薬事・食品衛生審議会
平成２２年度　第４回　血液事業部会運営委員会

議事次第

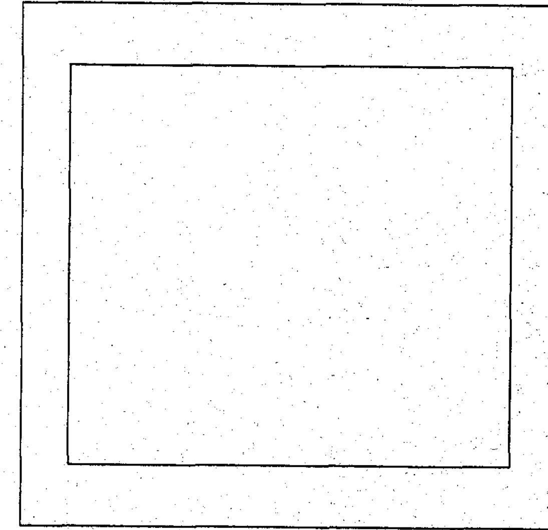
日時：平成２３年２月１８日（金）
　　　１０：００～１２：００
場所：中央合同庁舎５号館　厚生労働省
　　　専用第１２会議室（１２階）
　　　東京都千代田区霞が関１－２－２

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

配付資料：
座席表
委員名簿
資　料　１　平成２２年度第３回血液事業部会運営委員会議事要旨（案）
資　料　２　感染症定期報告について
資料３－１　供血者からの遡及調査の進捗状況について
資料３－２　血液製剤に関する報告事項について
資料３－３　献血件数及びＨＩＶ抗体・核酸増幅検査陽性件数
資　料　４　ＸＭＲＶに関する文献報告（続報）
資料５－１　献血血液の研究開発等での使用に関する指針（案）
資料５－２　献血血液の研究開発等での使用に関する指針（案）参考資料
資　料　６　英国滞在歴に関する制限緩和に伴う献血状況（報告）（日本赤十字社提出資料）
資　料　７　採血基準の改定に伴う準備状況（報告）（日本赤十字社提出資料）
資料８－１　フィブリノゲン製剤納入先医療機関の追加調査について（平成２３年１月２８日公表）
資料８－２　Ｃ型肝炎訴訟の和解について（平成２３年２月２日公表）

平成22年度第4回
薬事・食品衛生審議会薬事分科会
血液事業部会運営委員会
座席表

平成23年2月18日(金)
厚生労働省
専用第12会議室
10:00～12:00



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．佐川 公矯 （さがわ　きみたか）
　　久留米大学医学部付属病院臨床検査部教授

4．花井 十伍 （はない　じゅうご）
　　ネットワーク医療と人権理事

5．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

6．山口 照英 （やまぐち　てるひで）
　　（独）医薬品医療機器総合機構　生物系審査第一部
　　テクニカルエキスパート

（50音順、敬称略）

資料１

平成22年度第3回血液事業部会運営委員会議事要旨

日時：平成22年11月24日（水） 16:00～18:00
場所：中央合同庁舎5号館 厚生労働省 専用第12会議室
出席者：佐川委員長、大平、岡田、半田、山口各委員
（事務局）
三宅血液対策課長、安田血液対策企画官、難波江課長補佐
（採血事業者）
日本赤十字社血液事業本部 田所経営会議委員、日野副本部長、五十嵐臨床開発課長
（参考人）
倉恒関西福祉科学大学教授

議 題：
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2. 感染症定期報告について
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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

事務局及び日赤から、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後、下記のような意見が出された。

（血液製剤に関する報告事項関係）
○ 血液事業の体制がしっかりしてきたので、逆に血液以外の原因ではないかという症例が増えてきているということで、次は医療機関等での対策の充実が必要かと思う。

（献血件数及びHIV抗体・核酸増幅検査陽性件数関係）
○ HIV の保健所等の検査について、何かあったから充実するというのではなく、ベースラインの検査体制を各自治体はきっちり充実してほしい。
○ 検査目的の献血をしないよう献血に対する理解を一般の人たちに広く訴えかけて、献血からの感染リスクを減らしていく試みを続けていただきたい。

議題4及び議題5について

（慢性疲労症候群に対する献血制限の実施について）
岡田委員から「ＸＭＲＶに関する文献報告（続報）」について、事務局から「諸外国における慢性疲労症候群罹患者に対する献血制限について」、倉恒参考人から「日本における慢性疲労症候群について」説明後、日本における慢性疲労症候群の患者の方に対する献血制限について審議が行われ、以下のような結論が得られた。

① XMRV と慢性疲労症候群との関連性については、肯定する論文、否定する論文が出されており、未だ不明であること、
② 日本において、慢性疲労症候群の患者 100 名の血液を検査したところ、いずれからも XMRV が検出されなかったこと、
③ 献血は、健康でなければできないため、現在、慢性疲労症候群の症状がある方については実質的に献血制限がなされていること、
④ 既往歴まで含めた献血制限を実施した場合、患者及び家族への社会的な影響が及ぶ可能性があり、より慎重な対応が必要であること、

以上より、現時点では、献血者一人ひとりについて既往歴まで遡っての献血制限は実施せず、研究の動向を注視するとともに、新たな知見が得られた場合は運営委員会に報告すること。

（研究開発等における血液製剤の使用に関する指針の策定関係）
事務局から、「研究開発等における血液製剤の使用に関する指針の策定」について報告があり、下記のような意見が出され、次回までに事務局で指針の肉付けしたものを提出することとされた。
○ 期限内の血液を民間企業が研究開発のために使うということになると、今までの献血者が献血する動機づけの中で全くなかった新しいことであり、献血の枠組みそのものに抵触する話なので、かなり議論が必要ではないか。
○ 公衆衛生の向上のために、献血血液をどのように使っていくかということについて、テーマとして掲げてきちんと議論すれば、献血者の方たちに十分説明することで理解を得られるのではないかと考えている。

○ 疫学調査について、リスク評価をするための資料づくりとして、献血率が必要となるが、その際、輸血の安全性、有効性向上のためということで、ひとからげで了解を得られればいいが、新しい病原体が次々に出てきたときに、その度に献血者の了解をとることは不可能なので、リスク評価のための疫学調査については、血液の安全性向上のためということで、毎回了解を得なくてもできるようなシステムを盛り込んでほしい。(なお、現時点において、献血血液を輸血の有効性、安全性向上のために使用することについては、献血のときに了解をいただいていることを事務局から説明した。)

(血小板製剤に対する感染性因子低減化技術関係)

日赤から「血小板製剤に対する感染性因子低減化（不活化）技術の導入準備について」「血小板製剤に対する感染症因子低減化血小板の臨床試験の概要」及び「感染因子低減化技術導入に係る費用対効果分析の報告」について、事務局から「FDAプレゼンテーション」について報告がなされた。

(改定問診票関係)

日赤から「改定問診票」について報告がなされた。

(フィブリノゲン関係)

事務局から、フィブリノゲン製剤及び血液凝固因子製剤に関する公表等について報告がなされた。

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

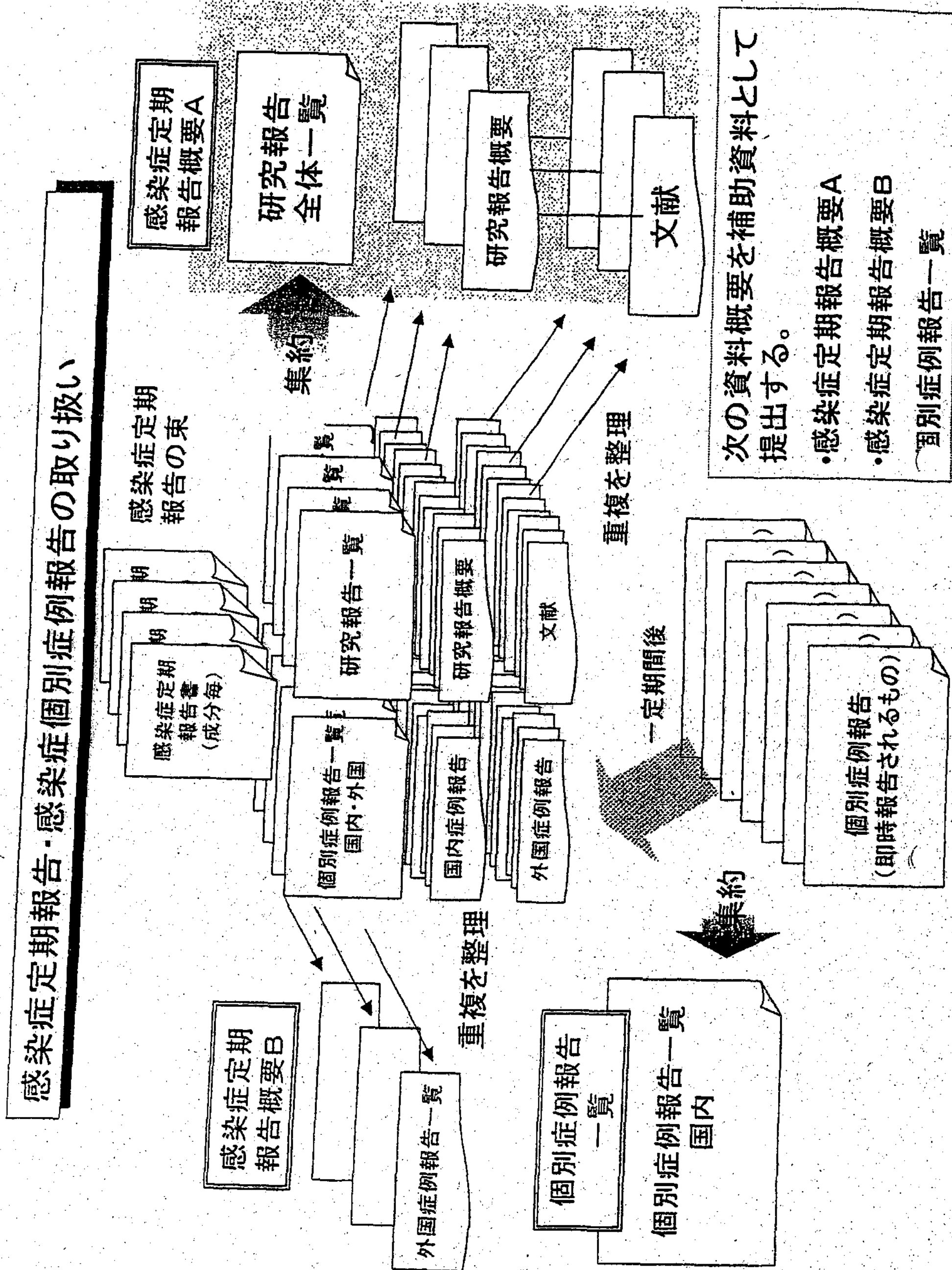
平成１６年度第５回
運営委員会確認事項
（平成１６年９月１７日）

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２３年２月１８日）

平成２２年９月１日受理分以降

Ａ　研究報告概要
Ｂ　個別症例報告概要

Ａ　研究報告概要

○　一覧表（感染症種類毎）
○　感染症毎の主要研究報告概要
○　研究報告写

研究報告のまとめ方について

１　平成２２年９月１日以降に報告された感染症定期報告に含まれる研究報告（論文等）について、重複している分を除いた報告概要一覧表を作成した。

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

感染症定期報告の報告状況(2010/9/1～2010/11/30)

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
100214	2010/9/29	100547	B型肝炎	J Infect Dis. 2010 Jul 15;202(2):192-201.	広範なB型肝炎ワクチン接種後の、米国におけるHBV感染の状況について傾向について検討された。1999-2006年と1988-1994年の2期間、米国健康・栄養調査において6歳以上を対象に、HBc抗体、HBs抗原及びHBs抗体の検査が実施された。罹患率の概算は加重及び年齢調整された。その結果、1999-2006年間の、年齢調整後のHBc抗体(4.7%)とHBs抗原(0.27%)の罹患率は、1988-1994年(各5.4%及び0.38%)であり、統計学的に違いはなかった。2期間のHBc抗体の罹患率は、6-19歳(1.9%から0.6%)、及び20-49歳(5.9%から4.6%)では減少したが、50歳以上では(7.2%及び7.7%)変化がなかった。1999-2006年のHBc抗体の罹患率は、非ラテンアメリカ系白人(2.8%)やメキシコ系アメリカ人(2.9%)より、非ラテンアメリカ系黒人(12.2%)と他の人種(13.3%)で高く、また米国出生(3.5%)より外国出生(12.2%)の方が高かった。米国出生の6-19歳(0.5%)では、人種や民族性による違いがなかった。米国出生と外国出生の子供では1988-1994年(1.0%対12.8%)より、1999-2006年(0.5%対2.0%)の方が小さかった。また、6-19歳では、56.7%がワクチンによる獲得免疫を持っていた。米国の子供におけるHBV罹患率の減少から、世界的及び国内のワクチン接種の効果が反映されているが、一方で、成人におけるHBV罹患率の状況はほとんど変わらず、およそ73万人(95%信頼区間、55万-94万人)の米国在住者は慢性的に感染していると説明している。	1
100214	2010/9/29	100547	B型肝炎	Pediatr Infect Dis J. 2010 May;29(5):465-7.	慢性B型肝炎患児の唾液中のB型肝炎ウイルス(HBV)の水平感染が伝播手段となっている可能性を検討するために、慢性B型肝炎患児を対象に唾液中及び血漿中のHBV量の関連性が検討された。デンマークにおいて2006年5月から2008年11月間で、慢性B型肝炎患児(0～16歳)46人由来の唾液と血液中のHBV-DNAをTaqManPCR法 にて定量した(検出感度は50 IU/mL)。その結果、本研究中にHBe抗原が陽性から陰性になった2人と、HBe抗原の状態が分からない1人を調査対象外とした25人(58%)がHBe抗原陽性で、18人(42%)がHBe抗原陰性であった。HBe抗原陽性の唾液中のHBV-DNA濃度は、HBe抗原陰性の血漿中のHBV-DNA濃度より高かった(39倍)。唾液がHBVの伝播手段になっていることが示唆された。	2
100214	2010/9/29	100547	B型肝炎	Transfusion. 2010 Jan;50(1):65-74. Epub 2009 Aug 26.	台湾において微量のB型肝炎ウイルス(HBV)DNAを検出目的とした個別検査とミニプール検査の有効性について報告された。台湾では、財政的な問題でルーチンの血液スクリーニングとしてNATの実施が制約されている。そこで、Ultrio分析(HBV、HCV、HIV)を用いて、実施可能な検査として個別供血検査(IDT)及び4本のミニプール(MP4)の実施成績を評価した。供血者10,290名(IDT 4210名、MP4 6080名)を対象に潜在的HBV陽性供血者(HBs抗原陰性/NAT陽性)を最高9カ月間、追跡調査した。Ultrio分析とHBs抗原検査結果が不一致の場合、さらにHBV抗体血清検査、代替NAT、HBV DNA定量検査ならびに塩基配列決定の解析を行った。その結果、再検査率は、IDT 0.55%とMP4 0.33%であった。HIVまたはHCV陽性症例は認められなかったが、潜在的HBV陽性例は12名(IDT 9名、MP4 3名)であった。そのうちの11名は、genotypeがB2であることが判明した。そのうちの10名は、追跡調査のために再来院し、ほとんどが潜在的HBV感染症(OBI)であると判明した。IDTの陽性率 9/4210(0.21%)はMP4の3/6080(0.05%)と比べ高いことから、台湾のようにOBIキャリアが多い地域においては、より高感度のNAT法で検査を実施することが有益であると説明している。	3
100214	2010/9/29	100547	B型肝炎	第46回日本肝臓学会総会; 2010 May 27-28; 山形	小児B型肝炎ウイルス(HBV)キャリア患者の感染経路・感染要因を解析し、現在のHBV感染予防対策の問題点について検討された。 施設1では32例、施設2では133例、施設3では22例の合計187例のHBVキャリアにおいて、男女比は1.43:1、診断時年齢は中央値2歳(0ヶ月～15歳)であった。1985年までに出生していた症例は102例で、母児感染59例(57.8%)、父子感染6例(5.9%)、輸血5例(4.9%)、水平感染31例(30.4%)、不明1例で母児感染が過半数を占めていた。一方、母児感染予防処置が導入された1986年以降に出生した症例は85例で、母児感染51例(60%)、父子感染13例(15.3%)、輸血2例(2.4%)、水平感染19例(22.4%)であった。母児感染の割合は1985年までに出生していた症例と変化なく、父子感染は増加した。母児感染のうち胎内感染が16例、予防処置実施中あるいは実施後にHBV感染が判明した症例が22例で、現在の予防法で防ぐことができなかった症例が合計38例(74.5%)であったが、予防処置の不完全施行や未施行によるものが8例(15.7%)存在した。父子感染や水平感染の症例でHBワクチンの投与症例はいなかった。HBV母児感染予防処置導入後も小児のHBVキャリアは発生している。母児感染のうち約15%は予防処置の不完全施行や未施行が原因であり、医療者の啓発を行うとともに、予防処置プロトコールを簡略な国際方式にすることにより完遂率が高まると思われる。また、父子感染・輸血を含めた水平感染例も4割を占めており、諸外国のように日本でも出生後早期にHBユニバーサルワクチンが導入されることが望まれる。胎内感染例については出生後の予防処置では防ぐことができず、HBVキャリア妊婦へのHBIGや抗ウイルス剤投与などを行うべきか、今後検討していく必要があると説明している。	4
100214	2010/9/29	100547	B型肝炎	第58回日本輸血・細胞治療学会総会; 2010 May 28-30; 愛知	2009年に全国の医療機関から報告された輸血関連感染症例(疑い例を含む)の解析結果と医療機関における「血液製剤等に係る遡及調査ガイドライン」(以下GL)に基づいた輸血前後の患者検体の検査実施状況等について報告された。2009年に医療機関より報告された症例を対象とし、献血者検体(献血者の保管検体等の個別NAT、当該製剤(使用済みバッグ)等の無菌試験等)と患者検体の調査により輸血との因果関係を評価した。また、医療機関における患者の輸血前後の検査の実施項目等を2007、2008年時と比較した。 その結果、10月末現在の報告数は82例(HBV 37例、HCV 21例、 細菌 20例、パルボB19 2例、HEV 1例、CMV 1例)であり、輸血との因果関係が高いと評価した症例は、HBV 5例、HEV 1例、及び細菌 1例であった。医療機関でのGLに基づく輸血前後の患者検体の検査実施数(輸血前:HBs抗原/HBs抗体/HBc抗体、輸血後:HBV-DNA)はHBV症例で2007年6例(8%)、2008年12例(20%)、2009年9例(24%)であった。またHCV症例では(輸血前:HCV-RNA or HCVコア抗原/HCV抗体, 輸血後:HCV-RNA or HCVコア抗原)2007年12例(29%)、2008年5例(12%)、2009年5例(24%)であった。細菌症例での医療機関における患者血培の実施数は、2007年27例(90%)、2008年43例(94%)、2009年20例(100%)であった。また、医療機関からの使用済みバッグの提供が2007年17例(57%)、2008年35例(76%)、2009年17例(85%)であった。以上よりGLが医療機関に浸透していることが推察された。	5

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100214	2010/9/29	100547	B型肝炎	第58回日本輸血・細胞治療学会総会; 2010 May 28-30; 愛知	スクリーニングNATのプール数の縮小効果について検討された。日本赤十字社では血液製剤等のHBV、HCV、HIVへの安全対策として1999年7月にプール検体(500本)によるスクリーニングNAT(AMP-NAT)を開始した。その後、プール検体数を50本、20本へと縮小し、2008年8月から検出感度向上を目的に新NATシステム(Taq-NAT)を導入した。2000年1月から2009年10月までの感染症報告症例のうち、輸血による感染を直接証明できた症例はHBV 91件、HCV 3件、HIV 1件であった。この原因となった輸血用血液の献血血液それぞれ 87献血、3献血、1献血を対象にし、当該献血時のスクリーニングNATをプール検体数別・試薬別に分類した。その結果HBV・HCV・HIV別に、50本プール前は8・0・0、 50本プール/AMP-NAT(2000年2月-2004年7月:4.5年間)は46・2・1、 20本プール/AMP-NAT(2004年8月-2008年7月:4年間)は30・1・0、20本プール/Taq-NAT(2008年8月-2009年10月:1.25年間)は3・0・0 であった。ウイルス増殖スピードの遅いHBVについて、プール検体数の縮小・試薬の検出感度向上により、輸血感染HBVの減少傾向が認められた。一方、ウイルス増殖スピードの速いHCV、HIVはスクリーニングNAT導入後約10年が経過した中で輸血感染HCVが3件、輸血感染HIVが1件と、NATの導入自体に効果があったことが推測された。2008年8月から導入された新NATシステムにより、更なる安全性向上に努めているところである。	6
100214	2010/9/29	100547	A型肝炎	www.47news.jp/CN/201004	国立感染研究所により、A型肝炎の患者が平成22年3月以降に増加していることが報告された。A型肝炎ウイルスに汚染した水や食材の摂取によって感染する可能性を懸念して、魚介類の十分な加熱など、注意を呼びかけている。4月18日までの合計(速報値)は121人で昨年の報告数115人を超えている。11日までの5週間の81人について、年齢は20～88歳、2例が劇症化し、うち1例が死亡した。福岡県、広島県などが多く、報告医師が推定した原因食材は「カキ」が45%と最も多かった。	7
100209	2010/9/28	100530	E型肝炎	Journal of Medical Virology 2010;82(1):69-76	感染動態を調査するために、HEVに自然感染した2匹の国産妊娠ブタの各同産仔(A群及びB群)を生後6か月まで研究した。母子移行IgG及びIgA抗体はA群から検出されたが、B群からは検出されなかった。生後30-110日において、全群の糞便からHEVが検出され、17匹については、生後40-100日にウイルス血症が出現した。系統発生分析によって、全群にHEV遺伝子型3に非常に近い塩基配列であることが示された。特異的なIgG及びIgAの血清レベルは、IgAが糞便で検出されなかったが、全群で同様であった。ウイルス血症と抗体陽転の開始は、A群で有意に遅れていた。糞便に排出されたウイルスの動態は両群で同様であった。感染動態の違いから、母性抗体がウイルス血症と抗体陽転開始を遅延させることが示唆された。定量的リアルタイムPCR解析の結果、糞便中のHEV RNAは約10^6copies/gであり、最初の排泄から10日後にHEV RNAのコピー数はピークに達することが明らかとなった。生後200日で、HEV RNAは13匹中3匹の内臓から検出された。ブタでのHEV自然感染について時間的経過を追った当該研究結果は、ブタからヒトへ感染する際のHEVの動態を理解するのに役立つであろう。	8
100206	2010/9/28	100527	パルボウイルス	Journal of General Virology 2010;91(2);541-544	パルボウイルスPARV4は、ヒト宿主のパルボウイルス科の種類として最近報告されたウイルスである。B型肝炎、C型肝炎あるいはHIV感染患者等の様々な集団由来の血漿、血清及び全血を用いて、定量PCR法により血中のPARV4の検出率が検討された。その結果、8検体がPARV4陽性であり、うち1検体は高コピー数を示した。高力価の血清は約5×10^8genome equivalents/mLであった。間接免疫蛍光法によって、PARV4抗体陽性が同定された2患者の血清を用いて、血清中の天然(native)PARV4を免疫電子顕微鏡下で可視化したところ、1患者由来の血清においてPARV4粒子が観察された。天然(native)のPARV4の可視化は、初めてのことである。	9

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100214	2010/9/29	100547	パルボウイルス	XXXIst International Congress of the ISBT;2010 Jun 26-July 1; Berlin,Germany	ヒトパルボウイルスB19(B19)DNA陽性血液製剤の感染性について検討された。2000年以降、ドイツのウルム研究所では、供血6～8週間後(血液製剤供給後)について、B19に対する供血者NATスクリーニングを実施している。レトロスペクティブな研究において、受血者を次の2群に分け(A群:B19ウイルス量≦10^5IU/mLの血液製剤受血者、B群:B19ウイルス量＞10^5IU/mLの血液製剤受血者)VP-1uゲノム領域の系統発生解析をB19DNA陽性供血者と受血者に対して行い、全ての試料に対して、IgM、IgG抗体を調べた。その結果、B19 DNAはB群の赤血球濃厚液受血者18名中9名に検出されたが、A群の受血者16名にはB19 DNAは検出されなかった。系統発生解析では、供血者と受血者間で同一ゲノム配列を示した。血液製剤によるB19伝播は、ウイルス濃度と中和抗体価に相関することが示唆されると説明している。	10
100214	2010/9/29	100547	ウイルス感染	Emerging Infectious Diseases 2010; 16 (5); 856-858 May 2010	2009年8月にテキサス州ダラスで採取した、ヒトスジシマカにおけるLa Crosseウイルス(LACV)について報告された。LACVは主にAedes triseriatusが媒介する、北アメリカでの小児脳炎の主要な原因である。しかし近年、LACV脳炎が南東部地域で増加し、南部でも報告されている。同時にアジアからの外来種であるヒトスジシマカが増加しているが、今までヒトスジシマカとLACV伝播の関連は不明であった。今回の調査で、テキサス州ダラスで採取したヒトスジシマカからLACVが検出され、これまで流行が確認されていた範囲外で、外来性の蚊に当該ウイルスが認められた。	11
100214	2010/9/29	100547	ウエストナイルウイルス	CDC/MMWR 2010 July 2	2009年の米国におけるウエストナイルウイルス(WNV)の流行状況について、米国疾病管理予防センター(CDC)が発表した。米国の38州の262郡と、コロンビア特別区から720症例のWNV感染症が報告された。そのうち386例(54%)が神経侵襲性疾患で、334例(46%)が非神経侵襲性疾患であった。WNV感染症での死亡者は全部で33人が報告され、そのうち32人が神経侵襲性疾患であった。神経侵襲性疾患のうち229例(59%)が脳炎、117例(30%)が髄膜炎、40例(10%)が急性弛緩性麻痺であった。急性弛緩性麻痺40例のうち、27例(68%)が脳炎または髄膜炎を併発した。WNVによる疾病を制御する上で、調査の継続、蚊の管理、蚊に対する防御用具、及び更に予防戦略を検討することが必要である。	12
100214	2010/9/29	100547	レトロウイルス(XMRV)	ABC Newsletter #15.2010 Apr 23;15	ニュージーランドの血液バンクでは慢性疲労症候群(CFS)の既往を持つ供血者の供血延期を開始し、オーストラリア当局は、供血ガイドラインの見直しを行っている。 ニュージーランドの決定は、前立腺癌と関連性があるXMRVが、健康集団と比較してCFS患者の血中に非常に多く認められたという調査を受けてなされた。他の科学者は、この結果を確認することができなかったが、米国保健当局は、CFSとXMRV間の関連の可能性について調査を行っており、カナダ血液サービスはすでにCFSの診断を受けた供血者からの供血を無期限延期としている。一方、オーストラリア赤十字血液サービスは、独自にリスク分析を行い、完全に回復するまでのCFS患者からの供血を延期することを現行のガイドラインで求めている。	13
100230	2010/10/26	100654	レトロウイルス(XMRV)	www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm223277.html	米国食品医薬品局生物製剤評価・研究センター及び米国国立衛生研究所臨床センターの研究者は、慢性疲労症候群(CFS)と診断された患者37例と健康血液ドナー44例由来の血液試料において、CFS患者由来の32例(87%)及び健康血液ドナー由来の3例(7%)に複数の異なるマウス白血病ウイルス(MLV)遺伝子配列を同定した。当該研究はMLV様ウイルスの遺伝的変異体であるXMRVがCFS患者の血液中に存在するとの過去の研究報告を支持し、CFSの診断と血液中のMLV様ウイルス遺伝子配列の存在との間に強い関連性があることを示している。さらにごく一部の健康血液ドナーにおいてMLV様ウイルス遺伝子配列が検出されている。CFSとの統計的な関連は強いものの、当該研究でレトロウイルスがCFSの原因であることが証明されたわけではない。	14

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100214	2010/9/29	100547	リフトバレー熱	ProMED-mail 20100513.1557	2010年5月10日の時点で南アフリカ保健省は、18人の死者を含む186人のリフトバレー熱(RVF)症例を報告している。主要な感染経路は、感染した家畜の血液や組織に触れることであるが、蚊に刺されることも感染原因となる。世界保健機関(WHO)は、南アフリカへの旅行に対して規制の勧告は行っていないが、特に農場や動物保護区に行く者は、動物組織や血液との接触を避け、未殺菌、非加熱ミルクや生肉の摂取をしないことを勧めている。全旅行者に対し、長袖長ズボンの着用や防虫剤、蚊帳を使用するなどして、蚊や吸血昆虫に刺されないよう注意を呼びかけている。また、ドイツ保健当局は、南アフリカ旅行から帰国したドイツ人の予備的診断ではRVFであったが、その後の追加検査により、この症例はRVFではなくリケッチア感染であったと報告した。	15
100236	2010/10/27	100663	Q熱	Clinical Infectious Diseases 50(11) 1433-1438 2010	2005年6月28日、イスラエル中央部の都市部で、全寮制高校の生徒及び職員の322名において多数の熱性疾患(発熱、頭痛、発汗など)症例が報告された。その後の調査で、その2週間前に大規模なQ熱アウトブレイクが発生していたことが分かった。Q熱疾患の危険因子特定するため、症例対照研究が実施された。2005年6月15日～7月13日の間に、303名中187名(62%)が体調不良の報告をしており、血清学的検査を実施した164名中144名(88%)に、*C.burnetii*感染が明らかとなった。Q熱感染の重大な危険因子は、学生であること、学校の食堂で定期的に食事をしたこと、6月の宗教上の休日期間並びにその前の週末に寮にいたことであった。PCR法により学食の空調から*C.burnetii*DNAが検出され、空調を介して病原体に空気感染したことが示唆された。	16
100236	2010/10/27	100663	アメリカ・トリパノソーマ症	J Infect Dis 201(9) 1308-1315 2010	Trypanosoma cruzi(T.cruzi)は媒介動物の糞便によって汚染された食物から経口感染する。アメリカ大陸での急性シャーガス病CDのアウトブレイク時において、ベネズエラでコホート疫学研究が実施された。曝露された1000名中103名に感染が確認され、そのうち75%に症状が認められ、20.3%が入院を必要とした。また59%は心電図異常を示し、44名(子供1名)に寄生虫血症が認められた。臨床的な特徴は媒介による感染で見られるものと異なっていた。子供は感染率が有意に高かった。疫学的な調査から、汚染した生グアバジュースが唯一の感染原因とされた。当該アウトブレイクは大都市部で主に若年齢を中心とした健康に問題のない集団における感染という、先例のない珍しいものであった。	17
100254	2010/10/28	100688	細菌感染	Agence France-Presse 2010/08/14	ベルギーにおいて、南アジア起源の薬剤耐性のsuperbugによる死亡1例が報告された。当該症例は、パキスタンを旅行中、交通事故にて脚を負傷し、パキスタンの病院で治療をうけ、ベルギーに帰国後死亡した。新規に同定されたNew Delhi metallo-β-lactamase 1(NDM-1)遺伝子を有する細菌に感染していた。2症例目はモンテネグロを旅行中に事故に遭い、入院後感染したが、ベルギーで治療をうけ回復した。	18
100202	2010/9/15	100453	細菌感染	The Lancet Infectious Diseases 10(9); 597-602; 2010 September	NDM-1(New Delhi metallo-β-lactamase 1)に起因するcarbapenem耐性腸内細菌(G(-))が問題となっており、インド、パキスタンおよび英国における多剤耐性腸内細菌におけるNDM-1の検出率を調査した。NDM-1が存在する分離株はChennaiで44、Haryanaで26、英国で37およびインドおよびパキスタンでは73株が分離された。NDM-1は大腸菌(36株)および肺炎桿菌(111株)で広く認められ、tigecyclineおよびcolistinを除くすべての抗生剤に強い耐性を示した。NDM-1陽性である英国人の多くは、一年以内にインドもしくはパキスタンに渡航歴があり、もしくは関連があった。	19
100202	2010/9/15	100453	細菌感染	毎日新聞 2010年8月17日	インド・パキスタンが発生源とみられ、ほとんどの抗生物質が効かない新種の細菌感染患者が欧州などで増えており、ベルギーでは2010年6月に最初とみられる死者が確認された。欧州メディアによると、英・仏・ベルギー・オランダ・独・米・カナダ・豪で感染が確認され、更なる拡大の恐れがある。Lancetの最新号に、特定の抗生物質を分解する酵素「NDM1」を作り出す遺伝子を持ち、ほとんどすべての抗生物質に対して耐性を持つ細菌について報告がある。	20

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
100236	2010/10/27	100663	真菌感染	PLoS Pathogens 6(4); e1000850; 2010 April	Cryptococcus gattiiは、従来、熱帯・亜熱帯性真菌と考えられていたが、1999年にカナダ、バンクーバー島で大流行し、現在においても隣接するカナダ本土ブリティッシュコロンビアや米国本土においてヒトや動物に感染し続けている。この大流行はVGII型、特にVGIIa/majorが原因であったが、加えて、オレゴンで新しい遺伝子型(VGIIc型)が出現した。MLST及びVNTR解析によって、新型VGIIc及びVGIIa/majorは、マクロファージやマウスに感染し、強毒性を示すことが分かった。	21
100263	2010/11/29	100734	クロイツフェルト・ヤコブ病	ANN NEUROL 2010;68:162-172	新規の孤発性プリオン蛋白質疾患の特性解析について報告された。プロテアーゼ感受性プリオン(PSPr)の新規の2遺伝子型、メチオニン同型接合(129MM)とメチオニン/バリン異型接合(129MV)が報告された。2008年に報告された新規のプリオン病の被験者11人はプリオン蛋白質(PrP)遺伝子のコドン129が全員バリン同型接合(129VV)であった。129MM、129MV、129VVの被験者15人について、罹患期間(22～45カ月)は129VVと129MVの被験者で有意に異なった。PrP電気泳動プロファイルと共に他のほとんどの機能は同様であったが、主な違いは疾患関連PrPのプロテアーゼ消化の感受性であり、129VVは感受性が高いが、129MVと129MMでは低いか、あるいは全くない。この違いにより可変プロテアーゼ感受性プリオン症(VPSPr)と呼ばれるようになった。被験者のPrP遺伝子コドン領域に変異はなかった。3つの129遺伝子型が全て関係し、区別でき、表現型として関係するので、VPSPrは2番目の孤発性プリオン蛋白質疾患になる。この特徴は1920年に報告したクロイツフェルトヤコブ病に似ていた。しかし、VPSPrは異常プリオン蛋白質の特性において典型的なプリオン病と異なり、恐らくゲルストマン・ストロイスラー・シャインカー疾患の亜型と類似している。	22
100206	2010/9/28	100527	異型クロイツフェルト・ヤコブ病	European Medicines Agency 2010/07/24	2003年2月に公表され、2004年6月に改訂されたクロイツフェルト・ヤコブ病と、血漿由来医薬品及び尿由来医薬品についてのCPMPの見解(EMEA/CPMP/BWP/2879/-02)の第2改訂版(案)であり、2010年9月30日まで意見を公募している。累積した疫学的エビデンスは、血液成分あるいは血漿由来製品による孤発性・家族性・医原性CJD感染を支持していない。ドナーの孤発性・家族性・医原性CJDが供血後に確認された場合、血漿由来製品の回収は妥当でないという以前からのCHMPの方針に変更はない。尿由来製品についてCJD、vCJDが感染したという疫学的なエビデンスはない。予防的措置として採血と同じドナーの選択基準を適用する。	23
100259	2010/11/25	100725	異型クロイツフェルト・ヤコブ病	Haemophilia 2010;16,305-315	英国の血液製剤による感染と遺伝性出血性疾患患者における英国の血液製剤による影響のリスクを低減する為の対策について報告された。vCJDの発生後、感染及び二次感染拡大のリスクを最小限に抑えるため、2004年に供血後にvCJDを発症したドナーから採取された血漿を含んでいるかどうかに関わらず、1980年から2001年までの間に英国でプールされた血液凝固因子製剤を投与された患者全員に予防措置が実施された。以降、英国におけるvCJDの新規症例は減少し、過去に関係する血液または血液製剤の投与を受けたvCJD患者は見つかっていない。しかし一般母集団における無症候性vCJD感染の有病率は不明であり、適切かつ有効なvCJDのスクリーニング試験はない。血友病患者において最近確認された1例目の無症候性のvCJD感染症例ならびにメチオニン/バリン異型接合患者におけるvCJD報告は、遺伝性出血性疾患患者を含む「リスクのある」集団において、継続調査が必要であることを示している。	24
100230	2010/10/26	100654	異型クロイツフェルト・ヤコブ病	Transfusion. 2010 May;50(5):1003-1006.	現在までに、後に変異型クロイツフェルト・ヤコブ病(vCJD)を発症した患者からの輸血によるvCJD感染例が4例報告されている。共通の供血者から輸血された可能性が示唆された症例は2例(症例A及びB)であった。症例Aは1989年に新生児特別治療室で4回の輸血歴があり、2006年、vCJDと診断されて6カ月後の18歳で死亡。症例Bは1993年6月と10月に2回の輸血歴があり、1998年にvCJDを発症し、41歳で死亡。合計103名の供血者の血液に曝露していた。症例Aと症例Bがいた両病院は同じ血液センターから供血血液の配給を受けていた。症例Bが曝露していた供血者103名中99名が症例Aへ輸血された後も、20年以上生存している。残りの4名はCJD以外の要因で死亡していた。vCJDを発症していない供血者から輸血を受けた症例AとBの2症例がvCJDを発症したことから、vCJD感染のパターンとして食事を通してBSEに感染した可能性も考えられる。	25

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
			2010. 7. 21	該当なし	
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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

○ワクチン接種世代の米国におけるB型肝炎ウイルス(HBV)感染の状況
目的:広範なB型肝炎ワクチン接種後の、米国におけるHBV感染の状況について傾向を評価すること。
方法:HBV感染と免疫の状況を調べるため、1999-2006年と1988-1994年の期間、米国健康・栄養調査の6歳以上の参加者で、HBc抗体、HBs抗原及びHBs抗体を検査した。罹患率の概算は加重及び年齢調整された。
結果:1999-2006年の期間中の、年齢調整後のHBc抗体(4.7%)とHBs抗原(0.27%)の保有率は、1988-1994年(それぞれ5.4%及び0.38%)と統計学的に違いはなかった。HBc抗体の保有率は、6-19歳(1.9%→0.6%;P<0.01)及び20-49歳(5.9%→4.6%;P<0.05)の間で減少したが、50歳以上(7.2%対7.7%)では変化がなかった。1999-2006年では、HBc抗体の保有率は、非ラテンアメリカ系白人(2.8%)やメキシコ系アメリカ人(2.9%)より、非ラテンアメリカ系黒人(12.2%)と他の人種(13.3%)で高く、そして、米国生まれ(3.5%)より外国生まれ(12.2%)の方が高かった。米国生まれの6-19歳の子供(0.5%)では、人種や民族性による違いがなかった。米国生まれと外国生まれの子供の相違は1988-1994年(1.0%対12.8%)より、1999-2006年(0.5%対2.0%)の方が小さかった。また、6-19歳では、56.7%がワクチンによる獲得免疫を持っていた。
結論:HBVの罹患率は米国の子供で減少した。それは世界的及び国内のワクチン接種の効果を反映している。しかし、成人の状況はほとんど変わらず、およそ730万人(95%信頼区間、550万-940万人)の米国在住者は慢性的に感染している。

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

広範なB型肝炎ワクチン接種後の米国におけるB型肝炎ウイルス罹患率を評価したところ、子供で罹患率が減少しており、ワクチン接種の効果を反映していることが分かったとの報告である。
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。

今後の対応

これまでの使用実績やバリデーション成績に鑑み本製剤の安全性は確保されており、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。なお、日本赤十字社では献血時のスクリーニング法としてより感度の高い化学発光酵素免疫測定法(CLEIA)および新NATシステムを導入した。

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MAJOR ARTICLE

The Prevalence of Hepatitis B Virus Infection in the United States in the Era of Vaccination

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Background. Our objective was to assess trends in the prevalence of hepatitis B virus (HBV) infection in the United States after widespread hepatitis B vaccination.
Methods. The prevalence of HBV infection and immunity was determined in a representative sample of the US population for the periods 1999–2006 and 1988–1994. National Health and Nutrition Examination Surveys participants ≥6 years of age were tested for antibody to hepatitis B core antigen (anti-HBc), hepatitis B surface antigen (HBsAg), and antibody to hepatitis B surface antigen (anti-HBs). Prevalence estimates were weighted and age-adjusted.
Results. During the period 1999–2006, age-adjusted prevalences of anti-HBc (4.7%) and HBsAg (0.27%) were not statistically different from what they were during 1988–1994 (5.4% and 0.38%, respectively). The prevalence of anti-HBc decreased among persons 6–19 years of age (from 1.9% to 0.6%; $P<.01$) and 20–49 years of age (from 5.9% to 4.6%; $P<.05$) but not among persons ≥50 years of age (7.2% vs 7.7%). During 1999–2006, the prevalence of anti-HBc was higher among non-Hispanic blacks (12.2%) and persons of "Other" race (13.3%) than it was among non-Hispanic whites (2.8%) or Mexican Americans (2.9%), and it was higher among foreign-born participants (12.2%) than it was among US-born participants (3.5%). Prevalence among US-born children 6–19 years of age (0.5%) did not differ by race or ethnicity. Disparities between US-born and foreign-born children were smaller during 1999–1996 (0.5% vs 2.0%) than during 1988–1994 (1.0% vs 12.8%). Among children 6–19 years of age, 56.7% had markers of vaccine-induced immunity.
Conclusions. HBV prevalence decreased among US children, which reflected the impact of global and domestic vaccination, but it changed little among adults, and ~730,000 US residents (95% confidence interval, 550,000–940,000) are chronically infected.

Hepatitis B virus (HBV) is a bloodborne and sexually transmitted virus. Each year, ~600,000 HBV-related deaths occur worldwide [1, 2], most of which result from the chronic sequelae of HBV infection [3–5]. Approximately 25% of persons who become chronically infected during childhood and ~15% of those who become chronically infected after childhood die from cirrhosis or liver cancer [2]. In the United States, before hepatitis B vaccines were licensed in 1982, 200,000–300,000 persons each year became infected with HBV [6]. Hepatitis B vaccination is the most effective measure to prevent HBV infection and its consequences. However, for persons already infected with HBV, antiviral agents are available that may prevent the serious sequelae of chronic liver disease, which highlights the importance of identifying infected individuals [7].

Patterns of HBV infection vary worldwide. Approximately 45% of the world's population live in regions that are highly endemic for HBV infection, where most infections are acquired perinatally or during early childhood [2, 8]. Another 43% live in regions of intermediate endemicity, where multiple modes of transmission (ie, perinatal, household, sexual, injection drug use associated, and health care associated) are important. In

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countries of low endemicity, most infections occur among adolescents and adults and are attributable to sexual and injection drug use exposures. In 1992, the World Health Organization set a goal for all countries to integrate hepatitis B vaccine into their childhood vaccination programs by 1997 [9].

In the United States, a country of low endemicity, a strategy to eliminate HBV transmission [10] was initiated in 1991, which includes universal vaccination of infants; screening of all pregnant women for HBV, with postexposure prophylaxis provided to infants born to infected women; catch-up vaccination of adolescents; and vaccination of adults who are at increased risk of infection [11, 12]. To assess US trends in the burden of HBV and to provide the first nationally representative analysis of the impact of hepatitis B vaccination, we compared the prevalence of HBV infection among National Health and Nutrition Examination Survey (NHANES) participants during 1999–2006 to that during 1988–1994 and measured the prevalence of vaccine-induced immunity among participants during 1999–2006.

METHODS

Study populations and sample design. NHANES is a series of surveys conducted periodically to obtain representative data on the health status of the US population. Participants are chosen using a complex, stratified, multistage sampling design to obtain a representative sample of the civilian, noninstitutionalized population. Our analyses include data from 1999–2006 (NHANES 1999–2006) and 1988–1994 (NHANES 1988–1994). Further details on the design and implementation of these surveys are described elsewhere [13, 14].

During the years evaluated, all ages were eligible to participate. Participants were interviewed at home and then visited a mobile examination center for additional interviews and a physical examination. Blood samples were collected for participants aged ≥6 years in NHANES 1988–1994 and aged >2 years in NHANES 1999–2006. Informed consent was obtained. Efforts were made to ensure participation; respondents were nominally remunerated for their time and travel expenses.

Laboratory methods. Serum samples from participants aged ≥6 years were tested for antibody to hepatitis B core antigen (anti-HBc) (NHANES 1988–1994: Corab radioimmunoassay [Abbott Laboratories]; NHANES 1999–2006: Ortho HBc ELISA [Ortho Clinical Diagnostics]) and, if results were positive, were tested for hepatitis B surface antigen (HBsAg) (NHANES 1988–1994: Ausria II [Abbott Laboratories]; NHANES 1999–2006: Auszyme [Abbott Laboratories]). Starting with NHANES 1999–2006, serum samples from participants aged >2 years were tested for antibody to hepatitis B surface antigen (anti-HBs) (Ausab [Abbott Laboratories]).

Definitions. Past or present HBV infection was defined as the presence of anti-HBc. Chronic HBV infection was defined as the presence of anti-HBc and HBsAg. For NHANES 1999–2006, persons with test results positive for anti-HBs and negative for anti-HBc were considered to have vaccine-induced immunity.

In NHANES 1988–1994, 25,733 (83.2%) of the participants aged ≥6 years were interviewed, of whom 23,527 (91.4% of those interviewed) were examined and 21,260 (90.4% of those examined) were tested for anti-HBc and HBsAg. In NHANES 1999–2006, 34,338 (79.8%) were interviewed, 32,534 (94.7% of those interviewed) were examined, and 29,828 (91.7% of those examined) provided serum samples. Analysis of vaccine-induced immunity included NHANES 1999–2006 participants aged ≥2 years tested for anti-HBs. Samples for participants aged 2–5 years were collected starting in NHANES 1999; participation rates in this age group were low, with samples available for 55.8% of 3592 examined children. In NHANES, race and ethnicity is categorized as non-Hispanic white (hereafter "NH-white"), non-Hispanic black (hereafter "NH-black"), Mexican American, or Other (which includes all other racial and ethnic groups, including Asians and other Hispanics). Age groups were 6–11, 12–19, 20–29, 30–39, 40–49, 50–59, and ≥60 years of age.

Statistical analyses. Prevalence estimates were weighted to represent the US population and to account for oversampling and nonresponse to the household interview and physical examination. Standard errors were calculated in SUDANN Statistical Analysis Software (Research Triangle Institute). Prevalence estimates were age-adjusted by the direct method using the age groups listed above to the 2000 US census population for comparisons across subgroups and between surveys [15]. Prevalence of vaccine-induced immunity was compared between the periods 1999–2002 and 2003–2006. Prevalence estimates of HBV infection and chronic infection for some subgroups, where noted in the tables, are based on a small number of persons with positive results and may be unstable. Statistical comparisons were evaluated using a t test for linear contrast procedure in SUDAAN. No adjustments for multiple comparisons were made.

RESULTS

Overall prevalence of past and present HBV infection and markers of immunity. The prevalence of past and present infection during the period 1999–2006 was 4.8% (95% confidence interval [CI], 4.3%–5.3%). Prevalence of chronic HBV infection was 0.28% (95% CI, 0.21–0.36%), which represents ~730,000 infected persons (95% CI, 550,000–940,000). Prevalence of markers of vaccine-induced immunity was 22.2% (95% CI, 21.3%–23.1%).

Prevalence of HBV infection increased with age, from 0.6% (95% CI, 0.2%–1.4%) among persons 6–11 years of age to 7.3% (95% CI, 6.2%–8.5%) among persons ≥60 years of age (Figure 1). Prevalence of vaccine-induced immunity was negatively correlated with age, ranging from 53.5% (95% CI, 50.8%–56.3%) among persons aged 6–11 years to 5.1% (95% CI, 4.3%–6.0%) among persons ≥60 years of age. Among the 2003 children 2–5 years of age who were tested, 57.3% (95% CI, 54.1%–60.4%) had test results that were positive for anti-HBs; the representativeness of that estimate is uncertain because of the low response rate in this age group.

Age-adjusted estimates of the prevalence of past and present HBV infection. The overall age-adjusted prevalence of past and present infection in NHANES 1999–2006 (4.7%) was lower than but was not statistically different from the prevalence in NHANES 1988–1994 (5.4%) (Table 1). However, among children 6–19 years of age, prevalence decreased significantly, from 1.9% to 0.6% ($P<.01$). Among adults, prevalence decreased significantly among those 20–49 years of age, from 5.9% to 4.6% ($P<.05$) but was unchanged among those ≥50 years of age.

In NHANES 1999–2006, age-adjusted prevalence of past and present infection was significantly higher among NH-blacks (12.2%; $P<.001$) and Others (13.3%; $P<.001$) than it was among NH-whites and Mexican Americans, and it was significantly higher among foreign-born persons (12.2%; $P<.001$) than it was among US-born persons (3.5%). Compared with NHANES 1988–1994, prevalence decreased significantly only among the Other (from 20.1% to 13.3%) and Mexican American (from 5.1% to 2.9%) race and ethnic groups. No significant change in sex-specific prevalence occurred; in NHANES 1999–2006, prevalence among male participants remained significantly ($P<.001$) higher than it was among female participants.

The age-adjusted prevalence of chronic HBV infection in NHANES 1999–2006 (0.27%) was lower but not statistically different than it was in NHANES 1988–1994 (0.38%) (Table 1). Among children 6–19 years of age, there was a 79% decrease in the age-adjusted prevalence of chronic infection, from 0.24% to 0.05%, which was not statistically significant. In NHANES 1999–2006, prevalence of chronic infection was lower among persons 6–19 years of age (0.05%) ($P<.001$) than it was among those 20–49 years of age (0.30%) or ≥50 years of age (0.38%), and it was lower among female participants (0.19%) ($P<.05$) than it was among male participants (0.35%). Chronic infection was more common among persons classified as Other (0.98%; $P<.001$) or NH-black (0.89%; $P\leq.001$) than it was among NH-whites (0.09%) and Mexican Americans (0.07%). Chronic infection among foreign-born participants (0.89%) decreased significantly ($P<.05$), compared with NHANES 1988–1994 (1.75%), but remained >5-fold higher than it was among US-born participants (0.16%; $P<.001$). The number of chronically infected persons identified in NHANES was small; estimates for some sparsely populated strata, where noted in the tables, have large confidence intervals and may be unstable.

Trends among children in past and present HBV infection. Among children, the age-adjusted prevalence of past and present infection among NH-blacks ($P<.05$) and Others ($P<.01$) decreased significantly across surveys. The decreases in these groups, which both had significantly ($P<.01$) higher prevalence than did NH-whites and Mexican Americans in NHANES 1988–1994, resulted in a narrowing of racial and ethnic disparities in NHANES 1999–2006, although the difference between the highest (NH-black) and lowest 2 groups (Mexican Americans and Other) remained significant ($P<.01$ and $P<.05$ respectively) (Table 2). Differences in prevalence between

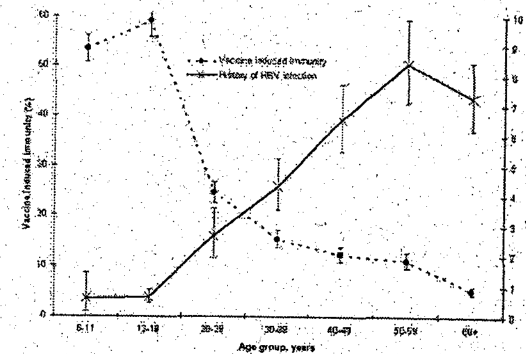

Figure 1. Crude prevalence of markers of hepatitis B virus (HBV) infection and vaccine-induced immunity by age, 1999–2006.

Table 1. Age Adjusted Prevalence of Hepatitis B Virus (HBV) Infection, by Selected Demographic Characteristics

	Past or present HBV infection					Chronic HBV infection		
	NHANES III (1988–1994)		NHANES 1999–2006					
Variable	Sample size[a]	Prevalence, % (95% CI)	Sample size[a]	Prevalence, % (95% CI)	P^b	NHANES III (1988–1994): Prevalence, % (95% CI)	NHANES 1999–2006: Prevalence, % (95% CI)	P^b
Overall	21,260	5.4 (4.8–6.1)	29,828	4.7 (4.2–5.2)	NS	0.38 (0.29–0.49)	0.27 (0.20–0.35)	NS
Age, years								
6–19	5679	1.9 (1.2–2.7)	12,004	0.6 (0.4–0.9)	<.01	0.24 (0.07–0.56)[c]	0.05 (0.02–0.11)[c,d]	NS
20–49	8857	5.9 (5.1–6.9)	9465	4.6 (3.9–5.3)	<.05	0.39 (0.25–0.60)	0.30 (0.21–0.42)	NS
≥50	6724	7.2 (6.2–8.3)	8359	7.7 (6.8–8.7)	NS	0.45 (0.21–0.84)[c]	0.38 (0.25–0.55)	NS
Race/ethnicity								
White, non-Hispanic	7963	3.0 (2.6–3.5)	12,075	2.8 (2.5–3.1)	NS	0.21 (0.09–0.41)[c]	0.09 (0.05–0.14)	NS
Black, non-Hispanic	6133	13.8 (12.4–15.3)	7302	12.2 (11.1–13.5)	NS	0.83 (0.59–1.14)	0.89 (0.57–1.33)	NS
Mexican American	6275	5.1 (3.8–6.6)	8094	2.9 (2.4–3.5)	<.01	0.15 (0.05–0.37)[c,d]	0.07 (0.01–0.25)[c,d]	NS
Other	889	20.1 (15.4–25.5)	2357	13.3 (10.9–16.0)	<.05	1.51 (0.83–2.51)	0.98 (0.57–1.56)	NS
Country of birth								
United States	17,301	3.9 (3.5–4.4)	24,291	3.5 (3.1–3.9)	NS	0.20 (0.12–0.30)	0.16 (0.11–0.23)	NS
Foreign born	3901	16.2 (12.8–19.9)	6528	12.2 (10.7–13.9)	<.05	1.75 (1.26–2.36)	0.89 (0.55–1.35)	<.05
Sex								
Male	10,088	6.4 (5.6–7.3)	14,523	5.6 (4.9–6.3)	NS	0.52 (0.35–0.74)	0.35 (0.25–0.48)	NS
Female	11,172	4.5 (3.8–5.4)	15,305	3.8 (3.2–4.4)	NS	0.23 (0.14–0.36)	0.19 (0.11–0.30)	NS

NOTE. CI, confidence interval; NHANES, National Health and Nutrition Examination Survey; NS, not significant.
[a] Stratum-specific sample sizes may not sum to total because of missing data.
[b] Determined by *t* test evaluating change across surveys.
[c] Estimate is small relative to its standard error (relative standard error >30%) and therefore may be unstable.
[d] Estimate based on ≤10 individuals with positive samples.

US and foreign-born children diminished as a result of greater decreases in prevalence among foreign-born children. The prevalence among foreign-born children (12.8%) in NHANES 1988–1994, which was almost 13-fold higher than that among US-born children (1.0%; $P<.01$), decreased to 2.0% in NHANES 1999–2006, compared with 0.5% ($P<.01$) among US-born children. Most notable was a >90% decrease among foreign-born Other children ($P<.001$).

Among US-born children, racial and ethnic disparities were reduced. In NHANES 1988–1994, prevalence was significantly higher among US-born NH-black children (2.1%; $P<.05$), compared with NH-whites (0.7%) and Mexican Americans (0.5%). In comparison, in NHANES 1999–2006, prevalence was similar among US-born children by race and ethnicity, ranging from 0.1% (Other) to 0.6% (NH-white). Race-specific estimates for some subgroups, as noted in Table 2, are based on <10 positive samples and may be unstable.

Trends among adults. The significant decrease in prevalence across surveys among persons 20–49 years of age ($P<.05$) reflected decreases among US-born and foreign-born participants, although only the decrease among US-born participants was statistically significant ($P<.05$). Prevalence remained significantly higher among foreign-born participants (10.3%) in NHANES 1999–2006 than among US-born participants (3.4%; $P<.001$) (Table 3). Among US-born adults, a pattern of decreasing prevalence was noted in all racial and ethnic groups, but only the decrease in prevalence among NH-blacks was statistically significant ($P<.05$). In NHANES 1999–2006, prevalence among US-born non-Hispanic NH-blacks (9.6%) remained higher ($P<.001$) than the prevalence among NH-whites and Mexican Americans. In contrast, prevalence among US-born Others no longer differed from that among US-born NH-whites or Mexican Americans. The decrease among foreign-born participants 20–49 years of age ($P<.05$) was seen among several racial and ethnic groups but was statistically significant only among Mexicans Americans ($P<.05$). The prevalence was ~3-fold higher among foreign-born Others (16.1%) than it was among US-born Others (5.6%; $P<.001$), a gap that appeared to widen, compared with NHANES 1988–1994, when prevalences among foreign-born and US-born Others were 21.3% and 17.4%, respectively.

In contrast to the trends among younger adults, the prevalence among persons ≥50 years of age in NHANES 1999–2006 (7.7%; 95% CI, 6.8%–8.7%) did not differ from that in NHANES 1988–1994 (7.2%; 95% CI, 6.2%–8.3%). Disparities by race and country of birth that were present in NHANES 1988–1994 (data not shown) remained unchanged in NHANES 1999–2006. In particular, prevalence remained unchanged and significantly higher among NH-blacks (21.7%; 95% CI, 19.2%–24.3%; $P<.001$) and Others (25.5%; 95% CI, 19.6%–32.1%;

Table 2. Age-Adjusted Prevalence of Past and Present Hepatitis B Virus Infection among Children 6–19 Years of Age, by Selected Demographic Characteristics

	NHANES III (1988–1994)			NHANES 1999–2006			
Variable	Sample size[a]	No. of children with positive results	Prevalence, % (95% CI)	Sample size[a]	No. of children with positive results	Prevalence, % (95% CI)	P^b
Overall	5679	77	1.9 (1.2–2.7)	12,004	81	0.6 (0.4–0.9)	<.01
Race and ethnicity							
White, non-Hispanic	1478	13	0.7 (0.4–1.3)[c]	3058	15	0.6 (0.3–1.2)[c]	NS
Black, non-Hispanic	1921	35	2.2 (1.4–3.3)	3830	44	1.0 (0.7–1.4)	<.05
Mexican American	2011	6	0.5 (0.1–1.3)[c]	4148	17	0.4 (0.2–0.7)[c]	NS
Other	269	23	10.3 (5.2–17.7)	968	5	0.4 (0.1–1.1)[c]	<.01
US born							
All	5022	50	1.0 (0.6–1.4)	10,474	44	0.5 (0.2–0.8)	<.05
White, non-Hispanic	1448	13	0.7 (0.4–1.3)	2963	13	0.6 (0.2–1.2)[c]	NS
Black, non-Hispanic	1840	31	2.1 (1.2–3.2)	3644	18	0.5 (0.3–0.7)	<.01
Mexican American	1581	4	0.5 (0.1–1.8)[c]	3079	12	0.4 (0.1–0.7)[c]	NS
Other	153	2	0.9 (0.0–5.8)[c]	788	1	0.1 (0.0–0.6)[c]	NS
Foreign born							
All	639	27	12.8 (6.7–21.4)	1529	37	2.0 (1.2–3.2)	<.01
White, non-Hispanic	28	0	0.0 (0.0–97.5)[c]	95	2	1.8 (0.3–5.9)[c]	NS
Black, non-Hispanic	74	4	5.3 (0.9–16.0)[c]	185	26	11.8 (5.9–20.3)	NS
Mexican American	421	2	0.5 (0.1–2.1)[c]	1069	5	0.3 (0.1–0.8)[c]	NS
Other	116	21	22.9 (12.6–36.3)	180	4	1.4 (0.3–3.9)[c]	<.001

NOTES. CI, confidence interval; NHANES, National Health and Nutrition Examination Survey; NS, not significant.
[a] Stratum-specific sample sizes may not sum to total because of missing data.
[b] Determined by *t* test evaluating change across surveys.
[c] Estimate is small relative to its standard error (relative standard error >30%) and therefore may be unstable.

$P<.001$), compared with NH-whites (4.7%; 95% CI, 3.9%–5.5%) and Mexican Americans (mean value, 6.0%; 95% CI, 5.0%–7.2%), and was significantly higher among foreign-born persons (22.8%; 95% CI, 19.4%–26.5%), compared with US-born persons (5.9%; 95% CI, 5.0%–6.9%; $P<.001$).

Age-adjusted prevalence of vaccine-induced immunity in NHANES 1999–2006. The age-adjusted prevalence of markers of vaccine-induced immunity in NHANES 1999–2006 was 22.9% (95% CI, 21.9%–24.0%), ranging from 56.7% (95% CI, 54.0%–59.3%) among children 6–19 years of age to 17.0% (95% CI, 15.8%–18.2%) among those 20–49 years of age to 7.5% (95% CI, 6.7%–8.3%) among persons ≥50 years of age (Table 4). Prevalence of vaccine-induced immunity increased significantly, from 20.5% during 1999–2002 to 25.2% during 2003–2006 ($P<.001$). This reflected significant increases in all age and racial and ethnic groups and among foreign-born and US-born participants. Comparing data from 1999–2002 with that from 2003–2006, the age-adjusted prevalence of vaccine-induced immunity increased from 52.7% to 60.5% among those 6–19 years of age, from 14.3% to 19.6% among those 20–49 years of age, and from 6.6% to 8.2% among those ≥50 years of age.

The prevalence of vaccine-induced immunity during 1999–2006 among children 6–19 years of age varied little by race and ethnicity, ranging from 53.6% (95% CI, 49.7%–57.6%) among NH-blacks to 59.7% (95% CI, 54.2%–65.0%) among Others and did not differ by sex. A significantly higher proportion of foreign-born children (63.7%; 95% CI, 59.3%–67.9.0%; $P<.01$) had evidence of vaccine-induced immunity, compared with US-born children (56.3%; 95% CI, 53.5%–59.1%), although the lowest prevalence in this age group occurred among foreign-born NH-blacks (51.2%; 95% CI, 41.7%–60.6%) (data not shown).

Among adults 20–49 years of age, prevalence was significantly higher among US-born persons (17.9%; 95% CI, 16.5%–19.4%; $P<.001$) than foreign-born persons (12.7%; 95% CI, 10.9%–14.6%) and higher among women (20.1%; 95% CI, 18.2%–22.0%; $P<.001$) than among men (13.8%; 95% CI, 12.6%–15.0%). Among adults ≥50 years of age, the age-adjusted prevalence of vaccine-induced immunity (7.5%; 95% CI, 6.7%–8.3%) did not differ by race and ethnicity or country of birth (data not shown) but was significantly higher among women (8.7%; 95% CI, 7.6%–9.9%; $P<.001$) than among men (6.1%; 95% CI, 5.2%–7.0%).

DISCUSSION

In this analysis of the most recent NHANES, conducted a decade after universal vaccination of US children against hepatitis

Table 3. Age-Adjusted Prevalence of Past and Present Hepatitis B Virus Infection among Persons 20–49 Years of Age by Selected Demographic Characteristics

Variable	NHANES III (1988–1994)		NHANES 1999–2006		
	Sample size[a]	Prevalence, % (95% CI)	Sample size[a]	Prevalence, % (95% CI)	P[b]
Overall	8857	5.9 (5.1–6.9)	9465	4.6 (3.9–5.3)	<.05
Race/ethnicity					
White, non-Hispanic	2724	3.3 (2.6–4.2)	4176	2.6 (2.2–3.1)	NS
Black, non-Hispanic	2825	13.8 (12.2–15.5)	2018	11.5 (9.6–13.6)	NS
Mexican American	2929	4.2 (3.1–5.6)	2398	2.2 (1.5–3.1)	<.01
Other	379	20.0 (14.4–26.7)	873	11.6 (8.8–14.8)	<.05
US born					
All	6564	4.5 (3.8–5.3)	6935	3.4 (2.9–4.0)	<.05
White, non-Hispanic	2604	3.2 (2.4–4.1)	3941	2.3 (1.9–2.8)	NS
Black, non-Hispanic	2601	12.5 (10.8–14.5)	1804	9.6 (8.0–11.4)	<.05
Mexican American	1283	4.3 (2.8–6.1)	826	2.3 (1.2–3.9)	NS
Other	76	17.4 (6.7–34.1)[c,d]	364	5.6 (2.6–10.2)[d]	NS
Foreign born					
All	2269	14.4 (11.0–18.3)	2530	10.3 (8.2–12.6)	NS
White, non-Hispanic	118	7.5 (3.4–14.1)[c]	235	8.1 (4.8–12.6)	NS
Black, non-Hispanic	207	28.1 (20.4–36.9)	214	25.8 (19.2–33.5)	NS
Mexican American	1645	4.3 (2.7–6.5)	1572	2.2 (1.4–3.3)	<.05
Other	299	21.3 (14.4–29.7)	509	16.1 (12.2–20.6)	NS

NOTE. CI, confidence interval; NHANES, National Health and Nutrition Examination Survey; NS, not significant.
[a] Stratum-specific sample sizes may not sum to total because of missing data.
[b] Determined by t test evaluating change across surveys.
[c] Estimate is small relative to its standard error (relative standard error >30%) and therefore may be unstable.
[d] Estimate based on <10 individuals with positive samples.

B began in 1991, we demonstrate a significant reduction of 68% in HBV infection prevalence among children, including those born in the United States and elsewhere. In addition, a 79% decrease in the prevalence of chronic infection in this age group, although based on a small number of children and not statistically significant, further suggests that substantial progress has been made in reducing the disease burden among children. NHANES, the only source of nationally representative information on the seroprevalence of hepatitis virus infections in the United States, has been critical to describing the burden of HBV infection and, for the first time with this report, determining how it is changing after implementation of a comprehensive national strategy to eliminate HBV transmission in the United States. Keeping in mind the limitations of estimates that are based on small numbers, extrapolation from these data suggests that the number of chronically infected children during 1999–2006 was ~29,000 (95% CI, 11,000–63,000), compared with ~122,000 (95% CI, 36,000–290,000) during 1988–1994. These decreases among children are likely due, in large part, to the incorporation of hepatitis B vaccination into domestic and global routine infant and childhood vaccination programs. A smaller yet significant decrease in the prevalence of HBV infection occurred among US-born adults 20–49 years of age. Among US-born and foreign-born adults aged ⩾50 years, HBV infection prevalence changed little over the decade. An estimated 730,000 US residents, mostly adults, had chronic HBV infection, which demonstrates the ongoing burden of HBV-associated disease.

The decrease in the prevalence of infection among children, which was primarily the result of large decreases among US-born NH-black and Other children and among foreign-born Other children, resulted in the elimination or narrowing of many disparities. Among US-born children, prevalence of HBV infection was uniformly low. Although the prevalence among foreign-born children continued to be higher than that among US-born children, it decreased by 84%, compared with data from the previous survey. Most strikingly, there was a >90% decrease among the foreign-born Other group, and the disparity between US-born and foreign-born children was reduced from 13-fold to 4-fold. These data provide a sense of the impact of vaccination here and abroad on preventing HBV infections among children living in the United States.

In the United States, the first recommendations for universal vaccination of children against hepatitis B were made in 1991 [10]. To prevent perinatal transmission of HBV, screening of pregnant women for HBsAg was recommended with the follow-up of infants born to infected women to ensure that they receive postexposure prophylaxis. "Catchup" vaccination of unvacci-

Table 4. Age-Adjusted Prevalence of Vaccine-Induced Immunity to Hepatitis B Virus (HBV) Infection by Selected Demographic Characteristics, 1999–2006

Variable	NHANES 1999–2006		NHANES 1999–2002		NHANES 2003–2006		
	Sample size[a]	Prevalence, % (95% CI)	Sample size[a]	Prevalence, % (95% CI)	Sample size[a]	Prevalence, % (95% CI)	P[b]
Overall	29,828	22.9 (21.9–24.0)	15,051	20.5 (18.7–22.4)	14,777	25.2 (24.2–26.3)	<.001
Sex							
Male	14,523	20.8 (19.8–21.8)	7290	18.8 (17.2–20.5)	7233	22.7 (21.7–23.8)	<.001
Female	15,305	25.0 (23.6–26.3)	7761	22.2 (20.0–24.5)	7544	27.8 (26.2–29.1)	<.001
Age, years							
6–19	12,004	56.7 (54.0–59.3)	6202	52.7 (48.1–57.3)	5802	60.5 (57.9–63.0)	<.01
20–49	9465	17.0 (15.8–18.2)	4701	14.3 (12.5–16.2)	4764	19.6 (18.1–21.2)	<.001
⩾50	8359	7.5 (6.7–8.3)	4148	6.6 (5.5–7.9)	4211	8.2 (7.3–9.3)	<.05
Race and ethnicity							
White, non-Hispanic, by age in years							
Overall	12,075	23.5 (22.2–24.8)	5910	21.0 (18.9–23.3)	6165	25.7 (24.4–27.0)	<.001
6–19	3058	56.7 (53.4–59.8)	1556	53.6 (48.5–58.7)	1502	59.3 (55.6–62.9)	NS
20–49	4176	18.0 (16.5–19.6)	2020	15.0 (12.8–17.4)	2156	20.9 (18.9–23.0)	<.001
⩾50	4841	7.7 (6.8–8.6)	2334	6.6 (5.3–8.2)	2507	8.5 (7.4–9.8)	<.05
Black, non-Hispanic, by age in years							
Overall	7302	21.4 (20.0–22.8)	3461	18.5 (16.5–20.6)	3841	24.0 (22.4–25.7)	<.001
6–19	3830	53.6 (49.7–57.6)	1849	46.5 (40.7–52.4)	1981	60.3 (56.6–64.0)	<.001
20–49	2018	15.5 (13.7–17.5)	934	13.1 (10.6–16.0)	1084	17.5 (15.1–20.2)	<.05
⩾50	1454	6.9 (5.6–8.5)	678	6.3 (4.5–8.5)	776	7.4 (5.5–9.8)	NS
Mexican American, by age in years							
Overall	8094	19.8 (18.3–21.3)	4408	17.9 (16.0–20.0)	3686	21.5 (19.5–23.6)	<.05
6–19	4148	57.0 (53.2–60.6)	2275	49.9 (44.5–55.3)	1873	63.5 (59.3–67.6)	<.001
20–49	2398	11.3 (9.6–13.3)	1291	10.7 (8.7–13.1)	1107	11.9 (9.3–14.9)	NS
⩾50	1548	5.8 (4.2–7.7)	842	5.7 (4.0–7.8)	706	5.8 (3.5–9.1)	NS
Other, by age in years							
Overall	2357	24.1 (21.9–26.4)	1272	21.9 (18.6–25.4)	1085	27.0 (24.5–29.7)	<.05
6–19	968	59.7 (54.2–65.0)	522	57.8 (49.1–66.2)	446	63.3 (57.7–68.6)	<.001
20–49	873	18.2 (15.0–21.6)	456	14.2 (10.1–19.3)	417	22.7 (18.7–27.1)	<.01
⩾50	516	7.4 (5.4–9.8)	294	7.5 (5.4–10.1)	222	7.1 (4.0–11.6)	NS
Country of birth							
US born	24,291	23.3 (22.1–24.6)	12,103	20.9 (18.8–23.1)	12,188	25.7 (24.6–26.8)	<.001
Foreign born	5528	22.1 (20.7–23.6)	2941	19.5 (17.6–21.5)	2587	24.8 (22.7–27.1)	<.001

NOTES. CI, confidence interval; NHANES, National Health and Nutrition Examination Survey; NS, not significant.
[a] Stratum-specific sample sizes may not sum to total because of missing data.
[b] Determined by t test evaluating change from 1999–2002 to 2003–2006.

nated adolescents was recommended in 1995 [16]. Vaccine coverage data indicate that, between 1993 and 2006, the percentage of children 19–35 months of age who received hepatitis B vaccine increased from 16% to 93% [17]. Coverage rates among adolescents 13–17 years of age have also increased substantially, to 81% in 2006 [18].

Considerable progress also has been made in implementing hepatitis B vaccination programs for children in other countries. As of December 2006, 164 (85%) of 193 World Health Organization member countries had introduced hepatitis B vaccination into their infant immunization schedules [19]. Of the 27 countries in the western Pacific, where HBV infection is endemic, 55% introduced infant hepatitis B vaccination by 1992 and, to date, 96% have integrated hepatitis B vaccine into their childhood immunization programs. Studies from Asian countries have documented the impact of these programs, including decreases in the prevalence of chronic infection and the incidence of hepatocellular carcinoma among children [20–22]. Of the 29 countries that have not yet integrated hepatitis B vaccination, 12 (41%) are in Africa, where endemicity remains high. The prevalence patterns among foreign-born children in NHANES appear to correlate with these global patterns of vaccination implementation, with dramatic decreases among the Other group, which includes those born in Asia.

Although patterns of markers of vaccine-induced immunity in NHANES 1999–2006 reflect the implementation of domestic and international vaccination programs, the results undoubtedly underestimate the true prevalence of vaccine-induced immunity, particularly that among children. Among persons who were vaccinated as infants or young children and responded to vaccination, 15%–45% have low or undetectable concentrations of anti-HBs 5–22 years after vaccination [8, 23–26]. However, evidence indicates that immunocompetent persons who respond to the vaccine remain protected against HBV even as anti-HBs levels become undetectable [27, 28]. Thus, prevalence of anti-HBs in NHANES underestimates the population level of vaccine-induced immunity by misclassifying participants who lost detectable anti-HBs as susceptible to HBV. Results from the National Immunization Survey and other surveys, which indicate high coverage among 19–35-month-old children and adolescents, provide a more complete reflection of coverage and immunity among US-born children [17].

The decreases in prevalence among younger US-born adults likely reflect the impact of several factors. Over the 18 years spanned by these NHANES surveys, the risk of HBV transmission has decreased, as evidenced by an 80% reduction in the incidence of acute hepatitis B cases since 1990[29]. This likely reflects the implementation of prevention strategies, such as improvements in infection control and screening of the blood supply, modified risk taking practices among high-risk groups, and the impact of targeted vaccination of adults at risk because of occupational or behavioral factors [30–32]. This decrease may also reflect the impact of programs to vaccinate adolescents [16]. This effect recently was documented among US military recruits, among whom anti-HBs prevalence ranged from 62% among those born during 1987–1988 to 27% among those born before 1982 [33].

Although substantial progress has been made in preventing HBV infection among children and young adults, NHANES indicates that the burden of chronic hepatitis B among adults remains large. Many disparities persist that reflect infections acquired over the participants' lifetimes. Among US-born adults, prevalence increased with age and was higher among NH-black and Other races and ethnicities. Of interest, prevalence decreased among young US-born adult Others, which could reflect an impact of vaccination programs targeting Asians of all ages [34–36]. As in previous surveys, HBV infection prevalence was significantly higher among foreign-born adults than it was among US-born adults, which reflected the level of endemicity in participants' countries or regions of origin. Foreign-born persons accounted for ~14% of the NHANES 1999–2006 population, which is similar to estimates from the US Census [37] that indicated that 12% of the US population was foreign-born. In NHANES 1999–2006, this group accounted for 43% of all chronic infections or ~317,000 (95% CI, 202,000–479,000) infections among foreign-born persons in the United States in 1999–2006.

The large burden of chronic HBV infection among adults demonstrated by NHANES highlights the need to improve screening programs and other efforts to identify chronically infected persons, most of whom remain asymptomatic until cirrhosis or end-stage liver disease develops. Limited data indicate that many persons with chronic infection are unaware of their infection status [38–40]. Screening and counseling programs are important to educate and medically manage infected patients to prevent liver disease progression and to identify and vaccinate susceptible contacts to interrupt further transmission [7].

There are limitations to the use of NHANES data to assess HBV prevalence. In NHANES, participants classify themselves with regard to race and ethnicity, but because the numbers of persons belonging to specific racial and ethnic groups other than non-Hispanic white, non-Hispanic black, or Mexican-American are not large enough to make stable prevalence estimates, the National Center for Health Statistics (NCHS), which oversees NHANES, groups these persons into a category of Other nonspecified race and does not release self-reported race data. Thus, the calculation of specific estimates for subgroups, such as Asians and Native Americans, is not possible. It is likely that prevalence among Asians is considerably higher than that reflected by the overall Other category, which includes populations which have lower prevalence of disease. Nevertheless, these groups are sampled in the NHANES population, and overall NHANES estimates reflect and are greatly influenced by the prevalence in these subgroups. A summary analysis provided by NCHS of unedited data, not publicly released, of participants' self-reported race and country of origin suggests that persons likely to be Asian represent ~3.3% (95% CI, 2.8%–3.8%) of the overall NHANES weighted sample and that ~71% of that group are foreign-born. These results may be subject to some error because of misclassification of Asian ethnicity based on unedited data but appear similar to US Census estimates [37], which characterize 4.4% of the US population as Asian, with 68% of this Asian population being born overseas. In addition, although composition of the Other category is not specified and varies somewhat across surveys, an estimated 30% of the group were classified as Asian based on the analysis of raw ethnicity and country of origin data, and the trends and patterns expected among the Asian population appear to be discernible in the results for the Other race and ethnic group.

Another limitation of NHANES is that it samples only from the noninstitutionalized civilian population of the United States. Thus, the overall estimate does not reflect infections among populations that include incarcerated persons, among whom HBV prevalence is known to be high. The prevalence of chronic HBV infection among the estimated 2.2 million

persons in US jails and prisons is ~2.0% [41], resulting in an estimated 44,000 persons with HBV infection in these settings and increasing the estimated number of chronically infected persons in the United States by 6%, to 774,000. Homeless persons, who also may have increased prevalence of infection, are also not included in NHANES [7].

In summary, this analysis of unique population-based data provides new evidence of the impact of domestic and global childhood hepatitis B vaccination programs on preventing HBV infections, while illustrating the remaining large burden of chronic HBV infection in the United States, which consists of ~730,0000 persons. These results are relevant to public health policy makers and highlight the importance of ongoing hepatitis B vaccination programs and of programs to identify persons with chronic HBV infection.

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研究報告の概要

○慢性B型肝炎患児の唾液中のB型肝炎ウイルス(HBV)DNA:唾液がHBV水平感染の伝播手段となっている可能性

目的:小児におけるHBVの水平感染の機序を検討するため、慢性B型肝炎患児46人の唾液中HBV量と血漿中HBV量を定量し、関連性を調べた。

対象および方法:デンマークにおいてB型肝炎は2000年から届出疾患となっている。2006年5月から2008年11月までに0〜16歳までの慢性B型肝炎患児(HBs抗原陽性)180人に手紙を送り、両親から同意が得られた46人について、6ヶ月あるいは12ヶ月ごとに唾液と血液を得た。HBV-DNAはTaqMan Assayにて定量した(検出感度は50 IU/mL)。

結果:本研究中にHBe抗原が陽性から陰性になった2人と、HBe抗原の状態が分からない1人を調査対象外とした。25人(58%)がHBe抗原陽性で、18人(42%)がHBe抗原陰性であった。HBe抗原陽性の子供の唾液に含まれるHBV-DNA濃度は、HBe抗原陰性の子供の血漿中より39倍高かった。

考察:唾液がHBVの伝播手段になっている。子供において血漿中のHBV量と唾液への分泌量は相関する。ユニバーサルワクチン接種が、児童間のB型肝炎の唾液による感染への懸念を軽減できる可能性がある。

使用上の注意記載状況・その他参考事項等

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
小児におけるHBVの水平感染の機序を検討するため、慢性B型肝炎患児の唾液中と血漿中のHBV量の関連性を調べたところ、HBe抗原陽性患児の唾液中に高値HBV-DNAを認め、児童間での唾液によるHBV水平感染の可能性が示唆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本製剤の安全性は確保されており、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。なお、日本赤十字社では献血時のスクリーニング法としてより感度の高い化学発光酵素免疫測定法(CLEIA)および新NATシステムを導入した。

(2)

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HEPATITIS B VIRUS DNA IN SALIVA FROM CHILDREN WITH CHRONIC HEPATITIS B INFECTION

IMPLICATIONS FOR SALIVA AS A POTENTIAL MODE OF HORIZONTAL TRANSMISSION

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Abstract: To explore the mechanism of horizontal transmission of hepatitis B virus (HBV) among children, we investigated the quantitative relationship between HBV in saliva and blood from 46 children with chronic hepatitis B.

We found high levels of HBV DNA in saliva of HBeAg (+) children, suggesting saliva as a vehicle for horizontal transmission of HBV among children.

Key Words: chronic hepatitis B, children, HBV DNA, saliva, horizontal transmission

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Hepatitis B virus (HBV) infection is a major global health problem and more than 350 million people worldwide are chronically infected. The course of hepatitis B infection is dependent on age at the time of infection. When infected perinatally, 90% of children become chronic carriers and 25% develop liver cirrhosis and are at risk for hepatocellular carcinoma. During childhood the HBV infected children are in a prolonged immune tolerance phase, and they constitute a silent infectious reservoir that may further maintain and spread HBV to susceptible individuals.

The most common routes of acquiring hepatitis B infection in adults are sexual contact and sharing injecting equipment. In childhood, perinatal and horizontal child-to-child transmission are the most common modes of transmission, but the mechanism of viral spread in horizontal transmission remains to be elucidated.[1–3]

Since 1992, WHO has recommended global vaccination against HBV, and by the end of 2006, 168 countries had implemented or were planning to implement a universal HBV immunization program for newborns, infants, and/or adolescents. Only 7 countries in Northern Europe have not yet implemented such a policy—Denmark, Finland, Iceland, the Netherlands, Norway, Sweden, and the United Kingdom.[4] These countries have adopted an at-risk strategy offering vaccination to individuals at high risk of infection.[5] The selective immunization strategy in Denmark includes immunization of staff and children at day-care centers before an HBsAg positive child is admitted. The Medical Officer of Health informs staff and parents before the vaccinations are given, and knowledge about the individual child with chronic hepatitis B infection is confidential. However, despite professional information, this strategy can cause social discrimination of the family and the child with chronic hepatitis B infection. The selective strategy in Denmark does not include hepatitis B vaccination before school entry, and parents are not obliged to inform the school that their child has chronic hepatitis B infection. For the parents of a child with chronic hepatitis B infection, this policy leads to fear of transmission of HBV from their child to unvaccinated children at school. The potential importance of saliva as a vehicle of spread is often a major concern, although transmission from saliva has not been documented except through percutaneous exposure (eg, a bite that breaks the skin).[6] Recent studies have shown that HBV DNA is present in saliva from infected adults and that there is a quantitative correlation between viral load in saliva and serum.[7,8]

The aim of this study was to explore the potential significance of saliva as a vehicle of transmission, and the quantitative relationship between HBV DNA in saliva and in plasma of children was determined.

MATERIALS AND METHODS

In Denmark, chronic hepatitis B infection has been a notifiable disease since the year 2000. All children nationwide, aged 0 to 16 years, notified with chronic hepatitis B ($n = 180$) were invited by letter to participate in the study during the period May 2006 to November 2008. The families of 46 children responded positively, and after written informed consent from the parents, 46 children were included in the study. Blood and saliva samples were obtained at the children's clinical visits every sixth or 12th month. The saliva samples were obtained using the saliva collection kit Oracol (Malvern Medical Developments, Worcester, United Kingdom). Blood was collected in EDTA tubes, spun, and separated into cells and plasma fractions. Purification of HBV DNA from plasma and saliva was performed using the MagNa Pure LC Instrument (Roche Applied Science, Penzberg, Germany). HBV DNA in plasma and saliva was quantitatively measured using the HBV TaqMan Assay as previously described.[9] The lower detection limit was 50 IU/mL. To monitor both loss and inhibition of the samples, a universal internal control consisting of a known number of Phocid herpesvirus type-1 particles was added to the samples, as previously described.[10] Corrections in viral load assessments were made if necessary. Data on the serological status (HBsAg, HBeAg, anti-HBeAg) were obtained from the children's clinical records. Statistical analyses were performed using mixed models with random intercepts with the statistical environment R-2.8.1 using the NLME package, taking into account repeated measurements on several of the patients. All HBV DNA values were log transformed by the natural logarithm prior to analysis, to ensure normality of standardized residuals.

RESULTS

A total of 46 HBsAg positive children were included in the study. Two children were excluded from the analyses as they converted from HBeAg (+) to HBeAg (−) during the study period, and one child was excluded due to unknown HBeAg status. Of those, 25 (58%) of the children were HBeAg (+) and 18 (42%) were HBeAg (−). Mean age at sample date was 10.2 years (SD ± 3.9 years). The number of samples collected ranged from 1 to 7 from each child. In total, we collected 117 plasma samples and 124 saliva samples from 43 children; 116 plasma and saliva samples were paired.

The geometric mean for HBV DNA in plasma from HBeAg (+) children was 41.9×10^6 IU/mL and 33.9×10^3 IU/mL in

TABLE 1. HBV DNA in Saliva and Plasma From Children With Chronic Hepatitis B Infection According to HBeAg Status

Subjects/Specimens	Log HBV DNA IU/mL	95% CI	*P*	Geometric Mean HBV DNA IU/mL	95% CI
HBeAg (+)					
Plasma	17.6	16.6–18.5		41.9×10^6	16.7×10^6 to 105.0×10^6
Saliva	10.4	9.5–11.4		33.9×10^3	13.0×10^3 to 88.4×10^3
HBeAg (−)					
Plasma	6.8	5.9–7.6		880	380–2038
Saliva	NA*			NA*	NA*
HBeAg (+) vs. HBeAg (−) in plasma	10.6	9.2–12.0	<0.001		
HBeAg (+) saliva vs. HBeAg (−) plasma	3.7	2.4–4.9	<0.001		

*All values below lower detection limit.

saliva, compared with 880 IU/mL in plasma from HBeAg (−) children. This showed a 39 times higher levels of HBV DNA in saliva from the HBeAg (+) children than in plasma from the HBeAg (−) children ($P < 0.001$). HBV DNA was undetectable in saliva from the HBeAg (−) children (lower detection limit 50 IU/mL). Results are shown in Table 1.

In 60% (50/84) of samples from HBeAg (+) children, HBV DNA levels in saliva were above 10^4 IU/mL, and in 33% (28/84) HBV DNA levels were above 10^5 IU/mL.

When analyzing the paired measurements of quantitative HBV DNA in plasma and saliva samples, we found a linear relationship between log HBV DNA in plasma and saliva of the HBeAg (+) children described by the equation:

$$\log \text{HBV DNA in saliva} = -6.63 + 0.92 \text{ times } (\log \text{HBV DNA in plasma})$$

The relationship is presented graphically online in Figure, Supplemental Digital Content 1, http://links.lww.com/INF/A417.

DISCUSSION

Saliva has been considered a potential source of HBV transmission, and HBV DNA has been detected in saliva from adults.[7,8] We studied paired saliva and plasma samples from 43 children with chronic hepatitis B and known HBeAg status. We found a high level of HBV DNA in saliva from the HBeAg (+) children. Of note, the levels of HBV DNA were 39 times higher in saliva from the HBeAg (+) children than it was in plasma from the HBeAg (−) children.

Our findings show that saliva is a source of HBV DNA. Assuming that HBV DNA levels reflect the number of infectious particles, saliva is a potential vehicle of spread of HBV. However, studies of the infectivity of HBV DNA in saliva are limited due to lack of available animal models and cell lines that support HBV infection. It is known that HBV can survive for at least 7 days outside the body, and that infection through close interpersonal contact within households is a common mode of transmission of HBV during early childhood in high endemic countries.[2,3] It is presumed that in these settings transmission occurs from skin lesions or by sharing blood contaminated objects, although a specific pathway of transdermal exposure is rarely identified.

A significant concern for children with chronic hepatitis B infection and their parents, is the risk of infecting unvaccinated children. Older children might experience anxiousness when sharing drinks and food with friends. Because not all countries routinely vaccinate children against hepatitis B, it is a dilemma affecting families in those countries.

In samples from the HBeAg (−) children, HBV DNA was not detectable in saliva (lower detection limit 50 IU/mL) and the levels of HBV DNA were low in plasma in this group (880 IU/mL). This confirms our knowledge that HBeAg (−) children are much less infectious than HBeAg (+) children. It is shown in Figure, Supplemental Digital Content 1, http://links.lww.com/INF/A417, that HBV DNA becomes detectable in saliva at a level where log HBV DNA in plasma is around 11, corresponding to a viral load in plasma of about 60×10^3 IU/mL. It has been discussed at what levels HBV DNA of a chronic carrier should be considered to be infectious. Various guidelines are used in the European countries for when health care workers are allowed to work with exposure prone procedures, based on knowledge of HBV DNA levels at which HBV transmission has occurred. In the United Kingdom and Ireland, a cut-off limit of 10^3 HBV DNA copies/mL (=185 IU/mL) is used; in the Netherlands it is 10^5 copies/mL (=18.5×10^3 IU/mL) and a European consensus group decided in 2003 for a cut-off level at 10^4 HBV DNA copies/mL (=1.9×10^3 IU/mL).[11,12]

The mean viral load in saliva from HBeAg (+) children in our study was 33.9×10^3 IU/mL and 33% of these children had HBV DNA levels more than 10^5 IU/mL. Provided that the saliva is contagious, these children should be considered as highly infectious.

We found a clear association between HBV viral load in plasma and saliva. Similar results have been shown in adults.[8] As discussed, we do not know whether the HBV DNA in saliva is infectious, but it has previously been demonstrated that inoculation of chimpanzees and gibbons with saliva from hepatitis B infected individuals caused an acute infection.[13,14] Today contact tracing of the transmission of HBV using epidemiological and molecular data can identify possible sources of infection.[15]

Infection with HBV in childhood has serious consequences, as most children become chronic carriers and are at increased risk of developing liver cirrhosis and hepatocellular carcinoma. We have an ethical duty on both individual and country level to protect children from an oncogenic virus when we have the means to do so. Universal immunization can be implemented during infancy and adolescence; vaccination of adolescents provides immunization at a time of increased high-risk behavior. However, vaccination of infants is preferable because immunization of this age group is better established, and children infected at this age are at high risk of acquiring chronic infection. Universal vaccination might alleviate the fear of saliva as a potential vehicle of trans-

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mission among children, and it is the only logical strategy to protect against HBV infection.

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SEQUENCE TYPES AND ANTIMICROBIAL SUSCEPTIBILITY OF INVASIVE *STREPTOCOCCUS PNEUMONIAE* ISOLATES FROM A REGION WITH HIGH ANTIBIOTIC SELECTIVE PRESSURE AND SUBOPTIMAL VACCINE COVERAGE

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Abstract: Multilocus sequence typing was carried out on 95 invasive pneumococcal isolates belonging to the most common 7 serotypes currently circulating in Taiwan. The study confirmed continued prevalence in Taiwan of a few global clones and sequence types (STs) since the mid-1990s and identified the recent emergence of ST320 (19A) and ST902 (6A). Antimicrobial nonsusceptibility was common in the predominant STs of serotypes 14, 19A, 19F, and 23F.

Key Words: sequence type, serotype, *Streptococcus pneumoniae*, antimicrobial susceptibility, pneumococcal conjugate vaccine, Taiwan

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Dissemination of multiple antibiotic resistant clones of *Streptococcus pneumoniae* across regions and countries is well documented.[1–5] Global clones and their variants, which have spread in Taiwan, include Spain[6B]-2, England[14]-9, Taiwan[19F]-14, Colombia[23F]-26, Spain[23F]-1, and Taiwan[23F]-15.[5,6] Recently, Hsieh et al, reported the emergence of invasive serotype 19A isolates in Taiwan among the 2007 invasive pneumococcal isolates.[3] To prevent pneumococcal infections, 7-valent pneumococcal conjugate vaccine (PCV7) is being widely used.[1,7] PCV7 has significantly reduced invasive pneumococcal diseases (IPD) caused by vaccine serotypes, but serotype 19A has dramatically increased in some countries, but not in others.[1,8] In Taiwan PCV7 was not available until October 2005.[3] The vaccine is now being used in the private sector, with a low penetration in the pediatric population.[3] The aim of this study was to determine the sequence types (STs) of common serotypes that caused IPD in Taiwan after the introduction of PCV7. We also analyzed antimicrobial susceptibility patterns of these isolates.

METHODS

The 95 IPD isolates were selected for sequence typing and antimicrobial susceptibility testing because their serotype distribution covered the most common 7 serotypes currently circulating in Taiwan.[3] All these pneumococcal isolates were identified as described previously by Hsieh et al.[6] An IPD isolate was from a positive blood culture or pleural fluid culture from a child with a consolidation pattern upon chest x-ray. IPD also included primary bacteremia without focus defined as a positive blood culture from a child with fever but without a focal lesion. These isolates were collected from Chang Gung Children's Hospital (CGCH) during 2005–2007. The age range of children was from 1 to 9 years, with a median of 4.5 years. Prior to all experiments, the *S. pneumoniae* isolates were cultivated in trypticase soy agar with 5% sheep blood (in CO_2 incubator). Antimicrobial susceptibility to penicillin, ceftriaxone, erythromycin and imipenem was assayed by E-test (AB Biodisk, Solna, Sweden) and interpretation was based on Clinical and Laboratory Standards Institute standards.[9] The susceptible, intermediately resistant, and resistant MIC interpretative breakpoints for penicillin (nonmeningitis criteria) were ≤2 μg/mL, 4 μg/mL, and ≥8 μg/mL, respectively.[9] Serotyping was performed by latex agglutination and confirmed by Quellung reaction (Statens Serum Institute, Copenhagen, Denmark). All the serotypes were double checked by a PCR method described earlier.[10] The nucleotide sequences of 450-bp internal regions

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研究報告の概要	使用上の注意記載状況・その他参考事項等
○台湾における微量のB型肝炎ウイルス(HBV)DNAを検出するための個別検査とミニプール検査の有効性 背景：財政的な制約は、現在も台湾の、ルーチンの血液スクリーニングとしてのNAT実施において、主要な問題となっている。実現可能な解決策を講じるため、TIGRISシステム(Novartis Diagnostics)のPROCLEIX ULTRIO(Ultrio)分析を用いて、個別供血検査(IDT)と4本のミニプール法(MP4)双方の実施成績を評価した。 試験デザインおよび方法：分析感度はWHO国際標準品により決定した。供血者10,290名(IDT 4210名、MP4 6080名)に検査を行った。潜在的HBV陽性供血者(HBs抗原陰性/NAT陽性)を最高9ヵ月間、追跡調査した。Ultrio分析とHBs抗原検査結果が不一致の場合、さらにHBV抗体血清検査、代替NAT、HBV DNA定量検査ならびに塩基配列決定の解析を行った。 結果：検出の95%検出限界(IU/mL)(95%信頼区間)は以下のとおり： ヒト免疫不全ウイルスType 1(HIV-1)18(12～34)、C型肝炎ウイルス(HCV)4.4(2.8～8.9)、HBV6.3(4.4～11)。再検査率は、IDT 0.55%とMP4 0.33%であった。HIVまたはHCV陽性症例は認められなかったが、潜在的HBV陽性例は12名(IDT 9名、MP4 3名)であった。そのうちの11名は、genotypeがB2であることが判明した。そのうちの10名は、追跡調査のために再来院し、ほとんどがオカルトHBV感染症(OBI)であると判明した。IDTの陽性率 9/4210(0.21%)はMP4の3/6080(0.05%)と比べ4倍高かった(p < 0.05)。 結論：MP4と比較したIDTの高い陽性率は、OBIキャリアが顕著である台湾のような地域で、高感度NAT法を実施する有益性を示している。	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
微量のB型肝炎ウイルス(HBV)DNAを検出するための個別NATとミニプールNATの有効性の評価を行い、オカルトHBVキャリアが多い台湾で、高感度NATの有益性が示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本製剤の安全性は確保されており、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。なお、日本赤十字社では献血時のスクリーニング法としてより感度の高い化学発光酵素免疫測定法(CLEIA)および新NATシステムを導入した。

The efficacy of individual-donation and minipool testing to detect low-level hepatitis B virus DNA in Taiwan

Meng-Hua Yang, Lei Li, Ying-Shen Hung, Cheng-Shen Hung, Jean-Pierre Allain, Kuo-Sin Lin, and Su-Jen Lin Tsai

BACKGROUND: Financial constraints are the main concern in implementing nucleic acid testing (NAT) as routine blood screening in Taiwan. The PROCLEIX ULTRIO assay (Ultrio) on the TIGRIS System (Novartis Diagnostics) was evaluated for its operational performance both for individual-donation testing (IDT) and in minipools of 4 (MP4) to develop a feasible solution.

STUDY DESIGN AND METHODS: Analytical sensitivity was determined by testing WHO international standards. We tested 10,290 blood donors, 4210 in IDT and 6080 in MP4. Potential hepatitis B virus (HBV) yield donors (hepatitis B surface antigen [HBsAg] negative/NAT reactive) were evaluated for up to 9 months' follow-up. Discordant results between the Ultrio assay and the HBsAg tests were further analyzed by HBV antibody serology, alternative NATs, HBV DNA quantification, and sequencing.

RESULTS: The 95% limits of detection in IU/mL (95% confidence interval) were as follows: human immunodeficiency virus Type 1 (HIV-1), 18 (12-34); hepatitis C virus (HCV), 4.4 (2.8-8.9); and HBV, 6.3 (4.4-11). The retest rates were 0.55% for IDT and 0.33% for MP4. No HIV or HCV yield cases were found, while there were 12 potential HBV yield cases, nine from IDT and three from MP4 testing. Eleven of them were successfully genotyped as B2. Ten of them returned for follow-up and mostly were determined as occult HBV infection (OBI). The IDT yield rate of 9 in 4210 (0.21%) was fourfold greater than the MP4 yield rate of 3 in 6080 (0.05%; $p < 0.05$).

CONCLUSION: The higher yield rate for IDT versus MP4 demonstrates the benefit to implement a more sensitive NAT strategy in regions having significant OBI carriers such as Taiwan.

Introduction of nucleic acid amplification testing (NAT) has been shown to result in the improvement of blood safety in many countries around the world.[1] NAT markedly reduces the window period (WP) defined as the time between infection and first detectable viral marker, compared to serologic assays. NAT can detect not only WP infections for human immunodeficiency virus (HIV), hepatitis C virus (HCV), and hepatitis B virus (HBV), but also occult HBV infection (OBI), which are missed by even the most sensitive hepatitis B surface antigen (HBsAg) tests. NAT has been introduced in North America, many European countries, Australia, New Zealand, and parts of Asia including Japan, Hong Kong, and Singapore. However, at the time of this study, it has not been implemented in Taiwan. While NAT screening for HIV-1 and HCV is more widespread than for HBV, the recent advancement of automated or semiautomated systems with multiplex tests has facilitated the simultaneous screening of all three viruses. The two assays currently commercially available are the Chiron PROCLEIX ULTRIO assay (Novartis Diagnostics, Emeryville, CA) and the Roche cobas MPX assay (Roche Molecular Systems, Pleasanton, CA).

Taiwan is an endemic area for HBV infection, with an HBsAg seroprevalence of 17.3% compared to 4.4% for HCV[2] and 0.012% for HIV.[3] Adoption of anti-hepatitis B core antigen (HBc) screening that correlates with HBV exposure, in many low-prevalence countries, resulted in the deferral of only a small number of donors. However, adding this safety measure in Taiwan, where anti-HBc seropositivity is reported to be 16% to 90% in the general population,[4,5] would defer far too many otherwise acceptable donors.

Taiwan has implemented widespread HBV vaccination since 1985 and adopted third-generation HBsAg blood screening tests to limit HBV infections. Nonetheless, one study[6] reported that at least 3% of the population carried occult HBV and hence transfusion-transmitted HBV (TTHBV) infections still occur underscoring the need for additional blood safety measures. Wang and coworkers[7] estimated that approximately 0.02% of donated blood in Taiwan could transmit HBV and predicted the HBV NAT yield to be 20-fold higher in Taiwan than in low-prevalent regions such as the United States. A more recent study[6] showed the rate of transfusion transmission of HBV in Taiwan to be 7- to 40-fold higher than that observed in low-prevalence countries with approximately 0.1% of the transfused recipients acquiring TTHBV. The same study showed that even some vaccinated children with low levels of anti-HBs developed HBV viremia posttransfusion, highlighting the continued threat of TTHBV despite the use of sensitive HBsAg blood screening and more than 20 years of HBV vaccination.[6]

While many recent evaluations of NAT systems in Asian populations have demonstrated their clinical utility, especially for HBV,[8-12] each country undertook evaluations of NAT, given the complexity and cost of NAT, in its own setting and determined which multiplex test is best suited to their circumstances. A recent pilot study[13] of minipool NAT screening of Taiwanese blood donors with an alternative technology showed yield rates 0.10 and 0.01% for HBV and HCV, respectively, that were higher than those observed in Hong Kong.[8]

The objective of this study was to evaluate both the performance of the Ultrio assay on the automated TIGRIS System under standard operational conditions and its ability to identify infectious units in seronegative Taiwanese blood donations (yield). A secondary objective was to determine which configuration of the Ultrio assay, individual donor testing (IDT) or minipool of 4 (MP4) testing, would provide the optimal combination of operational efficiency and blood safety in Taiwan.

ABBREVIATIONS: d = discriminatory (HBV, HCV, HIV-1 assay); IDT = individual donor testing; LOD(s) = limit(s) of detection; MP4 = minipool of 4; OBI(s) = occult hepatitis B virus infection(s); qPCR = quantitative polymerase chain reaction; S/CO = signal-to-cutoff; TTHBV = transfusion-transmitted HBV; WP = window period.

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MATERIALS AND METHODS

PROCLEIX ULTRIO assay

The Ultrio assay is an in vitro NAT utilizing transcription-mediated amplification for the qualitative detection of HIV-1 RNA, HCV RNA, and HBV DNA simultaneously in human plasma. The technology has been previously described.[14-16]

Analytical sensitivity

To verify the analytical sensitivity for detecting HIV-1, HCV, and HBV, diluted panels of World Health Organization (WHO) international standards (HIV-1 RNA International Standard 97/656, HCV RNA International Standard 96/798, and HBV DNA International Standard 97/746) were tested. The WHO international standards were obtained from the National Institute for Biological Standards and Control (NIBSC, Hertfordshire, UK) and panels were prepared at Acrometrix (Benicia, CA), by serially diluting the respective standard with nonreactive human plasma and storing aliquots at −80°C. Four sets of each WHO standard panel were prepared and tested, each set consisting of eight concentrations, with eight replicates of 1.5-mL aliquots for each concentration. The analytical ranges for each WHO standard were as follows: 0.23 to 30 IU/mL for HCV, 0.78 to 100 IU/mL for HIV-1, and 0.31 to 40 IU/mL for HBV. Aliquots were stored frozen at −20°C until testing. Eight replicates of each concentration were tested on each of three different days, to give a total of 24 replicates for each dilution of each virus. An additional eight replicates of all concentrations were tested with the PROCLEIX HIV-1, HCV, and HBV discriminatory assays (dHIV-1, dHCV, dHBV) on a fourth day. A Probit statistical model[17] was applied to the analytical sensitivity data and the 95% limit of detection (LOD) was calculated for the Ultrio assay and the discriminatory assays.

Operational performance

System reliability was assessed by computing the total sample invalid rate, the failed run rate for both IDT and MP4 testing, and the non-repeatable-reactive rate. A total of two reagent master lots were used in 64 test runs over 11 weeks by three operators.

Assay reproducibility

Signal-to-cutoff (S/CO) ratio results, including the means, standard deviations, and coefficients of variation (CV), from both assay controls and viral calibrators, were used to assess assay reproducibility. Data were taken from the routine testing runs only and did not include proficiency runs or runs of the WHO standards. Data were separately collected for the two master lots used in the study and the

three operators who performed the assays. Results from the operator who performed the fewest tests were combined with those of another operator for purposes of analysis.

Blood donor testing

A total of 10,290 different and consecutive blood donor specimens were collected at the Taipei Blood Center from August 13 to October 4, 2007. These blood donors had met the routine blood donation criteria established by Taiwan Health Authority and had consented to NAT screening of their blood. The study was conducted according to the regulatory guidelines in Taiwan and followed the Good Clinical Practice and Good Laboratory Practice Guidelines consistent with the principles originating in the Declaration of Helsinki. A separate BD VACUTAINER PPT plasma preparation tube (Becton Dickinson and Company, Franklin Lakes, NJ) was collected exclusively for NAT assay.

Routine serologic testing of donor specimens for HBsAg (Murex HBsAg v3.0, Abbott Diagnostics, Dartford, UK), anti-HCV (Murex anti-HCV v4.0, Abbott Diagnostics, Kyalami, South Africa), and *anti-HIV-1 and -2 (Murex HIV 1.2.O,* Abbott Diagnostics) was performed according to Taipei Blood Center's established standard operating procedures. Study specimens were linked to donors to permit follow-up evaluations.

Of the 10,290 specimens, 4210 were tested in IDT format and 6080 were tested in 1520 pools of MP4 format. MP4 testing was performed by pooling equal aliquots of plasma from four donation specimens. If a pool was reactive in the Ultrio assay, each specimen from the reactive pool was individually tested to identify the reactive specimen(s).

All Ultrio assay–reactive specimens, whether identified through IDT or MP4, were further tested with the discriminatory assays to determine specific viral activity. When the Ultrio assay was nonreactive and the donor specimen was seronegative, the testing was considered complete.

Supplemental serologic and alternative NAT

Donor specimens with discordant results between the Ultrio assay and the serologic tests of record were retested using specimens taken directly from the plasma unit. Supplemental serologic tests for HBV, HCV, and HIV were the HBsAg neutralization test (Quest *Diagnostics, San* Juan Capistrano, CA), anti-HCV recombinant immunoblot assay (Novartis Diagnostics, Emeryville, CA), and anti-HIV-1/2 Western blot (MP Diagnostics, Singapore), respectively. Additional supplemental serologic tests included anti-HBs (AxSYM, Abbott Diagnostics, Wiesbaden, Germany), anti-HBc Total and IgM (Quest Diagnostics), and anti-HCV (AxSYM, Abbott Diagnostics). Alternative NAT comprised two assays: the NGI HBV UltraQual assay (NGI, Los Angeles, CA), a polymerase chain reaction (PCR) assay with a 95% LOD of 0.9 IU/mL, and Cambridge University Laboratories quantitative (q)PCR assay (Cambridge, UK), with a 95% LOD of 20 IU/mL.[18]

For HIV, HCV, and HBV, the confirmed presence of viral genome without detectable viral antigen or specific antibody was identified as WP infection when follow-up samples confirmed seroconversion. For HBV, samples with the presence of DNA associated with anti-HBc and/or anti-HBs were defined as OBI.[19]

HBV nucleic acid sequencing and genotyping

Viral DNA was quantified from 500 µL of plasma.[20,21] In addition, after ultracentrifugation of 5 to 8 mL of plasma depending on the volume available, full-length HBV genome minus 50 bp in the precore region (approx. 3150 bp), pre-*S/S* region (approx. 1190 bp), and 300 bp in the basic core promoter/precore region were amplified using nested PCR. Amplified products were directly sequenced and those with sequences of greater than 1000 bp were phylogenetically analyzed.[20,21] Deduced amino acid sequences were compared to sequences of HBV strains of Genotypes B and C published in the GenBank database.

RESULTS

Analytical sensitivity

The 95% LOD for HIV-1, HCV, and HBV of the Ultrio assay and the corresponding discriminatory assays, as determined by Probit analysis, are shown in Table 1.

Assay reproducibility

For both reagent master lots used in the study the percent CVs for the reactive calibrators *was less than 5%. There* was 100% agreement between the expected and observed S/CO ratio results for the Ultrio assay controls. The three operators gave consistent and reproducible results (with no significant differences) for the reactive control specimens (data not shown).

TABLE 1. 95% LODs for Ultrio and discriminatory assays as determined by WHO panel tested by IDT

WHO panel	Assay tested	Estimated 95% LOD, IU/mL (95% CI)
HIV RNA 97/656	Ultrio*	18 (12-34)
	dHIV†	14 (8.1-48)
HCV RNA 96/798	Ultrio	4.4 (2.8-8.9)
	dHCV	8.5 (3.8-63)
HBV DNA 97/746	Ultrio	6.3 (4.4-11)
	dHBV	12 (5.6-69.1)

* Performed on 3 separate days with eight replicates per day, for a total of 24 replicates.
† Performed on 1 day with a total of eight replicates.

Operational performance

A total of 4210 donations in IDT and 6080 donations in 1520 pools of MP4 were tested with the Ultrio assay on the TIGRIS platform. A summary of the testing data is shown in Tables 2 and 3. The non-repeat-reactive rates were 0.07% for IDT and 0.13% for MP4. There were 23 invalid results among 4210 specimens tested IDT (0.55%) and 5 invalid results among the 1520 pools tested (0.33%). All invalid results were valid when the tests were repeated. The retest specimen rate of 0.27% was mostly a result of assay processing errors.

Seronegative donor specimens tested in IDT and MP4

Testing results for 10,290 donor specimens by serology and by Ultrio assay in IDT (4210) and in MP4 (6080) are *shown in Fig. 1A. None of the NAT-only-reactive samples* were discriminated as either HIV or HCV. Among the 4179 seronegative specimens tested in IDT, 10 were Ultrio assay reactive. Six of these were discriminated as HBV, while four were nonreactive in discriminatory testing. These 10 specimens were further analyzed; 9 of 10 were found to be positive for HBV by alternative PCR, viral load, or genotyping and were regarded as potential yield cases. The results are summarized in Table 4. For donor IDT-A9, it was considered an indeterminate result. IDT-A9 was initially Ultrio assay *reactive but no HBV, HCV, or HIV nucleic acid* detectable (data not shown). Among the 6044 seronegative specimens in MP4, three were reactive in the Ultrio assay and were all discriminated as HBV and were also reactive in the NGI HBV UltraQual assay. These three specimens were further studied as potential yield cases as summarized in Table 4. In total, there were 12 potential yields cases, nine from IDT and three from MP4. They were between ages of 30 and 63, with equal male-to-female ratio.

Follow-up study of potential yield cases

Among the 12 potential yield cases, 10 donors joined in the follow-up study; results of the samples are listed in Table 4. All index samples and follow-up samples were anti-HBc positive, except the index sample of donor MP4-A3. Donors IDT-A1 and IDT-A3 became HBV DNA negative a few months after the index donations.

The combination of molecular and serologic marker data allows further definition of the diagnostic phase of HBV infection (Table 4). The presence of anti-HBc in all but one index sample excluded preseroconversion WP infection and in four cases anti-HBs were also detected indicating resolved infection. In IDT-A9 where molecular confirmation was doubtful, the presence of anti-HBc did not particularly help the diagnostic process because 16% to 90% in the Taiwanese general population[4,5] carry this marker. The potential yield cases were genotyped as B2, except donor IDT-A10. This sample could not be amplified in any of the four different regions targeted, although the viral load tested by qPCR provided a positive result.

Finally, in 10 donors, at least one follow-up sample was obtained and this

TABLE 2. Summary of IDT and MP4 run results on Ultrio TIGRIS

Variable	IDT	MP4	Total
Number of individual donor samples	4,210	6,080	10,290
Total number of pools tested	4,210	1,520	5,730
Number of initially reactive pools	32	23	55
Initial reactive rate	0.76	1.51	0.96
Number of resolved pools	NA	21	21
Number of reactive donation(s) on discrimination assay	28	21	49
Non–repeat-reactive IDT/pools (%)	4 (0.09)	2 (0.13)	6 (0.1)
Total number of batches	21	24	45
Total invalid batch (%)	1* (4.76)	0 (0)	1 (2.22)
Total retested donor samples† (%)	23 (0.55)	5 (0.33)	28 (0.27)
Assay processing error	22	5	27
Internal control invalid	1	0	1

* Caused by negative control and HIV-1–positive control volume error.
† The retested donor samples resulted from invalid tests or invalid batch.
NA = not available.

TABLE 3. Summary of serology and Ultrio results observed

Result	IDT				MP4			
	HIV-1	HCV	HBV	Total	HIV-1	HCV	HBV	Total
Seropositive/Ultrio nonreactive	4	1	4	9	7	0	9	16
Seropositive/Ultrio reactive and discriminated	0	2	20	22	0	4	16	20
Seronegative/Ultrio reactive	0	0	6	6	0	0	3	3
Seronegative/Ultrio reactive and nondiscriminated				4*				0
Seronegative/Ultrio nonreactive				4169				6041

* Three confirmed reactive; one indeterminate result by alternative NAT (see Table 4).

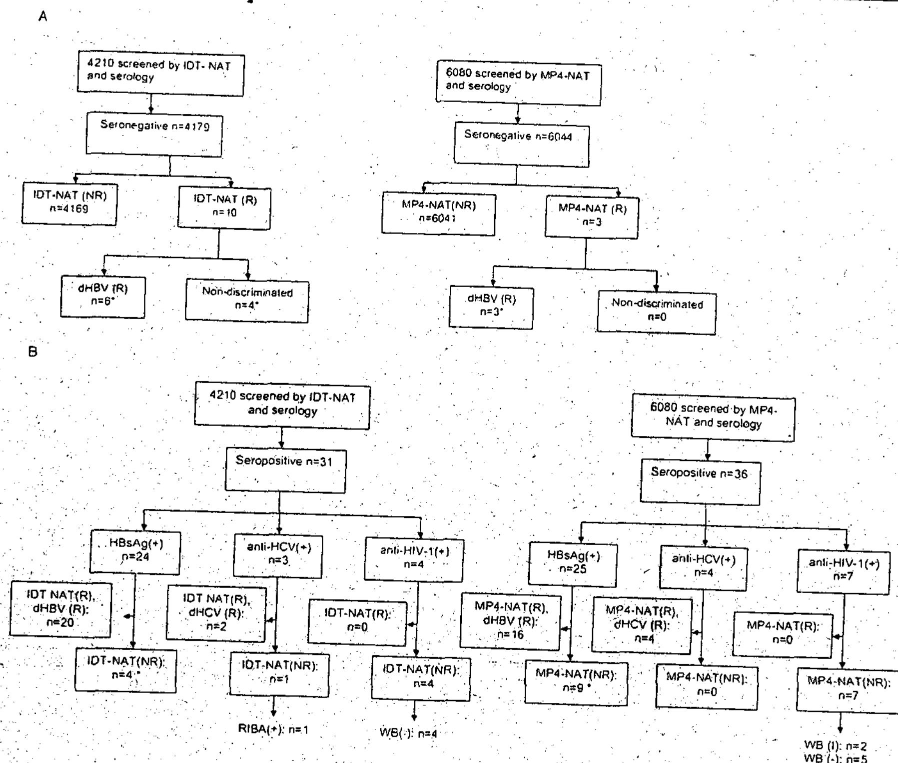

Fig. 1. (A) Results of seronegative donations screened by IDT and MP4 and serology. NR = nonreactive; R = reactive. *All these samples are discussed in Table 4. (B) Results of seropositive donations screened by IDT and MP4 and serology. NR = nonreactive; R = reactive. I = indeterminate; RIBA = recombinant immunoblot assay; WB = Western blot. *All these samples are discussed in Table 5.

refined the preliminary classification obtained on the basis of molecular and serologic results. In eight cases, the results obtained in the index samples were reproduced in the follow-up samples available confirming the diagnosis of OBI. In two cases, IDT-A3 and IDT-A6, a low level of anti-HBs was found in follow-up samples indicating cases of resolved infections with fluctuating levels of anti-HBs. In IDT-A5 HBsAg was detected in the follow-up sample together with the persistence of anti-HBc already present in the index sample. This profile suggested a chronic HBV infection with fluctuating, low-level, HBsAg. In MP4-A3, the follow-up sample became anti-HBc positive, while it was negative in the index donation. And the presence of HBV DNA in index donation of MP4-A3 suggested that this donor was a window case during that time.

Seroreactive donor specimens tested in IDT and MP4

Testing results for the 31 seroreactive donor specimens identified among the 4210 tested in IDT and for the 36 seroreactive specimens within the 6080 samples screened in MP4 are shown in Fig. 1B. Among the 31 specimens tested in IDT, 24 were HBsAg reactive, three were anti-HCV reactive, and three anti-HIV reactive. Twenty of the 24 HBV-seroreactive specimens were also dHBV reactive,

TABLE 4. Confirmation and possible status of HBV yield cases in Taiwan

Donor ID	Time (days)	HBV DNA: dHBV	HBV DNA: Alt PCR*	HBV DNA: Viral load (IU/mL)†	HBV DNA: Genotype‡	HBV serological markers: HBsAg (PRISM)	HBV serological markers: Anti-HBc§	HBV serological markers: Anti-HBs (mIU/L)¶	Possible HBV status of donors
IDT-A1	Index	R[5]	P	7	B2	N	P	N	OBI
	81		P			N	P	N	
	199		P			N	P	N	
	261		N			N	P	N	
IDT-A2	Index	R	P	15	B2	N	P	488	OBI
	85		P			N	P	367	
	276		P			N	P	434	
IDT-A3	Index	R	P	N	B2	N	P	N	OBI
	82		P			N	P	13	
	144		N			N	P	8	
	215		N			N	P	6	
IDT-A4	Index	R	P	48	B2	N	P	N	OBI
	77		P			N	P	N	
IDT-A5	Index	R	P	<5	B2	P**	P	N	CHBV††
	215		P			P	P	N	
IDT-A6	Index	R	P	<5	B2	N	P	N	OBI
	160		P			N	P	N	
						N	P	11	
IDT-A7	Index	NR	P	N	B2	N	P	N	OBI
	189		P			N	P	N	
IDT-A8	Index	NR	N	N	B2	N	P	86	OBI
	185		N			N	P	65	
	256		N			N	P	66	
IDT-A9	Index‡‡	NR	N	N	NA	N	P	P	Ind§§
IDT-A10	Index‡‡	NR	N	6.4	NA	N	P	P	OBI
MP4-A1	Index	R	P	9	B2	N	P	N	OBI
	175		P			N	P	N	
MP4-A2	Index‡‡	R	P	N	B2	N	P	N	OBI
MP4-A3	Index	R	P	N	B2	N	N	N	WP
	253		P			N	P	N	New infection

* Alternative PCR by NGI HBV UltraQual.
† Results of Cambridge qPCR with numbers indicating viral load in IU/mL. <5 indicates a signal too low to allow reliable quantification.
‡ HBV genotyped by sequencing.
§ The results correspond to IgG anti-HBc. All the anti-HBc IgM determinations were N.
¶ Anti-HBs is given either qualitative (P or N) or quantitative in mIU/L.
** HBsAg N by Abbott Murex (S/CO = 0.9) and Ortho Assays; P by PRISM in subsequent analysis.
†† Chronic HBV infection with low and fluctuating HBsAg level.
‡‡ Donor was lost to follow-up.
§§ Indeterminate result, possibly contamination or OBI.
N = negative; NA = not available; NR = not reactive; P = positive; R = reactive.

while four were nondiscriminated and were further investigated (Table 5).

Thirty-six seroreactive specimens (25 HBsAg, four anti-HCV, and seven anti-HIV) were involved in NAT MP4 testing. Sixteen of the 25 HBsAg-reactive specimens were dHBV reactive and were considered true positive while nine were not and were further investigated as shown in Table 5.

Of the seven anti-HCV–reactive specimens (three IDT and four MP4), six were HCV RNA reactive. One of the three IDT-reactive specimens was found to be dHCV nonreactive. Of the 11 anti-HIV–reactive specimens (four IDT and seven MP4), none were HIV RNA reactive and none were confirmed antibody positive by Western blot (see Fig. 1B).

DISCUSSION

In a region where up to 90% of the population has evidence of past exposure or ongoing infection for HBV,[5] undetected OBIs pose a great threat to blood safety. While NAT only yield cases may occur under a number of circumstances—1) acute infection in the WP, 2) tail end of a chronic HBV infection, 3) persistence of low-level HBV replication in the presence of anti-HBs, and 4) escape mutant not detected by current HBsAg assays[22,23]—for this discussion we restrict the term of OBI to refer to HBV infection with the presence of anti-HBc and/or anti-HBs with no other detectable HBV markers except for HBV DNA.[24] While the transfusion transmission risk is lower for OBIs than for WP infections,[25] OBIs numerically pose a more significant threat to the blood supply, especially in HBV-endemic countries.[1,26]

In Asia, Taiwan in particular, many reports indicated that HBV DNA could be present, generally at a low level, in HBsAg-negative but anti-HBc–positive blood donations.[4,7,27] The proportion of this type of blood donation (1%-7%) was considerably higher than in low-prevalence Western countries (0%-3.5%).[26,28-31] Identifying and

TABLE 5. Profile of the 13 Ultrio-nonreactive initially HBsAg-positive (Abbott Murex) specimens

Donor ID	HBV serologic markers: HBsAg: Murex (S/CO*)	PRISM	Neutralization	HBV antibodies: Anti-HBc	Anti-HBs*	HBV DNA: Ultrio IDT (Reactive/Total)	Ultrio dHBV	Alt PCR†
IDT-B1	28.56	P	P	P	N	NR (0/3)	NA	R
IDT-B2	10.89	P	P	P	N	NR (0/3)	NA	NR
IDT-B3	6.68	P	P	P	N	NR (0/3)	NA	NR
IDT-B4	3.56	P	P	P	N	R (1/3)	NR (0/3)	R
MP4-B1	50.66	P	P	P	N	R (3/3)	HBV	R
MP4-B2	3.47	P	P	P	N	R (1/3)	HBV	R
MP4-B3	1.59	P	P	P	N	R (2/3)	NR (0/3)	NR
MP4-B4	1.45	P	P	P	N	NR (0/3)	NA	R
MP4-B5	10.72	P	P	P	N	NR (0/3)	NA	NR
MP4-B6	1.32	N	Not confirmed	P	N	NR (0/3)	NA	NR
MP4-B7	1.12	N	Not confirmed	N	>1000	NR (0/3)	NA	NR
MP4-B8	2.22	N	Not confirmed	N	>1000	NR (0/3)	NA	NR
MP4-B9	1.00	N	Not confirmed	N	N	NR (0/3)	NA	NR

* Anti-HBs is given either qualitative (P or N) or quantitative in mIU/L.
† Alternative NAT: NGI HBV UltraQual assay used LOD = 0.9 IU/mL.
NA = not available; NR = nonreactive; R = reactive.

excluding such donations from the blood supply in Taiwan is important since it was demonstrated that this type of blood can be infectious by transfusion.[6,7,25] The risk of HBV transmission with the anti-HBc–"alone" blood has been reported to cover a wide range (0.4%-90%). In contrast, in a Japanese study, no donations containing both HBV DNA and anti-HBs were found infectious through transfusion.[25] However, a recent report from Slovenia presented two cases of HBV transmission by transfusion of an OBI unit containing low levels of anti-HBs.[32] Furthermore, vaccinated children with low levels of anti-HBs but relatively immunocompromised appeared to be susceptible to HBV infection after transfusion with HBsAg-negative blood products.[6] Therefore, on the basis of these studies, it appears important for blood safety in Taiwan that routine HBV NAT be implemented in addition to the current HBsAg screening.

Assay performance characteristics are critical to the interdiction of potentially infectious donations. A UK model, adjusted for test and processing errors, revealed that 22% of the risk of transfusion-transmitted infections (including HBV, HCV, and HIV) was the result of test failures and operational errors,[33] underscoring the need for a robust, reliable screening assay. The Ultrio assay in our hands had both a low invalid test rate of 0.27% and a low overall non–repeatable-reactive rate of 0.07% for IDT and 0.13% for MP4. These characteristics, along with its high assay sensitivity and specificity, provide a suitable system for routine screening of the blood supply in Taiwan.

The most critical assay attribute for detection of low-level viremia is analytical sensitivity. Our evaluation showed the Ultrio assay to be highly sensitive with 95% LODs of 18.41, 4.38, and 6.28 IU/mL for HIV-1, HCV, and HBV, respectively, and 13.97, 8.54, and 12.04 IU/mL for the respective discriminatory assays (Table 1). These results are consistent with the claims stated in the package insert (PROCLEIX ULTRIO assay, Package Insert IN0167EN rev. 2, 2004, Gen-Probe Inc., San Diego, CA) and with the findings of other investigators.[6,14,34,35]

While it was demonstrated that testing in plasma pools of small sizes was essentially as efficient as IDT for HIV-1 and HCV, pooling had a substantial impact on the efficacy of detecting low-level HBV DNA. Results presented in Tables 3 and 4 show that proportionally more HBV DNA–positive samples were identified among HBV-containing donations in IDT (87.9%) than in MP4 (67.9%). Our study provides an opportunity to determine the distribution of concordant and discordant blood donor samples between the two main HBV tests: HBsAg and HBV DNA. Among the HBV-containing donations, IDT identified 60.6% positive for both HBsAg and HBV DNA, 12.1% HBsAg only, and 27.3% HBV DNA only, whereas MP4 identified 57.2% positive for both HBsAg and HBV DNA, 32.1% HBsAg only, and 10.7% HBV DNA only. This distribution is similar to the data in our previous study[13] (58.6, 26.8, and 14.6%, respectively). Although the two testing populations in this study show different HBsAg-reactive rates (0.57% for IDT and 0.41% for MP4), they are not much different compared to the 0.48% reactive rate of Taiwanese donor population in 2007 (from Taiwan Blood Services Foundation annual report 2007). The distribution observed in an area like Taiwan, where HBV Genotypes B and C are prevalent, considerably differs from data generated in Ghana, West Africa, where Genotype E is prevalent and, tested with the Cambridge qPCR used in this study, 84% of samples were HBsAg and HBV DNA positive, 6% HBsAg only, and 10% DNA only.[36]

Additionally, the data presented in Table 5 suggest that some HBsAg-positive samples may carry an extremely low level of HBV DNA, below the LOD of most assays currently available for blood testing. This lack of sensitivity would be further compounded by any level of

pooling. Several options can be offered to address this issue in addition to IDT-NAT, such as extraction from larger plasma volume or concentration of viral particles by high-speed centrifugation.[36] Nevertheless, data of our study demonstrate that, at least for the time being, HBsAg and HBV DNA screening are complementary and that both are beneficial for the blood safety.

One important issue for NAT is the confirmation and characterization of yield cases to appropriately inform the implicated donors. As shown in Table 4, there are three successive levels of supplementary testing that can help to achieve this goal: 1) alternative NAT assays for HBV DNA, 2) detection of other HBV serologic markers to refine the HBV infection profile, and 3) testing follow-up samples to reach a suitable diagnosis. To verify potential (HBsAg-negative, NAT repeat-reactive) and probable (HBsAg-negative, NAT-reactive, and alternative NAT-reactive on an alternate specimen) yield cases, we subjected index samples to molecular analysis and genotyping and we tested follow-up specimens from these donors with six different serologic tests and three alternative NAT assays. Parts of the HBV genome (pre-core, pre-S, and S) along with the full genome were amplified in most index cases. All yield cases were Genotype B2, which is the predominant genotype in Taiwan.[37] The qualitative NAT (NGI HBV UltraQual) with a 95% LOD of 0.9 IU/mL detected HBV DNA in follow-up specimens from 9 of 11 potential yield donors, whereas a quantitative NAT with a LOD of 100 IU/mL (Quest Diagnostics) was not able to quantify DNA in any of the follow-up specimens, although it detected an HBV signal in six donors (data not shown). A third highly sensitive quantitative NAT with a LOD of 20 IU/mL (in-house PCR, Cambridge University Laboratories), only being used for testing the index donations, found HBV DNA levels ranging from less than 5 to 48 IU/mL, underscoring the assay sensitivity as a defining factor for the detection of DNA in these low-level specimens. In addition, nested amplification of multiple regions of the HBV genome after concentration by ultracentrifugation proved to be the most reliable and sensitive method of confirmation (Table 4). These data illustrate the need for alternative NATs with high assay sensitivity in confirming the presence of HBV DNA in donation samples.

The seroconversion of HBsAg and/or other HBV markers in a donor with a totally seronegative index donation distinguishes between WP infection and other diagnoses. In Case MP4-A3, anti-HBc is detected after 8 months (Table 4). Both HBV DNA and anti-HBs levels are known to fluctuate in some cases. Here, examples of such fluctuations are seen in Cases IDT-A1 and IDT-A3.

The HBV yield rate for the IDT Ultrio assay (0.21%) in Taiwan was about five times higher than was observed in Hong Kong[8] and was 10- to 100-fold higher than reported in countries with low HBV prevalence. The 12 yield cases, 10 of which were verified by NAT reactivity in follow-up specimens, are consistent with the finding of our previous study[13] on a different cohort of our donor population and with a different NAT system.

The results of this study could be used to estimate the impact of adding NAT for the whole blood donor population in Taiwan. HBV DNA screening by IDT together with HBsAg testing would initially identify 3919 confirmed donations per 500,000 donors tested. Comparing to current HBsAg screening alone, it will interdict 1069 additional infectious donations potentially transfused to more than 1000 recipients.

In summary, our study demonstrated that the great majority of our yield cases were of OBI and that these yield samples had very low viral load, necessitating the use of a highly sensitive NAT for detection. The yield rate observed with the IDT approach was higher than that observed with MP4 approaches in this study which confirmed the higher clinical utility of the more sensitive IDT approach. Implementation of HBV NAT screening, especially with the IDT format, shows promise in enhancing the safety of the blood supply in Taiwan.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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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

○小児B型肝炎キャリア187例の感染実態と現在のHBV感染予防対策の問題点

目的:小児B型肝炎ウイルス(HBV)キャリア患者の感染経路・感染要因を解析し、現在のHBV感染予防対策の問題点を明らかにする。

方法:大阪府立母子保健総合医療センター消化器・内分泌科(施設1)、大阪大学医学部小児科(施設2)、及び大阪府立急性期・総合医療センター小児科(施設3)に通院歴のあるHBVキャリア小児について後方視的に検討した。

結果:施設1では32例、施設2では133例、施設3では22例の合計187例のHBVキャリアが診療を受けていた。男女比は1.43:1、診断時年齢は中央値2歳(0ヶ月～15歳)であった。母児感染予防処置が行われるようになった1986年以前の出生児と、以後の出生児に分けて検討した。1985年までに出生していた症例は102例で、母児感染59例(57.8%)、父子感染6例(5.9%)、輸血5例(4.9%)、水平感染31例(30.4%)、不明1例で母児感染が過半数を占めていた。一方、1986年以降に出生した症例は85例で、母児感染51例(60%)、父子感染13例(15.3%)、輸血2例(2.4%)、水平感染19例(22.4%)であった。母児感染の割合は1985年までに出生していた症例と変化なく、父子感染は増加した。母児感染のうち胎内感染が16例、予防処置実施中あるいは実施後にHBV感染が判明した症例が22例で、現在の予防法で防ぐことができなかった症例が合計38例(74.5%)であったが、予防処置の不完全施行や未施行によるものが8例(15.7%)存在した。父子感染や水平感染の症例でHBワクチンの投与を受けていたものはいなかった。

結論:HBV母児感染予防処置導入後も小児のHBVキャリアは発生している。母児感染のうち約15%は予防処置の不完全施行や未施行が原因であり、医療者の啓発を行うとともに、予防処置プロトコールを簡略な国際方式にすることにより完遂率が高まると思われる。また、父子感染・輸血を含めた水平感染例も4割を占めており、諸外国のように日本でも出生後早期にHBユニバーサルワクチンが導入されることが望まれる。胎内感染例については出生後の予防処置では防ぐことができず、HBVキャリア妊婦へのHBIGや抗ウイルス剤投与などを行うべきか、今後検討していく必要がある。

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
B型肝炎ウイルス(HBV)母児感染予防措置導入以前と以後の小児HBVキャリア患者の感染経路・感染要因を比較・解析し、ユニバーサルワクチン導入の必要性を述べた報告である。 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本製剤の安全性は確保されており、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。なお、日本赤十字社では献血時のスクリーニング法としてより感度の高い化学発光酵素免疫測定法(CLEIA)および新NATシステムを導入した。

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JRC2010T-027

A72　肝臓　51巻 suppl. (1) (2010)

WS4-5　B型肝炎母子感染防止事業開始後に誕生した若年者におけるB型肝炎ウイルス感染についての検討

林 和彦, 片野義明, 後藤秀実
名古屋大学医学部消化器内科

【目的】1985年に厚生省B型肝炎母子感染防止事業による感染予防策が開始され, HBV感染者の新規発生が大幅に抑制された. しかしながら, 1985年以降に生まれた若年者にB型慢性肝炎(CHB)は存在しているが, その詳細は不明である. そこで1985年以降に誕生した若年者のCHBを対象に, その臨床的, ウイルス学的な解析を行った.

【方法】当院に通院中のCHB372例, 男220例, 女152例, 平均年齢43.9±1.4歳(3-86歳)である. 1985年以降(Young; Y群)と1960-1985(Middle; M群), 1960年以前(Old; O群)誕生の3群に分類して比較した. HBVsubgenotypeは, preS領域のダイレクトシークエンス法で判定した. S領域のダイレクトシークエンスでワクチンエスケープ変異(VEM)を検索した.

【成績】Y群12例, M群259例, O群101例とY群は全体の3.2%であった. Y群の感染経路は: 母子感染例が9例, 水平感染が3例. 母子感染では, ワクチン未接種例が2名で海外からの移民であった. 他の7例はすべて出産後に標準的な母子感染予防を施行したが, 感染が予防できなかった無効例であった. 水平感染例は, それぞれ手術, 鍼灸, 性行為が感染経路と推定された. Y群のHBVsubgenotypeは10例Ceで, BaとAeがそれぞれ1例であった. Baはフィリピンからの移民で, Aeは性行為感染後の慢性化例であった. M群, O群のgenotype Ceの分布は78.4%, 80.2%と同様であったが, genotype Aeの分布は6.9%, 1.9%とM群に広く感染していた. Y群のHBe抗原は11例陽性であり, すべてBCP/PC変異は野生型であった. Y群のG145Aなど既報のVEMはなかった. y群4例の全塩基配列を解析したが, 有意な変異は確認できなかった.

【考案】母子感染予防無効例にVEMを有するCHBはなくウイルス因子以外に, ホスト側の要因も重要であることが示唆された. また移民のHBV感染を認めており, 今後さらに海外交流が盛んになると推測するのでわが国も地球規模でのHBV予防対策が望まれる. 母子感染が75%を占めていたが, 水平感染例もあり, これら症例に対してはユニバーサルワクチンが有効と考える. また19歳での感染後の慢性化したgenotype Aeも存在し, M群のCHBにおいてgenotype Aeは増加しており, このhigh risk groupに対してもワクチンなどの対策が必要と思われる.

【結語】母子感染防止事業開始後に発症したCHBの多くは母子感染予防無効例で, 一部に移民や慢性化したgenotypeAeも存在した.

WS4-6　小児B型肝炎キャリア187例の感染実態と現在のHBV感染予防対策の問題点

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大阪府立母子保健総合医療センター消化器・内分泌科[1], 大阪大学大学院医学系研究科医学部小児科学[2], 大阪府立急性期・総合医療センター小児科[3]

【目的】小児B型肝炎ウイルス(HBV)キャリア患者の感染経路・感染要因を解析し, 現在のHBV感染予防対策の問題点を明らかにする.

【方法】大阪府立母子保健総合医療センター消化器・内分泌科(施設1), 大阪大学医学部小児科(施設2), および大阪府立急性期・総合医療センター小児科(施設3)に通院歴のあるHBVキャリア小児について後方視的に検討した.

【成績】施設1では32例, 施設2では133例, 施設3では22例の合計187例のHBVキャリアが診療を受けていた. 男児110例, 女児77例で男女比1.43:1と男児が多かった. 診断時年齢は中央値2歳(0ヶ月～15歳)であった. これらの症例の感染経路について, 母児感染予防処置が行われるようになった1986年以前の出生児と, 以後の出生児に分けて検討した. 1985年までに出生していた症例は102例で, 母児感染59例(57.8%)父子感染6例(5.9%)輸血5例(4.9%)水平感染31例(30.4%)不明1例で母児感染が過半数を占めていた. 母親のHBV感染の詳細が判明したものは8例だけで, うち5例がHBeAg陽性, 3例がHBeAb陽性であった. 父親については3例の情報があり, 全例HBeAg陽性であった. 一方1986年以降に出生した症例は85例で, 母児感染51例(60%), 父子感染13例(15.3%), 輸血2例(2.4%), 水平感染19例(22.4%)であった. 母児感染の割合は1985年までに出生していた症例と変化なく, 父子感染は増加していた. 母親のHBV感染の詳細が判明したものは29例あり, 全例HBeAg陽性であった. 父親については3例がHBeAg陽性, 1例がHBeAb陽性であった. 母児感染のうち胎内感染が16例, 予防処置実施中あるいは実施後にHBV感染が判明した症例が22例で, 現在の予防法では感染を防ぐことができなかった症例が合計38例(74.5%)であったが, 予防処置の不完全施行や未施行によるものが8例(15.7%)存在した. 父子感染や水平感染の症例で, HBワクチンの投与を受けていたものはいなかった.

【結論】HBV母児感染予防処置導入後も小児のHBVキャリアは発生している. 母児感染のうち約15%は予防処置の不完全施行や未施行が原因であり, 医療者の啓発を行うとともに, 予防処置プロトコールを簡略な国際方式にすることによって完遂率が高まると思われる. また父子感染・輸血を含めた水平感染症例も4割を占めており, 諸外国のように日本でも出生後早期にHBユニバーサルワクチンが導入されることが望まれる. 胎内感染例については, 出生後の予防処置では防ぐことができず, HBVキャリア妊婦へのHBIGや抗ウイルス剤投与などを行うべきか, 今後検討していく必要がある.

医薬品 研究報告 調査報告書

<table>
<tr><td>識別番号・報告回数</td><td></td><td></td><td>報告日</td><td>第一報入手日
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該当なし</td><td rowspan="3">総合機構処理欄</td></tr>
<tr><td>一般的名称</td><td colspan="2">人血清アルブミン</td><td rowspan="2">研究報告の公表状況</td><td rowspan="2">大塚裕司, 平力造, 百瀬俊也, 日野学. 第58回日本輸血・細胞治療学会総会; 2010 May 28-30; 愛知</td><td>公表国</td></tr>
<tr><td>販売名(企業名)</td><td colspan="2">赤十字アルブミン20(日本赤十字社)
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赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)</td><td>日本</td></tr>
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研究報告の概要

○2009年輸血関連感染症報告症例の解析と傾向

はじめに:2009年に全国の医療機関から報告された輸血関連感染症例(疑い例を含む)の解析結果と医療機関における「血液製剤等に係る遡及調査ガイドライン」(以下GL)に基づいた輸血前後の患者検体の検査実施状況等について報告する。

対象と方法:2009年に医療機関より報告された症例を対象とし、献血者検体(献血者の保管検体等の個別NAT、当該製剤(使用済みバッグ)等の無菌試験等)と患者検体の調査により輸血との因果関係を評価した。また、医療機関における患者の輸血前後の検査の実施項目等を2007、2008年時と比較した。

結果と考察:10月末現在の報告数は82例(HBV 37例、HCV 21例、細菌 20例、パルボB19 2例、HEV 1例、CMV 1例)であり、輸血との因果関係が高いと評価した症例は、HBV 5例、HEV 1例、及び細菌 1例であった。医療機関でのGLに基づく輸血前後の患者検体の検査実施数(輸血前:HBs抗原/HBs抗体/HBc抗体, 輸血後:HBV-DNA)はHBV症例で2007年6例(8%)、2008年12例(20%)、2009年9例(24%)であった。またHCV症例では(輸血前:HCV-RNA or HCVコア抗原/HCV抗体, 輸血後:HCV-RNA or HCVコア抗原)2007年12例(29%)、2008年5例(12%)、2009年5例(24%)であった。細菌症例での医療機関における患者血培の実施数は、2007年27例(90%)、2008年43例(94%)、2009年20例(100%)であった。また、医療機関からの使用済みバッグの提供が2007年17例(57%)、2008年35例(76%)、2009年17例(85%)であった。これらのことによりGLが医療機関に浸透していることが推察された。

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
2009年に全国の医療機関から報告された輸血関連感染症例(疑い例を含む)の解析結果と医療機関における「血液製剤等に係る遡及調査ガイドライン」(以下GL)に基づいた輸血前後の患者検体の検査実施状況等についての報告である。なお、2009年12月末現在までの報告数は98件(HBV 45例、HCV 26例、細菌 23例、パルボB19 2例、HEV 1例、CMV 1例)、報告中、輸血との因果関係が高いと評価した症例はHBV 7例、HEV 1例、細菌 2例となっている。医療機関でのGLに基づく輸血前後の患者検体の2009年の検査実施数は、HBV症例 9例(20%)、HCV症例 9例(35%)、細菌症例の血培実施数23例(100%)、また医療機関からの使用済みバッグの提供は20例(87%)となっている。	これまで本製剤を介してこの報告で輸血後感染が示唆された病原微生物の感染はない。除菌工程やモデルウイルスによるバリデーション成績に鑑み、本製剤の安全性は確保されており、特別の対応を必要としないが、今後も輸血感染症に関する新たな知見等について今後も情報の収集に努める。なお、日本赤十字社では献血時のスクリーニング法としてより感度の高い化学発光酵素免疫測定法(CLEIA)および新NATシステムを導入した。

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日本輸血細胞治療学会誌　第56巻　第2号

WS-1-2　2009年輸血関連感染症報告症例の解析と傾向

日本赤十字社血液事業本部
大塚裕司, 平　力造, 百瀬俊也, 日野　学

【はじめに】2009年に全国の医療機関から報告された輸血関連感染症例(疑い例を含む)の解析結果と医療機関における「血液製剤等に係る遡及調査ガイドライン(以下GL)」に基づいた輸血前後の患者検体の検査実施状況等について報告する.
【対象と方法】2009年に医療機関より報告された症例を対象とし, 献血者検体(献血者の保管検体等の個別-NAT, 当該製剤(使用済みバッグ)等の無菌試験等)と患者検体の調査により輸血との因果関係を評価した. また, 医療機関における患者の輸血前後の検査の実施項目等を2007, 2008年時と比較した.
【結果と考察】10月末現在の報告数は82例(HBV 37例, HCV 21例, 細菌20例, パルボB19 2例, HEV 1例, CMV 1例)であり, 輸血との因果関係が高いと評価した症例は, HBV 5例, HEV 1例及び細菌の1例であった.
医療機関でのGLに基づく輸血前後の患者検体の検査実施数(輸血前:HBsAg/HBsAb/HBcAb, 輸血後:HBV-DNA)はHBV症例で2007年6例(8%), 2008年12例(20%), 2009年9例(24%)であった. またHCV症例では(輸血前:HCV-RNA or HCVコア抗原/HCV-Ab, 輸血後:HCV-RNA or HCVコア抗原)2007年12例(29%), 2008年5例(12%), 2009年5例(24%)であった. 細菌症例では医療機関における患者血培の実施数は, 2007年27例(90%), 2008年43例(94%), 2009年20例(100%)であった. また医療機関からの使用済みバッグの提供が2007年17例(57%), 2008年35例(76%), 2009年17例(85%)であった. これらのことによりGLが医療機関に浸透していることが推察された. 今後, 患者検体の日赤への提供状況等を併せて調査し, 報告する予定である.

WS-1-3　献血由来の血漿分画製剤製造メーカーで実施したHEV-NAT検査と, 遡及調査により判明した輸血後E型急性肝炎の1症例

武蔵野赤十字病院輸血部[1], 東京都赤十字血液センター学術二課[2], 中央血液研究所感染症解析部[3]
森　威典[1], 清水隆弘[1], 中村圭太[1], 鈴木　光[1], 内田茂治[3], 長田　薫[1]

【はじめに】日本国内で輸血によるE型肝炎ウイルス(HEV)感染が2002年に初めて報告され, 現在まで数例の報告がある. 今回我々は, 献血由来の血漿分画製剤製造メーカーより, 原料血漿の受け入れ試験として実施したHEV核酸増幅検査(NAT)によってHEV-RNAが検出されたとの報告があり, 当該血液から製造された赤血球製剤の遡及調査により, 輸血後E型急性肝炎が判明した事例を経験したので報告する.
【症例】患者は74歳男性. 2008年5月に敗血症, 脊髄硬膜外膿瘍, ARDS, 急性腎不全などの重篤な病態にて当院救命救急科に入院. 6月～10月までにRCC-LR計40単位の輸血を必要とした. 8月下旬に一過性の急激なAST/ALTの上昇を認めたが, 重篤な病態にて種々の薬剤が投与されていることもあり, 原因の特定には至らなかった. 肝機能は11月には正常化し, 2009年1月に原疾患の回復により退院した. 2009年5月, 赤十字血液センターに血漿分画製剤製造メーカーより, 献血由来の血液からHEV-NATにてHEV-RNAが検出されたとの報告があった. そして, 当該血液由来のRCC-LRが当院に供給されたと遡及調査の依頼があり, 2008年8月22日に前記患者に輸血されていたことが判明した. 輸血前後の患者保管血漿によるHEV抗体, HEV-RNAが輸血前は陰性, 輸血後は陽性, さらに献血者および患者のウイルス塩基配列も完全に一致したため, 輸血によるHEV感染であることが証明された. よって, 患者は輸血によるHEV感染から急性肝炎を発症したものと考えられた.
【考察】本症例は血漿分画製剤製造メーカーによるHEV-NATと, 輸血前検体の保管により判明した輸血後E型急性肝炎であり, 検体保管の重要性を再認識した. また, 輸血後AST/ALTの急激な上昇が見られ, HBV, HCVの感染を認めないケースでは, HEV感染も要因の一つとして疑う必要があると思われた.

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2010. 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	平力造, 大塚裕司, 鈴木光, 百瀬俊也, 内田茂治, 日野学, 第58回日本輸血・細胞治療学会総会; 2010 May 28-30; 愛知	公表国 日本	
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

○スクリーニングNATのプール数の縮小効果について

はじめに：日本赤十字社では血液製剤等のHBV、HCV、HIVへの安全対策として1999年7月にプール検体(500本)によるスクリーニングNAT(試薬：AMPLINAT MPX(AMP-NAT))を開始した。その後、プール検体数を50本、20本へと縮小し、2008年8月から検出感度向上を目的に新NATシステム(試薬：TaqScreen MPX(Taq-NAT))を導入した。これらのプール数の縮小効果を医療機関から報告された感染症報告症例より検証した。

対象と方法：2000年1月から2009年10月までに医療機関より報告された感染症報告症例の内、輸血による感染を直接証明できた症例はHBV 91件、HCV 3件、HIV 1件であった。この原因となった輸血用血液の献血血液それぞれ87献血、3献血、1献血を対象にし、当該献血時のスクリーニングNATをプール検体数別・試薬別に分類した。

結果：献血血液の分類結果はHBV・HCV・HIV別に、50本プール前：8・0・0、50本プール/AMP-NAT(2000年2月-2004年7月：4.5年間)：46・2・1、20本プール/AMP-NAT(2004年8月-2008年7月：4年間)：30・1・0、20本プール/Taq-NAT(2008年8月-2009年10月：1.25年間)：3・0・0 であった。

考察：ウイルス増殖スピードの遅いHBVについて、プール検体数の縮小・試薬の検出感度向上により、輸血感染HBVの減少傾向が認められた。一方、ウイルス増殖スピードの速いHCV、HIVはスクリーニングNAT導入後約10年が経過した中で輸血感染HCVが3件、輸血感染HIVが1件と、NATの導入自体に効果があったことが推測された。2008年8月から導入された新NATシステムにより、更なる安全性向上に努めているところである。

使用上の注意記載状況・その他参考事項等

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
日本赤十字社で実施した、スクリーニングNATにおける段階的なプール検体数の縮小と、2008年8月からの試薬の検出感度向上による効果の検証である。日本赤十字社では、血清学的検査に加え、HBV、HCV、HIVについて20プールでスクリーニングNATを行い、陽性血液を排除している。また、「血液製剤等に係る遡及調査ガイドライン」(平成20年12月26日付薬食発第1226011号)に基づき、輸血感染症の調査を行っている。	日本赤十字社では、従来の凝集法と比べてより感度の高い、化学発光酵素免疫測定法(CLEIA)及び精度を向上させたNATシステムを導入している。これらの措置によって原料血漿への病原微生物の負荷が減少し、本製剤の安全性はより高まっている。今後も輸血感染症に関する新たな知見等について今後も情報の収集に努める。

⑥

日本輸血細胞治療学会誌 第56巻 第2号

WS-1-6 スクリーニングNATのプール数の縮小効果について

日本赤十字社血液事業本部
平 力造, 大塚裕司, 鈴木 光, 百瀬俊也, 内田茂治, 日野 学

[はじめに]日本赤十字社では血液製剤等のHBV, HCV, HIVへの安全対策として1999年7月にプール検体(500本)によるスクリーニングNAT(試薬：AMPLINAT MPX(AMP-NAT))を開始した. その後, プール検体数を50本, 20本へと縮小し, 2008年8月から検出感度向上を目的に新NATシステム(試薬：TaqScreen MPX(Taq-NAT))を導入した. これらのプール数の縮小効果を医療機関から報告された感染症報告症例より検証した.

[対象と方法]2000年1月から2009年10月までに医療機関より報告された感染症報告症例の内, 輸血による感染を直接証明できた症例はHBV(TT-HBV)91件, HCV(TT-HCV)3件, HIV(TT-HIV)1件であった. この原因となった輸血用血液の献血血液87献血, 3献血, 1献血を対象とし当該献血時のスクリーニングNATをプール検体数別・試薬別に分類した.

[結果]献血血液の分類結果はHBV・HCV・HIV別に, 50本プール前：8・0・0, 50本プール/AMP-NAT(2000年2月-2004年7月：4.5年間)：46・2・1, 20本プール/AMP-NAT(2004年8月-2008年7月：4年間)：30・1・0, 20本プール/Taq-NAT(2008年8月-2009年10月：1.25年)：3・0・0であった.

[考察]ウイルス増殖スピードの遅いHBVについて, プール検体数の縮小・試薬の検出感度向上によりTT-HBVの減少傾向が認められた. 一方, ウイルス増殖スピードの速いHCV・HIVは, スクリーニングNAT導入後約10年が経過した中でTT-HCVが3件, TT-HIVが1件とNATの導入自体に効果があったことが推測された. 2008年8月から導入された新NATシステムにより, 更なる安全性向上に努めているところである. 今後も, 安全対策の導入に際して, そのリスク評価を検討し, 輸血医療の安全性向上に資することとしたい.

WS-2-1 当院における貯血式自己血輸血の現状と問題点

安城更生病院血液輸血センター[1], 安城更生病院血液・腫瘍内科[2]
原田康夫[1], 山本喜之[1], 山本敦子[1], 伊藤達也[2]

【はじめに】自己血輸血は同種血輸血の副作用を回避し得る最も安全な輸血療法とされ, 近年, 積極的に導入されつつあるが, 返血実施のガイドラインは未だ確立されていない. そこで, 今回, 適正化に向けて当院における貯血式自己血の現状と問題点を検討したので報告する.

【対象と方法】2003年1月~2008年12月の貯血式自己血輸血の推移を診療科別に貯血数, 実施数, 同種血回避率, 廃棄率等の実施状況について科別解析を行った.

【結果】6年間の貯血式自己血採血総数は1204症例. 特に産科, 婦人科, 整形外科, 泌尿器科において年次増加が顕著であった. 自己血実施総数は1104症例で実施率は91.7%であった. 同種血併用総数は52件(うち30件は心臓血管外科), 同種血輸血回避率は95.3%で年間の変化は認められなかった. 科別廃棄率は0~35%に分布していた. 術後に実施された自己血輸血時の総平均Hb値は10.0g/dl(6.3g/dl~14.7g/dl)であったが, 中にはHb値未測定症例や, 比較的術後貧血が軽度な症例も含まれていた. 科別では産科9.5g/dl, 婦人科9.9g/dl, 整形外科9.9g/dl, 外科10.1g/dl, 血液内科10.4g/dl, 泌尿器科10.7g/dl, 心臓血管外科11.3g/dlであった. 使用時期別解析では, 術中使用率は74.5%, 術後使用では, 術後当日, 翌日で大半を占めたが最長は7日までであった. 科別術後実施率は, 心臓血管外科の0.5%~整形外科の75.5%の分布を示した.

【結語】当院の貯血式自己血輸血総数は年々増加傾向にある. しかしながら, その実施基準は未だ不統一であり, 廃棄状況にもばらつきが大きい. 術式や患者の状況によっても大きく左右されるが, 少なくとも, 各科ではHb値と実施時期に関して一定の基準策定が望まれる. 近年, 自己血輸血による副作用も注目されており, リスク, ベネフィットをより厳密に考慮した更に適正な自己血輸血の体制整備を進める必要がある.

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2010. 5. 11	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	47News. Available from: http://www.47news.jp/CN/201004/CN2010042901000540.html	公表国 日本	
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

○A型肝炎が増加、死亡例も 魚介類や水、注意呼び掛け
A型肝炎の患者が3月以降増加し、既に昨年1年間の患者数を超えたことが、国立感染症研究所の集計で分かった。劇症化し死亡したケースもあった。A型肝炎ウイルスに汚染された水や食材の摂取によって感染する。同研究所は「広い範囲で散発的な集団発生が起きている可能性がある。55歳未満はほとんどが抗体を持たず、高齢者は重症化しやすい」として、魚介類の十分な加熱など、注意を呼び掛けている。同研究所によると、今年の患者の報告数は3月上旬から増加、4月4日までの1週間では18人と、2007年以降では1週間当たりの人数が最多で、その後も多い状態が続いている。4月18日までの合計(速報値)は121人で昨年の報告数(115人)を超えた。4月11日までの5週間の患者81人をみると、年齢は20〜88歳、2例が劇症化し、うち1人が死亡した。福岡県、広島県などが多く、報告した医師が推定した原因食材は「カキ」が45%と最も多かった。

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

A型肝炎の患者が3月以降増加し、既に昨年1年間の患者数を超えたことが、国立感染症研究所の集計で分かったとの報告である。これまで、本剤によるHAV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HAV-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。

今後の対応

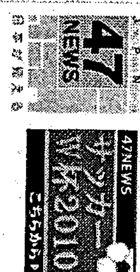
本製剤の安全性は確保されていると考えるが、今後もウイルスの検出や不活化する方策について情報の収集に努める。なお、日本赤十字社は、輸血後A型肝炎に対する対応として、問診で肝炎の既往があった場合、A型肝炎については治癒後6ヶ月間、家族に発症した人がいる場合は1ヶ月間献血不適としている。

⑦ 別紙2

MedDRA/J Ver.13.0J

2010年(平成22年)6月8日 [火曜日] 大安

JRC2010T-020



トップ　地域ニュース　共同ニュース　トピックス　コラム　スポーツ　エンタメ　マネー　動画　学び　特集　言葉ランキ

47NEWS > 共同ニュース > 記事詳細

ニュース詳細

| 特集 菅政権へ | 日本の実力 | ランキング

A型肝炎が増加、死亡例も 魚介類や水、注意呼び掛け

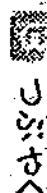
A型肝炎の患者が3月以降増加し、既に昨年1年間の患者数を超えたことが、国立感染症研究所の集計で29日分かった。劇症化し死亡したケースもあった。

A型肝炎ウイルスに汚染された水や食材の摂取によって感染する。同研究所は「広い範囲で散発的な集団発生が起きている可能性がある。55歳未満はほとんどが抗体を持たず、高齢者は重症化しやすい」として、魚介類の十分な加熱など、注意を呼び掛けている。

同研究所によると、今年の患者の報告数は3月上旬から増加、4月4日までの1週間は18人と2007年以降では1週間当たり最多で、その後も多い状態が続く。4月18日までの合計(速報値)は121人で昨年の報告数(115人)を超えた。

11日までの5週間の81人をみると、患者の年齢は20〜88歳。2例が劇症化し、うち1人が死亡した。福岡県、広島県などが多く、報告した医師が推定した原因食材は「カキ」が45%と最も多かった。

2010/04/29 17:26 【共同通信】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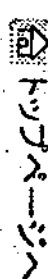
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医薬品
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識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	厚生労働省処理欄
				2010 年 2 月 4 日		
一般的名称	①②③④人血清アルブミン ⑤⑥乾燥濃縮人アンチトロンビンⅢ ⑦人ハプトグロビン ⑧乾燥濃縮人血液凝固第Ⅷ因子	研究報告の公表状況	Journal of Medical Virology 2010; 82(1): 69-76		公表国 日本	
販売名（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ノイアート（ベネシス） ⑧ハプトグロビン静注 2000 単位「ベネシス」（ベネシス） ⑨コンコエイト－HT（ベネシス）					

研究報告の概要	使用上の注意記載状況・その他参考事項等
ブタはヒトへＥ型肝炎ウイルス(HEV)を伝播するリザーバーと考えられる。 感染動態を調査するために、HEV に自然感染した国産ブタ 2 つの同産仔（A と B、10 匹の子豚を含む）を生後 6 箇月になるまで研究した。母子移行 IgG および IgA 抗体は同産 A 群の子豚からは検出されたが、B 群からは検出されなかった。生後 30-110 日のとき、全てのブタは糞便の中に HEV を排出し、17 匹は生後 40-100 日のときにウイルス血症を現した。系統発生分析では、全てのブタで HEV 遺伝子型 3 に非常に近い配列を示した。特異的な IgG と IgA の血清レベルは IgA は糞便では検出されなかったが、全てのブタで同様だった。興味あることに、ウイルス血症と抗体陽転の開始は、同産児 A 群のブタで有意に遅れた。糞便に排出されたウイルスの動態は、両方の同産児群で同様であった。感染動態の違いは、母性抗体がウイルス血症と抗体陽転開始を遅延させることを示唆した。 定量的リアルタイム逆転写酵素-ポリメラーゼ連鎖反応法分析において、糞便中の HEV RNA は約 10^6copies/g で最初の排泄から 10 日後にピークに達することが明らかになった。生後 200 日で、HEV RNA は、13 匹のブタの 3 つの内臓で見つかった。この研究の発見は、ブタにおける HEV 自然感染の動態、ブタから人へのウイルス伝播の管理に役立つことができる。	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。

報告企業の意見	今後の対応
ブタにおけるHEV自然感染の動態についての報告である。 万一、ヘパリンの原料であるブタ小腸粘膜にHEV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バリデーション試験成績から、ヘパリンの製造工程において十分に不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

ヘパリン

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Long-Term Shedding of Hepatitis E Virus in the Feces of Pigs Infected Naturally, Born to Sows With and Without Maternal Antibodies

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Pigs are presumed reservoirs for hepatitis E virus (HEV) transmission to humans. To examine infection kinetics, two litters of domestic pigs (A and B, each containing 10 piglets) infected naturally with HEV were studied until pigs were 6 months old. Maternal IgG and IgA antibodies were detected in litter A piglets, but not in litter B ones. All pigs shed HEV in feces when they were 30–110 days old, and 17 developed viremia at 40–100 days of age. Phylogenetic analysis revealed a highly close sequence of HEV genotype 3 in all pigs. The serum levels of specific IgG and IgA were similar in all pigs, although IgA was not detected in the feces. Interestingly, the onset of both viremia and seroconversion was delayed significantly in litter A pigs. The kinetics of fecal virus shedding was similar in both litters; shedding was not detected after the pigs were 120 days old. The differences in the infection kinetics between litters A and B suggested that maternal antibodies delayed the onset of viremia and seroconversion. Quantitative real-time reverse transcriptase-polymerase chain reaction revealed that HEV RNA in feces peaked 10 days after initial shedding of approximately $10^{6.0}$ copies/g. The viral load was much lower in the serum than in the feces. At 200 days of age, HEV RNA was found in the internal organs of 3 out of 13 pigs. These study findings improve the understanding of the dynamics of natural HEV transmission in pigs, which could help in controlling virus transmission from pigs to humans. *J. Med. Virol. 82:69–76, 2010.*
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KEY WORDS: swine; transmission; time course; epidemiology

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INTRODUCTION

Hepatitis E virus (HEV) is a causative agent of acute hepatitis in humans. HEV is a small non-enveloped single-stranded positive-sense RNA virus. Recently, HEV was classified as the sole member of the genus *Hepevirus* in the family *Hepeviridae* [Emerson and Purcell, 2003]. HEV isolates from mammals can be divided into at least four genotypes on the basis of complete sequence analysis [Lu et al., 2006]. Genotype 1 is distributed in Asia and Africa [Escribà et al., 2008; Sugitani et al., 2008], whereas genotype 2 is found in Mexico and Africa [Lu et al., 2006]. These two genotypes are transmitted to the human population via the fecal–oral route, and large human outbreaks have occurred in non-industrialized countries as a result of drinking water contaminated with feces [Jameel, 1999]. Genotype 3 has been detected in humans, domestic pigs, and several wild animals, and is distributed worldwide [Lu et al., 2006; Lewis et al., 2008; Lam et al., 2009]. Genotype 4 has been detected in humans and domestic pigs in Asian countries and Germany [Lu et al., 2006;

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Wichmann et al., 2008]. The genotypes 3 and 4 strains are considered to be zoonoses [Meng, 2005]. An HEV-related agent, the so-called avian HEV, has been detected in poultry but it does not seem to cause human infection [Huang et al., 2004].

Since the initial discovery of swine HEV in the USA [Meng et al., 1997], cases of HEV infection in pigs have been documented worldwide [Meng, 2005; Dalton et al., 2008]. Previous studies have shown the genetic similarity of swine and human HEV [Wang et al., 2000; Kabrane-Lazizi et al., 2001; Huang et al., 2002; Nishizawa et al., 2003; Takahashi et al., 2003; Yazaki et al., 2003; Ijaz et al., 2005], and have reported experimental cross-species infections from humans to pigs or from pigs to non-human primates [Meng et al., 1998; Halbur et al., 2001; Feagins et al., 2008; Ji et al., 2008]. All of these findings suggest that pigs are reservoirs of human HEV.

Epidemiological studies have revealed that HEV infections in pigs are ubiquitous, and that pigs over the age of 3 months have a high seroprevalence [Meng et al., 1999; Huang et al., 2002; Banks et al., 2004]. HEV shedding in feces has been observed in pigs of all ages, but is more frequently observed in 2–4 months old pigs as compared to slaughter-age (6-month old) or adult pigs [Meng et al., 1997; Yazaki et al., 2003; Cooper et al., 2005; Fernandez-Barredo et al., 2006; Seminati et al., 2008]. These results indicate that domestic pigs are infected easily with HEV at an early age, but that the majority of pigs stop shedding HEV RNA before they are 6 months old. Although many epidemiological studies have been conducted on this subject, longitudinal studies following individual pigs are limited [Meng et al., 1997; de Deus et al., 2008]. Of particular importance is the fact there have been no long-term quantitative analyses of virus shedding and serum antibody levels in individual piglets infected naturally with HEV.

In the present study, long-term follow-up characterization was performed until slaughter age of two litters of pigs infected naturally with HEV—one with HEV-specific maternal antibodies and the other without these antibodies—to investigate the dynamics of HEV RNA shedding in feces, as well as assess viremia, antibody levels, and the effect of maternal antibodies on HEV infection.

MATERIALS AND METHODS

Animals and Sample Collection

Twenty mixed-breed pigs, 10 born to sow A (litter A) and 10 to sow B (litter B), from a swine herd in Japan were followed up until they were 200 days old (day 200). The two litters were born on the same day in separate pens and raised together after day 30. They were separated again from day 83 till the end of the study.

The sera of sows A and B were collected before delivery and examined for HEV-specific IgG antibodies. During the study period, fecal and serum samples were collected every 10 days from each pig, and stored at −80°C until use. Thirteen pigs (five from litter A and eight from litter B) were euthanized on day 200, and tissue samples (liver, ileum, and colon), serum, bile, and intestinal contents (ileum, colon, and rectum) were collected and stored at −80°C before testing. The tissues were treated with RNAlater (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Euthanasia and tissue sampling were performed according to the Laboratory Animal Control Guidelines of Rakuno Gakuen University.

Enzyme-Linked Immunosorbent Assay for Detecting Anti-HEV Antibodies

The anti-HEV IgG antibodies in the sera collected before the delivery of the sows, the anti-HEV IgG and IgA antibodies in the serum samples, and the anti-HEV IgA antibodies in individual feces samples were detected using a commercial ELISA kit for the detection of hepatitis E antibodies (Viragent HEV-Ab kit; Cosmic Corporation, Tokyo, Japan) according to the manufacturer's instructions. Serum samples from five pigs in litter A and eight in litter B were used for detecting HEV-specific serum IgA. For detection of antibodies in feces, suspensions of 10% fecal matter in phosphate-buffered saline were prepared. The kit used a truncated recombinant HEV ORF2 protein expressed in silkworm pupae [Mizuo et al., 2002]. Rabbit anti-pig IgG or IgA antibodies coupled with horseradish peroxidase (Kirkegaard and Perry Laboratories, Gaithersburg, MD) were used as secondary antibodies. Antibody titres were recorded as index values and calculated according to the following formula:

$$\text{Index value} = \frac{\text{Optical density of sample}}{\text{Optical density of positive control}} \times 100.$$

RNA Extraction

Viral RNA was extracted from 140 µl of serum, bile, 10% fecal suspension, and a 10% suspension of the intestinal contents by using a QIAamp Viral RNA Mini Kit (Qiagen). The final elution was carried out using 50 µl of elution buffer. Viral RNA was extracted from the tissue samples with TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions.

Semi-Nested Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

To detect HEV RNA, semi-nested RT-PCR was performed. The 5′-terminal region of ORF1 was amplified using broadly reactive primers [Hagiwara et al., 2007]. For the first round of PCR, the sense primer HE61 (5′-CACRTATGTGGTCGAYGCCATGGAG-3′; R = A or G, Y = C or T) and the anti-sense primer HE51 (5′-GCCKRACYACCACAGCATTCG-3′; K = G or T) were used. This produced an expected fragment of 125 base pairs (bp). For the second round of PCR, the internal sense primer HE50 (5′-AAGGCTCCTGGCRTYAC-

WAC-3′; W = A or T) and the anti-sense primer HE51 were used, producing an expected fragment of 85 bp. Reverse transcription and first-round amplification were carried out using the OneStep RT-PCR Kit (Qiagen). In each reaction, 5-μl aliquots of viral RNA solution were used. The reactions were performed in an Eppendorf Mastercycler (Eppendorf, Hamburg, Germany) under the following conditions: reverse transcription at 50°C for 30 min, denaturation at 95°C for 15 min, 45 cycles of denaturation at 95°C for 15 sec each, annealing at 55°C for 30 sec, elongation at 72°C for 30 sec, and final elongation at 72°C for 7 min. After first-round PCR, 1 μl of the PCR product was amplified under the following conditions: 20 cycles of denaturation at 95°C for 15 sec each, annealing at 60°C for 30 sec, and elongation at 72°C for 15 sec, followed by final incubation at 72°C for 7 min. The amplified second-round PCR products were characterized using 2% gel electrophoresis. A PCR amplicon of 85 bp was determined to be HEV-specific.

Quantitative Real-Time RT-PCR

The copy number of HEV RNA was measured by quantitative real-time RT-PCR according to the technique developed by Jothikumar et al. [2006] with a slight modification. TaqMan® probe (5′-FAM-TGATTCCCAGCCCTTCGC-TAMRA-3′) was designed based on the sequence of the HEV ORF3 region (accession number AB481228) from litter A pig. Five microliters of extracted RNA (equivalent to 1.4 mg of feces or 14 μl of sera) was used per reaction. A 5 μl aliquot of RNA was amplified using the forward primer 5′-GGTGGTTTCTGGGGTGAC-3′ and the reverse primer 5′-AGGGGTTGGTTGGATGAA-3′ in a LightCycler (Roche, Basel, Switzerland) under the following conditions: reverse transcription at 50°C for 30 min, denaturation at 95°C for 15 min, and 45 cycles of amplification, each consisting of 1 sec at 95°C followed by 1 min at 60°C. Viral RNA copy numbers were calculated on the basis of the calibration curve constructed using standard RNA as described below, using LightCycler Software 4.0.

To construct a calibration curve for quantification, in vitro transcribed RNA from the HEV ORF3 region was collected from a cloned plasmid. The copy number of standard RNA was calculated using a spectrophotometer. Preliminary examination using in vitro transcribed RNA showed that the detection limit of quantitative real-time RT-PCR was $10^{3.8}$ copies/g of feces, $10^{2.6}$ copies/ml of serum, and $10^{3.6}$ copies/ml of tissue.

Sequence and Phylogenetic Analysis

Four fecal samples from four pigs in litter A and one fecal sample from sow A, all of which were found to be positive for HEV by nested RT-PCR, were subjected to sequence analysis. The ORF2 region of the viral RNA was amplified using the primers HE044 (5′-CAAGG-HTGGCGYTCKGTTGAGAC-3′; H = A, C, or T) and HE041 (5′-TTMACWGTCRGCTCGCCATTGGC-3′; M = A or C), as described previously [Mizuo et al., 2002]. The PCR products were sequenced directly using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA). Sequence analysis was performed using Genetyx-Windows version 7 (Genetyx Corp., Tokyo, Japan). The sequence alignment was generated by CLUSTAL W [Thompson et al., 1994]. The four nucleotide sequences of swine HEV isolates, named swJB-M3, -M5, -M8, and -M10, have been deposited in the GenBank sequence database under the accession numbers AB471965–AB471968. A phylogenetic tree was constructed using prototype sequences of genotype 1, 2, 3, and 4 obtained from GenBank and the neighbor-joining method [Saitou and Nei, 1987], on the basis of a 412-nucleotide partial sequence of the ORF2 region; the tree was drawn using the TreeView program [Page, 1996].

Statistical Analysis

The number of pigs shedding virus in feces, the number with viremia, and the time to seroconversion were compared between litters A and B by using the Wilcoxon rank-sum test. Statistical analysis was performed using the JMP 5.1.2 software (SAS Institute, Inc., Cary, NC). $P \leq 0.05$ was considered statistically significant.

RESULTS

Detection of Anti-HEV IgG and IgA in Serum and Feces

A total of 20 piglets were studied, 10 from litter A and 10 from litter B. Before delivery, sow A tested positive for IgG antibodies specific to the HEV ORF2 protein, but sow B did not. Figure 1 shows the levels of HEV-specific IgG and IgA in the sera of the piglets when they were 1–180 days old. The serum samples from litter A pigs tested positive for both IgA and IgG antibodies on day 1, with ELISA index values of 122.6 and 144.5, respectively; the levels of these antibodies in their sera decreased rapidly until day 50. In contrast, the serum levels of IgG and IgA in litter B pigs were significantly low on day 1, with ELISA index values of 17.4 and 27.5, respectively. The serum IgG levels in the litter B pigs remained low during days 1–50. Seroconversion began on day 60 in litter A pigs and on day 50 in litter B pigs, after the onset of viremia (Fig. 1). The Wilcoxon rank-sum test revealed that there was a significant difference between litters A and B pigs with respect to the time of IgG seroconversion ($P < 0.001$) (data not shown), that is, seroconversion occurred significantly earlier in litter B pigs. The antibody titres peaked on days 90 and 70 in litters A and B, respectively, and then decreased gradually till the end of the study.

HEV-specific fecal IgA to HEV were not detected during the study period (data not shown).

Detection of HEV RNA in Feces and Serum

Pig feces were examined for HEV RNA during days 30–110 by using semi-nested RT-PCR (Fig. 1). On day

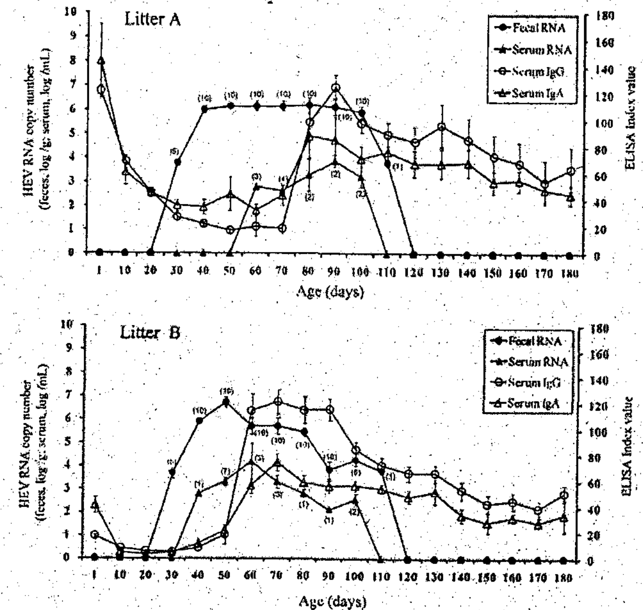

Fig. 1. Hepatitis E virus (HEV) shedding and seroconversion in two litters of pigs. The copy numbers of HEV RNA in feces and serum are shown, along with the enzyme-linked immunosorbent assay (ELISA) index values of anti-HEV IgG and IgA in the serum samples. HEV RNA copy numbers in feces and serum represent the average values among positive animals. The numbers of animals positive for fecal RNA and serum RNA are indicated in parentheses. The levels that were undetectable by quantitative real-time RT-PCR were approximated using the estimated detection limit ($10^{3.8}$ copies/g for fecal RNA, $10^{2.6}$ copies/ml for serum RNA). ELISA index value = (OD of sample/OD of positive control) × 100. Error bars represent standard error.

30; HEV RNA was detected in the feces of five pigs from each litter. During days 40–90, HEV RNA was detected in the feces of all 20 pigs. On day 100, all 10 of the pigs in litter A shed HEV RNA in their feces, but only 5 pigs from litter B did. On day 110, only one pig from each litter was found to be shedding HEV RNA in the feces. No HEV RNA was detected in feces after day 120.

The modified TaqMan® probe, designed to be specific to the present HEV strain, reacted strongly to every sample tested, indicating that the HEV detected in all of the litter A and B pigs belongs to the same strain. The dynamics of fecal shedding of HEV were quantitatively characterized by real-time PCR (Fig. 1). On day 30, HEV RNA could be detected in feces by semi-nested RT-PCR but not by real-time RT-PCR, indicating that the RNA copy number was below the detection limit of quantitative real-time RT-PCR ($10^{3.8}$ copies/g). On day 40, however, HEV RNA increased suddenly to $10^{6.0}$ copies/g in the feces from both litters. The pigs in litter A continued to shed large amounts of HEV RNA (approximately $10^{6.0}$ copies/g) until day 100, whereas the amounts of HEV RNA in the feces of litter B pigs decreased gradually. On day 110, the HEV RNA in the feces from both litters decreased to amounts below the detection limit of real-time RT-PCR.

During the study period, viremia was detected in 7 pigs in litter A and 10 in litter B. The onset of viremia occurred on day 60 in litter A pigs and on day 40 in litter B pigs (Fig. 1). The Wilcoxon rank-sum test showed that this difference between the time of onset of viremia in litter A and B pigs was statistically significant ($P = 0.024$; Fig. 1). Throughout the study, the amounts of HEV RNA in the serum were lower than those in the feces (Fig. 1). The highest serum HEV RNA titre was found on day 90 in a pig from litter A ($10^{4.2}$ copies/ml) and on day 60 in a pig from litter B ($10^{5.6}$ copies/ml).

Time Courses of Changes in Virus Shedding, Viremia, and Serum Antibody Titres

Based on the data obtained on virus shedding and antibody reaction in individual pigs (data of individual pigs not shown), the general time course of HEV infection in domestic pigs can be described as follows (data are expressed as mean (SD; range)): pigs begin to shed HEV in feces on day 30 (27.4; 0–70) after birth and

viremia and seroconversion of serum IgG and IgA occur 33.5 (7.0; 10–60) and 32.3 (7.4; 20–50) days, respectively, after the onset of HEV shedding in feces. HEV shedding in feces continues for 63.5 (7.4; 50–80) days, whereas viremia can appear transiently for 11.8 (12.9; 10–40) days. In this study, virus shedding in feces was observed in all pigs with high antibody titres, whereas viremia was observed in a total of 17 pigs, all of which had relatively low antibody titres. Serum IgG and IgA antibody levels peaked 8.5 (12.0; 0–30) and 6.2 (7.1; 0–20) days, respectively, after seroconversion. After peaking, they decreased gradually but remained detectable during the entire study period, even after the end of viremia and after the pigs stopped shedding virus in feces.

Sequence Analysis

Genomic sequencing of the ORF2 region of virus isolates from four piglets in litter A and from sow A revealed that the virus strains were identical. Phylogenetic analysis of the HEV isolates indicated that they belonged to HEV genotype 3 and that they were clustered with genotype 3_{us}, both of which are related to the strains of swine and human HEV found in the USA (Fig. 2) [Takahashi et al., 2003].

HEV RNA Detection in Tissue Samples From 200 Days Old Pigs

Of the 13 pigs (5 from litter A, 8 from litter B) euthanized on day 200, HEV RNA was detected in the internal organs of 3 pigs by semi-nested RT-PCR: in the gall bladder of one litter A pig, in the mesenteric lymph nodes and liver of one litter B pig, and in the hepatic and mesenteric lymph nodes of another litter B pig. According to real-time RT-PCR, in contrast, none of these samples tested positive for HEV RNA, indicating that the amounts of HEV RNA present in these samples were below the detection limit of real-time RT-PCR, that is, $10^{3.6}$ copies/g.

DISCUSSION

Although there a number of epidemiological surveys of HEV in pigs have been conducted, longitudinal studies of the time course of HEV infection in pigs infected naturally have been quite limited [Meng et al., 1997; de Deus et al., 2008]. The dynamic HEV life cycle in piglets infected naturally can only be evaluated through long-term follow-up studies with quantitative measurements of both HEV RNA and viral-specific antibodies in individual pigs from birth to slaughter. This is the first report on the quantitative dynamics of virus shedding in feces, viremia, and specific serum antibodies that were evaluated in a long-term follow-up study of pigs infected naturally with HEV.

Maternal antibodies, including IgG, IgA, and IgM, that are transmitted via the colostrum have been reported to protect piglets from infection by various pathogens [Andries et al., 1978]. Although maternal antibodies against HEV have been found in piglets born to HEV-positive sows, the protective role of these

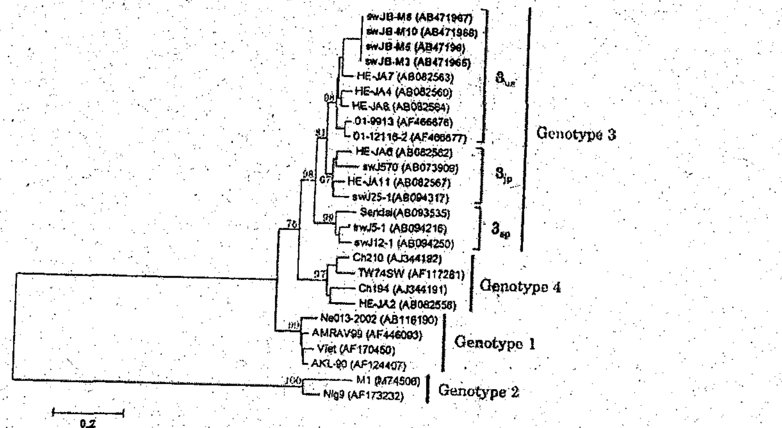

Fig. 2. Phylogenetic analysis of the nucleotide sequence of the ORF2 region of HEV (412 bp). Intra-genotypic classification of genotype 3 (3_{us}, 3_{sp}, and 3_{jp}) was done according to a previous report [Takahashi et al., 2003]. HEV isolates obtained in this study (swJB-M3, -M5, -M8, and -M10) are indicated in bold letters. Prototype sequences of genotype 1, 2, 3, and 4 from GenBank are given with their accession numbers. Phylogenetic tree was constructed using the neighbor-joining method. The bootstrap values (n = 1,000) are given for the major nodes.

maternal antibodies has not yet been determined [Meng et al., 1997; Kasorndorkbua et al., 2003]. In the present study, two litters of piglets—one with maternal antibodies and the other without—were studied to determine whether the presence of maternal antibodies affected HEV shedding. The results showed that virus shedding in feces occurred from days 30 to 110 in both litters, though a significant delay in the onset of both seroconversion and viremia was observed in the litter A piglets, which had maternal antibodies. Although serum IgG and IgA of litter B pigs were slightly reactive to the HEV antigen on day 1, as determined using ELISA, this reactivity was considered to be non-specific because of the presence of large quantities of maternal antibodies to various pathogens.

The similarity between the litters in terms of the kinetics of fecal virus shedding indicates that maternal antibodies do not protect piglets from primary HEV infections in the early days of their lives. Interestingly, another study has reported that maternal antibodies can have an immunosuppressive effect [Siegrist, 2003]. Although the immunological mechanisms responsible for such an adverse affect remain unclear, it is possible that in this study, maternal antibodies delayed the piglets' immune responses against HEV infection, causing the delay in seroconversion that was seen in the litter A piglets.

Since this study followed up domestic pigs raised under normal conditions, it was not obvious whether HEV infection in the two litters occurred under similar conditions. Therefore, it was difficult to determine the exact effect of maternal antibodies on the kinetics of HEV infection. Further studies are required to clarify the role of maternal antibodies.

In previous epidemiological studies, fecal and serum HEV RNA and serum antibodies have been used as markers of HEV infection [Meng et al., 1998; Cooper et al., 2005]. In the present study, fecal RT-PCR was far more sensitive than serum RNA testing in detecting HEV RNA. Indeed, all of the pigs shed high copy numbers of HEV RNA in feces for 70–80 days, whereas viremia appeared transiently the copy number of the RNA shed was low. In addition, viremia remained undetectable in three pigs. It is possible that the sampling schedule, particularly the 10-day intervals between sampling days, may have led to the low rate of serum RNA detection. Fecal RT-PCR, in contrast, does not appear to have the same limitations, and can be recommended as an indicator of current HEV infection based on early occurrence, high viral load, and long duration of HEV RNA in feces. It may prove especially useful in quarantine situations when pigs are introduced to another herd.

The reactivity of the modified TaqMan® probe used in this study, which was designed according to the sequence of HEV obtained from litter A pigs, to the present HEV suggested that all the pigs were infected with the same viral strain. The strain in question was found to belong to genotype 3_{us}, which is related to the HEV strains found in humans and pigs in the USA. It is one of the three clusters into which genotype 3 has been divided: the other two are 3_{sp} and 3_{jp} [Takahashi et al., 2003]. HEV genotypes 3 and 4, both of which have been reported in Japan [Takahashi et al., 2003], are considered to be zoonoses, causing hepatitis in humans; genotype 4 has been reported to cause a particularly severe form of hepatitis [Ohnishi et al., 2006]. Some phenotypic variations between genotypes 3 and 4 have been reported. Though the results of the present study contribute significantly to the understanding of the infection of HEV genotype 3 in pigs, further studies on genotype 4 and the other two sub-clusters of genotype 3 will be required to develop a conclusive strategy to control HEV infection in domestic pigs.

In this study, HEV RNA was detected in the liver, gall bladder, or lymph nodes of 3 of 13 pigs examined on day 200, that is, 3 months after the pigs had stopped shedding the virus. The prevalence of HEV RNA in pig livers at grocery stores in Japan and the USA has been reported as 2% and 11%, respectively [Yazaki et al., 2003; Feagins et al., 2007]. Furthermore, HEV isolated from pig livers at grocery stores in the USA was found to be infectious. This could create public health problems stemming from HEV contamination in slaughtered pigs, even if no HEV shedding is observed before slaughter. In addition, the long-term shedding of large amounts of virus, which was observed in this study, supports the idea that farm workers exposed to infected pigs could be infected directly because of a contaminated working environment [Zheng et al., 2006]. Controlling HEV infection on pig farms would therefore help decrease the likelihood of the disease being transmitted to people.

CONCLUSIONS

To understand the time course of HEV infection in domestic pigs, pigs infected naturally with HEV genotype 3 were followed up from birth to slaughter age. These pigs shed HEV in feces when they were 30–110 days old, and developed viremia when they were 40–100 days old. Seroconversion of anti-HEV IgG and IgA antibodies occurred 20 days after the onset of viremia. HEV RNA in feces peaked at approximately $10^{6.0}$ copies/g 10 days after the onset of fecal shedding. The kinetics of HEV infection seemed to be influenced by the presence of maternal antibodies. At day 200, 3 of 13 pigs (23%) still had detectable levels of HEV RNA in their livers, gall bladders, and/or lymph nodes, though they had stopped shedding the virus in feces. Although the amounts of HEV RNA in these tissues were low, the presence of HEV in the internal organs after the virus shedding has stopped could have important implications for the prevention of virus transmission to people through food. The time course of HEV infection revealed in this study will be very helpful in understanding the kinetics of HEV transmission from pigs to humans, and in developing a control strategy to prevent zoonotic HEV infection from pigs.

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番号 11

医薬品
医薬部外品　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2010年7月12日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	乾燥濃縮人血液凝固第Ⅷ因子		研究報告の公表状況	Journal of General Virology 2010; 91(2): 541-544	公表国 イギリス	
販売名（企業名）	コンコエイト-HT（ベネシス）					

研究報告の概要

パルボウイルス PARV4 は、ヒト宿主のパルボウイルス科の種類として最近記載された。
血中の PARV4 の保有率を調査するため、定量的 TaqMan PCR が開発され、様々な集団からのプラズマ、血清または全血について検討した。8つの検体が PARV4 陽性であった（高いコピー数が1つ）。高力価陽性血漿は約 5 × 10⁸genome equivalents/mL のウイルス量であった。間接免疫蛍光法で PARV4 抗体陽性確認された2つのヒト血清が高力価ヒト血漿で自然の PARV4 を視覚化する試みとして免疫電子顕微鏡に使用された。PARV4 粒子はこれら2つの血清のうち1つで観察された。
我々の知る限りでは、自然の PARV4 が可視化されたのはこれが初めてのことである。

使用上の注意記載状況・その他参考事項等

2. 重要な基本的注意
(2) 溶血性・失血性貧血の患者〔ヒトパルボウイルスB19の感染を起こす可能性を否定できない。感染した場合には、発熱と急激な貧血を伴う重篤な全身症状を起こすことがある。〕
(3) 免疫不全患者・免疫抑制状態の患者〔ヒトパルボウイルスB19の感染を起こす可能性を否定できない。感染した場合には、持続性の貧血を起こすことがある。〕
2. 重要な基本的注意
(1)略
1) 血漿分画製剤の現在の製造工程では、ヒトパルボウイルスB19等のウイルスを完全に不活化・除去することが困難であるため、本剤の投与によりその感染の可能性を否定できないので、投与後の経過を十分に観察すること。
5. 妊婦、産婦、授乳婦等への投与
妊婦又は妊娠している可能性のある婦人には、治療上の有益性が危険性を上回ると判断される場合にのみ投与すること。〔妊娠中の投与に関する安全性は確立していない。本剤の投与によりヒトパルボウイルス B19 の感染の可能性を否定できない。感染した場合には胎児への障害（流産、胎児水腫、胎児死亡）が起こる可能性がある。〕。

報告企業の意見

ヒト血清で PARV4 が免疫電子顕微鏡法により可視化された最初の報告である。
パルボウイルス４（PARV4）は、パルボウイルス科パルボウイルス亜科のどの属にも分類されないウイルスである。また、PARV4が発見されたのは2005年であり、PARV4及びその関連変異型であるPARV5の病原性は現時点では明らかではない。血漿分画製剤からの伝播事例は報告されていないが、英国で1970年代及び1980年代に製造された第Ⅷ因子製剤からPARV4が検出されている。万一、原料血漿にPARV4が混入した場合、CPVをモデルウイルスとしたウイルスバリデーション試験成績からは、PARV4の製造工程における不活化・除去が十分であるとはいえないので、今後注意深く追加情報をフォローする必要があると考える。

今後の対応

PARV4 に関する追加情報の入手に努める。

コンコエイト

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Short Communication

Parvovirus PARV4 visualization and detection

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The parvovirus PARV4 is the most recently described member of the family *Parvoviridae* that has a human host. To investigate the prevalence of PARV4 in blood, a quantitative TaqMan PCR was developed and plasma, sera or whole blood from a variety of population groups were examined. Eight samples were positive for PARV4, one at high copy number. The high-titre-positive plasma had an approximate viral load of 5×10^8 genome equivalents ml^{-1}. Two human sera, identified as PARV4 antibody-positive by indirect immunofluorescence, were used in immune electron microscopy to try to visualize native PARV4 within the high-titre human plasma. PARV4 particles were observed using one of these two sera. To our knowledge, this is the first time that native PARV4 has been visualized.

PARV4 is the most recently described member of the family *Parvoviridae* that has a human host (Jones *et al.*, 2005). It is currently a virus without any apparent disease association (Fryer *et al.*, 2007a). It was identified by a random amplification of nucleic acids extracted from a patient with acute virus infection who was co-infected with hepatitis B virus (HBV) (Jones *et al.*, 2005). Two further genotypes of PARV4 have now been described (Fryer *et al.*, 2006; Simmonds *et al.*, 2008). Very little is known about PARV4 and its biology. It was initially described as 'not closely related to any known parvoviruses' (Jones *et al.*, 2005). However, further work has shown that PARV4 is most similar to the recently discovered bovine and porcine HoKo viruses (Lau *et al.*, 2008) and that it groups together with these and also the more distantly related Myanmar erythrovirus (Hijikata *et al.*, 2001), another porcine virus.

The human parvovirus B19 can be present at very high titres in the blood of infected individuals. Plasma and whole-blood samples thought likely to harbour PARV4, namely samples being tested for hepatitis B or C or from human immunodeficiency virus (HIV)-positive patients, were therefore examined. For comparison, samples from UK blood donors were also tested. All samples analysed were anonymized. The frequency of detection of PARV4 in the UK blood-donor population is expected to be low, based on limited data from previously reported surveys (Fryer *et al.*, 2007b; Simmonds *et al.*, 2007; Schneider *et al.*, 2008), although large, formal studies have yet to be performed.

Nucleic acid was extracted from plasma, serum or whole blood, either manually using Qiagen blood kit spin columns or on a Qiagen BioRobot. A quantitative TaqMan PCR (Q-PCR) was designed with the aid of Beacon Designer 3 software (Premier Biosoft International) and optimized for open reading frame (ORF) 2 of PARV4. The Q-PCR was performed on an ABI 7500 platform (Applied Biosystems), using ABgene reagents, and was shown to have linearity of detection over the range 10^1–10^8 copies ml^{-1}, with a limit of sensitivity of 50 copies ml^{-1}. An oligonucleotide positive control of the target sequence was synthesized (Eurofins MWG Operon), but was subsequently replaced by a biological standard: a high-titre-positive plasma, once one had been identified. Murine cytomegalovirus was used as an internal extraction and amplification control. Samples tested and results are shown in Table 1. Q-PCR conditions were 95 °C for 15 min followed by 45 cycles of 95 °C for 15 s, 60 °C for 60 s. The TaqMan primers used were PWTPARV4.1F (5′-CCTCTCCGAGTCCATTAGCAGA-3′; 1937–1958) and PWTPARV4.1R (5′-GCTCCATACCTTTCAGCAGTTTC-3′; 2069–2047). The TaqMan probe was PWTPARV4-probe (5′-FAM-CGCCGCCGAGGACACCAGACAGT-TAM-3′; 1961–1983). Sequences are numbered according to GenBank accession no. AY622943.

In total, PARV4 DNA was detected in eight samples. Quantification of PARV4 in samples was initially carried out against a $\log_{10}$ dilution series of the oligonucleotide positive control and subsequently against a high-titre-positive control PARV4 plasma (designated plasma 129). This plasma had a viral load of 5×10^8 DNA copies ml^{-1} and was from a hepatitis C virus (HCV) RNA-positive, HCV antibody-negative patient. Viral loads of all eight positive samples are shown in Table 2. The four samples that had a viral load $\geq$760 copies ml^{-1} were amplified successfully for sequencing, but those with viral loads of $\leq$285 failed to amplify. Three samples (129, 135 and 342)

Table 1. Samples tested for PARV4 by Q-PCR

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; HIV-1, human immunodeficiency virus type 1; IVDU, intravenous drug user.

Population group tested	*n*	No. (%) PARV4-positive by Q-PCR
HCV antibody-negative, RNA-positive blood donors (HCV window phase)	94	3 (3.2)
Samples for routine HCV RNA testing	88	2 (2.3)
Samples for routine HBV DNA testing	140	2 (1.4)
HIV-1 proviral DNA-positive IVDUs	50	0*
Samples for routine HIV-1 RNA viral load testing	88	1 (1.1)*
UK blood donors – 20 pooled DNA extracts from 96 donors	–	0

*Overall detection frequency of 1 in 138 (0.7 %) in HIV-1-positive samples tested.

were amplified by using a semi-nested PCR to ORF2, initially with primers PARV4Seq1 (5′-CCGGAACC-TTCAAGTCAAGCCA-3′; 2465–2486) and PARV4Seq2 (5′-CCGCTCAAGGTCTGGTTCAACAA-3′; 3010–2988), followed by PARV4Seq1 and PARV4Seq3 (5′-CAAGG-TGGACTCCGACATCTGG-3′; 2954–2933). The resulting 490 bp fragments from these three samples were then sequenced with PARV4Seq1 and PARV4Seq3. All three were typed as PARV4 genotype 1. Sample 168 was also confirmed as PARV4 genotype 1 by sequencing with primers PVORF1F and PVORF1R (Fryer *et al.*, 2006). Sequence similarity was determined by using the FASTA program at http://www.ebi.ac.uk and searching the Viral Database.

For electron microscopy, 300 μl high-titre plasma 129 was centrifuged at 48 000 *g* for 45 min. The resultant pellet was resuspended in distilled water and stained with 1.5 % phosphotungstic acid (PTA), pH 6.6. Grids were examined in a Philips 420 transmission electron microscope fitted with an AMT XR60 digital imaging system. Parvovirus particles were not seen. Small, round, featureless virus particles, such as parvoviruses, however, can be extremely difficult to detect, particularly amongst the background debris of plasma or serum. Immune electron microscopy (IEM), a technique that has been employed successfully to detect other small viruses, including parvovirus B19 (Cossart *et al.*, 1975; Curry *et al.*, 2006), was used in a further attempt to visualize the native PARV4 particles. Two serum samples containing antibody to PARV4 had been identified in our laboratory on the basis of their reactivity in an indirect immunofluorescence test (R. P. Parry, unpublished data). These two antibody-positive sera were each mixed with an aliquot of high-titre plasma 129, incubated at room temperature for 1 h and centrifuged at 48 000 *g* for 45 min. Pellets were resuspended in distilled water and stained with 1.5 % PTA or 2 % methylamine tungstate, pH 6.6, and examined as described above. Parvovirus-like particles that had been aggregated into clumps by one of the sera were seen (Fig. 1a). The particles measured around 20–22 nm in diameter and were morphologically typical of parvoviruses. For comparison, recombinant PARV4 capsids expressed in *Sf*9 cells by baculovirus (PARV4 capsids provided by Dr Kevin E. Brown, Health Protection Agency) can be seen in Fig. 1(b). The recombinant capsids and the particles found in plasma 129 are similar in size and have the characteristic hexagonal appearance of parvoviruses. Stain has penetrated into several of the recombinant particles, as would be expected, whereas the particles from plasma 129 appear complete.

Table 2. Viral loads of PARV4-positive samples

Sample	Viral load (DNA copies ml^{-1})				
	PARV4	HCV	HCV genotype	HBV	HIV
129	5×10^8	2.70×10^3	2b		
135	760	1.22×10^6	3a		
168	4.6×10^3	1.05×10^5	3a		
A5	1	5.04×10^6			
C10	5	+		+	
342	3.4×10^3			+	+
490	170			+	
H10	285				+

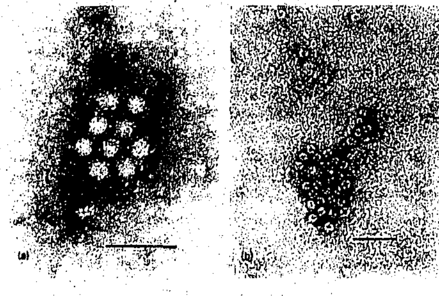

Fig. 1. Electron micrographs of parvovirus particles. (a) IEM of particles seen in plasma 129; antibody can be seen coating the particles. Stained with methylamine tungstate. (b) Recombinant viral capsids of PARV4, stained with PTA. Bars, 100 nm.

The antibody-aggregated clumps of particles observed in plasma 129 resembled the appearance of B19 virus when visualized by IEM. Plasma 129 and the two serum samples containing antibody to PARV4, however, were negative by PCR for B19 and human bocavirus, and it was concluded that the particles seen were PARV4.

Failure to detect virus particles with the second serum may have been related to the titre of the reagents. The sera were only tested at one dilution by immunofluorescence, but results from a prototype ELISA suggested that this second serum had a lower antibody titre to PARV4. For IEM purposes, the titre of PARV4 in plasma 129 was also low and probably near the limits of sensitivity for IEM detection. This may account for the fact that virus particles from this sample were not seen with PTA staining, rather than any difference between the stains.

PARV4 was detected at low frequency in samples from the blood of patients infected with HIV-1, HCV and HBV. In a study of the three human parvoviruses, B19, bocavirus and PARV4, in HIV-1-infected and non-infected individuals, Manning *et al.* (2007) established that a high proportion (70.8 %) of HIV-1-infected individuals harbour PARV4 in lymphoid and bone-marrow tissues, but none had viraemia. It is interesting to note that seven of the eight individuals in whom PARV4 was detected in the plasma were co-infected with hepatitis viruses (Table 1). The original discovery of PARV4 was in an intravenous drug user (IVDU) from the USA. The 94 HCV window-phase plasma samples analysed in our study for PARV4 were USA-sourced plasmas and the donors may have been remunerated financially. PARV4 was not detected in any of the UK blood donors tested.

These data contrast with those of a recent study in Thailand, which revealed PARV4 in sera both from IVDUs (8 %) and in blood donors (4 %) (Lurcharchaiwong *et al.*, 2008). Both of these figures are higher than those reported previously from the UK and elsewhere. It is again of interest that the majority of the PARV4-positive IVDUs in the Thai study, seven of eight (87.5 %), were HCV-co-infected; this may of course simply be coincidental, as the proportion of HCV positives within this group of IVDUs was very high (88.6 %). The determination of the prevalence of past infection with PARV4 in these different populations awaits the results of serological studies. Whether co-infection is a reflection of the natural history of the virus infection, a commonality of transmission routes or a consequence of underlying disease also awaits further elucidation.

The high viral load found in sample 129 (5×10^8 DNA copies ml^{-1}) suggests that this patient was experiencing active virus replication and may represent primary infection. The only other known high-level samples were from the original patient, which contained 6 log_{10} copies ml^{-1} (E. Delwart, personal communication), and from archived plasma pools with 6.58 log_{10} copies ml^{-1} (Fryer *et al.*, 2007b). It is not known whether the lower viral loads found in this (Table 2) and other studies represent virus replication, waning virus levels as antibody develops or a chronic virus carrier state. Fluctuating low levels of B19 DNA were observed in the plasma of 7.9 % of patients with congenital haemoglobinopathy. It has been postulated that this may be due to minor reactivation from sites of virus persistence (Lefrère *et al.*, 2005), which may also explain the 1 % of pregnant women (Lefrère *et al.*, 2005) and blood donors (Candotti *et al.*, 2004) who are B19 DNA-positive. A similar phenomenon may be occurring with PARV4. Further development of antibody assays and follow-up studies on PARV4-positive patients are required to investigate these hypotheses.

The high level of sequence conservation observed within the samples that tested positive for PARV4 is consistent with the findings of other groups. This argues for a recent evolutionary origin or a high conservation pressure. Manning *et al.* (2007) observed an apparent temporal shift in PARV4 genotypes, with genotype 1 representing the current 'modern' infection and genotype 2 the older strain. Study subjects positive for genotype 1 were all born after 1958 and those infected with genotype 2 were born between 1949 and 1956. A similar situation has recently been described for B19 variants, with genotype 1 superseding genotype 2 in the skin (Norja *et al.*, 2006). Demographic information on the patients and donors in our study was not available, as all samples were obtained in a random, anonymized manner.

The three genotypes of PARV4 now identified (Simmonds *et al.*, 2008) have not yet been related to any disease. However, 8 years elapsed between the discovery of B19 and its association with fifth disease (erythema infectiosum) (Anderson *et al.*, 1983). Our findings and those of others suggest that a parenteral transmission route is likely. It remains to be seen where PARV4 replicates and whether there are any disease associations.

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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
				2010. 7. 8	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	Houfar MK, Mayr-Wohlfart U, Sireis W, Seifried E, Schrezenmeier H, Schmidt M. XXXIst International Congress of the ISBT; 2010 Jun 26-Jul 1; Berlin, Germany	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

○ヒトパルボウイルスB19(B19)DNA陽性血液製剤の感染性
背景:2000年以降、ドイツのウルム研究所では、B19に対する供血者NATスクリーニングを供血6～8週間後(すなわち血液製剤供給後)に実施している。本研究において、輸血された血液製剤中のウイルス濃度との関連においてB19陽性血液製剤の感染性を評価した。
研究方法:後方視的研究において、受血者を次の2群に分けた:A) B19ウイルス量≦10^5IU/mLの血液製剤受血者;B) B19ウイルス量>10^5IU/mLの血液製剤受血者.
VP-1uゲノム領域の系統発生解析を、B19 DNA陽性供血者と受血者の対で実施した。また、すべての検体のIgM、IgG抗体を調べた。
結果:B19 DNAはB群の赤血球濃厚液受血者18名中9名に検出されたが、A群の受血者16名にはB19 DNAは検出されなかった(p=0.016)。系統発生解析では、供血者と受血者間で同一ゲノム配列を示した。
結論:血液製剤によるB19伝播は、ウイルス濃度と中和抗体価に相関することが分かった。

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

輸血された血液製剤中のヒトパルボウイルスB19(B19)濃度と感染性について評価を行ったところ、B19伝播は、ウイルス濃度と中和抗体価に相関することが分かったとの報告である。
パルボウイルスB19は脂質膜のない小型DNAウイルスである。これまで本製剤によるB19感染の報告はない。B19は耐熱性とされていたが最近、液状加熱で容易に不活化できることが明らかにされた。本製剤の製造工程には、当該工程が含まれている。また最終製品についてB19-NAT陰性であることを確認していることから、本製剤の安全性は確保されている。

今後の対応

日本赤十字社では、以前よりRHA法によるB19抗原検査を導入しウイルス量の多い血液を排除してきた。2008年からさらに感度の高い化学発光酵素免疫測定法(CLEIA)を導入し、10^6IU/mL以上のB19を含む血液を陽性と判定し排除するものであることから、現在は原料血漿プール中のウイルス濃度が10^4IU/mL以下となっている。今後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.13.0J

JRC2010T-028

Materials: Test results of all blood samples from 929 RR blood donors recorded during the 2006-2009 period obtained at CTTM by testing a total of 313,564 blood units were analyzed.
Results: In 929 RR donors, HCV predominated (51%) due to the use of combined anti HCV/HCV Ag-Ab test, followed by HIV (18.9%) and a comparable proportion of HBsAg and anti TP RR donors (14.4% and 15.8%, respectively). The HBsAg test yielded the highest rate of confirmed reactivity (42%), followed by syphilis-EIA (22.4%) and the lowest rate for HCV and HIV (4.4% and 1.1%, respectively). Table 1 summarizes the results of all RR donors analyzed during the study period, showing that 742 (79.9%) RR donors met the requirements for subsequent blood donation. In Table 2, next donations by these 742 donors are classified as follows: 475 (64%) presented for donation, 246 (51.8%) of them were seronegative and 229 (48.2%) showed repeat reactivity. Repeat reactivity was later recorded in 4 of 246 donors having continued with blood donation.

Table 1

2006-2009	HBV	HCV	HIV	SYPH.	Total	%
RR DONORS	134	473	175	147	929	100.0
Permanently deferred confirmed positive	56	21	2	33	112	12.0
Permanently deferred confirmed indetermined	3	28	2	9	42	4.5
Temporarily deferred/ To folow up	2	19	7	5	33	3.6
Free to donate/ Confirmed negative/flagged	73	405	164	100	742	79.9

Table 2

2006-2009	HBV	HCV	HIV	SYPH.	Total	%
Presented for donation	58	233	122	62	475	64.0
Next donations negative	33	119	65	29	246	51.8
Next donations reactive	25	1114	57	33	229	48.2

Conclusions: Testing for blood transmissible infections yielded nonspecific reactivity in the majority of 929 RR donors and repeat reactivity in nearly half of subjects (HBV 43%, HCV 48%, HIV 46% and syphilis 53%). None of RR donors developed infection (PCR negative) on follow up, whereas blood unit reactivity was recorded in 4 of 246 RR donors having continued with blood donation. Thus, the use of PCR on their reinclusion in the donor pool is justified. Further follow up in 229 blood donors scheduled for monitoring and additional testing resulted in permanent deferral in 108 and temporary deferral in 97 donors, whereas subsequent donation was approved in 24 donors.

P-0516

INFECTIVITY OF B19 DNA POSITIVE BLOOD PRODUCTS

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Background: Since 2000, blood donor screening for B19 by NAT at the Ulm Institute has been conducted 6-8 weeks post donation, i.e. after transfusion of cellular blood products whereas at the Frankfurt Institute all donations are screened before releasing any blood product. In the current study, we evaluated the infectivity of B19 positive blood products in relation to the virus concentration in the transfused blood component.
Study design: In a retrospective study, recipients were classified into two groups (A: transfused with blood products with B19 virus load less than 10^5 IU/ml; B: transfused with blood products with B19 virus load $>10^5$ IU/ml). Phylogenetic analyses were done for B19 DNA positive donor and recipient pairs in the variant VP-1u genome region. All samples were investigated for IgM and IgG B19 antibodies.
Results: B19 DNA was detected in 9 out of 18 recipients of red blood cell concentrates from group B whereas none out of 16 recipients from group A were B19 DNA positive (P = 0.016). Phylogenetic analysis demonstrated identical genome sequences between donors and recipients.
Conclusions: B19 transmission by cellular blood products correlates with the virus concentration as well as with the concentration of neutralizing antibodies. As a consequence, blood donor screening for B19 by mini-pool NAT should be implemented for all products in order to discard all donations with a high virus burden and to enable transfusion of B19 negative blood products for at-risk patients.

P-0517

WHO WORKSHOPS ON DEVELOPING NATIONAL SYSTEMS FOR 100% QUALITY-ASSURED SCREENING OF BLOOD DONATIONS FOR TRANSFUSION-TRANSMISSIBLE INFECTIONS

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Background: The provision of safe blood and blood products for transfusion or manufacturing use involves a number of processes, from the selection of blood donors and the collection, processing and testing of blood donations to its administration to patients. There is a risk of error in each process in this "transfusion chain" which can have serious implications for transfused patients. Thus, while blood transfusion can be life-saving, there are associated risks, particularly the transmission of bloodborne infections. It is the responsibility of governments to assure a safe and sufficient supply of blood and blood products for all patients requiring transfusion. However, in 2007, 41 countries are not able to screen all blood donations for one or more of the transfusion-transmissible infections (TTIs) – including HIV, hepatitis B, hepatitis C and syphilis.
Aim: The main aims of the WHO workshops are to: provide an opportunity for the sharing of experience among countries on the challenges and strategies in developing national systems for quality-assured blood screening; identify needs and areas of concern in strengthening national blood screening programmes; develop country action plans for priority activities for national blood screening programmes, and make recommendations to international organizations for supporting countries to meet their needs in achieving 100% quality-assured screening of donated blood.
Methods: WHO Blood Transfusion Safety Programme had organized two 3-day workshop on "Developing National Systems for 100% Quality-Assured Screening of Donated Blood for Transfusion-Transmissible Infections". The WHO document, "Recommendations on Screening Donated Blood for Transfusion-Transmissible Infections" were used as the basis of the training workshop.
Results: Sixty participants from 25 countries in the African, South-East Asian and Western Pacific regions attended the workshops. These represent the countries that were not able to screen all donated blood for major transfusion-transmissible infections or to perform screening within a quality system. Invited participants from each country will include the national blood programme manager and a senior laboratory manager in the blood transfusion service (medical/scientific/technical) who is involved in setting up national systems for the quality-assured screening of donated blood. The working methodology of the workshop will include country presentations, group work and the development of country plans.
Conclusions: The workshops were able to facilitate the sharing of experience among countries on the challenges and strategies in developing national systems for quality-assured blood screening; identify variations in screening strategies, practices and areas of concern of the countries; provide opportunity for participants to develop country action plans for priority activities to strengthen national blood screening programmes, and strengthen the strategies and capacity of international organizations and institutions to respond to countries' needs on policy and technical guidance in supporting countries to meet their needs in achieving 100% quality-assured screening of donated blood.

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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
				2010. 5. 7	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	EID Jnl. Vol.16 No.5	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)				米国	

研究報告の概要	使用上の注意記載状況・その他参考事項等
○ヒトスジシマカにおけるLa Crosseウイルス(LACV)(2009年米国テキサス州ダラス) 2009年8月にテキサス州ダラスで採取した、ヒトスジシマカにおけるLACVについて報告する。LACVは主にAedes triseriatusが媒介する、北アメリカでの小児脳炎の主要な原因である。しかし近年、LACV脳炎が南東部地域で増加し、南部でも報告されている。同時にアジアからの外来種であるヒトスジシマカが増加しているが、今までヒトスジシマカとLACV伝播の関連は不明であった。今回の調査で、テキサス州ダラスで採取したヒトスジシマカからLACVが検出された。これまで流行が確認されていた範囲外で、外来性の蚊に当該ウイルスが認められたことは、公衆衛生上の懸念である。	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
2009年8月にテキサス州ダラスで採取した、外来種であるヒトスジシマカからLa Crosseウイルスが検出されたとの報告である。 La Crosseウイルスはブニヤウイルス科の脂質膜を持つRNAウイルスである。これまで、本製剤によるLa Crosse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渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

La Crosse Virus in *Aedes albopictus* Mosquitoes, Texas, USA, 2009

Amy J. Lambert, Carol D. Blair, Mary D'Anton, Winnann Ewing, Michelle Harborth, Robyn Seiferth, Jeannie Xiang, and Robert S. Lanciotti

We report the arthropod-borne pediatric encephalitic agent La Crosse virus in *Aedes albopictus* mosquitoes collected in Dallas County, Texas, USA, in August 2009. The presence of this virus in an invasive vector species within a region that lies outside the virus's historically recognized geographic range is of public health concern.

La Crosse virus (LACV) is the most common cause of arthropod-borne, pediatric encephalitis in North America. A member of the California serogroup within the family *Bunyaviridae* and the genus *Orthobunyavirus*, LACV is enveloped and contains a negative-sense, tripartite genome with segments designated small (S), medium (M), and large (L). Cases of LACV-associated encephalitis, which can be fatal, occur within the geographic range of its principal vector, *Aedes triseriatus* mosquitoes. This native, tree-hole breeding mosquito is distributed throughout wooded regions east of the Rocky Mountains within the United States. Historically, most LACV-associated encephalitis cases have occurred in upper midwestern states, including Wisconsin, Illinois, Minnesota, Indiana, and Ohio (Figure 1). In recent years, LACV encephalitis activity has increased above endemic levels in regions of the southeastern United States, including West Virginia, North Carolina, and Tennessee (Figure 1) (*1*). In addition, recent cases of LACV encephalitis have been reported as far south as Louisiana, Alabama, Georgia, and Florida (Figure 1).

Ae. albopictus is an invasive mosquito species that was first discovered in Houston, Texas, in 1985 (*2*), having apparently arrived in the United States in a shipment of used tires from Asia (*3*). An opportunistic container-breeder, its vector competence for many arthropod-borne viruses (arboviruses), including LACV, and its catholic feeding habit have made the invasion of *Ae. albopictus* mosquitoes disconcerting to researchers, who have warned of the potential for an increased incidence of vector-borne diseases as a result (*4,5*). Since 1985, the geographic distribution of these mosquitoes has grown to include most of the southeastern United States. The concurrent increase in LACV encephalitis activity has led to speculation on the possible transmission of LACV by *Ae. albopictus* mosquitoes as an accessory mechanism to the historically recognized transmission by *Ae. triseriatus* mosquitoes (*6*). LACV has been isolated from *Ae. albopictus* mosquitoes in Tennessee and North Carolina in 1999 and 2000, respectively, during a period of greatly increased LACV activity in those areas (*6*). However, the role of this species in LACV transmission remains unknown.

We report the isolation of LACV from a pool of 3 *Ae. albopictus* mosquitoes collected outside the known geographic range of the virus, in Dallas County, Texas, on August 13, 2009 (Figure 1). This is one of only several isolations of LACV within the state; the first isolate was derived from a pool of *Ae. infirmatus* mosquitoes collected in Houston in 1970 (*7*). After the identification of LACV in the Dallas pool, an additional isolation of LACV was made from a mixed pool of 29 *Ae. albopictus* and 2 *Ae. triseriatus* mosquitoes collected in Fort Bend County, Texas, in October 2009 (Figure 1). The Fort Bend County location is relatively near the site of collection of the 1970 Texas LACV-positive pool and the known geographic distribution of LACV activity in southeastern Texas and Louisiana (Figure 1). Taken together, our results represent an unprecedented number of LACV findings within the state of Texas.

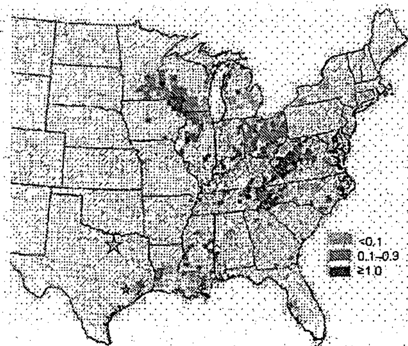

Figure 1. Geographic distribution of La Crosse virus (LACV) in accordance with the habitat range of *Aedes triseriatus* mosquitoes in the United States as inferred from the California serogroup virus neuroinvasive disease average annual incidence by county, 1996–2008. Incidence rates are shown in shades of blue. Dallas County and Fort Bend County locations of the 2009 LACV isolations from pools containing *Ae. albopictus* and *Ae. triseriatus* mosquitoes are indicated by green and red stars, respectively. Data and figure adapted from the Centers for Disease Control and Prevention website (www.cdc.gov/lac/tech/epi.html).

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The Study

As part of ongoing arbovirus surveillance efforts, the City of Dallas Vector Control Division collected 65 mosquitoes in a gravid trap at the edge of a wooded area near a residential district in Dallas County on August 13, 2009. Upon their receipt at the Texas State Department of Health Services, none of the mosquitoes was viable. The mosquitoes were sorted and identified by sex. Female mosquitoes were grouped into 3 pools by species: pool no. AR6318, consisting of 50 *Culex quinquefasciatus* mosquitoes, pool no. AR6319, consisting of 3 *Ae. albopictus* mosquitoes; and pool no. AR6320, consisting of 1 *Ae. triseriatus* mosquito.

Generated pools were macerated in 1.5 mL of bovine albumin diluent arbovirus medium followed by 2 rounds of centrifugation at 10,000 rpm for 5 min each. Between each round of centrifugation, a rest period of 15 min was used to facilitate pellet formation. After centrifugation, 50 µL of the resultant supernatant was injected onto BHK and Vero cells. These cells were incubated at 37°C and examined for cytopathic effect (CPE) over the next 10 days. At day 5 postinoculation, Vero cells inoculated with the supernatant derived from pool no. AR6319 (*Ae. albopictus*) demonstrated marked CPE. This condition represented a preliminary virus isolation-positive result. No CPE was observed in the BHK cells. Infected cells were then subjected to immunofluorescent antibody assays with antibodies directed against various arboviruses, followed by the use of fluorescein isothiocyanate–conjugated antimouse antibodies for detection. From these analyses, the isolate derived from pool no. AR6319 (*Ae. albopictus*) was determined to be a California serogroup virus. Furthermore, pool no. 6318 (*Cx. quinquefasciatus*) tested positive for West Nile virus, and pool no. 6320 (*Ae. triseriatus*) was negative for virus by the above described methods.

To further identify the California serogroup virus identified in pool no. AR6319 (*Ae. albopictus*), the pool and the Vero cell–derived isolate were sent to the Centers for Disease Control and Prevention in Fort Collins, CO, USA, for additional testing. Upon receipt of the samples in Fort Collins, a reverse transcription–PCR was performed to amplify cDNAs from all 3 segments of the orthobunyavirus genome by using the consensus oligonucleotide primers shown in the Table and conditions and methods previously described (*8*). Generated cDNAs were then subjected to nucleotide sequencing and BLAST (www.ncbi.nlm.nih.gov/BLAST) analyses; the results indicated that the pool and the isolate were positive for LACV S, M, and L segment RNAs.

Subsequently, a pool (AR8973) of 29 *Ae. albopictus* and 2 *Ae. triseriatus* mosquitoes collected in Fort Bend County, Texas on October 5, 2009, was identified as positive for LACV S, M, and L segment RNAs by using the same processing and characterization methods described above. After these analyses, full-length S, M, and L segment genomic sequences (GenBank accession nos. GU591164–9) were generated for LACV RNAs extracted from LACV-positive pools and Vero cell isolates by using oligonucleotide primers specific for the previously published LACV prototype genome (human 1960, GenBank accession nos. EF485030–2) and methods previously described (*9*).

Phylogenetic analyses of partial LACV M segment sequences (Figure 2) indicate that the LACVs present in the Texas 2009 pools are closely related to LACVs isolated from Alabama, Georgia, and New York of the previously described lineage 2 (*11*) and genotype C (*7*) designations. These findings suggest a likely southeastern ancestry for the Texas 2009 LACV isolates.

Conclusions

The presence of LACV in *Ae. albopictus* mosquitoes in Dallas County, Texas, in late summer 2009 represents the possible expansion of the geographic range of an endemic pathogen within this invasive mosquito species in the United States. The subsequent occurrence of LACV in Fort Bend County in October 2009 should be of concern to public health practitioners who have been alerted to the

Table. Orthobunyavirus consensus oligonucleotide primers used for amplification and sequencing of La Crosse virus partial S, M, and L segment cDNAs, Texas, 2009*

Targeted genomic regions	Name	Primer sequence (5′ → 3′)	Approximate amplicon size, bp
S segment nucleocapsid ORF	Cal S forward	GCAAATGGATTTGATCCTGATGCAG	210
	Cal S reverse	TTGTTCCTGTTTGCTGGAAAATGAT	
M segment 5′ terminus/glycoprotein ORF	Ortho M 5′ terminus	AGTAGTGTACTACC	410
	Ortho M ORF reverse	TTRAARCADGCATGGAA	
L segment 5′ terminus/polymerase ORF	Ortho L 5′ terminus	AGTAGTGTACTCCTA	550
	Ortho L ORF reverse	AATTCYTCATCATCA	

*Oligonucleotide primers designed against conserved regions of the orthobunyavirus genome. S segment primers appear in a previous publication (*8*). All primers were applied in singleplex reactions using methods described previously (*8*) with altered primer annealing conditions of 50°C for 1 min. S, small; M, medium; L, large; ORF, open reading frame.

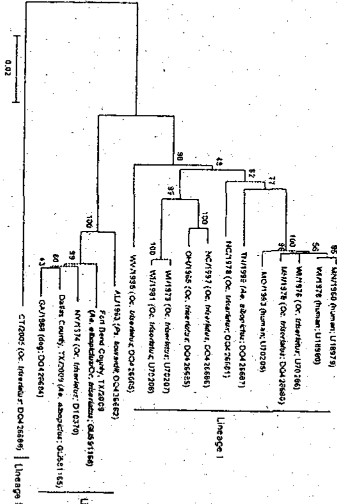

Figure 2. Phylogeny of La Crosse virus (LACV) medium (M) segment sequences of diverse origins. According to a limited availability of full-length sequences in GenBank, 1,663 nt of the M segment glycoprotein gene open-reading frame are compared. Isolate source and GenBank accession nos. appear after the isolate designation for each taxon. Sequences were aligned by ClustalW (10) and neighbor-joining and maximum-parsimony trees were generated by using 2,000 bootstrap replicates with MEGA version 4 software (10). Highly similar topologies and confidence values were derived by all methods and a neighbor-joining tree is shown. Scale bar represents the number of nucleotide substitutions per site. The 2009 Texas (TX) isolates group with strong support with lineage 2 viruses of the extreme south and New York (NY), which suggests a likely southern origin for LACV isolates. MN, Minnesota; WI, Wisconsin; Oc, *Ochlerotatus*; MO, Missouri; TN, Tennessee; Ae., *Aedes*; NC, North Carolina; OH, Ohio; WV, West Virginia; AL, Alabama; Ps., *psorophora*; GA, Georgia; CT, Connecticut.

presence of this pathogen near 2 major urban centers, Dallas and Houston. Of interest, San Angelo virus, which is serologically related to LACV, is known to occur in Texas and has been shown to replicate in and be transovarially transmitted by *Ae. albopictus* mosquitoes (12), although this virus has no known association with human disease. Cocirculation enables possible reassortment of genomic segments between LACV and San Angelo virus, a phenomenon that has been described for viruses of the California serogroup within *Ae. albopictus* mosquitoes (13) with unknown public health outcomes.

Ms Lambert is a research microbiologist at the Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases, Fort Collins, Colorado. Her primary research interests lie in the molecular characterization, detection, and evolution of viruses of the family *Bunyaviridae*.

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No. 11

医薬品 研究報告 調査報告書

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研究報告の概要	使用上の注意記載状況・その他参考事項等
○2009年の米国におけるウエストナイルウイルス（WNV）の流行状況 米国疾病管理予防センター（CDC）が発表した2009年の米国におけるWNVの流行状況である。米国の38州の262郡と、コロンビア特別区から720症例のWNV感染症が報告された。そのうち386例（54%）が神経侵襲性疾患で、334例（46%）が非神経侵襲性疾患であった。WNV感染症での死亡者は全部で33人が報告され、そのうち32人が神経侵襲性疾患であった。神経侵襲性疾患のうち229例（59%）が脳炎、117例（30%）が髄膜炎、40例（10%）が急性弛緩性麻痺であった。急性弛緩性麻痺40例のうち、27例（68%）が脳炎または髄膜炎を併発した。	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
2009年、米国におけるウエストナイルウイルス感染症例は38州及びコロンビア特別区から720症例が報告され、そのうち386例が神経侵襲性疾患であり、全体の死者は33人であったとの報告である。 ウエストナイルウイルスは脂質膜を持つRNAウイルスである。これまで、本剤によるウエストナイルウイルス感染の報告はない。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る。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国（入国）後4週間は献血不適としている。また、ウエストナイルウイルス感染の国内発生に備え、平成17年10月25日付血液対策課発事務連絡に基づき緊急対応の準備を進めているほか、厚生労働科学研究「献血血の安全性確保と安定供給のための新興感染症等に対する検査スクリーニング法等の開発と献血制限に関する研究」班と共同して対応について検討している。今後も引き続き情報の収集に努める。

JRC2010T-026

Morbidity and Mortality Weekly Report

Weekly / Vol. 59 / No. 25 July 2, 2010

West Nile Virus Activity — United States, 2009

West Nile virus (WNV) was first detected in the Western Hemisphere in 1999 in New York City and has since caused seasonal epidemics of febrile illness and neurologic disease across the United States, where it is now the leading cause of arboviral encephalitis (*1*). This report updates a previous report (*2*) and summarizes WNV activity in the United States reported to CDC in 2009. A total of 38 states and the District of Columbia (DC) reported 720 cases of WNV disease. Of these, 33 states and DC reported 386 cases of WNV neuroinvasive disease, for an incidence of 0.13 per 100,000 population. The five states with the highest incidence of WNV neuroinvasive disease were Mississippi (1.05 per 100,000), South Dakota (0.74), Wyoming (0.73), Colorado (0.72), and Nebraska (0.61). Neuroinvasive disease incidence increased with increasing age, with the highest incidence among persons aged ≥70 years. A total of 33 WNV deaths were reported, 32 from neuroinvasive disease. Calculating from the number of neuroinvasive disease cases and projections from 1999 serosurvey data, CDC estimated that 54,000 persons were infected with WNV in 2009, of whom 10,000 developed nonneuroinvasive WNV disease. The continuing disease burden caused by WNV affirms the need for ongoing surveillance, mosquito control, promotion of personal protection from mosquito bites, and research into additional prevention strategies.

WNV is a nationally notifiable disease. Data are reported to CDC through ArboNET, an Internet-based arbovirus surveillance system managed by state health departments and CDC (*2*). Using standard case definitions,* human WNV disease cases are classified as WNV neuroinvasive disease (e.g., meningitis, encephalitis, or acute flaccid paralysis) or WNV nonneuroinvasive disease (e.g., acute systemic febrile illness that often includes headache, myalgia, or arthralgia). Nonneuroinvasive disease reporting varies greatly by jurisdiction, depending on disease awareness, health-care–seeking behaviors, and testing practices. Therefore, this report focuses on WNV neuroinvasive disease cases, which are thought to be identified and reported more consistently because of the severity of the illness. In addition to human disease cases, ArboNET captures data on presumptively viremic blood donors (PVDs), veterinary cases, and WNV infections in sentinel animals (most commonly chickens), dead birds, and mosquitoes. Not all jurisdictions conduct nonhuman surveillance.

* Available at http://www.cdc.gov/ncphi/disss/nndss/casedef/arboviral_current.htm.

Human Surveillance

During 2009, a total of 720 cases of WNV disease were reported from 262 counties in 38 states and DC. Of these 720 cases, 386 (54%) were reported as WNV neuroinvasive disease and 334 (46%) as nonneuroinvasive disease. A total of 116 PVDs, identified through routine screening of the blood supply, also were reported. Of these PVDs, 92 (79%) were asymptomatic, 23 (20%) developed nonneuroinvasive disease, and one (1%) subsequently developed neuroinvasive disease. PVDs who developed symptomatic disease were included in disease case counts.

The 386 reported cases of neuroinvasive disease represented a rate of 0.13 per 100,000 population in the United States, based on July 1, 2009 U.S. Census population estimates (Figure 1). States reporting the most WNV neuroinvasive disease cases were Texas with 93 (24% of U.S. cases) and California with 67 (17%). Washington, which reported only two neuroinvasive disease cases in 2008, reported 26 (7%) cases in 2009. The five states with the highest incidence were Mississippi (31 cases, 1.05 cases per 100,000 residents), South Dakota (six cases, 0.74), Wyoming (four cases, 0.73), Colorado (36 cases, 0.72), and Nebraska (11 cases, 0.61) (Figure 2). WNV neuroinvasive disease peaked in the United States during mid-August, and 352 (91%) of the 386 cases were reported during July–September. This seasonality was consistent with trends observed over the preceding 10 years (*2*).

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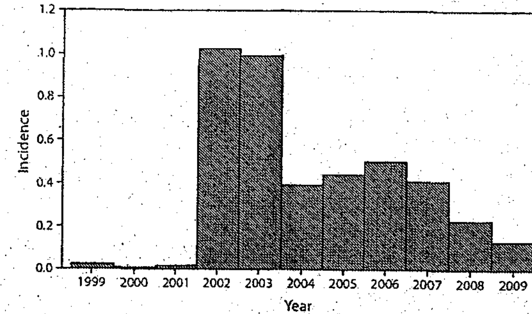
FIGURE 1. Annual incidence* of cases of West Nile virus neuroinvasive disease† — ArboNET, United States, 1999–2009§



* Per 100,000 population, based on July 1 U.S. Census estimates for each year.
† Meningitis, encephalitis, or acute flaccid paralysis.
§ N = 12,208 during 1999–2009; N = 386 in 2009

Of the 386 neuroinvasive disease cases, 226 (59%) occurred in males. The median age of patients was 60 years (range: 2–91 years), with increasing incidence among persons in older age groups (Figure 3). Overall, 368 (95%) patients with neuroinvasive disease were hospitalized, and 32 (8.3%) died (median age: 72 years; range: 19–89 years). A total of 229 (59%) neuroinvasive disease cases were classified as encephalitis, 117 (30%) as meningitis, and 40 (10%) as acute flaccid paralysis; 27 (68%) of the 40 cases classified as acute flaccid paralysis had coincident encephalitis or meningitis.

Serologic surveys indicate that for every case of WNV neuroinvasive disease there are approximately 140 infections and approximately 20% of infected persons develop nonneuroinvasive disease (*3*). Using the 386 reported neuroinvasive disease cases, CDC estimated that 54,000 infections and 10,000 cases of WNV nonneuroinvasive disease occurred in the United States in 2009. Only 334 nonneuroinvasive disease cases were reported to ArboNET in 2009, representing approximately 3% of the estimated number.

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Animal Surveillance

Of 298 reported veterinary cases of WNV disease, 275 (92%) occurred in equines and 23 (8%) occurred in other species: squirrels, 13; canines, eight; camelids, one; and deer, one. The equine cases were reported from 168 counties in 36 states, with 72 (26%) reported from Washington. The number of reported WNV-infected equines peaked during the first week of September.

In 2009, a total of 759 dead WNV-infected birds were reported from 141 counties in 25 states and the District of Columbia: California reported 515 (68%) dead birds. Of the 141 counties reporting WNV-infected birds, 92 (65%) counties in 19 states reported infected dead birds but no human disease cases. The number of reported WNV-infected birds peaked during the first week of September. Corvids (e.g., crows, jays, and magpies), which are targeted for surveillance by most states, accounted for 534 (70%) of the birds. Since 1999, WNV infection has been reported in 328 avian species, including two species, MacGillivray's warbler and tricolored blackbird, in which WNV was identified for the first time during 2009.

Mosquito Surveillance

In 2009, a total of 6,646 mosquito pools† from 351 counties in 40 states and DC were reported as testing positive for WNV. Among the WNV-positive pools, 4,987 (75%) had species of *Culex* mosquitoes thought to be the principal vectors of WNV (e.g., *Culex pipiens, Culex quinquefasciatus, Culex restuans, Culex salinarius*, and *Culex tarsalis*). Unidentified or other species of *Culex* mosquitoes made up 1,488 (22%) pools, and non-*Culex* mosquito species (e.g., *Aedes* sp., *Anopheles* sp., *Coquillettidia perturbans, Culiseta* sp., *Mansonia titillans, Psorophora columbiae*, and *Uranotaenia sapphirina*) made up 171 (3%) pools. Data from 2009 also included the first report of WNV infection in *Aedes epactius*, which was collected in Texas. The number of reported WNV-infected mosquito pools peaked during mid-August.

Reported by

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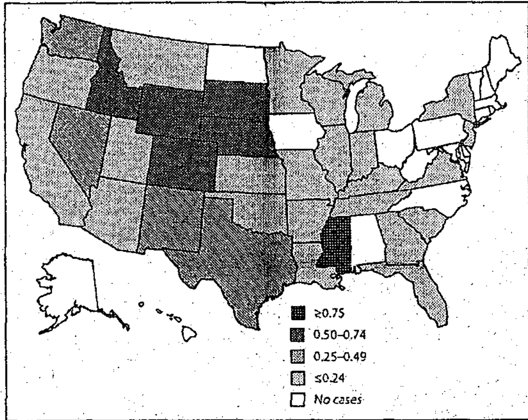
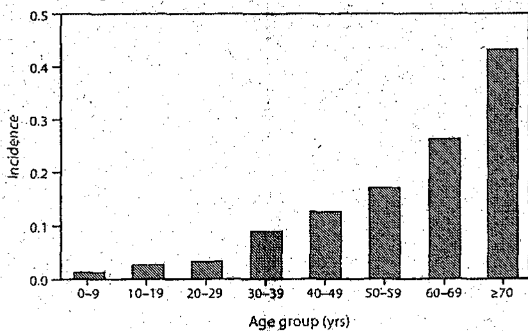
† A sample of mosquitoes (usually no more than 50) of the same species and sex, collected within a defined sampling area and period.

FIGURE 2. Incidence* of cases (N = 386) of West Nile virus neuroinvasive disease† — ArboNET, United States, 2009



* Per 100,000 population, based on July 1, 2009 U.S. Census estimates.
† Meningitis, encephalitis, or acute flaccid paralysis.

FIGURE 3. Incidence* of cases (N = 386) of West Nile virus neuroinvasive disease,† by age group — ArboNET, United States, 2009



* Per 100,000 population, based on July 1, 2009 U.S. Census estimates.
† Meningitis, encephalitis, or acute flaccid paralysis.

Editorial Note

Since introduced into the United States in 1999, WNV has become the leading cause of arboviral encephalitis in the country. However, in 2009, the reported incidence of WNV neuroinvasive disease in the United States was 0.13 per 100,000 population, the lowest recorded since 2001 (*2*). During

What is already known on this topic?

Since introduced into the United States in 1999, West Nile virus (WNV) has become the leading cause of arboviral encephalitis in the country.

What is added by this report?

In 2009, 386 cases of WNV neuroinvasive disease were reported in the United States, or 0.13 cases per 100,000 population, the lowest incidence recorded since 2001; however, CDC also estimated that 54,000 persons were infected with WNV in 2009, including 10,000 with nonneuroinvasive WNV disease.

What are the implications for public health practice?

The continuing disease burden caused by WNV affirms the need for ongoing surveillance, mosquito control, promotion of personal protection from mosquito bites, and research into additional prevention strategies.

2004–2007, WNV had appeared to reach a stable incidence of approximately 0.4 per 100,000, but incidence dropped to 0.2 per 100,000 in 2008 (*2*) and continued to decline in 2009. This trend might be attributed to variation in populations of vectors and vertebrate hosts, accumulation of immunity in avian amplifying hosts, human behavior (e.g., use of repellents and protective clothing), community-level interventions, reporting practices, or environmental factors (e.g., temperature and rainfall) (*4,5*).

In 2009, evidence of WNV human disease again was detected in all geographic regions of the continental United States. The highest incidence of WNV neuroinvasive disease continued to occur mainly in the west-central United States, likely because of the high efficiency of *Cx. tarsalis* as a WNV vector. Mississippi (31 cases, 1.05 cases per 100,000) continued to be among those states with the highest incidence of WNV neuroinvasive disease. Arizona, which had the second highest incidence of WNV neuroinvasive disease in 2008 (62 cases, 1.0 per 100,000), reported an 81% decrease in cases with 12 cases and an incidence of only 0.18 per 100,000 in 2009 (*1*). After reporting its first two neuroinvasive disease cases in 2008, Washington reported the seventh highest state incidence in 2009 (26 cases, 0.39 per 100,000). These findings illustrate the wide annual variability and focality of WNV transmission.

The findings in this report are subject to at least two limitations. First, ArboNET is a passive surveillance system that depends on clinicians to consider the diagnosis of an arboviral disease, obtain the appropriate diagnostic test, and report any positive results. Diagnosis and reporting likely are incomplete, leading to underestimation of the true incidence of disease. Second, arboviral surveillance programs, testing capacity, and reporting can vary by county, state, or region, affecting incidence estimates.

In the absence of an effective human vaccine, prevention of WNV disease depends on community-level *mosquito control and promotion of personal* protective measures. Such measures include use of mosquito repellents, barrier protection (e.g., long-sleeved shirts, long pants, and socks), avoiding outdoor exposure, or using personal protection from dusk to dawn. Household measures, such as window screens and covering or draining peridomestic water-holding containers can further decrease the risk for WNV exposure.

Additional information on prevention of WNV infection is available from CDC at http://www.cdc.gov/ncidod/dvbid/westnile/index.htm. An overview of current year WNV transmission activity is available at http://diseasemaps.usgs.gov/wnv_us_human.html.

Acknowledgments

This report is based, in part, on data provided by ArboNET surveillance coordinators in local and state health departments and ArboNET technical staff, Div of Vector-Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, CDC.

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医薬品 研究報告 調査報告書

			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2010. 4. 27	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	ABC Newsletter #15. 2010 Apr 23; 15.	公表国 ニュージーランド・オーストラリア	
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	使用上の注意記載状況・その他参考事項等
○ニュージーランドの血液銀行は慢性疲労症候群（CFS）の既往を持つ供血者の供血延期を開始し、オーストラリア当局は、供血ガイドラインの見直しを行っている ニュージーランドの決定は、前立腺癌と関連性があるXMRVが、健常集団と比較してCFS患者の血中に非常に多く認められたという米国の試験を受けてなされた。他の科学者は、この結果を確認することができなかったが、米国保健当局は、CFSとXMRV間の関連の可能性について調査を行っており、カナダ血液サービスはすでにCFSの診断を受けた供血者からの供血を無期限延期としている。一方、オーストラリア赤十字血液サービスは、独自にリスク分析を行い、完全に回復するまでのCFS患者からの供血を延期することを現行のガイドラインで求めている。	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
ニュージーランドの血液銀行は慢性疲労症候群の既往を持つ供血者の供血延期を開始し、オーストラリア当局は、供血ガイドラインの見直しを行っているとの報告である。 XMRVの病原性の有無は未だ定かではない。XMRVはマウス白血病ウイルスと類縁な脂質膜を持つ大型RNAウイルスである。この性状から本製剤の製造工程でウイルスが不活化・除去されると期待しうることから、本製剤の安全性は確保されていると考える。	注目すべきウイルスとして今後も引き続き、新たなウイルス等に関する情報の収集に努める。

(13)

MedDRA/J Ver.13.0J

JRC2010T-016

GLOBAL NEWS

Members of the Dutch Parliament met last week to discuss the cost of blood in that country, and one of their topics was the transparency of operations at Sanquin, the foundation responsible for managing the blood supply in the Netherlands. Last August, a benchmark report compared the price of blood products in a number of European countries, and it concluded that prices in the Netherlands were higher than those in Ireland, Belgium, France, and Finland. In response, the Minister of Health wrote a letter in which he indicated a number of steps that would improve transparency at Sanquin, and he also announced a follow-up study that would focus on the current law on blood supply. The meeting this week was also attended by representatives from patient organizations, donor organizations, physicians, the Plasma Proteins Therapeutics Association, the Dutch Red Cross, and Sanquin. (Source: *PPTA Leadership Briefing*, 4/16/10) ♦

INFECTIOUS DISEASE UPDATES

CFS

Blood banks in New Zealand will begin deferring any potential blood donor who has a record of chronic fatigue syndrome (CFS), and officials in Australia are reviewing donation guidelines there. The decision in New Zealand was made in the wake of a US research study that found xenotropic murine leukemia virus-related virus (XMRV), a virus that has been linked to prostate cancer, in the blood of far more people with CFS than the healthy population. Other scientists have been unable to confirm those results, but health authorities in the US are investigating the possible link between CFS and XMRV, and Canadian Blood Services (CBS) has already instituted a lifetime deferral for potential blood donors who have been diagnosed with CFS (see *ABC Newsletter*, 4/9/10). The national medical director for New Zealand's blood banks, Peter Flanagan, said the New Zealand Blood Service (NZBS) reviewed the issue at a meeting held earlier this month and decided that the present exclusion of blood from people still suffering from CFS or patients who had been diagnosed in the past two years "should be extended to also exclude donors who report ever having been diagnosed with chronic fatigue syndrome." He admitted that the decision was made despite a lack of good scientific data on the issue. Meanwhile, the Australian Red Cross Blood Service is conducting its own risk analysis, and it says existing donor guidelines require people with CFS to defer giving blood until they make a full recovery. It said it collects more than 500,000 blood donations each year, but only 70 donors with CFS have been deferred in the past two years. The blood service said in a statement that it "currently defers donors who suffer from [CFS and] before we can accept their blood again, they need to bring us a letter from their treating physician advising us that they are completely recovered." (Sources: www.stuff.co.nz, 4/21/10; www.heraldsun.com.au, 4/20/10) ♦

We Welcome Your Articles

We at the *ABC Newsletter* welcome freelance articles on any subject relevant to the blood banking community. Writers are encouraged to submit short proposals or unsolicited manuscripts of no more than 1,100 words. While ABC cannot pay for freelance pieces, the writer's name and title will be included at the end of the story, brief news item, or commentary. If proposing a story, please write a few paragraphs describing the idea and sources of information you will use, your present job and background, and your qualifications for writing on the topic. ABC staff cannot guarantee all stories will be published, and all outside writing will be subject to editing for style, clarity, brevity, and good taste. Please submit ideas and manuscripts to Editor Robert Kapler at rkapler@americasblood.org. You will be sent a writer's guide that provides information on style conventions, story structure, deadlines, etc.

別紙様式第２－１

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
一般的名称	－		研究報告の公表状況	http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm223277.htm http://www.fda.gov/BiologicsBloodVaccines/SafetyAvailability/ucm223232.htm	公表国 米国	
販売名(企業名)	－					

研究報告の概要

米国食品医薬品局生物製剤評価・研究センターおよび米国国立衛生研究所臨床センターの研究者が、慢性疲労症候群(CFS)と診断された患者37名と健常血液ドナー44名から採取した血液サンプルを検査したところ、CFS患者37名のうち32名のサンプル(87%)、および健常血液ドナー44名のうち3名のサンプル(7%)において複数の異なるマウス白血病ウイルス(MLV)遺伝子配列を特定した。

本研究は、MLV様ウイルスの遺伝的変異体であるXMRV(異種指向性マウス白血病ウイルス関連ウイルス)がCFS患者の血液中に存在することを明らかにした過去の研究結果を裏付けており、CFSの診断と血液中のMLV様ウイルス遺伝子配列の存在との間に強い関連性があることを実証している。さらに、ごく一部の健常血液ドナーにおいてMLV様ウイルス遺伝子配列が検出された。CFSとの統計的関連性は強いとはいえ、これらのレトロウイルスがCFSの原因であることを証明するものではない。XMRVやその他のMLV関連ウイルスがCFSを引き起こす可能性を確定するためには、今後も研究を継続する必要がある。

「MLVやXMRVは血液製剤や組織由来製剤によって伝播するか？」については、これらウイルスが血液やヒトの組織によって伝播する可能性があるかどうか、そして、これらのウイルスが疾患を引き起こすかどうかを調査するためには追加研究を行う必要がある。FDA、NIH、CDCおよびその他の科学機関の研究者は、血液中のXMRVやMLV関連ウイルスの検出用として多くの研究所が使用している試験の能力を検証するため、複数の研究を実施中である。これらの研究は、XMRVが血液や組織のレシピエントに伝播する可能性だけでなく、XMRVと疾患との関連性についてもより詳しく調べるために、感受性が高く、特異的なXMRV試験の開発や標準化を目的としている。

使用上の注意記載状況・その他参考事項等

重要な基本的注意
[患者への説明]
本剤の投与にあたっては、疾病の治療における本剤の必要性とともに、本剤の製造に際し感染症の伝播を防止するための安全対策が講じられているが、ヒト血液を原料としていることに由来する感染症伝播のリスクを完全に排除することができないことを、患者に対して説明し、理解を得るよう努めること。

報告企業の意見	今後の対応
慢性疲労症候群の原因である可能性があるXMRVやその他のMLVの血液からの検出に関する情報である。 現時点で疾患の原因として特定されておらず、検出法についても検討中との情報であった。	今後ともXMRVやその他のMLVに関する安全性情報等に留意していく。

(14)

NIHONSEIYAKU
2008-018

U.S. Department of Health & Human Services

FDA U.S. Food and Drug Administration

Home > News & Events > Newsroom > Press Announcements

News & Events

FDA Note to Correspondents

For Immediate Release: August 23, 2010
Media Inquiries: Shelly Burgess, 301-796-4651, shelly.burgess@fda.hhs.gov
Consumer Inquiries: 888-INFO-FDA

Study: Presence of murine leukemia virus related gene sequences found in CFS patients

Researchers have found murine leukemia viruses (MLV) related gene sequences in blood samples collected from patients diagnosed with chronic fatigue syndrome (CFS) and some healthy blood donors, according to a study published online today by the scientific journal Proceedings of the National Academy of Sciences (PNAS).

Investigators from the U.S. Food and Drug Administration's Center for Biologics Evaluation and Research and the National Institutes of Health Clinical Center, in collaboration with a physician scientist at Harvard Medical School, examined blood samples from 37 patients diagnosed with CFS and from 44 healthy blood donors.

MLV is a type of retrovirus known to cause cancer in mice. Several different MLV gene sequences were identified in samples from 32 of the 37 patients with CFS (87 percent) and 3 of the 44 (7 percent) healthy blood donors. Investigators performed DNA sequencing on all positively amplified samples to confirm MLV like gene sequences.

This study supports a previous investigation [Lombardi et al. Science October 23, 2009 326: 585] that showed XMRV, a genetic variant of MLV-like viruses, to be present in the blood of people with CFS. The study demonstrates a strong association between a diagnosis of CFS and the presence of MLV-like virus gene sequences in the blood. The study also showed that MLV-like viral gene sequences were detected in a small fraction of healthy blood donors. Although the statistical association with CFS is strong, this study does NOT prove that these retroviruses are the cause of CFS. Further studies are necessary to determine if XMRV or other MLV-related viruses can cause CFS.

A previous study, published in 2009, reported finding XMRV infections in a high percentage of CFS patients and a small percentage of healthy blood donors. However, several other studies from the United States (including a recent report from the Centers for Disease Control and Prevention), the United Kingdom, and the Netherlands have found no evidence of XMRV or other MLV-like viruses in the blood of people with CFS.

For more information:

- Murine Leukemia Virus Gene Sequence Study - Questions and Answers[1]
- Xenotropic Murine Leukemia Virus-related Virus - Overview[2] (CDC)
- Xenotropic Murine Leukemia Virus-related Virus - Questions and Answers[3] (CDC)

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Links on this page:

1. http://www.fda.gov/BiologicsBloodVaccines/SafetyAvailability/ucm223232.htm
2. http://www.cdc.gov/xmrv/index.html
3. http://www.cdc.gov/xmrv/questions-answers.html

FDA U.S. Food and Drug Administration

Vaccines, Blood & Biologics

New study on the detection of murine leukemia virus-related virus gene sequences in the blood of patients with chronic fatigue syndrome (CFS) and healthy blood donors - Questions and Answers

Questions and Answers

1. What are murine leukemia viruses?

Murine leukemia viruses (MLV) are retroviruses known to cause cancer in certain mice. In 2006, investigators found that a type of MLV, called xenotropic murine leukemia virus-relate virus (XMRV), could potentially infect humans. XMRV is one of a number of MLVs that appear to be transmitted to humans.

2. What is CFS?

Chronic fatigue syndrome (CFS) is a debilitating disorder defined solely by clinical symptoms and the absence of other causes. It's unknown what causes CFS.

3. Has MLV or XMRV previously been associated with CFS or other disease?

A previous study, published in the journal [Lombardi et. al. *Science* October 23, 2009 326: 585], reported finding XMRV in a high percentage of CFS patients and a small percentage of healthy blood donors. However, other studies conducted in the U.S., Netherlands, and UK did not detect evidence of XMRV or other MLV-related viruses in CFS patients.

XMRV was first identified in tissue samples from some prostate cancer patients in 2006. However, one subsequent study failed to find XMRV in prostate cancer tissues, and another *study found the virus only rarely in such tissues.*

4. What did the new study evaluate?

Investigators from the Food and Drug Administration's (FDA) Center for Biologics Evaluation and Research, the National Institutes of Health (NIH) Clinical Center, and Harvard Medical School have published a study in the scientific journal *Proceedings of the National Academy of Sciences* that examines the presence of MLVs in blood collected from two groups -- patients diagnosed with CFS and healthy blood donors.

This study tested blood samples collected from the New England area in the mid-1990s from 37 patients diagnosed with CFS, as well as samples from 44 healthy blood donors collectec in the Clinical Center Blood Bank, NIH, between 2003 and 2006. Investigators performed DNA sequencing on each sample that produced positive product for verification of MLV-like gene sequences. Diverse MLV gene sequences, similar to that of the recently discovered XMRV, were identified in samples from 32 of the 37 patients with CFS (86.5%) and 3 of the 44 (6.8%) healthy blood donors that were tested.

Follow-up samples were collected from 8 of the CFS patients in 2010, and 7 of these again tested positive for MLV-like gene sequences.

5. What did the new study conclude?

This study supports a previous investigation[Lombardi et al. *Science October 23, 2009 326: 585*]that showed XMRV, a genetic variant of MLV-like viruses, to be present in the blood of people with CFS. The study demonstrates a strong association between a diagnosis of CFS and the presence of MLV-like virus gene sequences in the blood. The study also showed tha MLV-like viral gene sequences were detected in a small fraction of healthy blood donors. Although the statistical association with CFS is strong, this study does NOT prove that these retroviruses are the cause of CFS. Further studies are necessary to determine if XMRV or other MLV-related viruses can cause CFS.

6. Are there studies that support different conclusions?

Some previous studies from the United States (including a study by the Centers for Disease Control and Prevention), the United Kingdom and the Netherlands reported finding no evidence of XMRV or other MLV-related infections in people with CFS. These different findings could be caused by a variety of factors (for example, difference in study populations); and underscore the need for additional studies and standardized methods.

7. Can MLV or XMRV be transmitted by blood or tissue products?

Additional research is needed to investigate the possibility that these MLV-related viruses and XMRV may be transmitted by blood or human tissue and are capable of causing disease. Investigators at FDA, NIH, CDC and other scientific institutions are in the process of conducting studies to verify the capabilities of the tests used by the different laboratories for the detection of XMRV or MLV-related viruses in blood. These studies are intended to develop and standardize a highly sensitive and specific XMRV test to better study its association with disease, as well as the possibility that XMRV can be transmitted to blood or tissue recipients.

8. What are the implications for blood donors?

At present, FDA does not have a donor policy specific to XMRV or other MLVs. There is currently no evidence that XMRV or MLVs are transmitted by transfusion in humans or that XMR' or other MLVs cause human disease. FDA regulations require that donors be in good health at the time of donation.

9. Does FDA agree with the AABB recommendation to discourage donation by people with history of CFS?

FDA does not object to the AABB recommendation. The AABB recommendation is consistent with a long-standing position of the Chronic Fatigue and Immune Dysfunction Syndrome (CFIDS) Association of America that individuals with CFS voluntarily should not donate blood.

10. How are the differences between the CDC and FDA study results being evaluated?

Differences in the results could reflect differences in the patient populations that provided the samples. Alternatively, undefined differences in the method of sample preparation could be contributing to the discordant test results. All of the scientists involved are working collaboratively to design experiments to quickly answer this scientifically puzzling question. An independent investigator at the National Heart, Lung, and Blood Institute (NHLBI) set up a test set of 36 samples, including known positives and presumed negatives. Both the FDA/NIH and CDC labs participated in this test, and the results showed that both labs were able to detect XMRV present at low levels in blinded samples. Additionally, the CDC laboratory provided 82 samples from their published negative study to FDA, who tested the samples blindly. Initial analysis shows that the FDA test results are generally consistent with CDC, with no XMRV-positive results in the CFS samples CDC provided (34 samples were tested, 31 were negative, 3 were indeterminate).

11. What do these findings mean to CFS patients and clinicians who treat them?

Although this study found MLV-like viral gene sequences in a high percentage of CFS patients, this does not prove that these retroviruses are the cause of CFS or of any other disease. Moreover, other studies have not found evidence of such retroviruses in patients with CFS. Further studies are necessary to determine if XMRV or other MLV-like viruses are reproducibly associated with CFS, and if so whether the virus is a causative agent or a harmless co-traveler. The different findings from various studies reinforce the need for more research--including careful analysis of other cohorts of CFS patients from different geographic regions, studies of larger populations of healthy people, and testing of transmissibility of the agents through blood transfusions in animal models. FDA, NIH, and CDC have and will continue to collaborate with other agencies and groups involved in this research.

Links on this page:

別紙様式第2-1

No. 15

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
			2010. 5. 18	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	ProMED 20100513.1557, 2010 May 13, 情報源: WHO Global Alert and Response (GAR) Disease Outbreak News	公表国 WHO	
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

○南アフリカのリフトバレー熱（RVF）
2010年5月10日の時点で南アフリカ保健省は、18人の死者を含む186人のRVF症例を報告している。主要な感染経路は、感染した家畜の血液や組織に触れることであるが、蚊に刺されることも感染原因となる。世界保健機関（WHO）は、南アフリカへの旅行に対して規制の勧告は行っていないが、特に農場や動物保護区に行く者は、動物組織や血液との接触を避け、未殺菌、非加熱ミルクや生肉の摂取をしないことを勧めている。そして、全旅行者に対し、長袖長ズボンの着用や防虫剤、蚊帳を使用するなどして、蚊や吸血昆虫に刺されないよう注意を呼びかけている。また、ドイツ保健当局は、4月に、南アフリカ旅行から帰国したドイツ人のRVF検査確定症例を報告したが、その後の追加検査により、この症例はRVFではなくリケッチア感染であったと報告した。

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

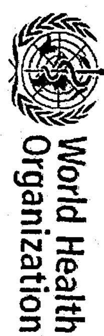
南アフリカでは2010年5月10日現在、18名の死者を含む186名のリフトバレー熱症例が報告されているとのことである。
リフトバレー熱ウイルスはブニヤウイルス科の脂質膜を持つウイルスである。これまで、本製剤によるリフトバレー熱ウイルス感染の報告はない。本製剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれていることから、本製剤の安全性は確保されていると考える。

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渡航歴の有無を確認し、帰国（入国）後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

⑮

JRC2010T-021



Rift Valley fever in South Africa- update 2

12 May 2010 -- On 11 May 2010 Bernhard-Nocht-Institute for Tropical Medicine in Germany reported that additional laboratory analyses conducted both in Germany and South Africa on the German tourist who was preliminarily diagnosed with Rift Valley Fever (RVF) following her return from South Africa, was in-fact infected with Rickettsia and not with RVF virus.

Rickettsia, commonly known as tick fever is a bacterium which can cause many diseases that are transmitted by blood-sucking parasitic arthropods such as fleas, lice and ticks. Symptoms of rickettsial infections include rash, fever, and flulike symptoms. African tick bite fever is caused by *rickettsia africae* and tends to be a milder illness, with less prominent rash and little tendency to progress to complicated disease. All rickettsial diseases respond to treatment with antibiotics such as doxycycline and tetracycline

As of 10 May, the Government of South Africa has reported 186 confirmed cases of RVF in humans, including 18 deaths, in Free S[illegible] Province, Eastern Cape Province, Northern Cape Province, Western Cape, and North West Province. RVF is a viral disease th[illegible] primarily affects animals (such as cattle, buffalo, sheep, goats and camels). The disease can also affect humans. The main mode of transmission of RVF is via direct or indirect contact with the blood or organs of infected animals. Human infections have also resulted from the bites of infected mosquitoes. There is evidence that humans may become infected by ingesting the unpasteurized or uncooked milk of infected animals.

WHO advises no international travel restriction to or from South Africa. However, WHO recommends that visitors to South Africa, especially those intending to visit farms and/or game reserves, avoid coming into contact with animal tissues or blood, avoid drinking unpasteurized or uncooked milk or eating raw meat.

All travelers should take appropriate precautions against bites from mosquitoes and other blood-sucking insects (including the use of insect repellents, wearing long-sleeved shirts and trousers, and sleeping under mosquito nets). Travel medicine professionals and travel medicine services should be aware of the current RVF situation in South Africa in order to provide advice and care accordingly.

For more information

Department of Health, South Africa

National Institute for Communicable Diseases (NICD)

Robert Koch Institute

Rift Valley fever: WHO fact sheet

Protection against vectors [pdf 548kb]
International Travel and Health

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別紙様式第2-1

No. 9

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2010. 5. 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	Amitai Z, Bromberg M, Bernstein M, Raveh D, Keysary A, David D, Pitlik S, Swerdlow D, Massung R, Rzotkiewicz S, Halutz O, Shohat T. Clin Infect Dis. 2010 Jun 1;50(11):1433-8.	公表国 イスラエル	
販売名（企業名）	新鮮凍結血漿「日赤」（日本赤十字社） 新鮮凍結血漿-LR「日赤」（日本赤十字社） 新鮮凍結血漿-LR「日赤」成分採血（日本赤十字社）					

研究報告の概要		使用上の注意記載状況・その他参考事項等
	○イスラエル中央部の都市の学校における大規模Q熱アウトブレイク 背景：2005年6月28日に、イスラエル中央部の都市部の、全寮制高校の生徒および職員322名に多くの発熱性疾患症例が報告された。その後の調査で、その2週間前のQ熱アウトブレイクが確認された。 方法：Q熱疾患の危険因子を特定するため、症例対照研究を行った。環境サンプルを採取し、*Coxiella burnetii*（*C.burnetii*）の感染源と伝播経路を確認した。 結果：2005年6月15日～7月13日の間に、303名中187名（62%）が体調の不具合を報告した。検査を実施した164名中144名（88%）に、*C.burnetii*感染の血清学的証拠が明らかとなった。学生であること、学校の食堂で定期的に食事をしたこと、6月の宗教上の休日期間ならびにその前の週末に寮生活を行ったことは、いずれもQ熱感染の重大なリスク因子であった。PCR検査により学食の空調から*C.burnetii* DNAが検出され、空調を介して病原体に空気感染したことが示唆された。 結論：インフルエンザのオフシーズンにおいて、インフルエンザ様疾患のアウトブレイクの調査を行う際には、*C.burnetii*感染を強く疑うことが必要である。	新鮮凍結血漿「日赤」 新鮮凍結血漿-LR「日赤」 新鮮凍結血漿-LR「日赤」成分採血 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
イスラエル中央部の都市の全寮制高校における大規模Q熱アウトブレイクの原因調査を行ったところ、学食の空気調節システムから*Coxiella burnetii* DNAが検出され、空気調節システムによる空気感染が示唆されたとの報告である。	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。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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MAJOR ARTICLE

A Large Q Fever Outbreak in an Urban School in Central Israel

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Background. On 28 June 2005, numerous cases of febrile illness were reported among 322 students and employees of a boarding high school located in an urban area in central Israel. Subsequent investigation identified a large outbreak of Q fever which started 2 weeks earlier. We describe the investigation of this outbreak and its possible implications.

Methods. We conducted a case-control study to identify risk factors for Q fever disease. Environmental sampling was conducted to identify the source and the mode of transmission of *Coxiella burnetii*, the infectious agent.

Results. Of 303 individuals, 187 (62%) reported being ill between 15 June and 13 July 2005. Serological evidence for *C. burnetii* infection was evident in 144 (88%) of the 164 tested individuals. Being a student, dining regularly at the school dining room, and boarding at school during a June religious holiday and the preceding weekend were all significant risk factors for contracting Q fever. *C. burnetii* DNA was detected using polymerase chain reaction on samples from the school dining room's air conditioning system, supporting contribution of the air conditioning system to the aerosol transmission of the infectious agent.

Conclusions. We report a large outbreak of Q fever in an urban school, possibly transmitted through an air conditioning system. A high level of suspicion for *C. burnetii* infection should be maintained when investigating point source outbreaks of influenza-like disease, especially outside the influenza season.

Q fever is a worldwide-distributed bacterial zoonosis caused by *Coxiella burnetii*. The most common reservoirs are domesticated ruminants, but other mammals, birds, and arthropods are also naturally infected [1, 2]. *C. burnetii* is often excreted in milk, urine, and feces of infected animals and is present in high numbers within the amniotic fluid and the placenta during parturition [2]. Viable bacterium may be present in the soil for months or years, and inhalation of contaminated aerosols is the major mode of transmission [2, 3]. In humans, acute infection may present as a self-limited influenza-like illness, hepatitis, and/or atypical pneumonia [4, 5]. About 60% of infections may be asymptomatic [4], especially among female persons [4, 6] and children aged <15 years [7].

Most reports of Q fever outbreaks are from rural areas and are associated directly or indirectly with farms or farm animals [2, 3]. Nevertheless, urban outbreaks have been described after exposure to slaughterhouses [8, 9], animal research laboratories [10], parturient cats [11], contaminated straw [12], and following wind-borne spread of *C. burnetii* from farmlands [13]. In some urban outbreaks, the source of the infection was never determined [14, 15].

In Israel during 1998–2004, the average annual incidence of Q fever was 0.6 cases 100,000 persons (20–70 cases per year) (Israel Ministry of Health, personal communication). Only a few outbreaks were reported, with the majority occurring in rural or adjacent areas following outbreaks of Q fever in livestock, and all were relatively limited in scale [15–17].

We report a very large urban outbreak of Q fever in a boarding high school in Israel. This outbreak is unique in its magnitude and setting, because there was no proximity to livestock or their products.

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METHODS

Epidemiologic investigation. On 28 June 2005, 2 reports of a possible outbreak of febrile illness in a religious boarding high school in the center of the largest urban area in Israel were received at the Tel Aviv District Health Department. Initial investigation identified a large outbreak of influenza-like illness which started 2 weeks earlier, had already peaked, and was later confirmed to be due to acute *C. burnetii* infection.

We conducted a case-control study to identify risk factors for contracting Q fever. All school students and employees were asked to fill out a short questionnaire, including demographic characteristics, medical history, school boarding history, in-school dining habits, and contact with pets at school. Those who reported being ill during the previous 2 months were asked to specify the date of onset of illness, duration, symptoms and signs, and use of health services. All students and employees were referred for Q fever testing. In several cases, primary practitioners were contacted for additional information. Regional and reference laboratories were queried about additional Q fever cases from the school surroundings during the same time period.

Human serologic testing. Serum samples were tested for antibodies to *C. burnetii* with use of several laboratory methods. Indirect immunofluorescent assays were performed at the Israeli Reference Laboratory for Rickettsial Diseases in Ness-Ziona [18]. Complement fixation tests were performed by the Tel Aviv Medical Center's Clinical Virology Unit with use of the standard complement fixation microtiter method (Lennette and Schmidt) [19]. Qualitative enzyme immunoassays were performed by Clalit Health Services community laboratories with use of the PANBIO Q fever DIP-S-TICKS test. Quantitative tests were performed in various laboratories in Western Europe.

Case definitions. A "clinical case" was defined as a patient with symptoms compatible with Q fever, with illness onset from 1 June through 31 July 2005 and no other likely cause for his/her illness.

A "confirmed case" was defined as anyone with immunoglobulin (Ig) M and IgG indirect immunofluorescent assay titers ≥100 to phase II antigen, or IgG titers ≥800 and IgM titers <100 in a "clinical case" that was tested at least 4 months after illness [8, 20]. Using complement fixation test, a phase II titer ≥256 was considered to represent a confirmed case.

A "probable case" was defined as phase II IgM titer ≥100 and IgG titer <100 by indirect immunofluorescent assay, a phase II titer <256 but ≥32 by complement fixation test, or a positive or borderline laboratory result of qualitative enzyme immunoassay or other quantitative tests. A "possible case" was defined as a "clinical case" with no serologic testing. A "non-case" (control) was defined as negative serologic results for Q fever.

Environmental and veterinary investigation. A comprehensive environmental inspection of the school grounds was conducted by environmental health inspectors, a veterinarian, and an air-conditioning system specialist for a possible source of infection. Two weeks after the last reported case, environmental samples were collected from the air-conditioning systems. The samples included 8 gauze pads that were used to swab the dining room's and synagogue's air-conditioning systems and 4 samples from the 2 fiberglass filters from the inlet of the dining room's air-conditioning unit. All samples were prepared for DNA extraction.

Serum samples of male and female feral cats trapped in the Tel Aviv area for routine neutering by municipality veterinarians were tested for Q fever by complement fixation test [21]. Samples that reacted nonspecifically were retested by indirect immunofluorescent assay (*C. burnetii* spot IF; BioMérieux). In addition, endometrial tissue proximal to the cervix was collected from each of the spayed female cats and was processed for DNA extraction.

DNA was extracted by use of the DNeasy DNA purification kit (Qiagen). Polymerase chain reaction (PCR) assay was performed as described by Stein and Raoult [22].

All tests were performed in the Kimron Veterinary Institute (Bet Dagan, Israel). Filter samples from the dining room's air-conditioning system were also sent to the Rickettsial Zoonoses Branch, Centers for Disease Control and Prevention.

Data analysis. Data were analyzed with Excel (Microsoft) and SPSS, version 10 (SPSS), software. The prevalence of possible risk factors for contracting Q fever in cases (confirmed cases with and without probable cases) and controls was compared using the Fisher's exact test. Odds ratio (ORs) and 95% confidence intervals (95% CI) were calculated. All significant risk factors were tested for colinearity.

RESULTS

The school setting. The school, a religious boarding high school for boys, is located in central Tel Aviv in a densely populated area. During June 2005, 271 students aged 14–20 years (mean age ± standard deviation, 16.9 ± 1.5 years) and 51 employees attended the school. Eighty-four students boarded at the school regularly. Some of the others, who resided in different cities in Israel, stayed over during certain weekends and holidays. A weekend occurred on 10–11 June 2005, and 12–13 June was a special Jewish holiday (Shavuot). The em-

ployees were mainly men (84%) aged 33–92 years (mean age ± standard deviation, 55.4 ± 13.8 years) from various cities in central Israel.

Outbreak description. Of the 322 individuals who attended the school during June 2005, 187 reported being ill from 1 June through 31 July 2005, including 179 (96%) students and 8 (4%) employees (19 individuals were excluded from further analyses because of lack of information). The clinical attack rate was 62% (70.5% and 16% among students and employees, respectively). Attack rates were similar in different grades and ranged between 67% and 74.5%.

Information on date of illness onset was available for 155 (83%) individuals. The epidemic curve (Figure 1) correlates to a point source epidemic. The earliest and the latest date of illness onset were 15 June and 13 July, respectively. The majority of cases reported onset during 19–26 June. Assuming an incubation period of 14–21 days [1, 2], the presumed exposure occurred around 5 June. The reported illness duration was 1–21 days (mean duration ± standard deviation, 7 ± 3 days).

The dominant clinical presentation (Table 1) was fever (98%), headache (90%), and weakness (80%). Only 21% had cough, and none reported symptoms consistent with hepatitis. One hundred forty-one individuals (79%) visited their primary practitioner during their illness. Thirty-one individuals underwent chest radiography examination, and 7 (4%) received a diagnosis of pneumonia. Five patients were hospitalized (2 students and 3 employees) for pneumonia ($n = 2$, 1 of which was a man aged 92 years, the oldest patient in our exposed population), perimyocarditis ($n = 1$), perimyocarditis and pneumonia ($n = 1$), and observation ($n = 1$). Duration of hospitalization ranged between 1–7 days. No deaths occurred. Only 3 individuals were treated with doxycycline during illness. Of note, no additional cases of acute Q fever were diagnosed in the neighborhoods surrounding the school during the same time period.

Serologic results. Results of serologic tests were available for 164 individuals (151 [59%] students and 13 [26.5%] employees). One hundred eight (66%) were "confirmed cases" (103 students and 5 employees), 36 (22%) were "probable cases" (35 students and 1 employee), and 20 (12%) were "non-cases" (13 students and 7 employees). Sixty-five individuals met the criteria for a "possible case" (63 students and 2 employees).

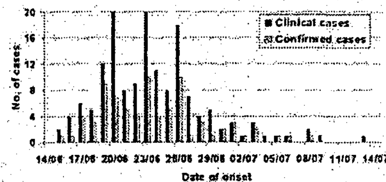

Figure 1. Epidemic curve of all clinical cases and confirmed symptomatic cases. Clinical cases were defined as individuals who reported symptoms compatible with Q fever with illness onset from 1 June through 31 July, with other etiologies ruled out. Confirmed symptomatic cases included any clinical case with positive serologic test results for Q fever.

Table 1. Symptoms of All Clinical Cases and Confirmed Symptomatic Cases

	No (%) of cases	
Symptom	All clinical cases	Confirmed cases
Fever	184 (98)	92 (98)
Headaches	166 (90)	85 (92)
Sweats	81 (49)	45 (53)
Weakness	145 (80)	78 (87)
Chills	60 (35)	36 (42)
Vomiting	30 (17)	22 (24)
Myalgia	39 (23)	22 (26)
Cough	38 (21)	22 (24)
Sore throat	42 (23)	23 (26)
Chest pain	21 (13)	13 (15)

Eighty-six percent and 81% of the confirmed and probable cases, respectively, were clinically ill. All of the non-cases were asymptomatic. The incubation period and the clinical presentation of the confirmed cases resembled that of all clinical cases (Figure 1 and Table 1).

The exact attack rate could not be determined, because everyone was not tested for Q fever; therefore, we estimated a range. The lower limit was 144/303 (47.5%), including confirmed and probable cases. The upper limit was 209/303 (69%), which also included the possible cases. This was based on the observation that all serologically tested clinical cases were either confirmed or probable cases.

The symptomatic to asymptomatic ratio among serologically positive individuals (85:15) is biased, because symptomatic individuals were more likely to be tested. Given that all tested symptomatic individuals had positive results, the numerators were more likely to be near 187 versus 116−20 (all symptomatic individuals vs the asymptomatic minus the seronegative individuals), which translates to a ratio of 66:34 or even higher.

Risk factors. Table 2 summarizes the prevalence of several possible risk factors in confirmed cases and controls. Being a student (OR, 11.09; 95% CI, 3.07–40.07), boarding at school during the June holiday (OR, 13.9; 95% CI, 4.45–43.45), and dining regularly at the school dining room (OR, 8.57; 95% CI, 2.05–35.79) were significantly associated with contracting Q fever. When probable cases were included in the univariate analysis, boarding at school during the weekend before the June holiday was also significantly associated with Q fever infection (OR, 3.18; 95% CI, 1.09–9.22). Because all of the above significant risks factors were statistically associated with each other, we did not perform multiple logistic regression analysis.

Table 2. Risk Factors for Acquiring Q fever

	No (%) of persons		
Factor	Cases	Controls	OR (95% CI)
Status in school (student vs employee)	103 (95)	13 (65)	11.09 (3.07–40.07)
Boarding at school on a regular basis	32 (30)	3 (15)	2.45 (0.67–8.95)
Boarding at school during Shavuot holiday	91 (92)	9 (45)	13.9 (4.45–43.45)
Boarding at school during the weekend before the holiday	48 (59)	6 (35)	2.67 (0.9–7.92)
Boarding at school during the weekend after the holiday	33 (41)	6 (35)	1.29 (0.43–3.83)
Eating at the school dining room (frequently vs seldom or never)	96 (96)	14 (74)	8.57 (2.05–35.79)
Contact with pets on school ground	0 (0)	0 (0)	

NOTE. CI, confidence interval; OR, odds ratio.

Environmental and veterinary investigation. Numerous stray cats were seen in the schoolyard, especially in proximity to the kitchen and the garbage cans which were located outside the dining room. The dining room had its own air-conditioning system, with inlet that drew air from the dining room and outlet that emitted the cooled air back to the room. The air-conditioning ducts were located on the dining room's roof and could be accessed by animal secretions. One of the 4 filter samples, as well as 1 of the 8 gauze swabs taken from the inlet of the dining room's air-conditioning unit, had positive results for Q fever by PCR. Similar positive PCR results were obtained by the Centers for Disease Control and Prevention on filter samples.

Serum samples of 65 feral cats were tested for Q fever serology. Nine cats (14%) had positive results; 2 (10%) of 20 were caught within a 2-km radius of the school, whereas the other 7 (15%) of 45 were from other parts of the city. Forty feline uterine specimens were tested by PCR, and all were found to have negative results.

DISCUSSION

We describe a Q fever outbreak that was unusual in its magnitude and place of occurrence. It represents 1 of the largest outbreaks described in the literature and the largest to occur in a densely populated urban area located far away from livestock farms [3]. The clinical attack rate was remarkably high (62%), with the serological attack rate estimated to be even higher (69%). This is a conservative estimate because asymptomatic individuals, who could have been serologically positive (if tested), were not included and the pre-existing immunity in this particular population was assumed to be very low (based on research that found 14% seropositivity to Q fever among adults residing in the Northern part of Israel, which is a more rural area) (A.K., unpublished data). The symptomatic to asymptomatic ratio was estimated to be 66:34, higher than that reported elsewhere (40:60) [1, 4].

The high attack rate and symptomatic to asymptomatic ratio might be explained by a large inoculum of bacteria and effective modes of transmission. The demonstration of the presence of *C. burnetii* by PCR in the samples from the dining room's air-conditioning system supports an effective aerosol transmission. A similar phenomenon was described in an outbreak in a cosmetics factory where all the exposed workers were symptomatic [23]. The high proportion of symptomatic infection can also be attributed to the male predominance of the exposed population [4, 6] and to the fact that none of the students were aged <14 years [7].

Notable is the low clinical attack rate among the school employees, compared with the students (16% vs 70.5%), which we think is attributable to their lower exposure to the infectious agent. An alternative explanation could be a higher pre-existing immunity among the employees. However, even if the pre-existing immunity was 14% (A.K., unpublished data), this would have changed the calculated clinical attack rate among employees by 2% only (from 16% to 18%).

The dominant clinical presentation was an influenza-like illness, and the working diagnosis of the majority of the primary physicians was a viral infection. Seven patients (4%) received a diagnosis of pneumonia, and none exhibited overt signs of hepatitis. Because of the delayed notification of the Tel Aviv District Health Department and the subsequent delay in the laboratory confirmation of *C. burnetii* infection, the outbreak investigation had little effect on the clinical management during the acute illness. Thus, laboratory and imaging tests were not conducted routinely but were rather conducted on the basis of clinical judgment, and only 3 individuals were treated with doxycycline.

Geographic variation in the clinical presentation of Q fever is well described [2]. In a recent review of 100 hospitalized patients with acute Q fever from Israel [24], the most common presentation was an acute febrile illness with few physical findings. Rare but severe manifestations of the disease are myocarditis and pericarditis, each described in ~1% of patients [1]. Two patients in the present study were hospitalized for myopericarditis. Thus, the clinical presentation in the present study is consistent with that described in the literature.

Most reported large Q fever outbreaks have occurred in or adjacent to rural areas as a result of direct or indirect exposure to infected livestock, especially to parturition products, as is the case in an outbreak in the Netherlands [25]. Urban outbreaks have been typically linked to farm animals that were brought to slaughterhouses [8, 9], animal research laboratories [10], urban farmers' markets [26], contaminated livestock products [23], or windborne aerosols carried long distance from neighboring farms engaged in outdoor lambing and calving [13]. Some urban outbreaks have been linked to parturient dogs [27] and cats [11, 28], and in some the source was never determined [14, 15].

The source of infection in the present outbreak was not clearly defined. However, the findings that being a student, dining at the school's dining room, and boarding during the June holiday were significantly associated with contracting the disease support the hypothesis that the transmission of the infection occurred in the dining room. The positive PCR results from the dining room's air-conditioning system further suggest that the air-conditioning system contributed to the aerosol transmission of the agent, although we could not prove whether the primary source of infection was the dining room or the air-conditioning system. The fact that the environmental samples were taken 2 weeks after the last reported case and mainly from the inlet of the air-conditioning system could explain why only 2 inlet samples of 12 total samples had positive results for *C. burnetii* by PCR. No new cases appeared a month after the initial case (Figure 1), and no other cases were diagnosed in the vicinity of the school, pointing to a limited exposure, both in time and space.

The air-conditioning system could have been contaminated by the numerous stray cats seen in the schoolyard. We were unable to demonstrate that cats from the school vicinity were more likely to be seropositive for Q fever than cats from different areas of the city. Nevertheless, the cat sampling showed that *C. burnetii* is endemic in feral cats in the school's surroundings. To our knowledge, no similar surveys were previously conducted among cats in Tel Aviv.

The magnitude of the present outbreak is impressive, given the yearly incidence of Q fever in Israel (0.6 cases 100,000 persons) and in comparison with other outbreaks described in nonrural areas. It demonstrates that *C. burnetii* can be effectively transmitted to a large number of people through a common exposure.

This outbreak raises the issue of underdiagnosis of Q fever, especially when a primary practitioner treats a sporadic case that manifests as an influenza-like illness. In our study, the working diagnosis of the majority of the physicians was a viral infection. This also implies that there could be a delay in outbreak investigations with implications on the probability of revealing their sources. A high index of suspicion is required when dealing with a relatively prolonged febrile disease, even with no history of exposure to farm animals. A cluster of febrile patients, especially if occurring outside the influenza season, should raise the possibility of Q fever, and rapid investigation into the etiology and source of infection should be made by public health authorities.

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Potential conflicts of interest. All authors: no conflicts.

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医薬品 研究報告 調査報告書

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新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社)</td><td></td><td></td></tr>
<tr><td>研究報告の概要</td><td colspan="5">○カラカス(ベネズエラ)の学校における、経口感染による急性シャーガス病(CD)の大都市でのアウトブレイク
背景:Trypanosoma cruzi(T. cruzi)は、媒介動物の糞便で汚染された食物によって経口感染する。経口感染による急性CDの小規模流行の疫学的・臨床的な特徴については、ほとんどわかっていない。
方法:学校コミュニティに影響を及ぼした急性CDのアウトブレイク時において、コホート疫学研究を実施した。症状と感染源を特定するため、統一的問診を計画した。すべての患者から心電図データを入手し、免疫酵素的および間接血球凝集検査によって、特異的血清抗体を評価した。一部の症例においては、寄生虫血症を直接的または培養、動物接種試験、PCR法により検査した。
結果:曝露された1000名中103名に感染が確認された。感染者のうち、75%に症状があり、その20.3%は入院を必要とした。また59%は心電図異常を示し、44名に寄生虫血症が認められ、子供1名が死亡した。臨床的な特徴は、ベクターを介した感染で見られるものとは異なった。子供は感染率が有意に高かった。疫学研究では、汚染した生グアバジュースが唯一の感染原因とされた。
結論:当該アウトブレイクは、大都市部で、主に若年齢を中心とした中流層の、健康に問題のない集団に感染するという、先例のない公衆衛生的非常事態を招いた珍しいものであった。迅速な診断と処理により、高い死亡率は回避された。しかしT. cruziの食物を介する感染は、現在認識されるより頻繁に起こる可能性がある。</td><td>使用上の注意記載状況・その他参考事項等
新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血

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</td></tr>
<tr><td colspan="3">報告企業の意見</td><td colspan="3">今後の対応</td><td></td></tr>
<tr><td colspan="3">ベネズエラの大都市におけるシャーガス病のアウトブレイクについての疫学研究を行ったところ、Trypanosoma cruziの食物を介する感染は、現在認識されるより頻繁に起こる可能性が示されたとの報告である。</td><td colspan="3">日本赤十字社は、輸血感染症対策として献血時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、シャーガス病の既往がある場合には献血不適としている。日本在住の中南米出身献血者については、厚生労働科学研究「献血血の安全性確保と安定供給のための新興感染症等に対する検査スクリーニング法等の開発と献血制限に関する研究」班と共同して検討する予定である。今後も引き続き情報の収集に努める。</td><td> </td></tr>
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MAJOR ARTICLE

Large Urban Outbreak of Orally Acquired Acute Chagas Disease at a School in Caracas, Venezuela

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(See the editorial commentary by Miles, on pages 1282–1284.)

Background. *Trypanosoma cruzi* oral transmission is possible through food contamination by vector's feces. Little is known about the epidemiology and clinical features of microepidemics of orally acquired acute Chagas disease (CD).

Methods. A case-control, cohort-nested, epidemiological study was conducted during an outbreak of acute CD that affected a school community. Structured interviews were designed to identify symptoms and sources of infection. Electrocardiograms were obtained for all patients. Specific serum antibodies were assessed by immunoenzimatic and indirect hemagglutination tests. In some cases, parasitemia was tested directly or by culture, animal inoculation, and/or a polymerase chain reaction technique.

Results. Infection was confirmed in 103 of 1000 exposed individuals. Of those infected, 75% were symptomatic, 20.3% required hospitalization, 59% showed ECG abnormalities, parasitemia was documented in 44, and 1 child died. Clinical features differed from those seen in vectorial transmission. The infection rate was significantly higher among younger children. An epidemiological investigation incriminated contaminated fresh guava juice as the sole source of infection.

Conclusions. This outbreak was unique, because it affected a large, urban, predominantly young, middle-class, otherwise healthy population and resulted in an unprecedented public health emergency. Rapid diagnosis and treatment avoided higher lethality. Food-borne transmission of *T. cruzi* may occur more often than is currently recognized.

The burden of illness associated with Chagas disease (CD) remains the second highest among all of the endemic tropical diseases in Latin America and results in an annual loss of >2 million disability-associated life years (DALYs) [1, 2]. Although Chile, Uruguay, and Brazil have been certified as free of vectorial transmission by domiciliary *Triatoma infestans* [1], eradication appears to be an impossible task because of the complexity of the zoonotic life cycle of its causative agent, *Trypanosoma cruzi*. In addition to vectorial transmission, other secondary mechanisms of infection include congenital, transfusional, organ transplantation–related, and oral transmission. A sparse number of outbreaks of orally acquired human CD have been reported from Brazil [3–7], Argentina [8], and Colombia [9].

Venezuela has a successful CD vector control program that is based on the improvement of rural housing and vector control [10, 11]. However, epidemiological data suggest a reemergence of the infection [12–14]. At the capital, Caracas, which is a densely populated cosmopolitan city surrounded by mountains covered by tropical forests, the local sylvatic triatomine vector, *Panstrongylus geniculatus*, has been recorded since 1920 [15]; it was reported inside the houses in 1986 [16] and captured in the wild or within households show-

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ing a high rate (76.1%) of *T. cruzi* infection [17]. However, vectorial transmission has not been reported in this city.

The current study describes the largest known outbreak of orally acquired CD to date in the American continent, which involved numerous children and personnel from an urban school in Caracas.

METHODS

On 6 December 2007, trypomastigotes of *T. cruzi* were detected on peripheral blood smears from a 9-year-old student (index case), who was admitted to the Hospital Universitario de Caracas (Caracas, Venezuela) with a 3-week history of fever of unknown origin (FUO). Twenty persons from the patient's school were hospitalized with similar symptoms and were later found to have circulating trypomastigotes and/or serological test results positive for CD. The municipal health authorities were contacted at once, and they reported an unexpected simultaneous sharp increase in medical consultations and absenteeism among school personnel from 30 October through 25 November 2007.

The center involved (Unidad Educacional "Andrés Bello") is located in the Municipality of Chacao, in the eastern part of Caracas, with predominantly middle-class inhabitants. All of the food and beverages consumed by the students and personnel were supplied by the same caterer that supplied other municipal schools, with the exception of breakfast, which was prepared under unsupervised sanitary conditions, located in a distant slum on the western mountain slopes of the city. A multidisciplinary task force was summoned to analyze the epidemiological situation with the aim of controlling the outbreak [18]. A case-control, cohort-nested, epidemiological outbreak study was designed to assess the extent of the outbreak and to identify possible sources of infection. Cases were classified as "suspected" or "confirmed" in accordance with a consensus document prepared by the interdisciplinary group, based on World Health Organization recommendations [19]. A suspected case patient was any person with an epidemiological link to the institution involved from 10 October through 1 November 2007 who developed FUO of >5 days duration and other clinical manifestations. A confirmed case patient was any suspected case patient or asymptomatic person with the epidemiological link who, in addition, exhibited blood parasites or specific anti-*T. cruzi* antibodies by 2 different serological techniques: enzyme-linked immunosorbent assay (ELISA) and indirect hemaglutination (IH) or ELISA and Western blot (WB) tests.

The study population consisted of all students, teachers, workers from the school, external persons involved with the preparation or transportation of food consumed in the school, and any person considered to be a "school contact" potentially at risk. Blood samples for diagnosis were initially collected from 11 December through 14 December 2007, as an emergency intervention, with the aim of identifying infected persons and immediately starting antiparasitic treatment of any individual affected by a severe, potentially lethal, acute illness in the context of a large outbreak that occurred at a critical time of the year (3 days before a prolonged Christmas and new year vacation). During a second sampling that was performed 6 weeks later, 21 January through 25 January 2008, all participants undertook a detailed clinical and epidemiological questionnaire on CD risk factors (eg, exposure to vectors, transfusions, infected relatives, contact with animal reservoirs, and ingestion of food and/or beverages in the school). Case patients were compared with control subjects from the same cohort of exposed individuals.

The study was performed under the supervision of the Ethical Committee of the Tropical Medicine Institute. Informed written consent was obtained from each participant or from their legal guardians.

For the first 43 symptomatic patients, fresh and Giemsa-stained peripheral blood smears were reviewed for trypomastigotes. In addition, 2 mL of blood were cultured in biphasic medium and checked periodically over at least 3 months. Mice were inoculated intraperitoneally with 300 μL of blood and examined each week [19].

All serum samples were screened for immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies against a crude extract of *T. cruzi* epimastigotes [20] with use of an ELISA developed in house [21] and an IH test [22]. The immunodiagnosis of CD was based on the positivity of at least 2 specific serological tests [19]. Those samples with ELISA results positive for IgG and negative IH results were also tested with WB tests [23].

A representative number of 150 blood samples were randomly evaluated by a polymerase chain reaction (PCR). For the DNA extraction, 5 mL of blood was mixed with an equal volume of 6M guanidine HCl /0.2M EDTA (GE) [24]. The amplification reactions were targeted to the 330–base pair minicircle fragment of the *T. cruzi* kinetoplastid DNA [25].

Conventional 12-lead electrocardiogram (ECG) recordings were obtained from confirmed or suspected case patients and treated with either benznidazole (Rochagan; Roche Laboratories) at a dosage of 6 mg/kg/day for 60 days or nifurtimox (Lampit; Bayer Laboratories) at a dosage of 8 mg/kg/day for 90 days [19, 26].

The dependent variable or main outcome was based on serological status. Epidemiological exposure was evaluated using χ^2 or the Student's *t* test depending on the binary or continuous independent distribution of the variable. Only variables significantly associated in the univariate regression were included in the multivariate regression, using $P < .05$ as the entry criteria. The relationship between risk factors and final outcome (*T.*

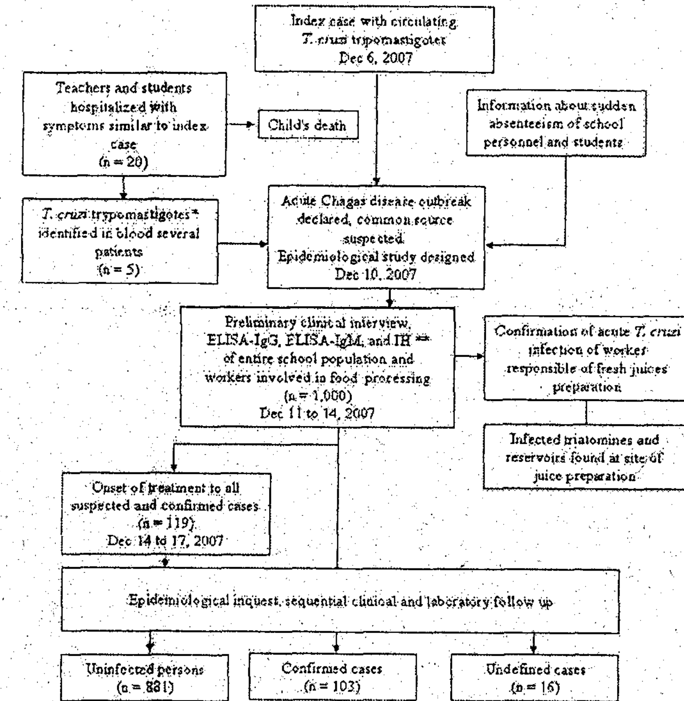

Figure 1. Study profile and major outcomes of the epidemiological investigation of the outbreak of acute Chagas disease, Caracas, Venezuela, 2007. *Parasitemia investigation by direct techniques. **Parasitemia, Western blot, and polymerase chain reaction tests performed on a more limited group of exposed individuals (see Methods). ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; IH, indirect hemaglutination; *T. cruzi*, *Trypanosoma cruzi*.

cruzi infection) was estimated by means of the paired odds ratio (OR), with 95% confidence intervals (CIs). Stata, version 6.0 for Windows (Stata), was used as the basic statistical software for all calculations.

RESULTS

Figure 1 depicts the general outline of the study. Because the outbreak occurred in a well-off urban area of the city with no current vectorial transmission, a food-borne mechanism was presumed to be the cause. Date of exposure was estimated to occur between 10 October and 25 October 2007, based on previous reports of orally acquired infections with documented incubation periods of 5–20 days [5].

The demographic characteristics of the entire exposed population ($n = 1000$) are shown in table 1. No statistically significant differences were found in the attack rates among the sexes. Although, as a whole, age was not associated with the main outcome, a more meticulous revision of age distribution of those infected revealed a bimodal distribution curve, with a reverse trend, in which the OR for CD decreased with age for children but increased with age for adults. As depicted in Tables 1 and 2, significantly different attack rates were observed among students and teachers in relation to their school attendance (morning vs afternoon shifts; 65 cases [17.9%] among 363 subjects vs 10 cases [2.6%] among 385 subjects; OR, 3.19 [95% CI, 2.1–4.8; $P < .001$). The difference between the attack rate among students of the morning shift (22.5%) and the attack rate among children of the afternoon shift (2.4%) was statis-

Table 1. Demographic Characteristics and Rates of Infection of 1000 Individuals Exposed to Infection at a Public School Community of Caracas, Venezuela, Affected by a Large Outbreak of *Orally Acquired Acute Chagas Disease in December 2007*

	No. (%) of exposed individuals (n = 1000)	
Variable	Study population	Infected subjects
Age		
≤18 years old	795 (79.5)	77 (9.6)
>18 years old	205 (20.5)	26 (12.6)
Sex		
Male	455 (45.1)	50 (10.9)
Female	545 (54.9)	53 (9.7)
Students		
Kindergarten	65 (8.7)	15 (23.1)
1st grade	63 (8.4)	13 (20.6)
2nd grade	54 (7.2)	7 (12.9)
3rd grade	61 (8.1)	9 (14.7)
4th grade	66 (8.8)	7 (10.6)
5th grade	92 (12.3)	9 (9.7)
6th grade	82 (10.9)	7 (8.5)
7th grade	96 (12.8)	8 (6.2)
8th grade	89 (11.9)	0 (0)
9th grade	79 (10.5)	4 (5.1)
Subtotal	747 (74.7)	77 (10.3)
Nonstudents		
Personnel	165 (16.5)	25 (15.2)
Food handlers	16 (1.6)	1 (6.2)
Other contact	72 (7.2)	0 (0)
Subtotal	253 (25.3)	26 (10.2)
Shift		
Morning	363 (36.3)	65 (17.9)
Afternoon	385 (38.5)	10 (2.6)
Both	252 (25.2)	28 (11.1)

tically significant ($P < .05$). Although the absolute number of infected children was higher (77 of 103 infected subjects), the maximum infection rate (15.2%) was observed among the school employees. One of the 16 workers who were involved directly in the preparation or transportation of luncheons showed evidence of acute *T. cruzi* infection, with serological test results positive for specific IgM and IgG (Table 1).

A significant positive correlation was found between ingestion of guava juice and risk of infection (OR, 3.5 [95% CI, 1.85–6.7]) (Table 2). The epidemiological interviews revealed that, except for the guava juice, all other beverages were made in the early morning. The guava fruits, in contrast, were boiled the night before and left to cool inside a large uncovered pot before blending in the morning. Once in the school, the juice was delivered to the morning shift, first to school personnel, then to kindergarten students, and then to students in ascending grades. Some personnel and students of the afternoon shift customarily consumed any remaining juice.

Of those infected, 75% were symptomatic, 20.3% required hospitalization, and a 5-year-old child died of acute chagasic myocarditis. Most patients reported fever that lasted >7 days, abdominal pain, headache, dry cough, and myalgia; to a lesser degree, they reported diarrhea, facial edema, malaise, arthralgias, dyspnea, and tachycardia (Table 3). In the univariate regression analysis, the following symptoms showed a significant association with a higher risk of serologically confirmed infection: fever, arthralgias, skin lesions (rash, erythema nodosum, or facial edema), and cardiovascular abnormalities. However, on the multivariate analysis, only fever and cardiovascular abnormalities showed statistical significance.

In 61 (59%) of the 103 confirmed cases, ≥1 abnormality was noticed on the ECG recordings. T wave abnormalities were significantly more common among patients ≤18 years of age, whereas supraventricular arrhythmias and microvoltages were predominant among adults (Table 4), who more frequently developed severe clinical cardiological manifestations that required hospitalization.

Among 1000 persons evaluated, 103 individuals had anti-*T. cruzi* IgG antibodies by ELISA, and 90 (87.3%) were also IgM positive. The specific IH test was concordant in 99 (96.1%) of 103 individuals, whereas the remaining 4 individuals had positive WB results.

Because of logistic constraints, parasitemia could be assessed in only 43 patients by parasitological methods. Of these, 13 (30.2%) had positive fresh-stained blood smear results, in vitro culture, or mice inoculation.

Sixteen individuals with ELISA results positive for anti-*T. cruzi* IgG antibodies but negative IH results nevertheless received a full course of antiparasitic treatment. During follow-up, they became IgG seronegative while remaining persistently negative according to both IH and WB results. Five such patients developed clinical signs, as well as ECG abnormalities. Because these patients did not fulfill World Health Organization criteria for the CD diagnosis, they were considered to have undefined cases (Figure 1).

Samples of 150 persons were randomly chosen to be tested by specific PCR targeted at the *T. cruzi* kinetoplastid DNA. The reaction was positive in 35 (79.5%) of 44 serologically confirmed cases. All 106 seronegative individuals tested were also negative by PCR. A collateral survey performed at the site where the incriminated juice was processed revealed the presence of infected *P. geniculatus* and domestic rats.

As part of an ongoing cooperative study with the Instituto López Neyra in Granada, Spain, 3 parasite isolates obtained from patients, as well as from 1 infected triatomine captured at the juice preparation site, were typed using *T. cruzi* ribosomal and mini-exon gene markers. Preliminary results revealed a great genetic homogeneity, with all of the isolates belonging to the *T. cruzi* I lineage. Furthermore, homology analysis of the

Table 2. Univariate and Multivariate Logistic Regression Analysis of Risk Factors Associated with *Trypanosoma cruzi* Transmission during an Outbreak of Orally Acquired Chagas Disease, Caracas, Venezuela, 2007

	Univariate analysis		Multivariate analysis	
Variable	OR (95% CI)	P	OR (95% CI)	P
Age				
≤18 Years	0.85 (0.79–0.91)	.01	0.7 (0.73–0.87)	.001
>18 Years	1.03 (1.0–1.07)	.02	1.03 (1.1–1.05)	.01
Worker vs student	1.3 (0.83–2.06)	.24	...	...
Shift (morning vs afternoon)	3.19 (2.1–4.8)	.001	4.7 (2.6–8.3)	.001
Any fresh beverage	2.17 (0.77–6.1)	.14	...	...
Guava juice	3.5 (1.85–6.7)	.001	3.2 (1.4–7.1)	.004
Passion fruit juice	0.95 (0.59–1.62)	.95	...	...
Melon juice	1.16 (0.76–1.7)	.47	...	...
Lemon-starch drink	1.03 (0.68–1.52)	.85	...	...
"Chicha"	0.77 (0.51–1.18)	.24	...	...
Oat meal drink	1.37 (0.9–2.0)	.13	...	...
Tamarind juice	0.6 (0.39–0.94)	.60	...	...
Mango juice	0.79 (0.5–1.1)	.26	...	...
Papaya juice	1.32 (0.8–2.0)	.19	...	...
Pineapple juice	0.72 (0.4–1.9)	.12	...	...

NOTE. CI, confidence interval; OR, odds ratio.

sequence of an amplified polymorphic mini-exon from *T. cruzi* RNA confirmed that all parasite isolates from the patients were identical, which was consistent with a common source of infection.

DISCUSSION

Thanks to a coordinated program in the Southern Cone countries, the transmission of CD has been successfully interrupted in Uruguay and Chile, as well as in at least 8 of the 12 states of Brazil in which CD is endemic [19, 27]. However, the persistence of numerous sylvatic foci and the wide distribution of vectors and reservoirs, together with a progressive reduction in the availability of the vector's natural source of blood (birds and mammals) in intervened forested areas, is driving originally wild triatomines to invade human dwellings [28, 29]. Once domiciliation has occurred, *P. geniculatus* may feed abundantly on domestic reservoirs, as well as on humans. As part of their nocturnal activity, vectors circulate widely inside the house and can thereby eventually contaminate unprotected food and beverages with their feces. There is also the possibility of trans-

Table 3. Univariate and Multivariate Logistic Regression Analysis According to Symptoms and Serological Test Results for 1000 Individuals Exposed during an Outbreak of Orally Transmitted Acute Chagas Disease in Caracas, Venezuela, 2007

		Serological test results		Univariate analysis		Multivariate analysis	
Symptom	No. (%) of subjects (n = 1000)	Percent positive/percent negative	P[a]	OR (95% CI)	P	OR (95% CI)	P
Fever	190 (19.0)	46.6/15.7	.001	4.6 (3.0–7.1)	.001	5.4 (3.9–9.6)	.001
Artralgias	18 (1.8)	6.6/1.2	.001	5.7 (2.1–15.4)	.001	3.3 (0.4–26.2)	.250
Skin lesions[b]	30 (3.0)	11.4/2.0	.001	6.2 (2.9–13.4)	.001	2.2 (0.7–6.9)	.140
Cardiovascular	4 (0.4)	1.9/0.2	.001	8.6 (1.2–62.0)	.030	4.3 (1.2–12.8)	.040
Gastrointestinal	84 (8.4)	11.4/8.0	.230	1.4 (0.7–2.8)	.240		
Respiratory	49 (4.9)	7.6/4.5	.170	1.7 (0.7–3.7)	.170	...	...
Unspecific	26 (2.6)	4.7/2.3	.140	2.0 (0.7–2.8)	.150		

NOTE. CI, confidence interval; OR, odds ratio.

[a] By χ^2 analysis.

[b] Rash, erythema nodosum, and facial edema.

Table 4. Basal Electrocardiogram (ECG) Abnormalities by Age Group for 61 Infected Patients from an Outbreak of Orally Acquired Acute Chagas Disease in Caracas, Venezuela, 2007

	Age group			
ECG abnormality	≤18 Years (n = 48)	>18 Years (n = 13)	Total	P[a]
ST abnormality	30	4	34	.028
T abnormality	39	1	40	<.001
Supraventricular arrhythmia	3	6	9	.002
Ventricular arrhythmia	2	0	2	.897
Microvoltage/decrease amplitud QRS	0	3	3	.007
QTc prolongation	2	0	2	.897
Fascicular block	3	2	5	.621
AV block	2	0	2	.897

[a] Yates corrected χ^2 analysis.

mission by food contamination with urine or anal secretions of infected marsupials [30].

The genetic homogeneity and lack of significant genetic intralineage polymorphism observed in all of the isolates thus far typed from the current outbreak is consistent with a common source of infection. Moreover, the confirmation of an acute infection in the woman responsible for the preparation of the juice lends further support to evidence that indicates short-term exposure, as do the logistic regression analysis results, which incriminated the guava juice as the possible source of contamination. We therefore postulate that, during the night, infected triatomines might have contaminated the unprotected pot where the guava juice was left before being blended in the early morning. Once the juice arrived at the school, it was first served to the teachers and afterwards served to the students, progressing from the lower to the higher grades of the morning shift. Any remaining juice was later shared by the teachers and students of the afternoon shift. This sequence of events could explain the relatively high attack rate observed among school personal (15.2%) and the significant decrease in the attack rate among students in the ninth grade (5.1%), compared with that among kindergarten students (23.1%). The significant difference in the attack rates found between students of the morning (22.5%) and afternoon shifts (2.4%) suggests that the concentration of the inoculum may have been different for both groups, perhaps reflecting a steady decrease in the survival of infecting metacyclic trypomastigotes [31].

Orally transmitted CD episodes have been described previously, all of which have been reported in South America [3–9, 32–34]. Distinctive epidemiological features included a lower number of infected persons (37 cases being the maximum number reported in any outbreak); relatively high lethality (up to 35.2%, with an average rate of 7.1%); a preponderance of cases occurring among adults; and occurrence in remote rural areas or in urban communities where fruits obtained from areas of endemicity, such as açai *(Euterpe oleracea)*, piassava *(Leopoldinea piaçaba)*, and sugar cane, were consumed. The present outbreak is unique in that it affected a large, predominantly young, healthy urban population and was associated with high rates of parasitemia and morbidity but a very low mortality rate (0.97%). The latter probably relates to prompt diagnosis and treatment. It is the first time that contaminated guava juice has been incriminated as the source of infection. Moreover, this represents a genuine urban oral CD outbreak, because the *T. cruzi* strain that was involved in the outbreak originated from an inner-city household, where peridomestic triatomines and rodent reservoirs allowed the maintenance of transmission.

One crucial problem was the overwhelming amount of clinical cases that required diagnostic confirmation. Serological testing with the ELISA was very useful for this purpose, and the assessment of both IgG and IgM anti–*T. cruzi* antibodies for all members of the exposed population enabled us to demonstrate the infection in the early phase. The concurrent onset of symptoms in most cases and the fact that specific IgM antibodies were demonstrated in a high percentage of cases (87.3%) further suggests that exposure to the infecting inoculum was recent [35] and singular or short-lived.

Of the 103 individuals in whom *T. cruzi* parasitemia was determined by parasitological methods and/or PCR, 44 (40.7%) had positive test results. This is probably one of the highest rates of parasitemia ever documented in any orally transmitted CD outbreak.

Although 75% of the infected individuals were symptomatic, the predominant clinical manifestations observed (fever, headache, and myalgias) are all highly unspecific. Indeed, dengue, mononucleosis, hepatitis, and intoxications were among the causes contemplated initially. Clinical findings such as facial edema, gingivitis, and dry cough are probably the consequence of the penetration of the parasite throughout the oral cavity, lips or pharyngeal mucosa. These latter manifestations, along

with other unexpected findings, such as erythema nodosum, anasarca, and lower limbs edema, are not described in vectorial transmission and even in prior reports of orally-acquired CD. They may be related to the host's immune inflammatory response conditioned by the genetics of each individual or by a high parasite load [36]. On the other hand, the findings of acute myocarditis were observed in an unusually high proportion (59%) of confirmed cases.

The diagnosis of acute CD requires a high index of suspicion by the clinician, especially when patients are seen away from the traditional areas of endemicity. In countries in which CD occurs, this condition must be considered in the differential diagnosis of FUO, because food-borne acute CD may occur more often than is currently recognized.

Progressive environmental changes that affect the ethology and ecology of potential *T. cruzi* reservoirs and vectors, together with an increase in human populations surrounded by intervened forests, have favored the urbanization and domiciliation of the cycle maintained by *P. geniculatus*, thus affecting the poor populations of the misery belts around most Latin American cities and middle-class populations, under the concept of the "edge-mediated effects" [37]. This new situation imposes necessary changes in the strategy of CD control programs, which until now have been limited to vector control activities in rural Latin American communities in areas of endemicity.

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別紙様式第 2-1
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医薬品
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一般的名称	①②③乾燥抗 HBs 人免疫グロブリン ④⑤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	Agence France-Presse /2010/08/14	公表国 パキスタン	
販売名 （企業名）	①ヘブスブリン筋注用 200 単位　（ベネシス） ②ヘブスブリン筋注用 1000 単位　（ベネシス） ③ヘブスブリン　（ベネシス） ④ヘブスブリン IH 静注 1000 単位　（ベネシス） ⑤静注用ヘブスブリン-IH　（ベネシス）					

研究報告の概要

ベルギーの男性が南アジア起源の薬剤耐性いわゆるスーパー細菌で死亡した。新たな健康の脅威からの最初の死亡報告である。
2 番目のベルギー人はモンテネグロに旅行中に事故に遭い、入院後感染したが、ベルギーで治療をうけ回復した。
最初の犠牲者はパキスタンへの旅行中に交通事故に遭い、脚を負傷し、パキスタンの病院で治療している間に耐性菌に感染したベルギー人で、帰国後の 6 月に死亡した。
彼は、最近同定された New Delhi metallolactamase-1(NDM-1)という、例えば E.coli のような通常のバクテリアを抗生物質耐性にする遺伝子を有するバクテリアに感染した。このバクテリアは昨年、インドで入院していたスウェーデン人の患者で最初に同定された。
このバクテリアの流行の中心はインドとパキスタンのようである、しかし、接触と旅行を通して伝播は広がっている。

使用上の注意記載状況・その他参考事項等

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

報告企業の意見	今後の対応
NDM-1 産生多剤耐性菌による死亡例の初めての報告である。 万一、ニューデリーメタロ-β-ラクタマーゼ-1(NDM-1)を産生する細菌が原料血漿に混入したとしても、除菌ろ過等の製造工程で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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BENESIS 2010-017

Belgian man dies of South Asian superbug, hospital reports

AGENCE FRANCE-PRESSE AUGUST 14, 2010

A Belgian man died from a drug-resistant so-called superbug originating in South Asia, a doctor said Friday, the first reported death from the new health threat.

A second Belgian was infected after being hospitalized after an accident during a trip to his native Montenegro, but recovered following treatment in Belgium, another expert said.

The first victim was infected while being treated in a hospital in Pakistan and died in June, Denis Pierard, a microbiologist from AZ VUB hospital in Brussels where the man had been treated, told Belgian media.

"He was involved in a car accident during a trip to Pakistan. He was hospitalized with a major leg injury and then repatriated to Belgium, but he was already infected," the doctor said.

Despite being administered colistin, a powerful antibiotic, the patient died, Pierard said.

He was infected by a bacteria that carried the newly identified gene New Delhi metallolactamase-1 (NDM-1) that makes ordinary bacteria such as E. coli resistant to antibiotics. It was first identified last year in a Swedish patient admitted to hospital in India.

Scientists fear the gene could easily migrate to other bacteria, making them antibiotic-resistant.

"The epicentre of the presence of this bacteria seems to be India and Pakistan, but it appears through contact and travel, its spread is becoming wider," said Youri Glupczynski from the University of Leuven.

British medical journal The Lancet reported this week that bacteria containing the NDM-1 gene had been found in 37 Britons who had received medical treatment in South Asia, and three cases have been reported in Australia. There have been two cases in Canada, one of them in B.C.

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別紙 3

番号 2

研究報告調査報告書

<table>
<tr><td>識別番号・報告回数</td><td></td><td></td><td></td><td>第一報入手日
：平成 22 年 8 月 18 日</td><td>新医薬品等の区分
：該当なし</td><td rowspan="3">総合機構処理欄</td></tr>
<tr><td>一般的名称</td><td colspan="2">－</td><td rowspan="2">研究報告の公表状況</td><td rowspan="2">－</td><td rowspan="2">公表国：
英国</td></tr>
<tr><td>販売名（企業名）</td><td colspan="2">－</td></tr>
<tr><td>研究報告の概要</td><td colspan="5">インド、パキスタン、英国の多剤耐性腸内細菌における NDM－1 の流行を調査した。その結果、チェンナイで 44 件、ハリヤナで 26 件、英国で 37 件、その他のインドとパキスタンで 73 件の NDM－1 が検出された。ほとんどが大腸菌（36 件）と肺炎桿菌（111 件）で見つかり、tigecycline と colistin 以外の抗生物質に耐性化していた。ハリヤナから分離された肺炎桿菌は同一だったが、英国とチェンナイの株は多様化していた。大部分が分離株のプラスミド上に NDM－1 があった。NDM－1 陽性だった多くの英国人が、1 年以内にインドかパキスタンに旅行したか、あるいはこれらの国と関連があった。</td><td>使用上の注意記載状況等・その他参考事項等</td></tr>
<tr><td colspan="3">報告企業の意見</td><td colspan="3">今後の対応</td><td></td></tr>
<tr><td colspan="3">本報告は，当該生物由来製品による感染症情報ではない．
本報告を“新規感染症”および“重大な感染症情報”と考え，報告する．</td><td colspan="3">今後も感染症情報の収集に努め，当該生物由来製品に係る情報を入手した場合には速やかに調査・報告を行い安全性の確保に努める．</td><td></td></tr>
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Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study

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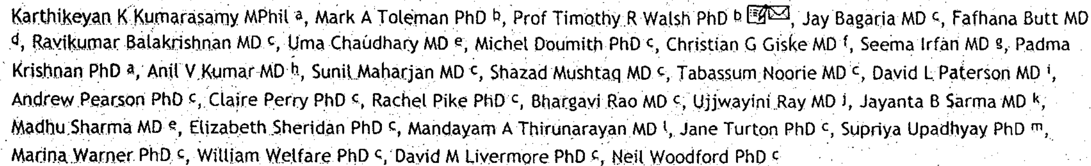

Karthikeyan K Kumarasamy MPhil [a], Mark A Toleman PhD [b], Prof Timothy R Walsh PhD [b], Jay Bagaria MD [c], Fafhana Butt MD [d], Ravikumar Balakrishnan MD [c], Uma Chaudhary MD [e], Michel Doumith PhD [c], Christian G Giske MD [f], Seema Irfan MD [g], Padma Krishnan PhD [a], Anil V Kumar MD [h], Sunil Maharjan MD [c], Shazad Mushtaq MD [c], Tabassum Noorie MD [c], David L Paterson MD [i], Andrew Pearson PhD [c], Claire Perry PhD [c], Rachel Pike PhD [c], Bhargavi Rao MD [c], Ujjwayini Ray MD [j], Jayanta B Sarma MD [k], Madhu Sharma MD [e], Elizabeth Sheridan PhD [c], Mandayam A Thirunarayan MD [l], Jane Turton PhD [c], Supriya Upadhyay PhD [m], Marina Warner PhD [c], William Welfare PhD [c], David M Livermore PhD [c], Neil Woodford PhD [c]

Summary

Background

Gram-negative Enterobacteriaceae with resistance to carbapenem conferred by New Delhi metallo-β-lactamase 1 (NDM-1) are potentially a major global health problem. We investigated the prevalence of NDM-1, in multidrug-resistant Enterobacteriaceae in India, Pakistan, and the UK.

Methods

Enterobacteriaceae isolates were studied from two major centres in India—Chennai (south India), Haryana (north India)—and

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those referred to the UK's national reference laboratory. Antibiotic susceptibilities were assessed, and the presence of the carbapenem resistance gene bla_{NDM-1} was established by PCR. Isolates were typed by pulsed-field gel electrophoresis of XbaI-restricted genomic DNA. Plasmids were analysed by S1 nuclease digestion and PCR typing. Case data for UK patients were reviewed for evidence of travel and recent admission to hospitals in India or Pakistan.

Findings

We identified 44 isolates with NDM-1 in Chennai, 26 in Haryana, 37 in the UK, and 73 in other sites in India and Pakistan. NDM-1 was mostly found among *Escherichia coli* (36) and *Klebsiella pneumoniae* (111), which were highly resistant to all antibiotics except to tigecycline and colistin. *K pneumoniae* isolates from Haryana were clonal but NDM-1 producers from the UK and Chennai were clonally diverse. Most isolates carried the NDM-1 gene on plasmids: those from UK and Chennai were readily transferable whereas those from Haryana were not conjugative. Many of the UK NDM-1 positive patients had travelled to India or Pakistan within the past year, or had links with these countries.

Interpretation

The potential of NDM-1 to be a worldwide public health problem is great, and co-ordinated international surveillance is needed.

Funding

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