WP reduction, using an HBV NAT assay system with a 50% limit of detection (LOD) of approximately 10 copies/mL, was independently calculated to be 17.9[13] and 14.9 days,[19] yielding a calculated WP for HBV by ID-NAT of 20.4 and 24.3 days, respectively. We previously estimated the 50% LOD to be 29 copies/mL and the infectious WP to be 24.3 days.[1] These reported WP estimates were all based on the assumption that one virus in a blood transfusion is enough to be infectious. The variation in the estimated lengths of the WPs in these reports is mainly caused by variation in the 95% and 50% detection limits observed in the analytical sensitivity studies. In this study the infectious WPs with Ultrio were considerably shorter because the refined transmission risk model of Weusten and colleagues[18] also brings the likelihood of infectivity of a low viral burden or the ID_{50} into the equation. Using a ID_{50} of approximately four HBV DNA copies (as found after recalibration of the viral load in a chimpanzee challenge plasma with a known infectivity titer[13] in multiple bDNA assays[15]), we estimated an infectious WP of 15.3 risk-day equivalents with the Ultrio assay and an additional 1.3 risk-day equivalents in the second anti-HBc–positive WP. Compared to the calculated infectious WP for HIV by ID-NAT (2.9 days) and the calculated infectious WP for HCV by ID-NAT (1.3 days),[15] it is evident that there is statistically a greater probability that a blood donation will be in an HBV infectious WP relative to those of HIV and HCV.

During the first 4 years of ID-NAT testing, SANBS detected 2523 HBV confirmed positive donations in 315,488 first-time donations, a prevalence of 0.80%. In the same period the incidence rate of acute HBV infections was 254 in 2,303,103 repeat donations (1:9067). In all 2,921,561 donations 3007 HBV infections were confirmed, yielding an infection rate of 0.10%. Of these confirmed positive HBV infections, 336 donations were detected by ID-NAT only and, of these, 149 were in the HBsAg-negative WP, 177 were OBIs, and 10 were unable to be classified. Therefore, for 149 WP donations interdicted by ID-NAT (rate, 1:19,600), only one donation was found to be in the pre–ID-NAT WP and caused infection in the recipient (1:2,900,000). We estimated the residual risk of a donation being in the first or second infectious WPs for HBV in South Africa to be 27.1 per million donations (1:36,800). This translates to 79.2 HBV transmission events in the 4-year ID-NAT screening period or an estimated 19.8 HBV transmissions per year.

Why is the estimated residual risk far higher using mathematical modeling than the observed transmission rate? Several reasons may account for the 80-fold lower observed transmission rate than the estimated WP transmission risk: 1) A proportion of approximately 40% of the transfusion recipients in South Africa have already been exposed to HBV and are immune;[24] 2) the younger population has, since 1995, been vaccinated in infancy against hepatitis B;[24] 3) low levels of HBV that escape ID-NAT screening can be easily neutralized by anti-HBs present in simultaneously administered blood products; 4) the infectious dose of HBV in ID-NAT–nonreactive WP donations may not lead to clinically recognized HBV infections in the majority of recipients since previous studies in prisoners showed that the incubation time and the occurrence of clinical hepatitis B was inversely correlated with the infectious dose;[42] 5) HBV infection in lookback programs may be underreported and underdiagnosed for several reasons among which is lack of pretransfusion sampling and fortuitous timing of HBV assays in posttransfusion samples; 6) the incubation time of HBV can be much longer when HBV is present in immune complexes;[21] and 7) possible reduction of the infectivity of HBV in stored RBC units.

Many studies have focused on the transmission risk posed by donors with chronic OBI.[23,43] So far, two lookback studies have been reported that indicate that the risk of HBV transmission by low viral load occult HBV carriers is 3% or less.[9,44] The HBV transmission case in this report is consistent with the premise that transfusion-transmitted HBV is greater than 10-fold more likely to occur when the donor is in a recent postexposure infectious WP compared to the presence of low-level viremia in OBI.[9] The risk of HBV transmission by ID-NAT–screened blood donations from donors with OBI is considered to be very low, but further systematic lookback studies are required to confirm this.

It has been suggested that the variation in analytical sensitivity of the current Ultrio test system may not only be HBV genotype dependent but also individual strain dependent.[17] In particular, a variation in relative sensitivities of HBV DNA detection on the Tigris system compared to the Roche s201 (Roche Molecular Systems) has been observed with Genotype A seroconversion panels.[17] This variation in sensitivity may have further contributed to the lack of detection of HBV DNA on the initial ID-NAT screening of the blood donation in this HBV transmission case. However, the comparison of the proportions of reactive results in the WP transmission sample did not demonstrate a large difference in sensitivity between the Ultrio, Ultrio Plus, and TaqScreen assays. The HBV transmission sample was not detectable 90% of the time in replicate Ultrio assays, but would also not be detectable 67% to 83% of the time by the Ultrio Plus and TaqScreen assays in ID-NAT configuration. Clearly, ID-NAT is not sensitive enough to prevent HBV transmission by donations in the early window phase. Further studies are required to determine whether ID-NAT is sensitive enough to prevent HBV transmission by donors with OBI. In the South African high-prevalence setting of hepatitis B a lookback program in recipients of donors, later identified as occult carriers, can only provide meaningful results if a control group of recipients that received blood from anti-HBc–negative donors is also subjected to the same lookback procedures and the proportion of anti-HBc–positive recipients in each group is compared to determine significant difference between the two groups. So far such a comparison study of prevalence of HBV markers, in particular anti-HBc, in recipients of blood from occult HBV carriers and a noninfected control group has not been undertaken.

This communication reports the first known case of transmission of HBV by blood that had been screened by ID-NAT and confirms that blood donations that test negative for HBV DNA may transmit the virus when the donation is in the infectious WP. Transmission was confirmed by the 99.7% sequence homology between the complete genome sequences of both the donor and the recipient viral strains. Our study has demonstrated that the introduction of ID-NAT has significantly enhanced the safety of the blood supply in South Africa, but does not completely eliminate the transmission risk.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest relevant to the manuscript submitted to TRANSFUSION.

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研究報告の概要

○25年間のユニバーサルワクチン接種プログラムによるB型肝炎感染の最小化

背景と目的:1984年に乳幼児へのユニバーサルワクチン接種プログラムが実施されるまで、台湾ではB型肝炎ウイルス(HBV)感染が非常に流行していた。ワクチン接種プログラムの開始から1年未満、5年後、10年後、15年後、20年後と、計5回の血清疫学調査がこれまでに行われた。

方法:30歳未満の各年齢集団から約100人ずつ、3,332人の被験者を登録した。これまでの調査同様、被験者は台北の学校や施設から任意で採用された。HBV血清マーカーにはHBs抗原、HBs抗体、HBc抗体が含まれた。HBc抗体陽性でHBs抗原陰性の被験者のみHBV DNAが測定された。

結果:HBs抗原、HBs抗体、HBc抗体の陽性率は2009年におけるプログラム開始後に出生した被験者と、1984年のベースライングループとの間で大きく異なった(それぞれ0.9%対10%、55.9%対24.5%、7.0%対28%)。この6回目の調査において、HBs抗原保有率がワクチン集団でさらに減少したことを示した。ワクチン無効者のうち86%は、母親がHBs抗原陽性であった。HBV DNAはHBc抗体陽性/HBs抗原陰性被験者の4.2%(6/142)から検出された。これらは全てHBVジェノタイプCであった。

結論:台湾における乳幼児ユニバーサルHBV予防接種プログラムに対する25年間の追跡調査が完了し、若年者へのワクチンの有効性が明確であることが分かった。HBs抗原陽性率が継続して減少していることは、HBV感染症の撲滅が現実となりつつあることを示唆している。母子感染はワクチン無効の主要な原因であるため克服が必要である。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン5%静注12.5g/250mL
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
台湾の25年間に亘る乳幼児対象ユニバーサルB型肝炎ワクチン接種プログラムの追跡調査において、ワクチン接種集団におけるHBs抗原陽性率は継続して減少していることが分かり、若年者に対するワクチンの有効性は明確であったとの報告である。なお、現在日本では、母子感染プログラムは実施されているが、多くの国で行われている全小児へのユニバーサルワクチン接種は行われていない。本情報は今後日本のHBV感染予防対策を検討する上で有益な情報である。	これまでの本製剤の使用実績やバリデーション成績に鑑み、特別の対応を必要としないが、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。

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Research Article

Minimization of hepatitis B infection by a 25-year universal vaccination program

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Background & Aims: Hepatitis B virus (HBV) infection was hyperendemic in Taiwan before the implementation of the universal infant hepatitis B immunization program, which was launched in 1984. Five previous seroepidemiologic surveys were conducted at 0, 5, 10, 15, and 20 years after the launch of the vaccination program.

Methods: We enrolled 3332 subjects younger than 30 years of age, with approximately 100 of them in each age cohort. Subjects were recruited voluntarily from schools and other institutions in Taipei, as in previous surveys. HBV seromarkers included hepatitis B surface antigen (HBsAg) and antibodies to HBsAg (anti-HBs) and hepatitis B core antigen (anti-HBc). HBV DNA levels were measured in anti-HBc positive/HBsAg negative subjects (anti-HBc only).

Results: The HBsAg, anti-HBs, and anti-HBc seropositive rates were very different between subjects born after the program in 2009 and the baseline group in 1984 (0.9% vs. 10%, 55.9% vs. 24.5%, and 7.0% vs. 28%, respectively). In this 6th survey, we showed that HBsAg prevalence further decreased in the vaccinated cohorts. A positive maternal HBsAg status was found in 86% of vaccine failures. Serum HBV DNA was detected in 4.2% (6/142) of anti-HBc positive/HBsAg negative subjects, with a low level of HBV DNA. All of these six subjects' HBV were genotype C.

Conclusions: The universal infant HBV immunization program in Taiwan has completed its 25-year follow-up and its efficacy in young adults is clear. The continued decrease in HBsAg prevalence suggests that the elimination of HBV infection is becoming a reality.

Keywords: Hepatitis B; Vaccination; Genotype; Occult infection.
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Abbreviations: HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; anti-HBs, antibodies to HBsAg; anti-HBc, antibodies to hepatitis B core antigen; HBeAg, hepatitis B e antigen; HCC, hepatocellular carcinoma.

Introduction

The world's first nationwide hepatitis B virus (HBV) universal vaccination program for infants was launched in Taiwan in July 1984 [1]. All infants were mandated to receive scheduled HBV vaccinations. In addition, infants born to hepatitis B e antigen (HBeAg)-positive mothers received hepatitis B immunoglobulin (HBIG) 24 h after birth [2]. Through January 2010, the coverage rate for infants who completed the 3-dose HBV vaccination program was estimated to be 96.5% (http://www.cdc.gov.tw/public/Data/097144257T1.pdf).

We have serially performed five seromarker surveys in the young people covered by the universal infant HBV vaccination program in Taipei City. The first survey was a baseline study conducted just before the launch of the universal program in 1984 [3], and the ensuing seroepidemiologic surveys were done every 5 years in 1989, 1994, 1999, and 2004 [4–7]. In children <15 years of age, the seroprevalence of hepatitis B surface antigen (HBsAg) declined from 10% [3] (pre-vaccination era) to 0.6% after 20 years of universal vaccination [7]. Consistent with the decrease in chronic HBV infection, the incidence of HBV-related pediatric hepatocellular carcinoma (HCC) in Taiwan also decreased [8]. From 1981 to 1994, the incidence of pediatric HCC declined from 0.52 to 0.13 per 100,000 person-years. In 2004, the incidence of HCC in the 6–9, 10–14, and 15–19 age groups decreased to 0.15, 0.19, and 0.16, respectively [9,10].

The 25-year universal infant HBV vaccination program has provided long-term protection (>20 years), and subjects who received a primary vaccination did not require a universal booster [11]. Though largely diminished, mother-to-infant transmission was not completely blocked by immunoprophylaxis [7]. The emerging escape mutants caused some concerns, but they did not increase the risk of chronic infection [12]. Occult HBV infection had recently attracted some attention. Even if subjects are HBsAg negative, they may harbor HBV DNA in serum or liver [13]. In addition to the risk of transmission of infection to others, persons with occult HBV infection are at risk of reactivation when they receive chemotherapy or immunosuppressive therapy [14,15].

This study will continue to monitor the HBV seroprevalence in children and adults in the same area that has been surveyed for the last 25 years. We aimed at eradicating HBV infection by targeting all possible sources of infections, including mother-to-infant transmission and occult HBV infection.

Patients and methods

Vaccination program

The HBV universal infant vaccination program was launched in Taiwan on July 1, 1984 [1]. Before July 1992, four doses of plasma-derived vaccine (Hevac B; Pasteur-Mérieux, Lyon, France, or its equivalent derivative, Lifeguard hepatitis B vaccine; Hsin-Chu, Taiwan) were given to infants at 0, 1, 2, and 12 months of age. After July 1992, three doses of the recombinant vaccine H-B-Vax II (5 μg/0.5 ml; Merck Sharp & Dohme, Rahway, NJ, USA) or Engerix-B (20 μg/1 ml; SmithKline Beecham, Rixensart, Belgium) were administered at <1 week, 1 month, and 6 months of age.

In addition, all pregnant women were screened for HBsAg. If HBsAg was positive, hepatitis B e antigen (HBeAg) would be checked. HBIG 0.5 ml (100 IU) was given within 24 h after birth to newborns of HBeAg positive carrier mothers. Details of the vaccination program have been previously described [2,16].

Study population

From January to December 2009, serum samples were collected from 3332 apparently healthy individuals (M:F = 1696:1636) younger than 30 years of age. All the subjects were recruited from schools, institutes, or workplaces in Taipei City, the same area in which the baseline and follow-up seroepidemiologic studies were conducted. The study protocol was approved by the National Taiwan University Hospital's Institutional Review Board.

The 3332 subjects consisted of 290 children <3 years of age enrolled from the well-baby clinic and nursery room of the Department of Pediatrics, National Taiwan University Hospital, and one day-care center; 453 children aged 3–6 years from four kindergartens; 715 children aged 6–13 years from two elementary schools; 595 adolescents aged 13–18 years from two middle schools and one high school; 406 college students aged 18–22 years from two universities; and subjects aged between 22 and 29 years including 495 graduate students from the graduate schools of our university and 378 new employees of two general hospitals and two local commercial companies.

All the subjects were voluntarily recruited through poster advertisements, or health staff invitations. Either the subjects themselves or their parents signed an informed consent and provided the vaccination history, which was recorded in a health booklet distributed to each newborn by the Department of Health (DOH), if available. Because the HBV vaccination program did not include all newborns until 1986, only the vaccination histories of those below 23 years of age (born after 1986) were included in the inquiries. Once an HBsAg positive carrier was detected, we not only checked their vaccination histories and family histories provided by the subjects themselves, but also verified their vaccination histories using the HBV vaccination database of the Department of Health and confirmed the family history by taking blood samples, if possible.

HBV serology

The presence of serum HBsAg, its antibody (anti-HBs), and hepatitis B core antibody (anti-HBc) was tested in all the subjects (Abbott Laboratories, North Chicago, IL, USA). Anti-HBs is considered positive if the titer is ≥ 10 m IU/ml. The maternal HBsAg status was also investigated, if indicated.

HBV DNA detection/quantification

To elucidate any occult HBV infection, we assayed HBV DNA levels of anti-HBc positive and HBsAg negative subjects, irrespective of the presence of anti-HBs. HBV DNA viral load measurements were performed as described previously [17]. Briefly, HBV DNA was extracted from 50 μl of serum and subjected to polymerase chain reaction (PCR). Real-time PCR measurement was performed using the LightCycler analysis software, version 3.5 (Roche Diagnostics Applied Science, Mannheim, Germany). HBV genotypes were analyzed by PCR using type-specific primers, as described previously [18].

Statistics

Differences in frequency between groups were examined using the Chi-square test with Yates' correction or the Fisher's exact test, where appropriate. A p value of <0.05 was deemed to indicate statistical significance. A trend test was used to evaluate the cohort study.

Results

The universal vaccination program significantly decreased HBV prevalence

HBsAg prevalence in this and five previous seroepidemiologic surveys showed a clear effect: subjects who were born after the implementation of the universal HBV vaccination program had a low seropositive rate compared to those born before the implementation (Fig. 1). The prevalence of HBsAg and anti-HBc in children <15 years of age was 0.5% (9/1651) and 2.9% (45/1550), respectively. The prevalence rate of HBsAg in the population ≤25 years of age, born in or after the year of the launch of the program, was 0.9% (Table 1). This is notably lower than that of subjects aged 26–30 years, who were born before the vaccination program (8.2%, 31/378) (p <0.0001, Chi-square test) (Table 1). Overall, HBsAg, anti-HBs, and anti-HBc seropositive rates were 0.9%, 55.9%, and 7.0%, respectively, in subjects born after the vaccination program (<25 years old) in 2009.

No increase in HBsAg prevalence with age by birth cohorts

Table 2 lists the HBsAg seropositive rates of each birth cohort born after the universal vaccination program, at different ages, in the five seroepidemiologic surveys conducted in 1989, 1994, 1999, 2004, and in the present study (2009) [3–7]. We did not include the cohort of those born from 1984 to 1986 when only neonates born to HBsAg-carrier mothers received HBV immunoprophylaxis. In the cohort born during the period 1987–1988, a trend towards a decrease of HBsAg positivity was observed 22 years after the primary vaccination. A similar trend was observed in all birth cohorts across all the surveys (Table 2). Though the HBsAg seropositivity did not increase in these birth cohorts, the anti-HBc seropositivity, which is a surrogate marker of natural infection, increased gradually in the longitudinal follow-up (Table 3).

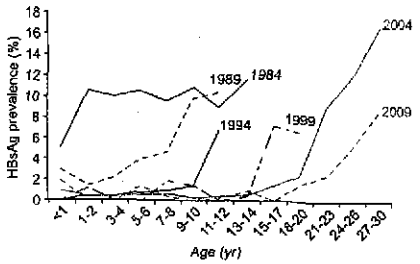

Fig. 1. Age-specific hepatitis B surface antigen seropositive rates in 1984, 1989, 1994, 1999, 2004, and 2009 in Taipei, Taiwan. In 1984, none of the subjects were under universal vaccination coverage. In 1989, only children <5 years of age were covered. Subsequently, children less than 10, 15, 20, and 25 years of age were covered by the universal vaccination in 1994, 1999, 2004, and 2009 respectively. Those who were born before the implementation of this program had a higher HBsAg carrier rate than those born after the implementation.

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Table 1. HBV seroprevalence 25 years after implementation of the universal vaccination in Taipei, Taiwan: HBsAg positive and anti-HBc positive rates (%).

Age (yr)	No. persons	HBsAg* (95% CI)	Anti-HBc alone (95% CI)	Anti-HBc + anti-HBs (95% CI)	Anti-HBs alone (95% CI)	Negativity (95% CI)*
<1	101	2.0 (1.7-2.3)	6.9 (6.4-7.4)	70.2 (69.4-71.2)	21.8 (21.0-22.6)	1.0 (0.8-1.2)
1-2	189	0	0	2.6 (2.5-2.8)	89.4 (89.1-89.7)	7.9 (7.6-8.2)
3-4	253	0	0	1.2 (1.1-1.3)	77.5 (77.2-77.8)	21.3 (21.0-21.6)
5-6	200	1.5 (1.4-1.6)	0	1.5 (1.4-1.6)	43.5 (43.0-44.0)	54.5 (54.0-55.0)
7-8	206	0.5 (0.4-0.6)	1.0 (0.9-1.1)	1.5 (1.3-1.6)	55.8 (55.3-56.3)	41.3 (40.8-41.7)
9-10	191	0	0	2.1 (1.9-2.2)	39.2 (38.8-39.8)	58.6 (58.1-59.1)
11-12	318	0.3 (0.3-0.4)	2.5 (2.4-2.6)	1.9 (1.8-2.0)	23.3 (23.0-23.5)	72.3 (72.1-72.6)
13-14	193	1.0 (0.9-1.1)	0.5 (0.4-0.6)	2.6 (2.4-2.7)	19.2 (18.8-19.6)	75.1 (74.7-75.6)
15-17	402	0.3 (0.2-0.3)	1.7 (1.7-1.8)	2.5 (2.4-2.6)	29.9 (29.8-30.1)	65.7 (65.4-65.9)
18-19	221	1.4 (1.3-1.5)	1.4 (1.3-1.5)	2.3 (2.1-2.4)	43.9 (43.5-44.3)	51.1 (50.7-51.6)
20-21	185	1.6 (1.5-1.8)	0	2.2 (2.0-2.3)	51.4 (50.8-51.9)	44.9 (44.3-45.4)
22-23	297	2.0 (1.9-2.1)	1.7 (1.6-1.8)	4.4 (4.2-4.5)	64.6 (64.3-65.0)	27.3 (27.0-27.6)
24	101	3.0 (2.7-3.3)	0	10.9 (10.3-11.5)	68.3 (67.4-69.2)	17.8 (17.1-18.6)
Total	2857	0.9 (0.9-0.9)	1.1 (1.1-1.1)	5.0 (5.0-5.0)	50.9 (50.9-50.9)	45.9 (45.8-45.9)
25	97	6.2 (5.7-6.7)	0	10.3 (9.7-10.9)	62.9 (61.9-63.9)	19.6 (18.8-20.4)
26-27	199	6.0 (5.8-6.3)	0	9.5 (9.3-9.8)	70.4 (69.9-70.8)	14.6 (14.2-14.9)
28-29	179	10.6 (10.3-11.0)	4.5 (4.2-4.7)	16.8 (16.4-1.2)	50.2 (48.7-50.8)	17.9 (17.5-18.3)

*All HBsAg positive subjects were anti-HBc positive.
*Negativity represents those subjects who were negative for all three HBV seromarkers, i.e. HBsAg, anti-HBc, and anti-HBs.
CI, confidence interval.

Sources of HBV infection in vaccine failures

We detected 25 HBsAg carriers born after the implementation of the universal infant vaccination program. All were confirmed to have received at least three doses of hepatitis B vaccine based on the subjects' own records and the DOH database. The histories of HBV infection in the families of these 25 subjects were investigated. One subject declined the inquiry and the family histories of two subjects could not be traced. Among the remaining 22 subjects, 17 had a HBsAg carrier mother (but not father), both parents of two of them were HBsAg positive, and two had a HBsAg carrier father. Only one subject had no family history of HBV infection, however, he did have a history of blood transfusion during early childhood. In other words, 86.4% (19/22) of the vaccination failures were likely related to the maternal HBsAg carrier status.

Detection of occult HBV infection

Among the subjects born after the launch of the universal vaccination program (n = 2954), 71 were anti-HBc positive and HBsAg negative. Twenty-one of these 71 subjects were anti-HBc positive and two of the 21 had detectable HBV DNA (4.7×10^3, and 2.7×10^3 copies/ml), while one of the remaining 50 subjects positive for both anti-HBc and anti-HBs was HBV DNA positive (2.0×10^3 copies/ml). All three were genotype C and born to HBsAg carrier mothers.

Among the 378 subjects born before the launch of the universal infant vaccination program, 71 were anti-HBc-positive and HBsAg-negative. Nine of the 71 were anti-HBc positive and anti-HBs negative. One of the nine subjects had detectable HBV DNA (mixed genotypes B and C, 3.8×10^3 copies/ml). Two of the remaining 62 subjects who were both anti-HBc and anti-HBs positive had detectable HBV DNA (1.3×10^3 and 1.6×10^3 copies/ml, respectively) (Table 4). Again, both were genotype C. The family histories of these three cases were not solicited.

Occult HBV infection was significantly more prevalent in subjects born before the implementation of the universal infant vaccination program than in those born after (3/378 vs. 3/2954, p = 0.003, Fisher's exact test). The frequency of occult infection

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Table 2. HBsAg seropositive rates in birth cohorts born after the vaccination program in five consecutive surveys conducted in 1989, 1994, 1999, 2004, and 2009.

Birth year	1989		1994		1999		2004		2009		p value*
	N (%)	OR (95% CI)	N (%)	OR (95% CI)	N (%)	OR (95% CI)	N (%)	OR (95% CI)	N (%)	OR (95% CI)	
1987-1988	3/205 (1.46)	1	3/371 (0.81)	0.55 (0.09-3.43)	0/296 (0)	0 (0-1.54)	71/4088 (1.74)	1.19 (0.39-3.60)	5/262 (1.91)	1.31 (0.34-5.02)	0.07
1989-1993	-	-	2/574 (0.35)	1	7/455 (1.54)	4.28 (0.81-29.9)	51/6298 (0.81)	2.33 (0.62-9.53)	6/595 (1.01)	2.91 (0.67-14.28)	0.19
1994-1998	-	-	-	-	1/241 (0.41)	1	8/1790 (0.45)	1.08 (0.17-8.57)	3/606 (0.50)	1.13 (0.16-10.82)	1.00
1999-2003	-	-	-	-	-	-	7/1479 (0.47)	1	2/479 (0.42)	0.88 (0-3.75)	1.00
2004-2008	-	-	-	-	-	-	1/110 (0.91)	1	2/560 (0.36)	0.39 (0.05-4.29)	0.42
2009	-	-	-	-	-	-	-	-	2/101 (1.98)	-	-

* Trend test.
OR, Odds' ratio; CI, confidence interval.

Table 3. Anti-HBc seropositive rates in birth cohorts born after the vaccination program in four consecutive surveys conducted in 1994, 1999, 2004, and 2009.

Birth year	1994		1999		2004		2009		p value*
	N (%)	OR (95% CI)	N (%)	OR (95% CI)	N (%)	OR (95% CI)	N (%)	OR (95% CI)	
1987-1988	9/342 (2.63)	1	3/301 (1)	0.37 (0.08-1.51)	188/4088 (4.60)	1.78 (0.92-3.47)	15/262 (5.73)	2.25 (0.99-5.11)	0.002
1989-1993	11/604 (1.82)	1	22/461 (4.77)	2.70 (1.24-6.00)	149/6298 (2.37)	1.31 (0.71-2.40)	30/595 (5.04)	2.86 (1.44-5.69)	<0.001
1994-1998	-	-	6/161 (3.73)	1	23/1790 (1.28)	0.34 (0.14-0.81)	25/639 (3.91)	1.05 (0.44-2.54)	<0.001
1999-2003	-	-	-	-	17/1479 (1.15)	1	11/479 (2.30)	2.02 (0.96-4.27)	0.07
2004-2008	-	-	-	-	-	-	12/564 (2.13)	-	-

*To avoid the interference by maternal-transferred anti-HBc effect, we excluded data obtained in the first year of life. A trend test was used.
OR, Odds' ratio; CI, confidence interval.

in the anti-HBc-alone and anti-HBc plus anti-HBs groups, irrespective of the year of birth, was 10% (3/30) and 2.7% (3/112) (OR: 5.6; 95CI: 1.2–26.5; Fisher's exact test, $p = 0.036$), respectively. This suggests that the presence of anti-HBs may indicate a more complete protection and a lower risk of HBV carrier status than anti-HBc alone.

Discussion

In this 2009 survey, the seropositivity rates of the subjects born after the program were as follows: HBsAg 0.9%, anti-HBs 55.9%, and anti-HBc 7.0%. In contrast, in the baseline 1984 study, HBsAg, anti-HBs, and anti-HBc positive rates were 10%, 24.5%, and 28%, respectively [3]. Therefore, the younger generations in Taiwan now enjoy a low HBV prevalence.

In the 2004 study, we found higher HBsAg (1.5%) and anti-HBc (4.0%) seropostive rates in subjects aged 15–17 years (1986–89 birth cohorts), and HBsAg and anti-HBc seropostive rates of 2.1% and 6.8%, respectively, in those aged 18–19 years (1984–86 birth cohort) [7]. The 15–17 year old group in the 2004 study (HBsAg seropositive rate, 1.5%) is the same cohort as the 20–21 age group in the 2009 study, which had an almost identical HBsAg seropositive rate (1.6%). The HBsAg seropositive rate of the 18–19 age group in 2004 was 2.1%, similar to the 2.0% in 2009. The relatively higher HBsAg seropositivity of these two cohorts may be explained by the lower coverage rates of HBV vaccination. A nationwide seroepidemiologic survey conducted in the 6-year old subjects in 1993 showed a hepatitis B vaccination coverage rate (≥3 doses of vaccine) of 88.7% in the entire country [16]. According to the data provided by the Taiwan CDC, the national coverage rate increased to 97% in 2002 and later on (http://www.cdc.gov.tw/public/Data/09714425771.pdf). The importance of extensive primary vaccination coverage cannot be overemphasized. Such good coverage in infants could not be replaced or compensated by the later boost program because the chronic infection status is established early in life, and only rarely in the adolescence or young adulthood.

The birth cohort analysis (Table 3) showed no increase in HBsAg seropositive rates in subjects aged >20 years. After the primary vaccination in infancy, a universal booster strategy may not be necessary since the HBsAg seropositivity is not increasing [19–22]. Beyond early childhood, HBV infection is usually associated with a favorable outcome. The risk of chronic HBV infection decreases markedly with increasing age, with approximately 25% of preschool children and 2.7% of university students infected [23,24]. One recent study showed that immune memory to HBV vaccines may have disappeared in some university students

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Table 4. Serum HBV DNA positivity rates in subjects with occult HBV infection: anti-HBc positive and negative HBsAg.

Positive serum HBV DNA	Anti-HBs (-) and anti-HBc (+)	Anti-HBs (+) and anti-HBc (+)	p value
Birth cohort*			
After (n = 2954)	2/21	1/50	0.15
Before (n = 378)	1/9	2/62	0.27

*2954 subjects were born after the implementation of the universal infant vaccination program in 1984 in Taiwan while 378 subjects were born before that point. These subjects were recruited from the general population as described in the "Study population" section.

[25]. This is consistent with our observation that anti-HBc seropositivity, but not that of HBsAg, increased after this age.

Mother-to-infant transmission remains the most important route of HBV acquisition in the post-vaccination era. A high vaccination coverage rate can rule out compliance as a concern for new HBsAg carriage in Taiwan. Intrauterine HBV infection [26] and a high maternal viral load [27] are possible underlying causes of vaccine failure in mother-to-infant transmission. Only a few horizontal transmitted infections remained in this study, perhaps through close family contact or occult infection.

The prevalence of occult HBV infection was about 0.1% (3/2954) in a population under universal vaccination program coverage. Occult HBV infection may cause problems in special circumstances, such as transfusion or organ donation. Once the majority of the donor population is born after the implementation of the universal program, we may exclude subjects with positive anti-HBc as donors to minimize the risk of occult HBV infection. This scenario is expected to occur in the near future, since most donors are young adults. The anti-HBc positive rate is now 6.2% in subjects aged <25 years, which is lower than that (80%) of the population not covered by the vaccination program [28].

To move toward the elimination of HBV infection, three issues must be addressed: (1) elimination of infectious sources, (2) interruption of all transmission routes, and (3) immunization of all susceptible individuals [29]. The program we describe here has successfully addressed the third issue. With respect to the first issue, the pool of infected people has been markedly reduced in the vaccinated population. An effective treatment strategy for the eradication of the virus from all the infected persons is now required. Most current treatments are unable to achieve this goal [30]. Concerning the second issue, the mother-to-infant transmission route must be interrupted, perhaps by administering antiviral agents to pregnant mothers [31]. In addition, patients with HBV infection, even the occult one, should be excluded from use in blood transfusion and organ transplantation [32]. The illicit drug user and sexual transmission routes should be handled by general preventive measures and immunization.

Genotype B is dominant in Taiwan and outnumbers genotype C at a 3:1 ratio [33]. However, genotype C was prevalent in the vaccination failure cases in our previous study [34]. This study demonstrated that genotype C predominated in occult HBV infections. HBeAg seroconversion in genotype C patients occurred decades later compared to those with other genotypes [33]. Genotype C-infected pregnant mothers tend to be HBeAg positive and have a high viremia; thus, these mothers are more likely to transmit the infection to their infants [34].

In conclusion, our data demonstrate that universal vaccination in infancy provides long-term protection against HBV infection. This strategy transformed a hyperendemic area into one of low prevalence. Elimination of this infection in the next several decades is therefore feasible. Mother-to-infant transmission remains the key route of vaccine failure that needs to be overcome. To achieve complete control of HBV infection, an effective strategy for detecting all the vaccination failure cases is needed, including occult infection, which would allow implementation of an appropriate treatment as early as possible.

Financial support

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Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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識別番号・報告回数			報告日	第一報入手日 2012 年 7 月 17 日	新医薬品等の区分	厚生労働省処理欄
一般的名称	①②③④⑤ポリエチレングリコール処理人免疫グロブリン ⑥⑦人免疫グロブリン		研究報告の公表状況		公表国 中国	
販売名（企業名）	①献血ヴェノグロブリン IH5%静注 0.5g/10mL　(ベネシス) ②献血ヴェノグロブリン IH5%静注 1g/20mL　(ベネシス) ③献血ヴェノグロブリン IH5%静注 2.5g/50mL　(ベネシス) ④献血ヴェノグロブリン IH5%静注 5g/100mL　(ベネシス) ⑤献血ヴェノグロブリン-IH ヨシトミ　(ベネシス) ⑥グロブリン筋注 450mg/3mL「ベネシス」　(ベネシス) ⑦グロブリン筋注 1500mg/10mL「ベネシス」　(ベネシス)			*Virology journal 2012; 9(2)*		

研究報告の概要

背　景

B型肝炎ウイルス(HBV)は最も重要なヒト病原体の一つで、食用動物でのその存在は、公衆衛生に重大な脅威である。疫学的研究は、ガボンとコンゴのヒト集団に相当する大型類人猿での HBV 感染の高い有病率を示した。更にまた、我々のチームはブタでの HBV の存在を発見し、食用動物における HBV 感染の可能性を示した。

ヒトが食用動物関連の HBV 変異型に感染した証拠は今のところないが、食用動物における HBV の存在は研究者と一般市民にはより大きな注目に値する。鶏は世界中でヒトによって広く消費されているが、鶏が HBV 感染に罹っているかどうかは明らかでない。本研究の目的は、HBV が鶏の血清及び肝臓に存在するかどうかを判断することであった。

結　果

血清検体の高いパーセンテージは、HBsAg (28.68%、37/129)、抗 HBs (53.49%、69/129) 及び抗 HBc (17.05%、22/129) が陽性であることが分かった、ところが HBeAg と抗 HBe の検体はそれぞれ 4.65% (6/129) と 9.3% (12/129) だけ検出した。129 の血清検体の僅か 3 つは、*HBsAg* と *HBeAg* が陽性だった。*TEM* によるこれらの血清検体の更なる分析は、HBV の完全で空のウイルス粒子と非常に類似したサイズと形態が 2 種類の粒子を含むことが分かった。HBV の Dane 粒子であるように見えた 40nm の直径のものは、そして他は 20nm の直径で、ヒト血清中の HBV の小さな球状粒子と類似していた。

免疫組織化学染色は、鶏からの肝臓組織が HBsAg と HBcAg に陽性であることを示した。顕微鏡下で、HBsAg は肝細胞の細胞質で発見された、一方 HBcAg は主に肝細胞の核の中に配布していた。その上、幾つかのリンパ球は HBV が鶏に病原性であることを示した門脈部と肝細胞の間で見つかった、そして HBV の複製はセクションで観察される肝炎障害に対して責任があるかもしれない。

193 の肝臓検体の内、僅か二つが同じ配列の HBV DNA を含むことが分かった。この DNA は、それぞれ既知の HBV 株 EF157291 (B 型肝炎ウイルス分離株 B3586-YKH94、完全なゲノム、日本) のヌクレオチド配列の 92.2%を、そして HBV 株 ABD14397 (B 型肝炎ウイルス分離株 38Y20HCC、日本) のヌクレオチド配列の 97.9%を共有していた。

議　論

（ 略 ）

使用上の注意記載状況・その他参考事項等

代表として献血ヴェノグロブリン IH5%静注 0.5g/10mL 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 抗体及び抗 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、ウイルス除去膜によるろ過処理及び pH3.9～4.4 の条件下での液状インキュベーション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。

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結　　論 結論として、HBV 抗原と抗体の高い有病率は鶏の血清と肝臓検体で見つかり、鶏での HBV 感染症を示した。 鶏で見つかった HBV がヒト HBV と同じであることが確認されることができるならば、鶏の HBV 感染は鶏、或いは鶏製品で働く人々に大変重要な危険性を意味するだろう。	
報告企業の意見	今後の対応
HBV は直径 42nm の球形をした DNA ウイルスで、ウイルス粒子は二重構造をしており、ウイルス DNA をヌクレオカプシドが包む直径約 27nm のコア粒子と、これを被うエンベロープから成り立っている。万一、原料血漿に HBV が混入したとしても、BVD 及び BHV をモデルウイルスとしたウイルスクリアランス試験成績から、本剤の製造工程において不活化・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。

グロブリン

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Detection of Hepatitis B virus in serum and liver of chickens

Jijing Tian[1], Kangkang Xia[1,2], Ruiping She[1*], Wenqui Li[1,3], Ye Ding[1], Jiande Wang[2], Mingyang Chen[1*] and Jun Yin[1]

Abstract

Hepatitis B virus (HBV) is one of the most important human pathogens. Its existence in food animals could present a significant threat to public health. The objective of this study was to determine if HBV is present in serum and liver of chickens. A total of 129 serum samples from broiler chickens were collected for the detection of HBV antigens and antibodies, and 193 liver samples were tested for HBV DNA sequence by PCR and for the existence of HBV antigens by immunohistochemistry. The overall prevalence of HBsAg, anti-HBs, anti-HBc was 28.68%, 53.49%, 17.05%, respectively, whereas HBeAg, anti-HBe were barely detectable. Three serum samples were found to be positive for both HBsAg and HBeAg. Further analysis of these samples with transmission electron microscopy (TEM) revealed two morphologic particles with 20 nm and 40 nm in diameter, which were similar to small spherical and Danes particles of HBV. The viral DNA sequence identified in two of the chicken livers shared 92.2% of one known HBV strain and 97.9% nucleotide sequence of another HBV strain. Our results showed the existence of HBV in chickens. This would present a significant risk to people who work with live chickens or chicken products if HBV found in chicken could be confirmed to be the same as human HBV.

Background

Hepatitis B virus (HBV) is one of the most important human pathogens. More than 350 million people worldwide are persistently infected with HBV and are at risk of developing chronic liver disease, cirrhosis and hepatocellular carcinoma [1]. While vertical transmission of HBV from mother to neonate always results in chronic hepatitis, infection during adulthood results in lifelong protective immunity [2]. Although measures such as vaccination have been taken for years, the prevalence of HBV has not been controlled effectively, and it is still a major threat to human health.

The HBV genome is a relaxed circular DNA of ~ 3 200 nucleotides and consists of full length of the negative strand and a shorter positive strand. Serologic markers of hepatitis B virus infection include both viral antigens (surface antigen, HBsAg, and e antigen, HBeAg) and antibodies (anti-HBs, anti-HBc, anti-HBe). HBsAg is the most frequently used to screen for the presence of HBV infection. The presence of HBeAg in a host's serum is associated with much higher rates of viral replication and enhanced infectivity [3]. Detection of all the serologic markers is meaningful for clinical diagnosis of HBV in human.

Infection of HBV has already been documented in non-human primates (NHPs)[4] such as chimpanzees [5,6] and gorillas [7,8] in sub-Saharan Africa; gibbons and orangutans in South-East Africa [7-9]. Epidemiological studies have shown a high prevalence of HBV infection in great apes, that is comparable to human population in Gabon and Congo [7]. Furthermore, our team has found the existence of HBV in swine [10], indicating the possibility of HBV infection in food animals. Although there is currently no evidence that human population have been or are infected with food animal-associated HBV variants, existence of HBV in food animals deserves greater attention from researchers and the general public. Chickens are widely consumed by people all over the world, but it is not clear whether chickens have HBV infection. The objective of this project was to determine if HBV is present in chickens.

Results

High percentages of the serum samples were found to be positive for HBsAg (28.68%, 37/129), anti-HBs

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Table 1 Detection of HBV Markers in Chicken Serum Samples

	Samples (n)	Positive Samples (n)	Positive Ratio (%)
HBsAg	129	37	28.68
anti-HBs	129	69	53.49
anti-HBc	129	22	17.05
HBeAg	129	6	4.65
anti-HBe	129	12	9.30
HBsAg + HBeAg	129	3	2.33

Table 2 Detection of HBsAg and HBcAg in Chicken Liver Samples By Immunohistochemical Staining (n = 193)

	HBsAg	HBcAg
Positive Samples (n)	106	86
Positive Ratio (%)	54.9	44.6

(53.49%, 69/129) and anti-HBc (17.05%, 22/129), whereas HBeAg and anti-HBe was detected only in 4.65% (6/129) and 9.3% (12/129) of the samples respectivel. Only three of the 129 serum samples were positive for both HBsAg and HBeAg (Table 1).

Further analysis of these serum samples with TEM found that they contained two types of particles, the size and morphology of which were very similar to complete and empty viral particles of HBV (Figure 1). The one with a diameter of 40 nm appeared to be HBV Dane particle; and the other, with a diameter of 20 nm, was similar to small spherical particles of HBV in human serum.

Immunohistochemical staining showed that liver tissues from chickens were positive for HBsAg and HBcAg (Table 2). Under the microscope, HBsAg was detected in cytoplasm of hepatocytes, while HBcAg was mainly distributed in the nucleus of hepatocytes. In addition, a number of lymphocytes were found in the portal area and among hepatocytes, indicating that HBV was pathogenic to chickens, and replication of HBV might be responsible for the hepatitis lesions observed in the sections (Figure 2).

Of the 193 liver samples, only two were found to contain HBV DNA (Figure 3) of the same sequence (Figure 4). This DNA shared 92.2% of the nucleotide sequence with the known HBV strain EF157291 (Hepatitis B virus isolate B3586-YKH94, complete genome, Japan)[11] and 97.9% of nucleotide sequence for the HBV strain AB014397 (Hepatitis B virus genomic DNA, complete sequence, isolate 38Y20HCC, Japan)[12], respectively (Table 3).

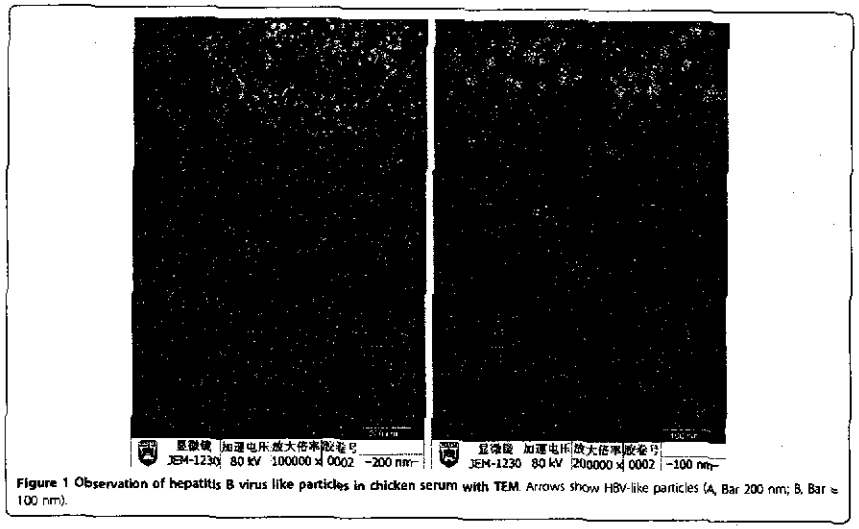

Figure 1 Observation of hepatitis B virus like particles in chicken serum with TEM. Arrows show HBV-like particles (A, Bar 200 nm; B, Bar = 100 nm).

Discussion

The present study is the first to report high prevalence of HBV infection in chickens, as indicated by the findings that 28. 68% and 53.49% of the chicken serum samples were positive for HBsAg and anti-HBs, and 54.9% and 44.6% of the liver samples were positive for HBsAg and HBcAg (Table 1, Table 2). Hepatitis B virus has been reported in other mammals (orthohepadnaviruses) and birds (avihepadnaviruses). Avihepadnaviruses have been reported in various duck species (WMHBV), grey herons (HHBV), geese (GHBV), Ross's goose (RGHBV), storks (STHBV), and cranes (CHBV) [13-15]. However, there were no reports of chicken HBV in the literature. How did the chickens get infected by HBV is unknown. Duck hepatitis B virus was the first of its kind to utilize an avian host where it was found in the bloodstream of an egg-laying duck. The virus was passed on from the infected duck to the egg resulting in congenital infection [16]. It is conceivable that the same could happen with HBV in chickens. The fact that different HB antigens and antibodies were detected in some of the chicken serum and liver samples but not in others might reflect different stages of HBV infections in the chickens. Interestingly, two types of viral particles, found in serum samples positive for both HBsAg and HBeAg, were similar to Dane particle and small spherical particle of human HBV in size and morphology, providing morphological evidence of HBV infection in chickens.

Immunohistochemistry staining is a common method for HBV detection [17]. Others reported that the marker and intensity of hepatocytes staining positive for HBsAg, as well as the cellular pattern of distribution, were related to HBV replication in patients with HBeAg-positive chronic hepatitis B (CHB). In general, HBsAg was located in the cytoplasm, whereas HBcAg was predominantly located in the nucleus in livers of HBV infection [18,19]. In this study, positive signals of HBsAg and HBcAg were both observed in liver specimens from chickens. Distribution pattern of the two antigens was

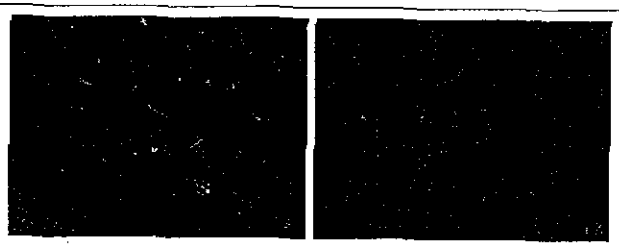

Figure 2 Immunohistochemical analysis of HBsAg and HBcAg in liver tissues of chickens. HBsAg was distributed mostly in cytoplasm of hepatocytes (A, 400x), and HBcAg was distributed mostly in the nucleus of hepatocytes (B, 400x).

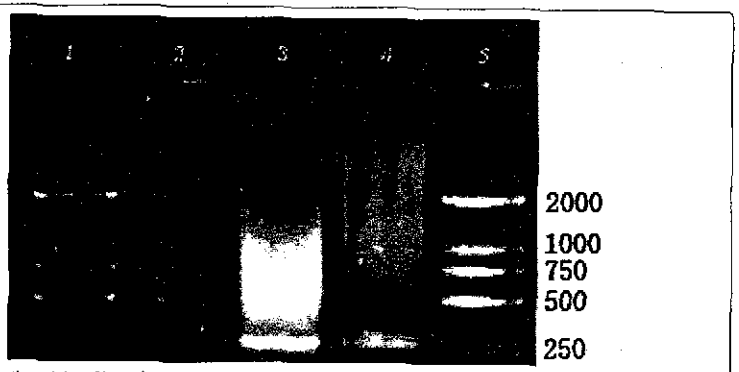

Figure 3 Identification of the PCR products ofHBV in livers of chickens by agarose gel electrophoresis. 1, 5: DL2000 DNA marker; 2: Negative control; 3, 4: Liver samples.

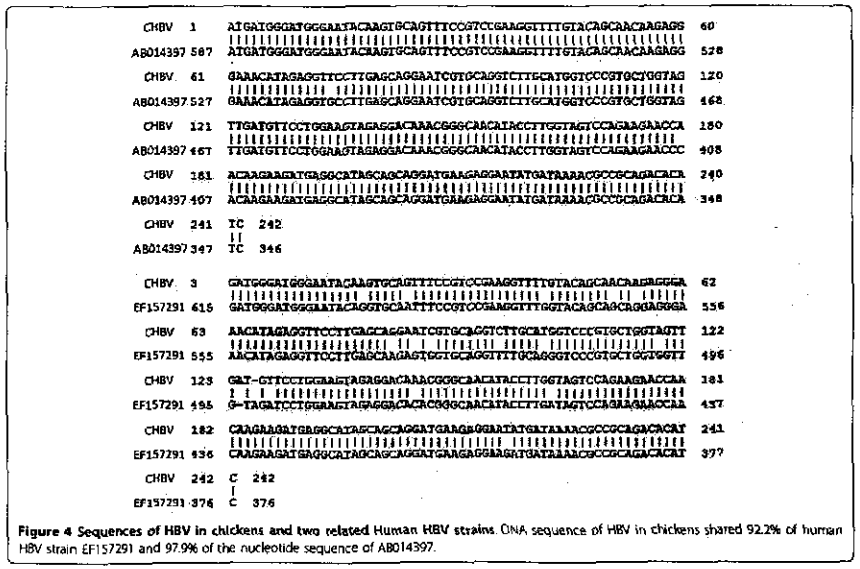

Figure 4 Sequences of HBV in chickens and two related Human HBV strains. DNA sequence of HBV in chickens shared 92.2% of human HBV strain EF157291 and 97.9% of the nucleotide sequence of AB014397.

the same as observed in HBV patients. Moreover, inflammatory signs observed in some of the liver samples such as accumulation of lymphocytes in the portal area and among hepatocytes suggest that HBV infection could lead to pathological changes in the liver of chickens. However, prevalence of HBsAg and HBcAg in liver was much higher than HBV DNA detected by PCR method. We found the same phenomenon in HEV (hepatitis E virus) infection in swine and chicken (data not show).

Although hepadnaviruses are usually host specific, HBV infections also occur frequently in chimpanzee, gorillas, gibbon and other ape populations in sub-Saharan Africa and South-east Asia where the HBV infection rate in apes was remarkably comparable to that of human population in these areas [5-9]. Scientists are concerned about the ability of HBV to cross species barriers. PCR detection in this study confirmed the existence of HBV in liver tissues of chickens. Although HBV DNA was detected in only two of the 193 liver samples, the DNA sequences from the two samples were identical, indicating the same HBV strain might be responsible for the HBV infection in chickens. It was not surprising that why HBV DNA was only detected in two liver samples that were positive for HBsAg and HBcAg by immunochemistry method, because HBV was only detectable during the incubation period and many of the chickens probably had passed that period.

Table 3 Comparison of Amplified Sequences from the HBV in Chickens with That from HBV EF157290 and AB014397*

Strain No.	ChickenHBV		EF157290	AB014397
ChickenHBV		100.0	92.2	97.9
	0.0		92.2	97.9
EF157290	8.3	8.3		91.5
AB014397	2.2	2.2	9.1	

*The identities (%) are in the right-up part of the table, and the divergences (%) are in the left-down part of the table.

Most HBV infection in human can be traced to neonatal transmission, drug-injection, sexual activity, or occupational exposure. Other, causes of infection, less frequent, include household contact, hemodialysis, transmission from a surgeon [20], and a receipt of organ or blood products. However, for more than 20%-30% of patients, no clear risk factors could be identified. The high homology of DNA sequence of HBV from chicken with the known human HBV strains is of concern from a public health point of view, because it raises the possibility that the HBV found in chicken could be the same or a variant of HBV that is responsible for hepatitis B in human. Our team has reported the presence of HBV in another food animal, the swine [10]. It is very common that people come into contact with food animals such as swine and chickens or food animal products. If chickens were infected by HBV, chicken meat could become a source of infection for people who work with it, especially when they have accidental cuts in their hands. However, it is premature to speculate that HBV infection in food animals such as chicken might contribute to the spread of HBV among human populations. Further research is needed to confirm how common HBV infection is in chickens and whether HBV can be passed from people to chickens and vice versa.

Conclusions

In conclusion, high prevalence of HBV antigens and antibodies was found in chicken serum and liver samples, indicating HBV infection in chickens. If the HBV found in chicken could be confirmed to be the same as human HBV, HBV infection in chicken would represent a very significant risk to people who work with chickens or chicken products.

Methods

Broiler chickens (42 days old) were processed in a slaughter house in Beijing, following the standard "Chicken Slaughtering Operation Procedures GB/T 19478-2004". Blood was drawn from the jugular vein immediately after the chickens were stunned by an electric shock. Serum was collected after blood samples were allowed to coagulate and centrifuged, and was kept frozen at -20°C until analysis. All serum samples were screened for hepatitis B serological markers (anti-HBc, HBsAg, anti-HBs, HBeAg, and anti-HBe) with respective enzyme-linked immunosorbent assay (ELISA) kits (SIIC Kinghaw Biotech Co. Ltd., Beijing, China) according to the manufacturer's recommendations. The absorbance was read at 450 nm (Multiscan Titertek MCC). Blank, negative and positive controls were included on each plate.

To obtain ultrastructural evidence for the presence of HBV-related viral particles in chickens, the three serum samples found to be positive for both HBsAg and HBeAg, were centrifuged at 4000 rpm for 10 min, then 0.01 M poly ethylene glycol 6000 (PEG6000) was added into the subsequent upper aqueous phase. After incubation overnight at 4°C, the serum was centrifuged at 20,000 rpm for 1 h, resuspended in PBS and stained for 1 min with 1% uranyl acetate. For the thin section study, the fixative used was 2.5% paraformaldehyde-glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). The sections were postfixed in 1% OsO4 for 1 h, and treated with 1% uranyl acetate, dehydrated in ethanol and embedded in Epon 812. All electron micrographs were obtained with JEV1230 transmission electron microscope (JEOL Ltd., Tokyo, Japan) at 80 kV.

Liver samples were collected when chickens were eviscerated. A portion of each liver sample was fixed in 2.5% (v/v) glutaraldehyde-polyoxymethylene solution immediately with the rest frozen at -80°C for the detection of HBV DNA sequence. The fixed liver tissue samples were dehydrated and embedded in paraffin wax. Serial paraffin sections (4 μm) were prepared and kept at 37°C for more than 12 h. The sections were immersed in three consecutive washings in xylene for 5 min to remove paraffin, and then hydrated with five consecutive washings with alcohol in descending order 100, 95, 80, 70, 50% and deionized water respectively. Sections were incubated for 15 min and blocked with 3% peroxide at room temperature for endogenous peroxidase ablation. The following steps were carried out in a moist chamber. Sections were incubated with blocking buffer (Zymed Laboratories Inc., San Diego, USA) containing 20% normal goat serum (Gibco) and 80% PBS (0.01 M, pH 7.4) at room temperature for 30 min. After discarding the goat serum, sections were incubated in primary monoclonal antibodies against HBsAg and HBcAg (Zhongshan Golden Bridge Biotech Co. Ltd., Beijing, China) diluted in PBS, for 2 h at 37°C. After rinsing for 3 times in PBS-T, sections were incubated with the goat anti-mouse IgG conjugated with HRP (Sigma) at 37°C for 40 min and rinsed 3 times in PBS-T. The specimens were incubated with 3,3-diaminobenzidin (DAB; Zymed Laboratories Inc) at room temperature for 10 min in the dark. Finally, sections were stained with hematoxylin for 5 min after rinsing for 3 times in PBS-T, dehydrated, and mounted with neutral gums. Sections for the negative control group were prepared by the same steps as described above but with the HBsAg and HBcAg antibodies replaced by PBS.

Liver tissues (50 mg) were homogenized in 450 μl of Tris/NaCl/EDTA. After addition of $NaDodSO_4$ (Sodium dodecyl sulfate) and proteinase K to a final concentration of 1% (wt/vol) and 1 mg/ml, respectively, the homogenates were incubated for 24 h at 42 – 48°C before they were extracted with a phenol- chloroform-isoamylol, 25:24:1 (vol/vol) mixture. The DNA was precipitated by adding 1/10 vol of 3 M NaOAc and 2 vol of 100% EtOH. After being centrifuged and washed with 70% ice-cold ethanol, the DNA was dried under vacuum at room temperature. It was redissolved in TE buffer and store at -20°C.

Two primers [21] were used to detect genetically divergent strains of HBV (Table 4). Amplification conditions for PCR was: 30 cycles of 94°C for 4 min; 94°C for 30 s, 58°C for 30 s, 72°C for 40 s; 72°C for 5 min. Negative (water) control was included in each detection to

Table 4 Primers Used in Detection of Hepatitis B Virus in Chicken Livers

	Sequence	Location	Product Size
HBVs-F1	5'-GAT GTG TCT GCG GCG TTT TA-3'	S gene	281 bp
HBVs-R1	5'- TTTTTCACCTCTGCCTAATCA-3'		

exclude the possibility of contamination and failure of amplification. PCR products were sequenced by BGI (Beijing Genomics Institute, China). The sequences were compared to two known Japan HBV strains [11,12] in the GenBank database over the Internet by using the NCBI BLAST server [22].

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Authors' contributions
JJT carried out the immunohistochemical staining and drafted the manuscript. KXX carried out the serological analysis of hepatitis B virus markers and PCR detection. WGL carried out the homology analysis. YD and MYC completed the transmission electron microscope investigations. JDW and JY did the pretreatment of animal samples. RPS carried out the design of the study and revision of the manuscript. JJT and KXX are joint first authors of this paper. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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医薬品　研究報告　調査報告書

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				2012. 8. 17	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	Komatsu H, Inui A, Sogo T, Tateno A, Shimokawa R, Fujisawa T. *J Infect Dis.* 2012 Aug;206(4):478-85. Epub 2012 Apr 16.	公表国 日本	
販売名（企業名）	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)					使用上の注意記載状況・その他参考事項等 新鮮凍結血漿-LR「日赤」 新鮮凍結血漿-LR「日赤」成分採血 新鮮凍結血漿-LR「日赤」120 新鮮凍結血漿-LR「日赤」240 新鮮凍結血漿-LR「日赤」480

研究報告の概要

○慢性B型肝炎ウイルス(HBV)感染症の子どもの涙はHBVを媒介する：ヒト型肝臓キメラマウスを用いた涙液による実験的HBV感染

背景：HBV保有者の唾液、尿、汗及び涙などの体液は、HBV感染源となりうる。

方法：慢性HBV感染症の子ども39人及び成人8人が研究に登録された。HBV DNAの定量にはリアルタイムPCRが用いられた。

結果：尿サンプルの73.7%(14/19)、唾液サンプルの86.8%(33/38)、涙液サンプルの100%(11/11)、汗サンプルの100%(9/9)にHBV DNAが検出された。平均HBV DNA量(±SD)は、尿4.3±1.1log copies/mL、唾液5.9±1.2log copies/mL、涙液6.2±0.7log copies/mL、汗5.2±0.6log copies/mLであった。血清検体と、唾液及び涙液のHBV DNAレベル間には有意な相関が見られた(r=0.88; P<0.001)。ある子どもの涙液検体をヒト肝細胞移植キメラマウスの血清はいずれもHBV DNA陽性であった。

結論：幼児の涙液検体におけるHBV DNAレベルは高かった。キメラマウスを用いて、涙液の感染性が確認された。高レベルのウイルス血症を有するHBV保有者の体液に直接接触することを防ぐ徹底的な対策が必要である。

報告企業の意見	今後の対応
慢性HBV感染患児の涙液のHBV DNAレベルは高いことが分かり、ヒト型肝細胞移植キメラマウスを用いた実験により涙液の感染性が示されたとの報告である。	日本赤十字社では、化学発光酵素免疫測定法(CLEIA)によりHBs抗原、HBc抗体検査を実施することに加えて、精度を向上させたNATシステムを導入し、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。なお、2012年8月より抗体検査の判定基準を強化し、HBc抗体(C.O.I.)1.0以上かつHBs抗体価200mIU/mL未満の感染既往を示す者の献血を不適としている。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。

MAJOR ARTICLE

Tears From Children With Chronic Hepatitis B Virus (HBV) Infection Are Infectious Vehicles of HBV Transmission: Experimental Transmission of HBV by Tears, Using Mice With Chimeric Human Livers

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(See the editorial commentary by Heiberg and Hogh, on pages 464–5.)

Background. Body fluids such as saliva, urine, sweat, and tears from hepatitis B virus (HBV) carriers are potential sources of HBV transmission.

Methods. Thirty-nine children and 8 adults who were chronically infected with HBV were enrolled. Real-time polymerase chain reaction was used for the quantification of HBV DNA.

Results. HBV DNA was detected in 73.7% of urine samples (14 of 19), 86.8% of saliva samples (33 of 38), 100% of tear samples (11 of 11), and 100% of sweat samples (9 of 9). Mean HBV DNA levels (±SD) in urine, saliva, tears, and sweat were 4.3 ± 1.1 log copies/mL, 5.9 ± 1.2 log copies/mL, 6.2 ± 0.7 log copies/mL, and 5.2 ± 0.6 log copies/mL, respectively. A statistically significant correlation was observed between the HBV DNA level in serum specimens and HBV DNA levels in saliva and tear specimens ($r = 0.88$; $P < .001$). Tear specimens from a child were injected intravenously into 2 human hepatocyte-transplanted chimeric mice. One week after inoculation, both chimeric mice had serum positive for HBV DNA.

Conclusions. The levels of HBV DNA in tear specimens from young children were high. Tears were confirmed to be infectious, using chimeric mice. Strict precautions should be taken against direct contact with body fluids from HBV carriers with high-level viremia.

Hepatitis B virus (HBV) infection causes acute and chronic liver diseases. Fortunately, HBV infection is a vaccine-preventable disease, and as of 2008, 177 countries (92%) have integrated HBV vaccine into routine infant immunization programs. However, Japan and northern European countries, where the endemicity of HBV is low, continue to implement an HBV immunization strategy that targets high-risk groups, rather than a universal vaccination program [1]. Nonetheless, HBV infection by sexual contact and household contact does occur in Japan [2–5]. Children with chronic HBV infection are usually asymptomatic and have high-level viremia. Therefore, it is believed that children with chronic HBV infection may be a major reservoir for spreading HBV to other close susceptible individuals [6–8]. This scenario would especially threaten the countries that adopt an "at-risk" immunization strategy [6, 9–13].

Body fluids such as saliva, semen, urine, sweat, and tears are also potential sources of HBV transmission. Several studies have reported that HBV DNA in these body fluids can be detected by polymerase chain reaction (PCR) [9–18]. Of these body fluids, however, only serum, saliva, and semen have been demonstrated to be infectious in humans or experimental animal models [19–21].

In this study, HBV DNA levels in urine, saliva, tears, and sweat were quantified by real-time PCR. Body fluid samples were collected from HBV-carrier children and HBV-carrier mothers. After quantification of HBV DNA levels for each specimen type, we evaluated the infectivity of tears from HBV carriers. Mice with severe combined immunodeficiency, carrying a urokinase-type plasminogen activator transgene controlled by an albumin promoter (uPA/SCID), and with transplanted human hepatocytes have recently been used as an appropriate animal model for studying viral hepatitis due to HBV and hepatitis C virus [22–24]. Using these mice, we evaluated whether tears from HBV-carrier children were infectious.

MATERIALS AND METHODS

Patients and Materials

Eligible patients were chronic HBV carriers who attended our outpatient clinic. Their chronic HBV infection status was routinely evaluated by blood examination. All of the patients were asymptomatic. Serum, urine, saliva, tears, and sweat samples were collected when possible from each patient.

Serum samples were collected in preparation tubes. Each urine sample was collected in a sterile plastic tube. Saliva, tear, and sweat samples were collected using an indicating FTA Micro Card (Whatman, GE Healthcare, Tokyo, Japan) and sterile foam-tipped applicators (Whatman). When children shed tears spontaneously, we collected tear samples using the FTA cards. Serum, urine, saliva, tear, and sweat specimens were collected on the same day. Informed consent was obtained from all patients or all patients' parents. This study was approved by the Research Ethics Committee of Eastern Yokohama Hospital.

HBV DNA Extraction and Real-Time PCR

HBV DNA in serum was measured by COBAS TaqMan HBV DNA test, version 2.0 (Roche Diagnostics, Tokyo, Japan). HBV DNA was extracted from 200 μL of urine, using the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany). HBV DNA was extracted from saliva, tear, and sweat specimens that were spotted on FTA cards, using QIAamp DNA Mini kit (QIAGEN). Three circles were punched from the FTA card by use of a single-hole paper puncher (Harris Micro Punch 3.00 mm, GE Healthcare) and were used for HBV DNA extraction. The extracted DNA was dissolved in 100 μL of elution buffer.

Quantification of HBV DNA in urine, saliva, tear, and sweat samples was performed using an in-house TaqMan real-time assay. The real-time PCR was performed using a genotype-independent method described previously [25]. PCR was performed in an MX3000P (Stratagene), and the results were analyzed with MxPro software (version 3.0). The lower limit of detection was >100 copies/mL. All assays were performed in duplicate with negative control samples. This assay was standardized using HBV DNA samples of known concentrations measured by the COBAS TaqMan HBV DNA test and recombinant plasmid controls. In this study, the standard of qualification is based on the result of COBAS TaqMan HBV DNA test. Therefore, the conversion factor between HBV copies/mL and HBV IU/mL is considered to be 5.82 copies/IU. Genotyping of HBV was determined by the PCR-Invader assay [26].

Tear Specimen for Experimental Transmission

For experimental transmission, a tear specimen was collected from a 10-month-old girl with chronic HBV infection. The source of her HBV infection was mother-to-child transmission due to the failure of prophylactic treatment. A total of 200 μL of tears were gently collected from her face when she cried, using a 1.0-mL syringe. The 200-μL tear specimen was diluted with 1300 μL of sterile saline, yielding a total volume of 1500 μL. The specimen underwent filter sterilization with a 0.2-μm filter.

Inoculation of Chimeric Mice With Livers Repopulated by Human Hepatocytes

Three male chimeric mice were purchased from PhoenixBio (Hiroshima, Japan). Human hepatocytes were imported from BD Bioscience (Woburn, MA). Of the 3 mice, 2 (mouse 101 and mouse 102) were inoculated once intravenously with 100 μL of the sterilized tear sample. The remaining mouse (mouse 103) was orally inoculated with 100 μL of the sterilized tear sample every 4 weeks. After inoculation, blood samples for real-time PCR assay were collected from the chimeric mouse every week.

HBV DNA Extraction From Mice Samples and Real-Time PCR

A total of 50 μL of whole blood samples were collected from the mice every week after inoculation, and serum was separated. Saliva and tear specimens were collected from chimeric mice, using FTA cards. HBV DNA was extracted from 20 μL of mouse serum, using SMI-TEST EX-R&D (Medical Biological Laboratories, Aichi, Japan). The extracted DNA was dissolved in 20 μL of nuclease-free water. HBV DNA was quantitatively measured using real-time PCR with the TaqMan PCR Core Reagent kit (Applied Biosystems, Foster City, CA). Real-time PCR was performed in a 25-μL reaction mixture containing 0.125 μL Ampli Taq Gold with 0.2 μM primers (forward primer: 5′-CACATCAGGATTCCTAGGAC C-3′ [nucleotides 166-186]; reverse primer: 5′-AGGTTGGTG AGTGATTGGAG-3′ [nucleotides 325-344]), 0.3 μM probe (5′-FAM-CAGAGTCTAGACTCGTGGTGGACTTC-TAMRA-3′ [nucleotides 242-267]), and 5 μL extracted DNA. The nucleotide position was based on GenBank accession number AB300361 (genotype C). After incubation for 2 min at 50°C and for 10 min at 95°C, the PCR cycling program underwent

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53 2-step cycles, one at 95°C for 20 seconds and the other at 60°C for 1 minute. TaqMan PCR was performed with an ABI Prism 7500 (Applied Biosystems). In this study, the volume of serum collected from each mouse was 20 μL, which is a very small amount compared with that used in human studies. Therefore, we considered the upper limit of detection of real-time PCR for a small-volume sample to be >10 000 copies/mL, which provided us with more reliable results. This assay was standardized using mouse HBV DNA samples of known concentrations and the recombinant plasmid controls, as previously described [27].

Immunostaining for HBV Surface Antigen (HBsAg) and HBV Core Antigen (HBcAg)

Immunostaining for HBsAg and HBcAg was performed on frozen sections, using the Ventana i VIEW DAB detection kit (Ventana Medical Systems, Tucson, AZ) and the Dako Envision kit (Dako, Tokyo, Japan), respectively. Primary monoclonal antibodies to HBsAg (Santa Cruz Biotechnology, CA), at a 1:100 dilution, and polyclonal antibodies to HBcAg (Dako), at a 1:500 dilution, were used. Liver tissue was taken from mice after they were euthanized, and the tissue was stored at −80°C.

Statistical Analysis

Categorical variables were compared between groups, using the *Yates corrected* χ^2 *test or the Fisher exact test. Noncategorical* variables were compared between groups by the Mann-Whitney *U* test. For analysis of the correlation between log HBV DNA level in serum and in saliva and tears, we used the Pearson correlation coefficient. All tests were 2-sided, and a *P* value of ≤.05 was considered to indicate statistical significance. All statistical analyses were performed with StatMate IV for Windows (Advanced Technology for Medicine & Science, Tokyo, Japan) and Microsoft Office Excel 2007.

RESULTS

Patients and Materials

Between August 2009 and September 2010, 39 children and 8 adults who were chronically infected with HBV were randomly enrolled in this study. Twenty-six subjects were male, and 21 were female; the mean age (±SD) was 12.4 ± 12.0 years, and the median age was 9 years (range, 0–47 years. The 47 HBV carriers fell into the following age groups: 0–5 years, n = 18 (16 were HBV e antigen [HBVeAg] positive); 6–10 years, n = 11 (9 were HBeAg positive); 11–19 years, n = 9 (7 were HBeAg positive); and 20–27 years: n = 9 (7 were HBeAg positive). Of the 47 patients with chronic HBV infection, 39 were positive for HBeAg. In addition, 39 patients had serum HBV DNA levels of ≥6 log copies/mL. One, 6, and 40 patients were infected with genotype A, genotype B, and genotype C, respectively. Serum samples were collected from all patients. From the 47 patients, we collected 19 urine samples, 38 saliva samples, 11 tear samples, and 9 sweat samples. One subject provided urine, saliva, and tears only; 3 provided urine, saliva, and sweat only; 10 provided urine and saliva only; 10 provided saliva and tears only; 1 provided urine and sweat only; 1 provided saliva and sweat only; 4 provided urine only; 13 provided saliva only; and 4 provided sweat only. Samples were collected individually at the same time. The characteristics of body fluid samples are shown in Table 1. There were no significant differences in sex, the number of patients with a serum HBV DNA level of >6 log copies/mL, and the prevalence of genotype C among patients supplying different types of samples. However, there was a significant difference in the age of patients supplying the different kinds of samples.

HBV DNA Detection in Body Fluids

All patients were positive for HBV DNA in serum by the COBAS TaqMan HBV DNA test. The levels of serum HBV DNA ranged from 2.1 log copies/mL to >9 log copies/mL. The median *HBV DNA level in serum was >9* log copies/mL. HBV DNA was detected in 73.7% of urine specimens (14 of 19), 86.8% of saliva specimens (33 of 38), 100% of tear specimens (11 of 11), and 100% of sweat specimens (9 of 9) (*P* = .07). In patients with a high viral load (ie, >6 log copies/mL), HBV DNA was detected in 85.7% of urine samples (12 of 14), 100% of saliva samples (32 of 32), 100% of tear samples (11 of 11), and 100% of sweat samples (9 of 9) (*P* = .24). Although the frequency of HBV DNA detection in urine was slightly lower than that in other body fluids, there were no significant differences in the frequency of HBV DNA detection among body fluids.

Quantification of HBV DNA From Body Fluids

Figure 1 shows the levels of HBV DNA in body fluids. Mean levels (±SD) of HBV DNA in urine, saliva, tears, and sweat specimens were 4.3 ± 1.1 log copies/mL, 5.9 ± 1.2 log copies/mL, 6.2 ± 0.7 log copies/mL, and 5.2 ± 0.6 log copies/mL, respectively. Levels of HBV DNA in urine were significantly lower than those in other body fluids. Levels of HBV DNA in body fluids from patients who had a high viral load (ie, >9 log copies/mL) in serum are shown in Figure 2. Mean levels (±SD) of HBV DNA in urine (n = 10 specimens), saliva (n = 23), tears (n = 8), and sweat (n = 8) were 4.4 ± 0.9 log copies/mL, 6.4 ± 0.9 log copies/mL, 6.4 ± 0.9 log copies/mL, and 5.3 ± 0.6 log copies/mL, respectively. Even after the HBV load in serum was well matched, the HBV DNA levels in urine specimens were significantly lower than those in saliva and tear specimens.

Although there was no significant difference in HBV DNA levels between saliva, tears, and sweat specimens from patients with high viral load in serum, the quantification of HBV DNA in saliva and tear specimens showed almost the same levels (Figure 2). Levels of HBV DNA in the 11 pairs of saliva and tear specimens are shown in Figure 3. Mean HBV DNA levels (±SD) in saliva and tear specimens were 6.1 ± 1.0 log copies/mL and 6.2 ± 0.8 log copies/mL, respectively. The levels of HBV DNA in tear specimens were as high as those in saliva specimens.

The association between the levels of HBV DNA in serum specimens and in saliva and tear specimens was evaluated. Because the upper detection limit of the COBAS TaqMan HBV DNA test was >9 log copies/mL, we used data from *patients in whom the levels of HBV DNA in serum ranged* from 2.9 to 8.8 log copies/mL. Data from 15 patients (15 serum samples, 15 saliva samples, and 3 tears samples) were available for the correlation analysis. A significant correlation was observed in the levels of HBV DNA between serum specimens and saliva and tear specimens ($r = 0.88$; $P < .001$) (Figure 4*A*). The relationship between HBV DNA in serum specimens and HBV DNA in saliva and tear specimens was described as follows: [log HBV DNA load in saliva and tear specimens] = −3.23 + 1.06 × [log HBV DNA load in serum specimens]. On the other hand, there was no significant

Table 1. Characteristics of Body Fluid Samples

Characteristic	Body Fluid				
	Urine (n = 19)	Saliva (n = 38)	Tears (n = 11)	Sweat (n = 9)	*P*
Male sex, no. (%)	10 (52.6)	23 (60.5)	6 (54.5)	7 (77.8)	.73
Age, years, median (range)	11 (1–40)	7 (1–33)	1 (0–3)	16 (8–40)	<.05[a]
HBV DNA in serum, no. (%)					
>6 log copies/mL	14 (73.7)	32 (84.2)	11 (100)	9 (100)	.13
Genotype C	14 (73.7)	33 (86.8)	9 (81.8)	9 (100)	.31

[a] Significant difference between urine and saliva, between urine and tears, between saliva and sweat, and between tears and sweat.

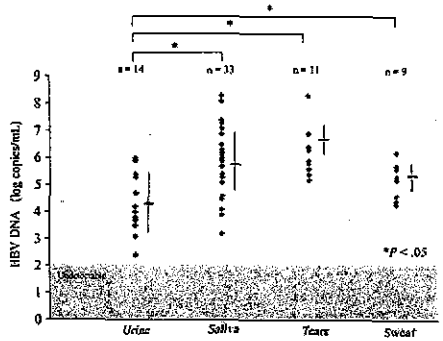

Figure 1. Hepatitis B virus (HBV) DNA levels in urine, saliva, tear, and sweat specimens from 47 patients. The levels of HBV DNA in urine samples were significantly lower than those in saliva, tear, and sweat samples (*P* < .05). The bar indicates the mean of the levels of HBV DNA. SDs are indicated by vertical bars.

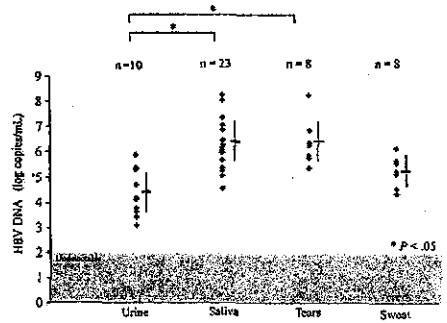

Figure 2. To adjust serum hepatitis B virus (HBV) DNA levels among groups, we show the HBV DNA levels in urine, saliva, tear, and sweat samples from patients whose levels of HBV DNA in serum were ≥9 log *copies/mL. Although a significant difference in HBV DNA levels between* urine and sweat specimens was not present, HBV DNA levels in urine specimens were significantly lower than those in saliva and tear specimens (*P* < .05). The bar indicates the mean of the levels of HBV DNA. SDs are indicated by vertical bars.

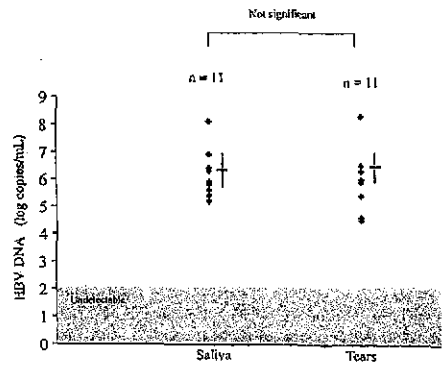

Figure 3. Hepatitis B virus (HBV) DNA levels in saliva and tear samples that were paired. Both groups showed the same HBV DNA levels. The bar indicates the mean of the levels of HBV DNA. SDs are indicated by vertical bars.

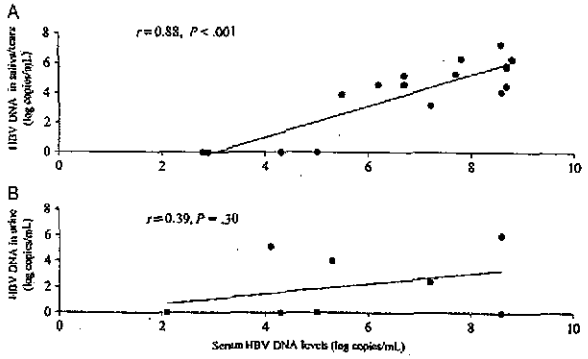

Figure 4. *A*, The association between hepatitis B virus (HBV) DNA levels in serum samples and saliva and tear samples ($n = 18$). Data from patients whose levels of HBV DNA in serum ranged from 2.9 to 8.8 log copies/mL were used for analysis. There was a significant correlation between HBV DNA levels in serum specimens and saliva and tear specimens ($r = 0.88$; $P < .001$). *B*, The association between HBV DNA levels in serum and urine samples ($n = 9$). Data from patients whose serum HBV DNA levels ranged from 2.1 to 9.0 log copies/mL were used for analysis. There was no significant correlation between HBV DNA levels in serum and urine specimens ($r = 0.41$; $P = .10$).

association between HBV DNA loads in serum and urine specimens (HBV DNA levels in 9 serum specimens ranged from 2.1 to 8.6 log copies/mL; $r = 0.39$; $P = .30$) (Figure 4*B*).

Transmission of HBV by Tears

The level of HBV DNA in tear specimens collected from a 10-month-old girl (genotype C; serum HBV DNA load, >9.0 log copies/mL) were 7.1 log copies/mL. The final concentration of HBV DNA in filter-sterilized tear specimens was 6.1 copies/mL.

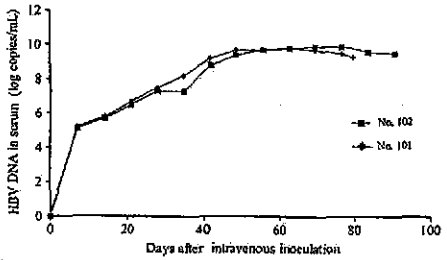

Figure 5. Hepatitis B virus (HBV) DNA levels in serum from chimeric mice after intravenous inoculation with tear specimens. The level of HBV DNA in a tear specimen collected from a girl with failure of immunoprophylaxis (HBV DNA load in serum, >9.0 log copies/mL) was 7.1 log copies/mL. After sterilization, the final concentration of HBV DNA in the tear sample was 6.1 copies/mL. One hundred microliters of the tear specimen was injected intravenously into chimeric mice.

A total of 100 μL of the filter-sterilized tear specimen was injected intravenously into 2 chimeric mice. One week after inoculation, both chimeric mice became positive for HBV DNA in serum (no. 101 had an HBV DNA level of 5.2 log copies/mL, and no. 102 had an HBV DNA level of 5.1 log copies/mL). The levels of HBV DNA in serum from the chimeric mice gradually increased with time. Seven weeks after inoculation, the levels of HBV DNA in serum from the chimeric mice increased to 9 log copies/mL and remained at this level thereafter (Figure 5). Saliva and lacrimal fluids were collected using FTA cards at day 80 (for mouse 101) and day 91 (for mouse 102). Although HBV DNA was extracted from a very small spot (1 pinched-out circle from the FTA card), the levels of HBV DNA were 4.4 log copies/mL (in saliva) and 4.5 copies/mL (in lacrimal fluids) in mouse 101 and 4.0 log copies/mL (in saliva) and 4.3 log copies/mL (in lacrimal fluids) in mouse 102. The remaining chimeric mouse (mouse 103) was orally inoculated with 100 μL of the filter-sterilized tear specimen. Unfortunately, we had to discontinue oral administration because of the deterioration of the mouse's health 35 days after inoculation. The chimeric mouse (mouse 103) had been inoculated orally twice (on days 0 and 28) before discontinuation. Real-time PCR performed 6 times (on days 0, 7, 14, 21, 28, and 35) detected no HBV DNA in serum.

Immunohistological Analysis of Liver Tissue for HBV Antigens

Immunohistochemical staining was performed on a liver specimen from the mouse with HBV viremia (no. 101). The hepatocytes were positive for HBsAg and HBcAg (Figure 6).

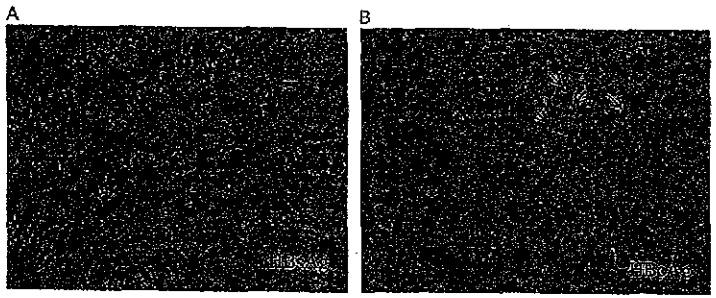

Figure 6. Immunohistological staining for liver tissue with antibodies to hepatitis B virus (HBV) surface antigen (HBsAg) and HBV core antigen (HBcAg). *A*, HBsAg was expressed on cytoplasmic membrane (original magnification ×400). *B*, HBcAg were expressed in nuclei of hepatocytes (original magnification ×400). Arrows indicate the nuclei of HBcAg-positive staining.

These findings indicated that HBV transmission from tears could be replicated in a human liver chimeric mouse model.

DISCUSSION

Although it has been reported that HBV DNA was detectable by PCR in tears from chronic HBV carriers [11, 17], tears have been considered to be low risk for HBV transmission. However, this study demonstrated that tears from children chronically infected with HBV were highly infectious. HBV DNA from serum could be detected in both chimeric mice 1 week after inoculation. Moreover, the levels of HBV DNA in serum continuously increased and reached the upper limit of the PCR assay 7 weeks after inoculation. A previous study showed that chimeric mice usually became positive for HBV DNA in serum 4 weeks after intravenous inoculation with serum from HBV carriers [28]. The levels of HBV DNA in tears used for this study were much higher than those in serum used in the previous study. Therefore, HBV DNA in serum from the chimeric mice became detectable quickly after inoculation.

Recent studies measuring HBV DNA in body fluids from HBV carriers have been conducted in the Netherlands, Sweden, and Denmark. Including the present study, all studies are from countries in which a selective HBV immunization program has been implemented [9–13]. Clearly, physicians from these countries are keen to know whether various body fluids might be sources of HBV transmission. Additionally, physicians are concerned that a vaccination strategy that focuses on at-risk groups is ineffective for prevention of HBV infection. Although recent studies have shown that HBV DNA in urine, saliva, tear, and sweat specimens from chronic HBV carriers was detectable by PCR, these studies did not show that body fluids from chronic HBV carriers were infectious in animal experiments. Approximately 30 years ago, the infectivity of semen and saliva from HBV carriers was proven by experimental transmission, using gibbons [20, 21]. Since then, no other body fluids have been evaluated for infectivity. This study is the first to confirm that tears are infectious sources of HBV.

Tears are presumed to originate from circulating blood. HBV DNA was first detected in tears in 1994 by PCR. In a previous study, tear specimens from 47.1% of HBV carriers (16 of 34) were positive for HBV DNA [17]. In 2006, a previous study measured HBV DNA in paired saliva and tear specimens. Of 7 patients with chronic HBV infection, 4 (57%) had tear specimens that were positive for HBV DNA. The levels of HBV DNA in tear specimens ranged from 0.2×10^3 to 1.4×10^4 copies/mL [11]. Compared with the previous study, the levels of HBV DNA in tears were relatively high in this study. There are 2 possible explanations for the difference in HBV DNA levels between these studies. First, the majority of the patients supplying tear samples in our study were very young children (median age, 1 year). Young children with chronic HBV infection are usually in the immunotolerant phase and have a high viral load. Second, the FTA card was effective at collecting body fluids and extracting DNA. Although the number of tear samples was small, this study demonstrates that tears, as well as saliva, contain a large amount of HBV DNA. Interestingly, HBV DNA in lacrimal fluid and saliva could also be detected in the chimeric mice. These findings suggest that tears, like saliva, have the potential to transmit HBV.

Among body fluids, the highest levels of HBV DNA are detected in blood. However, HBV DNA can also be detected in urine, saliva, tears, and sweat. In this study, HBV DNA was detected in a high proportion of body fluid samples. In addition, there was a statistically significant correlation in the

levels of HBV DNA between tear and saliva specimens and serum specimens, in which [log HBV DNA level in saliva and tear specimens] = −3.23 + 1.06 × [log HBV DNA level in serum specimens]. Similarly, previous studies reported that the levels of HBV DNA in saliva specimens were significantly related to the levels of HBV DNA in blood specimens. In this study, however, the levels of HBV DNA in urine specimens were not significantly associated with the levels of HBV DNA in serum specimens. The levels of HBV DNA in urine samples were significantly lower than those in saliva and tear samples. This finding is also consistent with that of a previous study [13]. We cannot provide any clear explanation why the levels of HBV DNA were lower than those in other body fluids. Further studies are required to study not only the infectivity of urine but also the mechanism of the reduction of the HBV DNA level in urine.

It has been known that the oral administration of serum from HBV carriers causes HBV infection [19]. After we confirmed the infectivity of tears through the intravenous route, tears were administered orally to a chimeric mouse. Although both transmission routes were investigated using the same sample, this study, like previous animal experiments [20, 21], failed to demonstrate that HBV infection occurred through an oral route; unfortunately, the period of observation was not sufficient to evaluate the infectivity of tears. We tried to detect HBV DNA in the liver of chimeric mouse 103 after discontinuation of oral administration of tear specimens, but HBV DNA was not detectable in the liver by real-time PCR (data not shown).

There are few studies that have measured the levels of HBV DNA in sweat specimens from chronically infected patients. A previous study quantified HBV DNA levels in Olympic wrestlers, who were negative for HBsAg but positive for HBV DNA in blood [14]. In the previous study, a statistically significant relation between the levels of HBV DNA in blood and sweat was observed. In the present study, all sweat samples were positive for HBV DNA. In addition, the levels of HBV DNA in sweat specimens were high (mean level [±SD], 5.2 ± 0.6 log copies/mL). Therefore, sweat from HBV carriers might also have the potential to cause horizontal HBV infection.

The US Centers for Disease Control and Prevention considers that the risk of transmission in child-care settings is very low [29–31]. However, Ireland, Norway, and Sweden have a policy that children should be immunized if another child in a day care center is positive for HBsAg. This study showed that various body fluids from young HBV carriers have a high concentration of HBV DNA. Previous studies have reported that 10% of HBV particles are infectious [32]. Therefore, all body fluids from HBV carriers should be considered to be infectious, and HBV vaccine should be recommended for day care staff.

In conclusion, HBV DNA was detected at high proportions in urine, saliva, tear, and sweat specimens from chronic HBV carriers. The levels of HBV DNA in saliva and tear specimens from young children were extremely high. In addition, tear samples from a child with chronic HBV infection were confirmed to be infectious, using chimeric mice. Although the HBV transmission risk between young children in nurseries or day care centers may be limited, strict precautions should be taken against contact with body fluids from HBV carriers with high-level viremia, especially in counties implementing an immunizing program focused on individuals at-risk for HBV infection.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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医薬品 研究報告 調査報告書

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研究報告の概要

○初期無症候性供血者集団におけるC型肝炎ウイルス感染の臨床的及び組織学的経過とその伝播様式に関する25年間の研究

背景: 合計738人のHCV抗体陽性の献血者が、15年間の研究中及び推定感染成立日から最高54年までリスク要因と経過に関して評価された。

方法: 真のHCV抗体陽性と偽陽性を区別するために第3世代リコンビナント免疫ブロット法(RIBA)が実施された。HCV PCRの結果により、被験者を慢性HCV感染者か治癒した者かに分類した。肝生検検体は、Ishak線維化スコアによりステージ化され、組織活動性指標によってグレード化された。

結果: 738人のHCV抗体陽性被験者のうち、469人(64%)がRIBA陽性、217人(29%)が陰性、52人(7%)が不確定であった。主な独立リスク因子は静注薬物使用(オッズ比[OR]、35.0;P<0.0001)、輸血(OR、9.9;P<0.0001)、及び静注薬物使用や輸血を否定した「麻薬吸入者」79人を含む経鼻コカイン使用(OR、8.5;P<0.0001)であった。分類・回帰木及びランダムフォレストによりこれらのリスク要因を確認した。合計384人(82%)のRIBA陽性供血者がHCV RNA陽性であった;そのうち185人(48%)からの肝生検検体において、33%に線維化は見られず、52%に軽度の線維化、12%に架橋線維化が見られた。感染後平均25年で2%に肝硬変が見られた。反復生検63件の解析結果は、8%が5年以上でIshakステージ2以上に進行したことを示した(平均進行、0.06 Ishakステージ/年)。

結論: 1990年以前の静注薬物使用と輸血は、HCV感染の有意なリスク因子である;経鼻コカイン使用は非経口伝播の潜在的な経路である可能性がある。HCV感染後平均25年の組織学的な経過は比較的軽度であった(85%は線維化が見られないか軽度であり、わずか2%のみが肝硬変になった。1/5近くが自然治癒した)。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血
新鮮凍結血漿-LR「日赤」120
新鮮凍結血漿-LR「日赤」240
新鮮凍結血漿-LR「日赤」480

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

5

報告企業の意見	今後の対応
無症候性供血者集団におけるHCV感染の臨床的及び組織学的経過と伝播様式に関する研究を行ったところ、1990年以前の主な感染リスク因子は静注薬物使用及び輸血であり、経鼻コカイン使用は潜在的な非経口感染経路である可能性が示唆された。また組織学的変化は比較的軽度であることが分かったとの報告である。	日本赤十字社では、HCV抗体検査を実施することに加えて、精度を向上させたNATシステムを導入し、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。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。

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MAJOR ARTICLE

A 25-Year Study of the Clinical and Histologic Outcomes of Hepatitis C Virus Infection and Its Modes of Transmission in a Cohort of Initially Asymptomatic Blood Donors

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Background. A total of 738 volunteer blood donors who were positive for anti-hepatitis C virus (HCV) were assessed for risk factors and outcomes for up to 15 years within the study and up to 54 years from the estimated onset of infection.

Methods. A third-generation recombinant immunoblot assay (RIBA) was performed to distinguish true from false anti-HCV reactivity. Findings of HCV polymerase chain reaction classified subjects as having chronic HCV infection or as having recovered. Liver biopsy specimens were staged by Ishak fibrosis score and graded by histologic activity index.

Results. Of 738 anti-HCV–positive subjects, 469 (64%) had positive RIBA results, 217 (29%) had negative results, and 52 (7%) had indeterminate results. Primary independent risk factors were injection drug use (odds ratio [OR], 35.0; $P < .0001$), blood transfusion (OR, 9.9; $P < .0001$), and intranasal cocaine use, including 79 "snorters" who repeatedly denied injection drug use or blood transfusion (OR, 8.5; $P < .0001$). Classification and regression tree and random forest analyses confirmed these risk factors. A total of 384 RIBA-positive donors (82%) were HCV RNA positive; of these, liver biopsy specimens from 185 (48%) showed no fibrosis in 33%, mild fibrosis in 52%, bridging fibrosis in 12%, and cirrhosis in 2% a mean duration of 25 years after infection. Analysis of 63 repeat biopsy specimens showed that 8% progressed ≥2 Ishak stages over 5 years (mean progression, 0.06 Ishak stages/year).

Conclusions. Injection drug use and blood transfusion before 1990 are dominant risk factors for HCV acquisition; intranasal cocaine use may be a surreptitious route of parenteral spread. After a mean of 25 years of HCV infection, histologic outcomes were relatively mild: 85% had no or mild fibrosis, and only 2% had cirrhosis. Nearly one-fifth spontaneously recovered.

While the frequency of new hepatitis C virus (HCV) infections in the United States has declined considerably in the past decade, the disease burden from cumulative infections over the past 50 years is substantial and is rising, as the long duration of individual infections allows evolution into cirrhosis and hepatocellular carcinoma (HCC) [1, 2]. The full spectrum of HCV-related outcomes is difficult to discern because milder disease often goes unrecognized, and slowly evolving chronic sequelae generally are not amenable to prospective ascertainment. The introduction of routine donor screening for antibody to HCV in 1990 afforded the opportunity to place otherwise unselected subjects into long-term follow-up to assess (1) the proportion of HCV-infected

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individuals who become chronic carriers, (2) the risk factors for HCV acquisition, (3) the long-term outcomes based on findings of liver histologic evaluations, and (4) the relationship of liver fibrosis to a variety of demographic, virologic, serologic, and biochemical indices. This cohort of anti-HCV–positive blood donors was previously analyzed in 1995 [3]. Reported herein is a further decade of data accrual, allowing for up to 15 years of follow-up within the study and a mean interval of 25 years from the time of a parenteral exposure that presumably initiated the infection to the time of liver biopsy.

METHODS

Screening and Confirmation of HCV Infection

At study initiation, a first-generation enzyme immunoassay (EIA) was used by the American Red Cross (ARC; EIA1.0; Ortho Diagnostics, Raritan, NJ) and the National Institutes of Health (NIH) (EIA1.0; Abbott Laboratories, Abbott Park, IL) to screen donated blood for HCV antibodies. A more sensitive second-generation EIA (EIA2.0; Ortho Diagnostics and Abbott Laboratories) was introduced in 1992. The specificity of anti-HCV-positive reactions was tested by second and/or third-generation recombinant immunoblot assays (RIBA HCV 2.0 SIA; Chiron, Emeryville, CA) [4]. Donors who were RIBA positive were considered to have confirmed antibody to HCV and, thus, to have been infected with HCV. Donors who were RIBA negative were considered to have false-positive antibody reactivity by EIA and, thus, not to have been infected with HCV. The HCV antibody status of donors with an indeterminate result of RIBA could not be determined, and donors with this result underwent testing for HCV RNA but were otherwise excluded from analysis.

At least 1 sample from each participant was tested in duplicate for HCV RNA, using reverse-transcription polymerase chain reaction (PCR; COBAS Amplicor HCV Test, version 2.0; Roche, Branchburg, NJ; limit of detection, 100 IU/mL) [3]. Quantitative serum HCV RNA levels were measured by the COBAS Amplicor HCV Monitor Test, version 2.0 (Roche), and genotyping was performed by the INNO-LiPA 2.0 assay (Innogenetics, Ghent, Belgium). Samples that were obtained prior to licensure of these PCR assays were retrieved from frozen storage for later testing.

Enrollment of Participants

Volunteer blood donors from the Greater Chesapeake and Potomac Region of the ARC and from the NIH Department of Transfusion Medicine who tested anti-HCV positive on replicate testing and provided informed consent were enrolled beginning in August 1990. The study protocol was approved by the ARC and NIH institutional review boards and has been reviewed annually.

Initial Evaluation and Follow-up

On the initial visit, the donor was interviewed by a physician, who completed an extensive questionnaire that recorded demographic characteristics, blood donation history, sexual history, and past medical history, including assessments of alcohol use, illicit drug use, and other potential risk factors for HCV infection. Participants completed a second questionnaire in private about illicit drug use. A physical examination was performed, and samples for biochemical and hematologic blood tests were obtained at baseline. A physician performed an interim history and physical examination annually, and laboratory testing was repeated biannually for the duration of study. RIBA-positive subjects continue to be followed up in this ongoing study; donors who were repeatedly RIBA negative and HCV RNA negative were discharged from the study after 6–12 months of follow-up because they were considered to have been unexposed to HCV and to have had false-positive results of EIA.

Histologic Evaluation of Liver Biopsy Specimens

Biopsy specimens were obtained from 185 HCV RNA-positive, treatment-naive participants. All specimens were read by the same hepatic pathologist (D.E.K.) without knowledge of the patient's clinical history. A biopsy specimen was considered adequate if it contained >10 portal tracts. The extent of liver fibrosis was scored from 0 to 6, using the Ishak fibrosis scale (0 = no fibrosis, 1–2 = portal fibrotic expansion, 3–4 = bridging fibrosis, and 5–6 = cirrhosis) [5]. Necrosis and inflammation were graded using a modification of the histologic activity index (HAI) on a scale of 0–18 [6, 7].

Mortality Follow-up

Vital status, date of death, and cause-specific mortality from 1990 through 2005 were obtained by searching the National Death Index (National Center for Health Statistics, Hyattsville, MD).

Statistical Analysis

Analyses were performed with standard statistical packages (SPSS v15.0 for Windows, SPSS, Chicago, IL; SAS v9.2, SAS Institute, Cary, NC). Only data collected prior to HCV treatment were included. Associations between categorical variables were assessed with the unadjusted χ^2 statistic or a 2-tailed Fisher exact test.

Univariate analysis of variance was used to assess associations between continuous variables and group status. When normality could not be assumed, the Kruskal-Wallis test was used. The Bonferroni method was used to adjust for multiple comparisons. When an a priori order in the group status was assumed, the Jonckheere nonparametric test for trend was used, with a 2-sided P value. For comparisons of proportions to population proportions, the exact binomial test was used.

A multivariate logistic regression model was used for analysis of risk factors. A forward selection method with a

significance level of 0.05 for entry was used, and odds ratios (ORs) with 95% confidence intervals were calculated based on the profile likelihood. Subjects who were EIA positive but RIBA negative and HCV RNA negative were used as controls in the risk factors analysis. Results of the forward logistic regression analysis were confirmed using backward and stepwise selection methods. Classification and regression tree (CART) and random forest analyses were used to confirm the findings of logistic regression and were performed with R statistical computing language.

RESULTS

Enrollment, Demographic Characteristics, and RIBA Status

Seven hundred and thirty-eight anti-HCV–positive blood donors were enrolled: 692 (94%) were enrolled from the ARC, 36 (5%) were enrolled from the NIH, and 10 (1%) were enrolled from other blood centers. A total of 454 anti-HCV–positive ARC donors were enrolled from 1990 through 1994 (11% of all anti-HCV–positive ARC donors in the region); 238 ARC donors were enrolled from 1995 through 2005 (43% of all anti-HCV–positive donors). Correspondingly, 28 anti-HCV–positive NIH donors (84% of the total detected) were enrolled from 1990 through 1994, and 8 (17% of the total) were enrolled from 1995 through 2005.

Demographic data on 1 040 713 blood donors who donated at the ARC between 1990 and 2005 were compared with the 692 ARC donors enrolled in the study. The populations had a similar sex distribution, but study participants were older (41.4 vs 38.0 years; $P < .01$), more likely to be African American (14% vs 8.9%; $P < .01$), and less likely to be first-time donors (23.8% vs 76.5%; $P < .01$).

Demographic data on 14 400 NIH volunteer blood donors who donated during the study period were compared to data for the 36 who were enrolled. Enrolled donors were similar with respect to sex and African American race (13.9% vs 6.5%; $P = .16$), were younger (41.9 vs 49.0 years; $P < .01$), and were less likely to be first-time donors (13.9% vs 80.1%; $P < .01$). Study participants from the ARC and the NIH were compared, and there were no differences in sex, age, African American race, or first-time donor status.

Among anti-HCV–positive blood donors, 469 (64%) were positive by the third-generation RIBA, 217 (29%) were negative, and 52 (7%) had indeterminate results. Characteristics of RIBA-positive and RIBA-negative individuals are compared in Table 1.

Table 1. Characteristics of Blood Donors, by Hepatitis C Virus (HCV) Antibody Status

	Results of Third-Generation RIBA[a]		
Characteristics	Positive (n = 469)	Negative (n = 217)	P
Age, mean ± SD (years)	40 ± 10	44 ± 12	<.001
Female sex	215 (46)	96 (44)	.742
Race			
White	372 (79)	190 (88)	.010
African American	85 (18)	15 (7)	<.001
No college education	212 (45)	49 (23)	<.001
First-time donor	145 (31)	18 (8)	<.001
History of STD	127 (27)	22 (10)	<.001
MSM	19 (4)	1 (0.5)	<.007
ALT level, mean (IU/L)[b]	54	22	<.001

Data are no. (%) of donors, unless otherwise indicated.

Abbreviations: ALT, alanine aminotransferase; MSM, men who have sex with men; RIBA, recombinant immunoblot assay; STD, sexually transmitted disease.

[a] All subjects had positive anti-HCV findings by enzyme immunoassay (EIA). Donors with positive results of RIBA are considered to have been infected with HCV. Donors with negative results of RIBA are considered to have false-positive anti-HCV findings by EIA.

[b] At initial evaluation, ALT level was elevated in 48% of RIBA-positive donors, compared with 6% of RIBA-negative donors ($P < .001$).

Risk Factors Analysis

Independent risk factors for HCV infection in the multivariate logistic regression analysis (Table 2) included, in order of entry into forward and stepwise logistic regression models, intranasal cocaine use (OR, 6.4; $P < .0001$), blood transfusion prior to 1991 (OR, 9.9; $P < .0001$), history of injection drug use (IDU; OR, 35.0; $P < .0001$), sexual promiscuity (>5 partners/year, history of sexually transmitted disease, exchanging sex for drugs or money, or a combination of these factors; OR, 2.3; $P < .001$), ear piercing (OR, 1.8; $P < .01$), and occupational exposure to human blood (OR, 3.8; $P = .018$). Ear piercing was a significant risk factor in 70 (28%) of 253 RIBA-positive men ($P < .0001$) but not in women.

Among 292 RIBA-positive subjects who snorted cocaine, 213 (73%) also reported a history of IDU or blood transfusion prior to 1991; 79 (27%) who snorted cocaine repeatedly denied IDU or other parenteral risk factors both in personal interviews and on a questionnaire. Among 70 men who had ear piercing as a risk factor, 67 (96%) had also snorted cocaine, had received a blood transfusion prior to 1991, had a history of IDU, or had a documented needlestick exposure to human blood.

The CART analysis identified the same risk factors for HCV infection and in the same order of importance as did the forward, backward, and stepwise logistic regression methods, except that CART did not identify occupational exposure as an important variable. Random forest analysis confirmed the importance of the risk factors identified by logistic regression; occupational exposure and sex were ranked as least important. CART and random forest analyses confirmed

Table 2. Multivariate Logistic Regression of Risk Factors for Hepatitis C Virus Infection

	Results of Third-Generation RIBA		Multivariate Logistic Regression Analysis[b]	
Risk Factor[a]	Positive, no. (%) (n = 469)	Negative, no. (%) (n = 217)	Odds Ratio (95% CI)	P
IDU	195 (42)	2 (1)	35.0 (10.4–218.0)	<.0001
Blood transfusion	126 (27)	17 (8)	9.9 (5.6–18.3)	<.0001
Intranasal cocaine use	292 (62)	23 (11)	6.4 (3.8–11.2)	<.0001
Intranasal cocaine use without IDU or blood transfusion	79 (49)[c]	20 (10)[c]	8.5 (4.9–15.1)	<.0001
Occupational exposure	27 (6)	5 (2)	3.8 (1.3–12.4)	.0176
Sexual promiscuity	243 (52)	48 (22)	2.3 (1.4–3.7)	.0006
Males[d]	148 (58)	36 (30)	3.3 (2.1–5.3)	<.0001
Females[e]	95 (44)	12 (13)	5.5 (3.0–11.2)	<.0001
Ear piercing	273 (58)	86 (40)	1.8 (1.2–2.8)	.0088
Males[d]	70 (28)	1 (<1)	45.9 (9.9–815)	.0002
Females[e]	203 (95)	85 (89)	...	NS
Male sex	254 (54)	121 (56)	...	NS
Tattooing	104 (22)	9 (4)	...	NS
Acupuncture	29 (6)	5 (2)	...	NS

Data are ordered by odds ratio.

Abbreviations: CI, confidence interval; IDU, injection drug use; NS, nonsignificant; RIBA, recombinant immunoblot assay.

[a] Order of entry into the model (forward selection method): (1) intranasal cocaine use, (2) blood transfusion, (3) IDU, (4) sexual promiscuity, (5) ear piercing, and (6) occupational exposure.

[b] Ellipses indicate that the risk factor did not meet criteria for entry into the model.

[c] Results from analysis of a subset of 361 subjects who denied IDU and blood transfusion (163 RIBA positive, 198 RIBA negative).

[d] Results from analysis of a subset of 375 males (254 RIBA positive, 121 RIBA negative).

[e] Results from analysis of a subset of 311 females (215 RIBA positive, 96 RIBA negative).

findings of the subset analyses of 361 subjects, presented in Table 2.

Survey of Intranasal Cocaine Use

Six hundred and ninety-two donors completed a detailed survey on cocaine use. Of 273 RIBA-positive subjects who used intranasal cocaine, 236 (86%) had shared straws or other snorting devices, 87 (32%) had experienced epistaxis during or after intranasal use, and 67 (25%) observed epistaxis in others with whom they were sharing materials. Longer duration of intranasal cocaine use was associated with positive RIBA results ($P = .01$) but not with detection of HCV RNA. Intranasal cocaine use was a significant independent risk factor for HCV infection, whether analyzed in the entire population ($P < .0001$) or in the subset of 79 who snorted cocaine but denied IDU and blood transfusion ($P < .0001$; Table 2).

Detection of HCV RNA by PCR and Follow Up of HCV RNA–Positive Subjects

Among 469 RIBA-positive blood donors, 384 (82%) were HCV RNA positive, and 85 (18%) were repeatedly HCV RNA negative. Of the 85 RIBA-positive, HCV RNA–negative donors, RIBA was performed a mean of 7 times over a mean period of 2.75 years, during which results remained persistently positive. The longest interval of RIBA-positive, HCV RNA–negative status documented in this study was 9.7 years. Patients who were RIBA positive, HCV RNA negative on at least 2 occasions were presumed to have been exposed to HCV and spontaneously recovered. All 217 RIBA-negative donors and 52 persistently RIBA-indeterminate donors tested negative for HCV RNA. Among RIBA-positive subjects, age, sex, and race were not significantly different between those who were HCV RNA positive and those who were HCV RNA negative: mean age, 40.2 versus 38.6 years ($P = .18$), male sex, 54% versus 53% ($P = .81$), and white race, 78% versus 85% ($P = .19$).

At the time the database was frozen for analysis, 257 of 384 HCV RNA–positive subjects (67%) were still being actively followed; 95 (37%) were treated for HCV infection. Since this was a natural history study, outcomes in these patients were only analyzed up to the time that treatment was initiated.

Among 258 HCV RNA–positive repeat blood donors, 65% were donating potentially HCV-infected blood for >10 years, and 42% had donated ≥10 times.

ALT Levels and Clinical Liver Disease in HCV RNA–Positive Subjects

Elevated alanine aminotransferase (ALT) levels were found at initial evaluation in 214 HCV RNA–positive subjects (56%).

Table 3. Pattern of Mean Alanine Aminotransferase (ALT) Level Elevations Among Hepatitis C Virus RNA–Positive Blood Donors

ALT Level, Mean	No. (%)	No. (%) Biopsied	Mean HAI		No. (%) Severe Fibrosis[a]	
Normal[b]	127 (33)	42 (33)	6.19		3 (7)	
Elevated	255 (67)	142 (55)	7.50		24 (17)	
1–2 × ULN	185 (48)	94 (51)	7.08	$P < .001$[c]	13 (14)	$P < .001$[d]
2–5 × ULN	64 (17)	45 (70)	8.20		10 (22)	
>5 × ULN	6 (2)	3 (50)	10.33		1 (33)	

Abbreviations: HAI, histologic activity index; ULN, upper limit of normal.

[a] Defined as Ishak stage 3–6 (bridging fibrosis or cirrhosis).

[b] Persistently normal in 57 of 127 donors (45%). Of these 57, 7 (12%) underwent biopsy, with none having severe fibrosis.

[c] There is a significant trend of increasing HAI with increasing ALT group.

[d] There is a significant trend of severe fibrosis with increasing ALT group.

Over an average follow-up of 5.7 years, the mean ALT level was 62 U/L (range, 13–344 U/L), compared with 22 U/L in 354 among HCV RNA-negative subjects ($P < .001$). Fifty-seven HCV RNA–positive subjects (15%) had persistently normal ALT levels; 7 (12%) underwent biopsy, with all having an Ishak fibrosis score of ≤1. The pattern of mean ALT level elevations is shown in Table 3.

Fifty-one of 384 HCV RNA–positive patients (13%) had physical signs of chronic liver disease: icteric sclerae was detected in 6, spider angiomata in 40, collateral venous circulation in 1, palmar erythema in 9, splenomegaly in 2, and encephalopathy in 1; none had ascites.

Extent of Liver Disease at Biopsy

On initial liver biopsy of 185 chronically infected subjects, 61 (33%) had no fibrosis, 97 (52%) had mild fibrosis, 23 (12%) had bridging fibrosis, and 4 (2.2%) had cirrhosis (Figure 1). One patient developed HCC. Associations with liver fibrosis are shown in Table 4. Both age at infection and duration of HCV infection were extrapolated from the reported date of probable exposure, specifically, blood transfusion prior to 1991, the first year of IDU, or the date of a well-defined needlestick exposure; 125 (69%) of 185 biopsied patients had these defined risk factors. On the basis of these risk exposures, the mean age at the onset of HCV infection was 21 years (range, birth to 59 years), and the mean duration between infection and the last liver biopsy specimen obtained in the study was 25 years (range, 9–43 years). Within this time frame, the duration of infection was not associated with increasing severity of fibrosis.

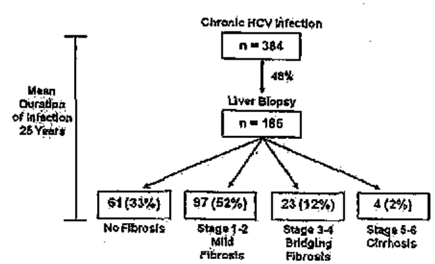

Figure 1. Stage of liver fibrosis among 185 hepatitis C virus (HCV)–positive patients undergoing initial liver biopsy. After a mean of 25 years based on the interval since a known parenteral exposure, 85% had no or minimal fibrosis, 2% had cirrhosis, and 12% had bridging fibrosis that might progress to cirrhosis.

Sex, race, education level, alcohol use at the time of HCV diagnosis, and total peak alcohol use were not significantly associated with the stage of fibrosis; body mass index was positively correlated with worsening fibrosis ($P = .005$; Table 4). Patients with bridging fibrosis or cirrhosis had higher HAI scores than those with mild or no fibrosis ($P < .001$). Elevated levels of serum markers of liver inflammation were highly associated with increasing stage of fibrosis (ALT and aspartate aminotransferase levels, $P < .002$; lactate dehydrogenase level, $P = .010$). The alpha-1 fetoprotein level was only significant when one patient who developed HCC was included ($P = .006$).

Among biopsied patients, 182 (98%) were genotyped, and 136 (76%) were genotype 1; 111 (60%) of biopsied patients had quantitative HCV RNA load measured, with a mean level of 3.32×10^6 copies/mL (range, 1.14×10^3 to 4.81×10^7 copies/mL; median, 1.20×10^6 copies/mL). Neither genotype nor HCV load were associated with a more severe stage of liver fibrosis.

Extent of Liver Disease at Repeat Liver Biopsy

Sixty-three patients (34%) underwent a second biopsy after a mean interval of 4.6 years between biopsies. Over that interval, 21 (33%) had fibrosis that increased by at least 1 Ishak stage (5 increased by at least 2 stages), 34 (54%) had no change, and 8 (14%) showed a decrease of ≥1 Ishak stage (Figure 2). The

Table 4. Characteristics of 185 Hepatitis C Virus–Positive Patients Who Underwent Liver Biopsy, Staged by Ishak Fibrosis Score

	Score				
Characteristic	0	1	2	3–6	P
Patients (no. [%])	[illegible]	[illegible]	33 (18)	[illegible]	
Age at infection, mean (years)[a]	19.3	21.7	21.4	24.5	.037
Age at initial biopsy, mean (years)	40.4	43.3	42.3	[illegible]	[illegible]
Duration of infection, mean (years)[a]	23.9	25.9	24.5	24.0	.555
Sex					
Male	26 (28)	33 (35)	21 (22)	14 (15)	.264
Female	[illegible]	[illegible]	[illegible]	[illegible]	
Race					
White	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
African American	5 (25)	10 (50)	1 (5)	4 (20)	
[illegible]					
Current use	5	7.1	6	6.5	.993
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Total peak use (no. drinks/year × total years)	7910	11 476	11 388	18 663	.174
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
HAI inflammation, mean	6.1	7.5	7.3	9.3	<.001
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Peak ALT level, mean (IU/L)	113	124	138	142	.006
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Peak AST level, mean (IU/L)	70	80	81	102	<.001
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Total bilirubin level, mean (mg/dL)	0.6	0.7	0.7	0.7	.064
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Platelet count, mean (platelets/mm^3)	248	229	240	188	<.001
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Partial thromboplastin time, mean (s)	27.8	28.7	27.4	28.7	.928
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Alkaline phosphatase level, mean (IU/L)	77	72	79	87	.289
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Creatine kinase level, mean (IU/L)	124	134	132	104	.909
[illegible]					
Quantitative RNA load, mean (× 10^6 copies/mL)	3.43	3.17	4.58	1.96	.189
Genotype					
1	46 (34)	49 (36)	22 (16)	19 (14)	.414
2	[illegible]	[illegible]	[illegible]	[illegible]	
3	1 (14)	2 (29)	1 (14)	3 (43)	
4	[illegible]	[illegible]	0	[illegible]	
6	0	1	0	0	
Mixed	0	[illegible]	[illegible]	0	

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGTP, γ-glutamyl peptidase; HAI, histologic activity index.

[a] For 125 subjects, inferred from the date of blood transfusion, the first year of injection drug use, or the date of a well-defined needlestick exposure.

[b] $P = .024$ after excluding data for 1 study subject, who had hepatocellular carcinoma.

mean progression rate between biopsies was 0.06 Ishak stages per year (0.28 for patients whose fibrosis increased).

Treated Versus Untreated HCV RNA–Positive Subjects

Among 384 HCV RNA–positive subjects, 95 (25%) were treated with interferon with or without ribavirin, or with pegylated interferon plus ribavirin. Compared with those who were not treated, subjects who received treatment were of similar age (40.2 vs 40.1 years; $P = .97$) and similar sex (48% vs 56% were male; $P = .18$). However, subjects who received treatment were more likely to be white (89% vs 74%; $P < .01$) and had a higher mean ALT level (78.5 vs 56.4; $P < .01$).

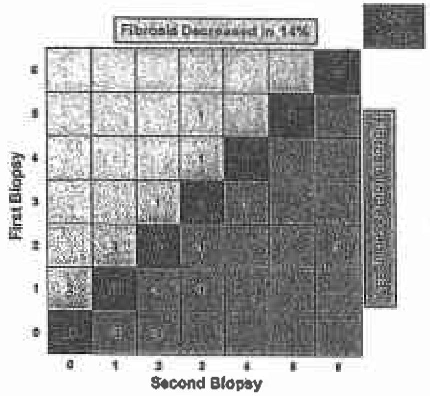

Figure 2. Fibrosis progression among 53 hepatitis C virus (HCV)–positive patients undergoing repeat liver biopsy. After a mean interval between biopsies of 4.6 years, 54% showed no change in interval biopsies, 14% showed lessened fibrosis, and 33% showed increased fibrosis by at least 1 stage; 5 (9%) increased by ≥2 stages.

When liver histologic findings were compared between biopsied subjects who were and those who were not treated, treated subjects had a higher mean Ishak fibrosis stage (1.65 vs 0.87; $P < .01$) and HAI (7.90 vs 6.64; $P < .01$).

Mortality

From 1990 to 2005, there were 28 deaths (4%) in the total study population, and 22 (79%) of those who died were HCV RNA positive (OR, 3.5; $P < .01$). The mean age at death was 51 years. Among those chronically infected with HCV, only 2 (9%) died from liver-related causes, one from HCC and the other from complications of cirrhosis.

DISCUSSION

Informed decisions for the treatment of chronic HCV infection require knowledge of the natural history of the disease because the key issue is not which drug or dosage to use, but whether treatment is indicated. Given that current treatments are arduous, expensive, and fraught with adverse events, and given that fibrosis progression is neither linear nor inevitable, one has to balance the probability of disease progression against the near certainty of deleterious drug-induced side effects. Early retrospective studies overestimated the severity of chronic HCV infection by focusing on those with established chronic liver disease while excluding the much larger number of silent infections [8–10]. This study prospectively followed asymptomatic individuals found to be anti-HCV positive at the time of blood donation. Although the study is biased by limiting enrollment to volunteer blood donors, we believe it provides a valid model for assessing transmission patterns in low-risk populations, the rate of spontaneous recovery in immunocompetent individuals, and the long-term outcomes of HCV infection.

Epidemiologic comparisons between RIBA-positive and RIBA-negative (EIA false-positive) controls demonstrated striking differences. Although all donors denied IDU at the time of donation, in subsequent private interviews with a physician, 41% of RIBA–positive donors admitted to IDU at some point in their life, compared with only 1% of RIBA-negative controls; none were current drug addicts. Thus, even in a presumed low-risk population, IDU was the greatest risk factor for HCV acquisition, with an OR of 35.0 ($P < .0001$). Unexpectedly, intranasal cocaine use was an additional strong independent risk factor in a multivariate logistic regression analysis and in CART and random forest analyses. Although intranasal cocaine use often overlapped IDU, there were 79 RIBA-positive subjects who snorted cocaine and repeatedly denied IDU or blood transfusion, and cocaine snorting remained a strong independent risk in this subset (OR, 8.5; $P < .0001$). Although one can never be certain of the veracity of IDU denial, there is plausibility to the concept that cocaine snorting might transmit HCV, in that (1) 86% of those who snorted admitted to the shared use of snorting devices, a previously implicated risk factor [11, 12]; (2) cocaine is known to denude mucous membranes, allowing direct access to blood vessels; (3) HCV RNA has been detected in nasal secretions [13]; (4) approximately 30% of subjects who snorted either experienced or observed nosebleeds during shared intranasal cocaine use; and (5) anti-HCV positivity was significantly associated with the duration of cocaine use. Thus, intranasal cocaine use may be a covert parenteral route of viral transmission, a route that might be applicable to human immunodeficiency virus and hepatitis B virus infection, as well as to HCV infection.

Over a mean interval of 25 years from onset of infection to liver biopsy, only 14% had severe histologic outcomes, and only 2% had cirrhosis; 85% had no or minimal fibrosis. Other studies have shown a similarly low proportion of severe histologic outcomes during the first 2–3 decades of HCV infection [14–17]. Further, this low incidence of severe outcomes is a worst-case scenario because biopsied patients had higher average ALT levels than nonbiopsied subjects, as there was reluctance to biopsy the approximate 30% who had normal or low-level ALT elevations. Thus, although this study has a selection bias based on the propensity to biopsy and treat those with the most severe clinical or biochemical profiles, this bias would be in the direction of observing more severe histologic outcomes rather than the relatively mild outcomes actually

observed. If subjects with spontaneous recovery (15%) are factored into the outcome analysis, the number of acutely infected patients who progress to severe outcomes would be proportionately less. However, there is a further caveat to this outcome analysis. Despite a mean duration of follow-up of 25 years between the time of probable exposure and the time of the last available biopsy, patients biopsied in this study were still relatively young and had not reached the 30–40-year disease duration that seems critical to fibrosis progression in HCV infection [18]. Indeed, the histologic progression observed between 5-year-interval biopsies in one-third of our patients portends worse outcomes for some in the ensuing decades. Nonetheless, it is probable that those who have shown no or little fibrosis progression over 25 years will have a nonprogressive or slowly progressive course that will provide time for more effective and safer therapies to emerge and induce sustained virologic responses that appear tantamount to cure [19]. Clearly, a subset of patients will have progressive histologic deterioration either because treatment was not accessed or because antiviral therapy failed to achieve a sustained virologic response. Identification of silent HCV carriers and access to treatment remain major public health hurdles, but among treated subjects the number who will not achieve a sustained virologic response has been reduced dramatically with the recent licensure of protease inhibitors [20, 21]. Since the majority of HCV-infected individuals will not be treated in the near term, continued long-term follow-up is critically needed to provide better estimates of clinical and histologic outcomes after ≥3 decades of HCV infection, although one small study has shown relatively benign outcomes even after a mean observation period of 45 years [22]. Although the prognosis for the individual patient with HCV infection can be increasingly optimistic, the global burden of this disease is staggering on the basis of the sheer magnitude (estimated 100 million) of those who are already chronically infected.

Notes

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研究報告の概要

○臓器移植レシピエントにおけるE型肝炎ウイルス感染、オランダ
2000年～2011年、オランダ最大の臓器移植センターで、臓器移植を受けた1200人の生存レシピエントに対してRT-PCRを用いたHEV RNA調査を行った。そのうち12人(心臓移植5人、肺移植1人、肝臓移植3人、腎臓移植1人、複数臓器移植2人)のHEV感染が判明し、11人は慢性感染症であった。患者の年齢中央値は56.9歳(19.9～63.5歳)、9人(75%)が男性であった。慢性HEV感染症の全患者で肝酵素レベルが上昇し、ビリルビン値は患者の45.5%で上昇した。HEV RNA検出は、ALTレベルの上昇と同時かまたはその後に続いた。HEV RNA陽性時からIgMが検出されるまでの期間の中央値は32日、IgGが検出されるまでの期間は平均124日であった。11人のHEV感染患者のサンプルから分離したウイルスは全てジェノタイプ3であった。HEV感染の原因が市中感染か院内感染かは分からなかった。慢性HEV感染患者において、RNAが検出されてからIgM及びIgGが検出されるようになるまでに期間があるので、高いALT値を示す臓器移植患者におけるHEV感染症の診断はRNAの検出によって確認されるべきである。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン5%静注12.5g/250mL
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

報告企業の意見

臓器移植患者1200人に対してHEV RNA調査を行ったところ、12人のHEV感染が判明、そのうち11人は慢性HEV感染症であることが分かったとの報告である。
HEVは脂質膜のないRNAウイルスである。本剤の製造工程にはコーン分画及び液状加熱の2つのウイルス除去・不活化工程が含まれている。最近ある遺伝子型のHEVは耐熱性であるとの成績が発表され、液状加熱の有効性に一部疑念を生じている。しかし、血漿分画製剤で最も長い歴史を持つアルブミンにはHEVの侵淫度が遥かに高い過去から現在に至るまで世界的にHEV感染例がないとの疫学的事実があること、最終製品についてHEV-NAT陰性を確認していることから、本剤の安全性は確保されていると考える。

今後の対応

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高値の献血血液を排除している。また、厚生労働科学研究「経口感染によるウイルス性肝炎(A型及びE型)の感染防止、病態解明、遺伝的多様性及び治療に関する研究」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。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。

Hepatitis E Virus Infection among Solid Organ Transplant Recipients, the Netherlands

Suzan D. Pas, Rob A. de Man, Claudia Mulders, Aggie H.M.M. Balk, Peter T.W. van Hal, Willem Weimar, Marion P.G. Koopmans, Albert D.M.E. Osterhaus, and Annemiek A. van der Eijk

We screened 1,200 living heart, lung, liver, and kidney transplant recipients for hepatitis E virus infection by reverse transcription PCR. In 12 (1%) patients, hepatitis E virus infection was identified; in 11 patients, chronic infection developed. This immunocompromised population is at risk for hepatitis E virus infection.

Hepatitis E virus (HEV) can cause acute or chronic infection in humans. Four genotypes have been identified in humans. HEV genotype 3 predominantly infects pigs and deer, but is also recognized as a zoonotic agent. As awareness increases, more reports of HEV infection among humans, especially immunocompromised persons, have been published (*1*,*2*).

Analysis of exposure histories of persons with HEV genotype 3 infections has demonstrated its underdiagnosis, and a source was not identified for most cases (*3*). Because HEV has been reported as a cause of liver disease in solid organ transplant (SOT) recipients (*4*), we screened all living recipients of SOTs during 2000–2011 at Erasmus Medical Center, the largest SOT center in the Netherlands, for HEV RNA. This study was designed to identify SOT recipients with acute or chronic HEV infection.

The Study

A cross-sectional study was performed of all living adult SOT recipients for whom serum or EDTA-plasma samples were available in the Erasmus Medical Center biobank (stored at –20°C and –80°C, respectively, and collected during previous routine visits to the outpatient clinic; complete methods are described in detail in the online Technical Appendix, wwwnc.cdc.gov/EID/pdfs/11-1712-Techapp.pdf). Some recipients eventually had been referred to peripheral hospitals. A Laboratory Information Management System database search was performed for availability of the most recent follow-up sample. Thirty-nine HEV RNA–positive samples in the center's biobank from non-SOT patients were genotyped and used as reference for phylogenetic analysis. Samples were screened for HEV RNA by using real-time reverse transcription PCR (RT-PCR) (*5*) with primers detecting all 4 genotypes and validated according to International Standards Organization guidelines 9001 and 15189 (www.iso.org/iso/search.htm). HEV IgM and IgG were detected by using the PE2 HEV-IgM and IgG ELISA (Wantai Biological Pharmacy Enterprise Co., Ltd., Beijing, People's Republic of China). A case of HEV infection was defined by the following criteria: an HEV RNA–positive sample, confirmed either by presence of HEV IgM or IgG or HEV RNA in sequential samples. Chronic infection was diagnosed by retrospective testing of stored samples and defined as HEV RNA positive for >6 months. We retrospectively tested samples from HEV RNA–positive patients so the antibody kinetics and viremia levels could be studied. For calculating phylogenetic relationships, HEV open reading frame (ORF) 1 sequences were generated with primer set MJ-C (*6*). All viral sequences were deposited into GenBank (accession nos. JQ015399–JQ015448).

The 1,200 SOT recipients consisted of 259 heart transplant (HTX), 53 lung transplant (lungTX), 300 liver transplant (LTX), 574 kidney transplant (NTX), and 14 multiple SOT recipients (4 HTX–NTX, 1 lungTX–NTX, and 9 LTX–NTX). Twelve HEV-infected patients were identified: 5 HTX, 1 lungTX, 3 LTX, and 1 NTX recipients and 2 multiple SOT-recipients (1 HTX-NTX and 1 LTX–NTX). For 11 patients, HEV infection was chronic (Table 1). The median age of the HEV-infected patients was 56.9 years (range 19.9–63.5 years); 9 (75%) were men. In 10 HEV patients, immunosuppression was achieved by using prednisolone and tacrolimus, combined with mycophenolate mofetil (n = 3) or everolimus (n = 2). Two patients received regimens of cyclosporine and prednisolone or mycophenolate mofetil and prednisolone.

Table 1. Overview of HEV infections among SOT recipients, the Netherlands, 2000–2011*

SOT group	No. recipients	HEV infections, no. (%)	
		Confirmed	Chronic
HTX	259	5 (1.9)	5 (1.9)
LungTX	53	1 (1.9)	1 (1.9)
LTX	300	3 (1.0)	3 (1.0)
NTX	574	1 (0.2)	1 (0.2)
Multiple SOT†	14	2 (14.3)	1 (7.1)
Total	1,200	12 (1.0)	11 (0.9)

*HEV, hepatitis E virus; SOT, solid organ transplant; HTX, heart transplant; lungTX, lung transplant; LTX, liver transplant; NTX, kidney transplant.
†9 NTX–LTX, 4 NTX–HTX, and 1 NTX–lungTx.

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All patients who had chronic HEV infection had elevated liver enzyme levels; bilirubin levels were elevated in 45.5% of the patients (Table 2). Although it proved difficult to identify abnormal liver functions uniquely related to the HEV infection, HEV RNA detection always coincided with or was followed by an increase in alanine aminotransferase. Apparently no overt clinical symptoms were associated with infection; however, such symptoms are difficult to recognize in immunosuppressed SOT recipients. Inflammation compatible with viral hepatitis was shown in 8 of 9 patients with chronic infection for whom liver biopsy specimens were available. Other findings were F0–F2 fibrosis, steatosis 1–2 (Brunt classification), cholestasis, and Councilman bodies.

Samples from all 12 HEV patients were tested for HEV RNA and HEV IgM and IgG. One infection was traced to 2003 (lungTX), 1 to 2008 (NTX), 1 to 2009 (multiple SOT recipient, NTX–HTX), 7 to 2010 (5 HTX, 1 LTX and 1 multiple SOT recipient, NTX–LTX) and 3 to 2011 (all LTX). Among the patients, 1 LTX recipient had an acute HEV infection and cleared the virus within 6 days. Because HEV IgM and IgG were detected 4 years before HEV RNA detection, both reactivation and reinfection should be considered. The median span of HEV RNA-positive time period of chronic HEV cases was 16 months (range 6–55) with a median peak cycle threshold value of 20.0 (range 16.7–26.6). HEV RNA was detected during viremia (median cycle threshold value 19.9, range 15.5–28.3) in feces from 8 patients with chronic illness.

To assess the value of diagnostic techniques for detection of HEV infection in SOT recipients, we studied antibody kinetics (HEV IgM and IgG) and viremia. The median time from RNA positivity to IgM detection was 32 days (range 0–826 days). Five patients had detectable HEV IgM at the time of HEV RNA positivity. In 1 case, no HEV IgM was detected. HEV IgG titers were detectable an average of 124 days later than HEV RNA (range 0–826 days). HEV IgG was absent in 2 samples, and in 4 samples, HEV IgG was detectable when HEV RNA was detected. The median time between transplantation and first HEV RNA-positive result was –0.3 to 20.0 years (median 1.99 years).

Viruses isolated from samples from 11 HEV-infected patients were all within the genotype 3 group. Because no ORF1b sequences from the Netherlands were available in GenBank, ORF1b sequences were determined from samples from non-SOT HEV-infected patients in the Netherlands (Figure). No indications for a common or nosocomial source of HEV transmission were found.

Conclusions

Recent HEV infections in SOT recipients (*4*,*7*–*9*) prompted us to perform a survey among SOT recipients admitted to the largest transplantation center in the Netherlands. Our findings showed that they are at risk for HEV infection. Nine of 12 case-patients were treated postoperatively with a tacrolimus-based regimen, which has been associated with increased risk for HEV infection (*9*).

The cross-sectional RT-PCR screening detected 12 HEV infections but could not provide information about previously acquired and cleared HEV infections. Real-time RT-PCR screening was performed for 2 reasons. First, because a patient received immunosuppressive drugs, specific antibodies against HEV might be absent. Second, ELISAs have been developed to detect antibodies to genotypes 1 (Myanmar) and 2 (Mexico) and might not be sensitive enough to detect antibodies to genotype 3 or 4 (*10*). Information about results of serologic assays to validate HEV genotype 3 is limited, and seroprevalence measured can vary with the assays used (*11*–*13*). Furthermore, independent studies found that sensitivity and specificity of HEV RNA assays from laboratories in the Netherlands (S.D. Pas and B. Hogema, unpub. data) and other European countries (*14*) differ greatly. Therefore, international standardization should be encouraged.

Although the observed 1% of HEV-infected SOT recipients may seem low, HEV infection may be life threatening in immunocompromised patients. Misdiagnosis of HEV infection as drug-induced liver injury or autoimmune hepatitis has been reported (*15*); empirical treatment of these misdiagnoses by raising immune suppression would exacerbate the condition. Temporary reduction of immunosuppression resulted in immune-mediated control and clearance of HEV in 30% of cases (*9*).

Table 2. Parameters in chronic HEV infections among SOT recipients, the Netherlands, 2000–2011*

Parameter	Median	Range	ULN (F/M)
Peak alanine aminotransferase, U/L	301	81–909	30/40
Peak aspartate aminotransferase, U/L	172	66–1016	30/36
Peak gamma-glutamil transferase, U/L	299	72–1746	34/49
Peak bilirubin, μmol/L	16	5–100	16/16
Peak HEV RNA, cycle threshold values	20.0	16.7–26.6	NA
Period of HEV RNA positivity, mo	16	6–55	NA
Time between SOT and first HEV RNA–positive result, mo	2.0	–0.3 to 20.1	NA
Time of HEV RNA positivity before HEV IgM positive, d	32	0–826	NA
Time of HEV RNA positivity before HEV IgG positive, d	124	0–826	NA

*HEV, hepatitis E virus; SOT, solid organ transplant; ULN (F/M), upper limit of normal (female/male); NA, not applicable.

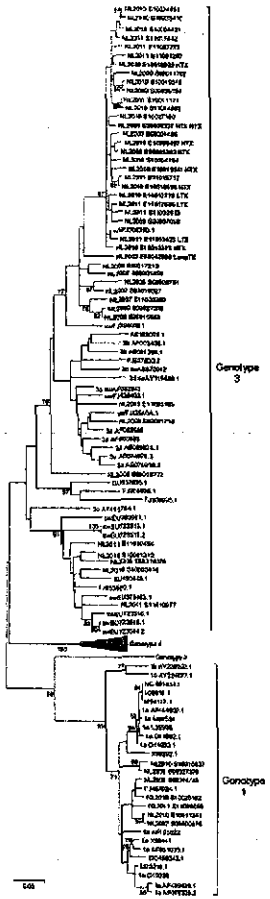

Figure. Phylogenetic tree of hepatitis E virus (HEV) open reading frame (ORF) 1 sequences, including HEV infections, the Netherlands, 2000–2011. Phylogenetic relation of a 306-bp ORF1 region was calculated by using maximum-likelihood, Kimura 2-parameter analysis with bootstrapping (n = 1,000). HEV sequences originating in the Netherlands are indicated as NL with year of isolation and isolate number (GenBank accession nos. JQ015389–JQ015448). Boldface indicates virus strains of chronic HEV-infected solid organ transplant recipients identified in this study. Scale bar indicates number of nucleotide substitutions per site. HTX, heart transplant; NTX, kidney transplant; LTX, liver transplant; lungTX, lung transplant.

This study also found that in patients with chronic HEV infection, HEV RNA was detected an average of 32–124 days before HEV IgM and IgG, respectively. Therefore, in SOT recipients with elevated liver enzymes (alanine aminotransferase), the diagnosis of HEV infection should be considered and verified by detection of HEV RNA.

This systematic survey of HEV infections among SOT recipients in a major transplant center shows that this population is at risk for HEV infection. Given the consequences of HEV infection, SOT recipients with liver function impairment of unknown etiology should be tested for HEV RNA.

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Ms Pas is a scientific researcher heading a molecular diagnostic team at the Department of Virology, Erasmus Medical Center, Rotterdam, the Netherlands. Her research interests include drug-induced resistance of viral hepatitis, and development and evaluations of molecular diagnostic assays used in the clinical laboratory setting.

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医薬品 研究報告 調査報告書

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研究報告の概要

○スウェーデン、ドイツ及び米国からの血漿供血におけるB型肝炎ウイルスRNAの陽性率

ドイツ、スウェーデン及び米国からの165,010血漿供血において、96供血までのミニプール血漿中のB型肝炎ウイルス(HEV)RNAの存在をRT-PCRアッセイを用いて調査した。スウェーデンから95,835本、ドイツから18,100本の供血がスクリーニングされ、それらのうちスウェーデンからの12本、ドイツからの4本がHEV RNA陽性であった。実際のミニプールを考慮すると、HEV陽性供血の割合はスウェーデンの供血において1:7986、ドイツの供血において1:4525であった。対照的に、米国からの51,075供血においてHEV陽性供血は検出されなかった。HEV RNA陽性血液は製剤原料から除外された。サンプルのうち12本は分子学的及び血清学的分析により特徴づけられた。HEV株は全てジェノタイプ3であり、BLAST検索では、イノシシからのHEV株とドイツ人供血者から得られたサンプル6、7の間で、密接なRNA配列一致があった。ウイルス量は3.2-5.7 log10IU/mL HEV RNA間で変動し、日本人献血者での報告と同様の範囲であった。大部分のサンプルはウィンドウ期供血であり、異なるバッチのキットを使用した繰り返し検査により、1人のウイルス血症サンプルのみIgM陽性、もう1人はIgG陽性であることが確定した。3供血においては、最初は陽性であったが、繰り返し検査により陰性となった。このような曖昧な結果は、HEV血清学的分析の標準化に関する問題点となる。検査された12本のウイルス血症供血のうち3本のみ、ALTレベルの上昇がわずかに見られた(80−110 IU/L)。これはALTによる血液/血漿スクリーニングが、HEVウイルス血症供血者の除外の方法として信頼できないことを示している。

ヨーロッパでの多数のHEV陽性供血は多くの無症候性感染を示唆し、血液のHEV汚染の結果は更なる調査を必要とする。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン5%静注12.5g/250mL
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

報告企業の意見

スウェーデン、ドイツ及び米国からの血漿供血165,010本においてHEV RNAの存在を検査したところ、スウェーデンとドイツの供血においてそれぞれ1:7986、1:4525の割合でHEV RNA陽性であり、米国の供血は全て陰性であることが分かったとの報告である。

HEVは脂質膜のないRNAウイルスである。本剤の製造工程にはコーン分画及び液状加熱の2つのウイルス除去・不活化工程が含まれている。最近ある遺伝子型のHEVは耐熱性であるとの成績が発表され、液状加熱の有効性に一部疑念を生じている。しかし、血漿分画製剤で最も長い歴史を持つアルブミンにはHEVの侵淫度が遥かに高い過去から現在に至るまで世界的にHEV感染例がないとの疫学的事実があること、最終製品についてHEV-NAT陰性を確認していることから、本剤の安全性は確保されていると考える。

今後の対応

日本赤十字社では、厚生労働科学研究「経口感染によるウイルス性肝炎(A型及びE型)の感染防止、病態解明、遺伝的多様性及び治療に関する研究」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。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。

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Vox Sanguinis

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LETTER

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Vox Sanguinis

Occurrence of hepatitis E virus RNA in plasma donations from Sweden, Germany and the United States

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Dear Editor,

We have investigated 165 010 plasma donations from Germany, Sweden and the United States for the presence of hepatitis E virus (HEV) RNA in plasma mini-pools of up to 96 donations using a proprietary internally controlled real-time RT-PCR assay; the 95% cut-off of the assay is ~250 IU/mL as determined by dilution of the WHO International Standard for HEV RNA [1]. From Europe, 95 835 Swedish and 18 100 German donations were screened, of these, 12 Swedish and four German donations were positive for HEV RNA. Allowing for actual mini-pool size, the rate of HEV-positive donations was 1:7986 and 1:4525 for the Swedish and German donors, respectively. In contrast, no HEV-positive donations were identified in 51 075 donations from the United States. Whenever mini-pools were positive for HEV RNA, individual positive donations were resolved and excluded from pharmaceutical production; 12 of the samples were characterized by molecular and serological analysis (Table 1). Analysis of the HEV strains revealed genotype 3 in all cases. Genotyping was performed by amplification of the ORF2/3 region of the HEV genome using the OneStep RT-PCR kit (Qiagen GmbH, Hilden, Germany) and the forward primer 5′-GGGTGGAATGAATAA CATGT and reverse primer 5′-AGGGGTTGGTTGGATGAA or 5′-GGGCGCTGGCMCTGGTCACGCCAAG. Amplification products were sequenced directly; all sequenced strains were distinct from each other (accession numbers JN995562-JN995573). The closest RNA sequence matches from BLAST searches were between an HEV strain from a wild boar (accession number FJ705359) and samples 6 and 7 obtained from German donors. Viral loads varied between ~3·2–5·7 $\log_{10}$ *IU/ml HEV RNA and are in a similar range* reported for Japanese blood donors [2]. Anti-HEV IgM and anti-HEV IgG in the individual donations were determined using EIAs from Wantai (Wantai, Beijing, China). The majority of samples were window-period donations. Only one viraemic sample was positive for IgM, another for IgG (confirmed by repeat testing using different kit lots). Three samples were initially reactive; however, repeat testing using different kit batches gave negative results. Such

Table 1 Summary of characterization of HEV RNA-positive plasma donations

Sample code	IgM*	IgG†	ALT‡	Viral load $\log_{10}$ IU/ml§	Country of origin
1	+/−	−	Neg.	3·22	Sweden
2	+	+/−	Neg.	3·26	Germany
3	−	−	Neg.	5·35	Germany
4	−	−	Neg.	4·39	Sweden
5	−	+	Neg.	4·95	Sweden
6	+/−	−	Elevated	4·54	Germany
7	−	−	Neg.	4·19	Germany
8	−	−	Neg.	4·76	Sweden
9	−	−	Neg.	3·86	Sweden
10	−	−	Elevated	4·64	Sweden
11	−	−	Elevated	3·20	Sweden
12	−	−	Neg.	5·68	Sweden

ALT, alanine transaminase.

*Positive samples (+) defined as S/Co ≥1 (according to the kit specifications); equivocal samples (+/−) gave an S/Co ≥1 on initial round of testing and S/Co <1 on repeat testing using alternative batches of kit. Negative samples (−). Positive control for IgG was performed using the WHO International Reference Reagent for anti-HEV IgG (95/584).

†> 80 IU/L.

‡RNA titres determined by real-time RT-PCR in comparison to the WHO International Standard for HEV RNA - code number 6329/10 [1].

equivocal results highlight some of the problems with standardization of HEV serological assays. Alanine transaminase (ALT) levels were slightly elevated (80–130 IU/l) in only 3 of 12 viraemic donations tested. This indicates that screening of blood/plasma by ALT is not a reliable measure for exclusion of HEV viraemic donors.

Our results are in accordance with a recent investigation from the UK detecting 6 of 880 mini-pools (with mini-pool comprising 48 donors) positive for HEV RNA [3]. Considering a ratio of 1:4525 viraemic donations in Germany, the limited viraemic titre of some donations, and a size of ~3500 donations per plasma fractionation pool, this could explain why we found ~10% of large plasma pools for fractionation from Germany positive for HEV RNA [4]. We agree with Ijaz *et al.* [3] that such high numbers of HEV-positive blood donations in Europe suggest many subclinical infections [5], and the consequences of HEV contamination of blood and plasma warrants further investigation.

Acknowledgements

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No. 9

医薬品 研究報告 調査報告書

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				2012. 4. 21	該当なし	

一般的名称	人血清アルブミン	研究報告の公表状況	公表国	使用上の注意記載状況・その他参考事項等
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン5%静注12.5g/250mL(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)	Lavoie M, Sharp CP, Pépin J, Pennington C, Foupouapouognigni Y, Pybus OG, Njouom R, Simmonds P. Emerg Infect Dis. 2012 Apr;18(4):680-3. doi: 10.3201/eid1804.110628.	カナダ	赤十字アルブミン20 赤十字アルブミン25 赤十字アルブミン5%静注12.5g/250mL 赤十字アルブミン20%静注4g/20mL 赤十字アルブミン20%静注10g/50mL 赤十字アルブミン25%静注12.5g/50mL 血液を原料とすることに由来する感染症伝播等

研究報告の概要

○ヒトパルボウイルス4感染、カメルーン
2009年にHCV調査のために採取されたカメルーン人の血清サンプルを用いてヒトパルボウイルス4(PARV4)抗体陽性率の調査を行った(60－102歳;中央値70歳、n=451)。その結果、79人(17.5%)がPARV4抗体を有していた。PARV4抗体陽性はマラリア予防薬の静脈注射、結核の非経口治療、避妊薬の筋肉注射に関連があった。また、高齢者よりも60－64歳の初老の人に陽性者が多かった。これはウイルス暴露の経年的変化や、PARV4抗体価が次第に弱まっていき、最終的に偽陰性となることなどが考えられる。また左腕のワクチン接種跡(天然痘ワクチン)が欠如している人は接種跡がある人よりも陽性者が多かった。天然痘ワクチン接種後に跡が残らなかった事は、PARV4感染への感受性と関連した免疫特性を反映している可能性がある。抗体陽性率は性別、HCV抗体、HBc抗体、トレポネーマ抗体の存在と相関しなかった。
今回の調査はある程度のPARV4非経口感染を示唆する。

報告企業の意見	今後の対応
カメルーンの60歳以上の人を対象にヒトパルボウイルス4(PARV4)抗体陽性率についての調査を行ったところ、PARV4抗体陽性者は抗マラリア薬の静脈注射や結核の非経口治療、避妊薬の筋肉注射を受けた人などに見られ、ある程度のPARV4非経口感染が示唆されたとの報告である。 パルボウイルス4は脂質膜を持たない小型のDNAウイルスである。PARV4のヒトにおける病原性の有無は未だ明らかではないが、仮に原料血漿にPARV4ウイルスが含まれていたとしても、平成11年8月30日付医薬発第1047号に沿った類縁モデルウイルスによるプロセスバリデーションで除去・不活化されることが検証されており、本製剤の安全性は確保されていると考える。	本剤の安全性は確保されていると考えるが、念のため今後も情報収集に努め、今後とも輸血用血液及び血漿分画製剤の安全性向上のために努力する。

MedDRA/J Ver.15.0J

Human Parvovirus 4 Infection, Cameroon

Myriam Lavoie, Colin P. Sharp, Jacques Pépin, Christopher Pennington, Yacouba Foupouapouognigni, Oliver G. Pybus, Richard Njouom, and Peter Simmonds

In a post hoc analysis of samples collected in 2009, we determined seroprevalence of parvovirus 4 (PARV4) among elderly Cameroonians. PARV4 seropositivity was associated with receipt of intravenous antimalarial drugs, intramuscular streptomycin, or an intramuscular contraceptive, but not hepatitis C virus seropositivity. Findings suggest parenteral acquisition of some PARV4 infections.

Human parvovirus 4 (PARV4), also known as partetravirus, was identified in 2005 from the plasma of an intravenous drug user (IDU) (*1*). In separate studies that used PCR, PARV4 was subsequently documented in autopsy tissues from IDUs and persons with hemophilia; in bone marrow aspirates from patients with AIDS; and in the blood of transplant recipients, hemodialysis patients, and infants in Ghana (*2–5*).

In 2007, 199 (32.4%) of 626 adults tested in Burkina Faso, Democratic Republic of the Congo, and Cameroon were seropositive by first-generation serologic assay for PARV4 (*6*). In South Africa, prevalence was 36% among HIV-infected blood donors but only 4% among their HIV-seronegative counterparts (*6*). Although PARV4 presence in IDUs and hemophilia patients suggests parenteral transmission (*7,8*), this route has not yet been studied and other modes of transmission have not been ruled out. The pathogenicity of PARV4 remains unclear, but PARV4 DNA recently was found in the cerebrospinal fluid of 2 children from India who had unexplained encephalitis (*9*).

During 2010, to investigate the epidemiology of PARV4 in Africa, we tested for PARV4 antibodies in serum samples collected during a 2009 study of a defined population of elderly Cameroonians among whom prevalence of hepatitis C virus (HCV) infection was high. Previous exposures to parenteral and sexual risk factors had been documented for this population (*10–12*), indicating that this population had been excessively exposed to improperly sterilized syringes and needles and that the main risk factor for HCV was the administration of intravenous antimalarial drugs, mostly before 1960.

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DOI: http://dx.doi.org/10.3201/eid1804.110628

The Study

The ethics committees of the Cameroonian Ministry of Health and the Centre Hospitalier Universitaire de Sherbrooke (Sherbrooke, Quebec, Canada) approved the 2009 study and 2010 follow-up specimen testing. The study was conducted in Ebolowa, southern Cameroon (*10*). Inclusion criteria were age ≥60 years and consent. Exclusion criteria were dementia or inability to communicate. With cooperation from community leaders, we visited a convenience sample of houses to identify participants. We obtained venous samples from participants and gathered sociodemographic data and information about past intravenous treatment for any disease, past parenteral treatment for infectious diseases, transfusions, scarifications, and circumcision. Vaccine scars were documented.

We performed PARV4 IgG detection on each sample in replicate by indirect ELISA by using baculovirus-expressed viral protein 2 and control antigens (*8*); arbitrary unit (AU) values were calculated relative to a control sample. Because of a high background reactivity observed for this cohort, we additionally stipulated that for positive samples, the optical density ratio (ODR) of viral protein 2 to control must be >1.2; ODRs below this threshold were considered negative.

Serologic assays for HCV and treponemal antibodies were described in the original study by Pepin et al. (*10*). We detected antibodies against hepatitis B core antigen (HBcAg) by using AxSYM (Abbott, Montreal, Quebec, Canada) and analyzed data by using Stata 10.0 (StataCorp LP, College Station, TX, USA). Proportions were compared by using either the χ^2 or Fisher exact test. Variables associated with PARV4 seropositivity in univariate analysis were tested in logistic regression models through nonautomated forward selection, continuing until no other variable reached significance. Each variable was then eliminated to assess its effect by using likelihood ratio tests. We retained in the final model variables that enhanced the fit at the $p<0.05$ level.

The study comprised 451 persons 60–102 years of age (median 70 years); 56% were HCV seropositive, 74% had antibodies against *Treponema* (10), and 95% were anti-HBcAg seropositive. Seventy-nine (17.5%) persons carried PARV4 antibodies.

PARV4 antibodies were more prevalent among persons 60–64 years of age than among older persons (Table 1). Prevalence did not vary by sex or by presence of anti-HCV, anti-HBcAg, or treponemal antibodies. The prevalence of anti-PARV4 increased, but not significantly, with

Table 1. Prevalence of human parvovirus 4 by patient characteristics, Cameroon, 2009*

Characteristic	No. virus positive/ no. tested (%)	p value
Age, y		0.04
60–64	32/125 (26)	
65–69	13/96 (14)	
70–74	17/103 (17)	
≥75	17/127 (13)	
Sex		0.15
M	25/178 (14)	
F	54/273 (20)	
HCV serologic results		0.61
Negative	29/178 (16)	
Positive	47/252 (19)	
Anti-HBcAg		1.00
Negative	3/21 (14)	
Positive	76/430 (18)	
Treponemal antibodies		0.06
Absent	28/119 (24)	
Present	51/332 (15)	
Intravenous treatment for malaria		0.04
No	29/216 (13)	
Yes	50/235 (21)	
Intravenous treatment for other diseases		0.93
No	42/239 (18)	
Yes	37/212 (17)	
No. past intravenous treatments		0.38
0	12/88 (14)	
1–3	33/208 (16)	
≥4	25/116 (22)	
Unknown	9/41 (22)	
Tuberculosis		0.04
No	72/433 (17)	
Yes, treated with oral drugs only	4/12 (33)	
Yes, treatment included streptomycin	3/6 (50)	
Transfusion		0.09
No	76/408 (19)	
Yes	3/43 (7)	
Depo-Provera injections†		0.006
No	50/268 (19)	
Yes	4/5 (80)	
Scarifications		0.88
No	30/165 (18)	
Yes	49/286 (17)	
Vaccine scar, left arm		0.005
Absent	17/53 (32)	
Present	61/397 (15)	
Vaccine scar, right arm		0.76
Absent	27/165 (16)	
Present	51/284 (18)	
Circumcision (males only)		0.74
Medical	9/73 (12)	
Traditional	16/105 (15)	

*HCV, hepatitis C virus, HBcAg, hepatitis B core antigen.
†Pharmacia & Upjohn Company, New York, NY, USA.

exposure to intravenous treatments in general. Receipt of intravenous antimalarial drugs was associated with PARV4 seropositivity, which was also more frequent among persons treated for tuberculosis and among the few women who had received injections of the contraceptive Depo-Provera (Pharmacia & Upjohn Company, New York, NY, USA). PARV4 seropositivity was not associated with treatments delivered by injection against yaws, syphilis, leprosy, or trypanosomiasis (data not shown) or with sexually transmitted infections. PARV4 seropositivity was less common among persons who had a vaccine scar on the left arm.

In multivariate analysis (Table 2), PARV4 seropositivity was associated with younger age, intravenous receipt of antimalarial drugs, and parenteral receipt of antituberculosis treatment (the latter was of borderline significance) and was less common among persons with a left-sided vaccine scar. In that model, Depo-Provera injections were associated with PARV-4 seropositivity among women (adjusted odds ratio 17.27, 95% CI 1.57–189.78; p = 0.02).

To confirm that associations were not biased by assay sensitivity, we conducted a secondary analysis that excluded 81 borderline PARV4-negative persons (AU >0.5 and ODR <1.2) and 35 borderline PARV4-positive persons (AU 0.5–2.0, ODR >1.2) (Table 2). The same factors as in the main analysis were associated with PARV4 seropositivity; receipt of intravenous antimalarial drugs was not significant in the smaller sample.

Conclusions

We retrospectively analyzed samples obtained during a study of elderly Cameroonians from an area where HCV infection was hyperendemic and in which we had collected much information about potential parenteral modes of transmission of blood-borne viruses but less information about other routes (*10*). Because this was a cross-sectional study, the time sequence of exposure routes and PARV4 infection could not be determined. Thus, our results should be considered exploratory.

The sensitivity, specificity, and ability of our assay to identify seroconversions are comparable to those of PCR-based methods for determining active infections and past exposure (*7–9,13*). Exclusion of samples showing low antibody levels that might represent nonspecific reactivity had little effect on the analysis of risk factors.

The results provide some evidence for parenteral transmission of PARV4 in the study community. As was HCV infection (*10*), PARV4 infection was associated with receipt of intravenous antimalarial therapy. This risk factor was found for half of the population we studied, whereas intramuscular Depo-Provera and streptomycin were administered to few patients. In univariate analysis, PARV4 seropositivity was also more common in patients treated with oral antituberculosis drugs. Although the seroprevalence of PARV4 increased with past exposure to intravenous treatments in general, this finding was not statistically significant because antibodies against PARV4 were common among persons who reported no such treatments. This finding, and the lack of association between PARV4 and HCV seropositivity, suggests that other, nonparenteral modes of transmission existed.

Table 2. Correlates of study participants and human parvovirus 4 infection in multivariate analysis, Cameroon, 2009

Participant characteristic	All participants		After exclusions*	
	Adjusted odds ratio (95% CI)	p value	Adjusted odds ratio (95% CI)	p value
Age group, y				
60–64	2.21 (1.13–4.31)	0.02	2.88 (1.16–7.17)	0.02
65–69	1.01 (0.46–2.24)	0.98	1.20 (0.39–3.70)	0.76
70–74	1.16 (0.54–2.46)	0.71	1.51 (0.55–4.16)	0.42
>75	1.00		1.00	
Tuberculosis				
No	1.00		1.00	
Yes, treated with oral drugs only	2.09 (0.58–7.54)	0.26	2.91 (0.63–13.51)	0.17
Yes, treatment included streptomycin	5.21 (0.99–27.37)	0.05	20.96 (1.67–262.99)	0.02
Vaccine scar, left arm				
Absent	1.00		1.00	
Present	0.37 (0.19–0.71)	0.003	0.32 (0.13–0.78)	0.01
Intravenous treatment for malaria				0.06
No	1.00		1.00	
Yes	1.92 (1.13–3.24)	0.015	1.98 (0.97–4.03)	0.06

*After exclusion of 81 participants with borderline negative results and 35 with borderline positive results.

PARV4 seropositivity was more common in persons 60–64 years of age than in older persons. This finding has 3 potential explanations. First, exposure to the virus might have fluctuated over time. Second, titers of antibodies against PARV4 might progressively wane, eventually leading to false negative results. Third, PARV4 infection might increase long-term risk for death, although this explanation seems unlikely.

Absence of a vaccine scar on the left arm was associated with PARV4 seropositivity. Historical and epidemiologic data suggest that in Cameroon, the left side was used for smallpox vaccine and the right side for *Mycobacterium bovis* BCG (*14*,*15*). Failure of scar development after smallpox vaccination might reflect immunologic characteristics associated with greater susceptibility to PARV4 infection.

Our findings suggest that some parenteral transmission of PARV4 occurred among elderly Cameroonians, but parenteral transmission might not have been the main route of infection. The association with past tuberculosis, although perhaps coincidental, is intriguing and deserves further study.

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医薬品 研究報告 調査報告書

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研究報告の概要

○血友病の被験者におけるヒトパルボウイルス4の一次感染についてのウイルス学的、臨床的な特徴:ウイルス不活化凝固因子製剤による高頻度な伝播

背景:ヒトパルボウイルス4(PARV4)は、静注薬物常習者及び非ウイルス不活化凝固因子製剤に曝露した血友病患者を含む非経口曝露歴を持つ人々において、新たに発見されたパルボウイルスである。血漿由来ウイルス不活化凝固因子製剤の治療を受けている血友病患者に対するPARV4の潜在的伝播を調査するため、血友病患者の大規模集団(194人、7～16歳)におけるPARV4抗体の陽転化について、5年以上の期間、スクリーニングを行った。

研究計画及び方法:Hemophilia Growth and Development Studyコホートに登録の血友病患者194人に、ウイルス不活化凝固因子製剤のみの治療の開始時及び5年間の調査終了時に、PARV4抗体スクリーニング検査を実施した。研究期間中に抗体陽転化している被験者から、中間時点でのサンプルが採取され、抗体陽転化の時期の絞り込み及びIgM応答、急性ウイルス血症の持続期間、臨床症状を調査するためにスクリーニングが行われた。

結果:研究開始時のPARV4抗体陽性率は44%であった。観察期間中、9人の被験者(そのうち7人はHIV陽性)においてPARV4抗体が陽転化した(発生率, 1.7%/年)。感染した被験者は比較的長期のウイルス血症期間(平均7カ月)及び感染急性期に弱い一過性のIgM応答を示した。有機溶媒/界面活性剤や液状加熱、乾燥加熱により不活化された凝固因子製剤は感染性があった。最も共通する臨床症状は発疹及び肝障害であった。

結論:この研究によって、PARV4はウイルス不活化処理に耐性を持つ輸血感染性病原体であることが確認された。血漿由来血液製剤を使用する人々において、今もなお定期的に感染が発生する可能性が懸念される。治療を受けている個人におけるPARV4の発生率及びPARV4感染に関連する疾患についての早急な評価が必要とされる。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン5%静注12.5g/250mL
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

9

報告企業の意見	今後の対応
血友病患者における大規模調査によって、ウイルス不活化処理済みの血漿由来凝固因子製剤におけるパルボウイルス4(PARV4)の感染性が示され、PARV4はウイルス不活化に耐性を持つ輸血感染性病原体であることが確認されたとの報告である。 パルボウイルス4は脂質膜を持たない小型DNAウイルスのため、ナノフィルトレーション工程のない凝固因子製剤ではウイルス除去は困難と考えられるが、本製剤では仮に原料にPARV4様ウイルスが含まれているとしても、平成11年8月30日付医薬発第1047号に沿った類縁モデルウイルスによるプロセスバリデーションで除去・不活化されることが検証されており、本製剤の安全性は確保されていると考える。	本剤の安全性は確保されていると考えるが、念のため今後も情報収集に努め、今後とも輸血用血液及び血漿分画製剤の安全性向上のために努力する。

MedDRA/J Ver.15.0J

JRC2012T-019

TRANSFUSION COMPLICATIONS

Virologic and clinical features of primary infection with human parvovirus 4 in subjects with hemophilia: frequent transmission by virally inactivated clotting factor concentrates

Colin P. Sharp, Alice Lail, Sharyne Donfield, Edward D. Gomperts, and Peter Simmonds

BACKGROUND: Human parvovirus 4 (PARV4) is a newly discovered parvovirus prevalent in injecting drug users and other groups with histories of parenteral exposure including persons with hemophilia exposed to non-virally inactivated clotting factor concentrates. To investigate its potential ongoing transmission to persons with hemophilia treated with plasma-derived, virally inactivated clotting factors, we screened a large cohort of persons with hemophilia for antibody seroconversion to PARV4 over a 5-year observation period.

STUDY DESIGN AND METHODS: Samples from 195 persons with hemophilia enrolled in the Hemophilia Growth and Development Study cohort were screened for PARV4 antibodies at the start and end of a 5-year period of treatment with exclusively virally inactivated clotting factor concentrates. Samples collected at intermediate time points from subjects seroconverting over the study period were screened to narrow down the seroconversion time and investigate immunoglobulin (Ig)M responses, duration of acute viremia, and clinical presentations.

RESULTS: PARV4 seroprevalence at the outset of the study was 44%. Over the observation period, nine subjects (seven human immunodeficiency virus positive) seroconverted for anti-PARV4 (incidence, 1.7%/year). Infected subjects showed relatively prolonged durations of viremia (mean, 7 months) and weak, transient IgM responses during acute infections. Clotting factors inactivated by solvent/detergent or by wet or dry heat were infectious. The most common clinical presentations were rashes and exacerbation of hepatitis.

CONCLUSION: This study identifies PARV4 as a transfusion-transmissible agent that is resistant to viral inactivation. Of concern, infections may still regularly occur in those exposed to plasma-derived blood products. Urgent evaluation of the incidence of PARV4 in treated individuals and disease associations of PARV4 infections is required.

A new parvovirus was recently identified by molecular virus discovery methods from plasma from a human immunodeficiency virus (HIV)-infected injecting drug user (IDU).[1] Genetically, the virus termed human parvovirus 4 (PARV4) was found to be distinct from existing genera within the family *Parvoviridae*, although viruses showing 61% to 63% sequence similarity to PARV4 have since been described in pigs and cows.[2] It has recently been proposed to assign all of these viruses as members of a new parvovirus genus, *Partetravirus*. PARV4 is now one of four parvoviruses known to infect humans. Others include the erythrovirus, B19V, transmitted by respiratory routes, highly prevalent, and generally associated with mild disease on acute infection. Adenoassociated viruses, which are acquired frequently during childhood, persist lifelong but are thought

ABBREVIATIONS: HGDS = Hemophilia Growth and Development Study; IDU(s) = injecting drug user(s); PARV4 = human parvovirus 4.

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to be entirely nonpathogenic. Human bocavirus Type 1 has been implicated in severe respiratory disease in children (reviewed in Allander et al.[3]), while the genetically distinct Types 2 through 4 are more typically recovered from fecal samples and may be an additional cause of enteric disease in children and adults.[4,5]

Several investigations, subsequent to the discovery of PARV4, have established that infections with PARV4 are likely acute resolving without long-term viremia. However, as also described for B19V and adenoassociated viruses,[6-8] viral DNA sequences can be detected in tissues likely lifelong after exposure.[9-11] The epidemiology and transmission of PARV4 differs strikingly from other parvoviruses. Through the use of autopsy tissue to record past infection, high rates of PARV4 exposure were identified among IDUs in the United Kingdom and Italy.[9,11,12] Infection frequencies were higher in those who are HIV seropositive but almost absent in low-risk, hepatitis C virus (HCV)-negative/HIV-negative control populations.

To facilitate further investigations of PARV4 exposure, we developed a serologic assay for PARV4 antibodies, using baculovirus-expressed VP2 proteins as antigen in an indirect enzyme-linked immunosorbent assay (ELISA).[13] Larger-scale screening confirmed associations between PARV4 infection and parenteral routes of exposure (IDUs) in the United Kingdom and United States, lower infection frequencies in HIV-infected gay men, and a complete absence in the general, low-risk population. This study also presented the first evidence for PARV4 infection through the use of clotting factor concentrates; 15 from 35 persons with hemophilia exposed to non–virally inactivated Factor VIII/IX concentrates were PARV4 seropositive, in contrast to only 1 from 35 sibling controls occupying the same household.

Although the epidemiology of PARV4 in Western countries is relatively well established, PARV4 infections are much more common and likely transmitted by different routes in sub-Saharan Africa. A high rate of PARV4 viremia likely associated with primary infection was recorded among children aged between 1 and 2 years in rural Ghana.[14] Using the VP2 serology assay, we recorded seroprevalences of 25% to 37% in the Cameroon, Burkina Faso, and Democratic Republic of the Congo.[15] A more recent study described a 10% anti-PARV4 seroprevalence among children with a mean age of 4.5 years in Southern India; acute infections of two individuals in the latter study were associated with severe encephalitis, representing an important although likely rare potential disease association of PARV4.[16]

Given the evidence for a predominantly parenteral route of PARV4 infection in Western countries, we have quantified the risk of infection through treatment of persons with hemophilia with plasma-derived blood products previously shown to be the source of widespread infection with HIV-1 and HCV in this patient group. Samples collected at the start and end of a 5-year observation period were assayed for anti-PARV4 to identify individuals infected during treatment with virally inactivated clotting factor concentrates. The cohort design provided samples at 6-month intervals enabling dates of infection to be narrowed down in seroconverting over the study period. The virologic and clinical analysis of the infection events, along with analysis of the types of clotting factor administered provided a detailed characterization of the infection risk of PARV4 in this vulnerable patient group and its clinical outcomes.

MATERIALS AND METHODS

Study subjects

Samples from 194 persons with hemophilia were obtained from the Hemophilia Growth and Development Study (HGDS) cohort.[17] Members of the group with hemophilia were born between 1972 and 1982, were between 7 and 16 years of age at study entry, and were between 10 and 21 years at the time study samples were taken. All HGDS study subjects with hemophilia had used non–virally inactivated clotting factor concentrate at some time before enrolment. Nine or more infusions over that period, or 100+ U/kg body weight of factor per year over the 2 years were required for eligibility. Paired samples from each subject, one each from the time of enrollment and one from the end of the study 5 years later, were used for the initial screening and samples from intermediate time points of approximately 6-month intervals were obtained from subjects showing evidence of PARV4 immunoglobulin (Ig)G seroconversion. Follow-up history was collected and a physical exam conducted at 6-month intervals. These were focused on physical growth and development, immunologic, neurologic, and neuropsychological functioning. Blood samples were collected at each of these visits, portions of which were placed in a central repository for subsequent testing. The human subjects committees of collaborating institutions approved the HGDS, informed consent was obtained from parents or legal guardians, and informed consent or assent was obtained from all participants, in compliance with the human experimentation guidelines of the US Department of Health and Human Services and in accordance with the Declaration of Helsinki.

PARV4 ELISA

Samples were tested using the previously described ELISA protocol and analysis variables[13] based on recombinant PARV4 capsid protein produced in a baculovirus expression system. Anti-PARV4 IgM was measured using a modification of the previously described protocol in which the anti-human IgG:horseradish peroxidase (HRP) conjugate

was replaced with a goat anti-human IgM:HRP conjugate (AbD Serotec, Oxford, UK). The IgM assay cutoff was calculated as for the IgG assay[13] (optical density [OD] of the mean plus 2 standard deviations of negative controls [IgG-negative, low-risk group]).

TABLE 1. PARV4 serologic status at start and end of study period*

Subjects	PARV4 status†		
	Positive/positive	Negative/positive	Negative/negative
Total	84 (43)	9 (5)	101 (52)
HIV-positive	61 (46)	7 (5)	65 (49)
HIV-negative	23 (38)	2 (3)	36 (59)

* Data are reported as number (%).
† PARV4 serologic status at start/end of study period.

Detection of PARV4 DNA by polymerase chain reaction

DNA was extracted from 200 µL of serum using a DNA blood mini kit (QIAamp, Qiagen, West Sussex, UK) according to manufacturer's instructions. Samples were screened using a nested polymerase chain reaction (PCR) for PARV4 DNA and other members of the *Partetravirus* genus, including all currently identified PARV4 genotypes to single copy sensitivity. First-round reactions were performed with 5 µL of extracted DNA using reagents (GoTaq, Promega, Southampton, UK) according to manufacturer's instructions with the outer primer pair (PARV4_OS TGA AYC AGA CCT TGA RCG SCC and PARV4_OAS CCM CCH AWC CAY TGA GCT TTW ACT TT). Second-round reactions were performed using 1 µL of first-round product as template and the inner primer pair (PARV4_IS TAY AAT TAT GTT GGT CCT GGT AAT CC and PARV4_IAS GGT AAR ACC TGY GAW ADT TGA ACA TC). Semiquantitation of viral DNA load was performed using 10-fold dilutions of extracted serum DNA in 0.05 µg/µL salmon testes carrier DNA (Sigma, Gillingham, UK) as template for the nested PCR. Endpoint titers of 50% positivity in replicate reactions were calculated using the Reed-Muench formula.[18]

Direct sequencing of PCR products and sequence analysis

Positive second-round PCR amplicons were sequenced in both directions using the inner sense and inner antisense primers used in the second round of amplification. Sequencing was carried out using a cycle sequencing kit (BigDye Terminator v3.1, Applied Biosystems, Paisley, UK) according to manufacturer's instructions. Sequences were read at The Gene Pool facility (University of Edinburgh, Edinburgh, UK) and analyzed using computer software (SSE v1.0, P. Simmonds, Edinburgh, UK).

RESULTS

HGDS cohort

A total of 194 subjects with hemophilia were sampled at 6-month intervals over a 5-year period between 1989 and 1994. Subjects were 7 to 16 years on entry, and all had received non–virally inactivated clotting factor concentrate before enrollment. A total of 133 (69%) were seropositive for HIV-1, and all but four were seropositive for HCV at enrollment. To estimate exposure of the group to PARV4, samples collected at enrollment were screened for anti-PARV4 VP2 IgG ELISA (Table 1). A total of 84 from 194 subjects (43%) were anti-PARV4 positive. PARV4 exposure was not significantly associated with HIV infection, 61 from 133 (46%) HIV-positive subjects were anti-PARV4 positive compared to 23 from 61 (38%) of HIV-negative subjects ($p = 0.35$ by Fisher's exact test; not significant).

Infection with PARV4 during study period

Samples collected at the end of the 5-year study period, during which time all subjects received only virally inactivated clotting factor concentrates, were similarly screened for PARV4 antibodies to identify further PARV4 infections. All subjects considered seropositive for PARV4 at the start of the study remained seropositive. However, of the initially anti-PARV4–negative group, nine subjects became anti-PARV4 positive (Table 1). Of these, seven were HIV positive and two were HIV negative (7/72 compared to 2/38; $p = 0.5$). Four further subjects showed small sustained increases in serologic reactivity during the study period to levels ranging from 0.5 to 2 units. As other tests were negative, these four subjects have been considered to be uninfected for the purpose of analysis. No seroconversions for HIV-1 occurred over the study period.

From the nine subjects showing evidence of primary infection, further samples taken at approximately 6-month intervals were tested by ELISA (Table 2) and used to narrow the time window of primary infection. Seroconversion occurred between 1989 and 1991 in the seven HIV-positive subjects and between 1992 and 1993 in the HIV-negative subjects (Fig. 1). Samples from or immediately preceding the time of seroconversion were analyzed for anti-PARV4 VP2 IgM. Two of the HIV-positive subjects (subjects "B" and "E") showed a clear IgM reactivity concurrent with the first IgG-positive sample. The short-lived nature of the IgM response was confirmed by testing samples collected at subsequent time points from the positive individuals, all of which were found to show no reactivity.

Direct detection of PARV4 viral DNA by PCR

To estimate the duration of PARV4 viremia during acute infection, DNA extracted from the serum of seroconvert-

TABLE 2. Time course for anti-PARV4 seroconversion and other markers of acute infection during study period

Subject	Exam date	PARV4 IgG arbitrary units (cutoff 0.5)	PARV4 IgM OD ratio (cutoff 1.3)	PARV4 PCR	Viral titer (DNA copies/mL)
A	July 1989	0.03	NT	NT	
	March 1990	0.00	NT	–	
	October 1990	0.00	0.99	+	10^4
	March 1991	48.55	1.03	–	
	July 1991	32.66	NT	NT	
B	May 1989	0.00	NT	NT	
	October 1989	0.20	NT	NT	
	May 1990	0.00	1.00	–	
	November 1990	30.87	1.44	+	10^2
	May 1991	100.00	0.99	–	
C	June 1989	0.00	1.12	–	
	January 1990	64.28	1.22	+	10^4
	November 1990	100.00	NT	–	
	August 1991	100.00	NT	NT	
D	July 1989	0.15	NT	NT	
	May 1990	0.00	NT	NT	
	September 1990	0.00	NT	–	
	March 1991	0.00	1.10	+	10^{10}
	October 1991	100.00	1.01	–	
E	March 1990	0.18	NT	NT	
	August 1990	0.00	1.03	–	
	April 1991	29.47	1.67	+	$<10^3$
	October 1991	24.42	0.92	–	
	April 1992	27.30	NT	NT	
F	May 1989	0.00	NT	–	
	December 1989	0.12	1.06	–	
	June 1990	18.22	1.18	+	$<10^3$
	October 1990	5.16	NT	–	
	June 1991	3.14	NT	NT	
G	July 1992	0.24	NT	–	
	December 1992	0.00	1.00	+	$<10^3$
	August 1993	100.00	1.05	+	$<10^3$
	June 1994	95.10	NT	–	
H	September 1992	0.00	1.16	–	
	March 1993	27.77	1.14	+	10^9
	September 1993	28.55	NT	–	
	September 1994	18.76	NT	NT	
I	January 1990	0.12	NT	NT	
	June 1990	0.22	1.21	–	
	February 1991	48.99	1.01	+	10^5
	June 1991	78.69	NT	–	
	December 1991	76.79	NT	NT	

NT = not tested.

ing subjects was screened by nested PCR. As with IgM screening, samples from and immediately preceding seroconversion were screened. Viral DNA could be found in all of the seroconverters. In six of the nine PCR-positive subjects, the sample from the time of seroconversion was positive; in two of the nine, the sample immediately before the conversion sample was positive; and in the remaining subject there was a sustained detection at both the time point before and at seroconversion for antibody. The predicted resolution of viremia was confirmed by testing of further samples from time points before and after positive results and demonstrating that these further samples were all negative.

Amplicons from PCR-positive subject samples were sequenced using both second-round primers. All samples were confirmed to be PARV4 and identified as Genotype 1 (Subjects F, G, and H) and Genotype 2 (Subjects A-E and I). Viral titers were assessed by semiquantitative PCR using 10-fold dilutions of serum DNA template in quadruplicate. As the PCR used has been shown to be sensitive to a single copy, the limiting dilution results were used to calculate semiquantitative plasma viral titers (Table 2). Despite the relatively wide sampling intervals in the study, intense viremia was detected in two of the seroconverting subjects (Subject D, 10^{10} DNA copies/mL; Subject H, 10^9 DNA copies/mL).

Clinical presentations concurrent with PARV4 infections

Treatment histories, blood chemistry, and clinical data from physical examinations during sampling visits were available from each seroconverting subject (Table 3). A wide range of different manufactured clotting factor concentrates administered in the 6 months before seroconversion were associated with PARV4 infection, including both solvent/detergent (S/D)- and heat-treated materials. The absence of HIV-1 seroconversions over this period is consistent with a greater resistance of PARV4 to the virus inactivation methods used in this period.

Persons with hemophilia presented with a range of symptoms and signs during the period of anti-PARV4 seroconversion. For the HIV-positive subjects, many of these likely relate directly to comorbidities associated with HIV-1 infection (for example, the lymphadenopathy of Subjects B and F was also noted in previous examinations) and hemophilia (such as joint problems, etc.). Disregarding these, the only repeatedly observed clinical presentations were rashes (Subjects D, F, and I) and unexplained hepatitis in the two HIV-negative subjects, Subjects G and H, although these were not associated with significant elevation in alanine aminotransferase or aspartate aminotransferase levels during the period of acute PARV4 infection (alanine aminotransferase values of 18, 26, 16, and 17 international units [IUs]/mL for the four test samples listed in Table 2 from Subject G and 58, 98, 85, and 34 IU/mL for the four listed samples from Subject H). No clinically presenting cases of meningitis or encephalitis or neurologic abnormalities (other than associated with hemophilia complications) were found in the cohort. No measured variables on clinical chemistry (liver enzymes, biochemistry) or hematologic (cell counts, hemoglobin) testing showed consistent associations with acute infection with PARV4 (data provided at http://www.virus-evolution.org/Downloads/Trans-2011-0496/).

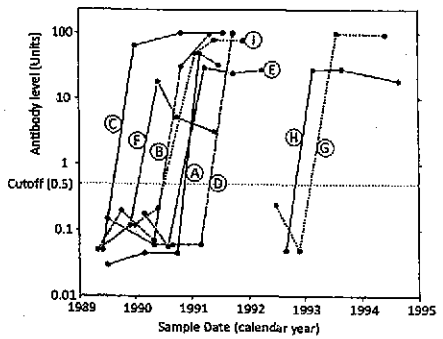

Fig. 1. Antibody levels in sequential samples. PARV4 anti-VP2 levels in sequential samples from the nine seroconverting subjects. Subjects A through F and I were HIV infected; the later seroconversions (H, G) were HIV uninfected.

DISCUSSION

This study documents, for the first time, the occurrence and virologic features of acute infection in persons with hemophilia with PARV4 during a period when treatment was restricted to virally inactivated clotting factor concentrates. Both the initial seroprevalence (43%) and the measured incidence of infection over this period (nine infections among 110 initially PARV4-uninfected individuals) is consistent with previous reports for much higher rates of PARV4 infection in this group than the background population and sibling controls[13] that can best be explained by their parenteral exposure to plasma-derived blood products.

The association of viremia with IgG seroconversion furthermore provides convincing evidence of the sensitivity and specificity of the anti-PARV4 ELISA. As discussed previously,[13] the use of baculovirus-derived VP2 antigens from PARV4 along with control antigens from mock-infected *Spodoptera frugiperda* 9 cells creates a resilient serologic assay with low levels of nonspecificity, as dem-

TABLE 3. Clinical background and presentations at primary infection

Subject	Blood product type*	Inactivation†	HIV	Presentation‡
A	M1	DH	Positive	Sore throat, fever
B	M2 and M3	SD/WH	Positive	Enlarged LNs (also pre)
C	M4	WH	Positive	Enlarged LNs
D	NR-AI	SD or WH	Positive	Rash, shingles
E	NR-heat treated	WH or DH	Positive	None
F	M5-AI	SD	Positive	Rash, enlarged LNs (also pre)
I	M4	WH	Positive	Rash (also pre), pneumonia
G	M1 and M3	WH	Negative	Hepatitis exacerbation
H	M6-AI and M1	SD/WH	Negative	Hepatitis exacerbation

* Manufacturer code (M1-M6; NR = not recorded) and type of clotting factor concentrate administered in the 6 months before seroconversion for PARV4. AI = monoclonal antibody affinity isolated preparations.
† Inactivation method used: DH = dry heat (typically 80°C); WH = wet heat (pasteurization; typically 60°C); SD = S/D inactivation.
‡ Clinical presentation in examination at first time point after seroconversion for PARV4. LNs = lymph nodes.

onstrated by the complete absence of serologic reactivity in control populations in Edinburgh and France without histories of parenteral exposure. The sensitivity of the assay is demonstrated by the uniformly high and rapid rises in serologic reactivity in acutely infected individuals (Fig. 1); PARV4 antibody levels in the initial positive samples ranged from 18 to more than 100 antibody units, where the cutoff for the assay (based on variance of negative controls) was set at 0.5. Using an assay format similar to the one in the current study, Lahtinen and coworkers[19] have similarly documented high and rising optical densities, codetection of IgM, and viremia over at least a 4-week period in two HIV-positive individuals likely exposed to PARV4 through needle-sharing injecting drug use. As for B19V,[20] the performance of VP2-based assays is likely enhanced by the spontaneous folding of expressed protein into virus-like particles that reproduces both linear and conformational epitopes exposed during PARV4 infection.

The association of seroconversion with intense PARV4 viremia recapitulates what occurs during primary infections with the erythrovirus B19V. For the latter, virus levels peak over a period of a week during primary infection, then decline to low levels that may persist for several weeks or months after primary infection at levels of approximately 10^2 to 10^3 IUs/mL. Although the relatively wide spacing of study samples precluded detailed analysis of the virologic events during acute PARV4 infections, the observation of two individuals with high-level viremia (10^9-10^{10} DNA copies/mL) and several more with lower levels (<10^5-10^4 copies/mL) is consistent with the pattern of intense viremia and relatively slow resolution observed in B19V.

As well as documenting the natural history of PARV4 infection, the study provides clear evidence for the infectivity of clotting factor concentrates that had been virally inactivated using methods effective for the enveloped viruses, HIV-1, HCV, and hepatitis B virus.[21] The nine infections that occurred during the study period originated from treatment with a variety of different clotting factors that reflects the diversity of manufacturers' blood products at that time. While it might be anticipated that PARV4, along with B19V and hepatitis A virus, might be resistant to S/D-based inactivation methods, PARV4 was additionally resistant to pasteurization and other heat treatments. Without knowing the viral loads of PARV4 in the source plasma used to make the various blood products, it is difficult to conclude whether these viral inactivation steps reduced PARV4 infectivity. An incidence of nine infections in 110 exposed individuals in 5 years is lower than the incidence of PARV4 infection before study enrolment, where 84 from 194 persons with hemophilia became infected. With a mean age at enrolment of 11.5 years, this amounts to an annualized incidence of 3.7%, over twice that observed in the study period (1.6%). However, contributing to the lower incidence in the study period may have been reductions in the numbers of PARV4-viremic donors, coinciding with the introduction of screening for HIV-1 screening and latterly for HCV, both associated with IDU risk behaviors shared with PARV4. Viral inactivation steps have therefore led to, at best, a modest reduction in the infection risk of plasma-derived clotting factors for PARV4.

The inactivation resistance of PARV4 resembles that of B19V, where treatment-associated infections have continued throughout the period of use of virally inactivated clotting factors. Both B19V and canine parvovirus used a model virus in previous virus inactivation experiments that has been previously shown to be resistant to S/D inactivation methods and moderately resistant to heat inactivation.[22,23] For B19V, the only effective step to prevent transmission is screening source plasma units for B19V DNA, thereby preventing high-titer donations from contamination plasma pools used subsequently for manufacturing, restricted in Europe to 10^4 IUs/mL.[24]

The ongoing risk of transmission of PARV4 to recipients of blood and blood products requires as yet undetermined information on its incidence and viremia frequency in blood and plasma donors, the effectiveness of predonation exclusion of individuals with identifiable risk factors for PARV4 infection (such as current injecting drug use), its cooccurrence with HCV and HIV-1 infections that would lead to donation exclusion, its partitioning into plasma components, the presence and titer of neutralizing antibody from other plasma units, and finally, its resistance to currently used viral inactivation procedures (that are more effective than those used during the study period). Although much of the information required for this risk assessment is currently undetermined (particularly donor incidence and viral loads), detection frequencies of PARV4 DNA in recently collected commercial source plasma pools from several sources have been found to be relatively low (4%; 14 from 351[25]) and generally with low viral loads (<200 DNA copies/mL). PARV4 DNA similarly contrasts with B19V in being infrequently detectable in clotting factor concentrates; in recent studies, 0% to 9% of recently manufactured solvent- or heat-inactivated concentrates were a PARV4 DNA positive.[25-27]

On the other hand, increased infectivity may originate from a likely much lower frequency of comanufactured PARV4 antibody–positive plasma units. In the case of B19V, virus neutralization has been considered an important factor in restricting the infectivity of both pooled plasma and blood products to those with relatively high viral loads (>10^7 IUs/mL[28]). This protective effect may not extend to a plasma-donating population with a low seroprevalence of anti-PARV4.

The final factor in the evaluation of PARV4 as a potential threat to blood product safety is its capability for causing disease in acutely or chronically infected individuals. Acute infections have been particularly problem-

atic to investigate; those few that have been identified to date were usually HIV coinfected with a series of comorbidities that complicate clinical assessments.[1,19] It is difficult to draw analogies with other parvoviruses because their clinical presentations vary considerably; B19V causes mild disease and rash, but can cause fetal damage as well as aplastic anemia in those with existing hematologic disease. In contrast, the recently discovered human bocaviruses have been associated with respiratory disease in young children, and for HBoV Type 2, gastroenteritis. Disease presentations in animal parvoviruses are similarly diverse.

A recent study describes acute PARV4 infections in association with severe encephalitis in two children in Southern India,[16] although its exact causative role remains to be firmly established. Certainly, despite close and lifelong clinical monitoring, no similar occurrences of neurologic presentations have been recorded in persons with hemophilia who have been exposed to PARV4, although as described, coinfection with HIV-1 may complicate clinical assessments. Given the extremely high incidence of PARV4 infection in young children in sub-Saharan Africa and likely Southern and Southeast Asia, severe neurologic disease of the severity described by Benjamin and coworkers[16] must be rare. Among the nine infected persons with hemophilia in the current study, seven were HIV coinfected and their clinical presentations of lymphadenopathy and possibly rash were potentially HIV related. It was, however, intriguing to note the occurrence of acute hepatitis in the two HIV-negative subjects at the time of primary infection. While PARV4 DNA sequences have been detected in liver and the virus may be hepatotropic,[10] it is possible that hepatitis symptoms originated from an exacerbation of chronic HCV infection rather than a de novo infection.

In summary, PARV4 is a transfusion-transmissible agent that is resistant to viral inactivation. PARV4 may still be regularly transmitted by plasma-derived blood products; determining the frequency with which this occurs and disease associations of PARV4 during primary and long-term infection both require urgent evaluation.

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CONFLICT OF INTEREST

None.

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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
				2012年9月3日	該当なし。	
一般的名称	別紙のとおり。	研究報告の公表状況	Flu News & Spotlights, 2012; August 31		公表国 米国	
販売名（企業名）	別紙のとおり。					

研究報告の概要

問題点：CDCは新たに12例の人に対するH3N2vインフルエンザ感染とともに、オハイオ州から報告のあった初めての死亡例について発表した。

CDCは新たに12例のH3N2vインフルエンザ感染とともに、オハイオ州から報告のあった初めての死亡例について発表した。患者は、複数の基礎疾患を持つ高齢者で、祭りに出品されていたブタに直接暴露していた。このウイルスのヒト-ヒト感染は限られており、散発的に発生しているが、集団における持続的な伝播は認められていない。今年の夏、当局は重大なインフルエンザ合併症に対するリスクが高い人に、ブタあるいはブタの囲い場を避けるよう勧めている。

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。

10

MedDRA/J ver. 15.0

別紙

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤人免役グロブリン、⑥乾燥ペプシン処理人免疫グロブリン、⑦乾燥ペプシン処理人免疫グロブリン、⑧乾燥スルホ化人免疫グロブリン、⑨乾燥スルホ化人免疫グロブリン、⑩乾燥スルホ化人免疫グロブリン、⑪乾燥スルホ化人免疫グロブリン、⑫乾燥スルホ化人免疫グロブリン*、⑬乾燥濃縮人活性化プロテインC、⑭乾燥濃縮人血液凝固第Ⅷ因子、⑮乾燥濃縮人血液凝固第Ⅷ因子、⑯乾燥濃縮人血液凝固第Ⅷ因子、⑰乾燥濃縮人血液凝固第Ⅸ因子、⑱乾燥濃縮人血液凝固第Ⅸ因子、⑲乾燥濃縮人血液凝固第Ⅸ因子、⑳乾燥濃縮人血液凝固第Ⅸ因子、㉑乾燥濃縮人血液凝固第Ⅸ因子、㉒乾燥濃縮人血液凝固第Ⅸ因子、㉓乾燥抗破傷風人免疫グロブリン、㉔抗HBs人免疫グロブリン、㉕トロンビン、㉖フィブリノゲン加第ⅩⅢ因子、㉗フィブリノゲン加第ⅩⅢ因子、㉘乾燥濃縮人アンチトロンビンⅢ、㉙ヒスタミン加人免疫グロブリン製剤、㉚人血清アルブミン*、㉛人血清アルブミン*、㉜乾燥ペプシン処理人免役グロブリン*、㉝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ガンマーグロブリン筋注450mg/3mL「化血研」、⑤ガンマーグロブリン筋注1500mg/10mL「化血研」、⑥献血静注グロブリン“化血研”、⑦献血グロブリン注射用2500mg「化血研」、⑧献血ベニロンーⅠ静注用500mg、⑨献血ベニロンーⅠ静注用1000mg、⑩献血ベニロンーⅠ静注用2500mg、⑪献血ベニロンーⅠ静注用5000mg、⑫ベニロン*、⑬注射用アナクトC2,500単位、⑭コンファクトF注射用250、⑮コンファクトF注射用500、⑯コンファクトF注射用1000、⑰ノバクトM注射用250、⑱ノバクトM注射用500、⑲ノバクトM注射用1000、⑳ノバクトM静注用400単位、㉑ノバクトM静注用800単位、㉒ノバクトM静注用1600単位、㉓テタノセーラ筋注用250単位、㉔ヘパトセーラ筋注200単位/mL、㉕トロンビン“化血研”、㉖ボルヒール、㉗ボルヒール組織接着用、㉘アンスロビンP500注射用、㉙ヒスタグロビン皮下注用、㉚アルブミン20%化血研*、㉛アルブミン5%化血研*、㉜静注グロブリン*、㉝アンスロビンP1500注射用
報告企業の意見	インフルエンザウイルスは70～120nmの球形または多形性で、核酸は8本の分節状マイナス一本鎖RNA、エンベロープを有し、エンベロープの表面に存在する赤血球凝集素（HA）とノイラミダーゼ（NA）の抗原性により16種類のHA亜型および9種類のNA亜型に分類される。 今回の報告は、インフルエンザA（H1N1）09pdm由来のM遺伝子を有するブタインフルエンザA（H3N2）の変異株（H3N2v）への感染者における初めての死亡例報告であるが、患者は複数の基礎疾患を有しており、農業見本市で直接ブタと接触していた。また、同変異株の人での集団伝播は認められていない。 上記製剤の製造工程には、冷アルコール分画工程、ウイルス除去膜ろ過工程、加熱工程等の原理の異なるウイルスクリアランス工程が導入されており、各工程のウイルスクリアランス効果は「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）」に基づく、モデルウイルスを用いたウイルスプロセスバリデーションにより確認されている。今回報告したブタインフルエンザA（H3N2v）ウイルスのモデルウイルスには、エンベロープの有無、核酸の種類等から、ウシウイルス性下痢ウイルス（BVDV）が該当すると考えられるが、上記工程のBVDVクリアランス効果については上記バリデーションにより確認されている。また、これまでに上記製剤によるブタインフルエンザA（H3N2v）への感染報告例は無い。 以上の点から、上記製剤はインフルエンザ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

INF2012-001

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CDC Reports More Cases, Hospitalizations and Nation's First H3N2v-Associated Death

The information contained in this web update reflects the situation at the time of posting. It may not reflect the current situation. Please see Influenza A (H3N2) Variant Virus Outbreaks (/flu/swineflu/h3n2v-outbreak.html) for the most recent information related to H3N2v.

Today, CDC is reporting 12 additional cases of H3N2 variant virus (H3N2v) infection, as well as the first H3N2v-associated death, which was reported by the state of Ohio. The death occurred in an older adult with multiple underlying health conditions (/flu/about/disease/high_risk.html) who reportedly had direct exposure to pigs in a fair setting. While limited person-to-person spread of this virus has been detected and likely continues to occur sporadically, no sustained community transmission has been found. CDC is monitoring this situation and working with states to respond to these evolving outbreaks. The agency continues to urge people at high risk from serious flu complications to stay away from pigs and pig arenas at fairs this summer.

"We're saddened to hear about the death of one person in Ohio associated with the current H3N2v outbreaks," says CDC's Dr. Lyn Finelli. "Like with seasonal flu, we have been – and continue to be – particularly concerned about people with factors that put them at high risk of serious complications if they get the flu. These people should absolutely not have contact with pigs or visit pig arenas at fairs this summer." Dr. Finelli is Lead for the Surveillance and Outbreak Response Team in CDC's Influenza Division.

High risk factors for serious flu complications include: being younger than 5 years (especially children younger than 2 years), or 65 and older, pregnancy, and certain chronic medical conditions like asthma, diabetes, heart disease, weakened immune systems, and neurological or neurodevelopmental conditions. A full list of high risk conditions (/flu/about/disease/high_risk.html) is available on the CDC seasonal flu website.

"Anyone with a high risk factor should not only avoid pigs and pig arenas at fairs, but they should also seek prompt medical attention if they get flu-like symptoms, especially if they have pig exposure, but even in the absence of pig exposure," Finelli says.

CDC has issued information for clinicians on H3N2v (/flu/swineflu/h3n2v-clinician.html); guidance which underscores the importance of rapid antiviral treatment of influenza, including H3N2v virus infection, in high risk patients. The H3N2v virus is susceptible to the influenza antiviral drugs oseltamivir (Tamiflu ®) and zanamivir (Relenza ®).

For seasonal flu, CDC recommends (/flu/takingcare.html) that it is best that people with high risk conditions who develop flu-like symptoms contact their doctor, tell them about their symptoms, and remind them about their high risk status. For the current H3N2v outbreaks, if high risk people have exposure to pigs, it's especially important that they tell their doctor about this exposure.

"Like with seasonal flu," Finelli says, "prompt antiviral treatment in a high risk person can mean the difference between having a milder illness versus a very serious illness that could result in a hospital stay or even death," says Finelli. "This message is critical not only for people who are at high risk, but for America's doctors who are treating these patients. We want their suspicion for H3N2v to be high right now. Ask patients with influenza-like-

illness if they have pig exposure, but regardless of whether they do, if they have a high risk factor, treat them empirically with antivirals for influenza without waiting for testing results."

The 12 new cases reported this week are from the states of Minnesota (1), Ohio (3), *Pennsylvania (1), and Wisconsin (7). Cumulative totals for 2011 and 2012 by state are* available in the H3N2v case count table (/flu/swineflu/h3n2v-case-count.htm) .

Symptoms of H3N2v have been consistent with seasonal influenza and can include some or all of the following: fever, cough, sore throat, runny or stuffy nose, muscle or body aches, headaches and fatigue. Like with seasonal flu, it's possible that not everyone will have a fever. This may be particularly true in elderly people or people with weakened immune systems, whose bodies may not mount as effective an immune response to the virus infection.

Found in U.S. pigs in 2010 and humans in July 2011, this H3N2v virus appears to spread more easily from pigs to people than other variant influenza viruses. Most reported cases to date have occurred in people who are exhibiting or helping to exhibit pigs at fairs this season after close and prolonged contact with pigs. "So far more than 90 percent of cases have occurred in people who are exhibiting or helping to exhibit pigs, or who are family members of these people. That is why our message is so targeted," says Finelli. CDC has developed recommendations and materials for people attending fairs this summer and is working with states as well as organizations like 4-H National Headquarters and the *International Association of Fairs and Expositions to disseminate these* messages and materials (/flu/swineflu/h3n2v-audiences.htm) .

CDC also has developed supplemental H3N2v guidance for schools (/flu/swineflu/h3n2v-schools.htm) . Last year, there was at least one outbreak of H3N2v in a day care setting in the fall and CDC believes it possible that localized outbreaks of H3N2v, particularly in schools or day cares, may occur as the weather turns colder and schools across the country are underway. "The guidance document is a heads up for schools to be aware of, and on the look-out for, illness with this virus," Finelli explains.

"It's important to remember that this is an evolving situation that could change quickly." Finelli concludes, "We're constantly looking at our data and re-evaluating."

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Content source: Centers for Disease Control and Prevention, National Center for Immunization and Respiratory Diseases (NCIRD)

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別紙様式第２－１ 2

医薬品 研究報告 調査報告書

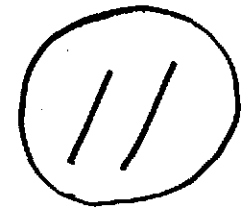
識別番号・報告回数			報告日	第一報入手日 2012年9月14日	新医薬品等の区分 該当なし。	総合機構処理欄
一般的名称	別紙のとおり。	研究報告の公表状況	MMWR.2012;61:741-746		公表国 メキシコ	使用上の注意記載状況・その他参考事項等
販売名（企業名）	別紙のとおり。					記載なし。

研究報告の概要

問題点：高病原性インフルエンザA（H7N3）の大規模な家禽感染が続くメキシコにおいて、家禽飼育場の勤務者が同ウイルスに感染した。

メキシコでは高病原性インフルエンザA（H7N3）の大規模な家禽感染が続いているが、家禽飼育場の勤務者から同ウイルスの感染事例が報告された。1例は、インフルエンザA（H7N3）が検出された家禽飼育場に勤務する32歳の女性で2012年7月7日に結膜炎と診断され、患者の目から得られた検体による核酸増幅検査の結果、インフルエンザA（H7）陽性であった。もう1例は、同じ飼育場に勤務する上記患者の親類であり、2012年7月10日に結膜炎を発症し、患者の目から得られた検体による核酸増幅検査の結果、インフルエンザA（H7）感染が判明した。両症例とも特に重大な症状に至ることなく回復している。

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。



MedDRA/J ver. 15.1

別紙

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤人免役グロブリン、⑥乾燥ペプシン処理人免疫グロブリン、⑦乾燥ペプシン処理人免疫グロブリン、⑧乾燥スルホ化人免疫グロブリン、⑨乾燥スルホ化人免疫グロブリン、⑩乾燥スルホ化人免疫グロブリン、⑪乾燥スルホ化人免疫グロブリン、⑫乾燥スルホ化人免疫グロブリン*、⑬乾燥濃縮人活性化プロテインC、⑭乾燥濃縮人血液凝固第Ⅷ因子、⑮乾燥濃縮人血液凝固第Ⅷ因子、⑯乾燥濃縮人血液凝固第Ⅷ因子、⑰乾燥濃縮人血液凝固第Ⅸ因子、⑱乾燥濃縮人血液凝固第Ⅸ因子、⑲乾燥濃縮人血液凝固第Ⅸ因子、⑳乾燥濃縮人血液凝固第Ⅸ因子、㉑乾燥濃縮人血液凝固第Ⅸ因子、㉒乾燥濃縮人血液凝固第Ⅸ因子、㉓乾燥抗破傷風人免疫グロブリン、㉔抗 HBs 人免疫グロブリン、㉕トロンビン、㉖フィブリノゲン加第ⅩⅢ因子、㉗フィブリノゲン加第ⅩⅢ因子、㉘乾燥濃縮人アンチトロンビンⅢ、㉙ヒスタミン加人免疫グロブリン製剤、㉚人血清アルブミン*、㉛人血清アルブミン*、㉜乾燥ペプシン処理人免役グロブリン*、㉝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ガンマーグロブリン筋注 450mg/3mL「化血研」、⑤ガンマーグロブリン筋注 1500mg/10mL「化血研」、⑥献血静注グロブリン “化血研”、⑦献血グロブリン注射用 2500mg「化血研」、⑧献血ベニロン－Ｉ静注用 500mg、⑨献血ベニロン－Ｉ静注用 1000mg、⑩献血ベニロン－Ｉ静注用 2500mg、⑪献血ベニロン－Ｉ静注用 5000mg、⑫ベニロン*、⑬注射用アナクトＣ2,500 単位、⑭コンファクトＦ注射用 250、⑮コンファクトＦ注射用 500、⑯コンファクトＦ注射用 1000、⑰ノバクトＭ注射用 250、⑱ノバクトＭ注射用 500、⑲ノバクトＭ注射用 1000、⑳ノバクトＭ静注用 400 単位、㉑ノバクトＭ静注用 800 単位、㉒ノバクトＭ静注用 1600 単位、㉓テタノセーラ筋注用 250 単位、㉔ヘパトセーラ筋注 200 単位/mL、㉕トロンビン “化血研”、㉖ボルヒール、㉗ボルヒール組織接着用、㉘アンスロビンＰ500 注射用、㉙ヒスタグロビン皮下注用、㉚アルブミン 20％化血研*、㉛アルブミン 5％化血研*、㉜静注グロブリン*、㉝アンスロビンＰ1500 注射用
報告企業の意見	インフルエンザウイルスは 70～120nm の球形または多形性で、核酸は 8 本の分節状マイナス一本鎖 RNA、エンベロープを有し、エンベロープの表面に存在する赤血球凝集素 (HA) とノイラミダーゼ (NA) の抗原性により 16 種類の HA 亜型および 9 種類の NA 亜型に分類される。 今回の報告は高病原性インフルエンザ A (H7N3) の大規模な家禽感染が続くメキシコにおいて、家禽飼育場の 2 名の勤務者が同ウイルスへの感染により結膜炎を発症したとの報告であるが、重大な症状に至ることなく回復している。 上記製剤の製造工程には、冷アルコール分画工程、ウイルス除去膜ろ過工程、加熱工程等の原理の異なるウイルスクリアランス工程が導入されており、各工程のウイルスクリアランス効果は「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）」に基づく、モデルウイルスを用いたウイルスプロセスバリデーションにより確認されている。今回報告したブタインフルエンザ A (H7N3) ウイルスのモデルウイルスには、エンベロープの有無、核酸の種類等から、ウシウイルス性下痢ウイルス (BVDV) が該当すると考えられるが、上記工程の BVDV クリアランス効果については上記バリデーションにより確認されている。また、これまでに上記製剤によるブタインフルエンザ A (H7N3) への感染報告例は無い。 以上の点から、上記製剤はインフルエンザ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

INF2012-002

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Morbidity and Mortality Weekly Report (*MMWR*)

Notes from the Field: Highly Pathogenic Avian Influenza A (H7N3) Virus Infection in Two Poultry Workers — Jalisco, Mexico, July 2012

Weekly
September 14, 2012 / 61(36);726-727

During June–August 2012, Mexico's National Service for Health, Safety, and Food Quality reported outbreaks of highly pathogenic avian influenza (HPAI) A (H7N3) virus in poultry on farms throughout the state of Jalisco (*1,2*). This report describes two cases of conjunctivitis without fever or respiratory symptoms caused by HPAI A (H7N3) virus infection in humans associated with exposure to infected poultry.

Patient 1. On July 7, a poultry worker aged 32 years complaining of pruritus in her left eye was examined at a clinic in Jalisco. Physical findings included redness, swelling, and tearing. Conjunctivitis was diagnosed; the patient was treated symptomatically and recovered fully. Because the patient had collected eggs in a farm where HPAI A (H7N3) virus was detected, the Institute for Epidemiological Diagnosis and Reference, Mexico, tested ocular swabs from both of her eyes for influenza A (H7) by real-time reverse transcription–polymerase chain reaction (rRT-PCR), and embryonated chicken eggs were inoculated for viral isolation. The swab material was positive for influenza A (H7) virus by rRT-PCR and virus was isolated from each eye. These findings were reported to the World Health Organization on July 19, and full genome sequences (CY125725–32) were uploaded to GenBank. The virus was closely related by nucleotide sequence to previously reported HPAI A (H7N3) viruses collected during poultry outbreaks in Jalisco with sequences available in GenBank (JX397993, JX317626).

Patient 2. A man aged 52 years, who was a relative of patient 1 and worked on the same farm, developed symptoms consistent with conjunctivitis on July 10 and sought care at a local clinic on July 13. He was treated symptomatically and recovered without sequelae. When public health authorities became aware of this patient, they obtained eye swabs, which were tested by rRT-PCR, revealing influenza A (H7).

Mexico has continued its efforts to contain poultry outbreaks in affected areas in Jalisco. Those efforts include quarantining affected farms, culling infected birds, vaccinating uninfected birds, and disinfecting contaminated areas. Government agencies also have provided personal protective equipment to farm personnel and are conducting active surveillance for influenza-like illness (ILI) and severe acute respiratory illness at two sentinel sites near the outbreak.

Avian influenza A viruses are designated as HPAI or low pathogenicity avian influenza (LPAI) based on molecular characteristics of the virus and the ability of the virus to cause disease and mortality in birds (*3*). To date, only influenza A (H5) and (H7) subtypes have been described as HPAI. Influenza A (H7) subtype viruses have been detected in wild birds in many parts of the world and can cause outbreaks in poultry. Influenza A (H7) infection in humans is uncommon, but can occur after direct contact with infected birds, especially

during outbreaks of influenza A (H7) virus among poultry (*4*). Illness can include conjunctivitis without fever, upper respiratory tract symptoms, or both (*4,5*), and severity can range from mild to fatal (*4*). In the United States, avian influenza outbreaks in poultry are rare, but they are detected and reported sporadically. In the United States, only two cases of illness with LPAI A (H7) virus infection are known to have occurred in humans, both of whom recovered (*6,7*).

The conjunctivitis cases in Jalisco most likely represent HPAI A (H7N3) virus transmission from infected poultry to humans through direct contact. United States agricultural, public health, and clinical personnel should be aware of these poultry outbreaks with transmission to humans in a neighboring country. Persons working with poultry known or suspected to be infected with influenza A viruses should use appropriate personal protective equipment, including face masks, gloves and eye protection (e.g., goggles). Clinicians and epidemiologists should consider avian influenza A virus infection in patients who have conjunctivitis or ILI and have contact with poultry in areas with known avian influenza outbreaks. Clinicians who suspect avian influenza A virus infections in humans should obtain a conjunctival or respiratory specimen, or both, depending on signs and symptoms, and submit samples to a national, regional, or state public health laboratory to enable specific influenza testing. Clinicians also should consider early empiric antiviral treatment of suspected cases with a neuraminidase inhibitor (*8,9*). Public health officials should survey family members and contacts of infected persons to find cases of human-to-human transmission.

Reported by

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番号8

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 2012年8月20日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①② 乾燥抗HBs人免疫グロブリン ③ 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	cdc.gov/ncidod/dvbid/westnile/index.htm/2012/08/17	公表国 アメリカ	
販売名（企業名）	① ヘプスブリン筋注用200単位　（日本血液製剤機構） ② ヘプスブリン筋注用1000単位　（日本血液製剤機構） ③ ヘプスブリンIH静注1000単位　（日本血液製剤機構）					

研究報告の概要

2012年、これまでに43州でヒト、トリ、蚊におけるウエストナイルウイルス感染が報告されている。26人の死亡を含むヒトにおけるウエストナイルウイルス疾患合計:693症例が米CDCへ報告されている。その内、406症例(59%)が神経侵襲性疾患（例えば、髄膜炎や脳炎など）、287症例(41%)が非神経侵襲性疾患である。
2012年、これまでに報告された693症例は、ウエストナイルウイルスが1999年に米国において初めて検出されて以降、2012年8月第2週を通じてCDCへ報告されたウエストナイルウイルス疾患症例の数が最多である。80%を超える症例が6州(テキサス州、ミシシッピ州、ルイジアナ州、オクラホマ州、サウスダコタ州とカリフォルニア州)からの報告で、全症例の約半数がテキサス州から報告されている。

報告企業の意見	今後の対応
ウエストナイルウイルス（West Nile virus：WNV）は、フラビウイルス科フラビウイルス属に属し、大きさは40～60nmのエンベロープを有するRNAウイルスである。血清学的には日本脳炎ウイルス群に含まれ、蚊によって媒介される。 FDAは、2005年6月の業界向けガイダンス改訂版において、「FDAは全ての血漿分画製剤について現在行われているウイルス低減工程を再調査した。現在行われている方法は、WNVと分類上関連しているフラビウイルスを不活化することがバリデートされている。」と評価し、CPMPもまたポジションステートメントにおいて、血漿分画製剤の製造工程でWNVは不活化・除去されると評価している。万一、原料血漿にWNVが混入しても、BVDをモデルウイルスとしたウイルスクリアランス試験成績から、本剤の製造工程において不活化・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

代表としてヘプスブリンIH静注1000単位の記載を示す。
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抗体陰性であることを確認している。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。

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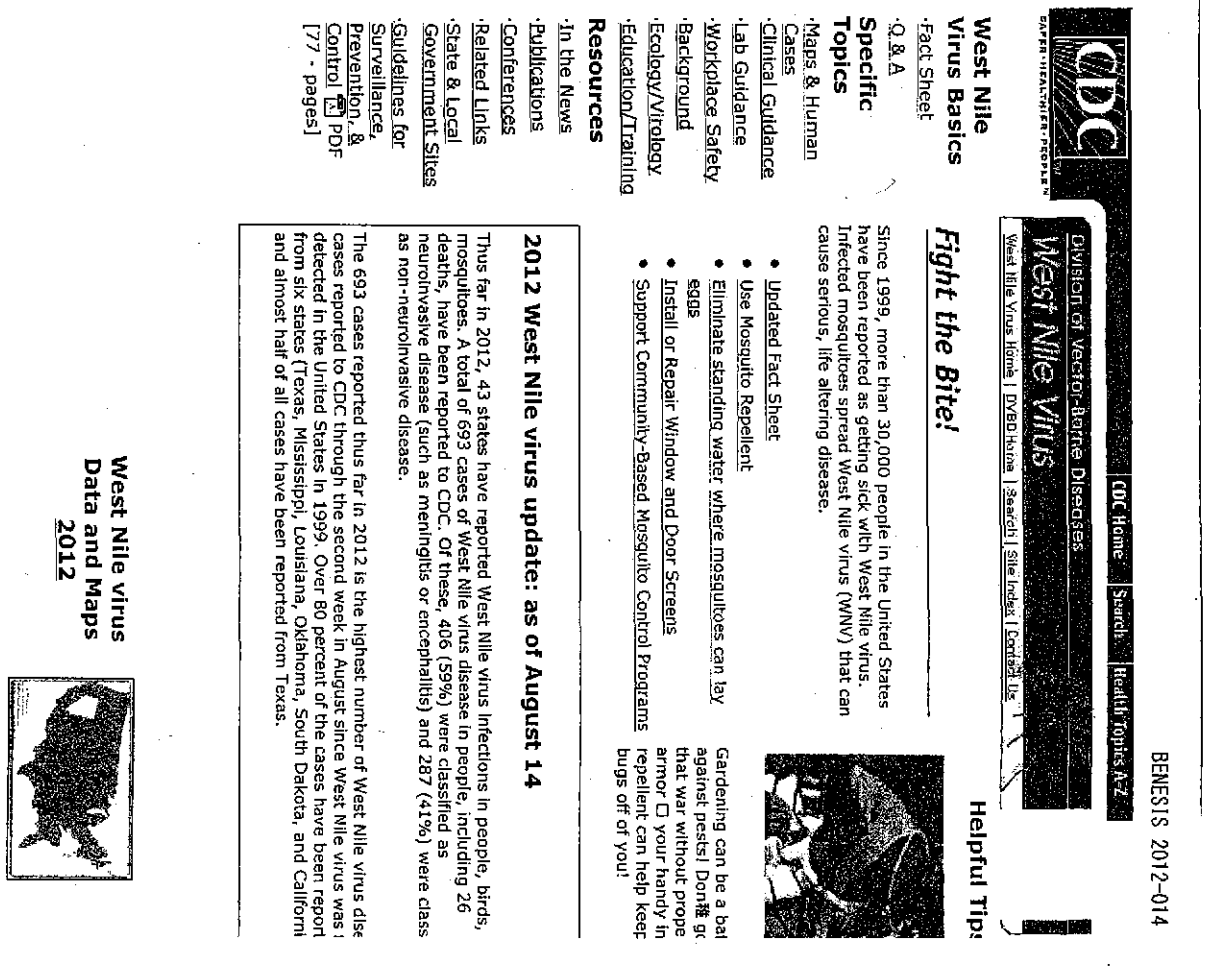
ヘプスブリン

BENESIS 2012-014



別紙様式第2-1

No. 14

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
				2012. 8. 17	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	Stramer SL, Linnen JM, Carrick JM, Foster GA, Krysztof DE, Zou S, Dodd RY, Tirado-Marrero LM, Hunsperger E, Santiago GA, Muñoz-Jordan JL, Tomashek KM. Transfusion. 2012 Aug;52(8):1657-66. doi: 10.1111/j.1537-2995.2012.03566.x. Epub 2012 Feb 17.	公表国 米国	
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)					

研究報告の概要

○2007年、プエルトリコでのデング熱アウトブレイクの期間中、RNAの確認された供血者におけるデングウイルス血症とデング熱輸血感染の検出

背景: 2007年、プエルトリコにおいて合計10,508件のデング熱疑い症例が報告された。供血はデングウイルス(DENV)-RNAについて検査され、RNA陽性供血の受血者は輸血感染の評価のために追跡された。

研究デザイン及び方法: 2007年の供血サンプルは保管され、DENV RNAについてTMA法により個別に検査された;サブセットはエンハンスTMA(eTMA)分析によってさらに検査された。TMA(eTMAを含む)が繰り返し反応(RR)した場合に、TMA反応性が立証できたと見なされた。TMA-RRサンプルは全て定量的DENVタイプ特異的RT-PCRと、ELISAによるDENV IgM抗体検査が行われた。RT-PCRで陽性となったサンプルは、蚊の細胞培養により感染性がさらに検査された。TMA-RR供血由来製剤の受血者は追跡された。

結果: 検査された15,350サンプルのうち29件がTMA-RRであり、529件当たり1件の割合であった(0.19%)。蚊での培養により感染性を示した12サンプルにおいて、RT-PCRによりウイルス力価10^5～10^9copies/mLでDENV 1型、2型及び3型が検出された。6件のTMA-RRサンプルはIgM陽性であった。TMA-RR供血の受血者29人のうち3人が検査された。10^8copies/mLのDENV-2を含む赤血球を輸血されたプエルトリコの受血者1人が輸血3日後に発熱し、デング出血熱に進行した。受血者はRT-PCRでDENV-2陽性であった;供血者と受血者の両方が同一のエンベロープ配列を有していた。

結論: 初めて記録された重篤なデング熱の輸血感染に加え、プエルトリコの供血者に高い割合でウイルス血症が検出されたことは、介入に関する更なる調査が必要である。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血
新鮮凍結血漿-LR「日赤」120
新鮮凍結血漿-LR「日赤」240
新鮮凍結血漿-LR「日赤」480

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
2007年のプエルトリコでのデング熱アウトブレイクの期間中、供血者にデングウイルス血症が高い割合で検出され、ウイルス血症供血者由来の血液を輸血された受血者にデング出血熱が発生したとの報告である。	日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

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Dengue viremia in blood donors identified by RNA and detection of dengue transfusion transmission during the 2007 dengue outbreak in Puerto Rico

Susan L. Stramer, Jeffrey M. Linnen, James M. Carrick, Gregory A. Foster, David E. Krysztof, Shimian Zou, Roger Y. Dodd, Lourdes M. Tirado-Marrero, Elizabeth Hunsperger, Gilberto A. Santiago, Jorge L. Muñoz-Jordan, and Kay M. Tomashek

BACKGROUND: In 2007, a total of 10,508 suspected dengue cases were reported in Puerto Rico. Blood donations were tested for dengue virus (DENV) RNA and recipients of RNA-positive donations traced to assess transfusion transmission.

STUDY DESIGN AND METHODS: Blood donation samples from 2007 were maintained in a repository and tested individually for DENV RNA by transcription-mediated amplification (TMA); a subset was further tested by an enhanced TMA (eTMA) assay. TMA-reactive samples were considered confirmed if TMA (including eTMA) was repeat reactive (RR). All TMA-RR samples were tested by quantitative, DENV type–specific reverse transcriptase–polymerase chain reaction (RT-PCR) and for anti-DENV immunoglobulin (Ig)M by enzyme-linked immunosorbent assay. Samples positive by RT-PCR were further tested for infectivity in mosquito cell culture. Patients receiving components from TMA-RR donations were followed.

RESULTS: Of 15,350 donation samples tested, 29 were TMA-RR for a prevalence of 1 per 529 (0.19%). DENV Types 1, 2, and 3 with viral titers of 10^5 to 10^9 copies/mL were detected by RT-PCR in 12 samples of which all were infectious in mosquito culture. Six TMA-RR samples were IgM positive. Three of the 29 recipients receiving TMA-RR donations were tested. One recipient in Puerto Rico transfused with red blood cells containing 10^8 copies/mL DENV-2 became febrile 3 days posttransfusion and developed dengue hemorrhagic fever. The recipient was DENV-2 RNA positive by RT-PCR; both the donor and the recipient viruses had identical envelope sequences.

CONCLUSIONS: High rates of viremia were detected in blood donors in Puerto Rico coupled with the first documented transfusion transmission of severe dengue disease, suggesting that further research on interventions is needed.

Dengue is a disease caused by four related RNA viruses of the genus *Flavivirus*, dengue virus (DENV)-1, -2, -3, and -4.[1] However, not all DENV infections result in clinically apparent disease. Approximately 75% of all DENV infections are asymptomatic, including those among adults.[2-6] Each DENV type is capable of causing the full spectrum of disease from nonspecific, acute febrile illness to severe disease including dengue hemorrhagic fever (DHF) and dengue shock syndrome. Approximately 5% of patients with dengue develop severe disease, which is thought to occur more commonly among those with second or subsequent infections.[7] Infection with one DENV-type produces lifelong immunity against that DENV-type and short-term (≤2 months) cross-protection against infection with the other three DENVs.[1,3,7] Therefore, an individual may have up to four DENV infections in their lifetime.

DENVs are primarily transmitted from person to person through the bite of an infected *Aedes aegypti* or *Aedes albopictus* mosquito.[8] DENV replicates in humans for 3 to 14 days before symptom onset. Infected persons can transmit DENV to mosquitoes as early as 1 to 2 days before symptoms develop and throughout the approximately 7-day viremic period.[8] Because of this, and the fact that viremia can be high titer (in excess of 10^7 viral RNA copies/mL) even among those who remain asymptomatic, DENV may be transfusion transmitted.[10-12] Cases of dengue after receipt of blood products or donor organs or tissue and after occupational exposure in a health care setting have been reported.[13-17] However, the true incidence of transfusion-transmitted dengue is unknown because many infections are asymptomatic or result in mild, nonspecific febrile illness that may not be recognized as transfusion acquired, and if a case is suspected, transfusion transmission (vs. vector-borne transmission) is difficult to prove in recipients in dengue-endemic countries. Moreover, there is no surveillance for such events, and diagnostic services to investigate infections and their sources are often not widely available in many endemic countries.[18]

Dengue is a major public health problem in the tropics; an estimated 50 million cases occur annually and 40% of the world's population lives in areas with DENV transmission.[19-22] Dengue is not endemic in the continental United States, Hawaii, or Alaska;[23-25] however, several dengue outbreaks with local transmission have occurred in Texas,[26,27] Hawaii,[28,29] and Florida[30,31] in the past decade. Dengue is endemic in the US territories of Puerto Rico, the Virgin Islands, and American Samoa, and millions of US travelers are at risk as dengue is the leading cause of febrile illness among travelers returning from the Caribbean, Latin America, and South Central/Southeast Asia.[32,33]

In 2007, there was a large, islandwide dengue outbreak in Puerto Rico with 10,508 reported cases.[34] It was the largest outbreak in Puerto Rico in nearly a decade and only the second outbreak to involve the simultaneous transmission of all four DENVs (although DENV-3 predominated followed by DENV-2). The 2007 outbreak was notable for the reappearance of DENV-1 and DENV-4 after nearly a decade of absence and an increase in disease severity compared with the 1994 to 1995 and 1998 outbreaks. It was in this context that we tested blood donations for DENV RNA to determine the rate of donors presenting with DENV RNA positivity and viremia as assessed by infection in mosquito cells; we also evaluated recipients of RNA-positive units to determine if transfusion transmission could be documented.

ABBREVIATIONS: ARC = American Red Cross; DENV(s) = dengue virus(-es); DHF = dengue hemorrhagic fever; ED = emergency department; eTMA = enhanced transcription-mediated amplification; IR = initially reactive; MAC-ELISA = immunoglobulin M–capture enzyme-linked immunosorbent assay; PDSS = passive dengue surveillance system; RR = repeat reactive; S/CO = signal to cutoff; TMA = transcription-mediated amplification.

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TRANSFUSION 2012;52:1657-1666.

MATERIALS AND METHODS

General approach

Over 28,000 EDTA plasma samples collected in plasma preparation tubes (PPT, Becton Dickinson, Franklin Lakes, NJ) from blood donations to the Puerto Rico region of the American Red Cross (ARC) during the dengue outbreak in 2007 (June-December) were retained frozen in a repository. After the dengue season, and the number of available samples by week were assessed relative to the epidemic, selected samples (focusing on the peak weeks of the epidemic) were batch tested for DENV RNA using transcription-mediated amplification (TMA; Gen-Probe, San Diego, CA). Samples were TMA tested individually with initially reactive (IR) samples retested by TMA in duplicate. TMA repeat-reactive (RR) samples were considered positive.[10] TMA-RR samples were diluted 1 to 16 in plasma screened negative for all infectious disease markers including DENV RNA, and the dilutions were retested using the same TMA assay in singlet. All DENV RNA testing was perfotmed during 2008 at Gen-Probe. Virologic, infectivity, and serologic testing performed on all TMA-RR samples at the Dengue Branch of the Centers for Disease Control and Prevention (CDC) in Puerto Rico included qualitative and quantitative DENV type-specific real-time, reverse transcriptase–polymerase chain reaction (RT-PCR), mosquito (*A. albopictus*) cell culture (C6/36 cells), and anti-DENV immunoglobulin (Ig)M-capture enzyme-linked immunosorbent assay (MAC-ELISA).[35-37] Hospitals receiving components from TMA-RR donations were contacted for recipient follow-up including elicitation of a history of illness, administration of a risk factor questionnaire, and submission of a serum sample to the Dengue Branch, CDC, for diagnostic testing for evidence of DENV infection including RT-PCR, MAC-ELISA, and anti-DENV IgG ELISA. Due to the retrospective nature of the study, recipient contact only occurred at 1 year or more after transfusion. The institutional review board of the ARC approved the study.

DENV TMA assay

The DENV TMA assay used for this study is based on the same technology as blood screening assays (PROCLEIX, Novartis Vaccines and Diagnostics, Emeryville, CA) for the RNA components of the human immunodeficiency virus (HIV) and hepatitis C virus (HCV) TMA assay (Ultrio assay, Gen-Probe, San Diego, CA; and Novartis Vaccine and Diagnostics) and that of the West Nile virus (WNV) assay (Gen-Probe/Novartis), both of which have been licensed by the US Food and Drug Administration. The DENV TMA assay is a research qualitative nucleic acid test for the detection of DENV RNA, which includes target capture and TMA,

followed by chemiluminescent detection of DENV RNA. The assay design most closely resembles the PROCLEIX WNV assay including the same base reagent formulations (with dengue-specific oligonucleotides) and processed on the automated system (TIGRIS, Novartis) utilizing software that performed all cutoff calculations and validity criteria using the same interpretative algorithms as the WNV assay. In a comparative study of DENV TMA and RT-PCR, TMA was 10 to 100 times more sensitive than RT-PCR and could detect RNA in up to 80% of clinical cases that were RT-PCR negative.[38] The DENV TMA assay can detect all four DENV types to below 20 copies/mL.[11,39] A subset of donations that tested TMA nonreactive (n = 8684) was retested by an enhanced TMA (eTMA). Based on internal Gen-Probe results, the eTMA assay is more sensitive than the routine TMA used in this study. The eTMA assay showed 95% detection at 14.9, 18.3, 13.0, and 16.4 copies/mL DENV-1 (95% confidence interval [CI], 11.7-20.4), DENV-2 (95% CI, 14.4-24.7), DENV-3 (95% CI, 10.3-17.6), and DENV-4 (13.0-22.2), respectively.

Recipient tracing

After hospital or transfusion service notification of the distribution of potentially infectious DENV RNA–containing components, recipients of TMA-RR donations were traced, consented, and tested for evidence of DENV infection after transfusion. Evidence of current or past DENV infection required the presence of DENV RNA and/or IgM and IgG antibodies in follow-up samples from the recipient with signs and symptoms consistent with dengue infection from the recipient's chart review. Consenting recipients also completed the questionnaire regarding DENV clinical history and risk factors. Serum samples from RNA-positive recipients and their respective donations were inoculated into cultured C6/36 cells and the presence of virus was confirmed by RT-PCR and indirect immunofluorescence. Isolates were further propagated and viral RNA was extracted from culture supernatant using the Universal BioRobot 16 System (Qiagen, Valencia, CA). The BioRobot Universal System automates and integrates all the instrumentation, software, purification and enzyme-related steps required for high-throughput molecular applications including RNA purification from blood. The envelope glycoprotein (E) gene was amplified and sequenced; sequence data were restricted to the E gene open reading frame (1485 bp). GenBank accession numbers were obtained. Evolutionary distances were computed and several E gene sequences from GenBank were included in the phylogenetic tree to support tree topology. Multiple sequence alignment was performed using ClustalW. Evolutionary distances were inferred using neighbor-joining trees.

RESULTS

2007 dengue outbreak in Puerto Rico

During the 2007 dengue season in Puerto Rico, 10,508 suspected cases of dengue, or 2.9 cases per 1000 population, were reported to the passive dengue surveillance system (PDSS). The PDSS is collaboratively operated by the Puerto Rico Department of Health and the CDC, Dengue Branch. By law, dengue fever, DHF, and/or dengue shock syndrome are reportable conditions in Puerto Rico and suspected cases are reported via PDSS along with submission of a serum sample for free dengue diagnostic testing. All four DENV types were in circulation in 2007 with a total of 3293 (33%) processed samples confirmed positive for DENV. DENV-3 and DENV-2 were detected most often (62 and 31%, respectively). More than 50% (52.5%) of reported cases were hospitalized, one-third (31.8%) had hemorrhage, 2.2% had DHF, and there were 44 reported deaths.[34] A repository of 28,277 samples from blood donations collected in Puerto Rico from June 1 to December 31, 2007, was created during this outbreak.

DENV TMA repeat reactivity and overall prevalence of DENV RNA among blood donations

Of 15,350 samples randomly selected from Peak Weeks 32 to 49 for DENV RNA testing by TMA, 28 were TMA-IR and 25 were TMA-RR for a positive rate of 1 per 614 (0.16%; Fig. 1). The 25 TMA-RR samples included DENV-1, -2, and -3 detected by DENV type–specific RT-PCR. Of the 25 TMA-RR units, 14 (56%) were reactive at a 1-to-16 dilution and 12 (48%) had RNA titers of 10^5 to 10^9 copies/mL (Table 1). All 12 samples with quantifiable RNA infected mosquito cell cultures of which nine (75%) were detectable at a 1-to-16 dilution. Six of 25 TMA-RR units were IgM positive of which only two of the six had quantifiable virus and infected mosquito cells in culture.

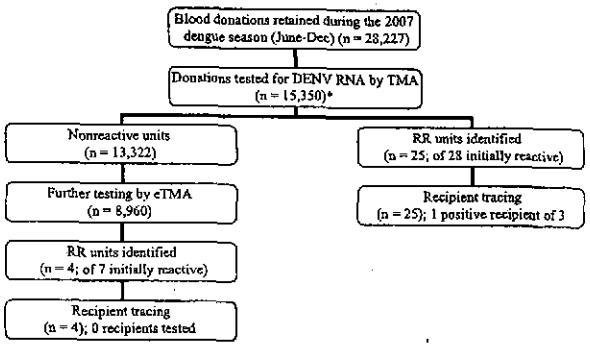

Fig. 1. DENV blood donation screening algorithm and TMA screening results including the 29 TMA RR blood donations. *TMA specificity based on IR samples that did not repeat as reactive = 15,315/15,321 = 99.96% (95% CI: 99.93-99-99).

TABLE 1. DENV TMA-RR blood donations collected in Puerto Rico in 2007

	S/CO by TMA*			S/CO by eTMA			CDC testing			
Unit	Initial	Retest	1:16	Initial	Retest	1:16	Serotype†	Viral load (copies/mL)	C6/36‡	Anti-DENV IgM
1	27.75	38.99	38.91	87.16	88.52		DENV-2	1.12×10^9	Pos	Neg
2	32.34	33.30	31.14				DENV-2	5.08×10^8	Pos	Pos
3§	33.30	37.38	35.39	91.10	83.09		DENV-2	1.35×10^9	Pos	Neg
4	37.66	39.16	40.26	*87.13*	*88.32*		*DENV-3*	7.25×10^7	*Pos*	*Neg*
5	40.29	27.03	36.10	82.29	92.04		DENV-3	1.37×10^7	Pos	Neg
6	32.73	35.03	34.99				DENV-3	1.18×10^7	Pos	Neg
7	33.91	32.87	33.89				DENV-3	7.67×10^8	Pos	Neg
8	31.97	30.59	0.17				DENV-1	4.49×10^6	Pos	Neg
9	19.14	13.94	0.21				DENV-2	2.82×10^6	Pos	Pos
10	33.10	38.68	40.31	87.86	89.91		DENV-3	6.39×10^5	Pos	Neg
11	31.25	33.56	27.75				DENV-3	3.50×10^6	Pos	Neg
12	5.68	20.55	1.16	29.48	21.59		DENV-3	1.00×10^6	Pos	Neg
13	34.81	37.21	32.97	76.16	32.72			$<10^3$	Neg	Neg
14	23.38	31.07	13.29	31.25	31.18			$<10^3$	Neg	Neg
15	14.23	23.26	7.32	28.59	3.28			$<10^3$	Neg	Pos
16	13.14	25.77	0.07	29.26	12.51			$<10^3$	Neg	Neg
17	11.51	5.63	0.04					$<10^3$	Neg	Neg
18	8.17	16.58	0.03					$<10^3$	Neg	Neg
19	6.84	8.91	0.20					$<10^3$	Neg	Pos
20	5.06	4.12	1.37	29.96	8.61			$<10^3$	Neg	Neg
21	3.37	4.95	0.83					$<10^3$	Neg	Pos
22	2.95	25.28	0.03					$<10^3$	Neg	Pos
23	8.20	1.40	0.13					$<10^3$	Neg	Neg
24	4.46	0.01	0.21	24.80	0.08			$<10^3$	Neg	Neg
25	1.02	2.29	0.13	28.01	0.01			$<10^3$	Neg	Neg
26‖	0.45			26.38	27.55	0.02		$<10^3$	Neg	Neg
27‖	0.17			26.18	30.99	0.02		$<10^3$	Neg	Neg
28‖	0.30			25.31	29.11	0.03		$<10^3$	Neg	Neg
29‖	0.50			24.34	17.85	0.05		$<10^3$	Neg	Neg

* TMA reactive when the S/CO ratio is 1.00 or greater.
† Serotype-specific, real-time RT-PCR.
‡ C6/36 = the mosquito cell line used for infectivity studies.
§ Unit 3 was involved in a transfusion transmission.
‖ Four TMA nonreactive samples were eTMA reactive.
Bold text indicates positive values.

Seven of 8684 TMA-nonreactive donations were eTMA IR and four were eTMA RR (Fig. 1 and Table 1). In addition, 13 of 25 TMA-RR donations with sufficient volume were retested by eTMA and all were reactive (Table 1) with high signal-to-cutoff (S/CO) ratios. Of the four additional eTMA-RR donations that tested nonreactive by TMA, none was confirmed by PCR, all were eTMA nonreactive at a 1-to-16 dilution, none infected mosquito cells in culture, and none contained IgM; however, all of the confirmatory methods have lesser sensitivity than TMA.[39] Thus, the four eTMA-RR donations were combined with the 25 TMA-RR donations for a total study yield of 29 RNA-reactive donations (further referred to as TMA-RR) of which nearly 80% lacked IgM.

Combined, 35 IRs and 29 RRs were identified from 15,350 tested samples, resulting in a DENV RNA prevalence during the 2007 outbreak season of 1 per 529 (0.19% or 18.9 per 10,000) and an overall TMA specificity based on IR samples that did not repeat of 99.96% (15,315/15,321; 95% CI, 99.93-99.99; Fig. 1). TMA-RR (including eTMA-RR) donors were detected between July and November, which encompassed the majority of the outbreak period (Fig. 2). Figure 2 also provides the number of cases reported by week of illness onset to the PDSS and the laboratory diagnoses of these cases.

Recipient tracing

Information on all 29 recipients of TMA-RR donations was obtained but serum samples for diagnostic testing were available from only three recipients (Fig. 1 and Table 2); pretransfusion samples were not available from any recipient. Two recipients consented to be tested and both had testing done nearly 2 years posttransfusion. MAC-ELISA was negative for anti-DENV IgM and anti-DENV IgG ELISA was also negative. These two additional recipients had received red blood cells (RBCs) prepared from a

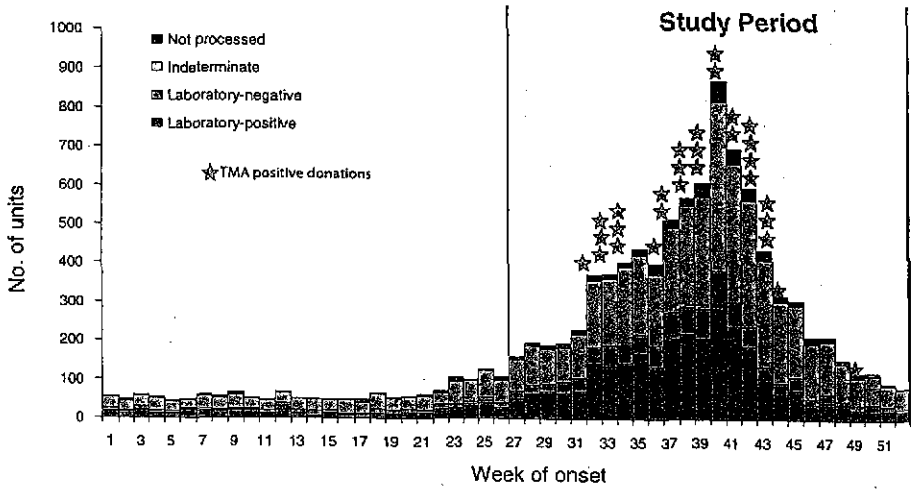

Fig. 2. Number of suspected dengue cases by laboratory outcome reported weekly during 2007 and the week in which TMA-RR blood donors were identified; the study period is indicated as that between the vertical lines.

TABLE 2. Recipient tracing results for 29 recipients who received DENV TMA-RR donations

Unit	Serotype	Viral load (copies/mL)	Component type	Recipient information
1	DENV-2	1.12×10^9	PP	Unit discarded
2	DENV-2	5.08×10^8	RBCs	Died within 3 weeks after transfusion, unrelated to dengue
3*	DENV-2	1.35×10^8	RBCs	DHF 3 days after transfusion; donor-recipient sequencing confirmed
4	DENV-3	7.25×10^7	RBCs	None
5	DENV-3	1.37×10^7	RBCs	Followed for 6 weeks; no s/s suggestive of dengue
6	DENV-3	1.18×10^7	RBCs	Died same day as transfusion
7	DENV-3	7.67×10^6	RBCs	None
8	DENV-1	4.49×10^6	RBCs	None
9	DENV-2	2.82×10^6	RBCs	None
10	DENV-3	6.39×10^5	RBCs	None
11	DENV-3	3.50×10^5	RBCs	Died within 7 months after transfusion, unrelated to dengue
12	DENV-3	1.00×10^5	RBCs	Followed for 2 months; no s/s suggestive of dengue
13		$<10^5$	RBCs	None
14		$<10^5$	RBCs	Died without s/s suggestive of dengue
15		$<10^5$	RBCs	None
16		$<10^5$	RBCs	None
17		$<10^5$	RBCs	Died 1 day after transfusion; no s/s suggestive of dengue
18†		$<10^5$	RBCs	Antibody (IgM/IgG) negative on follow-up 26 months after transfusion
19†		$<10^5$	RBCs	Antibody (IgM/IgG) negative on follow-up 23 months after transfusion
20		$<10^5$	RBCs	Unit discarded
21		$<10^5$	RBCs	None
22		$<10^5$	RBCs	Died within 3 weeks after transfusion, unrelated to dengue
23		$<10^5$	RBCs	None
24		$<10^5$	RBCs	Unit discarded
25		$<10^5$	RBCs	Discharged 6 days posttransfusion; no s/s suggestive of dengue
26‡		$<10^5$	RBCs	None
27‡		$<10^5$	RBCs	None
28‡		$<10^5$	RBCs	None
29‡		$<10^5$	RBCs	None

* Unit 3 was involved in a transfusion transmission.
† Units 18 and 19 went to recipients who were subsequently tested for DENV antibody (IgG/IgM).
‡ Donations detected as RR by eTMA.
PP = plateletpheresis unit; s/s = signs/symptoms.

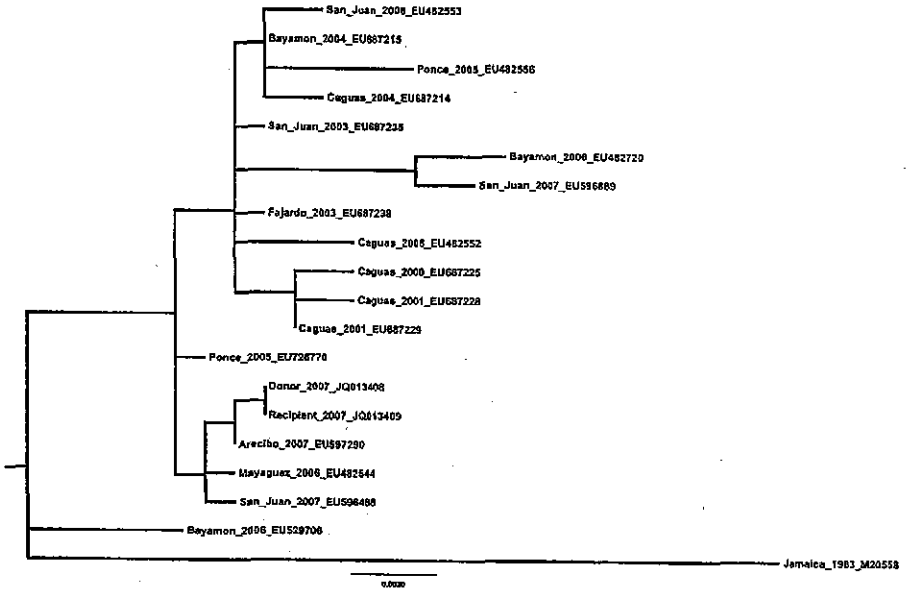

Fig. 3. Maximum likelihood phylogeny of complete E gene sequences (1485 bp) obtained from 19 endemic clinical isolates obtained between 2000 and 2007. Taxa label indicates the geographical region, collection date, and GenBank accession number. Caguas is the region where the city of Cidra is located, which is where the recipient resided. Isolates marked in red represent the sequences obtained from the blood donor and recipient. Pairwise distance shows a 100% similarity between these two sequences and less than 99% from the other sequences represented in the tree. The ML tree was rooted using the Jamaica 1983 E gene sequence.

blood unit in which the plasma contained fewer than 1000 DENV RNA copies/mL, one of which was from an IgM-positive donor (Tables 1 and 2; Donor Samples 18 and 19). In addition, neither recipient developed signs or symptoms consistent with DENV infection after transfusion. A third recipient in Puerto Rico who was transfused with RBCs from a unit containing 10^8 copies of DENV-2/mL of plasma became febrile 3 days posttransfusion and developed DHF (see Case report). The attending physician suspected dengue in the recipient and sent a serum sample for diagnostic testing on Day 5 after onset of illness that was positive for DENV-2 by RT-PCR. No other transmissions were detected.

The implicated donor unit was from a 31-year-old female who did not report any dengue-related symptoms before or after donation and was healthy on the day of donation. She had donated once previously in 2002 without event. Her unit was collected on September 13, 2007; testing showed that her plasma contained 1.35×10^8 copies/mL of DENV-2 and was IgM negative (Tables 1 and 2). RBCs prepared from the unit were transfused into a recipient who subsequently tested DENV-2 positive by RT-PCR (see Case report).

Frozen aliquots of the donation and recipient samples were available for further study. The sequence of 1485 bp corresponding to the DENV-2 envelope gene confirmed DENV-2 in both the donor and the recipient viruses and showed 100% sequence identity between the two viruses. Figure 3 shows the maximum likelihood analysis of the donor-recipient pair along with other DENV-2 isolates from various geographic areas representing different dengue outbreaks. Sequencing of viral isolates focused on the Caguas region of Puerto Rico including Cidra where the recipient resided. The sequencing results demonstrate that the virus from the donor and the recipient were identical and differed from other viruses found in the region where the recipient resided.

Case report

On September 26, 2007, an 80-year-old man with bronchial asthma, chronic hypertension, chronic obstructive

pulmonary disease, moderate tricuspid regurgitation, and myelodysplastic syndrome characterized by refractory anemia with ringed sideroblasts was admitted for symptomatic anemia. He was given 2 units of RBCs early in the morning of September 27, 2007. During the second transfusion, the patient became confused and pulled the line out of his arm, contaminating the floor and resulting in loss of half of the unit (i.e., he received 160 of 291 mL of the DENV-2 TMA-RR unit). The transfusions were otherwise uncomplicated; there were no transfusion-associated reactions and the patient's vital signs and electrocardiogram remained stable throughout. The patient was discharged to his home in central Puerto Rico that same evening.

On September 30, 2007, the recipient returned to the hospital's emergency department (ED) with complaints of general malaise and "not feeling good" since hospital discharge. The patient reported having chills, polyarthralgia, dry cough, headache, and fever since that morning (approx. 72 hr after transfusion). His hematocrit (Hct) was 35.5% and it had been stable since discharge but his creatinine and blood urea nitrogen were slightly elevated from baseline at 1.8 and 25.5 mg/dL. In the ED triage, the recipient had a temperature of 37.8°C, heart rate of 83 bpm, respiratory rate of 20 bpm, a blood pressure of 117/56 mmHg, and SaO_2 of 88% on room air. He appeared to be acutely ill but was alert, active, oriented, and not in any acute respiratory distress. The physical exam was unremarkable except for dry mucous membranes, minimal coarse rhonchi over the right lung field and bibasilar crackles, and a 2/6 systolic ejection murmur at the left sternal border. The recipient was given 0.9% normal saline intravenously, 3 L of oxygen by nasal canula, and respiratory treatments with ipratropium bromide and a β2-adrenergic agonist. He was readmitted with a presumptive diagnosis of health care–associated pneumonia for which he was given vancomycin and cefepime for 7 days. Blood and urine cultures collected in the ED and the initial chest radiograph were negative. A repeat chest radiograph on October 2 showed a right upper lung infiltrate.

Despite treatment with antibiotics, the recipient continued to have fever until the early morning of October 3, during which time his platelet (PLT) count and white blood cell count progressively declined from 183,000 and 4600 cells/mm³ respectively, at admission to 40,000 and 1800 cells/mm³. As a result, the diagnosis of dengue was considered and a serum sample was sent to the CDC's Dengue Branch for diagnostic testing where it tested DENV-2 positive by RT-PCR. In response to his low absolute neutrophil count, filgastrim, a granulocyte–colony-stimulating factor, was added to his treatment regimen. In the 48 hours after defervescence, the patient was noted to have episodes of hypotension (i.e., systolic blood pressure <90 mmHg) even though he had not had any antihypertensive medications since admission. In response, the patient was given intravenous volume replacement with 0.9% normal saline. At the same time, his serum albumin declined from 4.1 to 3.0 g/dL, and he developed large hematomas at injection sites. Even though the patient had no clinically significant bleeding detected, he met the criteria for DHF, namely, he had a fever for 5 days, thrombocytopenia, hemorrhagic manifestations, and plasma leakage as evidenced by development of hypotension and hypoalbuminemia after defervescence. The recipient received 1 unit of pheresis PLTs for a PLT count of 10,000 cells/mm³ on October 6 and 1 unit of RBCs for a Hct of 24.6%. The remainder of the hospital course was uneventful and he fully recovered from DHF. He received 1 unit of RBCs before being discharged to home on October 11, 2007.

DISCUSSION

This study demonstrates a high frequency of blood donations with plasma DENV TMA-RR (1:529) during the 2007 dengue season in Puerto Rico. Of the 29 TMA-RR units, nearly 80% lacked IgM; nearly half had high viral loads and were capable of infecting mosquito cells in culture, proving that these donations were viremic and could pose a risk to recipient safety. However, fewer than half of the TMA-RR units could be detected in a 1-to-16 dilution, the common pool size used for TMA for other viruses (HIV, HCV, HBV, and WNV); predictably, those detected at a 1-to-16 dilution also had high viral loads. Since the infectious dose of DENV by transfusion is not known, and underlying susceptibility of recipients will vary, all RNA-positive units should be considered potentially infectious. Transfusion transmission was documented in this study, which was the first to document transfusion-transmitted DENV resulting in significant clinical illness.

Studies in Brazil, Honduras, and Puerto Rico have demonstrated the presence of DENV RNA and viremia among blood donations using TMA to detect viral RNA.[10,11] In one study, 9 of 2994 (0.37%) plasma specimens from Honduras in 2004 to 2005 and three of 4858 (0.06%) archived plasma specimens from Brazil in 2003 tested positive although none of 5879 archived plasma specimens collected by the Australian Red Cross Blood Services in 2005 was positive.[11] In a prior study in Puerto Rico, 12 of 16,521 (0.07%) archived unlinked plasma specimens collected by the ARC between September 20 and December 4, 2005, were TMA-RR in a year where 6039 cases of dengue-related disease were reported versus 10,508 reported cases in 2007.[10] In that study, as in our study, fewer than half of the TMA-RR samples confirmed by type-specific RT-PCR or were viremic as demonstrated by mosquito culture. However, both RT-PCR and mosquito cell culture are less sensitive than TMA.[39]

Modeling studies estimating the DENV transfusion transmission risk in the absence of testing have been performed in various geographic areas. These include an estimated average risk during a dengue outbreak in 2004 in Cairns, Queensland, Australia, of 0.5 per 10,000;[40] a range of risk of 1.6 to 6 per 10,000 during 2005 in Singapore;[41] and, most recently in Puerto Rico, an average estimated risk of viremic donations of 7.0 per 10,000 over a 16-year period from 1995 to 2010.[42] Of note, the modeled estimated risk of viremic donations in Puerto Rico in 2007 was identical to the 29 TMA-RR donations observed in this study, with a 95% tolerance interval for the modeled estimate of 29 of 11 to 52. The modeled finding may be an overestimate based on the fact that not all RNA-positive donors will be viremic and infectious.[42]

There have been reports of DENV transmission through transfusion or transplantation.[12-14] The first published case of transfusion-transmitted dengue occurred in Hong Kong in 2002. The donor became symptomatic 1 day after donation and one recipient of RBCs developed dengue-related illness 3 days after transfusion; the patient subsequently seroconverted. Both the donor and the recipient had DENV-1 RNA identified in their blood by RT-PCR.[13] More recently, a second cluster of DENV transfusion transmission was identified in Singapore in which the donor became symptomatic 1 day after donation and two recipients (one of RBCs and the other of fresh-frozen plasma) developed dengue-related illness and seroconverted; the third recipient (of PLTs) was asymptomatic but developed IgM and IgG antibodies. The donor and the two symptomatic recipients were positive for DENV-2 RNA.[14] In addition, DHF was reported 5 days after receipt of a kidney transplant from an infected donor in Singapore[43] and dengue was reported in a bone marrow recipient in Puerto Rico in which DENV-4 was isolated from blood and tissues 4 days after transplant.[44] Moreover, seven instances of nosocomial transmission of dengue have been reported: six through needle stick injuries[45-49] and one through contact of infectious blood with the mucous membranes of a laboratory worker.[50]

Based on the results from this and the earlier studies,[10,11] it is clear that DENV RNA–containing donations occur and interventions should be considered. One intervention that the ARC implemented for collections during the 2009 dengue season in Puerto Rico included the use of a predonation question regarding dengue-related symptoms coupled with the use of an enhanced postdonation information sheet encouraging donors to call back if dengue-like symptoms developed (persistent fever and any of the following: headache, eye pain, muscle aches, joint or bone pain, new rash, bleeding from the nose or gums, or bruising easily). However, these measures would be predicted to be ineffective due to the fact that 53% to 87% of DENV infections are asymptomatic;[51,52] in fact, during the time of use, only one donor reported postdonation symptoms. Due to the fact that TMA has not been available for blood donation screening, serologic testing for DENV using a commercial NS1 antigen ELISA (Bio-Rad, Paris, France)[53] was implemented in March 2010; however, the clinical sensitivity of the NS1 antigen assay has been demonstrated to be 3- to 10-fold less sensitive than TMA by testing blood donations from the same DENV outbreak year.[54,55] For screening of donated blood, assays targeting DENV RNA are the preferred approach.

There may be several reasons why only a very limited number of dengue transfusion transmissions have been reported including: 1) recipient immunity from homotypic serotypes or recent heterotypic serotype immunity; 2) the infectious dose required for transfusion transmission may be higher than expected; and 3) clinical illness after transfusion may not be recognized as dengue, or if recognized, it may be incorrectly assumed to be mosquito acquired.[42] In any event, in an endemic area, the focus of public health is mosquito control versus the investigation of potential DENV transfusion transmission. Undoubtedly there are more DENV transfusion transmissions than have been documented, our case only being the third cluster reported. It seems likely that more infections resulted from the TMA-RR units identified by this study because not all recipients of such units were tested. Further, not all donations were tested during the 2007 dengue season in Puerto Rico. Therefore, the transmission of DENV-2 to one recipient through transfusion that was confirmed through this study represents the minimum level of transfusion transmission that occurred during the 2007 season in this dengue-endemic area. Based on the results of infecting mosquito cells in culture, in which a viral load of 10^5/mL was able to cause infection, 12 of 29 TMA-RR units contained infectious virions and hence were a risk to recipients. Since these 12 units were identified from 15,350 donations screened, this translates to a transfusion transmission risk of 1 per 1279 or approximately 0.1% of donations during the epidemic season in a dengue-endemic area. Results from this study indicate the need for additional research into the best strategies for preventing dengue transmission via blood transfusion in endemic areas and determining how such strategies should be implemented in nonendemic areas where dengue has recently been introduced.

CONFLICT OF INTEREST

None of the authors had a conflict of interest.

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研究報告の概要

○血液透析患者の口腔及び血液中のヒトヘルペスウイルス8

サウジアラビアでは、移植関連のカポジ肉腫(KS)罹患率が高く、腎疾患患者と健康な者の間でヒトヘルペスウイルス8(HHV-8)感染の割合に差がある。血液透析治療を受けている患者間でのHHV-8経口感染が腎疾患患者における高いKS罹患率の原因であると仮定された。血液透析患者72人の血漿中の抗HHV-8 IgG抗体及びCD45(+)末梢血液細胞におけるHHV-8 DNA感染率が、供血者178人、妊婦60人と比較された。血液透析患者と健康な被験者間での抗HHV-8 IgG抗体検出率は16.7%対0.4%(P<0.001)、HHV-8 DNA検出率は4.2%対0.4%(P<0.05)だった。HHV-8 DNAは血液透析中患者の口腔内サンプルを用いて、またHHV-8ウイルス量は患者の唾液を用いて測定された。HHV-8 DNAが口腔内から検出された患者5人の唾液中のウイルス量は、8,600ゲノム当量/mlから119,562,500ゲノム当量/ml(平均24,009,360)にまで分布した。最終的にHHV-8サブゲノムシークエンスが実施され、口腔内のHHV-8は、4人がジェノタイプC2、1人はジェノタイプA1及びC2に属していたことが示された。血液透析患者の口腔内のHHV-8は高ウイルス量で、多様性があるようだ。従って、血液とともに唾液はHHV-8感染を媒介し、おそらく血液透析患者における高いHHV-8感染リスクとなり、腎移植後の免疫抑制に続くKSの原因となると考えられる。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン5%静注12.5g/250mL
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
血液透析患者の口腔内から高濃度のヒトヘルペスウイルス8(HHV-8)が検出され、唾液がHHV-8を媒介し、腎移植後のカポジ肉腫の原因となることが示唆されたとの報告である。 ヘルペスウイルスは、脂質膜を持つ直径120-200nmの大型DNAウイルスである。これまで、本製剤によるヘルペスウイルス感染の報告はない。本製剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれていることから、本製剤の安全性は確保されていると考える。	日本赤十字社では献血者保護の観点から問診時に腎臓病の有無を尋ね、治癒後1年以内は献血延期としている。この対応により血液透析患者の献血はないと考える。今後も情報の収集に努める。

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Human Herpesvirus 8 Shedding in the Mouth and Blood of Hemodialysis Patients

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In Saudi Arabia, the prevalence of transplantation-associated Kaposi's sarcoma (KS) is high, and there is disparity in the prevalence rates of human herpesvirus 8 (HHV-8) infection between patients with renal disease and the general population. It was hypothesized that oral HHV-8 transmission among patients undergoing hemodialysis treatment contributes to the high prevalence of infection in renal disease patients. The detection rates of anti-HHV8-IgG in plasma and HHV-8-DNA in CD45(+)-peripheral blood cells of 72 hemodialysis patients were compared first with those of 178 blood donors and 60 pregnant women. Between the hemodialysis patients and the apparently healthy people sampled, the detection rate of anti-HHV-8-IgG was 16.7% versus 0.4% ($P < 0.001$) and that of HHV-8-DNA was 4.2% versus 0.4%, ($P < 0.05$). HHV-8 DNA was determined in oral samples and the HHV-8 viral load measured in saliva of patients undergoing hemodialysis. The amount of virus shed into saliva ranged between 8,600 and 119,562,500 (mean: 24,009,360) genome-equivalents/ml among the five patients in whom oral HHV-8 DNA was detected. Finally, HHV-8-subgenomic sequencing was conducted which showed that orally shed HHV-8 in four patients belonged to genotype C2, and in one patient to genotypes A1 and C2. HHV-8 shed in the mouth of hemodialysis patients may be extensive and diverse. Oral fluid in addition to blood is thus a likely vehicle for transmission of HHV-8, possibly contributing to the high risk of HHV-8 infection in patients undergoing hemodialysis and to KS following immunosuppression after renal transplantation. *J. Med. Virol. 84:792–797, 2012.* © 2012 Wiley Periodicals, Inc.

KEY WORDS: Kaposi's sarcoma; human herpesvirus 8; hemodialysis; saliva; nosocomial transmission

INTRODUCTION

Post-transplantation Kaposi's sarcoma (KS) is prevalent in Saudi Arabia, and accounts for over 70% of all *neoplasms observed in renal-allograft recipients* [Qunibi et al., 1988; al Sulaiman and al Khader, 1994]. KS is associated causally with infection by KS-associated human herpesvirus, also known as human herpesvirus 8 (HHV-8). HHV-8 is transmissible by *oral fluid as well as blood* [Teo, 2006; Al Otaibi et al., 2007]. HHV-8 infection does not seem to be highly endemic in the Saudi Arabian general population, since the seroprevalence of anti-HHV-8 in apparently healthy people has been reported to range between 1.7% and 7%, contrasting with 7–28% in patients with chronic renal failure [Qunibi et al., 1988; Almuneef et al., 2001; Alzahrani et al., 2005]. Studies of HHV-8 shedding in body fluids of hemodialysis patients, *especially those from the Middle East, have not, to date,* been reported. The present study investigated the prevalence of HHV-8 infection in such patients, and determined the extent of HHV-8 shedding in the oral fluid in addition to blood. Shedding from specific sites of the oral mucosa and in the oral adnexae was examined. Qualitative and quantitative methods were applied, as well as sequence-diversity determinations of the HHV-8 genome.

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Nucleotide sequence accession numbers: Sequences determined in this study have been deposited in EMBL Nucleotide Sequence Database (accession numbers FN908823 to FN908844).

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PATIENTS, MATERIALS, AND METHODS

Study samples were collected from 72 patients with chronic renal failure undergoing hemodialysis at the Riyadh Military Hospital, Saudi Arabia, during the months of March, April, and May 2004. No oral or cutaneous KS was evident in any of the patients with chronic renal failure. For comparison with a sample of apparently healthy people, 178 blood donors and 60 pregnant women attending the hospital were sampled. Approval for the investigation was secured from the Ethical Committee of the Riyadh Military Hospital and informed consent by participants was obtained. All participants for whom the demographic features are listed in Table I were of Saudi nationality and were seronegative for IgG antibody to the human immunodeficiency viruses. Clinical data for the participants were obtained from computer-based and standard medical records and then documented onto standardized forms, which were linked to patient samples by numerical code. Data entry and analysis were performed using the SPSS for Windows (Statistical Package of Social Science) software, version 12.0. Frequency distributions and cross-tabulation tables were constructed to analyze the relationship between HHV-8 infection and the patients' demographic and clinical characteristics, using, where appropriate, logistical regression, Chi-squared, or Fisher's exact test.

Matched oral and blood samples were obtained from all the patients with chronic renal failure; blood samples only were obtained from the blood donors and pregnant women. An enzyme immunosorbent assay (Advanced Biotechnologies, Inc., Columbia, MD) was utilized for the detection of plasma anti-HHV-8 IgG. This uses, as antigen, a whole viral extract from the KS-1 cell line which was derived from an EBV/HIV-uninfected patient with primary effusion lymphoma (PEL). The test detects antibody to the majority of HHV-8 structural proteins [Said et al., 1996; Chatlynne et al., 1998]. The CD45(+) (pan-leukocyte)-subset of peripheral blood, whole-mouth saliva (supernatant fraction), parotid saliva, buccal mucosa exfoliates and palatal exfoliates were collected, and processed for HHV-8-DNA amplification as described previously [Beyari et al., 2003; Al Otaibi et al., 2007; Al Otaibi et al., 2009]. A 211-bp segment from the KS330 region of open-reading frame (ORF) 26 of the HHV-8 genome, and a 247-bp segment from ORF K1 that encompasses the highly variable V1 region (called the K1/V1 segment) were amplified by nested PCR [Al Otaibi et al., 2007]. Extracts were considered positive for HHV-8 DNA only when PCR yielded products from both KS330 and K1/V1 segments. A quantitative, fluorescence-based, real-time PCR assay [Stamey et al., 2001] targeting ORF 25 was applied to extracts of HHV-8-DNA-positive whole-mouth saliva specimens. In HHV-8-DNA-positive oral and blood samples, consensus K1/V1 nucleotide sequencing was conducted initially. To search for and characterize minority HHV-8 strains, the samples were then subjected to nested PCR for K1/V1 using the EXPAND High-Fidelity PCR System (Roche Diagnostics, Indianapolis, IN), after which clones from each amplificate were generated and the sequences of K1/V1 inserts in 16 clones, picked at random, were determined, as described previously [Al Otaibi et al., 2007].

RESULTS

Table I summarizes the basic demographics, the plasma anti-HHV-8-IgG detection rates and HHV-8-DNA detection rates in CD45(+)-cells and in oral samples among the three study groups. Anti-HHV-8-IgG was detected in the plasma of 12 patients with chronic renal failure (designated CRFs 3, 10, 11, 15, 23, 24 33, 46, 48, 56, 57, and 64), 1 blood donor (designated BD 16) and none of the pregnant women; the anti-HHV-8-IgG detection rate was significantly higher in patients with chronic renal failure (17%) than in the blood donor and pregnant women groups combined (0.4%) ($P < 0.001$). HHV-8 DNA was amplified from CD45(+)-cells of three patients with chronic renal failure (designated CRFs 23, 24, and 57), all of whom were anti-HHV-8-seropositive. HHV-8 DNA was found in CD45(+)-cells of the one seropositive blood donor (designated BD 16), and none of the pregnant women. The HHV-8 DNA detection rate in CD45(+)-cells was significantly higher among patients with chronic renal failure (4.2%) than in the blood donor and pregnant women groups combined (0.4%) ($P < 0.05$).

Among CRFs 23, 24, and 57, HHV-8 DNA was amplified from both blood and oral compartments: for CRF 23 and CRF 57, in CD45(+)-cells and whole-mouth saliva; and for CRF 24, in CD45(+)-cells, whole-mouth saliva, buccal mucosa exfoliates, and palatal exfoliates. In two other patients with chronic renal failure (designated CRFs 10 and 56), HHV-8 DNA was amplified from whole-mouth saliva but not from CD45(+)-cells or other oral samples (Table II). These two patients were also anti-HHV-8-seropositive. The HHV-8 viral load in whole-mouth saliva of CRFs 10, 23, 24, 56, and 57 ranged between 8,600 and 119,562,500 (mean: 24,009,360) genome-copies/ml (Table II). The viral load in whole-mouth saliva obtained from CRF24, in whom HHV-8 DNA was found in both buccal mucosa exfoliates and palatal exfoliates, was notably high.

For BD 16, the K1/V1 sequence in CD45(+)-cells could be assigned to A1. The K1/V1 sequences in whole-mouth saliva of CRFs 10, 23, 56, and 57, and those in CD45(+)-cells of CRFs 23, 24, and 57 could be assigned to genotype C2. For CRF 24, the whole-mouth saliva and palatal exfoliate samples carried K1/V1 sequences that belonged to C2; however, in the buccal exfoliate sample, the majority sequences belonged to C2 whereas the minority sequences (estimated to comprise 13% of the total population) belonged to A1 (Table II). No identical K1/V1 sequences were observed between any two HHV-8 infected patients with chronic renal failure.

The proportion of males included in the blood donor and pregnant women groups combined (176/238; 74%) was significantly higher ($P = 0.026$, Fisher's exact test) than the proportion of males in the chronic renal failure group (43/72; 60%). The mean age of the chronic renal failure group was higher than that of the blood donor and pregnant women groups combined ($P < 0.001$, t-test). However, no statistically significant differences in anti-HHV-8-IgG or HHV-8 DNA detection rates in blood or oral samples according to sex, age, status of infection with hepatitis B or C viruses, duration of hemodialysis, or whether or not previous renal transplantation had been conducted were found. Anti-HHV-8-IgG and HHV-8 DNA were not detected in the patients receiving prednisolone or mycophenolate mofetil (Table III).

TABLE I. Demographic Features, Anti-HHV-8-IgG, and HHV-8-DNA Detection Rates Among Patients With Chronic Renal Failure Receiving Hemodialysis, Blood Donors, and Pregnant Women Sampled in Study

Group	No.	Gender		Age			No. with anti-HHV-8 IgG in plasma (%, 95% CI)	No. with HHV-8 DNA in CD45(+) blood cells (%, 95% CI)	No. with HHV-8 DNA in any oral sample (%, 95% CI)
		Male	Female	Mean (year)	SD	Range (year)			
CRF	72	43	29	57	14	23–83	12 (16.7, 8.9 to 27.3)	3 (4.2, 0.1 to 11.7)	5 (6.9, 2.2 to 15.5)
BD	178	176	2	30	7	18–51	1 (0.6, 0 to 3.1)	1 (0.6, 0 to 3.1)	—
PW	60	0	60	27	7	17–43	0	0	—

CRF, patients with chronic renal failure; BD, blood donors; PW, pregnant women; SD, standard deviation; 95% CI, exact 95% confidence interval; —, not determined.

DISCUSSION

Previous studies of HHV-8 infection in hemodialysis patients were confined exclusively to determining the prevalence of anti-HHV-8 antibodies [Almuneef et al., 2001; Andreoni et al., 2001; Hsu et al., 2002; Di Stefano et al., 2006; Bergallo et al., 2007]. In the present study, conducted among Saudi Arabian patients with chronic renal failure receiving hemodialysis, the extent and diversity of oral HHV-8 shed in saliva and oral cells were investigated, in addition to anti-HHV-8 seroprevalence determination. Higher detection rates of HHV-8 DNA and plasma anti-HHV-8-IgG were found in the hemodialysis patients compared to a sample of apparently healthy people (blood donors and pregnant women, which for purposes of analysis were combined as a single group). This result supports previous data showing the comparatively high prevalence of plasma anti-HHV-8 in patients with chronic renal failure when compared to controls from the same country [Qunibi et al., 1998; Regamey et al., 1998; Hsu et al., 2002; Bergallo et al., 2007; Caterino-de-Araujo et al., 2007; Lonard et al., 2007; Jalilvand et al., 2011].

Logistic regression was undertaken to control for the possible influence of differing age profiles between the two groups which confirmed that the relationship of interest was not materially modified by the observed differences in age. Moreover, the antibody detection rate in the hemodialysis group was much higher (>40-fold) relative to the blood donor and pregnant women groups combined; it would seem unlikely that such a substantial difference could be attributed solely to patient age. The effect of chronic renal disease and uremic immunodeficiency, exacerbated by the protracted rounds of dialysis [Haag-Weber and Horl, 1993; Descamps-Latscha and Chatenoud, 1996], are other possible contributory factors, although these were not evaluated specifically in the current study.

Earlier studies conducted by our group have found evidence of HHV-8 shedding from the oral mucosa of

TABLE II. Distribution of HHV-8 Genotypes in Peripheral Blood and Oral Samples of Patients With Chronic Renal Failure and Blood Donors

Patient	Sample	Amount HHV-8 shed (genome-equivalents/ml)	HHV-8 genotype carried
CRF 10	WMS	151,500	C2[a]
CRF 23	CD45(+) blood cells	—	C2[a]
	WMS	8,600	C2[a]
CRF 24	CD45(+) blood cells	—	C2[a]
	WMS	119,562,500	C2[a]
	Buccal exfoliate	—	C2[a] and A1[b]
	Palatal exfoliate	—	C2[a]
CRF 56	WMS	241,000	C2[a]
CRF 57	CD45(+) blood cells	—	C2[a]
	WMS	83,200	C2[a]
BD 16	CD45(+) blood cells	—	A1[a]

CRF, chronic renal failure; BD, blood donor; WMS, whole-mouth saliva; —, not determined.
[a]All or majority K1V1 sequences.
[b]Minority K1/V1 sequences.

TABLE III. Plasma Anti-HHV-8 Antibody and HHV-8 DNA Status of Patients With Chronic Renal Failure According to Demographic and Clinical Characteristics

Characteristic	No.	Positive plasma anti-HHV-8, no. (%)	Positive HHV-8-DNA,[a] no. (%)
Gender			
Female	29	7 (24.1)	3 (10.3)
Male	43	5 (11.6)	2 (4.7)
P-value for difference		0.204	0.386
Age (years)			
21–30	2	0	0
31–40	9	0	0
41–50	11	0	0
51–60	18	4 (22.2)	1 (5.6)
61–70	21	5 (23.8)	3 (14.3)
71–80	9	3 (33.3)	1 (11.1)
81–90	2	0	0
P-value for difference		0.255	0.955
Duration of dialysis			
≤5 years	36	4 (11.1)	2 (5.6)
>5 years	36	8 (22.2)	3 (8.3)
P-value for difference		0.343	1.000
Previous renal transplant(s)			
Yes	8	0	0
No	64	12 (18.8)	5 (7.8)
P-value for difference		0.337	1.000
Hepatitis B[b]			
Positive	4	0	0
Negative	68	12 (17.6)	5 (7.4)
P-value for difference		1.000	1.000
Hepatitis C[c]			
Positive	11	1 (9.1)	0
Negative	61	11 (18)	5 (8.2)
P-value for difference		0.677	1.000
Prednisolone			
Not administered	68	12 (17.6)	5 (7.4)
Administered	4	0	0
P-value for difference		1.000	1.000
Mycophenolate Mofetil			
Not administered	71	12 (16.9)	5 (7)
Administered	1	0	0
P-value for difference		1.000	1.000
Total	72	12/72 (16.7)	5/72 (7)

[a]HHV-8 DNA positivity in blood, oral, or both samples.
[b]According to seropositivity for hepatitis B surface antigen.
[c]According to seropositivity for anti-hepatitis C virus IgG.

KS patients [Cook et al., 2002a,b; Beyari et al., 2003; Al Otaibi et al., 2007], while others have found evidence of HHV-8 infection in the oral mucosa of immunocompetent individuals [Duus et al., 2004]. Prompted by these findings, an investigation into whether hemodialysis patients might also be shedding the virus orally, thereby possibly contributing to horizontal HHV-8 infection in the hemodialysis milieu, was conducted. Sampling of buccal and palatal exfoliates as well as whole-mouth saliva showed that whole-mouth saliva samples yielded higher PCR amplification rates than buccal or palatal exfoliates, thus likely reflecting virus shed from locations distant from the sampling sites or from oral sites other than the buccal mucosa or hard palate. Of 12 patients identified to be seropositive for anti-HHV-8, 3 were determined to carry HHV-8 DNA in blood, while 5 patients (including the 3 in whom HHV-8 DNA was detected in blood) were found to carry HHV-8 DNA in their oral samples (Table I). To detect possible intra-unit HHV-8 transmission in patients with chronic renal failure, the hypervariable region (K1/V1) was analyzed and compared between patients undergoing hemodialysis. Previous studies have documented intra-unit hepatitis C virus transmission in hemodialysis units, with phylogenetic analysis revealing clustering between patients who were dialyzed during the same shift and in the same area [Hmaied et al., 2007]. In the current study, however, phylogenetic analysis revealed that none of the HHV-8-infected patients with chronic renal failure carried identical K1/V1 sequences.

The findings of the present study indicate that oral HHV-8 shedding in the hemodialysis patients occurred more frequently than systemic shedding. Furthermore, by applying quantitative methods, it was determined that during a given episode, oral HHV-8 shedding can be extensive (Table II). The viral load in whole-mouth saliva of four of the five patients who were oral HHV-8 shedders was determined to exceed 10^5 genome-copies/ml. Previously, similar results were found among renal-allograft recipients with a history of KS [Al Otaibi et al., 2007], and a renal-allograft recipient who had been given immunosuppressants [Al Otaibi et al., 2009]. The single patient with chronic renal failure (CRF 24) in whom HHV-8 DNA was detected in both buccal and palatal exfoliates showed the highest whole-mouth saliva viral load DNA (approaching 1.2×10^7 genome-copies/ml). The marked diversity of HHV-8 variants in his oral compartment is consistent with this patient having acquired the strains from previous, multiple transmission events [Beyari et al., 2003].

The findings of the present study show that oral fluid may also be a vehicle of HHV-8 transmission. In an environment where multiple patients receive dialysis concurrently, repeated opportunities exist for person-to-person transmission of infectious agents shed from the mouth, directly or indirectly via contaminated devices, equipment, supplies, environmental surfaces, or hands of personnel. Any item taken to a patient's dialysis station could become contaminated with blood and other body fluids thereby serving as a vehicle of transmission to other patients [CDC, 2001]. Contact transmission is the most important route by which pathogens are transmitted in health-care settings such as hemodialysis units. Contact transmission occurs most commonly when microorganisms from a patient are transferred to the hands of a health-care worker and then to another patient. Less commonly, environmental surfaces (e.g., bed rails, countertops) can become contaminated and serve as fomites; transmission can occur when a worker touches the surface who then touches a patient or when a patient touches a contaminated surface. Contact transmission can be prevented by hand hygiene, glove use, and frequent disinfection of environmental surfaces [CDC, 2001].

In hemodialysis units, policies and practices should be periodically reviewed and monitored to ensure that infection control practices are implemented and followed rigorously [Sehulster and Chinn, 2003; Rebmann and Barnes, 2011]. Training and educating staff members and patients regarding infection control practices, including personal and hand hygiene, should be implemented regularly [CDC, 2001]. Maintaining and regularly updating these infection control practices routinely for all patients in the hemodialysis setting will reduce opportunities for direct and indirect patient to patient transmission of infectious agents, including HHV-8 infection, which if acquired may, in turn, potentiate the development of KS should the patients subsequently undergo kidney transplantation and then receive immunosuppressive drugs.

Prospective studies evaluating the incidence of HHV-8 infection, together with phylogenetic analysis, in patients with chronic renal failure given hemodialysis treatment would determine the risk of intra-unit blood and oral transmission of HHV-8 infection in hemodialysis units more definitively. Further studies would then be needed to clarify the specific factors responsible for transmission of HHV-8 among hemodialysis patients and to evaluate the effect of the current recommendations on prevention and control of infections in this setting. Particularly in the Middle East, implementation of additional measures preventing HHV-8 transmission from oral fluid as well as blood might be required to control HHV-8 infection and reduce the incidence of post-renal-transplantation KS.

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No. 12

医薬品 研究報告 調査報告書

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研究報告の概要

○ヒトでの致死的な感染症と関連するアンデスウイルスの新しい株、ボリビア中央部
南アメリカでヒト疾患と関連しているハンタウイルスの遺伝的多様性を調べるため、2008年～2009年にボリビア中央部のチャパレで発熱患者の血液サンプルを検査した。ハンタウイルス属RNAは死亡した1人を含む3人の患者に認められた。3人の患者からのS及びMセグメントの部分的RNA配列はアンデスウイルス系統に最も密接に関連していたが、既報告株とは異なっていた(90%以下のヌクレオチド一致)。チャパレ住民間での抗ハンタウイルスIgG抗体調査は人口の12.2%が過去に1つ以上のハンタウイルスへ曝露していたことを示し、農業従事者間で最も罹患率が高かった。ハンタウイルス株へ曝露する人が多いことと、結果的に生じる疾病が重大であることから、この新しいハンタウイルスの宿主、浸淫地域、及び公衆衛生への影響を決定するための更なる研究が必要とされる。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン5%静注12.5g/250mL
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
ボリビア中央部のチャパレで死亡した1人を含む3人から、以前報告されていたアンデスウイルスとは異なる、ハンタウイルスの新しい株が検出されたとの報告である。 ハンタウイルスは脂肪膜を有するRNAウイルスである。これまで、本製剤によるハンタウイルス感染の報告はない。本製剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれていることから、本製剤の安全性は確保されていると考える。	日本赤十字社では、輸血感染症対策として問診時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

15

Novel Strain of Andes Virus Associated with Fatal Human Infection, Central Bolivia

Cristhopher D. Cruz, Brett M. Forshey, Efrain Vallejo, Roberto Agudo, Jorge Vargas, David L. Blazes, Carolina Guevara, V. Alberto Laguna-Torres, Eric S. Halsey, and Tadeusz J. Kochel

To better describe the genetic diversity of hantaviruses associated with human illness in South America, we screened blood samples from febrile patients in Chapare Province in central Bolivia during 2008–2009 for recent hantavirus infection. Hantavirus RNA was detected in 3 patients, including 1 who died. Partial RNA sequences of small and medium segments from the 3 patients were most closely related to Andes virus lineages but distinct (<90% nt identity) from reported strains. A survey for IgG against hantaviruses among residents of Chapare Province indicated that 12.2% of the population had past exposure to ≥1 hantaviruses; the highest prevalence was among agricultural workers. Because of the high level of human exposure to hantavirus strains and the severity of resulting disease, additional studies are warranted to determine the reservoirs, ecologic range, and public health effect of this novel strain of hantavirus.

Hantaviruses (family *Bunyaviridae*, genus *Hantavirus*) are trisegmented negative-strand RNA viruses in which the small (S), medium (M), and large (L) genomic segments encode for the nucleocapsid protein (N), 2 envelope glycoproteins (Gn and Gc), and the viral polymerase, respectively. Hantaviruses are maintained in rodent reservoirs, and human exposure typically results from inhalation of aerosols from infectious urine or feces, although human-to-human transmission of Andes virus (ANDV) has also been described (*1*). Human hantavirus infection in South America is often associated with rapid onset of severe disease manifestations, such as respiratory failure and cardiac dysfunction referred to as hantavirus pulmonary syndrome (HPS) and case-fatality rates ≥50% (*2,3*). Despite the public health effects, in most cases of human infection, the precise etiologic agent is not identified. Thus, the extent of genetic diversity and geographic distribution of distinct hantavirus strains is not well understood.

Since the first identification of HPS in 1993, many new hantaviruses have been described throughout North, Central, and South America. Studies of rodent reservoirs in South America have identified an increasingly complex picture of hantavirus diversity and ecology (*2,4*). Unique strains of hantavirus have been identified in rodents in Venezuela (*5,6*), Peru (*7*), Brazil (*8–10*), Argentina (*11–13*), Paraguay (*14,15*), and Chile (*11,16*), many of which have also been associated with human illness. In Bolivia, the first hantavirus identified was Río Mamoré virus (RIOMV), which was isolated from a pygmy rice rat (*Oligoryzomys microtis*) (*17*) but has not been associated with human disease. In 1997, a Laguna Negra virus (LNV) variant was identified in an HPS patient in Chile who had traveled extensively in Bolivia (*18,19*). An ecologic assessment of reservoir hosts identified the large vesper mouse (*Calomys callosus*) as reservoir host of LNV in Bolivia (*20*). The association of ANDV (Nort lineage) and Bermejo virus (BMJV) with 2 HPS cases in southern Bolivia in 2000 documented the first human infection by BMJV (*21*).

To further describe the diversity of hantavirus strains associated with human disease in Bolivia, we screened febrile patients reporting to 2 health centers in Chapare Province for serologic and molecular evidence of hantavirus infection. We describe the clinical signs and symptoms and genetic characterization (partial S and M segment) of a novel strain of hantavirus in 3 patients, including 1 who died. In addition, we report results of a survey to determine the prevalence of previous hantavirus exposure in the region.

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Materials and Methods

Study Site and Human Participant Issues

Patients were recruited when they reported acute febrile illness (≤7 days) at the Hospital San Francisco de Asis or Centro de Salud Eterezama (16°55′S, 65°22′W; 265 m above sea level), located in the Chapare Province of the Department of Cochabamba in central Bolivia (*22*) (Figure 1). Chapare is a rural province with tropical rainforests surrounding the Chapare River, the main waterway of the region. The health centers are located in the towns of Villa Tunari and Eterezama, which had 2,632 and 2,001 inhabitants, respectively, at the time of the 2001 census (*23*).

Study protocols were approved by Servicio Departamental de Salud Santa Cruz, and Colegio Medico de Santa Cruz. Study protocols (NMRCD.2000.0008 and NMRCD.2005.0002) were also approved by the US Naval Medical Research Unit Institutional Review Board in compliance with all US Federal regulations governing the protection of human subjects. Written consent was obtained from patients ≥18 years of age. For patients <18 years of age, written consent was obtained from a parent or legal guardian. Written assent was also obtained from patients 8–17 years of age.

A survey for prior exposure to arenaviruses and hantaviruses was conducted in Chapare Province during April 25–May 2, 2005, after a reported outbreak of febrile illness and hemorrhagic fever in the region (*24*). Adults (≥18 years of age) were invited to participate in the study. Blood samples (10 mL) were collected by venipuncture for screening of antibodies against hantaviruses, and demographic data were collected for risk factor analysis in assorted villages in Chapare Province (Figure 1). Data included age, occupation, self-reported exposure to rodents, house construction materials, and recent health history.

Serologic Analyses

Serum samples from febrile patients were screened for IgM against ANDV or LNV antigens by ELISA. In brief, 96-well plates were coated with anti-human IgM, human serum samples (1:100 dilution) were added, and plates were incubated for 1 h at 37°C. Wells were subsequently incubated with ANDV or LNV antigen for 1 h at 37°C. Viral antigens were recognized by the addition of hyperimmune mouse ascitic fluid for 1 h at 37°C and incubation with horseradish peroxidase–conjugated anti-mouse IgG for 1 h. Finally, substrate (2,2′-azino-bis-[3-ethylbenzthiazoline-6-sulfonic acid]; Kirkegaard and Perry, Inc., Gaithersburg, MD, USA) was added, and optical density at a wavelength of 405 nm was measured by using a spectrophotometer. Patient serum specimens were also screened for IgM against a panel of arboviral pathogens, including dengue viruses, yellow fever virus, and Venezuelan equine encephalitis virus. Virus culture and identification was attempted in African green monkey Vero cell cultures by indirect immunofluorescence assay and Sin Nombre virus (SNV) polyclonal antibodies, as described for arboviruses (*22*).

Figure 1. Location of Villa Tunari, Department of Cochabamba, Bolivia, the area where patients with hantavirus infection were recruited. The constitutional (Sucre) and administrative (La Paz) capitals of Bolivia are shown for reference.

For the seroprevalence study, serum samples from healthy participants were screened by indirect ELISA for IgG against SNV antigen (Centers for Disease Control and Prevention, Atlanta, GA, USA). Serum samples were diluted 1:100 and incubated in SNV recombinant antigen–coated wells and then with horseradish peroxidase–conjugated mouse anti-human IgG (1:8,000 dilution). Finally, substrate (2,2′-azino-bis-[3-ethylbenzthiazoline-6-sulfonic acid]) was added, and absorbance was measured at 405 nm with a Dynex ELISA MRX Revelation absorbance reader (Dynex Technologies, Chantilly, VA, USA). Samples with optical densities greater than the mean of 5 negative controls plus 5 SD at a 1:100 dilution were considered positive.

Molecular Analyses

After serologic screening, RNA was extracted from whole blood and serum samples of patients positive for hantavirus IgM by using the QIAamp Viral RNA Mini Kit (QIAGEN, Valencia, CA, USA). A 1-step reverse transcription PCR (RT-PCR) was performed by using the Access RT-PCR system (Promega, Madison, WI, USA). Nested PCRs were performed by using the FastStart PCR Master (Roche, Indianapolis, IN, USA). Initial screening was performed by using primers specific for the S segment as described (*20*). Additional primers were designed on the basis of preliminary sequences to generate additional S segment coding region sequence (forward: HANSF3 5′-TGGATGTTAATTCCATCGA-3′ and reverse: HANSR4 5′-GATAATGTTTCGTGCTTTCA-3′; forward: HANF0001 TAGTAGTAGACTCCTTGAGAAGCTACT and reverse: HANTASR2 TAGTATGCTCCTTGAR AAGC). A 1,287-bp region of the S segment was generated, which included positions 43–1329 of the full-length S segment of ANDV strain Chile R123 (*25*).

For the M segment, RT-PCR and nested PCR were performed by using specific primers (*18*), which generated a 1,330-bp sequence of the M segment that included positions 1678–3007 of the full-length M segment of ANDV strain Chile R123. RT-PCR amplicons were purified by agarose gel electrophoresis and sequenced directly by using the Big Dye Terminator v3.1 Cycle Sequencing Kit on a 3100 Avant-Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Phylogenetic Analysis

S segment and M segment sequences (submitted to GenBank under accession nos. JF750417–JF750422) were compared with sequences from other members of the genus *Hantavirus*, including Puumula virus strain Umea (Genbank accession nos. S segment: AY526219, M segment: AY526218), RIOMV strain HTN-007 (S: FJ532244, M: FJ608550), SNV strain NMH10 (S: L25784, M: L24783), El Moro Canyon virus strain RM97 (S: U11427, M: U26828), Choclo virus (S: DQ285046, M: DQ285047), Caño Delgadito virus (S: DQ285566; M: DQ284451), Pergamino virus (PRGV; S: AF482717, M: AF028028), ANDV strain AH-1 (S: AF324902, M: AF324901), ANDV strain CHI7913 (S: AY228237, M: AY228238), ANDV strain Chile-9717869 (S: AF291702, M: AF291703), Maciel virus strain 13796 (MACV; S: AF482716, M: AF028027), Catacamas virus (CATV; S: DQ256126, M: DQ177347), Paranoa virus (S: EF576661), Oran virus (S: AF482715, M: AF028024), LNV (S: AF005727, M; AF005728), BMJV (S: AF482713, M: AF028025), Lechiguanas virus strain 22819 (S: AF482714, M: AF028022), ANDV strain Hu39694 (S: AF482711, M: AF028023), Playa de Oro virus (S: EF534079, M: EF534082), Neembucu virus (S: DQ345763), Alta Paraguay virus (S: DQ345762), Itapua virus (S: DQ345765), Araraquara virus (ARAV; S: AF307325, M: AF307327), Araucaria virus strain HPR/03–99 (S: AY740630), Jabora virus strain Akp8048 (S: JN232080), Juquitiba virus strain Olfo_777 (S: GU213198), and Castelo dos Sonhos virus (CASV; S: AF307324, M: AF307326).

Sequences were aligned by using ClustalW (www.clustal.org) with manual adjustments, and pairwise genetic distances were calculated by using MEGA4.0 (*26*). For phylogenetic analysis, maximum-likelihood (ML) and Bayesian approaches were used. ML phylogenetic trees were estimated by using PhyML (*27,28*) and 100 bootstrap replications to place confidence intervals at nodes. Phylogenetic reconstructions were also conducted in MrBayes version 3.1 (*29,30*) under the general time reversible + Γ + proportion invariant model of evolution, with 1 million Markov Chain Monte Carlo generations, and sampling every 100 generations with a burn-in of 25,000. Puumula virus S and M segments were included as outgroups in the phylogenetic reconstructions.

Results

Patient Identification

During January 2008–June 2009, serum samples from 372 febrile patients reporting to clinics in Chapare Province, Bolivia (Figure 1) were tested for serologic evidence of recent infection by ≥1 hantaviruses. Of these 372 patients, 199 (53.5%) were male patients with a median age of 31 years (range 7–95 years). IgM against ANDV (n = 8) or LNV (n = 1) antigen was identified in acute-phase or convalescent-phase samples from 9 (2.4%) patients. No evidence of recent arbovirus infection was detected in these samples. Of the 9 patients with IgM against hantaviruses, 7 (77.8%) were male patients with a median age of 32 years (range 15–49 years). Three of the 9 patients were positive for hantavirus RNA.

Patient 1 (FVB0554) was an 18-year-old man (student) from the town of Pedro Domingo Murillo, Bolivia, who came to Hospital San Francisco de Asis in January 2008. He reported 7 days of fevers, chills, and malaise. Other symptoms included oliguria, arthralgias, myalgias, bone pain, headache, and retroocular pain. Gastrointestinal (abdominal pain, diarrhea, nausea, emesis, and icterus) and respiratory (cough, dyspnea, and cyanosis) manifestations were also prominent. The patient died the next day. IgM against LNV antigen (reciprocal titer 1,600) was detected in a serum sample collected before his death.

Patient 2 (FVB0640) was a 27-year-old man (agricultural worker) from Samuzabety, Bolivia, who came to Hospital San Francisco de Asis in March 2008. The patient had a temperature of 39.9°C and reported 8 days of fever, chills, and malaise. Other symptoms included cough, arthralgias, myalgias, bone pain, headache, and retroocular pain. On examination, multiple cutaneous manifestations were noted, including petechiae, purpura, a maculopapular rash, and a diffuse erythematous rash. The patient was hospitalized for 4 days and recovered. IgM against ANDV was detected in an acute-phase serum sample (reciprocal titer 6,400); no convalescent-phase sample was obtained.

Patient 3 (FVB0799) was a 49-year-old man (farmer) from Flor de San Pedro, Bolivia, who came to Hospital San Francisco de Asis in June 2009. He reported 4 days of fever, chills, and malaise. Other symptoms included arthralgias, myalgias, bone pain, abdominal pain, headache, cough and dyspnea. The patient survived. IgM against ANDV was detected in an acute-phase serum sample (reciprocal titer 6,400); no convalescent-phase sample was available for additional analysis.

Molecular Analyses

Viral sequences generated from samples from the 3 patients were highly conserved over the gene regions analyzed; >99.6% pairwise nucleotide identity in the S segment (3–5-nt differences) and >99.2% pairwise nucleotide identity in the M segment (1–10-nt differences). Nucleotide sequences were compared with those of hantavirus strains available in GenBank (Table 1). In pairwise comparisons of S segment gene sequences, we observed the highest identity with CASV (*31*), which showed 89.3% identity at the nucleotide level and 98.6% identity at the amino acid level, although only limited sequence (643 nt) was available for comparison. In comparison with other Western Hemisphere hantaviruses for which more extensive sequences were available (1,287 nt) 75.8%–84.1% nucleotide sequence identity and 85.3%–97.7% amino acid identity were observed, and the highest similarity was with members of the species *Andes virus* (Table 1).

In pairwise comparisons of M segment gene sequences, the highest nucleotide identity (83.3%) was observed in comparison with CASV. Similar amino acid identities were observed with CASV (95.1%), Oran virus (95.3%), Lechiguanas virus (95.0%), and ANDV Hu39694 (95.3%) (Table 1). Viral sequences amplified from patient samples were more distantly related to LNV, Caño Delgadito virus, and Maporal virus; all showed <80% pairwise identity at the nucleotide level and <90% pairwise identity at the amino acid level (Table 1).

Table 1. Percent pairwise nucleotide and amino acid identity between select Western Hemisphere hantaviruses and virus sequences amplified from patients from central Bolivia*

Virus strain	Country	S segment (1,287 bp)		M segment (1,330 bp)	
		Nucleotide	Amino acid	Nucleotide	Amino acid
PRGV	Argentina	81.4	94.6	80.8	93.0
ANDV AH1	Argentina	83.5	96.0	81.7	93.9
ANDV Hu39694	Argentina	82.9	97.4	81.7	95.3
MACV	Argentina	81.7	94.2	80.2	91.4
BMJV	Argentina	83.7	97.7	80.2	93.9
LECV	Argentina	84.1	97.4	81.1	95.0
ORNV	Argentina	83.5	97.4	80.3	95.3
CASV†‡	Brazil	89.3	98.6	83.3	95.1
PARV	Brazil	82.9	95.3	NA	NA
ARAV§	Brazil	84.0	94.9	79.5	93.2
JABV	Brazil	77.3	88.6	NA	NA
ARCV	Brazil	82.2	95.8	NA	NA
ANDV 9717869	Chile	83.5	96.0	80.7	93.7
ANDV CHI7913	Chile	82.7	95.6	81.1	92.8
CATV	Honduras	76.9	88.1	76.0	86.2
PDOV	Mexico	77.4	87.4	75.8	85.4
CHOV	Panama	78.9	89.3	77.8	88.0
NEMV	Paraguay	84.9	97.0	NA	NA
ALPV	Paraguay	80.3	89.3	NA	NA
ITAPV	Paraguay	81.7	95.8	NA	NA
JUQV‡	Paraguay	82.5	95.5	NA	NA
LNV	Paraguay	79.4	90.2	79.2	90.5
RIOMV	Peru	80.1	90.0	80.6	91.4
SNV NMH10	USA	76.5	87.2	76.0	86.2
ELMCV RM97	USA	76.8	83.9	73.8	82.8
MAPV	Venezuela	79.6	91.1	77.8	89.8
CADV	Venezuela	75.8	85.3	74.3	83.1

*S, small; M, medium; PRGV, Pergamino virus; ANDV, Andes virus; MACV, Maciel virus; BMJV, Bermejo virus; LECV, Lechiguanas virus; ORNV, Oran virus; CASV, Castelo dos Sonhos virus; PARV, Paranoa virus; ; NA, sufficient sequence not available for comparison; ARAV, Araraquara virus; JABV, Jabora virus; ARCV, Araucaria virus; CATV, Catacamas virus; PDOV, El Moro Canyon virus; CHOV, Choclo virus; NEMV, Neembucu virus; ALPV, Alta Paraguay virus; ITAPV, Itaporanga virus; JUQV, Juquitiba virus; LNV, Laguna Negra virus; RIOMV, Rio Mamoré virus; SNV, Sin Nombre virus; ELMCV, El Moro Canyon virus; MAPV, Maporal virus; CADV, Caño Delgadito virus.
†S segment sequence comparison was limited to the homologous 999 bp (JUQV) or 643 bp (CASV) available from GenBank.
‡M segment sequence comparison was limited to the homologous 1,246 bp available from GenBank.

To further explore genetic relationships between the novel viral sequences and previously described hantaviruses, we conducted ML and Bayesian analyses on the basis of S segment and M segment nucleotide sequences. Similar results were obtained for ML and Bayesian approaches (Figure 2). Viral sequences derived from patient samples grouped with other strains of ANDV (www.ncbi.nlm.nih.gov/ICTVdb/index.htm); formed a clade with ARAV, MACV, PRGV, and other ANDV strains; and formed a subclade with CASV (Figure 2). Similar tree topologies for other strains of ANDV were obtained on the basis of analysis of S segment and M segment sequences. Genetic differences between CASV and the novel sequences were well supported by posterior probabilities (Figure 2) and ML bootstrap values.

Prevalence of IgG against Hantaviruses among Humans in the Chapare Region

To determine the extent of human exposure to hantaviruses in the region, we screened serum samples from residents of villages in Chapare Province for IgG against SNV antigen. A total of 500 participants >18 years of age residing in villages in the region were enrolled during April 25–May 2, 2005 (Table 2). Participants had a median age of 31 years (range 18–99 years); 54.9% were women (Table 2).

Sixty-one (12.2%; 95% CI 9.3%–15.1%) had IgG against SNV antigen (Table 2), and the highest prevalences were in the towns of Samuzabety (18.6%) and San Gabriel (17.2%). No differences were observed between sexes or among different age groups (Table 2). The highest prevalence of IgG against SNV was among agricultural workers (15.0%) and housewives (13.5%) (Table 2). No differences in seropositivity were observed for participants with differing house construction materials or quality.

Discussion

We demonstrated the association of a novel genotype of ANDV with fatal human infection in central Bolivia and extended the known genetic diversity of hantaviruses circulating in South America. One fatal case occurred among the 3 patients described, which was consistent with high mortality rates observed with infections with ANDV lineages in neighboring Brazil and Argentina (*3*). The International Committee on Taxonomy of Viruses has provided guidelines for the demarcation of hantaviruses (www.ictvdb.org/Ictv/index.htm), which include a ≥7% difference in amino acid identity in comparison with previously identified complete S segment and M segment gene sequences, a ≥4-fold difference in results of 2-way cross-neutralization tests, and occupation of a unique ecologic niche, such as a different primary rodent reservoir. As with other hantavirus strains, attempts to isolate virus in tissue culture were unsuccessful; thus, cross-neutralization studies could not be conducted. Genetic criteria, amino acid and nucleotide comparisons, and phylogenetic analysis clearly support inclusion of this strain in the species *Andes virus*.

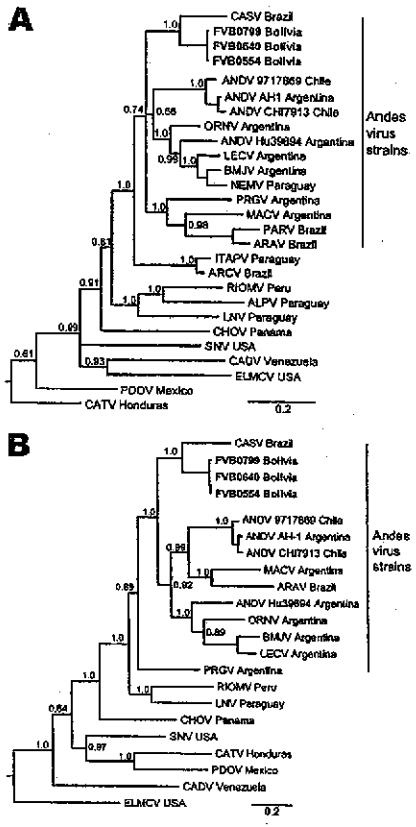

Figure 2. Phylogenetic analysis of hantaviruses from the Western Hemisphere on the basis of partial A) small and B) medium segments. Novel strains described in this study are indicated in boldface. Depicted phylogenetic reconstructions are based on Bayesian inference conducted in MrBayes (*29,30*). Posterior probabilities are indicated at relevant nodes. Scale bars indicate nucleotide sequence divergence. CASV, Castelo dos Sonhos virus; ANDV, Andes virus; ORNV, Oran virus; BMJV, Bermejo virus; LECV, Lechiguanas virus; BMJC, Bermejo virus; NEMV, Neembucu virus; PRGV, Pergamino virus; MACV, Maciel virus; PARV, Paranoa virus; ARAV, Araraquara virus; ITAPV, Itaporanga virus; ARCV, Araucaria virus; RIOMV, Río Mamoré virus; ALPV, Alta Paraguay virus; LNV, Laguna Negra virus; CHOV, Choclo virus; SNV, Sin Nombre virus; CADV, Caño Delgadito virus; ELMCV, El Moro Canyon virus; PDOV, El Moro Canyon virus; CATV, Catacamas virus.

Table 2. Characteristics of patients tested for IgG against Sin Nombre virus, central Bolivia*

Characteristic	No. positive/no. tested (%)
Sex	
M	28/224 (12.5)
F	32/273 (11.7)
Age, y	
18–30	28/244 (11.5)
31–50	28/207 (13.5)
>50	4/43 (9.3)
Occupation	
Agricultural worker	25/167 (15.0)
Housewife	26/193 (13.5)
Student/teacher	3/57 (5.3)
Health professional	0/20 (0)
Other/unknown	7/62 (11.3)
Village	
Eterazama	13/116 (11.2)
Isinuta	6/71 (8.5)
Primero de Mayo	1/ 20 (5.0)
Samuzabety	13/70 (18.6)
San Gabriel	5/ 29 (17.2)
San Julian	2/24 (8.3)
Urkupina	2/22 (9.1)
Other	19/148 (12.8)
Total	61/500 (12.2)

*Complete demographic data were not available for all participants.

No guidelines have been proposed for demarcation of viruses below the species level, and there does not appear to be consensus on the designation of novel genotypes. We observed the highest identity with CASV, which has been associated with human illness near the border of the Mato Grosso and Pará States of Brazil (*31,32*), ≈1,500 km from the Chapare study sites. We observed ≈11% and 17% divergence at the nucleotide level for the S segment and M segment, respectively, in comparison with CASV. However, the true difference between the strains might be underestimated because higher nucleotide and amino acid conservation was observed among ANDV strains overall in the limited S region available for comparison (14%), relative to other genome regions (16%). No other hantavirus was found to be <15% divergent at the nucleotide level in the S segment or <18% divergent in the M segment. If one considers that the strains identified in our study segregate with other strains of ANDV but are genetically distinct, we provisionally propose to name this novel genotype Tunari virus (TUNV), after the town of Villa Tunari, where all 3 patients sought medical attention.

On the basis of data in this report, we found that human hantavirus exposure is common in the Chapare Province. Including the 3 TUNV cases, in 2008 and 2009, >2% of febrile cases analyzed had evidence of recent hantavirus infection, which is consistent with the 4% of febrile cases reported for the region in 2005 and 2006 (*33*). In addition, the 2005 serosurvey of healthy persons indicated that a high percentage (≈12%) of adults had evidence of exposure to ≥1 hantaviruses at some time in their lives.

The extent of exposure we found is similar to that reported in neighboring Brazil, which was 13.3% in Maranhao State in northeastern Brazil (*34*) and 14.6% in southern Brazil (*35*), and in northern Argentina, which was 6.5%–17.1%, depending on the population studied (*13,36*). Occupational exposure appears to be a risk factor because the highest antibody prevalence and 2 of 3 TUNV cases were identified among agricultural workers. We did not observe an age-dependent increase in antibody prevalence among adults sampled, a finding also reported in southern Brazil (*35*). There are several possible explanations for this observation, including relatively recent emergence of hantaviruses in the region, high mortality rates among infected persons, and preponderance of risk for exposure during early adulthood.

Broad antigenic cross-reactivity that prevents discrimination among diverse hantavirus groups is 1 of the major limitations of ELISA-based serologic studies, whether used in screening febrile patients for IgM against hantaviruses or healthy persons for IgG against hantaviruses. Co-circulation of heterologous hantaviruses has been described in Bolivia in rodent reservoirs and in ill persons. RIOMV has been identified in the pigmy rice rat (*Oligoryzomys microtis*) in Bolivia (*17*). In 2000, HPS cases associated with BMJV and ANDV strain Nort were identified along the southern border of Bolivia with Argentina (*21*). LNV had been amplified from an HPS patient in Chile with recent travel history to Bolivia (*19*). In addition to these cases are many additional suspected cases of HPS in Bolivia for which no specific virus was identified. Of the 246 reported cases from 2007 through 2010, a total of 74 occurred in the Department of Cochabamba (*37*). Future studies with more specific serologic assays are necessary to determine the true extent of TUNV circulation in this population.

In this study, we made no effort to incriminate the reservoir host for TUNV. The only hantavirus reservoir identified in South America is rodents of the subfamily *Sigmodontinae*. *Oligoryzomys* spp. rodents appear to be the principal reservoirs for most ANDV variants, including CASV (*32,38*). In addition to *Oligoryzomys* spp. rodents, ANDV variants have been identified in *Akodon* spp. (PRGV), *Necromys* spp. (MACV and ARAV), and *Bolomys* spp. (MACV) rodents. Potential reservoir species are abundant in Bolivia, including *Oligoryzomys* spp., *Akodon* spp., and *Calomys* spp. (LNV) rodents. Increased rodent population density has been associated with the emergence of hantavirus infection in humans (*4*). Therefore identifying the TUNV reservoir host and understanding its

ecology could lead to interventions for reducing human exposure.

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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	総合機構処理欄
				2012. 6. 22	該当なし	
一般的名称	人血清アルブミン		研究報告の公表状況	Boone I, Wagner-Wiening C, Reil D, Jacob J, Rosenfeld U, Ulrich R, Lohr D, Pfaff G. Euro Surveill. 2012 May 24;17(21). pii: 20180.	公表国 ドイツ	
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン5%静注12.5g/250mL(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)					

研究報告の概要

○ドイツのバーデン－ヴュルテンベルク州における2011年10月からのヒトハンタウイルス感染症報告数の増加

2011年10月から2012年8月、852件のヒトハンタウイルス(プーマラウイルス)感染症がドイツで報告され、そのうち580件(68%)がバーデン－ヴュルテンベルク州で発生した。症例数は2007年及び2010年のアウトブレイクの時よりも早く増加し始め、例年の10月－4月期における報告数の中で最多であった。

プーマラウイルスは保有宿主であるハタネズミの排泄物への曝露によりヒトに伝播し、2～4週間の潜伏期後に流行性腎症を引き起こす。ドイツでの2001年～2011年のハンタウイルス感染年間届出数は72～447件(中央値235件;2007年と2010年のアウトブレイクを除く)であった。2012年第17週の最新報告数は87件であり、歴史的な最多報告数である2007年第22週の96件にほぼ到達している。

症例数急増の原因は不明であるが、気候要因と、恐らく2011年のブナの繁茂によるハタネズミの増加に関連すると推定されている。2012年夏季期間にさらなる症例数増加が見込まれるため、予防対策のためのさらなる情報が必要とされる。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン5%静注12.5g/250mL
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

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報告企業の意見

ドイツで例年の同時期に比べヒトハンタウイルス(プーマラウイルス)感染症が急増しており、その大部分がバーデン－ヴュルテンブルク州からの症例であるとの報告である。

ハンタウイルスは脂質膜を有するRNAウイルスである。これまで、本製剤によるハンタウイルス感染の報告はない。本製剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれていることから、本製剤の安全性は確保されていると考える。

今後の対応

日本赤十字社では、輸血感染症対策として問診時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

MedDRA/J Ver.15.0J

JRC2012T-018

RAPID COMMUNICATIONS

Rise in the number of notified human hantavirus infections since October 2011 in Baden-Württemberg, Germany

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From October 2011 to April 2012, 852 human hantavirus infections were notified in Germany, of which 580 (68%) were in Baden-Württemberg. Case numbers started to rise earlier than they did before the previous outbreaks in 2007 and 2010, and are the largest ever reported in this state during October to April of any year. The early rise could be due to a beech mast year in 2011, followed by an early and massive reproduction of the reservoir bank vole populations during winter 2011 and spring 2012.

Outbreak description

From October 2011 to April 2012 (reporting weeks 40 2011 to week 17 2012, *ending 27 April 2012*), 852 cases of hantavirus infections meeting the national case definition [1,2] were notified in Germany (cumulative incidence: 1.04 per 100,000 population) [3]. Of these, 580 cases (68%) originated in the southern federal state of Baden-Württemberg (cumulative incidence: 5.4 per 100,000 population) (Table). This count exceeds the number of cases observed during the months October to April that preceded the outbreaks in 2007 (172 cases) and in 2010 (327 cases) in the same state (Table). *We report on this ongoing outbreak in Baden-Württemberg, taking into consideration cases notified from October 2011 to April 2012.*

Background

Puumala virus is the predominant human pathogenic hantavirus species in western, central and northern Europe [4]. It is transmitted to humans by exposure to excreta of its rodent reservoir, bank voles (*Myodes glareolus*) [5]. After an incubation period of usually *two to four weeks [6], typical clinical manifestations* include a sudden onset with fever, headache, back pain and gastrointestinal symptoms. Renal involvement is prominent and manifests initially as oliguria and later as marked polyuria (nephropathia epidemica) [7]. Only 30% of Puumala virus infections occur with typical clinical signs, resulting in high under-reporting [8]. There is currently no specific antiviral treatment [9]. *Recommended prevention measures focus on the* avoidance of exposure and inhalation of potentially contaminated dust [9].

In Germany, laboratory-confirmed cases of hantavirus infections have been notifiable since 2001 [1,10]. Between 2001 and 2011, the number of annual notifications ranged from 72 to 447, with a median of 235, except for two outbreaks in 2007 (1,688 cases) and 2010 (2,107 cases) [11]. From November 2011 to February 2012, *the Robert Koch Institute observed an increase* in the number of cases notified in Germany compared with the mean in the same period in the five preceding years, from 2006/2007 to 2010/2011. Some 64% of these cases were reported from Baden-Württemberg [11].

Figure 1 represents the temporal distribution of cases in Baden-Württemberg from reporting week 40 in 2011 until reporting week 17 in April 2012, in comparison *with the outbreak periods of 2006–2007 and 2009–2010.* The current outbreak period 2011–2012 is characterised by an early increase of cases, which started already in October 2011. In the last reported week in 2012 (week 17), the number of cases (n=87) has almost reached the historical weekly maximum of the 2007 outbreak year (96 cases in week 22).

Figure 2 shows the geographical distribution of cases in Baden-Württemberg. Some 45% of all cases (n=580) *were reported from five of the 44 counties of Baden-*Württemberg. These counties are in the central part of the state, comprising the city of Stuttgart (n=65; incidence: 10.7 per 100,000 population), Tübingen (n=34; incidence: 15.4 per 100,000 population),

TABLE

Number and incidence of notified cases of hantavirus infection in each federal state, Germany, 2001–2012

State	2001–2006		2007		2008	2009	2010		2011		2012	
	Number of cases (incidence per 100,000 population)											
	Median annual number of cases (range)	Median annual incidence (range)	Winter[a]	Annual	Annual	Annual	Winter[a]	Annual	Winter[a]	Annual	Winter[a]	2012 until week 17[b]
Baden-Württemberg	87.5 (22–164)	1.02 (0.55–1.54)	172 (1.6)	1,090 (10.14)	74 (0.69)	83 (0.77)	327 (3.04)	998 (9.28)	61 (0.57)	128 (1.19)	580 (5.39)	501 (4.66)
Bavaria	23.5 (12–61)	0.24 (0.14–0.49)	38 (0.31)	296 (2.36)	41 (0.33)	21 (0.17)	86 (0.68)	437 (3.49)	45 (0.36)	46 (0.37)	65 (0.52)	43 (0.34)
Berlin	1 (0–2)	0.03 (0–0.06)	1 (0.03)	1 (0.03)	3 (0.09)	0 (0)	0 (0)	3 (0.09)	0 (0)	0 (0)	0 (0)	0 (0)
Brandenburg	0 (0–3)	0 (0–0.12)	1 (0.04)	4 (0.16)	3 (0.12)	0 (0)	0 (0)	2 (0.08)	2 (0.08)	6 (0.24)	4 (0.16)	0 (0)
Bremen	0 (0–1)	0 (0–0.15)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.15)	1 (0.15)	0 (0)	1 (0.15)	1 (0.15)
Hamburg	0 (0–1)	0 (0–0.06)	0 (0)	3 (0.17)	1 (0.06)	2 (0.11)	1 (0.06)	0 (0)	1 (0.06)	1 (0.06)	0 (0)	0 (0)
Hesse	10.5 (4–34)	0.21 (0.08–0.56)	3 (0.05)	27 (0.44)	12 (0.2)	4 (0.07)	31 (0.52)	174 (2.87)	10 (0.16)	13 (0.21)	23 (0.38)	19 (0.31)
Mecklenburg-Vorpommern	4 (1–8)	0.23 (0.23–0.46)	2 (0.12)	11 (0.65)	11 (0.66)	12 (0.73)	8 (0.48)	11 (0.67)	5 (0.3)	5 (0.3)	5 (0.3)	1 (0.06)
Lower Saxony	8 (3–75)	0.13 (0.04–0.94)	12 (0.16)	93 (1.17)	18 (0.23)	16 (0.2)	34 (0.43)	123 (1.55)	18 (0.23)	23 (0.29)	48 (0.6)	36 (0.45)
North Rhine-Westphalia	29.5 (18–143)	0.17 (0.11–0.79)	22 (0.12)	124 (0.69)	61 (0.34)	32 (0.18)	57 (0.32)	156 (0.87)	24 (0.13)	62 (0.35)	86 (0.49)	60 (0.34)
Rhineland-Palatinate	2.5 (2–10)	0.07 (0.05–0.25)	1 (0.02)	11 (0.27)	4 (0.1)	1 (0.02)	2 (0.05)	28 (0.7)	4 (0.09)	7 (0.17)	11 (0.27)	9 (0.22)
Saarland	0 (0–1)	0 (0–0.09)	0 (0)	2 (0.19)	0 (0)	0 (0)	0 (0)	1 (0.1)	0 (0)	0 (0)	1 (0.1)	1 (0.1)
Saxony	1 (0–2)	0.02 (0–0.05)	0 (0)	5 (0.12)	1 (0.02)	0 (0)	2 (0.05)	3 (0.07)	1 (0.02)	3 (0.07)	7 (0.16)	6 (0.14)
Saxony-Anhalt	1.5 (0–3)	0.08 (0.04–0.12)	0 (0)	3 (0.12)	1 (0.04)	1 (0.04)	1 (0.04)	6 (0.26)	1 (0.04)	1 (0.04)	4 (0.17)	4 (0.17)
Schleswig-Holstein	1.5 (0–7)	0.04 (0–0.25)	3 (0.11)	10 (0.35)	6 (0.21)	9 (0.32)	6 (0.22)	11 (0.39)	7 (0.25)	6 (0.21)	3 (0.11)	1 (0.04)
Thuringia	2 (0–14)	0.12 (0.04–0.6)	1 (0.04)	8 (0.35)	7 (0.31)	0 (0)	4 (0.18)	63 (2.82)	4 (0.17)	4 (0.18)	14 (0.62)	11 (0.49)
Total	205 (72–447)	0.28 (0.17–0.54)	256 (0.31)	1,688 (2.05)	243 (0.3)	181 (0.22)	559 (0.68)	2,017 (2.47)	184 (0.23)	305 (0.37)	852 (1.04)	693 (0.85)

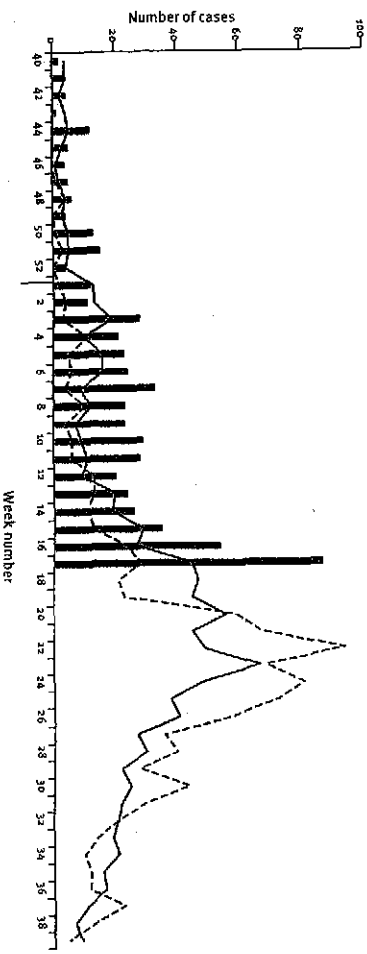
[a] Winter is used to describe the period from week 40 of the preceding year to week 17, a period that coincides with the annual influenza season in Germany.

[b] Ending 27 April 2012.

Source: Robert Koch Institute [2], as of 16 May 2012.

FIGURE 1

Cases of hantavirus infection by week of reporting, Baden-Württemberg, Germany, October (week 40) 2011–April (week 17) 2012 and weeks 1–39 for outbreak years 2007 and 2010, and from week 40 in 2006 and 2009 (years preceding outbreaks)

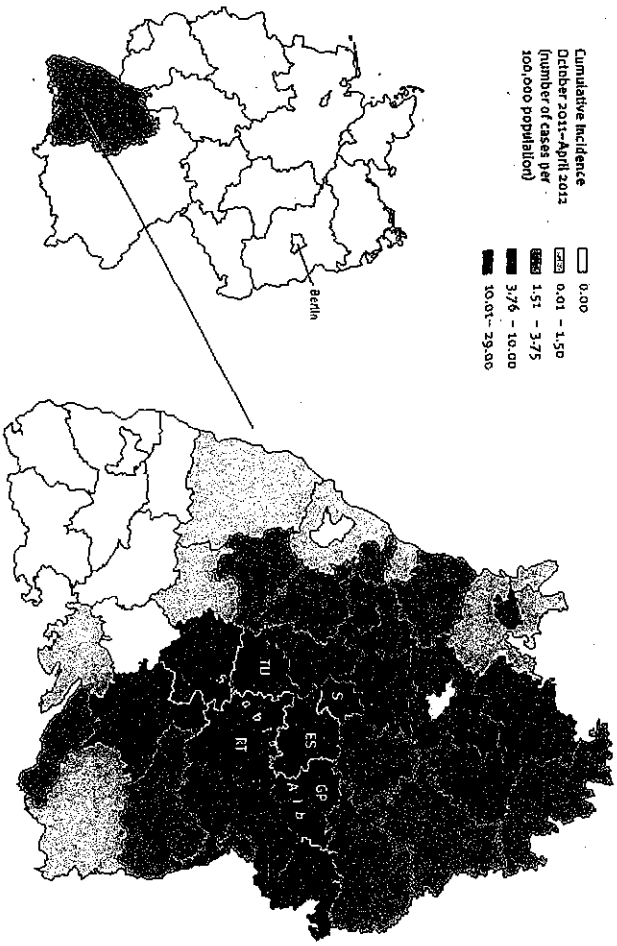


The bars show the number of cases reported during 3 October 2011 to 27 April 2012 (n=580). The broken line shows the number of cases from week 40 2006 to week 39 2007. The continuous line shows the number of cases from week 40 2009 to week 39 2010.

Source: Robert Koch Institute [2], as of 16 May 2012.

FIGURE 2

Geographical distribution of cases of hantavirus infection, by county and cumulative incidence, Baden-Württemberg, Germany, 3 October (reporting week 40) 2011–27 April (week 17) 2012 (n=580)



ES: Esslingen; GP: Göppingen; RT: Reutlingen; S: Stuttgart; TU: Tübingen.

Source: Robert Koch Institute [2], as of 16 May 2012.

Esslingen (n=53; incidence: 10.3 per 100,000 population), Reutlingen (n=62; incidence: 22.1 per 100,000 population) and Göppingen (n=71; incidence: 28.1 per 100,000 population). The last four counties are located in a hantavirus-endemic area lining the Swabian Alb, a low limestone mountain range covered by small forests and fields. Within all five counties, the cases were clustered in several municipalities (data not shown).

Of all the cases notified in Baden-Württemberg, 72% were male (418 of 578 cases with information on sex reported). The highest incidences were observed among persons between 20 and 59 years (Figure 3).

On the basis of preliminary data, the most common symptoms reported were fever (86%), renal impairment (75%), headache (51%) and back pain (23%). Some 69% of all cases were hospitalised. Where indicated (in 52% of the hospitalised cases), the median length of stay in hospital was five days (range: 1–20). No deaths were reported.

Information related to potential exposure was available for 39% of the cases. Most frequently mentioned were cutting and piling wood, spending time in a forest for leisure (hiking, hunting) or forestry work, contact with rodents or rodent excreta, especially during cleaning in barns, sheds, attics, cellars, garden houses, garages, etc.

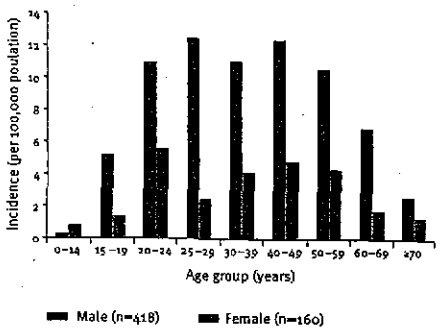
Discussion

Previous outbreaks of hantavirus infection in Baden-Württemberg in 2007 and in 2010 started in the first months of the year and peaked from May to June [11]. The early and intense increase in case numbers since October 2011 is without precedence. Early in 2012, the public was informed of the outbreak and recommended prevention measures [12,13] via media releases published state-wide on 13 January and 9 March 2012. Updated releases were also disseminated to local community-based media and physicians. However, data on the public's knowledge and the effectiveness of preventive measures against Puumala virus infections are lacking and are the subject of a separate study.

The causes for the early increase of case numbers remain unclear. Current hypotheses relate the rising incidence of Puumala virus infections to changes in the population density of bank voles, due to climatic factors [12] and possibly to the beech mast in 2011. During mast years, deciduous trees produce exceptionally high quantities of seeds, an important food source for bank voles [14]. Mast years and hantavirus outbreaks appear to be associated [15,16]. In Baden-Württemberg, the beech mast years of 2006 and 2009 were followed by outbreaks of human hantavirus infections in 2007 and 2010. Last year (2011) was again an exceptional mast year [17], followed by a remarkably mild winter [18]. This may have promoted winter survival and reproduction of bank vole populations.

FIGURE 3

Cumulative incidence of cases of hantavirus infection by age group and sex, Baden-Württemberg, Germany, 3 October (reporting week 40) 2011–27 April (week 17) 2012 (n=578)*



* Cases with information on sex reported.
Source: Robert Koch Institute [2], as of 16 May 2012.

Since spring 2010, the Julius Kühn-Institute (Federal Research Centre for Cultivated Plants) and Friedrich-Loeffler-Institute (Federal Research Institute for Animal Health) have been conducting monitoring studies in an area of Böblingen County, Baden-Württemberg, an endemic region for hantavirus. Trapping results showed a peak mean bank vole population density of 63±46 individuals per hectare (N±standard error/ha) in October 2011. In April 2012, the mean bank vole population density had increased to 76±23/ha (D. Reil, unpublished data). This study indicated considerable recruitment of bank voles, either through winter reproduction or migration. Serological and molecular studies in bank voles from this monitoring site demonstrated a continuous presence of Puumala virus during 2010 and 2011 and an increased Puumala virus seroprevalence in spring 2012 (U.M. Rosenfeld, unpublished data).

We anticipate a further increase in cases numbers during summer 2012. This necessitates additional public service information on prevention measures. Further studies have been initiated to correlate habitat factors of the bank vole reservoir with human exposure and behavioural data, to better understand the reasons for this early increase in case numbers. They will also examine possibilities for preventive measures that can be more efficiently communicated – and are at the same time effective and acceptable – to the population at risk.

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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	総合機構処理欄
				2012. 7. 6	該当なし	
一般的名称	人血清アルブミン		研究報告の公表状況		公表国	
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン5%静注12.5g/250mL(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)			Promed 20120713.1200936	WHO	

研究報告の概要

○幼児における原因不明の致死性疾患ーカンボジア:WHOの声明
カンボジアで多くの幼児に影響を及ぼした疾患及び死亡の調査によると、保健省に報告された大部分の症例の原因は、手足口病(HFMD)の重症型であるという結論に至った。合計31人の患者サンプルが、カンボジアのパスツール研究所において検査された。これらのサンプルの多くがHFMDの原因であるエンテロウイルス71(EV-71)陽性であった。いくつかのサンプルは、ヘモフィルスインフルエンザB型及びブタレンサ球菌を含む他の病原体の陽性反応も示した。適切なサンプルを採取する前に一部の患者は亡くなったため、全患者の検査を行う事は不可能であった。
調査では合計78症例が確認された。これらのうち基準を満たした61症例(そのうち54人は死亡)に焦点を当てて調査を行った。これによると、大部分の患者は3歳以下で、異なる14州から報告があり、数人の患者は慢性状態であることが分かった。症例のうちの多くは、発症中のある時期にステロイド治療が行われた。ステロイド治療はEV-71患者の症状を悪化させるようであった。WHOや関連機関の援助を受け、保健省は2012年4月以降の入院患児の疾患数や死亡数の調査を継続するとともに、保健センターに全てのHFMD患者を報告するよう指示し、EV-71によるHFMD患者に見られる神経・呼吸器症候群のサーベイランスを強化した。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン5%静注12.5g/250mL
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

17

報告企業の意見	今後の対応
カンボジアで多くの幼児に影響を及ぼした致死性疾患の原因は、エンテロウイルス71(EV-71)による手足口病の重症型であるという結論に至った。 EV-71は、脂質膜のないRNAウイルスである。これまで、本剤によるEV-71感染の報告はない。本剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つ以上の異なるウイルス除去・不活化工程が含まれていることから、本剤の安全性は確保されていると考えられる。	日本赤十字社では、発熱などの体調不良者を献血不適としている。また、手足口病の場合は完全に治癒して一定期間が経過するまで採血不可としている。 本剤の安全性は確保されていると考えるが、念のため今後も情報収集に努める。

MedDRA/J Ver.15.0J

ProMED-mail |

Published Date: 2012-07-13 16:15:03
Subject: PRO/EDR> Undiagnosed illness, fatal, child - Cambodia (07): WHO statement
Archive Number: 20120713.1200936

UNDIAGNOSED ILLNESS, FATAL, CHILD - CAMBODIA (07): WORLD HEALTH ORGANISATION STATEMENT
****
A ProMED-mail post
http://www.promedmail.org
ProMED-mail is a program of the
International Society for Infectious Diseases
http://www.isid.org

Date: Fri 13 Jul 2012

urce: World Health Organisation (WHO), CSR, Disease Outbreak News [edited]
http://www.who.int/csr/don/2012_07_13/en/index.html

Severe complications of HFMD caused by EV-71 in Cambodia

The investigation into the illnesses and deaths in Cambodia, which mainly affected very young children, concluded that a severe form of hand, foot and mouth disease (HFMD) was the cause in the majority of cases reported to the Ministry of Health.

Samples from a total of 31 patients were obtained and tested for a number of pathogens by Institut Pasteur du Cambodge. Most of these samples tested positive for enterovirus 71 (EV-71) which causes HFMD. A small proportion of samples also tested positive for other pathogens including _Haemophilus influenzae_ type B and _Streptococcus suis_. It was not possible to test all the patients as some of them died before appropriate samples could be taken.

The investigation included:

- a thorough review of the hospital records of the patients from Kantha Bhopa hospital as well as from other hospitals;
- laboratory tests;
- active follow-up with the affected families by the local Rapid Response Teams (RRT); and
- evaluation of the data from the national surveillance system.

A total of 78 cases were identified. These included the initial 62 cases reported by Kantha Bopha hospital, and cases reported from other hospitals. Of these, the investigation focused on 61 cases that fitted a specific criteria (the case definition), and of which 54 had died. The investigation revealed that most of the cases were under 3 years of age, from 14 different provinces, with some suffering from chronic conditions. A significant number of cases had been treated with steroids at some point during their illness. Steroid use has been shown to worsen the condition of patients with EV-71.

The Ministry of Health, with support from WHO and partners, which included Institut Pasteur du Cambodge and the US

JR.C2012T-023

Centers for Disease Control and Prevention, conducted the investigation following reports from Kantha Bopha Children's hospital of unusual numbers of illness and deaths among children hospitalised since April 2012.

In response to this event, health centers have been instructed by the Ministry of Health to report all patients with HFMD. In addition, the Ministry of Health, assisted by the WHO, has begun enhanced surveillance for neuro-respiratory syndrome, a key syndrome observed among patients with severe HFMD caused by EV-71. It is expected that the enhanced surveillance will identify occasional new cases of the severe form of the disease in the coming months.

In addition, the Ministry of Health is developing guidelines and training courses for staff to manage patients with mild and severe forms of HFMD. A campaign to raise awareness on the prevention, identification and care of children with HFMD is underway.

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Communicated by:

ProMED-mail Rapporteur Marianne Hopp

[This statement from the WHO now acknowledges that human enterovirus 71 infection has been associated with a *neuro-respiratory syndrome observed among a proportion of young children hospitalised with with severe HFMD.* The investigation revealed that most of the cases were under 3 years of age and had been referred from 14 different provinces. Inappropriate treatment with steroids many have contributed to the deaths of some of the children.

In the absence of an appropriate vaccine, or specific medication, attention must now shift to treatment options and general improvements in child care and social conditions. It seems likely that this situation is not confined to Cambodia, but is a common problem wherever enterovirus 71 is prevalent (e.g., see: Hand, foot & mouth disease - Viet Nam (11), ProMED-mail archive number 20120712.1199237).

The HealthMap interactive map of Cambodia can be accessed at: http://healthmap.org/r/2gds. - Mod.CP]

See Also

Undiagnosed illness, fatal, child - Cambodia (06): pathogen mix 20120711.1198162
Undiagnosed illness, fatal, child - Cambodia (05): EV71 treatment options 20120711.1197882
Undiagnosed illness, fatal, child - Cambodia (04): EV71, WHO 20120709.1195264
Undiagnosed illness, fatal, child - Cambodia (03): EV71 20120708.1193960
Undiagnosed illness, fatal, child - Cambodia (02) 20120707.1193413
Undiagnosed illness, fatal, child - Cambodia: RFI 20120704.1190037
Hand, foot & mouth disease - China 20120707.1193364
Hand, foot & mouth disease - Worldwide 20120701.1186614]

.....cp/ejp/lm

別紙様式第 2-1
番号 7

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2012 年 7 月 10 日	新医薬品等の区分	厚生労働省処理欄
一般的名称	①②③④⑤ポリエチレングリコール処理人免疫グロブリン ⑥⑦人免疫グロブリン		研究報告の公表状況	WHO/2012/07/09	公表国 カンボジア	
販売名（企業名）	①献血ヴェノグロブリン IH5%静注 0.5g/10mL	（ベネシス）				
	②献血ヴェノグロブリン IH5%静注 1g/20mL	（ベネシス）				
	③献血ヴェノグロブリン IH5%静注 2.5g/50mL	（ベネシス）				
	④献血ヴェノグロブリン IH5%静注 5g/100mL	（ベネシス）				
	⑤献血ヴェノグロブリン-IH ヨシトミ	（ベネシス）				
	⑥グロブリン筋注 450mg/3mL「ベネシス」	（ベネシス）				
	⑦グロブリン筋注 1500mg/10mL「ベネシス」	（ベネシス）				

研究報告の概要

カンボジアにける診断未確定の病気ー最新情報

2012 年 7 月 9 日ー診断未確定の病気の継続調査の一環として、カンボジア王国の保健省は全ての疑わしい入院症例の見直しを終えた。この最終報告は 2012 年 4 月から 7 月 5 日までの間に、更に 2 症例を加え、影響を受けた子供の総数は 59 人になり、52 人が死亡した。症例の年齢は 3 箇月から 11 歳までの範囲で、大多数が 3 歳未満であった。全体の男女比は、1.3:1 であった。
多くの症例は適切なサンプルを採取する前に死亡したため、入手できなかった。最新の検査結果によれば、サンプルの多くは、手足口病（HFMD）を引き起こすエンテロウイルス 71（EV-71）に対して陽性反応を示した。
EV-71 ウイルスは一般に一部の患者の中で重症合併症を引き起こすことが知られていた。その上、デング熱とブタ連鎖球菌を含む幾つかの他の病原体は、幾つかの検体で確認された。検体は H5N1 と他のインフルエンザウイルス、SARS 並びにニパに対して陰性であることが分かった。臨床、検査室及び疫学的情報に一致する更なる調査は進行中で、数日以内に結論を出す見込みである。
WHO とカンボジアのパスツール研究所及び米国疾病管理予防センターを含む パートナーは、この事象で保健省を援助している。政府は市民に良い衛生実践の認識（頻繁な手洗いを含む）も強化している。

手足口病に関する若干の事実：

（ 略 ）

現在、HFMD に利用できる特別な治療はない。患者は沢山の水や他の体を飲むべきで、症状の治療を必要としてもよい。医療提供者は症状に従って患者を治療し、ステロイドの使用を差し控えるよう忠告される。

使用上の注意記載状況・その他参考事項等

代表として献血ヴェノグロブリン IH5%静注 0.5g/10mL 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 抗体及び抗 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、ウイルス除去膜によるろ過処理及び pH3.9～4.4 の条件下での液状インキュベーション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。

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報告企業の意見	今後の対応
本報告の後、7 月 13 日付で WHO は「エンテロウイルス 71（EV-71）が原因の手足口病の重度の合併症」と結論付けた。 エンテロウイルス（enterovirus：EV）はピコルナウイルス科のエンベロープのない一本鎖 RNA ウイルスである（大きさは 18～25nm と 27～30nm）。腸管内で増殖するウイルスの総称のため、腸管ウイルスともいい、経口或いは糞口感染する。万一、EV が原料血漿に混入したとしても、EMC をモデルウイルスとしたウイルスクリアランス試験成績から、本剤の製造工程において不活化・除去されると考えている。 デングウイルス（dengue virus）は、フラビウイルス科フラビウイルス属に属する直径 40～50nm のエンベロープを有する球形の RNA ウイルスで、血清型の違いから D1、D2、D3、D4 の 4 種類があり、主としてネッタイシマカによって媒介される。万一、原料血漿にデング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クリアランス試験成績から、本剤の製造工程において不活化・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。

グロブリン

BENESIS 2012-011

World Health Organization

Global Alert and Response (GAR)

Undiagnosed illness in Cambodia - update

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9 JULY 2012 - As part of the continuing investigations into the undiagnosed illness, the Ministry of Health of the Kingdom of Cambodia is finalizing the review of all suspected hospitalised cases. This final review added an additional two cases between April to 5 July 2012, making the total number of children affected to be 59. Of these, 52 have died.

The age of the cases range from three months to 11 years old, with the majority being under three years old. The overall male: female ratio is 1.3:1.

Laboratory samples were not available for the majority of the cases as they died before appropriate samples could be taken.

Based on the latest laboratory results, a significant proportion of the samples tested positive for enterovirus 71 (EV-71), which causes hand foot and mouth disease (HFMD). The EV-71 virus has been known to generally cause severe complications amongst some patients.

Additionally, a number of other pathogens, including dengue and *streptococcus suis* were identified in some of the samples. The samples were found to be negative for H5N1 and other influenza viruses, SARS and Nipah.

Further investigations into matching the clinical, laboratory and epidemiological information are ongoing, and are likely to be concluded in a few days.

WHO and partners, which include Institut Pasteur du Cambodge and US Centers for Disease Control and Prevention, are assisting the Ministry of Health with this event.

The Government is also reinforcing awareness of good hygiene practices to the public, which includes frequent washing of hands.

Some facts on hand foot and mouth disease:

Hand foot and mouth disease (HFMD) is a common infectious disease of infants and children. The symptoms commonly observed include fever, painful sores in the mouth, and a rash with blisters on hands, feet and also buttocks.

HFMD is most commonly caused by coxsackievirus A16, which usually results in a mild self-limiting disease with a few complications. HFMD is also caused by enteroviruses, including enterovirus 71 (EV71) which has been associated with serious complications in certain groups, and may cause deaths.

Related links

A Guide to clinical management and public health response for hand, foot and mouth disease (HFMD)

Hand, foot and mouth disease information sheet

HFMD mainly occurs amongst children under 10 years old. The usual period from infection to onset of symptoms is 3-7 days.

The disease usually begins with fever, poor appetite, malaise, and frequently with a sore throat. One or two days after fever onset, painful sores develop in the mouth. They begin as small red spots that blister and then often become ulcers. They are usually located on the tongue, gums and inside of the cheeks. A non-itchy skin rash develops over 1-2 days with flat or raised red spots, some with blisters. The rash is usually located on the palms of the hands and soles of the feet, and may also appear on the buttocks and/or genitalia. A person with HFMD may not have symptoms, or may have only the rash or only mouth ulcers. In a small number of cases, children may experience a brief febrile illness, present with mixed neurological and respiratory symptoms and succumb rapidly from the disease.

HFMD virus is contagious and infection is spread from person to person by direct contact with nose or throat discharges, saliva, fluid from blisters, or the stool of infected persons. Infected persons are most contagious during the first week of the illness but the period of communicability can last for several weeks. HFMD is not transmitted from pets or other animals. HFMD should not be confused with the different disease in animals called foot-and-mouth disease.

Presently, there is no specific treatment available for HFMD. Patients should drink plenty of water or other liquids and may require treatment of *the symptoms*.

Health care providers are advised to treat patients according to their symptoms and to refrain from using steroids.

別紙様式第 2-1
番号 9

医薬品
医薬部外品　　研究報告　調査報告書
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識別番号・報告回数			報告日	第一報入手日 2012 年 7 月 23 日	新医薬品等の区分	厚生労働省処理欄
一般的名称	①②③④⑤ポリエチレングリコール処理人免疫グロブリン ⑥⑦人免疫グロブリン					
販売名 （企業名）	①献血ｸﾞｪﾉｸﾞﾛﾌﾞﾘﾝ IH5%静注 0.5g/10mL　(ベネシス) ②献血ｸﾞｪﾉｸﾞﾛﾌﾞﾘﾝ IH5%静注 1g/20mL　(ベネシス) ③献血ｸﾞｪﾉｸﾞﾛﾌﾞﾘﾝ IH5%静注 2.5g/50mL　(ベネシス) ④献血ｸﾞｪﾉｸﾞﾛﾌﾞﾘﾝ IH5%静注 5g/100mL　(ベネシス) ⑤献血ｸﾞｪﾉｸﾞﾛﾌﾞﾘﾝ-IH ﾖｼﾄﾐ　(ベネシス) ⑥ｸﾞﾛﾌﾞﾘﾝ筋注 450mg/3mL「ﾍﾞﾈｼｽ」　(ベネシス) ⑦ｸﾞﾛﾌﾞﾘﾝ筋注 1500mg/10mL「ﾍﾞﾈｼｽ」　(ベネシス)		研究報告の公表状況	Chinese Journal of Zoonoses 2012; 28(5): 442-448	公表国 中国	

研究報告の概要

ヒツジ痘はヒツジ痘ウイルス及びヤギ痘ウイルスによって起こる、小型反芻動物のウイルス性伝染病である。分類学的には、ヒツジ痘ウイルス(sheeppox virus:SPPV)、ヤギ痘ウイルス(Goatpox virus:GTPV)及びランピースキン病ウイルス(Lump skin disease virus:LSDV)は何れもポックスウイルス科カプリポックスウイルス属に属する。ヒツジ痘ウイルス及びヤギ痘ウイルスは小型反芻動物の家畜に感染する疾病であるが、通常はヒトには感染しない。ヒトが罹患動物との接触によりヒツジ痘ウイルスに感染することは極めて稀であり、臨床徴候診断に基づくヒトのヒツジ痘症例報告はあるが、これらの症例は病原学的な確定診断の根拠が乏しいため、結論には慎重を期さなければならない。

2010 年 8 月、重慶市彭水県で一連のヒツジ痘ウイルスのヒトへの感染発生が報告され、この伝染病発生の診断を明確にするため、調査担当者は一部患者及び罹患ヒツジの検体を採取し、ヒツジ痘ウイルスに対する一連の検査測定を行い、このウイルスの感染によるものであることの実証を試みた。その結果をここに以下の通り報告する。

1. 材料及び方法

（ 略 ）

2. 結　　果

2.1　感染発生の概況

重慶市彭水県は 2010 年 8 月、一連のヒツジ痘のヒト集団感染が疑われる状況が発生し、その原因はヒツジ痘に罹患したヤギに直接接触したためである疑いがあることを報告した。調査による実証：2010 年 7 月、彭水県石柳郷が四川省より黒ヤギ 640 頭を購入したところ、間もなく繁殖農家が一部のヤギの眼部、頭部及び腹部に痘疹が出現していることを発見し、更に死亡が相次いだ。累計発生率は罹患ヤギ 548 頭(86%)、死亡 172 頭(27%)で、ヤギの病死率は 31%となった。畜産局の職員は調査の過程で、繁殖農家の中に、罹患ヤギとの接触後に皮疹が出現した人が複数いることを発見し、直ちに衛生部門に対し関連状況を通知した。衛生部門の調査によると、本事例では計 34 人が発症し、うち男性 23 人、女性 11 人、年齢は最低が 1 歳、最高が 52 歳で平均年齢は 27 歳、農民 20 人、学生・生徒 12 人、未就園児 2 人だった。症例のうち 31 例は 3 つの行政村の 14 戸の繁殖農家に分布しており、3 例のみ、繁殖農家 2 戸の近隣住民だった。11 戸では 2 例以上の症例が発生していた。繁殖農家 17 戸のうち 16 戸で罹患ヤギ、或いは死亡ヤギが発生し、14 戸でヒトのヒツジ痘症例が発生していた。全ての症例で罹患ヤギとの接触歴があり、未接触者の発症はなかった。症例の発症期間は 7 月 15 日～9 月 1 日の間だった。彭水県ではそれまでにヒツジ痘のヒトへの感染症例の報告はなかった。

2.2　ウイルスの分離

検体接種細胞 9 試料のうち、罹患ヤギ 2 から採取した 1 検体のみ細胞病変が出現し、この検体を細胞に接種した後、培養第 1 代の 4

使用上の注意記載状況・
その他参考事項等

代表として献血ヴェノグロブリン IH5%静注 0.5g/10mL 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 抗体及び抗 HTLV-1 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、ウイルス除去膜によるろ過処理及び pH3.9～4.4 の条件下での液状インキュベーション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。

日目に細胞集簇が形成され、4 日目に細胞融合が出現し、細胞質は粗くなり、光透過性が低下した。第 2 代接種培養では 2 日目に細胞集簇が形成され、その後明らかな細胞融合が出現した。ヒツジ痘ウイルス特異的プライマーA95/B7 を用いて PCR 増幅検査及び配列測定を行い、ヤギ痘ウイルス分離陽性を実証した。

2.3　ウイルス核酸検査

ヒツジ痘のヒト感染疑い症例の水疱液検体 5 検体及び罹患ヤギ 4 検体に対し、ヒツジ痘ウイルス A29L 遺伝子の特異的プライマー CPVS/CPVA を用いて PCR 検査を行ったところ、9 検体で何れも 413bp の特異的バンドが検出され、結果は何れも陽性だった。ヒツジ痘ウイルス P32 遺伝子に対応する特異的プライマーA95/B7 を用いてヒトの 5 検体を検査したところ、症例 4 の検体は陰性、他の 4 症例の検体は陽性だった。また、ヒトのグリセルアルデヒドリン酸デヒドロゲナーゼ遺伝子に対応する GAPDH1 及び GAPDH2 プライマーを内部対照として使用することで、ヒトの 5 検体で何れも特異的な 226bp の DNA 断片増幅に成功し、これにより抽出した核酸がヒト組織由来のものであり、核酸検査結果が有効であることを実証した。

2.4　配列測定

増幅強度の比較的高い症例 2、症例 3 及び罹患ヤギ 2 の Vero E6 細胞ウイルス陽性分離物に対し、プライマーA95/B7 を用いてヒツジ痘ウイルスの P32 遺伝子の増幅を行うとともに配列決定を行い、その結果を BLAST により解析したところ、2 症例の水疱液検体及びヤギの Vero E6 細胞ウイルス分離物の増幅ウイルス核酸配列と、ヤギ痘ウイルスの G20-LKV ウイルス株の間には 99%の相同性が見られたため、今回のヒトのヒツジ痘感染はヤギ痘ウイルスによるものであると判定した。配列決定によって得られたヒト症例の 2 本の P32 遺伝子配列と、罹患ヤギのウイルス分離物の遺伝子配列との相同性は 100%であり、このことで今回発生したヤギとヒトのヤギ痘の病因は同一のウイルスであることが明らかとなった。

2.5　ヤギ痘ウイルスの P32 遺伝子核酸配列の進化系統解析

GenBank からカプリポックスウイルス属メンバーの P32 遺伝子 842bpDNA 相同性配列を収集し、ソフトウェアの BioEdit を使用して配列の編集を行い、MEGA により ClustalW を使用して多重配列比較を行い、進化系統樹ファイルを作成するとともに、Neighbor-Joining 法により進化系統樹を作成した。進化系統樹により、重慶市彭水県で得られたヤギ痘ウイルスは、中国広西チワン族自治区柳州市柳江県で 2003 年に流行したウイルス株及び、ベトナムで 2005 年に流行したウイルス株と進化距離が比較的近く、2009 年に重慶市及び甘粛省で確認されたウイルス株との進化距離も比較的近いことが分かり、更にカザフスタンを起源とする G20-LKV ウイルス株と同属の、進化上で独立したヤギ痘ウイルスの分枝上に位置することがわかった。

3. 考　　察

近年、ヒツジ痘は中国でたびたび発生し、養羊業にとって大きな危害となっている。この疾病は国際獣疫事務局が報告を義務付けている疾病の一つであり、中国ではⅠ類動物疾病に指定されている。中華人民共和国農業部獣医公報によると、2000 年～2009 年上半期の間で地理上の分布を見ると、先ず中国の北西部地域、次に華中地域、華南地域がヒツジ痘発生の集中した地域となっている。2000 年～2009 年の政府資料及び公開発表された文献によると、中国では北京市、重慶市、遼寧省、山東省、河南省、チベット自治区においてはこの期間中にヒツジ痘発生の報告はなかったが、その他の省・自治区では全て発生が報告されていた。北西部地域の甘粛省、青海省、寧夏回族自治区及び湖南省、南西部地域の雲南省、華中地域の湖南省、華東地域の福建省は発生の報告が比較的多かった。華北地域で発生の報告が比較的多かったのは内モンゴル自治区、山西省、河北省だった。発生の報告が最も多い地域はそれぞれ、甘粛省、青海省、寧夏回族自治区、新疆ウイグル自治区となった。ヒツジ間でヒツジ痘が広く発生している状況により、ヒツジ痘ウイルスのヒトへの感染リスクは増大している。

ヒツジ痘のヒトへの感染症例の報告は稀であり、報告された症例も病原学的な診断根拠が乏しい。中国の吉林省、雲南省、天津市、陝西省、山東省、貴州省等ではこれまでにヒツジ痘のヒト感染症例が報告されているが、それらの症例は何れも臨床診断、或いは病理学的診断によるものであり、特異的な病原学的診断根拠はなかった。今回重慶市彭水県で発生した感染では、疫学調査を合わせて実施し、ヒツジ間での感染発生とヒト間での感染発生の時期及びヒトのヒツジ痘症例と罹患ヒツジのウイルスの核酸配列比較結果に

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基づくことで、今回発生したヒツジ痘の集団感染が、外部の省から購入した黒ヤギによって持ち込まれたヤギ痘ウイルスによるものであり、ヒトが直接ヤギと接触することによりヤギ痘ウイルスに感染し、続いて集団感染を引き起こしたものと確定した。疫学調査の結果により、全ての症例で罹患ヤギとの接触歴があり、未接触者に発症者はいないこと、曝露集団が罹患ヤギと接触した際に何れも個人防護を行っていなかったこと、また何れも毎回有効な手洗いを行っていなかったことが分かった。このため、直接罹患ヤギと接触したことが、ヤギ痘ウイルス感染の主な原因であると言える。

報告企業の意見	今後の対応
ヒツジ痘ウイルスはポックスウイルス科コルドポックスウイルス亜科カプリポックスウイルス属に属する線状の 2 本鎖 DNA をゲノムとして持つ DNA ウイルスで、そのビリオンは 220～450nm×140～260nm×140～260ｎｍの煉瓦状ないし卵形で、エンベロープを有する。ヤギ痘ウイルスと極めて近縁もしくは同一で、核内及び細胞質内両方に封入体を形成する。万一、本剤の原料血漿中にヒツジ痘ウイルス又はヤギ痘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クリアランス試験成績から、本剤の製造工程において不活化・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。

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一起人感染羊痘病毒疫情的实验室诊断

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摘 要:目的 对2010年重庆彭水县一起人感染羊痘病毒疑似病例进行病原学检测,以明确诊断。方法 对患者进行流行病学调查,采用羊痘病毒特异性引物对5例患者的5份痘疹液标本和来自3只病羊的眼分泌物、痘疱液、痘痂块共4份标本进行PCR检测,并采用VeroE6细胞进行病毒分离;对扩增出的DNA片断进行测序,经BLAST进行序列比对分析。结果 报告的34个病例均与病羊有接触史。1份病羊痘疱液标本山羊痘病毒分离阳性。9份标本的羊痘病毒A29L基因扩增出413bp片段扩增结果均为阳性。使用A95/B7扩增羊痘病毒P32基因,5份人的痘疹液标本中,4份为PCR扩增阳性。2份病例标本及1份病羊的病毒分离物P32基因扩增产物的DNA片段序列同具有100%的同源性,3条序列与山羊痘病毒CQ-LKV株序列具有99%的同源性。系统进化树显示:重庆市彭水县获得的山羊痘病毒与我国广西柳江2003年流行的病毒和越南2006年流行的毒株进化距离较近,与2009年重庆及甘肃的病毒株也有较近的进化距离。结论 重庆市彭水县人感染羊痘病疫情系由人直接接触病羊而感染山羊痘病毒所致。

关键词:疫情;山羊痘病毒;病毒分离;核酸扩增
中图分类号:R373 文献标识码:A

Laboratory diagnosis on an outbreak of human cases infected with Goatpox Virus in Chongqing

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ABSTRACT: In 2010, an outbreak of 34 suspected cases of human infected with capripox virus was reported in Pengshui County, Chongqing, China. Nine specimens, including vesicle fluid, eye discharge and scab, were collected from 5 human cases and 3 capripox infected goats, respectively. Viral DNA was detected by PCR with capripox virus specific primers, while virus isolation was performed for all specimens on VeroE6 cells. PCR amplification revealed that all specimens were positive for A29L gene. Additionally, one virus was isolated from eye discharge of an infected goat. For the P32 gene, 4 of 5 human cases were positive. Sequence analysis of three P32 amplicons from 2 patients and 1 goat showed 100% similarity. Phylogenetic analysis also showed that all three sequences clustered within goatpox virus, with close relationship with a goatpox virus isolating from Liujiang of Guangxi, China in 2003 and a Vietnam-originated goatpox virus in 2006. In conclusion, an outbreak of human cases infected with goatpox virus was confirmed by several approaches in our laboratory. This investigation suggested that close contact with infected goats imported from Sichuan had caused these clustered human infections, that routine quarantine on imported livestock should be implemented immediately, and that relevant health education is required for those farmers.

KEY WORDS: outbreak; goatpox virus; virus isolation; nucleic acid amplification

羊痘病是由绵羊痘病毒和山羊痘病毒引起的小型反刍动物的病毒性传染病。在分类学上,绵羊痘病毒(*sheeppox virus*,SPPV)和山羊痘病毒(*Goatpox virus*,GTPV),以及牛皮肤病病毒(*Lump skin disease virus*,LSDV)同属于痘病毒科羊痘病毒属。绵羊痘病毒和山羊痘病毒感染家畜养小型反刍动物疫病,但通常不会感染人类。人因接触患病动物而感染羊痘病毒的情况极为罕见,虽然有基于临床体征诊断的人羊痘病例报告,但由于这些病例缺乏病原学的确诊依据,结论需谨慎对待。2010年8月,重庆市彭水县报告1起人感染羊痘病疫情,为明确该起疫情的诊断,调查人员采集了部分患者及病羊标本,进行了针对羊痘病毒的一系列检测,证实系由该病毒感染所致,现将结果报告如下。

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1 材料与方法

1.1 标本来源及病例情况 共采集5例疑似人感染羊痘病例的痘疹液标本5份,见表1。来自3只病羊的标本有:病羊1,眼分泌物1份;病羊2,痘疱液1份;病羊3,痘疱液1份,痘痂块1份;共4份标本。所有标本由彭水县疾病预防控制中心采集送检。

表1 5例疑似人感染羊痘病例痘疹样本

Tab.1 Specimens information of 5 suspected cases of human infectedwoth capripoxvins capripoxi

病例	性别	年龄	职业	类型	接触羊的时间	出疹部位	出诊日期	采样日期
1 谭某	女	48	农民	丘疹、水疱疹	2010-7-15	手指、手背、手掌、前臂、上臂、大腿内侧、大腿外侧、大腿前侧、小腿	2010-8-3	2010-9-2
2 赵某	男	28	农民	丘疹、水疱疹	2010-7-15	大腿内侧、大腿外侧、大腿前侧、小腿	2010-8-1	2010-9-2
3 罗某	男	38	农民	丘疹、水疱疹	2010-7-15	手指、前臂、上臂、大腿内侧、大腿外侧、大腿前侧、小腿、胸腹	2010-8-3	2010-9-2
4 周某	男	1	散居儿童	丘疹、水疱疹	2010-7-15	前臂、上臂、面部躯部	2010-8-1	2010-9-2
5 鱼某	女	69	农民	水疱疹	2010-7-15	手指、手背、前臂、大腿内侧、大腿外侧、大腿前侧、胸腹、背	2010-7-20	2010-9-2

1.2 仪器和试剂 PCR扩增仪(2700型PCR仪)为美国ABI公司产品,病毒RNA/DNA提取试剂盒(TaKaRa Mini Best Viral RNA/DNA Extraction Kit Ver4.0)购自大连宝生物工程有限公司。PCR试剂(GoTaq Hot Start Green Master Mix,M612B)购自美国Promega公司。VeroE6细胞由中国疾病预防控制中心病毒病预防控制所提供。

1.3 病毒分离 取痘疱液标本和眼分泌物标本,分别加入双抗和庆大霉素(终浓度为2 000单位/mL和1 000单位/mL),痘痂块样本按体积比1∶5加入RPMI1640维养液后匀浆,5 000 r/min离心15 min,取上清加入双抗和庆大霉素(终浓度为2 000单位/mL和1 000单位/mL),4 ℃冰箱过夜。取处理后的样本液各100 μL同时加入200 μL细胞维持液,接种到已长成单层的VeroE6细胞管中,37 ℃吸附2 h,弃去样本液,加入1 mL维持液培养,连续观察记录细胞病变,盲传2代,每代7～10 d。对出现病变的细胞,采用PCR检测羊痘病毒核酸,然后测序鉴定。

1.4 病毒核酸扩增 使用的PCR扩增羊痘特异性引物和人GAPDH对照引物见表2。

表2 核酸扩增使用的引物

Tab.2 Primers used for PCR amplification

引物名称	引物序列	扩增产物长度(bp)	扩增区域	文献
羊痘特异性引物				
CPVS	5′-AACTGCCGTCAATGAAGAATGG-3′	413	A29L	1
CPVA	5′-TTTCAAAGCTTGTTTTTAACGTRGG-3′			
A95	5′-CACGGATCCATG GCAGAT ATC CCA TTA-3′	1 024	P32	2
B7	5′-AAC AAG CTT ACT CTC ATT GGT GTT CGG-3′			
对照引物				
GAPDH1	5′-GAA GGT GAA GGT CGG AGT-3′	226		
GAPDH2	5′-GAA GAT GGT GAT GGG ATT TC-3′			

扩增条件:PCR反应体系总体积为50 μL,其中包括2×PCR Master Mix buffer 25 μL,上、下游引物(CPVS和CPVA、A95和B7、GAPDH1和GAPDH2,浓度为10 μmol/L)各3 μL,样本DNA模板10 μL,dH_2O 9 μL。反应条件:95 ℃ 2 min;95 ℃ 30 s,55 ℃ 60 s,72 ℃ 60 s,35个循环;72 ℃ 5 min。

1.5 DNA序列测定及分析 选取PCR扩增后,经凝胶电泳检测,条带清晰且反应强度较高的样本DNA产物2份和病毒培养物PCR产物1份,回收特异性片段,用扩增引物进行双向测序。测序结果用DNAStar软件及BLAST服务器

进行序列比对分析，并进行进化树分析。

2 结 果

2.1 疫情概况 2010年8月，重庆市彭水县报告一起聚集性人感染羊痘病疑似疫情，疫情疑为人直接接触患羊痘病的山羊所致。调查证实：2010年7月，彭水县石柳乡从四川省购入黑山羊640只，养殖户很快发现部分羊的眼、头及腹部出现泡疹，并相继出现死亡，累计发生病羊548只(86%)，死亡172只(27%)，羊病死率为31%。畜牧局人员在调查过程中发现养殖户家中有多人在接触病羊后出现皮疹，即将有关情况通知卫生部门。经卫生部门调查发现，本次事件共发病34人，其中男性23人，女性11人；年龄最小1岁，最大52岁，平均27岁；农民20人，学生12人，散居儿童2人。31例病例分布在3个行政村的14户养殖户中，另有3例为2户养殖户的邻居；11户中有2例以上病例发生。17户养殖户中16户有病羊及死羊发生，14户发生人羊痘病例。所有病例均与病羊有接触史，未接触者中无发病，病例的发病时间在7月15日—9月1日期间。彭水县当地既往无人感染羊痘病例报告。

2.2 病毒分离 共9份标本接种细胞，仅有采自病羊2的1份标本出现细胞病变，该标本接种细胞后，在培养第1代的第4 d出现细胞聚集成簇，第4 d出现细胞融合，胞浆变粗透光性降低。第2代接种培养的第2 d开始出现细胞聚集成簇，之后开始出现明显的细胞融合。经用羊痘病毒特异性引物A95/B7进行PCR扩增检测和序列测定，证实为山羊痘病毒分离阳性。

2.3 病毒核酸检测 5份疑似人羊痘病例的疱疹液标本和4份病羊标本经使用针对羊痘病毒A29L基因的特异性引物CPVS/CPVA进行PCR检测，9份标本均检测到413 bp的特异性条带，结果均为阳性。使用针对羊痘病毒P32基因的特异性引物A95/B7检测5份人的标本，病例4的标本为阴性，其余4份人病例样本为阳性。同时，使用针对人磷酸甘油醛脱氢酶基因的GAPDH1和GAPDH2引物做为内对照，5份人的样本均成功扩增出特异性226 bp DNA片段，证实提取的核酸来自人体组织样本，核酸检测结果有效。

2.4 序列测定 对扩增强度较高的病例2、病例3，以及病羊2的VeroE6细胞病毒阳性分离物，使用引物A95/B7扩增羊痘病毒P32基因并进行测序，测序结果经BLAST分析表明：2个病例疱疹液标本以及羊的VeroE6细胞病毒分离物中扩增的病毒核酸序列与山羊痘病毒G20-LKV病毒株具有99%的同源性，判定本次人感染羊痘病由山羊痘病毒所致。测序获得的2条人病例的P32基因序列与病羊的病毒分离物的基因序列同源性为100%，说明此次引起羊和人山羊痘病的病原为同一病毒。

2.5 山羊痘病毒部分P32基因核酸序列系统进化分析 由GenBank中收集羊痘病毒属成员P32基因842 bp DNA同源序列，经BioEdit软件进行序列编辑，由MEGA软件采用ClustalW方法进行多重序列比对，生成系统进化树文件，并用Neighbor-Joining法生成进化树(图1)。系统进化树显示，重庆市彭水县获得的山羊痘病毒与我国广西柳江2003年流行的病毒、越南2005年流行的毒株进化距离较近，与2009年重庆及甘肃的病毒株也有较近的进化距离，并与来源于哈萨克斯坦的G20-LKV毒株同属在进化上独立的山羊痘病毒分支。

图1 重庆市羊痘病毒P32基因部分核酸序列系统进化树分析

Fig.1 Phylogenetic tree based on partial P32 DNA sequences of capripox virus

3 讨 论

近年来，羊痘疫情在我国时有发生，对养羊业危害严重。该病被世界动物卫生组织列为法定必须报告的疾病，我国将其列为Ⅰ类动物疾病。根据中华人民共和国农业部兽医公报显示，2000—2009年上半年从地理分布来看中国的西北地区，其次是华中地区，华南地区是羊痘疫情集中地区。2000—2009年的官方资料和公开发表的文献显示中国除北京、重庆、辽宁、山东、河南、西藏在这期间没有羊痘疫情报道，中国其他省区都有羊痘疫情报道。西北的甘肃、青海、宁夏和湖南、西南的云南，华中的湖南，华东的福建是报告疫情较多的省份；华北地区报道疫情较多的是内蒙古、山西、河北。报告疫情最多的地区分别为甘肃、青海、宁夏和新疆维吾尔族自治区[8]。羊间羊痘疫情的广泛存在增加了人感染羊痘病毒的风险。

(下转第448页)

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人感染羊痘病例罕有报道，且报告病例缺乏病原学诊断依据。在我国的吉林、云南、天津、陕西、山东、贵州等省虽曾有人感染羊痘病例报道，但报道的病例均为临床诊断或病理学诊断，无特异性病原学诊断依据[4-10]。此次重庆市彭水县发生的疫情，结合流行病学调查，根据羊间疫情和人间疫情的发生时间，以及人羊痘病例和病羊的病毒核酸序列比对结果，确定本次发生的羊痘病聚集性疫情是由于当地从外省引进黑山羊，黑山羊携带山羊痘病毒，发生羊痘病，人在直接接触羊后，发生山羊痘病毒感染，继而引起聚集性人羊痘病疫情。流行病学调查结果显示：所有病例均与病羊有接触史，未接触者中无人员发病；暴露人群与病羊接触时均未进行个体防护，均未能做到每次有效洗手。因此，直接与病羊接触是发生人山羊痘病毒感染的主要原因。

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医薬部外品　研究報告　調査報告書
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識別番号・報告回数			報告日	第一報入手日 2012年5月7日	新医薬品等の区分	厚生労働省処理欄
一般的名称	①②③④人血清アルブミン ⑤⑥乾燥濃縮人アンチトロンビンⅢ ⑦人ハプトグロビン ⑧乾燥濃縮人血液凝固第Ⅷ因子		研究報告の公表状況	Archives of Virology (Arch. Virol.) 2012; 157(3): 521-524	公表国 中国	
販売名 (企業名)	①献血アルブミン25%静注5g/20mL「ベネシス」(ベネシス) ②献血アルブミン25%静注12.5g/50mL「ベネシス」(ベネシス) ③献血アルブミン5%静注5g/100mL「ベネシス」(ベネシス) ④献血アルブミン5%静注12.5g/250mL「ベネシス」(ベネシス) ⑤ノイアート静注用500単位(ベネシス) ⑥ノイアート静注用1500単位(ベネシス) ⑦ハプトグロビン静注2000単位「ベネシス」(ベネシス) ⑧コンコエイトーHT(ベネシス)					

研究報告の概要

緒　言

ブタ・サイトメガロウイルス（PCMV）は、ヘルペスウイルス科に属するβヘルペスウイルスである。PCMVは封入体鼻炎、体重増加不良及び生まれたばかりの子豚の死亡を引き起こす。群において、感染の主要部位は鼻甲介と残りの上気道である傾向がある、そして生後3週齢未満のブタは肺炎、或いは全身感染を患う可能性がある。PCMV感染はヒトの同種移植において重要な問題でもある。ヒト・サイトメガロウイルスは全ての人々で普遍し、全ての新生児の最高1%を感染させる。ブタ・サポウイルス（SaVs）は、ヒトと動物で急性胃腸炎を引き起こすことができる重要な腸内病原体である。SaVsは実験的な条件下においてブタで下痢を引き起こすことができる。更に、SaVsはヒトにも感染することができ、ヒトSaVsの有病率は世界的に0.3%～9.3%にわたる。SaVsは高齢者や他の年齢層におけるウイルス性胃腸炎同様に、乳幼児における急性胃腸炎の重要な原因である。中国の小児におけるウイルス性胃腸炎の約19%はSaVsに起因すると推定された。

過去10年で、湖南省は大きな社会経済発展を遂げ、生活水準と生活様式の変化において大幅な増加をもたらした。これはますますブタが集中的に増加する結果となった。その上、PCMVとSaVsは広範な分布を持ち、ブタの群れでのそれらの有病率は過小評価されるかもしれない（全体的に経済的損失と同様に重大な健康問題をもたらすことがある）。より重要なことに、SaVsはヒトにも感染することができ、世界的に急性胃腸炎を引き起こしている重要な腸内病原体と考えられる。従って、ブタでのPCMVとSaVs感染の調査は、湖南省のヒトと動物における両ウイルスの予防と管理のための重要な意味を持っている。しかし、ブタでのPCMVとSaVsに対する抗体は限定された国、或いは地域のみで報告されている、そして中国のブタでのPCMVとSaVsの抗体陽性率の調査は報告されていない。

本調査の目的は、湖南省の集約農場からブタでのPCMVとSaVsの抗体陽性率を調べることであった。結果は、この省のブタでのPCMVとSaVs感染の改善された管理のための基盤を提供しなければならない。

材料と方法

（ 略 ）

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静注用500単位の記載を示す。
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。

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血清検体の収集と準備

合計：500の血液検体は、湖南省における10の代表的な行政地域に分布する集約農場のブタから、2005年5月から2010年10月の間に採取された。各農場で育てられるブタの数は約1000～5000の範囲であった。サンプリング前に、ブタはそれらの健康状態を判定するために診察を受けた。それぞれのブタに関する情報（例えば年齢、病歴、成長ホルモンと体重）が集められた。全てのブタは十分な配合飼料（高エネルギーと高たん白質）を与えられ、配合飼料の量は体重によって決められた。何れのブタもサイトメガロウイルス、或いはサポウイルスに対して予防接種を受けていなかった。各年齢層から健康なブタがランダムに選ばれ、血液検体が各々のブタから採取された。全ての血液検体は個々にラベルをつけられ、湖南農業大学獣医学部（湖南省長沙市）の検査室に輸送される間冷やされた。それから、血液検体は10分間、1,000Gで遠心分離され、血清が集められ、凍結され、そして使用まで-20℃で保存された。

血清学的検査

検査結果は、450nm波長の光学濃度（OD）値として表わされた。陽性コントロール（PCX）と陰性コントロール（NCX）の平均値は、2つの別々のウェルの平均値を計算することによって得られた。S/P値に基づき、各検体の分類は以下の通りだった：S/P<0.3、PCVM陰性(-)；S/P>0.4、PCVM陽性(+)；0.3<S/P<0.4、PCVM疑い（±）、そしてS/P<2.0、SaVs陰性（-）；S/P>2.1、SaVs陽性(+)；2.0<S/P<2.1、SaVs疑い（±）。

（ 略 ）

結果と考察

PCMVとSaVに対する抗体は、それぞれ間接gB-ELISA及びVP1-ELISAによって検査されたブタの96.40%(482/500)と63.40%(317/500)で検出された。ブタでのPCMV及びSaV感染の抗体陽性率は、それぞれ56.36%～72.50%、94.74%～98.48%の範囲であった。異なる地理的場所からのブタの抗体陽性率の間で有意差はなかった。湖南省の10の代表的な行政地域の全てにおけるブタでのPCMVの抗体陽性率は90%以上で、最高抗体陽性率（98.48%）は郴州であった。湖南省の10の代表的な行政地域の内の7箇所におけるブタでのSaV抗体陽性率は60%以上で、最高抗体陽性率（72.50%）は長沙であった。

我々の本調査の前に中国におけるブタでのPCMV及びSaV感染の抗体陽性率に関する報告はなかった；ブタでの症例報告と予備調査だけが記録されていた。これはおそらく必要とした疫学調査を行うために必要な検出試薬の不足によるものである。本研究で分かった全体的な抗体陽性率はPCMVの96.40%とSaVsの63.40%で、それは中国におけるPCMVとSaVsの頻繁な循環を示し、PCR手法を用いての以前の結果と一致している。PCMVの全体的な抗体陽性率はカナダで報告されたものと類似していたが、日本のそれより低かった。これは異なる動物の権利保護と農業慣習に起因している可能性がある。SaVsの全体的な抗体陽性率はベネズエラで報告されたものと類似していた。

本研究において、これらの違いは統計的に有意でなかったにも拘わらず、繁殖雌ブタが最高PCMV抗体陽性率（96.67%）で、続いて授乳ブタ（95.0%）、肥育ブタ（91.67%）、そして離乳ブタ（85.0%）であった。繁殖雌ブタでのSaVsの最高抗体陽性率は83.33%で、続いて離乳ブタ（68.75%）であった。我々の調査は、離乳ブタがSaVs感染のより高い抗体陽性率（68.75%）であることを示した。この結果は、VLP ELISA手法を用いた以前の研究（10週齢と12週齢のブタから採取した検体で報告されたゼロ陽性）と一致していなかった。

雌ブタでのPCMVの抗体陽性率は、雌ブタからその子供達へのPCMVの垂直感染による感染の指標であると仮定される。現在の調査において、雌ブタでのPCMVの抗体陽性率は96.67%で、この仮説を支持する。PCMVの感染はミルクを通して、子宮内感染を通して、そして周産期間の感染を通して起こり得るが、ウイルスが体液を通して排出されるので、感染の最も一般的な経路は口腔咽頭と生

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殖管を経由している。本調査において、PCMVの抗体陽性率はブタの他のカテゴリーにも広く分配され、PCMVの感染の最も一般的な経路が水平感染であることを確認した。非常に限られたデータはSaVsの感染に関して利用できる、そして最近の知見において、SaVsの感染の主要経路が糞口であると結論をだした、しかし嘔吐と汚染食品も感染性かどうかはまだ分かっていない。

結論として、現在の調査の結果はPCMV及びSaV感染の両方が湖南省のブタで非常に流行していることを示しているが、このひどい状況は過去に殆ど注意を払われていなかった。従って、中国のこの省と他の地域におけるブタでのPCMV及びSaV感染を予防管理のために、総合的な管理戦略と措置を適用することが避けられない。

報告企業の意見	今後の対応
ブタ・サイトメガロウイルス（porcine cytomegalovirus:PCMV）は、ブタ・ヘルペスウイルス2型（suid herpesvirus 2；SuHV-2）とも呼ばれ、DNAウイルスのヘルペスウイルス科のβヘルペスウイルス亜科に属し、エンベロープを有する大きさは150～200nmの球状粒子である。万一、ヘパリンの原料であるブタ小腸粘膜にPCMVが混入したとしても、PRVをモデルウイルスとしたウイルスクリアランス試験成績から、ヘパリンの製造工程において不活化・除去されると考えている。 サポウイルス（sapovirus:SaV）は感染性胃腸炎の原因ウイルスの一つで、ノロウイルス（Norovirus）と同じRNAウイルスのカリシウイルス科（Caliciviridae）に属し、エンベロープを有しない大きさは約38nmの球状粒子である。万一、ヘパリンの原料であるブタ小腸粘膜にSaVが混入したとしても、HPV1及びPPVをモデルウイルスとしたウイルスクリアランス試験成績から、ヘパリンの製造工程において不活化・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

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Seroprevalence of porcine cytomegalovirus and sapovirus infection in pigs in Hunan province, China

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Abstract The seroprevalence of porcine cytomegalovirus (PCMV) and sapovirus (SaV) infections in pigs was investigated in Hunan province, China, between May 2005 and October 2010. A total of 500 pig serum samples collected from 10 representative administrative regions in Hunan province were evaluated for antibodies against PCMV and SaV using enzyme-linked immunosorbent assay (ELISA). The overall seroprevalence of porcine cytomegalovirus and sapovirus in pigs was 96.40% (482/500) and 63.40% (317/500), and the seropositivity of 10 herds we surveyed varied, ranging from 94.74% to 98.48% and 56.36% to 72.50%, respectively. The highest prevalence was found in breeding sows (96.67% for PCMV and 83.33% for SaVs). The results of the present survey indicated that infections with porcine cytomegalovirus and sapovirus are highly prevalent in pigs in Hunan province, China.

Introduction

Porcine cytomegalovirus (PCMV) is a betaherpesvirus belonging to the family *Herpesviridae*. PCMV causes inclusion body rhinitis, poor weight gain and death of newborn piglets [11, 14]. In herds, a major site of infection tends to be the turbinates and the rest of the upper respiratory tract, and pigs under three weeks of age may suffer from pneumonia or generalized infection [8]. PCMV infection is also an important problem for human allotransplantation [7]. Human cytomegalovirus is ubiquious in all populations, and infect up to 1% of all newborns [23, 26]. Porcine sapoviruses (SaVs) are important enteric pathogens that can cause acute gastroenteritis in humans and animals. SaVs can cause diarrhea in pigs under experimental conditions [1]. Moreover, SaVs are also transmissible to humans, and the prevalence of human SaVs ranges from 0.3% to 9.3% worldwide [22]. SaVs are an important cause of acute gastroenteritis in infants and young children, as well as viral gastroenteritis in the elderly or other age groups [9, 15, 21]. It is estimated that approximately 19% of viral gastroenteritis in children in China is caused by SaVs [25].

In the past decade, Hunan province has undergone major socio-economic development, and this has resulted in a huge increase in living standards and lifestyle changes. This has led to more and more pigs being raised intensively. In addition, PCMV and SaVs have global distribution, and their prevalence in pig herds might be underestimated, which can result in significant health problems as well as economic losses globally [4, 5]. More importantly, SaVs can also infect humans and is considered a significant enteropathogen causing acute gastroenteritis worldwide [9, 15, 21, 28]. Therefore, investigation of PCMV and SaV infection in pigs has important implications for the prevention and control of both viruses in humans and animals in Hunan province. However, antibodies against PCMV and SaVs in pigs have been reported in only a limited number of countries or regions [1, 3, 24, 27], and no survey of the seroprevalence of PCMV and SaVs in pigs in China has been reported.

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The objective of the present investigation was to examine the seroprevalence of PCMV and SaVs in pigs from intensive farms in Hunan province. The results should provide a foundation for the improved control of PCMV and SaVs infection in pigs in this province.

Materials and methods

The study province

Hunan province is situated in the central eastern part of mainland China, between the northern latitudes of 25° to 30° and eastern longitudes of 109° to 114°. The climate is humid subtropical monsoon with an average annual temperature of 16-18°C. The average annual rainfall ranges from 1200 to 1700 mm. Hunan province is divided in to 14 administrative regions (cities), with the city of Changsha as its capital.

Collection and preparation of serum samples

A total of 500 blood samples were collected between May 2005 and October 2010 from pigs in intensive farms that are distributed in 10 representative administrative regions in Hunan province (Table 1). The numbers of pigs reared on each farm ranged from 1000 to 5000, approximately. Before sampling, pigs were subjected to clinical examination to determine their health status. Information about each pig, such as age, medical history, growth hormones, and weight were collected. All pigs were provided with sufficient concentrate mixture (high energy and high protein). The quantity of concentrate mixture was determined by body weight. None of the pigs were vaccinated against cytomegalovirus or sapovirus. Healthy animals from each age group were selected randomly, and one blood sample was collected from each animal. All of the blood samples were labelled individually and cooled during transport to the laboratory at the College of Veterinary Medicine, Hunan Agricultural University (Changsha, Hunan Province). Blood samples were then centrifuged at 1,000 *g* for 10 min, and serum was collected, frozen, and stored at −20°C until use.

Table 1 Seroprevalence of porcine cytomegalovirus and sapoviruses in pigs in Hunan province as determined by ELISA

Source of serum	No. examined	No. positive PCMV/SaVs	Prevalence (%) PCMV/SaVs
Yueyang	50	48/34	96.00/68.00
Yiyang	40	39/26	97.50/65.00
Shaoyang	55	53/31	96.36/56.36
Henyang	64	62/39	96.88/60.94
Yongzhou	60	58/36	96.67/60.00
Changsha	40	38/29	95.00/72.50
Xiangtan	38	36/26	94.74/68.42
Loudi	42	40/27	95.23/64.29
Huaihua	45	43/31	95.56/68.89
Chenzhou	66	65/38	98.48/57.58
Total	500	482/317	96.40/63.40

Serological examination

Antibodies against PCMV and SaV were detected by indirect gB-ELISA and VP1-ELISA methods, respectively [17, 18]. Briefly, a 96-well ELISA plate was coated with PCMV- or SaV-specific antigen, and 100-μl diluted serum samples were then added to the test wells. Positive control sera were collected from piglets that were experimentally infected with PCMV or SaV. Negative control sera were collected from newborn piglets born to sows that were not infected.

The results of the test were expressed as the optical density (OD) value at a wavelength of 450 nm. The mean values for the positive control (PCX) and the negative control (NCX) were obtained by calculating the average value for two separate wells. Based on the S/P value, classification of each sample was as follows: S/P< 0.3, negative (−); S/P>0.4, positive (+); 0.3< S/P<0.4, suspicious (−/+) for PCVM, and S/P<2.0, negative (−); S/P>2.1, positive (+); 2.0< S/P<2.1, suspicious (−/+) for SaVs.

Statistical analysis

Differences in the PCMV and SaV seropositivity in pigs from different geographical localities were analyzed using the chi square test in SPSS for Windows (Release 17.0, standard version, SPSS Inc., Chicago, IL, USA).

Results and discussion

Antibodies to PCMV and SaV were detected in 96.40% (482/500) and 63.40% (317/500) of the pigs examined by indirect gB-ELISA and VP1-ELISA, respectively. The seroprevalence of PCMV and SaV infection in pigs ranged from 56.36% to 72.50% and 94.74% to 98.48%, respectively (Table 1). There was no significant difference between the seroprevalence in pigs from different geographical locations ($p>0.05$, data not shown). The seroprevalence of PCMV in pigs in all of the 10 representative administrative regions in Hunan province was more than 90%, and the highest seroprevalence (98.48%) was in Chenzhou (Table 1). The SaV seroprevalence in pigs in seven of the 10 representative administrative regions in Hunan province was more than 60%, and the highest seroprevalence (72.50%) was in Changsha (Table 1).

There were no reports on the seroprevalence of PCMV and SaV infection in pigs in China prior to our present investigation; only case reports and preliminary research in pigs have been documented [13, 16, 19, 25]. This is likely due to the scarcity of the detection reagents required to undertake the needed epidemiological surveys. The overall seroprevalence found in this study was 96.40% for PCMV and 63.40% for SaVs, which is consistent with previous results using a PCR approach [13, 14, 19, 25], indicating the frequent circulation of PCMV and SaVs in China. The overall seroprevalence of PCMV was similar to that reported in Canada [3] but lower than that in Japan [27]. This may be attributed to different animal-welfare and husbandry practices. The overall seroprevalence of SaVs was similar to that reported in Venezuela [1].

In the present study, breeding sows had the highest PCMV seroprevalence (96.67%), followed by suckling pigs (95.0%), fattening pigs (91.67%), and weanling pigs (85.0%) (Table 2), although these differences were not statistically significant ($P>0.05$). The highest seroprevalence of SaVs in breeding sows was 83.33%, followed by weaning pigs (68.75%) (Table 3). Our investigation indicated that weanling pigs had a higher seroprevalence of SaVs infection (68.75%). This result was not consistent with that of a previous study using a VLP ELISA approach [10], in which zero positivity was reported in the samples collected from pigs 10 and 12 weeks of age.

It is hypothesized that the seroprevalence of PCMV in sows is an indicator of infection because of vertical transmission of PCMV from sows to their offspring. In the present investigation, the seroprevalence of PCMV in sows was 96.67%, which supports this hypothesis. Although transmission of PCMV can occur through milk, through intrauterine transmission, and through transmission during the perinatal period [5, 6, 12], the most common routes of transmission are via the oropharyngeal and genital tracts, because the virus is excreted through body fluids [20]. In the present investigation, the seroprevalence of PCMV was also widely distributed among other categories of pigs, confirming that the most common route of transmission of PCMV is horizontal transmission. Very limited data are available regarding the transmission of SaVs, and in a recent review, it was concluded that the major route of transmission of SaVs is fecal-oral [2], but it is not yet known whether vomit and contaminated food are also infectious.

In conclusion, the results of the present survey indicate that both PCMV and SaV infections are highly prevalent in pigs in Hunan province, but this severe situation has received little attention in the past. Therefore6, it is imperative to apply integrated control strategies and measures to prevent and control PCMV and SaV infections in pigs in this province and elsewhere in China.

Table 2 Seroprevalence of porcine cytomegalovirus in pigs of different categories in Hunan province as determined by ELISA

Category	Approximate age	Weight (kg)	No. examined	No. positive	Prevalence (%)
Breeding sows	Adult females	120-150	60	58	96.67
Suckling pigs	1-3 weeks	7-9	60	57	95.0
Weanling pigs	4-10 weeks	12-17	60	51	85.0
Fattening pigs	12-18 weeks	55-70	60	55	91.67
Total			240	221	92.08

Table 3 Seroprevalence of porcine sapovirus in pigs of different categories in Hunan province as determined by ELISA

Category	Approximate age	Weight (kg)	No. examined	No. positive	Prevalence (%)
Breeding sows	Adult females	120-150	42	35	83.33
Weanling pigs	1-3 weeks	12-17	96	66	68.75
Total			153	96	66.01

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別紙様式第2-1

No. 26

医薬品 研究報告 調査報告書

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研究報告の概要

○高いトランスアミナーゼレベルの献血者から分離された新規DNA配列

目的: 日本では、急性肝炎の10−20%の原因は不明である。この研究は、非A-E型肝炎の原因となっている病原体を確かめるために行われた。

方法: ALT値が上昇した500人の献血者からの血清サンプルが、RNAヘリカーゼの保存領域から設計されたプライマーを用いてPCRによりスクリーニングされた。得られた配列のウイルス特性について調査された。

結果: 4つの血液サンプルに9496 bpの新しいDNA配列が含有されていることが分かった。これをKIs-Vと称する。KIs-Vは制限酵素*Sal*I及び*Bst*XIに反応した。ローリングサークル増幅法でKIs-V DNAが大量に増幅された。ショ糖密度勾配遠心法において、KIs-Vは1.158 g/cm3の密度であった。有機溶媒処理はKIs-Vの密度を上昇させた。KIs-Vはヒト白血球DNAから検出されなかった。連続濾過により、KIs-Vは30-50nmの粒子であることが示唆された。*in silico*分析より、KIs-Vは13のORFを含むことが分かり、既報告のいかなるウイルスタンパク質とも相同性を示さなかった。1つの遺伝子は、DNAポリメラーゼ領域に類似性を示した。転写開始及びCpGアイランドの強いシグナルが確認された。KIs-Vのヌクレオチド構成は複製開始点と終点を含む環状DNAゲノムの特徴を示した。予備研究で、KIs-Vは高いALT値を示すE型肝炎ウイルス抗体陽性者において度々検出された。

結論: 新しい配列のKIs-Vは、高いALT値を示す献血者から分離された。KIs-Vはエンベロープを有する新しい分類の二本鎖環状DNAゲノムであることが示された。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血
新鮮凍結血漿-LR「日赤」120
新鮮凍結血漿-LR「日赤」240
新鮮凍結血漿-LR「日赤」480

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

21

報告企業の意見	今後の対応
ALT値が高い献血者から、新しい分類のウイルスに属すると思われるKIs-Vが分離されたとの報告である。	今後も情報の収集に努める。

Hepatology Research

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Original Article

Novel DNA sequence isolated from blood donors with high transaminase levels

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Aim: In Japan, the etiology of 10–20% of cases of acute hepatitis remains undarified. This study was conducted to verify the agent causing non-A–E hepatitis.

Methods: Serum samples from 500 blood donors with elevated alanine aminotransferase (ALT) levels were screened by polymerase chain reaction using primers constructed from conserved areas of RNA virus helicase. The sequence obtained was investigated for viral properties.

Results: Four blood samples were found to contain a novel DNA sequence of 9496 bp, which was designated KIs-V. KIs-V was sensitive to the restriction enzyme *Sal*I and *Bst*XI. Rolling-circle amplification produced an excessive amount of KIs-V DNA. In sucrose density gradient ultracentrifugation, KIs-V banded at a 1.158-g/cm^3 density. Detergent treatment increased the density of KIs-V. There was no KIs-V DNA amplification from human leukocyte DNA. Serial filtration suggested that KIs-V was included in a 30–50-nm size particle. *In silico* analysis revealed that KIs-V contained 13 potential genes, none of which showed homology to any viral proteins reported. One gene showed similarity to a DNA polymerase domain. Strong signals for transcription initiation and a CpG island were identified. The nucleotide composition of KIs-V showed a characteristic feature of circular DNA genomes that contain a replication origin and a terminus. In a preliminary study, KIs-V was frequently identified among hepatitis E virus antibody positive individuals with elevated ALT levels.

Conclusion: A new sequence KIs-V was isolated from blood donors with elevated ALT levels. It was suggested that KIs-V is a double-stranded circular DNA genome derived from a novel category of enveloped viruses.

Key words: alanine aminotransferase, hepatitis virus, non-A–E hepatitis, RNA helicase

INTRODUCTION

VIRAL HEPATITIS CONSTITUTES a disease category of vital importance not only because of its high morbidity and mortality but also because of its high prevalence and transmissibility. To date, a causal relationship between viruses and hepatitis has been established for hepatitis A, B, C, D and E. A number of serological and/or genetic methods have been proposed for the detection of these viruses in the sera obtained from patients with hepatic dysfunction. Some of them have been successfully implemented for clinical diagnosis and blood screening. In 10–20% of patients, however, the etiology of acute hepatitis remains unclarified despite the technological advances in molecular biology.[1,2] Representational difference analysis[3] was used to detect the GB virus[4] and the TT virus.[5] Non-specific polymerase chain reaction (PCR) analysis was also used to detect novel single-stranded DNA fragments.[6] In contrast to these sophisticated but laborious methods, we attempted to directly detect a new sequence using primers constructed from a conserved portion of RNA virus helicase.[7–9] Applying this method to blood samples from volunteer blood donors with high transaminase levels, we successfully detected a novel sequence that was deemed to be a part of an enveloped DNA virus. In this article, we describe the characteristics of the novel sequence obtained and its homology with those of known organisms.

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METHODS

Blood samples and testing for hepatitis virus

BLOOD SAMPLES WERE obtained from the blood donors who were negative for hepatitis B virus (HBV)-DNA and hepatitis C virus (HCV)-RNA but were disqualified because of high alanine aminotransferase (ALT) levels (>60 IU/L). The method for nucleic acid amplification tests for HBV and HCV has been described elsewhere.[10] Anti-hepatitis E virus antibody (HEVAb) was measured by IgG/IgM anti-HEV EIA (Institute of Immunology, Tokyo, Japan). HEV-RNA was detected by reverse transcription (RT)–PCR following the method of Mizuo *et al.*[11] The whole study program on the identification of novel viruses among blood donors was approved by the Japanese Red Cross ethics committee.

Screening for and determination of nucleotides with novel sequences

Nucleic acids were extracted from 0.1 mL of plasma samples using an extraction kit (Smitest ExR&D; Genome Science, Fukushima, Japan) and were reverse transcribed (50°C, 30 min; 94°C, 15 min) using a reverse transcriptase (SuperScript III; Invitrogen, Tokyo, Japan). PCR was performed for 35 cycles (94°C, 30 s; 55°C, 30 s; 72°C, 2 min) using primers IA-3 and IV-3 (Table 1) that were constructed according to the amino acid (a.a.) sequence of the consensus domain of helicase of positive-stranded RNA viruses.[7–9] Five hundred blood samples were thus screened, and a novel sequence obtained was designated KIs. It was first determined that the KIs sequence had a DNA nature (see below). Employing the primer walking technique, unknown portions of the KIs sequence were amplified by PCR without RT using primers constructed from a portion of the KIs sequence and the kit primers (DNA Walking SpeedUp Premix Kit-II; Seegene, Seoul, South Korea). The extension and determination of the sequence was repeated, and the sequence thus obtained was designated KIs-V.

In the screening or experiments that required the confirmation of KIs-V, nested PCR was performed using primers that were constructed on the basis of the KIs-V sequence: 101-C and N101-B for the first-round PCR

Table 1 Primers used in this study

Target sequence	Names	Polarity	Sequences	Positions (nt)
KIs	IA-3	Sense	5′-CCIACIGGIAGIGGIAARAGCAC-3′	
	IV-3	Antisense	5′-CTICCMGTCCGICCICCSCCYTG-3′	
KIs-V	101-C	Sense	5′-CAACACCGCAATCACAAAGT-3′	3007–3026
	N101-B	Antisense	5′-AACATTGAAACGTCATGTCC-3′	3445–3464
	KS-2	Sense	5′-CTCGTCTCGTCGTCATCGTA-3′	3082–3101
	N101-D	Antisense	5′-CATTTGCTCCCGCTGGAGATG-3′	3365–3385
	KIV-13	Sense	5′-CAATGAGATTGGATGGACGA-3′	9195–9214
	101-TT	Antisense	5′-CCGTGAAGCTCTTGGCAAAG-3′	321–340
	KIV-14	Sense	5′-CGTGTACTAACTATACTGAC-3′	9255–9274
	KIV-2	Antisense	5′-CACTCGTCCATTATACCGCT-3′	121–140
	KIV-5	Sense	5′-AGAACGGTGACGACATAAAC-3′	457–476
	101-Su	Sense	5′-ATGGCCTATTCTCAATCACC-3′	5715–5734
	101–29	Sense	5′-ACCTGCGCCTGAGGCTACCA-3′	6888–6907
	101–45	Sense	5′-TGGGTAAAGTATGCAGAGTTGT-3′	8863–8884
	101-T	Antisense	5′-TCGATCCCCTTTCGGTACGT-3′	1383–1402
	101-6R	Antisense	5′-CACGCGATTCCCATATCCCT-3′	8789–8808
	X-7	Antisense	5′-TGGGAGTATGGAGTCGACAT-3′	5331–5350
	X-3	Antisense	5′-GTTGGGAAGAGGTTGATTGT-3′	4091–4110
	101–22	Sense	5′-GCTCAACGAACTCGCCTCGA-3′	4888–4907
HBV		Sense	5′-TCGTGTTACAGGCGGGGTTT-3′	192–211
		Antisense	5′-CGAACCACTGAACAAATGGC-3′	685–704
HCV		Antisense	5′-AACACTACTCGGCTAGCAGT-3′	246–265
		Sense	5′-CTGTGAGGAACTACTGTCTT-3′	45–64
B19V	425	Sense	5′-CAGTATCAGCAGCAGTGGTGGTG-3′	1956–1978
	426	Antisense	5′-GGGATTAGAAGCTCCCACATGGC-3′	2360–2382

(34 cycles); KS-2 and N101-D for the second-round PCR (25 cycles) (Table 1) (94°C, 30 s; 55°C, 30 s; 72°C, 2 min). The KIs-V nucleotide positions were numbered in this paper according to the sequence index that was registered in GenBank (see below) in which the beginning of the index was arbitrarily assigned.

Characterization of KIs-V

Nucleic acids were extracted from a KIs-V-positive plasma and divided into three tubes. The first aliquot (0.1 mL) was digested with 4 units of deoxyribonuclease I (DNase I; Takara Shuzo, Shiga, Japan) at 37°C for 1 h and then heated at 95°C for 10 min to inactivate the enzyme. The second aliquot was digested with RNase (Nacalai Tesque, Kyoto, Japan) at 37°C for 1 h and heat-inactivated. The third aliquot was not treated with any of these enzymes. From each of the three samples above, nucleic acid was extracted and subjected to nested RT–PCR. The RT–PCR products were separated and visualized by electrophoresis.

Inverted PCR was performed to examine whether KIs-V was a circular DNA using two primer combinations that were constructed for the sequences near both ends of the extended sequence: KIV-13 and 101-TT; KIV-14 and KIV-2 (Table 1). The circular structure of KIs-V was further confirmed employing rolling-circle amplification (RCA) using an Illustra TempliPhi DNA Amplification kit (GE Healthcare Science, Piscataway, NJ, USA). In brief, DNA extracted from KIs-V-positive plasma was amplified overnight at 30°C using a bacteriophage Phi29 DNA polymerase and random hexamer primers. The amplification product was subjected to nested PCR using the primers for KIs-V. The same RCA experiment was conducted using two sets of primers, each of which consisted of five primers constructed from the KIs-V sequence (sense primers 101-C, KIV-5, 101-Su, 101–29, 101–45; antisense primers N101-B, KIV-2, 101-T, 101-6R, X-7; Table 1).

Enzyme digestion experiments were performed using two restriction enzymes specific for double-stranded DNA, *SalI* and *BstXI*. The *SalI* consensus cleavage site was identified within the sequence, and primers were constructed for both sides of the cleavage site. DNA extracted from plasma containing 10^7 copies/mL KIs-V was treated with varying concentrations of *SalI* (Takara Shuzo) at 37°C for 90 min and then heat-inactivated. DNA was extracted from the *SalI*-treated and non-*SalI*-treated samples and subjected to PCR using the primer pairs constructed. For the digestion with *BstXI*, a diluted sample containing 10^5 copies/mL KIs-V was used. A plasma sample containing 10^4 copies/mL human parvovirus B19 (B19V), a single-stranded DNA virus, was included as a control. Extracted DNA was digested with *BstXI* (Takara Shuzo) at 45°C for 1 h. The primers used for PCR were constructed so that they cross over the *BstXI* cleavage site in the KIs-V sequence and authentic B19V sequence (GenBank accession no. M13178-1a).

To elucidate the approximate size of the putative particle containing the KIs-V sequence, a filter passage experiment was carried out. The pore sizes of the filters used were 0.2, 0.1, 0.05 and 0.03 μm (Nuclepore Track-Etch Membrane; Whatman Japan, Tokyo, Japan). KIs-V-positive plasma diluted at a concentration of approximately 10^3 copies/mL was loaded onto the filter with a 0.2-μm pore size. After removing an aliquot for PCR, the effluent from this filter was loaded onto the filter with the second larger pore size, namely, a filter with a 0.1-μm pore size. This sequential loading was repeated until effluent was obtained from the filter with a 0.03-μm pore size. As a reference, HBV-containing plasma was also subjected to the same sequential filtration. Tenfold titration series were prepared from each effluent, from which DNA was extracted and subjected to PCR using KIs-V-specific or HBV-specific primers (Table 1). The end titration value with positive PCR was evaluated as the viral concentration in the effluent.

Ultracentrifugation in sucrose density gradient

A plasma cocktail (0.4 mL) containing KIs-V, HBV and HCV was layered onto a stepwise density gradient consisting of 1.0 mL of 60% (w/v) sucrose and 0.4 mL each of 50%, 40%, 30% and 20% sucrose in Tris-HCl buffer (50 mM, pH 8.0, TE buffer) containing 1 mM ethylenediamine tetraacetic acid in a 4.4-mL tube. The tube was overlaid with 1.4 mL of TE buffer and centrifuged at 256 760 *g* for 16.5 h at 10°C in a Beckman SW60Ti rotor (Beckman, Palo Alto, CA, USA). The tube was pierced at the bottom, and 200-μL fractions were collected, for which PCR analysis of KIs-V-DNA, HBV-DNA and HCV-RNA was performed. The primers used for determining HBV or HCV are shown in Table 1.

To explore whether KIs-V is lipid-enveloped, KIs-V-positive samples were either treated or not treated with 0.1% Nonidet P-40 in TE buffer for 5 min. The samples were then ultracentrifuged, and the distribution pattern of KIs-V was determined as described above.

Sequence analysis

The protein coding regions and transcription promoter sites were predicted using the MolQuest web interface programs FGENESV0 and TSSG, respectively (www.molquest.com). Protein coding regions were predicted assuming the eukaryote genome genetic code. The transmembrane regions of the predicted proteins were inferred using the TMHMM web server ver. 2.0 (www.cbs.dtu.dk/services/TMHMM/). Homology search (BLAST) and conserved domain search (CD-search) were conducted through the NCBI web server (www.ncbi.nlm.nih.gov/).

The GC skew plot was generated using an in-house Perl script. The cumulative values of (#G – #C) / (#G + #C) were plotted with a window size of 2 kb and 100-bp intervals, where #G and #C represent the numbers of G and C nucleotides, respectively, in the window on the sense strand.

RESULTS

Detection of KIs and KIs-V from donors with high ALT levels

PLASMA SAMPLES FROM 12 out of 500 donors with more than 60 IU/L ALT were found to be RT–PCR-positive using the primers IA-3 and IV-3. Eight of the 12 samples were either positive for the GB virus or contaminated with bacteria and were excluded. The remaining four samples, three from male donors and one from a female donor, were subjected to further analysis. The ages of the four donors ranged 30–49 years. Their ALT levels were between 61 and 82 IU/L. The DNA sequences of the amplification products from the four donors were identical and were designated KIs. The length of KIs was unexpectedly short (210 bp: nt 3170–3379) (Fig. 1) and, to our surprise, KIs did not show homology with the consensus sequence of RNA virus helicase even though it was obtained using primers constructed from RNA virus helicase.

During the initial screening, it was noticed that there was little difference in PCR yield with or without prior RT, suggesting a DNA nature of the sequence. To confirm this, nucleic acids extracted from a KIs-positive sample was treated either with DNase or RNase and then subjected to RT–PCR. Nucleic acids treated with RNase, not DNase, were amplified by RT–PCR, indicating that the novel sequence obtained was a DNA (data not shown).

After several cycles of extension of the KIs sequence using the primer walking method, inverted PCR was carried out using two primer combinations that were constructed near both ends of the extended sequence. PCR produced sequences connecting both ends with concordant sequences (Fig. 2). The total length of the sequence was determined to be 9496 nucleotides from this experiment. The sequence was designated KIs-V and registered in GenBank with the accession number AB550431. Using the primer sets for the nested PCR for KIs-V (101-C and N101-B, KS-2 and N101-D), screening was repeated for the 500 blood samples, which resulted in the identification of 16 samples, including the four samples described above, to be KIs-V-positive.

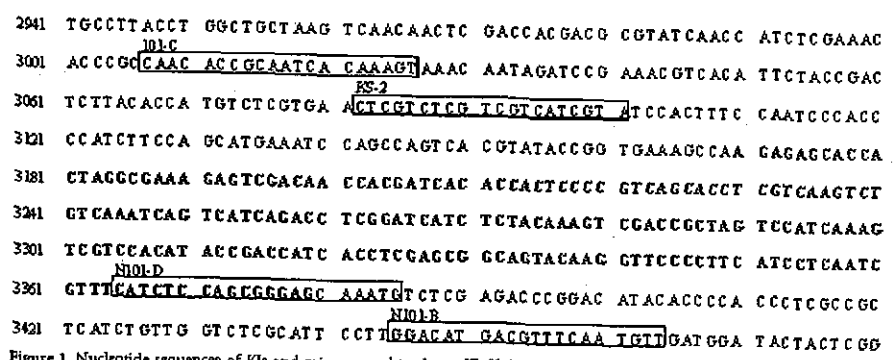

Figure 1 Nucleotide sequences of KIs and primers used to detect KIs-V. Bold characters indicate the KIs sequence that was first detected using the primers constructed from the consensus domain of helicase of positive-stranded RNA viruses. Primers used to detect KIs-V are shown in boxes: first-round polymerase chain reaction (PCR) primers, 101-C and N101-B; second-round PCR primers, KS-2 and N101-D.

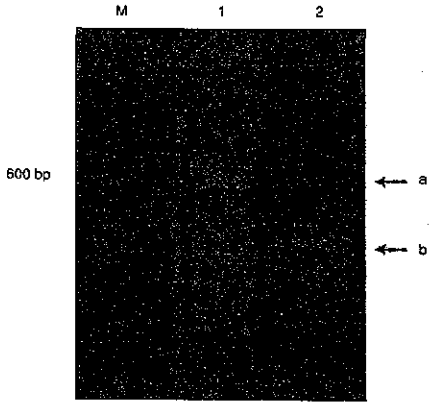

Figure 2 Electrophoresis of products of inverted polymerase chain reaction (PCR) using primers constructed near both terminals of KIs-V. The primers used are KIV-13 (nt 9195–9214) and 101-TT (nt 321–340) for lane 1 and KIV-14 (nt 9255–9274) and KIV-2 (nt 121–140) for lane 2. Bands indicated by arrow a in lane 1 and arrow b in lane 2 show the polynucleotides that connect both ends of the extended sequence. The bands were excised from the gel and subjected to PCR and sequencing, which revealed that they had a concordant sequence. The nucleotide lengths of band a and band b were assumed to be 641 bp and 381 bp, respectively, on the basis of the whole sequence of KIs-V. Lane M shows the DNA ladder marker by every 100 bp with the bold line indicating 600 bp.

Along with the whole KIs-V sequence from one of the four samples, primer pairs were serially constructed, targeting overlapping sequences of approximately 500 bp length each. PCR with the primer pairs was carried out for the other three KIs-positive samples. It was revealed that the whole sequence obtained from each of the three samples was identical to that of the first sample: the four blood donors possessed KIs-V with completely identical sequences. The concentration of KIs-V in the four donor samples was between 10^6 and 10^8 copies/mL, as verified by limiting dilution assay.

To confirm that KIs-V is not a DNA derived from human host genomes, PCR was performed for genomic DNA from leukocytes obtained from two KIs-V-negative individuals. Using the nested primers described above, no amplification was achieved in any of them, indicating KIs-V being of non-host origin (data not shown).

Properties of KIs-V

The overnight amplification of a KIs-V-positive plasma sample with RCA using random hexamer primers produced a large amount of DNA that was confirmed to be derived from KIs-V by PCR analysis (data not shown). The same results were obtained in the two series of RCA that employed KIs-V sequence-based primers. These findings together with those from inverted PCR indicate that KIs-V forms a circular DNA in the sample.

Nine cleavage sites for the restriction enzyme *SalI* were identified in the KIs-V sequence: nt 688, 1349, 3193, 3279, 5333, 6496, 8013, 8135 and 8748. PCR was performed targeting restriction sites nt 3193 and 3279 using primer pairs KS-2 (nt 3082–3101)/X-3 (nt 4091–4110) and 101-C (nt 3007–3026)/N101-B (nt 3445–3464). The amplification product was not obtained from a sample treated with 10 U/μL *SalI* solution (Fig. 3a).

Four cleavage sites for *BstXI* were identified in the KIs-V sequences: nt 423, 2052, 4991 and 7550. Primers that crossed the cleavage site nt 4991 were prepared: 101–22 (nt 4888–4907) and X-7 (nt 5331–5350). The pretreatment of KIs-V DNA with 10 U/μL *BstXI* abrogated the production of polynucleotides by PCR using the above primers (Fig. 3b left). In the B19V sequence, two cleavage sites for *BstXI* were identified: nt 2033 and nt 4730. Targeting nt 2033, primers were constructed: 425 (nt 1956–1978) and 426 (nt 2360–2382) (Table 1). The pretreatment of B19V-DNA with *BstXI* did not change the density of the bands of PCR products (Fig. 3b right). Overall, these findings indicate that KIs-V forms a double-stranded DNA structure.

Regarding the effect of DNase treatment on PCR, the DNase treatment (4U DNase I at 37°C for 1 h) of KIs-V-positive plasma not before but after DNA extraction using a proteinase-containing reagent abrogated the amplification of KIs-V, indicating that KIs-V DNA present in plasma is encapsulated by a proteinase-sensitive substance (data not shown).

We next carried out the serial passage of KIs-V-containing plasma through filters of various pore sizes. Because of the blocking of the filter, we were often unable to recover the full volume loaded in the effluent. It is nevertheless evident that the 0.05-μm filter passed the KIs-V sequence freely, whereas the 0.03-μm filter trapped it completely, as no KIs-V amplification was obtained using neat effluent from the 0.03-μm filter (Table 2). These findings indicate that the size of the putative particle containing the KIs-V sequence is between 30 and 50 nm. As a reference, the same experi-

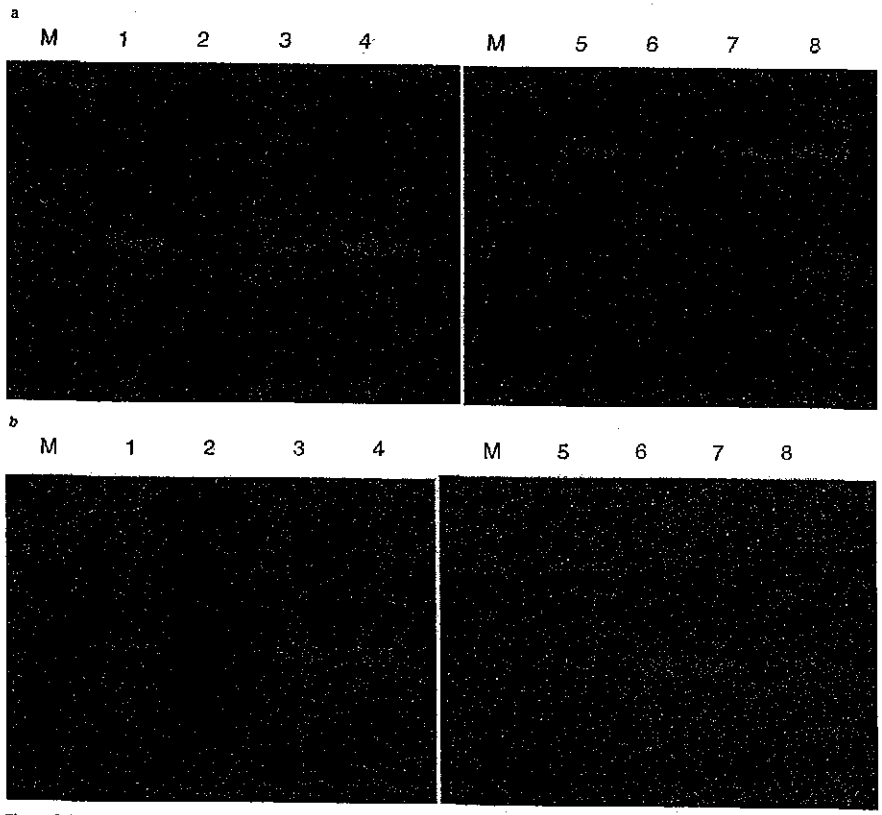

Figure 3 Digestion of DNA with restriction enzymes *SalI* (a) and *BstXI* (b) that are specific for double-stranded DNA. (a) DNA extracted from plasma containing 10^7 copies/mL KIs-V was digested with *SalI* and amplified using primers 101C and N101B (left) or KS-2 and X-3 (right). There are two *SalI*-cleavage sites between the sense primer and the antisense primer in both primer combinations. The concentrations of *SalI* were 10 U/μL for lanes 2 and 6, 1 U/μL for lanes 3 and 7, and 0.25 U/μL for lanes 4 and 8. Lanes 1 and 5 show the control without the enzyme. (b) DNA extracted from a sample containing 10^5 copies/mL KIs-V (left) and DNA from plasma containing 10^4 copies/mL human parvovirus B19 (B19V) (right) were digested with *BstXI* and electrophoresed after polymerase chain reaction (PCR). Primers used for PCR are X-7 and 101–22 for KIs-V and 425 and 426 for B19V. There is one *BstXI*-cleavage site between the primers in both the KIs-V and B19V sequence. The concentrations of *BstXI* were 10 U/μL for lanes 2 and 6, 1 U/μL for lanes 3 and 7, and 0.25 U/μL for lanes 4 and 8. Lanes 1 and 5 show the control without the enzyme. Lane M shows DNA ladder marker by every 100 bp with the bold line indicating 600 bp.

Table 2 Filter passage performance of KIs-V-positive plasma

Pore size of filters (μm)	0.2	0.1	0.05	0.03
KIs-V				
Loaded volume (mL)	5	4	2.3	1.7
Effluent volume (mL)	4	2.3	1.7	0.8
End titer†	1000	1000	1000	0‡
HBV				
Loaded volume (mL)	5	4	2.5	2
Effluent volume (mL)	4	2.5	2	1
End titer	1000	1000	1000	0

Plasma containing either KIs-V or hepatitis B virus (HBV), the concentration of which was adjusted to approximately 10^3 copies/mL, was loaded onto the filter with 0.2-μm pores and the effluent of this filter was loaded onto the filter with the second larger pore size.
†Maximum plasma dilution that yielded positive polymerase chain reaction (PCR) result.
‡Neat effluent of the filter did not yield positive PCR result.

ment was performed using HBV-containing plasma. It was revealed that the 0.03-μm filter, not the 0.05-μm filter, trapped HBV, which is in agreement with the Dane particle being 42 nm in diameter, confirming the filter performance.

A plasma cocktail containing KIs-V, HBV and HCV was fractionated by ultracentrifugation in a sucrose density gradient. KIs-V appeared in the fractions with a peak at 1.158 g/cm³ as revealed by PCR, while HBV and HCV appeared in those with peaks at 1.23 and 1.024 g/cm³, respectively (Fig. 4). When KIs-V-positive samples were treated with detergent and subjected to gradient ultracentrifugation, the peak density of the fractions containing the KIs-V sequence shifted from 1.158 g/cm³ for the non-treated sample to 1.210 g/cm³ for the treated sample, indicating that KIs-V is lipid-enveloped (Fig. 5).

In silico analysis of KIs-V sequence

Potential protein coding regions in the KIs-V sequence were searched for using a computer program, which predicted 13 potential genes. The hypothetical genes were numbered from genes 1 to 13 according to the sequence index (Table 3). Ten and three genes were encoded in the *sense and complementary strands*, respectively. Among them, the longest open reading frame (ORF) potentially encoded an 860-a.a.-long protein (gene 13) and the second longest ORF potentially encoded an 815-a.a.-long protein (gene 6). Detailed annotations are summarized in Table 3.

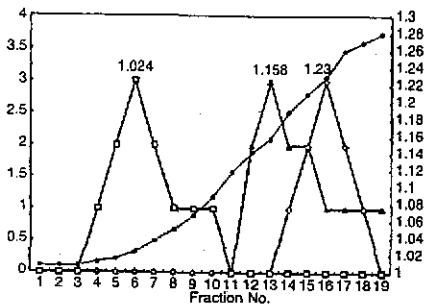

Figure 4 Sucrose density gradient analysis of KIs-V. A cocktail of plasma sample containing KIs-V, hepatitis B virus (HBV) and hepatitis C virus (HCV) was subjected to sucrose density gradient ultracentrifugation, and 19 fractions were collected. The relative amounts of the polymerase chain reaction (PCR) products for each virus are shown in a composite figure. The left axis shows the intensity of the gel band of PCR products for HBV (◇), HCV (□) and KIs-V (▲). The right axis shows sucrose density (g/mL, ●).

Although no viral proteins showed homology to the predicted genes, 10 predicted proteins showed low to high homologies to proteins encoded in bacteria or fungi. In particular, genes 10 and 13 showed high

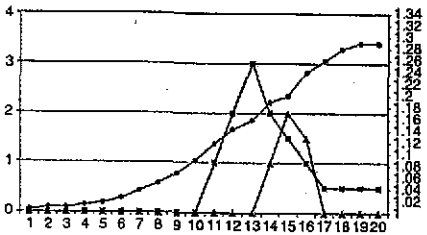

Figure 5 Sucrose density gradient analysis of detergent-treated and non-detergent-treated KIs-V. A detergent (NP-40)-treated and non-detergent-treated KIs-V sample were subjected to sucrose density gradient ultracentrifugation, and 20 fractions were collected. The relative amounts of the polymerase chain reaction (PCR) products of each sample are shown in a composite figure. The left axis shows the intensity of the gel band of PCR products for non-detergent-treated KIs-V (■) and that for detergent-treated KIs-V (▲). The right axis shows sucrose density (g/mL, ●).

Table 3 *In silico* analysis of KIs-V sequence

Gene ID	Strand	Start	End	Length (aa)	PI†	MW‡ (kD)	Best BLAST hit, Identity (%), E-value	CD-search§	TMHMM¶
1	+	76	450	124	4.76	13.4441	YP_003797582, 33%, 3.1	No hit	No hit
2	–	594	1361	255	10.57	29.2631	XP_659885, 39%, 0.42	No hit	No hit
3	+	1592	1870	92	7.81	9.1172	YP_288237, 34%, 0.28	No hit	68–88
4	+	3070	3669	199	9.92	21.2769	ZP_04094369, 26%, 0.97	No hit	No hit
5	+	3826	4293	155	10.44	18.0216	No hit	No hit	No hit
6	+	3831	6278	815	6.63	86.5631	No hit	PRK14971	No hit
7	+	5233	6054	273	11.45	32.3655	ZP_07063563, 23%, 6.0	No hit	106–124
8	+	6224	6484	86	10.93	10.3484	No hit	No hit	No hit
9	+	*6611*	*6880*	*89*	*12.81*	*10.3323*	*XP_001527475, 30%, 7.4*	*No hit*	*No hit*
10	+	6877	7872	331	9.51	36.6633	XP_567444, 40%, 6e-37	No hit	No hit
11	+	7888	8610	240	10.33	25.9111	ADV24399, 28%, 0.004	No hit	No hit
12	–	8476	8784	102	10.28	11.9779	XP_741105, 40%, 9.8	No hit	No hit
13	–	9455	2541	860	10.20	92.5745	ADV24464, 33%, 3e-67	cl02694	No hit

†Predicted isoelectric point.
‡Predicted molecular weight.
§Conserved domain search result.
¶Prediction of transmembrane domains.

homologies to hypothetical proteins encoded in *Cryptococcus*. We also searched for putative functional domains using the CD-search program.[12] Gene 6 and gene 13 showed similarities to a DNA polymerase domain (GenBank accession no. PRK14971) and the LCCL domain (GenBank accession no. cl02694), respectively. The TMHMM program predicted that two hypothetical proteins encoded by gene 3 and gene 7 harbored short transmembrane regions.[13]

We next investigated the pattern of nucleotide composition skew in the KIs-V sequence. In many circular bacterial and viral genomes, the bias of nucleotide G *relative to C is observed around the origin and terminus* of replication, which is possibly due to a strand-specific mutation rate. Previous studies have shown that in the GC skew plot, a plot of cumulative values of the bias, a valley and a peak would correspond to the replication origin and terminus of a circular genome, respectively.[14] In contrast, a single valley would be observed in a linear genome. In the KIs-V sequence, a distinct valley and a peak were found around nt 7000 and nt 2000, respectively (Fig. 6), indicating that the replication origin and the replication terminus were located around nt 7000 and nt 2000, respectively.

Using the TSSG program trained for predicting mammalian promoter sites,[15] we found that a region around the TATA box on the sense strand starting from nt 5438 had strong signals for transcription initiation. In addition, the adjacent region on the complementary strand showed weak signals for transcription initiation. This region also contained a CpG island spanning from nt 4717 to nt 5194, which contains more than 50% GC composition and more than 0.6 observed/expected CpG numbers. Another large CpG island was found in the region between nt 455 and nt 2008 with the same criteria.

KIs-V prevalence among blood donors

All four KIs-V-positive samples were found to be positive also for HEVAb by enzyme-linked immunosorbent assay although they were negative for HEV-RNA by

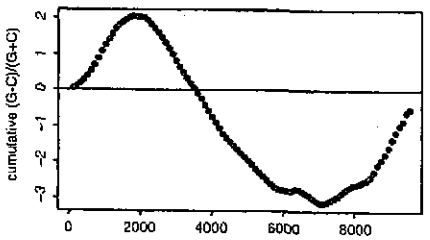

Figure 6 GC skew plot of KIs-V sequence. The cumulative scores of (G – C) / (G + C) in the 2000-bp-long window were calculated at 100-bp intervals. The center of the windows was plotted on the abscissa.

Table 4 Relationship between KIs-V, HEVAb, and ALT level

HEVAb	ALT ≤60		ALT >60	
	Negative	Positive†	Negative	Positive†
Number of samples investigated	120	196	100	100‡
Number of samples positive for KIs-V	1	0	1	36§

†All of the hepatitis E virus antibody (HEVAb)-positive samples were negative for HEV-RNA.
‡Male, 84; female, 16.
§Male, 30; female, 6.
ALT, alanine aminotransferase.

RT–PCR. Twelve other KIs-V-positive samples detected among the 500 samples using nested PCR were also HEVAb-positive. These findings prompted us to investigate the relationship between HEV and KIs-V. Five hundred and sixteen blood samples were newly obtained from blood donors and categorized into four groups by ALT level (either ≤60 or >60) and the presence or absence of HEVAb. Each of the four groups consisted of 100 or more blood samples. None of the 296 HEVAb-positive samples contained HEV-RNA. It was revealed that 36 out of 38 KIs-V-positive samples were found in the group with a high ALT level as well as positive for HEVAb (Table 4). The ALT level in the KIs-V-positive samples ranged 61–226 with the mean value being 88.3. There was no difference in male-to-female ratio between KIs-V-positive and KIs-V-negative samples.

DISCUSSION

A NOVEL DNA sequence designated KIs was detected in blood donors with ALT levels of more than 60 IU/L using primers constructed from conserved areas of RNA virus helicase. Because the consensus domain of helicase is widely shared by viruses including HCV and the GB virus,[9] we examined whether it was shared by unknown viruses associated with liver diseases, and screening by RT-PCR was carried out using primers constructed on the basis of the a.a. sequence of the helicase. The sequence obtained was 210-bp long and showed no homology with the consensus sequence of the helicase, suggesting that the sequence of the constructed primers was not inherent in the helicase. Possibly, the primer sequences with multiple positions permissive of alternative nucleotides decreased their hybridizing specificity, resulting in the coincidental acquisition of matching to a novel sequence.

Although there was seemingly no implication of RNA helicase in KIs, we concentrated on exploring its viral property. Using the primer walking method, we extended KIs to produce a sequence of 9496 nucleotides designated KIs-V. The experiments examining the effect of RT and the digestion with either DNase or RNase on the PCR yield confirmed that KIs-V is a DNA. Treatment with the restriction enzyme *SalI* or *BstXI* revealed that KIs-V is a double-stranded DNA. The RCA experiment[16] using either random hexamer primers or KIs-V-sequence-based primers yielded an excessive amount of DNA having the KIs-V sequence. Inverted PCR using the primers constructed near both terminals of the sequence produced the connecting sequence. These results indicate that KIs-V has a circular form.

It may be argued that a plasmid has similar characteristics to those described above. Direct treatment, however, of the KIs-V-positive sample with DNase did not affect the PCR yield, whereas the pretreatment with proteinase-containing extraction reagent followed by DNase digestion abrogated the PCR amplification of KIs-V. This indicates that KIs-V is present in plasma not as a nucleic acid per se but as an organism encapsulated by a protein. Moreover, the filter passage experiment indicates that KIs-V is integrated in a particle-like organism having a 30–50 nm size. It is inconceivable that a plasmid with bp of less than 10 000 has the filtration property presented here, namely, non-passage through a 0.03-μm pore filter.

The density of KIs-V was determined as 1.158 g/cm^3, indicating that KIs-V is derived from a microorganism with its inherent density. Detergent treatment of the sample increased the peak density of KIs-V, indicating that KIs-V is lipid-enveloped. As no amplification was achieved with any of the leukocyte DNA samples, KIs-V was considered not to be a sequence ubiquitously present in human hosts. All of the findings presented above indicate that KIs-V is a sequence derived from an organism, most likely a virus, having a double-stranded circular DNA as its genome in a lipid-enveloped structure.

There was no difference in the KIs-V nucleotide sequence between the four samples investigated. This may indicate that KIs-V is an agent with a very low sequence variation. KIs-V seems to be a genome belonging to a new category as there were no sequence homologies between KIs-V and any other viral genomes registered in the public databases. Our sequence analysis, however, supported the idea that KIs-V is likely a viral genome having humans as a host. First, the GC skew plot showed that KIs-V is indeed a circular DNA that has a replication origin and a terminus. Second, the human promoter prediction program found strong signatures of the mammalian promoter in the KIs-V sequence. Third, a CpG island was found near the promoter region, which is a typical feature of mammalian genomes, suggesting that KIs-V is a circular DNA using mammalian transcription machineries.

Although we identified a putative microorganism with a novel sequence from blood donor samples with high ALT levels, the ALT levels recorded among them were relatively moderate with values between 61 and 82 IU/L, which might raise a question about its relevance to hepatotoxicity. In the second screening study, however, KIs-V was identified almost exclusively among the HEVAb-positive individuals with moderately elevated ALT levels. It may be that KIs-V has a common mode of entry into humans with HEV. For example, HEV infection in developed countries is associated with the intake of underdone meat.[17,18] KIs-V may, in that sense, share the contamination spectrum in foods with HEV. On the other hand, the finding that KIs-V is frequently found among people with elevated ALT levels suggests that KIs-V has a hepatopathological nature indeed, causing liver dysfunction directly or indirectly. Because KIs-V was found among otherwise healthy blood donors, it may be either that it usually causes asymptomatic acute hepatic cell injury with a moderately elevated ALT level or that it has an etiological association with chronic asymptomatic hepatic cell dysfunction.

We have presented data that indicate the viral property of KIs-V: (i) it has a protein capsule; (ii) it is lipid-enveloped; (iii) it has a diameter between 30 and 50 nm; (iv) it has an inherent density of 1.158 g/cm^3; and (v) it has a replication origin and a terminus in the sequence with a putative mammalian transcription machineries. Despite all of these findings, it is yet to be determined whether KIs-V is indeed a viral genome. To unequivocally clarify this issue, it will be necessary to carry out infection experiments using appropriate animal models. It also has to be determined whether KIs-V causes acute hepatitis or whether KIs-V carriage leads to chronic hepatic dysfunction with a moderately elevated ALT level. It is also possible that the moderate hepatic dysfunction observed among KIs-V-positive individuals is a secondary finding during the clinical course of a KIs-V-associated illness that mainly targets organ(s) other than the liver. Studies are now in progress to collect clinical data from patients from varied clinical categories with hepatic dysfunction.

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