薬事・食品衛生審議会薬事分科会

血液事業部会運営委員会 委員名簿

1．大平 勝美 （おおひら　かつみ）
　　はばたき福祉事業団理事長

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

3．花井 十伍 （はない　じゅうご）
　　ネットワーク医療と人権理事

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

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

6．山口 照英 （やまぐち　てるひで）
　　（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）

薬事分科会血液事業部会運営委員会規程

（目的）

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

（所掌）

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

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

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

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

四　その他部会で調査審議する事項のうち特別の事項についての状況

（委員会への所属）

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

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

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

（委員長の選任）

第４条　委員会に委員長を置き、委員会に属する委員等の互選により選任する。

２　委員長は、委員会の事務を掌理する。

３　委員長に事故があるときは、委員会に属する委員等のうちから委員長があ

薬事・食品衛生審議会

平成２３年度　第１回　血液事業部会運営委員会

議 事 次 第

日時：平成２３年６月２７日（月）
１４：００～１６：００
場所：弘済会館 ４階 萩

議題：

1.委員長の選出及び委員長代理の指名
2.議事要旨の確認
3.感染症定期報告について
4.血液製剤に関する報告事項について
5.日本赤十字社からの報告事項について
6.その他

配付資料：

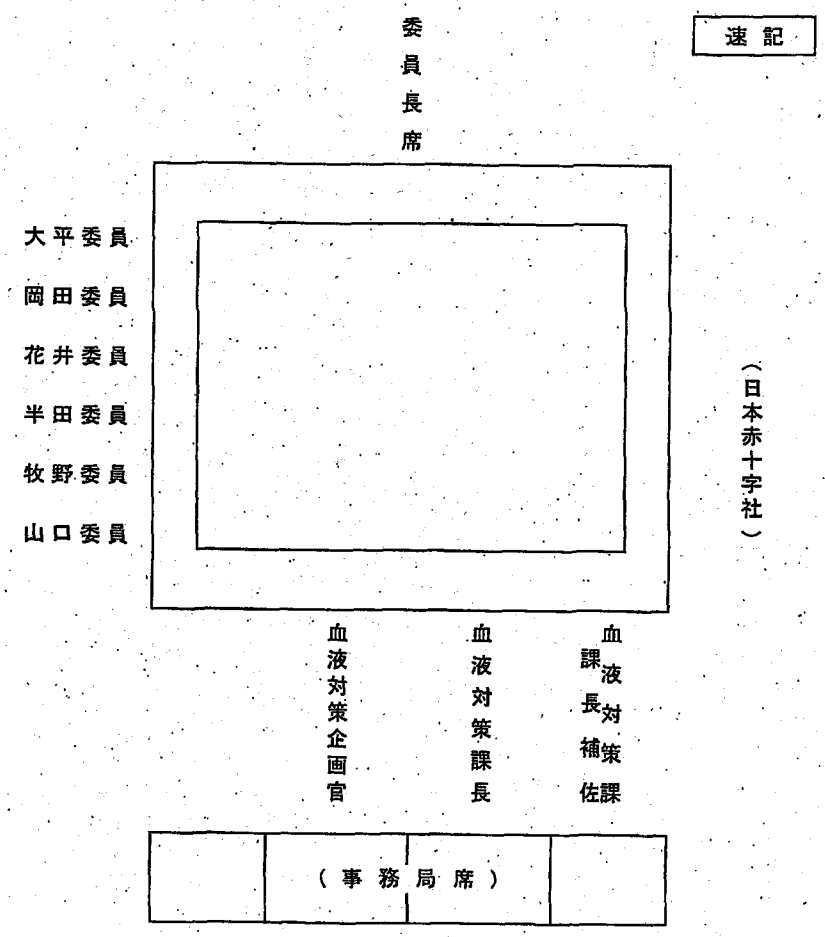
座席表
委員名簿

資　料　１　平成２２年度第４回血液事業部会運営委員会議事要旨（案）
資　料　２　感染症定期報告について
資料３－１　供血者からの遡及調査の進捗状況について
資料３－２　血液製剤に関する医療機関からの感染症報告事例等について
資料３－３　献血件数及びＨＩＶ抗体・核酸増幅検査陽性件数
資　料　４　ＸＭＲＶに関する文献報告（続報）
資　料　５　東日本大震災への対応（日本赤十字社提出資料）
資　料　６　改正採血基準の実施状況（日本赤十字社提出資料）
資料７－１　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６月２７日公表）
資料７－２　国立病院等におけるフィブリノゲン製剤投与に係る診療録等の精査状況等の調査結果について（平成２３年６月２７日公表）
資料７－３　平成２２年度フィブリノゲン製剤納入医療機関への訪問調査の結果について（平成２３年６月２７日公表）
資料７－４　平成２３年度フィブリノゲン製剤納入先医療機関訪問調査について（平成２３年６月２７日公表）

平成23年度第1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6月27日(月)
弘済会館
萩
14:00 ～ 16:00

速記



Press Release

追加資料2
日本赤十字社
Japanese Red Cross Society

平成23年6月17日

各 位

日 本 赤 十 字 社
田辺三菱製薬株式会社

日本赤十字社と田辺三菱製薬株式会社の血漿分画事業の統合の検討開始に関する基本合意について

本日、日本赤十字社（本社：東京都港区、社長 近衞 忠煇）と田辺三菱製薬株式会社（本社：大阪市中央区、代表取締役社長 土屋 裕弘）は、日本赤十字社理事会および田辺三菱製薬株式会社取締役会の承認の下、平成24年4月1日を目途として、日本赤十字社の血漿分画事業部門と田辺三菱製薬株式会社の完全子会社で、血漿分画製剤の製造販売会社である株式会社ベネシスとの統合に向けた検討を開始することに合意しましたのでお知らせいたします。

1．事業統合の趣旨および目的

我が国では、血液製剤の安全性の向上、安定供給の確保等によって、国民の保健衛生の向上に資することを目的として、平成15年7月に「安全な血液製剤の安定供給の確保等に関する法律」（血液法）が施行されています。本法律では倫理性、国際的公平性の観点に立脚し、その基本理念の一つとして血液製剤の国内自給の確保と安定供給が定められています。さらに、世界保健機関が加盟国に対して国内自給の達成を目的とした国家的、効率的かつ持続可能な血液事業を求めるなど、国内自給達成が国際的な要請となっており、また、世界的な血液製剤の供給不足が生じた際に国民の生命身体を守るという安全保障の観点からも国内自給達成が不可欠です。

しかし、現状では、我が国における血漿分画製剤の国内自給は達成されておらず、特にアルブミン製剤については58.7%（平成22年）であり、国内製造が全く行われていない製剤もあります。その主な理由としては、国内各メーカーの生産規模が全世界を市場とする海外競合メーカーに比べて小さく、製造コストを含め事業の効率化にも限界があることなどが考えられます。

このような状況の下、日本赤十字社と田辺三菱製薬株式会社は、日本国内における血漿分画事業の安定的継続という国民の負託に応えるためには、血漿分画事業の統合が大きな選択肢であることで一致し、その可能性を模索してまいりました。また、本年3月8日、厚生労働省の薬事・食品衛生審議会薬事分科会血液事業部会にて審議・了承された「血漿分画製剤の供給のあり方に関する検討会 中間報告」における提言内容も、これまでの両社の協議の方向性と整合することから、新法人の設立に向けた具体的な検討を開始することで基本合意に達しました。

なお、この新法人設立は、スケールメリットを生かした経営によって生産段階および供給段階でのコストを低減し、事業の健全性を確保することによって、血液製剤の国内自給達成をめざすものであります。

Press Release

日本赤十字社
Japanese Red Cross Society

2．事業統合後の姿

この度、日本赤十字社と田辺三菱製薬株式会社が、互いの血漿分画事業を統合して設立する新法人は、献血者の善意に基づき無償で得られた血液を原料とした血液製剤の国内自給の達成という公益性の高い目標のために取り組む、営利を目的としない法人とします。また、血漿分画製剤の国内必要原料を一括して処理できる能力を持つ大規模アルコール分画工場の新設を行い、効率的な生産体制によって血液製剤の国内製造における中核的役割を担うことをめざします。

3．基本合意の骨子

（1）基本合意書の締結

両社は本日、血漿分画事業の統合に向けて基本的な事項に関する合意書を締結いたしました。

（2） 事業統合に関する基本的な合意事項

ア．統合の形態

両社の血漿分画事業について、新法人が拠出又は事業譲渡を受ける方法による事業統合をめざします。

イ．新法人名

検討中

ウ．設立年月日

平成24年4月1日（予定）

エ．事業内容

血漿分画製剤およびその関連品の製造販売ならびに領布

オ．所在地

本部：東京都（予定）

カ．代表者

検討中

キ．本統合による業績の規模

両社血漿分画製剤売上高合算約370億円（平成21年度薬価ベース）

4．統合推進体制

両社は、この経営統合を円滑かつ速やかに実現していくため、本日、統合推進委員会を発足させました。

5．その他

開示すべき未決定の事項につきましては、今後、決定次第お知らせ致します。

以 上

【本件に関するお問い合わせ先】
日 本 赤 十 字 社　血液事業本部 広報推進担当　TEL：03-3437-7507
田辺三菱製薬株式会社　広報部　TEL：06-6205-5211

らかじめ指名する者が、その職務を代理する。

（委員会の開催）

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

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

（議決）

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

（議事の公開）

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

（雑則）

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

附則

この規程は、平成１５年７月３０日から施行する。

資料１

平成22年度第4回血液事業部会運営委員会議事要旨

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
出席者：佐川委員長、大平、岡田、花井、半田各委員
（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、石川副本部長、日野副本部長、五十嵐臨床開発課長

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

（審議概要）

<u>議題1について</u>

議事要旨に関する意見等については、事務局まで連絡することとされた。

<u>議題2について</u>

感染症定期報告について、事務局から説明後、質疑応答がなされた。

<u>議題3について</u>

事務局及び日赤から、供血者からの遡及調査の進捗状況、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）

○ 血液製剤の供給先の医療機関に対して、血液製剤による副作用あるいは感染症の可能性が疑われた場合には、すぐに連絡するように、一層の周知をする必要がある。

（献血件数及び HIV 抗体・核酸増幅検査陽性件数関係）

○ 検査目的の献血をしないよう国全体として HIV 検査体制の充実が必要である。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。

○ HIV 検査については、保健所等において匿名検査を実施しているが、ノーマライゼーションとして、医療機関全体で検査を行うことによって検査機会を増やすことも検討すべき。

議題4及び議題5について

（XMRV に関する文献報告関係）

岡田委員から、「XMRV に関する文献報告」について報告後、XMRV については、現時点では、血液事業という観点から緊急的な対応は必要ないが、引き続き情報収集を行って、新たな知見等が得られれば、この場で報告していただき、対応を検討することとされた。

（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、事務局が修正案を作成して、3月の血液事業部会で審議する方針となった。さらに、そこでのご意見を踏まえて修正したものをパブリックコメントにかけてご意見をいただくことで了承いただいた。

（英国滞在歴に関する制限緩和に伴う献血状況について）

日赤から、緩和された制限によって受け入れ対象となった献血受付者数の実数の推移及びこれに係る関連広報の展開について、報告がなされた。

（採血基準の改正に伴う準備状況について）

日赤から、初回献血者の採血副作用の防止対策など、採血基準の改正に伴う準備状況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６月２７日）

平成２２年１２月１日受理分以降

Ａ　研究報告概要

Ｂ　個別症例報告概要

感染症定期報告・感染症個別症例報告の取り扱い

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１２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（2010/12/1～2011/2/28）

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No
100271	2010/12/15	100768	E型肝炎	Lancet. 2010 Sep 11;376(9744):895-902. Epub 2010 Aug 20.	健康成人における組換えE型肝炎ワクチンHEV239(Hecolin; Xiamen Innovax Biotech,Xiamen,China)の有効性及び安全性を多施設共同無作為二重盲検プラセボ対照試験（第III相）により評価した。中国の江蘇省で16～65歳の健康成人において、HEV239群とプラセボ群を1:1に無作為割付けを行い、HEV239群にはHEV239ワクチンを、プラセボ群にはB型肝炎ワクチンを0、1、6カ月に1回（全3回）筋注投与し、19カ月間追跡調査された。主要評価項目は3回目の投与後31日目から12カ月の間のE型肝炎の予防効果とされた。参加者はHEV239群（n=56,302）またはプラセボ群（n=56,302）に無作為割付けされ、うち、全3回の接種を受けたHEV239群48,693名（86%）とプラセボ群48,663名（86%）が有効性の一次分析にかけられた。参加者のうち11,165名がE型肝炎ウイルスIgGの検査を受け、そのうち5,285名（47%）が抗体陽性であった。3回目の投与30日後から12カ月間に、プラセボ群の15名がE型肝炎を発症したのに対し、HEV239の有効性は100.0%（95% CI 72.1-100.0）であった。HEV239に関連する重篤な有害事象は示されなかった。以上よりHEV239は、中国の一般集団におけるE型肝炎の予防に有効であると結論づけている。	1
100295	2011/2/22	100916	E型肝炎	Vox Sanguinis 2010,99(Suppl.1) 1-516	血漿分画プールにおけるE型肝炎ウイルス（HEV）の広範囲な分布について、血漿分画プール中のHEV RNAの存在と抗HIV IgG抗体の濃度から検討された。その結果、約10%の血漿分画プールがHEV RNA陽性で、陽性プールの地理的分布はヨーロッパ、北アメリカ、東南アジアの数カ国を含み、広範囲にわたっていた。同定された株の系統発生解析により、genotype4がアジアのプールに限定されていたのに対し、genotype3はヨーロッパと北アメリカのプールで確認された。IgG抗HEV抗体とHEV RNAの間で相関関係は見られず、抗HEV抗体の濃度はアジアのプールでより高かった。以前HEV RNAが日本と中国の供血者で確認されたのに対し、血漿分画プールにおけるHEV RNAの分析はこれまで報告されたことがない。血漿分画製剤のHEVのリスクを判定するために、更なるプールのHEV RNA量を測定する調査が現在行われている。	2
100268	2010/12/1	100746	レトロウイルス(XMRV)	Proc Natl Acad Sci U S A. 2010 Sep 7;107(36):15874-9.	慢性疲労症候群(CFS)患者と健康な供血者の血液におけるマウス白血病ウイルス（MLV）関連ウイルス遺伝子配列の検出について報告された。最近、末梢血のPCR分析から健康成人218例中8例（3.7%）に対してCFS患者101例中68例（67%）と高率に異種指向性マウス白血病ウイルス関連ウイルス(XMRV)由来DNAが検出されたという研究報告があったが、続く4件の研究報告では、CFS患者の血液から当該DNAは認められていない。今回、CFS診断基準を満たす患者37名からの末梢血単核細胞由来DNA 41検体を調べ、37名中32名にMLV関連ウイルスgag 遺伝子配列を認めたが、健康ボランティア供血者では44名中3名にしか認められなかった。PCR分析システムまたは臨床検体において、マウスDNA混入の証拠は得られていない。全てのXMRVが遺伝的に近縁というこれまでの報告知見に対して、著者らは遺伝的に多様なグループのMLV関連ウイルスを確認した。CFS患者由来のgagおよびenv 配列は、XMRVよりも多指向性マウス内在性レトロウイルスに近縁であり、さらに、同種指向性のMLV配列とは近縁性が低かった。MLV関連ウイルスと同一の強い関連性があるかどうか、これらウイルスがCFS発症において原因的役割を担っているかどうか、ならびにこれらが血液供給の脅威となるかについては、さらなる研究が必要である。	3
100278	2010/12/27	100803	レトロウイルス(XMRV)	ABC Newsletter #31.2010 Aug 27	MLV-related Virusと慢性疲労症候群（CFS）との関連を検討した研究について、及び英国での処置について報告した。PNAS誌では、健康成人からの供血者44名の6.8%において、MLV-like viralの塩基配列が認められたと報告されている。Science誌では、CFS患者101例中68例（67%）にXMRVが検出され、健康成人218例中8例（3.7%）にXMRVが検出されたと報告されている。ABC NewsletterではXMRVは前立腺癌と関連について報告されているが、最新の7月1日のRetrovirologyでは、これまでの結果を確認できなかったとしている。PNAS誌とScience誌の報告に基づいて、11月1日以降、英国ではCFS/ME(筋痛性脳脊髄炎)の罹患歴のある患者由来の献血を永久に延期した。	4

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No
100271	2010/12/15	100768	パルボウィルス	Transfusion. 2010 Aug;50(8):1712-21	関連供血者と受血者のサンプル分析により確認された、赤血球輸血によるパルボウイルスB19(B19)感染について報告された。受血者のB19 DNAについてはPCR法、B19 IgG抗体についてはEIA法を用いて検査を行った。輸血に関連しない感染を除外するため、B19 DNA陽性受血者の輸血前サンプル及び関連供血者サンプルのB19 DNA、IgG、IgM抗体を検査した。感染の確認のためにDNA配列解析と系統発生解析を行った。その結果、受血者869人中14人(1.6%)がB19 DNA陽性であったが、受血者869人中1人(0.12%)が輸血による感染と確定された。この受血者は、急性感染した供血者からの赤血球(1単位中5×10^{10} IU のB19 DNAを含む)及び他の供血者3名からの赤血球(1,320 IUのB19 IgG抗体を含む)を輸血されていた。感染率(0.12%)は低いが、米国において毎年数百～数千例の感染症が発症する可能性がある。ほとんどの場合無症候性であるが、新生児や免疫不全状態、溶血状態にある者の場合、重篤になることがある。	5
100271	2010/12/15	100768	ウエストナイルウイルス	J Infect Dis. 2010 Nov 1;202(9):1354-61.	供血者スクリーニングによりウイルス血症と特定された者におけるウエストナイル熱の特徴について報告された。2003年6月から2008年にかけて米国赤十字は、初回検査でウエストナイルウイルス(WNV)RNA陽性となった供血者1,436名について、さらに経過観察と追加検査を行ったところ、821名の供血者がWNV感染症であると確認され、残りは未確定または偽陽性であった。WNV感染症の症状を576名の初期WNV感染者と、未感染者の間で比較したところ、WNV感染者の26%に、8症状(新しい発疹、全身の虚脱感、頭痛、重篤な筋肉痛、関節痛、発熱、悪寒、眼痛)のうち少なくとも3症状が存在すると推定された。症状を有する患者の半数近くが治療を求めたが、医療機関の認識不足のためWNV感染の診断を受けた者はわずか5%であった。また、女性および高いウイルス量の者は他の被験者より症状が発現する可能性が高かった。	6
100288	2010/12/1	100746	ウイルス感染	Am J Trop Med Hyg. 2010 Sep;83(3):714-21.	ボリビアとペルーにおけるヒトへのGuaroaウイルス(GROV)感染について報告された。GROVはコロンビアで1959年に初めてヒトから分離された。その後、ブラジル、コロンビア、パナマの発熱患者および蚊からウイルス分離株が採取されたが、ヒトの疾患とウイルスの関連性は不明であった。ボリビアとペルーの発熱疾患患者からGROV14株が分離され、また3症例でIgMセロコンバージョンが確認された。ペルーのIquitos居住者の抗GROV抗体陽性率は13%であり、林業、漁業、油田労働等の就職者において陽性率が高かった。代表的なGROV分離株の遺伝子学的特性からは、ボリビアとペルーの株の特性である単一系統グループを形成しており、以前にブラジルとコロンビアで分離された株とは異なる可能性が示された。本試験で、GROVが中南米の熱帯地域における発熱疾患の原因であることが確認された。	7
100271	2010/12/15	100768	ウイルス感染	Science. 2010 Oct 1;330(6000):20-21.	中国中央部における新型致死性ウイルスの特定について報告された。中国中央部でこの3年間、夏になると数百人が高熱と胃腸障害をきたし、多くの患者が多量出血し、ある地域では患者の30%近くが死亡した。ヒト類粒球アナプラズマ症が疑われたが、テキサス大学医学部のダニ媒介性疾患の専門家が新型のブニヤウイルスを特定した。その後の研究によりこのウイルスは重症発熱性血小板減少症候群(SFTS)ウイルスと命名され、ブニヤウイルス科フレボウイルス属に分類された。しかしこのウイルスの感染による致死率や、媒介生物はまだ分かっていない。	8

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No
100288	2010/12/1	100746	HTLV	Blood. 2010 Aug 26;116(8):1211-9	無症候性ヒトT細胞白血病ウイルスⅠ型(HTLV-1)キャリアにおける日本国内の前向き調査:無症候性HTLV-1キャリアにおける成人T細胞白血病(ATL)発症のリスク因子は、現在も不明である。近年、HTLV-1プロウイルス量は、ATLの重要な予測因子として評価されているが、少数の小規模前向き試験が実施されているだけである。2002年～2008年に、登録された無症候性HTLV-1キャ2002年～2008年に、登録された無症候性HTLV-1キャリア1,218名(男性426名、女性792名)を前向きに評価した。リア1,218名(男性426名、女性792名)を前向きに評価した。登録時のプロウイルス量は、女性と比べ男性(末梢血単核細胞(PBMCs)100あたりの中央値1.39 vs 2.10;P<0.001)、40歳以下の集団に対し年齢40～49歳および50～59歳の集団(それぞれP = 0.02, 0.007)、ATL家族歴を有さない集団に対し有する集団(PBMCs 100あたりの中央値1.33 vs 2.32;P=0.005)の方が、有意に多かった。14名の登録者が顕在性ATLへ進行した。この登録者の登録時のプロウイルス量は多かった(範囲:PBMCs 100あたり4.17～28.58)。登録時のプロウイルス量が4コピー以下の登録者は、ATLを発症しなかった。また多変量Cox解析では、プロウイルス量だけでなく、高年齢、ATLの家族歴、他疾患治療時における初回HTLV-1検査が、ATLの進行の独立リスク因子であることが示された。	9
100271	2010/12/15	100768	チクングニヤウイルス感染	http://medical.nikkeibp.co.jp/leaf/mem/pub/hotnews/inf/201010/516836.html	日本の蚊で伝播する新種ウイルス感染症 チクングニヤ熱が4類感染症に指定された。2010年10月1日、厚生科学審議会感染症分科会感染症部会において、アジア・アフリカ諸国での流行が問題となっているチクングニヤ熱を感染症法における4類感染症に追加することが合意された。4類感染症に指定されることで、患者を診断した医師には速やかな届け出が義務づけられる。さらに今回の部会では、チクングニヤ熱を検疫の対象となる感染症(検疫感染症)に追加することも合意された。チクングニヤ熱は日本国内に広く生息するヒトスジシマカの媒介で伝播する。急性期の患者における血中のウイルス量は多く、当該患者を刺した蚊を介してさらに感染者が広まる危険性がある。そのため感染症部会は、日本に持ち込まれたチクングニヤ熱が、国内で広まる可能性は十分高いと危惧している。	10
100295	2011/2/22	100916	新型インフルエンザ(H1N1)	Biologicals 38,2010,652-657	2009年のインフルエンザAウイルスH1N1の世界的流行が未だ猛威をふるっているが、このアウトブレイクによる血漿分画製剤の安全性への影響についてほとんど報告されていない。血漿分画製剤の安全性を評価するため、製造で使用される特定のウイルスクリアランス工程におけるウイルス除去効果が調査された。本研究では、インフルエンザAウイルスH1N1株A/NWS/33(H1N1)をモデルウイルスとした。アルブミンの製造工程では、パスツリゼーションだけでなくフラクションⅣ分画によりH1N1は不活化された。また、静注用免疫グロブリンの製造工程で、フラクションⅢ分画によって沈殿物中にH1N1は除去され、低pHインキュベーションにより完全に不活化された。第Ⅷ因子の製造工程では、0.3%リン酸トリ-n-ブチルと1.0%トリトンX-100を用いたS/D処理で1分以内に完全に不活化され、また98℃乾燥加熱でも10分以内に不活化された。アンチトロンビンⅢの製造工程においても、ViresolveNFPフィルターでH1N1は除去され、パスツリゼーション処理で不活化された。以上の結果から、H1N1は一般に用いられるウイルスクリアランス工程により、効果的に不活化・除去されことがわかった。	11
100288	2010/12/1	100746	デング熱	Am J Trop Med Hyg. 2010 Sep;83(3):664-71.	中国におけるデング熱の報告。中国では大規模アウトブレイクが1978年に既に報告されており、1978から2008年までに計655,324症例(うち610例が死亡)が報告された。1990年代以降、デング熱の流行は、南部沿岸地域から北部や西部にまで拡大している。中国本土において、この20年間でデング熱ウイルスの主要な伝播媒介動物であるヤブカ属の生物学的行動および媒介能が大きく変化してきており、これはおそらく都市化の加速や地球温暖化によるものである。また、人口増加や頻繁な海外旅行もデング熱流行の増加要因となる。デング熱制御への方法は、媒介蚊のコントロール、流行を予測する迅速ウイルス発見システムの確立、地域に密着した教育、そして安全かつ有効なワクチンを開発することである。	12

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100271	2010/12/15	100768	デング熱	ProMED 20100927.3506	アジア地域、オーストラリア、ヨーロッパ、南アメリカのデング熱更新情報について:フィリピンIloilo市では、2010年1月1日～9月18日までに4,825例(死亡27例)。台湾Kaohsiung市では、2010年これまでに227例。ベトナムでは、2010年これまでに55,400例。タイでは、2010年1月～9月11日までに75,852例(死亡87例)。インドネシアJakarta地域では、2010年1月～9月までに1,565例。マレーシアでは2010年これまでに死亡107例(前年死亡70例)。香港では、2010年これまでに43例。ネパールChitwanで7,000例以上の症例(少なくとも死亡19例/月)。インドDelhiでは、95症例以上、蚊媒介性疾患は計2,916例。インドBiharでは陽性518例(死亡6例)。パキスタンKarachiでは、2010年これまでに356例(216例が陽性)。オーストラリア(クイーンズランド州北部)では、4例が陽性、別8例が判定待ち。モナコでは、カリブから戻った18歳居住者に感染が認められた。フランスCorsicaでは、アジア旅行者の3例に症状が発現。フランス(アルプ・マリティーム)では2例。プエルトリコでは、2010年これまでに死亡23例。ホンジュラスでは、2010年これまでに死亡68例。ブラジルRoraimaでは、テング熱4型が12例に増加;ブラジルSao Pauloでは、4;100例(死亡17例)。パラグアイでは、2010年これまでの疑い症例数21,443例、確定症例数が13,678例。	13
100271	2010/12/15	100768	サルモネラ	ProMED 20100915.3343	アイルランドでアヒル卵を摂取することによるサルモネラ症計24例が報告され、アイルランドで近年記録された食中毒の中でも最大規模の流行となっている。感染者の年齢層は生後5カ月～80歳にわたり、最近の症例は裏庭や個人農場から得られたアヒル卵を摂取したことから感染に至っている傾向がある。これを考慮してアイルランド当局では、2010年9月14日、アヒル卵の安全な摂取法に関する助言を行った。また、アヒル卵に触った後の手洗い等の衛生管理を継続することの重要性について警告している。サルモネラネズミチフス菌DT8感染の症状は、嘔吐や下痢による軽度の症状から生命を脅かす疾患に変化してきている。乳児や妊婦、高齢者や病人は最も危険にさらされているため特に注意が必要である。この食中毒の集団発生に鶏卵は関係していない。	14
100276	2010/12/15	100774	コレラ	MMWR Vol. 59	ハイチでコレラのアウトブレークが発生し、2010年10月27日時点で303名の死亡が報告された。2010年10月21日Vibrio cholerae 01-serotype Ogawa-biotype El Torが同定された。2010年10月27日現在で4,722人が発症し、うち303名に死亡が報告された。ほとんどの症例がArtibonite Departmentで報告されているが、首都のあるQuest Departmentを含む他の地域にも症例が認められている。	15
100268	2010/12/1	100748	細菌感染	Lancet Infect Dis. 2010 Sep;10(9):597-602.	インド、パキスタン、英国での新規抗生物質耐性メカニズムの出現:New Delhi metallo-β-lactamase 1(NDM-1)によるカルバペネム耐性グラム陰性腸内細菌は、世界的に重大な健康問題となる可能性があるため、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陽性率を調査した。チェンナイ(南インド)、ハリヤーナー(北インド)で腸内細菌の分離株を対象として抗生物質の感受性を評価し、カルバペネム耐性遺伝子blaNDM-1の存在がPCRによって明らかになった。分離株はパルスフィールドゲル電気泳動法で、またプラスミドは、S1ヌクレアーゼ分解およびPCRによって解析された。英国患者については、インドまたはパキスタンへの渡航および最近の入院の有無を調査した。NDM-1産生分離株は、チェンナイ44株、ハリヤーナー26株、英国37株、その他のインド、パキスタン地域で73株が確認された。NDM-1は、ほとんどがEscherichia coli(36株)とKlebsiella pneumoniae(11株)に見つかり、チゲサイクリンとコリスチン以外のの抗生物質に高度耐性があった。ハリヤーナーから分離したK.pneumoniae分離株は遺伝的に均一であったが、英国およびチェンナイの分離株は遺伝的多様性を示した。ほとんどの分離株は、プラスミド上にNDM-1遺伝子を有し、英国およびチェンナイ分離株では受容株に容易にプラスミドが伝達されたが、ハリヤーナーの分離株は伝達性がなかった。英国のNDM-1陽性患者の多くは、前年にインド、パキスタンへの渡航歴があるか、これらの国と関連があった。	16

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No
100268	2010/12/1	100748	細菌感染	www.washingtontimes.com/news/2010/sep/7/japan-confirms-its-first-case-of-new-superbug-gene/	日本初のsuperbug(NDM-1を産生する、新たなタイプの多剤耐性菌)症例が報告された。細菌を薬剤耐性菌に変化させる新たなNew Delhi metallo-β-lactamase 1(NDM-1)遺伝子が日本で初めて、インドで治療を受けた50歳代日本人男性に確認された。この遺伝子はほとんどすべての抗生物質に耐性となるよう細菌を変化させる。この遺伝子は主に病原性大腸菌で見られ、他のタイプの細菌に容易に伝播することが出来るDNA構造を有している。当該男性はインドで内科治療を受け、帰国後の2009年4月に入院した。男性がインドで受けた治療は公表されなかった。男性は入院中に高熱を出したが、2009年10月に退院した。病院はsuperbug(NDM-1を産生する、新たなタイプの多剤耐性菌)を疑い検体を保管、検査し、NDM-1遺伝子の検出について、厚生労働省に届け出た。院内感染は認められていない。日本初のNDM-1症例確認後、厚生労働省は全国調査を開始した。	17
100271	2010/12/15	100768	細菌感染	USA TODAY. Available from: http://www.usatoday.com/yourlife/health/medical/2010-09-17-1Asuperbug17_ST_N.htm	抗生物質の機能を一時的に阻害する酵素Klebsiella pneumoniae carbapenamase(KPC)を産生する遺伝子を備えた強力な薬剤耐性菌が米国の35州を超える病院で報告されている。この細菌は重篤な疾患患者を襲い、死亡率は全症例の30～60%に及ぶ。米国疾病管理予防センター(CDC)によると、New Delhi metallo-β-lactamase 1(NDM-1)は米国では稀であり、KPCの方がはるかに一般的で、現在では米国の半分以上の州で報告されているという。この細菌に対する唯一の薬にポリミキシンがあるが、腎臓に有毒であるため数年前からほとんど使用されていない。従って予防は極めて重要である。2009年3月にCDCは予防に関する新しいガイドラインを示し、特にカルバペネム耐性菌感染症と診断される患者を治療する医師は、ガウンと手袋を着用して自身を守るとともに、他の患者への感染を防がなくてはならないとしている。	18
100271	2010/12/15	100768	感染	MMWR Vol. 59 No. 36	2009年12月14日、ミシシッピー州で、同一ドナーからの腎臓移植レシピエント2名が移植により脳炎を発症した可能性があると米国疾病管理予防センター(CDC)に報告された。CDCはドナーの剖検脳組織からアメーバを発見し、その後、ドナー及びレシピエント2名由来の検体における検査により、バラムチア・アメーバ性肉芽腫性脳炎(GAE)の伝播が確認された。これはBalamuthia mandrillarisに起因する稀な疾患である。レシピエント2名のうち1名(31歳女性)は死亡し、もう1名(27歳男性)は右腕、両脚、視力に後遺症があるが生存している。同一ドナーから心臓移植と肝臓移植を受けたレシピエントには感染の徴候は見られていない。ドナー(4歳)はインフルエンザA感染症を発症後、急性散在性脳脊髄炎で死亡したと推定される。これは臓器移植によるバラムチア感染症の初めての報告である。	19
100278	2010/12/27	100803	異型クロイツフェルト・ヤコブ病	Journal of Pathology 2010;10(1002):2767-2767	英国で採取された扁桃検体におけるリンパ網内性プリオンタンパクの大規模な免疫組織化学検査(IHC)について報告された。英国ではBSE流行の結果、2010年7月5日までにvCJD173例に至っている。扁桃検体63007検体について病因のプリオン蛋白(PrP^{res})に対するEIAを行ったが陰性であった。最もリスクのある1961-1985年生まれの検体とコントロールを含めた別検体について、PrP^{res}、抗プリオン抗体ICMS35及びKG9を用いてIHCを行ったところ、扁桃検体9160中1検体が陽性であり、英国人口100万人あたりプリオン蛋白関連疾患109人の検出率であった。この結果はIHCによってvCJD陽性であるかを見当づけることが出来ると説明している。	20
100278	2010/12/27	100803	異型クロイツフェルト・ヤコブ病	FDA/Vaccines, Blood & Biologics/2010/10/26	米国内採取血漿から製造されたヒト血漿由来第Ⅷ因子(pdFVⅢ)の使用に伴うvCJD罹患リスクとの定量的リスク評価ドラフトの2010年最新版が報告された。pdFVⅢリスク評価モデルで得られた結果から、①米国で製造されたpdFVⅢからのvCJD感染リスクは非常に低いと考えられるが、0ではないこと、②血漿プールがvCJDに汚染されている可能性は低いこと、③vCJD原因物質への曝露の可能性があること、又非常に低いながらも潜在的な感染リスクがあること、④一般的なvCJDリスク、あるいは個々の患者への真のリスクを正確に評価することは不可能であること、⑤感染リスクに影響を及ぼす最も重要な因子が製造ステップでのvCJD原因物質のクリアランス、個々の患者がどの程度の量の製剤を用いるか、及び変数として用いた英国供血者集団におけるvCJDの発生率であることが示唆されている。	21

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Articles

Efficacy and safety of a recombinant hepatitis E vaccine in healthy adults: a large-scale, randomised, double-blind placebo-controlled, phase 3 trial

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Summary

Background Seroprevalence data suggest that a third of the world's population has been infected with the hepatitis E virus. Our aim was to assess efficacy and safety of a recombinant hepatitis E vaccine, HEV 239 (Hecolin; Xiamen Innovax Biotech, Xiamen, China) in a randomised, double-blind, placebo-controlled, phase 3 trial.

Methods Healthy adults aged 16–65 years in Jiangsu Province, China were randomly assigned in a 1:1 ratio to receive three doses of HEV 239 (30 μg of purified recombinant hepatitis E antigen adsorbed to 0·8 mg aluminium hydroxide suspended in 0·5 mL buffered saline) or placebo (hepatitis B vaccine) given intramuscularly at 0, 1, and 6 months. Randomisation was done by computer-generated permuted blocks and stratified by age and sex. Participants were followed up for 19 months. The primary endpoint was prevention of hepatitis E during 12 months from the 31st day after the third dose. Analysis was based on participants who received all three doses per protocol. Study participants, care givers, and investigators were all masked to group and vaccine assignments. This trial is registered with ClinicalTrials.gov, number NCT01014468.

Findings 11 165 of the trial participants were tested for hepatitis E virus IgG, of which 5285 (47%) were seropositive for hepatitis E virus. Participants were randomly assigned to vaccine (n=56 302) or placebo (n=56 302). 48 693 (86%) participants in the vaccine group and 48 663 participants (86%) in the placebo group received three vaccine doses and were included in the primary efficacy analysis. During the 12 months after 30 days from receipt of the third dose, 15 per-protocol participants in the placebo group developed hepatitis E compared with none in the vaccine group. Vaccine efficacy after three doses was 100·0% (95% CI 72·1–100·0). Adverse effects attributable to the vaccine were few and mild. No vaccination-related serious adverse event was noted.

Interpretation HEV 239 is well tolerated and effective in the prevention of hepatitis E in the general population in China, including both men and women age 16–65 years.

Funding Chinese National High-tech R&D Programme (863 programme), Chinese National Key Technologies R&D Programme, Chinese National Science Fund for Distinguished Young Scholars, Fujian Provincial Department of Sciences and Technology, Xiamen Science and Technology Bureau, and Fujian Provincial Science Fund for Distinguished Young Scholars.

Introduction

Every year, 13 000–26 000 deaths are estimated in patients with chronic liver disease in industrialised countries.[1] In a continuing hepatitis E epidemic in Uganda, that has caused illness in more than 10 196 people and 160 deaths, mortality was 13% in children.[2] At least four genotypes of hepatitis E viruses have been identified.[3] Genotypes 1 and 2 were isolated from human beings and are mainly seen in developing countries. Genotypes 3 and 4 are zoonotic, with pigs being the principal reservoir; they have been identified in many sporadic cases and limited foodborne outbreaks mainly affecting middle-aged and elderly men.[4–6] Nevertheless, all hepatitis E virus associated with human diseases can be considered as belonging to one serotype.[7]

Two recombinant vaccines have undergone phase 2 clinical trials. One of the vaccines was produced in

別紙様式第2-1

No. 8

医薬品 研究報告 調査報告書

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販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)				

研究報告の概要

○健常成人における組換えE型肝炎ワクチンの有効性及び安全性:多施設共同無作為二重盲検プラセボ対照試験(第Ⅲ相)

目的:組換えE型肝炎ワクチンHEV239(Hecolin; Xiamen Innovax Biotech,Xiamen,China)の有効性と安全性を、無作為二重盲検プラセボ対照第Ⅲ相試験により評価すること。

方法:中国の江蘇省で16～65歳の健常成人において、HEV239群とプラセボ群を1:1に無作為割付けを行い、HEV239群にはHEV239ワクチンが、プラセボ群にはB型肝炎ワクチンが0、1、6か月に1回ずつ全3回、筋注投与された。参加者は19か月間追跡調査された。プライマリーエンドポイントとして3回目の投与後31日目から12か月の間のE型肝炎の予防効果を観察した。

所見:参加者はHEV239群(n=56,302)またはプラセボ群(n=56,302)に無作為割付けされ、そのうち、問題なく全3回の接種を終えることができたHEV239群48,693名(86%)とプラセボ群48,663名(86%)が有効性の一次分析にかけられた。試験参加者のうち11,165名がE型肝炎ウイルスIgGの検査を受け、そのうち5,285名(47%)が抗体陽性であった。

3回目の投与30日後から12か月間に、15名のプラセボ群参加者がE型肝炎を発症したが、3回の投与後のHEV239の有効性は100.0%(95% CI 72.1-100.0)であった。HEV239に関連する重篤な有害事象は示されなかった。

結論:HEV239は、中国の一般集団におけるE型肝炎の予防に有効である。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
中国で組換えE型肝炎ワクチンHEV239の多施設無作為二重盲検プラセボ対照第Ⅲ相試験を実施したところ、中国の一般集団におけるE型肝炎の予防に有効であったとの報告である。	日本赤十字社では、ALT高値の輸血血液を排除している。また、厚生労働科学研究「経口感染する肝炎ウイルス(A型、E型)の感染防止、遺伝的多様性、および治療に関する研究」班と共同して、献血者におけるHEV感染の疫学調査を行っている。今後もHEV感染の実態に関する情報の収集及び安全対策に努める。

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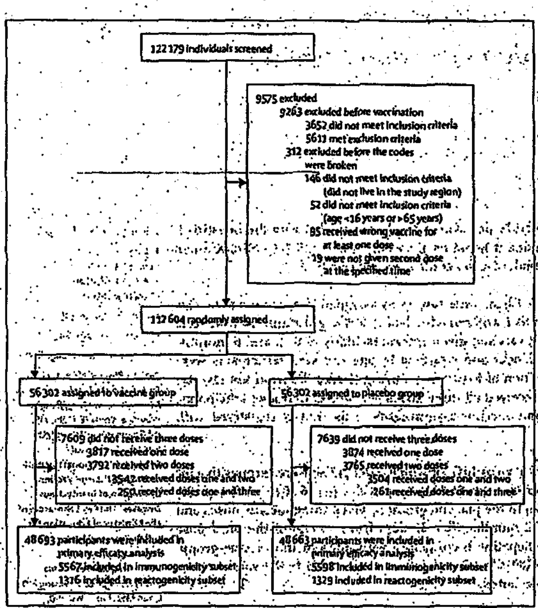

Figure 2: Trial profile
The webappendix lists reasons for exclusion (p 30) and for non-completion (p 31).

See Online for webappendix

For the protocol see http://nidvd.xmu.edu.cn/HEV/protocol/Protocol_phase_3_100509.pdf

insect cells and was safe and immunogenic in young men (mean age 25·2 years; SD 6·25), providing 95% protection against hepatitis E in Nepal, where only genotype 1 hepatitis E virus had been isolated.[7] The results were encouraging but two questions remained to be answered. The first related to the safety and efficacy of the vaccine in the general population, especially in women and elderly people. The second related to the efficacy of a vaccine originally derived from hepatitis E virus genotype 1 against disease caused by heterogenic hepatitis E virus. The other candidate vaccine, HEV 239 (Hecolin; Xiamen Innovax Biotech, Xiamen, China), was produced in bacterial cells and was safe and efficacious against infection with hepatitis E virus in seronegative participants in a phase 2 trial.[8]

We undertook a randomised, double-blind, placebo-controlled, phase 3 trial to assess the efficacy and safety of HEV 239 in the general population. The trial included men and women from age 16 to 65 years, with or without antibodies against hepatitis E, from a region where both genotypes 1 and 4 cocirculate with the zoonotic genotype 4 predominating.

Methods

Study design and participants

This double-blind, randomised, placebo-controlled trial was done between August, 2007, and June, 2009, in Dongtai County, Jiangsu Province, China. On October, 2007, after enrolment in one township (Quindong), and before enrolment in ten other townships, the protocol was modified so that each of the 10 000 participants in one of the ten townships (Anfeng) had serum samples collected on day 0 and month 7 to assess the level of antibody protection through long-term follow-up. Independent ethics committee approval was obtained from the Ethics Committee of the Jiangsu Provincial Centre for Disease Control and Prevention (JSCDC), and the study was done in accordance with the principles of the Declaration of Helsinki, the standards of Good Clinical Practice, and Chinese regulatory requirements as stipulated by the Chinese Food and Drug Administration.

The study was designed by JSCDC and Xiamen University. Study staff at JSCDC were responsible for data collection. A sentinel hepatitis surveillance system was set up to identify incident hepatitis cases as they presented. Serial serum samples obtained from study participants were independently tested by the Chinese National Institute for the Control of Pharmaceuticals and Biological Products (NICPBP). A contract research organisation (PPD-Excel PharmaStudies, Beijing, China) monitored and ensured that the trial was done in compliance with the protocol, evaluated progress, verified that the rights of the participants were protected, and ensured that data were complete, accurate, and verifiable from source data. An independent data and safety monitoring board (DSMB) was set up to oversee the trial and ensure the safety of participants and the integrity of the data. The DSMB reviewed the clinical and laboratory data to confirm the diagnosis of hepatitis E before the group assignment (ie, vaccine *vs* placebo) of trial participants was broken.

Men and women were eligible for enrolment if they were healthy, aged 16–65 years, and understood the study procedures (detailed eligibility criteria are described in webappendix pp 2–3). Written informed consent was obtained from all participants.

Vaccination

The preparation of HEV 239 vaccine is described elsewhere.[19] The vaccine contains 30 μg of the purified antigen adsorbed to 0·8 mg aluminium hydroxide suspended in 0·5 mL buffered saline. A licensed hepatitis B vaccine (Beijing Tiantan Biologic, Beijing, China) containing hepatitis B virus surface antigen in 0·5 mL aluminium hydroxide, was given as placebo. Vaccine doses and placebo doses were repackaged by Innovax under Good Manufacturing Practice conditions for identical appearance, but labelled with two letters each according to a random assignment. Three doses of vaccine or placebo were given intramuscularly at 0, 1, and 6 months.

Randomisation and masking

Trained local health-care workers enrolled the participants, and some of these health-care workers interviewed participants to assess adverse events and possible acute hepatitis later in the trial. An independent statistician prepared a permuted-block 1:1:1:1 randomisation list (with 20 codes to a block) using SAS software. The randomisation list was concealed and transferred into an immunisation management computer program through which participants were stratified by age and sex, and assigned vaccine codes. The study-group and vaccine code assignments were masked from all participants, carers, and investigators (or monitors). The integrity of the masking process was confirmed by the investigators and DSMB before the assignment of study group and vaccine codes was finally revealed. Health-care workers from JSCDC assigned participants to the study groups; they did not have any further involvement in the trial.

A subset of participants from one township was selected for active surveillance of adverse events (reactogenicity subset). Serum samples before immunisation were obtained from these participants and those from another township to establish the baseline concentration of hepatitis E virus IgG and for assessment of immunogenicity (immunogenicity subset). Fingerprint scanners and digital photographs were used to identify and track participants throughout immunisation, blood collection, and follow-up.

	Vaccine group	Placebo group
Randomised participants*	56 302	56 302
Men	24 511 (43·5%)	24 567 (43·6%)
Age (years)	44·14 (11·40)	44·13 (11·40)
Age group (years)		
16–20	2520 (4%)	2480 (4%)
21–30	4598 (8%)	4653 (8%)
31–40	12 684 (23%)	12 688 (23%)
41–50	18 292 (32%)	18 310 (33%)
51–60	14 644 (26%)	14 657 (26%)
61–65	3564 (6%)	3514 (6%)
Per-protocol population† (three doses)	48 693	48 663
Male to female ratio	0·74	0·74
Mean age (years)	44·72, SD 11·09	44·68, SD 11·10
Immunogenicity subset‡	5567	5598
Male to female ratio	0·64	0·65
Mean age (years)	45·22 (10·75)	45·25 (10·82)
Anti-HEV prevalence	47·76% (46·44–49·09)	46·91% (45·60–48·23)
GMC (Wu/mL)	0·14 (0·13–0·14)	0·13 (0·13–0·14)
Reactogenicity subset§	1316	1329
Men	524 (39·8%)	561 (42·2%)
Age (years)	44·70, SD 11·23	44·93, SD 11·10

Data are number (%), mean (SD), or mean (95% CI). HEV=hepatitis E virus. GMC=geometric mean concentration. *All randomised participants who received at least one dose of vaccine or placebo. †Per-protocol population denotes all randomised participants who received three doses of vaccine or placebo. ‡Participants in the immunogenicity subset were from two townships additionally investigated for antibody response to vaccination. §Participants in the reactogenicity subset were from one township visited regularly at home by investigators to assess adverse events.

Table 1: Baseline characteristics of participants

Hepatitis surveillance

Participants with suspected hepatitis were identified through an established active hepatitis surveillance system comprising 205 sentinels, including 162 community clinics, 30 private clinics, 11 central hospitals located in the townships, and two central hospitals in the city of Dongtai (webappendix p 32). A case of hepatitis was defined as a patient presenting with constitutional symptoms such as fatigue, loss of appetite, or both for longer than 3 days with alanine aminotransferase (ALT) exceeding 2·5-times the upper limit of normal range. Patients with abnormal concentrations of ALT were tested at first presentation by JSCDC for hepatitis A virus IgM, surface antigen of hepatitis B virus, hepatitis B virus core protein IgM, hepatitis C virus immunoglobulin, and hepatitis E virus IgM. Paired serum samples were obtained from these patients at the time of presentation and 2–6 weeks later. Serial samples were sent to the NICPBP to test for hepatitis E virus IgM and IgG, hepatitis E virus RNA, and hepatitis A virus IgM. The DSMB reviewed the clinical and laboratory results and confirmed the diagnoses of hepatitis E before unblinding. To be defined as an acute hepatitis E patient, a participant needed to fulfil three conditions: acute illness lasting for at least 3 days; abnormal serum ALT concentration 2·5-times the upper limit of normal range or greater; and positive hepatitis E virus IgM and RNA, ≥4-times increase in hepatitis E virus IgG, or both.

Laboratory measurements

The tests for hepatitis E virus IgM were done by use of two commercial assays in parallel (Beijing Wantai, Beijing, China; MP Biomedicals, Singapore).[11,20–23] The assay for hepatitis E virus IgG used antigen more truncated than that in the vaccine antigen (Beijing Wantai, China).[20,21] Hepatitis E virus IgGs were further quantified and expressed in WHO units per mL (Wu/mL; webappendix p 2). The lower limit of hepatitis E virus IgG quantification was 0·077 Wu/mL.[24] For the analysis, the antibody concentration in samples negative for hepatitis E virus IgG were arbitrarily set at 0·0385 Wu/mL. Serum samples of patients with detectable hepatitis E virus IgM or a two times or greater rise of hepatitis E virus IgG concentration in paired samples were tested for hepatitis E virus RNA.[7] Serum samples were taken before the first vaccine dose and 1 month after the third dose from participants in the immunogenicity subset to establish concentration of hepatitis E virus IgG. Antibody concentration of 0·077 Wu/mL or greater was deemed to be a positive finding. Antibody response was defined as a greater than four-times increase of hepatitis E virus IgG in an individual's paired sera. All reagents were supplied by Beijing Wantai Biological Pharmacy Enterprise, Beijing, China.

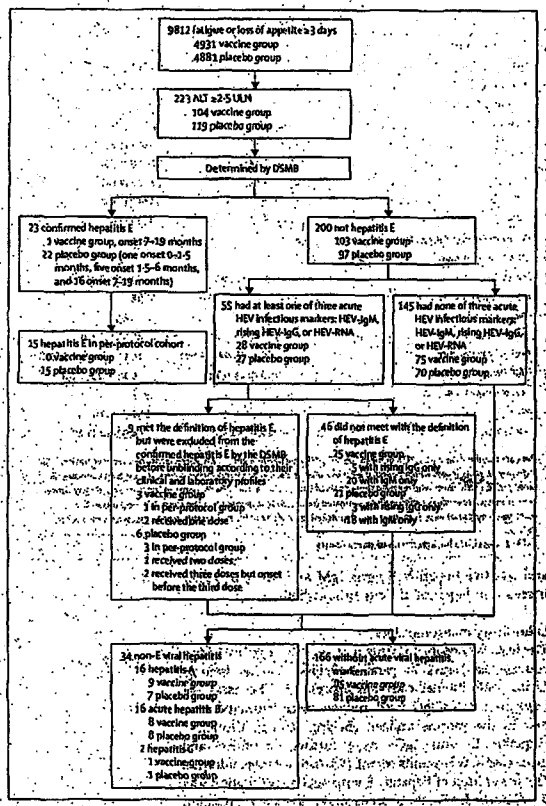

Figure 2: Flowchart of surveillance and certification of acute hepatitis E
Sentinel hepatitis surveillance system was set up to monitor study participants for development of acute hepatitis (webappendix p 32). A case of acute hepatitis was defined as a participant who presented with constitutional signs, such as fatigue, nausea for at least 3 days, and alanine aminotransferase (ALT) exceeding 2·5 times the upper limit of normal range (ULN). Clinical and laboratory findings were assessed by an independent data and safety monitoring board (DSMB). The DSMB reviewed clinical and laboratory data to confirm the diagnosis of hepatitis E before the group assignment of trial participants was broken. HEV=hepatitis E virus.

For more on the Medical Dictionary for Regulatory Activities see http://www.meddramsso.com/

Adverse events

After each dose, participants were observed for 30 min for immediate adverse reactions. Participants in the reactogenicity subset were visited at home by investigators at 6 h, 24 h, 48 h, 72 h, 7 days, 14 days, and 28 days after each dose, and observed or reported adverse effects, if any, were recorded on safety diary cards. Other participants were asked to report any adverse events to nearby clinics within 1 month after each dose. Additionally, investigators reviewed all records of admission to hospital and death to identify trial participants. Any serious adverse events were recorded throughout the study by use of the Medical Dictionary for Regulatory Activities (version 12.0).

Statistical analysis

We estimated that the incidence of hepatitis E for adults aged 16–65 years would be about four cases per 10 000 person-years (webappendix p 1). On the assumption of a vaccine efficacy of 70%, a two-group continuity-corrected χ^2 test with a one-sided significance level of 0·05 would have a power of 80% to detect a difference in incidence with 41 277 participants per group. To compensate for dropouts, 50 000 participants per group were needed.

Prespecified outcome analyses were done in eligible participants who had received at least one dose of either vaccine, and in those who received all of the three doses of the vaccines. The primary endpoint was prevention of hepatitis E in participants who received three doses of vaccine (ie, the per-protocol population) during the 12 months from the 31st day after receipt of the third dose. Vaccine efficacy and 95% CIs were calculated on the basis of the identified difference between the vaccine group and the placebo group and the accrued person-time. An exact conditional procedure was used to evaluate vaccine efficacy under the assumption that the numbers of patients with hepatitis E in the vaccine and placebo groups were independent Poisson random variables.[9] For robustness, efficacy was also assessed by use of a Cox proportional hazard model, and a log-rank test was used to compare the cumulative incidence of hepatitis E between the study groups.

Adverse events were summarised for all vaccination visits as frequencies and percentages according to study group. Proportions of events and 95% CIs (unadjusted for multiplicity) were compared between the groups by use of two-sided Fisher's exact test.

Data analysis was done with SAS software version 9.1. All reported p values are two-sided with an α value of 0·05.

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

122 179 people from 11 townships attended the enrolment visit between August and October, 2007. 112 604 participants fulfilled the eligibility requirements, were randomly assigned to the study group, and received at least one dose of vaccine or placebo. 97 356 participants received all three doses of vaccine or placebo and were included in the analysis of the primary endpoint (figure 1). Table 1 shows the baseline characteristics of study participants.

The DSMB confirmed 23 cases of hepatitis E before unblinding (figure 2); details of each case are listed in webappendix pp 4–24. Compared with the general study population, patients with hepatitis E were older (mean 51·3 years, SD 8·2, median 53, range 36–63) and more likely to be men (male-to-female ratio 2·3). The mean maximum serum ALT concentration of patients with hepatitis E was 30·8 times upper limit of normal range (SD 29·3; 18·1, 2·5–96·9), and the mean duration of illness was 57·1 days (SD 39·8; 41, 9–125). 15 patients were admitted to hospital for a mean of 14·4 days (SD 14·5; 10, 9–66). All 23 patients tested positive for hepatitis E virus IgM; 22 were positive for hepatitis E virus RNA, and 14 had a 4 times or greater increase in hepatitis E virus IgG. Of the 13 patients whose viruses were isolated for sequencing, 12 had genotype 4 and one had genotype 1. Of the eight patients who received all three doses and whose viruses underwent sequencing, all had genotype 4.

In the primary analysis population 15 participants developed hepatitis E during the 12 months from the 31st day after receipt of the third dose; all 15 were in the placebo group (table 2). Vaccine efficacy against hepatitis E was 100·0% (95% CI 72·1–100·0), and protection extended to all participants throughout the 12 months. Five participants developed hepatitis E during the 14 days after the second dose and before the third dose; all were in the placebo group. Vaccine efficacy after two doses was 100·0% (9·4–100·0).

Most randomised participants who received at least one dose of vaccine or placebo were followed up for 19 months from the beginning of the study, and a small proportion of participants were followed up from month 7 of the study. There were 23 cases of hepatitis E during the follow-up, one in the vaccine group (the participant received one

	Follow-up (month of study)	Vaccine group			Placebo group			Vaccine efficacy (95% CI)	p value
		Number of participants/ person-years at risk	Number of cases	Incidence (per 10 000 person-years)	Number of participants/ person-years at risk	Number of cases	Incidence (per 10 000 person-years)		
Per-protocol									
Whole group (three doses)	7–19	48 693/48 594·6	0	0·0	48 663/48 555·1	15	3·1	100·0% (72·1–100·0)	<0·0001
Men	7–19	20 662/20 616·1	0	0·0	20 709/20 660·0	11	5·3	100·0% (60·1–100·0)	0·001
Women	7–19	28 031/27 978·5	0	0·0	27 954/27 895·1	4	1·4	100·0% (−51·0 to 100·0)	0·045
Age 16–49 years	7–19	30 374/30 299·5	0	0·0	30 355/30 276·9	6	2·0	100·0% (15·13–100·0)	0·014
Age 50–65 years	7–19	18 319/18 295·2	0	0·0	18 308/18 278·2	9	4·9	100·0% (49·4–100·0)	0·003
First 6 months of follow-up	7–13	48 693/23 981·9	0	0·0	48 663/23 965·8	6	2·5	100·0% (15·12–100·0)	0·014
Second 6 months of follow-up	14–19	48 693/24 612·8	0	0·0	48 663/24 589·3	9	3·7	100·0% (49·4–100·0)	0·003
First two doses subset	1·5–5	54 986/20 202·1	0	0·0	54 973/20 196·8	5	2·5	100·0% (9·1–100·0)	<0·0001
Population receiving at least one dose	7–19	56 302/56 104·7	1	0·2	56 302/56 081·2	16	2·9	93·8% (59·8–99·9)	0·0001
Modified subset one (all participants received at least one dose)	0–19	56 302/87 354·2	1	0·1	56 302/87 323·2	22	2·5	95·5% (66·3–99·4)	<0·0001
Modified subset two (participants in reactogenicity subset were excluded because of lacking follow-up data during 0–6 months)	0–19	54 986/86 040·4	1	0·1	54 973/86 003·4	21	2·4	95·2% (64·6–99·4)	<0·0001

Person-years at risk is the cumulative follow-up years of at risk participants at the indicated timepoint. Number of at risk participants is the initial number of participants entered in the study–(cumulative hepatitis E cases+participants who had dropped out of study).

Table 2: Efficacy of recombinant hepatitis E vaccine

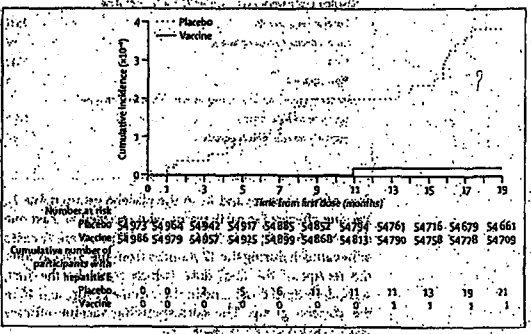

Figure 3: Cumulative incidence of hepatitis E
Cumulative incidence in each group at an indicated time=(cumulative number of cases/cumulated follow-up time (ie, at risk participants ×10 000). Number at risk=initial number of participants entered in the study–(cumulative hepatitis E cases+participants who had subsequently dropped out of study). The difference between the groups was significant (p=0·001 by log-rank test).

	Number of adverse events (rate, 95% CI)		p value*
	Vaccine group	Placebo group	
Reactogenicity subset			
Number of participants who received more than one dose	1316	1329	..
Solicited local adverse events within 72 h after each dose			
Local adverse events	177 (13·5%, 11·65–15·41)	94 (7·1%, 5·75–8·59)	<0·0001
Local adverse events ≥grade 3	2 (0·2%, 0·02–0·55)	0 (0·0%, 0·00–0·28)	0·248
Pain	136 (10·3%, 8·74–12·11)	73 (5·5%, 4·33–6·86)	<0·0001
Pain ≥grade 3	0 (0·0%, 0·00–0·28)	0 (0·0%, 0·00–0·28)	..
Swelling	30 (2·3%, 1·54–3·24)	8 (0·6%, 0·26–1·18)	<0·0001
Swelling ≥grade 3	2 (0·2%, 0·02–0·55)	1 (0·0%, 0·00–0·28)	0·248
Itch	20 (1·5%, 0·93–2·34)	13 (1·0%, 0·52–1·67)	0·210
Itch ≥grade 3	0 (0·0%, 0·00–0·28)	0 (0·0%, 0·00–0·28)	..
Solicited systemic adverse events within 72 h after each dose			
Systemic adverse events	267 (20·3%, 18·15–22·56)	263 (19·8%, 17·68–22·03)	0·748
Systemic adverse events ≥grade 3	7 (0·5%, 0·21–1·09)	4 (0·3%, 0·08–0·77)	0·356
Fever	245 (18·6%, 16·55–20·83)	239 (18%, 15·95–20·16)	0·674
Fever ≥grade 3	6 (0·5%, 0·17–0·99)	3 (0·2%, 0·05–0·66)	0·341
Headache	14 (1·1%, 0·58–1·78)	8 (0·6%, 0·26–1·18)	0·191
Headache ≥grade 3	1 (0·1%, 0·00–0·42)	0 (0·0%, 0·00–0·28)	0·498
Fatigue	28 (2·1%, 1·42–3·06)	20 (1·5%, 0·92–2·31)	0·230
Fatigue ≥grade 3	1 (0·1%, 0·00–0·42)	0 (0·0%, 0·00–0·28)	0·498
Total vaccinated cohort minus the reactogenicity subset			
Number of participants who received more than one dose	54 986	54 973	
Solicited local adverse events within 72 h after each dose			
Local adverse events	1532 (2·8%, 2·65–2·93)	1051 (1·9%, 1·8–2·03)	<0·0001
Local adverse events ≥grade 3	61 (0·1%, 0·08–0·14)	27 (0·1%, 0·03–0·07)	<0·0001
Pain	1143 (2·1%, 1·96–2·20)	754 (1·4%, 1·28–1·47)	<0·0001
Pain ≥grade 3	1 (0·0%, 0·00–0·01)	0 (0·0%, 0·00–0·01)	1·000
Solicited systemic adverse events within 72 h after each dose			
Systemic adverse events	1068 (1·9%, 1·83–2·06)	1045 (1·9%, 1·79–2·02)	0·617
Systemic adverse events ≥grade 3	60 (0·1%, 0·08–0·14)	63 (0·1%, 0·09–0·15)	0·786

(Continues on next page)

dose of the vaccine) and 22 in the placebo group (table 2). Vaccine efficacy for participants who received at least one dose was 95·5% (95% CI 66·3–99·4). There were 17 cases of hepatitis E during the 12 months from the 31st day after the receipt of the final dose (which could be the first, second, or third dose), one in the vaccine group, and 16 in the placebo group. The corresponding vaccine efficacy was 93·8% (95% CI 59·8–99·9%).

Figure 3 shows the cumulative incidence of hepatitis E in participants who were followed up for 19 months from the beginning of the study. The difference between the vaccine group and the placebo group was significant (p<0·0001).

Most adverse events were mild. Rates of serious adverse events were similar in the vaccine and placebo groups during the entire follow-up, and none were deemed by the DSMB to relate to vaccination (table 3 and webappendix pp 25–28). Participants in the reactogenicity subset were regularly interviewed by investigators after receipt of each dose to assess adverse events (table 3). In this subset, the proportion of all solicited local adverse events identified within 72 h after each dose was greater in the vaccine group (13·5%) than in the placebo group (7·1%; p<0·0001). The vaccine group also had a greater proportion of adverse reactions attributed to pain, swelling, and itching at injection sites, which were the most common local adverse events. The proportion of systemic adverse events were similar for both groups (20·3% *vs* 19·8%). On the basis of reports by participants not in the reactogenicity subset, the proportion of solicited local adverse events was higher in the vaccine group than in the placebo group (2·8% *vs* 1·9%) and the rates of solicited systemic adverse events were not significantly different between the two groups (table 3).

Serum samples were taken from 11 165 participants before vaccination and 1 month after receipt of the third dose. 5494 (98·7%) of 5567 participants in the vaccine group had an increase in antibody concentration in the samples after vaccination of four times or more from that of the corresponding samples before vaccination. In the samples after vaccination, geometric mean concentration in these participants rose from 0·14 Wu/mL to 19·0 Wu/mL (95% CI 18·6–19·4). By contrast,

	Number of adverse events (rate, 95% CI)		p value*
	Vaccine group	Placebo group	
(Continued from previous page)			
Total vaccinated cohort			
Number of participants who received more than one dose	56 302	56 302	..
Unsolicited events within 30 days after each dose†			
All	6771 (12·0%, 11·76–12·3)	6724 (11·9%, 11·68–12·21)	0·666
≥Grade 3	839 (1·5%, 1·39–1·59)	792 (1·4%, 1·31–1·51)	0·241
Serious adverse events within 30 days after each dose‡			
All	248 (0·4%, 0·39–0·50)	245 (0·4%, 0·38–0·49)	0·892
Admission to hospital	238 (0·4%, 0·37–0·48)	233 (0·4%, 0·36–0·47)	0·817
Disability	0 (0·0%, 0·00–0·01)	0 (0·0%, 0·00–0·01)	..
Death§	10 (0·0%, 0·01–0·03)	12 (0·0%, 0·01–0·04)	0·670
Serious adverse events during period from month 2 to month 6 and from month 7 to month 19†			
All	1423 (2·5%, 2·40–2·66)	1430 (2·5%, 2·41–2·67)	0·894
Admission to hospital	1328 (2·4%, 2·23–2·49)	1336 (2·4%, 2·25–2·50)	0·875
Disability	0 (0·0%, 0·00–0·01)	0 (0·0%, 0·00–0·01)	..
Death	95 (0·2%, 0·14–0·21)	94 (0·2%, 0·13–0·20)	0·942

Grade 3 pain, headache, and fatigue were defined as prevention of normal activities; grade 3 swelling was defined as a diameter of more than 30 mm; grade 3 itch was defined as body itch; and grade 3 fever was defined as temperature greater than 39·0°C. Symptoms with frequency more than 1% in any group are listed. The webappendix details all serious adverse events (pp 25–28). *p values are two-sided and were calculated by Fisher's exact test. †Unsolicited adverse events included any adverse events that happened from day 4 to day 30 after each dose and any adverse events within 3 days after each dose but had not been listed in the diary card for registering solicited adverse events. Most often unsolicited adverse events in the study included upper respiratory tract infection, headache, fever, and gastritis. ‡The data and safety monitoring board did not deem any of the serious adverse events to be related to vaccination. §22 participants died within 30 days after each vaccination. Of the ten participants in the vaccine group that died, eight died as the result of an accident, one died of a cerebral haemorrhage, and one died of liver cancer after 10 years with chronic hepatitis B. Of 12 participants in the placebo group that died, six died as the result of an accident, three died of myocardial infarction, two died of cerebral haemorrhage, and one died of stomach cancer.

Table 3: Safety outcomes

119 (2·1%) of 5598 participants in the placebo group showed an antibody response and all the episodes were subclinical infection.

Discussion

In our trial, efficacy of recombinant hepatitis E vaccine during the 12 months from the 31st day after the receipt of the third dose was 100·0% (95% CI 72·1–100·0), and protection was noted across all age and sex subgroups. Vaccination was also beneficial under less than perfect circumstances—ie, when participants did not receive all three doses. Vaccine efficacy after two doses was 100·0% (95% CI 9·1–100·0). Therefore, during a hepatitis E outbreak, or for travellers to an endemic area, protection can be quickly obtained by two vaccine doses given within 1 month.

Side-effects were few and mild and no serious adverse events related to vaccination. HEV 239 is unlikely to induce rare vaccine-related serious adverse events, because the large number of participants in the study affords a power of 85% to detect rare serious adverse events if the rate in the vaccine group is 0·03% and the rate ratio is 5·0 (webappendix p 29).

The study site is endemic for infection with hepatitis E virus, with nearly half the participants tested on day 0 being seropositive. The infection rate in the placebo group was 2·1% during the period from 0 months to 7 months. However, most of the infections seemed to be subclinical, and incidence of hepatitis E was estimated to be about four per 10 000 person-years (webappendix p 1). The reason for the low attack rate is unknown. In developed countries where the zoonotic hepatitis E genotype 3 predominates, emerging data showed a moderate hepatitis E virus seroprevalence but rare autochthonous cases of hepatitis E.[3] These findings suggest that the low attack rate might be a common feature of both zoonotic genotypes, relating to a low-level, but nevertheless widespread, exposure in areas where these viruses are prevalent.

Animal studies showed that HEV 239, which is produced with a genotype 1 isolate, confers protection against both genotypes 1 and 4.[22] 12 of 13 patients with hepatitis E who were typed by sequencing, had genotype 4, all in the placebo group. Therefore, our study substantiates that the vaccine cross protects against genotype 4 in human beings, and the cross protection probably extends to other genotypes as well, given that they belong to the same serotype as the vaccine strain.

Data suggest that individuals with chronic liver disease should be prioritised for hepatitis E vaccination to prevent serious damage from infection.[43] However, because we excluded this group, additional study is needed to assess the benefits of HEV 239. Another limitation was the lack

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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 2010年11月25日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	人C1-インアクチベーター		研究報告の公表状況	Widespread distribution of hepatitis E virus in plasma fractionation pools Baylis A., Koc O., Nübling C.M., Blümel J.: Paul-Ehrlich-Institut., Langen, Germany Vox Sanguinis 99 (Suppl.1): p320 JUL 2010	公表国 ドイツ	
販売名（企業名）	①ベリナートP ②ベリナートP静注用500 （CSLベーリング株式会社）					

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
背景：E型肝炎ウイルス（HEV）は、急性肝炎を引き起こす。HEV genotypes 1およびgenotypes 2は、人に限定され、発展途上国での流行と関連しているのに対して、genotypes 3およびgenotypes 4は人畜共通で、発展途上国と先進工業国の両方で人と他のいくつかの動物（特にブタ）を感染させる。 HEVは通常、汚染された水と食物を介して感染する。しかしながら、輸血は、フランス、イギリス、日本を含む数カ国で報告された症例による別の感染経路である。日本と中国の供血者の調査において、HEV RNAは、ALT値が上昇する場合もしない場合もほぼ107-108コピー/mL程度の高ウイルス量が異なる頻度で検出された。 目的：血漿分画プールのHEV RNAの存在、および、IgG抗HEV抗体の濃度を調べるため。 方法：血漿プールのサンプルは、ORF3を標的にして一回の反応につき10コピーのDNAを検出することができるRT-PCR法で、HEV RNAの分析がなされた。血漿サンプルは、COBAS AmpliPrep（Roche Diagnostics GmbH、ベンツベルク、ドイツ）とTotal Nucleic Acid Isolation kitを用いて抽出された。陽性対照は、HEV陽性ヒト血漿およびブタサンプルである。 HEV RNA陽性のプールは、ウエスタンブロッティングおよびELISAにより、IgG抗HEV抗体が調べられた。 結果：約10%のプールは、HEV RNA陽性だった。陽性プールの地理的分布は、ヨーロッパ、北アメリカ、東南アジアの数カ国を含む広範囲にわたっていた。プール中で同定された株の系統発生解析により、genotype4がアジアのプールに限定されていたのに対し、genotype3はヨーロッパと北アメリカのプールで確認されたことが証明された。 IgG抗HEV抗体とHEV RNAの間で相関関係はみられなかった。抗HEVの濃度は、全般にアジアのプールでより高かった。 結論：以前HEV RNAが日本と中国の供血者で確認されたのに対し、血漿分画プールにおけるHEV RNAの分析はこれまで報告されたことがない。血漿分画製剤のHEVのリスクを判定するために、更なるプールのHEV RNA量を測定する調査が現在行われている。	

報告企業の意見	今後の対応
血漿分画製剤によるHEV伝播は報告されていない。 仮にHEVが存在する場合にも、液状加熱（パスツリゼーション）による製造工程により低減することから、本剤のHEVに関する安全性は確保できると考える。	今後とも新しい感染症に関する情報収集に努める所存である。

②

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of a hepatitis E case in the vaccine group, meaning that the protective antibody concentration could not be assessed. Further analysis of our serology data might provide important information on the vaccine's efficacy against subclinical infection. Both our study and the previous phase 2 study of the vaccine produced in insect cells showed substantial short-term protection; however, the duration of this protection needs further assessment.

In our trial, we found the vaccine well tolerated and efficacious for a general adult population. Further studies are needed to assess the safety and to support the benefits of the vaccine for pregnant women and for people younger than 15 years or older than 65 years.

Contributors

All authors contributed towards acquisition of data or statistical analyses, or interpretation of data, writing and revising the report, and final approval. F-CZ and JZ contributed equally to this work.

Hecolin study group

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Conflicts of interest

Y-LX and Y-ML are employees of the Xiamen Innovax. The other authors declare that they have no conflicts of interest.

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別紙様式第2-1

No. 20

医薬品 研究報告 調査報告書

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研究報告の概要

○慢性疲労症候群(CFS)患者と健康な供血者の血液におけるマウス白血病ウイルス(MLV)関連ウイルス遺伝子配列の検出

CFSは原因不明の重篤な全身疾患である。最近、CFS患者の末梢血に高率に異種指向性マウス白血病ウイルス関連ウイルス(XMRV)由来DNAが検出されたという研究報告があったが、続く4件の研究報告は、それを証明できていない。

今回、CFS診断基準を満たす患者37名からの末梢血単核細胞由来DNA 41検体を調べ、37名中32名にMLV関連ウイルスgag遺伝子配列を認めたが、健常ボランティア供血者では44名中3名にしか認められなかった。PCR分析システムまたは臨床検体において、マウスDNA混入の証拠は検出されなかった。およそ15年後に得られた検体において、gag陽性患者8名中7名が再度陽性となった。全てのXMRVが遺伝的に近縁という報告知見に反して、遺伝的に多様なグループのMLV関連ウイルスを確認した。CFS患者に由来するgagおよびenv 配列は、XMRVよりも多指向性マウス内在性レトロウイルスに近縁であり、さらに、同種指向性のMLV配列とは近縁性が低かった。MLV関連ウイルスと同一の強い関連性があるかどうか、これらウイルスがCFS発症において原因的役割を担っているかどうか、ならびにこれらが血液供給の脅威となるかについては、さらなる研究が必要である。

使用上の注意記載状況・その他参考事項等

解凍赤血球濃厚液「日赤」
照射解凍赤血球濃厚液「日赤」
解凍赤血球-LR「日赤」
照射解凍赤血球-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見

慢性疲労症候群患者37名の末梢血単核細胞由来DNAを調べたところ、32名にマウス白血病ウイルス関連ウイルスgag遺伝子配列を認めたが、健常者ボランティアでは44名中3名にしか認められなかったとの報告である。

今後の対応

日本赤十字社では、献血の問診時に献血者の健康状態を把握・確認している。平成22年5月18日に開催された平成22年度第1回血液事業部会運営委員会において、XMRVとCFSとの関連について、現時点で緊急的な対応をとる必要はないものの、引き続き情報収集を行い、新たな知見等が得られれば、本委員会において対応を検討することとされた。今後も引き続き、情報の収集に努める。

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factor VIII injection, the presence of vCJD infectivity in the blood cannot be excluded. This has led to major concerns that a pool of infectious symptomless individuals could exist in the blood donor population leading to further cases of transfusion related person to person disease contamination. The estimated femtomolar sensitivity level required to detect PrP^{TSE} in the plasma of a donor in the pre-clinical phase of infection has been a caveat towards the development of blood screening assays.

Aim: The objective is to develop a sensitive and specific test that enables the confirmation of a PrP^{TSE} positive human blood sample.

Methods: The assay comprises three major steps: (i) selective capture of PrP molecules from human plasma by ligand-coated beads for PrP^{TSE} enrichment and removal of inhibitors present in plasma;(ii) in vitro amplification of PrP^{TSE} by Protein Misfolding Cyclic Amplification (PMCA), in which minute amounts of the captured PrP^{TSE} are amplified in a cyclic process using brain of humanised transgenic mice overexpressing PrP^{MET129} as substrate; (iii) detection by Western Blot of the amplified PrP^{TSE} after proteinase K digestion.

Optimisation of the assay was performed with human plasma samples spiked with serial 10-fold dilutions of 10% vCJD infected brain homogenate.

Results: After capture of PrP^{TSE} from spiked plasma and amplification by three rounds of PMCA, the sensitivity of PrP^{TSE} detection was improved by 5log in comparison with western blot detection only.

Donor	Time to symptoms	Highest temperature	Length of symptoms	Symptoms score	Influenza A (H1N1)PCR
1	4 hours	39.4	2 days	4	Negative
2	30 hours	37.6	4 days	13	Negative
3	12 hours	N.A.	2 days	9	Negative
4	30 hours	39.2	4 days	4	Negative
5	32 hours	39.2	4 days	4	Negative
6	45 hours	N.A.	4 days	3	Negative
7	1 hours	38.3	5 days	5	Negative
8	8 hours	N.A.	3 days	4	Negative
9	48 hours	N.A.	6 days	2	Negative
10	8 hours	38.8	2 days	4	Negative
11	38 hours	N.A.	2 days	4	Negative
12	1 hours	N.A.	3 days	4	Negative
13	27 hours	N.A.	2 days	5	Negative
14	24 hours	38.5	3 days	4	Negative
15	36 hours	40	3 days	6	Negative

Summary/conclusions: We have developed a test that combines a concentration step with the in vitro amplification of PrP^{TSE}, in order to reach the sensitivity required for the detection in blood. The future prospects are: (i) to perform epidemiological studies on at risk population samples from recipients who have received multiple RBC transfusions; (ii) to analyse for confirmation any repeatedly positive result that would be obtained after screening of blood donations by rapid tests.

P-0619

WIDESPREAD DISTRIBUTION OF HEPATITIS E VIRUS IN PLASMA FRACTIONATION POOLS

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Background: Hepatitis E virus (HEV) causes acute hepatitis. HEV genotypes 1 and 2 are restricted to humans and associated with epidemics in developing countries, whilst genotypes 3 and 4 are zoonotic and infect humans and several other animals, particularly swine, in both developing and industrialized countries. HEV is normally transmitted via contaminated water and food; however blood transfusion is an alternative route of infection with cases reported in several countries including France, the UK and Japan. In surveys of Japanese and Chinese blood donors, HEV RNA has been detected at varying frequency, both in the presence and absence of elevated levels of ALT, with the highest viral loads in the order of 10^7–10^8 copies/ml.

Aim: To investigate plasma fractionation pools for the presence of HEV RNA and to examine the levels of anti-HEV IgG.

Methods: Samples of plasma pools were analysed for HEV RNA using an RT-PCR method targeting ORF3 and capable of detecting 10 DNA copies per reaction. Plasma samples were extracted using the COBAS AmpliPrep (Roche Diagnostics GmbH, Penzberg, Germany) and the Total Nucleic Acid Isolation kit according to the manufacturer's instructions. Positive controls included HEV positive human plasma and swine samples. Pools positive for HEV RNA were examined for anti-HEV IgG by Western blotting and ELISA.

Results: Approximately 10% of pools were positive for HEV RNA. Positive pools were from a widespread geographic distribution, including several countries in Europe, North America and South East Asia. Phylogenetic analysis of the strains identified in the pools demonstrated that genotype 3 viruses were identified in Europe and North America whilst the genotype 4 viruses were restricted to the Asian pools. No correlation was observed between anti-HEV IgG and HEV RNA. Levels of anti-HEV levels were in general greater in the Asian pools.

Conclusions: Whilst HEV RNA has previously been identified in Japanese and Chinese blood donors, analysis of plasma fractionation pools for HEV RNA has not previously been reported. Further investigations are underway to determine the HEV RNA loads in pools in order to determine the risk of HEV in plasma derived products.

P-0620

HTLV LOOKBACK IN NHS BLOOD AND TRANSPLANT REVEALS THE EFFICACY OF LEUCODEPLETION

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Background: Leucodepletion was introduced within NHS Blood and Transplant (NHSBT) in 1998. HTLV screening of blood donations commenced in 2002. The NHSBT national computer database was introduced into blood centres in a phased fashion in the late 1990s. HTLV lookback was performed for previous donations from donors who tested HTLV positive after screening was introduced. Lookback was confined to donations included on the national database.

Aim: Lookback was performed to identify transfusion recipients who had been infected with HTLV through transfusion of cellular blood components from donors who were later identified as HTLV positive.

Methods: Lookback was performed according to established procedures, which were used for the HCV lookback in the 1990s. Previous donations were identified for all HTLV positive donors, and the fate of all cellular components was traced. Recipient hospitals were asked to trace the fate of the components, and to send details of living recipients to NHSBT. The clinician (generally the general practitioner) responsible for the care of the identified recipient was then contacted by the blood service, and notification of the recipient was carried out. Recipients were offered further advice and testing for evidence of HTLV infection. All details were recorded on a database which linked information about donors, donations, blood components and recipients.

Results: Lookback was performed on 413 cellular (red cell and platelet) components issued from 56 blood donors: 83% of components were traced at hospitals to an identified recipient and 182 (53%) of the recipients were known to be dead at the time of reporting. A total of 110 recipients (69% of the living recipients) were tested for HTLV: 80 had received leucodepleted and14 "buffy coat reduced" components, while 16 had received components without any white cell reduction measures. Only six recipients (5.5% of those tested) were positive for HTLV-1. One further recipient was HTLV-2 positive, but all donors were infected with HTLV-1 so this was not judged to be a transfusion-transmission. Overall, infection was present in 5/16 recipients who received non-leucodepleted components compared with 1/80 leucodepleted and 0/14 with buffy coat reduced components. The infected recipients associated with non-leucodepleted components received platelet pools (2) and non-leucodepleted red cells (3) transfused on day 6, 8

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Detection of MLV-related virus gene sequences in blood of patients with chronic fatigue syndrome and healthy blood donors

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Contributed by Harvey J. Alter, May 25, 2010 (sent for review March 23, 2010)

Chronic fatigue syndrome (CFS) is a serious systemic illness of unknown cause. A recent study identified DNA from a xenotropic murine leukemia virus-related virus (XMRV) in peripheral blood mononuclear cells (PBMCs) from 68 of 101 patients (67%) by nested PCR, as compared with 8 of 218 (3.7%) healthy controls. However, four subsequent reports failed to detect any murine leukemia virus (MLV)-related virus gene sequences in blood of CFS patients. We examined 41 PBMC-derived DNA samples from 37 patients meeting accepted diagnostic criteria for CFS and found MLV-like virus *gag* gene sequences in 32 of 37 (86.5%) compared with only 3 of 44 (6.8%) healthy volunteer blood donors. No evidence of mouse DNA contamination was detected in the PCR assay system or the clinical samples. Seven of 8 *gag*-positive patients tested again positive in a sample obtained nearly 15 y later. In contrast to the reported findings of near-genetic identity of all XMRVs, we identified a genetically diverse group of MLV-related viruses. The *gag* and *env* sequences from CFS patients were more closely related to those of polytropic mouse endogenous retroviruses than to those of XMRVs and were even less closely related to those of ecotropic MLVs. Further studies are needed to determine whether the same strong association with MLV-related viruses is found in other groups of patients with CFS, whether these viruses play a causative role in the development of CFS, and whether they represent a threat to the blood supply.

xenotropic murine leukemia virus-related virus | murine leukemia virus-like virus | viral gag gene sequence | polytropic | mouse mitochondria DNA PCR

Chronic fatigue syndrome (CFS) is a debilitating disorder defined solely by clinical symptoms (1) and the exclusion of other diseases; its distribution is wide and its cause is unknown. In many instances, the illness starts suddenly with an infectious-like syndrome. A number of objective immunological and neurological abnormalities have been found more often in patients with CFS than in healthy controls or in patients with other fatigue-inducing illnesses (2). Various microbial and viral infections have been implicated as possible triggers of CFS, including *human herpesvirus-6*, Epstein–Barr virus, *enteroviruses*, parvovirus B19, and the bacteria that cause Lyme disease and Q fever (2). However, no single agent has been associated with a large fraction of cases.

A recent study reported that a high percentage of patients with CFS are infected with a mouse leukemia retrovirus that has been designated xenotropic murine leukemia virus-related virus (XMRV) (3), a virus first identified in samples of human prostate cancer tissue about 4 y ago (4). However, two subsequent studies failed to find an infectious murine leukemia virus (MLV)-related virus in German prostate cancer patients (5, 6), and four recent studies from Europe and the United States have failed to detect XMRV or an MLV-related viral gene sequence in the blood of CFS patients using PCR (7–10).

In the mid-1990s, we obtained serum and whole-blood samples from CFS patients for the investigation of possible mycoplasmal infections (11). Whole-blood, peripheral blood mononuclear cell (PBMC), and plasma samples from 37 CFS patients in the mycoplasma studies were maintained in frozen storage at −80 °C. Twenty-five patients were from an academic medical center and 12 were referred by community physicians. Repeat blood samples were obtained from the academic medical center patients: *four samples were obtained 2 y later and similarly kept in frozen storage*, eight were obtained ~15 y later, in 2010, and processed for XMRV/MLV-related virus testing without being frozen.

By nested PCR assays targeting the MLV-related virus *gag* gene, using both the previously described primer sets (3, 4) and an in-house–designed primer set with highly conserved sequences from different MLV-like viruses and XMRVs, we examined DNA prepared from the blood samples of these 37 CFS patients for the presence of MLV-like virus *gag* gene sequences. In addition, RNA was prepared from the deep-frozen plasma samples of these patients and analyzed by RT–PCR assay. DNA extracted from frozen PBMC samples of 44 healthy volunteer blood donors was tested in parallel.

Results

MLV-Related Viral *gag* Gene Sequences Detected in the Blood of CFS Patients. By nested PCR assays, targeting the mouse retrovirus *gag* gene using either the previously reported PCR primer sets (first round: 419F/1154R; second round: GAG-I-F/GAG-I-R) (3, 4) or our in-house–designed PCR primer set (first round: 419F/1154R; second round: NP116/NP117) (Fig. 1), we detected a high frequency of MLV-related virus *gag* gene sequences in patients with CFS. The NP116/NP117 is an internal primer set with highly conserved sequences in different MLV-like viruses and XMRVs (Fig. S1). After the first round of nested PCR using primer set 419F/1154R, gel electrophoresis revealed positive PCR-amplified products with the predicted size of ~730 bp in 21 of 41 PBMC or whole-blood samples from 37 CFS patients (Fig. 1*A*). The nested PCR results produced by the second round of amplification using either the internal primer set GAG-I-F/GAG-I-R (with a predicted size of an ~410-bp product) or the internal primer set NP116/NP117 (with a predicted size of an ~380-bp product) were essentially identical. Overall, samples from 32 of 37 (86.5%) CFS patients revealed positive amplification products with the correct predicted sizes in the nested PCR (Fig. 1*B*). Of the 25 CFS patients who had been rigorously evaluated at the academic medical center, 24 (96%) were positive. On repeated testing 2 y later of four of the academic center patients, all four remained positive. On repeated testing of eight academic center patients ~15 y later (in 2010), seven remained positive. All PCR products with the correct predicted size were retrieved from the gel and analyzed by DNA sequencing. Their DNA sequences were all confirmed to be those of MLV-related virus *gag* genes. The alignments of all of the sequences obtained from PCR products of ~730 bp are shown in Fig. S1. All of the positive PCR products amplified from the CFS patients' samples using primer set 419F/1154R were 746 bp in length. All of the positive PCR products amplified from the patients' blood samples using primer set GAG-I-F/GAG-I-R and primer set NP116/NP117 were 413 and 380 bp in length, respectively.

In 42% of samples, we also detected and sequence-confirmed the presence of MLV-related viral RNA in the frozen plasma samples of these CFS patients, using an RT–PCR assay (Fig. 1*C*). With one exception, all of the patients who tested positive for viral RNA *gag* gene sequences in the plasma samples also tested positive in the DNA prepared from PBMCs and/or whole blood. On the other hand, only about half of the patients with MLV-related virus *gag* gene sequences detected in PBMC DNA also had viral *gag* RNA sequences detected in the plasma.

MLV-Related Viral *gag* Gene Sequences Detected in the Blood of Healthy Volunteer Blood Donors. DNA originating from 44 healthy volunteer blood donors was tested in parallel by nested PCR (Fig. 2). The nested PCR testing using the MLV-related virus *gag* gene-specific primer sets could produce many side products from human DNA (Figs. 1 and 2). We sequenced all of the PCR-amplified DNA bands from the 44 control samples of blood donors having molecular sizes close to that of the predicted PCR products from the target XMRV *gag* gene in the first round of PCR (Fig. 2*A*) and in the second round of nested PCR (Fig. 2*B*). After the first round of amplification in nested PCR, a positive PCR product with the predicted size of ~730 bp was found in PBMC DNA from 1 of 44 blood donor controls (*lane 4*, Fig. 2*A*). This ~730-bp PCR product amplified from the blood donor (BD22) was confirmed by DNA sequencing as an MLV-related virus *gag* gene sequence of 745 bp (Fig. S1). Overall, we found 3 of 44 (6.8%) blood donors' PBMCs (BD22, BD26, and BD28) to be positive for the MLV-related virus *gag* gene sequences by completing both rounds of nested PCR (Fig. 2*B* and Fig. S2).

MLV-Related Viral *env* Gene Sequences Detected in the Blood of a CFS Patient and a Healthy Blood Donor. PBMC DNA from all of the CFS patients and healthy blood donors was also tested by PCR, targeting various regions of the MLV-related viral *env* gene. The MLV-related viral *env* gene segment of 240 bp was amplified and confirmed by sequencing from one healthy donor (BD-26) by a semi-nested PCR using the primer set 5922F/6273R in the first round of amplification and 5922F /6173R in the second round of amplification. The MLV-related viral *env* gene segment of 206 bp was amplified and confirmed by sequencing from 1 CFS patient by a nested PCR using primer set 5922F/6273R in the first round of amplification and 5942F/6159R in the second round of amplification (*SI Materials and Methods*).

Phylogenetic Analyses of MLV-Related Virus *gag* and *env* Gene Sequences. Multiple sequence alignment (MSA) and phylogenetic analysis of the MLV-related virus *gag* gene sequences amplified from 21 CFS patient samples and one blood donor (BD22) are shown in Fig. S1 and Fig. 3*A*, respectively. There were three different MLV-related retroviral *gag* gene sequences identified by PCR in the blood samples of the CFS patients and a fourth variant was detected in blood donor BD22. The sequences in all four variants were more closely related to the sequences of polytropic mouse endogenous retroviruses (mERVs) than to those of XMRVs. Although variations were observed, the majority (18/21, 86%) of CFS patient samples had the same viral *gag* gene sequence (CFS type 1), whereas 2/21 had a different, but similar, viral *gag* gene sequence (CFS type 2), and a third distinct sequence (CFS type 3) was found in the remaining CFS case. Phylogenetic analysis using the 746-nt sequences obtained revealed that CFS type 1, CFS type 2, and CFS type 3 formed a cluster that is clearly separable from the cluster formed by the newly reported XMRVs (Fig. 3*A*). Interestingly, the 745-nt virus *gag* gene sequence of donor BD22 (with a 1-nt deletion in the alignment) could not be included in

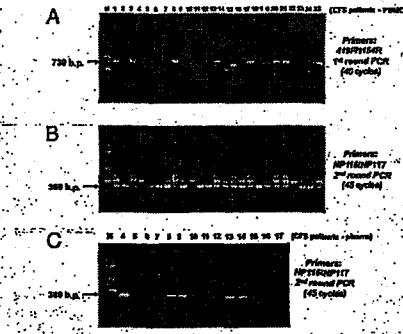

Fig. 1. MLV-related *gag* gene sequences detected in blood DNA from CFS patients. (*A*) Results of PBMC DNA from CFS patient samples 1–25 (of 41 samples examined) amplified after the first round of nested PCR using a previously published primer set (419F/1154R): targeting *gag* gene. (*B*) Results of PBMC DNA from the 25 CFS samples after completing the second round of nested PCR using an in-house–designed PCR primer set (NP116/NP117). (*C*) MLV-related *gag* gene RNA sequences are detected in plasma of CFS samples by RT-nested PCR. Results of RT-nested PCR for RNA derived from the plasma samples of CFS patients 4–17 are shown. The positions of expected sizes of the "positive" PCR amplicons are indicated by arrows. M, DNA ladder size marker. All positive PCR amplicons with the expected size have been confirmed by DNA sequencing.

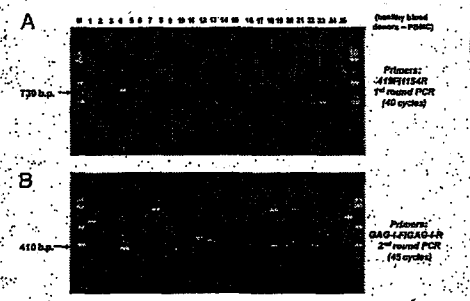

Fig. 2. MLV-related *gag* gene sequences detected in normal blood donors by nested PCR. (*A*) Results of PBMC DNA from blood donors 1–25 (of 44 donors examined) amplified after the first round of nested PCR using primer set 419F/1154R. Lane 4: PBMC DNA from BD22 has a positive target PCR amplicon confirmed by sequencing. (*B*) Results of PBMC DNA from the 25 normal blood donors after the second round of nested PCR using PCR primer set GAG-I-F/GAG-I-R (4). Sequencing of the PCR bands with size ~413 bp revealed that lane 4 (BD22), lane 7 (BD26), and lane 9 (BD28) were MLV-like virus *gag* gene sequences; lane 8 (BD27) was a human sequence. The positions of expected sizes of the positive PCR amplicons are indicated by arrows. M, DNA ladder size markers.

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The authors declare no conflict of interest.

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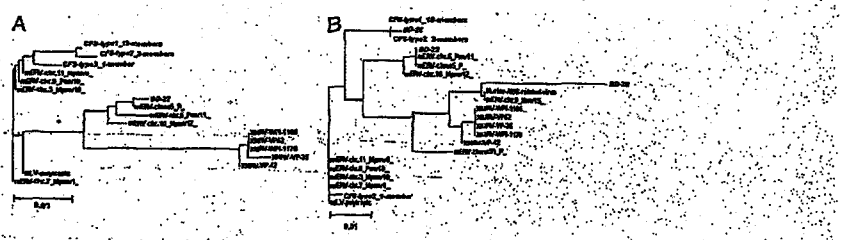

Fig. 3. Phylogenetic trees corresponding to the MSAs shown in Figs. S1 and S2 were generated by the ClustalW2 program using the neighbor-joining method (*Materials and Methods*). (*A*) Phylogenetic analysis based on the 746-nt *gag* gene nucleotide sequences amplified from blood samples of CFS patients and BD-22 of the corresponding MSA in Fig. S1. (*B*) Phylogenetic analysis based on the 380-nt *gag* gene sequences amplified from blood samples of CFS patients and healthy blood donors using the primer set NP116/NP117 of the corresponding MSA in Fig. S2.

either the cluster of CFS type 1/CFS type 2 or the cluster of XMRVs. The viral *gag* gene sequences of CFS type 3 and blood donor BD22 appear to be phylogenetically more closely related to polytropic mERVs or modified polytropic mERVs (Fig. 3*A*).

Sequence analysis of the shorter fragments of the viral *gag* gene amplified from blood of 36 out of 41 CFS patient samples and 3 out of 44 blood donor samples after the second round of nested PCR similarly confirms that there are significant variations among the MLV-like *gag* gene sequences. Fig. S2 shows sequence alignment of the 380-nt segments of viral *gag* genes amplified from three blood donors (BD22, BD26, and BD28), patients with CFS types 1–3, XMRVs, and other closely related mERVs/polytropic MLVs. As an example, the viral *gag* gene sequence identified in BD28, but not sequences of BD22 and BD26, has a prominent 21-nt deletion that is uniquely present in polytropic mERV clone 51 (Fig. S2). In phylogenetic analysis, the 380-nt segment of the *gag* gene sequence found in BD26 appears to be closely related to those of CFS types 1 and 2. However, similar to the analytic result with the 746-nt product, the 380-nt *gag* gene sequences of BD22 and BD28 again cannot be included in either the cluster of CFS type 1/CFS type 2 or the cluster of XMRVs (Fig. 3*B*). On the other hand, when the protein sequences coded by the *gag* gene sequences identified in the CFS patients and blood donors are compared with those of a wider range of exogenous and endogenous MLVs, they are most similar to those of polytropic MLVs and XMRVs (Fig. S3). A ClustalW Gag protein tree again reveals that MLV-like virus *gag* proteins have much more similarity to those of modified-polytropic and polytropic mERVs or to those of XMRVs, but are very different from those of exogenous ecotropic MLVs (Fig. 4).

The sequence alignment and the phylogenetic analysis of the MLV-related virus *env* gene sequences obtained from both the CFS patient and healthy blood donor revealed that they were also more closely related to those of polytropic or modified polytropic MLVs than to those of XMRVs (Fig. S4).

Testing for the Presence of Mouse DNA in Patient and Blood Donor Samples Positive for MLV-Like *gag* Gene Sequences. Mouse DNA contains endogenously many closely related proviruses of MLVs. Hence, contamination of the blood samples or reagents by mouse DNA could have produced falsely positive PCR results. Although we took great precautions to prevent potential contamination in the laboratory, and although multiple negative controls were always included in each assay, we took additional steps to confirm that no mouse DNA had contaminated the assays or the clinical samples prepared in this study. We estimated that there were about 200–1,800 mitochondrial DNA (mtDNA) copies per mammalian cell. A highly sensitive PCR assay targeting mouse-specific mtDNA was developed (*Materials and Methods*) to exclude any possible minute mouse DNA contamination in the assay system and in the clinical samples with positive amplified *gag* gene products.

The first round of the semi-nested PCR (40 cycles) used primer set mt15982F/mt16267R and could detect 10 fg of mouse DNA in the presence of 35 ng of human background DNA. By comparison, when studied in parallel under the same assay conditions, the first round of nested PCR (40 cycles) against the MLV *gag* gene required ~10 pg of mouse DNA to detect viral gag gene sequences (Fig. S4). Thus, the first round of mouse-specific mtDNA PCR assay could detect a positive mtDNA signal at a mouse DNA concentration 1,000-fold below the concentration required to detect a positive *gag* signal. The second round of mouse mtDNA semi-nested PCR, using primer set mt16115F/mt16267R, could consistently amplify the target 153-bp amplicon from 2.5 fg of mouse DNA mixed with 35 ng of human DNA (Fig. 5*B*). By comparison, the second round of the nested *gag* gene PCR produced positive ~400-bp amplicons from 500 fg of mouse DNA mixed with 35 ng of human background DNA in the reaction. Thus, the second round of mouse mtDNA semi-nested PCR had a sensitivity hundreds-fold higher than that of the second round of the MLV *gag* gene nested PCR in amplifying mouse DNA.

Using this highly sensitive PCR assay for mouse-specific mtDNA, we examined all of the blood samples that were found positive for MLV-like virus *gag* gene sequences from both CFS patients and healthy controls for evidence of mouse DNA contamination. PBMC DNA (30–40 ng) from the CFS patients

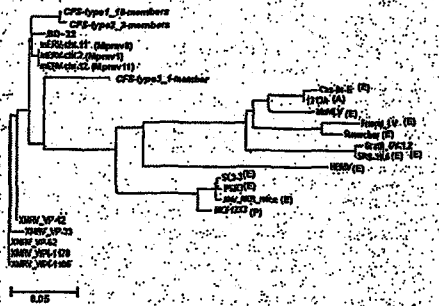

Fig. 4. Phylogenetic analysis of protein sequences based on the alignment shown in Fig. S3. CFS types 1, 2, and 3 and BD-22 and MLVs *gag* protein sequences are compared. Gag protein sequences starting from the AUG initiation codon are aligned with those of relevant endogenous as well as exogenous MLVs. Sequences of MLVs are referred to as polytropic (P), ecotropic (E), amphotropic (A), or modified polytropic (Mpmv). MoMLV, Moloney murine leukemia virus; HEMV, hortulanus endogenous murine virus.

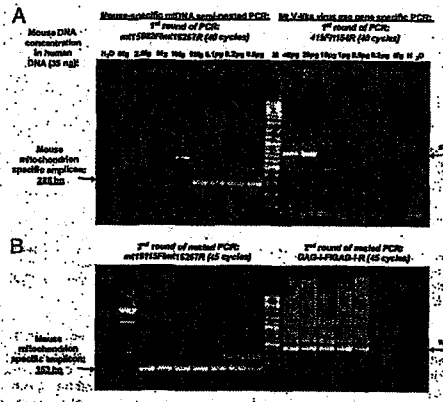

Fig. 5. Comparison of sensitivity in amplifying mouse DNA by the semi-nested PCR targeting mouse-specific mtDNA and by the nested PCR targeting MLV-like virus *gag* gene. Serial dilutions of mouse spleen DNA (from 40 pg to 2.5 fg) were spiked into 35 ng of total human PBMC DNA and compared in parallel for the mouse DNA detection sensitivity of the two PCR assays. (*A*) In the first round of the mtDNA-specific PCR assay, 10 fg or more of mouse DNA could be detected in the presence of 35 ng of human DNA by producing the 286-bp target product. In the first round of MLV *gag* gene nested PCR assay, 10 pg or more of mouse DNA could be detected in the presence of 35 ng of human DNA by producing the ~730-bp target product. (*B*) In the second round of mouse-specific mtDNA semi-nested PCR, the 153-bp target amplicon could consistently be amplified from 2.5 fg of mouse DNA. In the second round of *gag* gene-specific nested PCR, the 413-bp target product could be amplified from 0.5 pg or more of mouse DNA. Lane 0 fg: 35 ng of human DNA without spiking any mouse DNA. Lane H_2O: No DNA template. M: 100-bp DNA ladder mix. Primers and PCR cycle numbers used in each round of amplification for both of the assays are shown at the top of each gel.

and the healthy blood donors, as well as serial dilutions from 50 to 1 fg of mouse DNA mixed with 35 ng of human DNA as the positive templates, were tested in parallel. No mouse DNA was found in the PCR mix nor in the blood samples of CFS patients and blood donors that tested positive for the MLV-like virus *gag* gene sequences. Fig. 6 shows the results of the two rounds of mouse-specific mtDNA semi-nested PCR testing in DNA from PBMCs of four CFS patients (patients 8, 17, 20, and 25) with positive 746-bp amplicons in the first round of the nested PCR targeting the MLV-like virus *gag* gene, as well as from three blood donors (BD22, BD26, and BD28) who tested positive and two donors (BD21 and BD23) who tested negative for MLV-like *gag* gene sequences.

Discussion

Detection of MLV-Related Nucleic Acid Sequences. Our laboratory detected MLV-related virus *gag* gene sequences in DNA from PBMC and whole-blood samples from 32 of 37 (86.5%) CFS patients, compared with 3 of 44 (6.8%) volunteer blood donors, using a two-round nested PCR. Following only one round of PCR amplification, 21 of the 41 CFS patients' DNA samples were found positive compared with only 1 of 44 donor samples. In every instance throughout these studies, the "positive" result by PCR (an amplicon of the predicted size) was confirmed by sequencing.

In four CFS patients from whom two samples were obtained, 2 y apart, the *gag* gene sequences were detected on both occasions. Further, *gag* gene sequences were still detectable in seven of eight CFS patients from whom fresh samples were obtained ~15 y after they were initially found to be MLV *gag* gene positive. In one *gag*-positive CFS patient and one *gag*-positive blood donor, MLV-related *env* gene sequences also were detected by PCR. However, we were unable to PCR amplify and determine the MLV-related *env* gene sequences in the majority CFS patients, possibly because of the low copy number and the greater genetic variability in the *env* gene compared with the *gag* gene.

In the CFS patients, plasma samples revealed MLV-related virus *gag* gene sequences in 42% when tested by RT–PCR for viral RNA. Whereas all but one patient whose plasma tested positive for viral RNA also tested positive in PBMCs for viral DNA, only half of the cases in which MLV-related virus *gag* gene sequences were detected in PBMCs had detectable RNA sequences in plasma. Thus, accurate determination of the prevalence of these agents in patients and donors requires cellular DNA for analysis.

Sequence Variability. Previous reports of XMRV isolates from patients with CFS and with prostate cancer and from individuals in different geographic locations have described very similar nucleic acid sequences (3, 4, 12), a feature believed to be a unique characteristic of XMRVs (13). However, our analysis revealed three different types of MLV-related virus *gag* gene sequences in CFS patients. In all three groups, the sequences were more closely related to the sequences of polytropic mERVs than to XMRVs and were more distant from the sequences of ecotropic MLVs (Fig. 3). Moreover, viral *gag* gene sequences with significant variations from both the cluster of CFS type 1/CFS type 2 and the cluster of XMRVs were identified in at least two blood donors (BD22 and BD28); phylogenetic analysis revealed the latter sequences to be more closely related to those of polytropic or xenotropic mERVs (Fig. 3*B*). It is unclear whether the sequence variations of the viral genes identified in the CFS patients and healthy blood donors have any significance in viral pathogenesis or disease development.

The MLV-like virus *gag* gene sequences in the CFS patients and blood donors had a deletion of 9 nt in the 5′ *gag* leader region and did not have the 24-nt deletion in this region reported in XMRVs. Internal deletions of 9 nt similar to what we have identified in the CFS patients and the blood donors are known to be present in the region that encodes the glycosylated Gag protein (GlycoGag) in some infectious endogenous (ecotropic) MLVs and exogenous (xenotropic) MLVs, such as AKV and DG-75 (14, 15). Many previous studies have shown that the nonstructural GlycoGag of MLVs plays a critical role in viral pathogenesis and in vivo infectivity (16–19). In this context, the MLV-like virus *gag* gene sequences identified in most of our CFS patients (both CFS type 1 and CFS type 2) appear to have an intact GlycoGag in-frame with the matrix and are consistent with the gene sequences of infectious MLVs. Unfortunately, the sequences presently obtained in the study are still a bit short and lack the alternative start codon CUG. Slight extension of the 5′ leader sequence will be needed to confirm the intact nature of GlycoGag.

Could Our PCR Results Have Been Falsely Positive? Voisset and coauthors (20) recently reviewed the pitfalls encountered in the identification of new retroviruses ("rumor viruses"). False-positive results can occur for a variety of reasons. Viral gene sequence-specific PCR primers can nonspecifically amplify nucleic acid sequences that differ from the target sequence. For this reason, we sequenced every positive PCR product (every amplicon of the predicted size) and confirmed MLV-related gene sequences in every instance.

Although contamination must always be a concern for any PCR-based study, several pieces of evidence argue against the

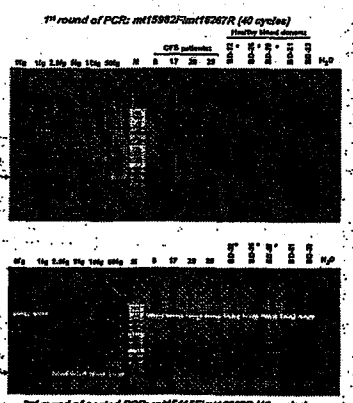

Fig. 6. Testing of CFS patients' and healthy blood donors' samples positive for MLV-like *gag* gene sequences for the presence of mouse DNA contamination using the semi-nested PCR assay targeting *mouse-specific mtDNA*. Serial dilutions of mouse DNA were spiked into 35 ng of human DNA and used as the controls of the assay sensitivity. The first round of mouse mtDNA semi-nested PCR (*A*) detected 10 fg of mouse DNA, and the second round of the semi-nested PCR (*B*) detected 2.5 fg of mouse DNA in the presence of 35 ng human background DNA. No evidence of mouse DNA contamination could be found by either round of mouse mtDNA semi-nested PCR in the PBMC DNA (35 ng) of CFS patients (patients 8, 17, 20, and 25); three blood donors (BD-22, BD-26, BD-28) tested positive and two blood donors (BD-21 and BD-23) tested negative for the MLV-like virus *gag* gene sequences. Healthy blood donors' samples positive for MLV-like *gag* gene sequences are labeled by asterisks. Lane 0: 35 ng of human DNA without spiking any mouse DNA. Lane H_2O: No DNA template. M: 100-bp DNA ladder mix.

possibility that the PCR products identified in our study are the result of laboratory contamination. First, every clinical sample that tested positive for the MLV-like virus *gag* gene sequences was tested for evidence of mouse DNA contamination using a semi-nested PCR for mouse-specific mtDNA that was exponentially more sensitive in detecting mouse mtDNA than MLV-related *gag* sequences (Fig. 5). Any detection of MLV *gag* that was caused by contamination with mouse DNA also would have detected mouse mtDNA by PCR, thereby identifying the *gag* result as falsely positive. In fact, no positive signal was detected by the mtDNA semi-nested PCR assay in any of the reaction mixtures or in the DNA of clinical samples examined in the study, thus excluding possible contamination by mouse DNA.

Second, we addressed the possibility that the clinical samples or the assay system might have been contaminated. The blood samples were obtained in clinical laboratories that never worked with mice or retroviral vectors and were drawn through sterile needles into vacuum tubes that remained unopened until testing. The laboratory in which PCR testing was performed also had never worked with murine cells, tissues or serum samples, or MLV vectors. Finally, because repeated entry into samples would increase the chance of contamination, we emphasize that sample vials from both patients and normal donors had never been entered before our testing.

Third, there were at least six different MLV-related *gag* gene sequences amplified from the blood samples of CFS patients and blood donors. Typically, contamination would be manifest as the same sequence in all or most samples. Moreover, the sequences that we observed all had significant variations from the previously reported exogenous MLVs or viral vectors. Furthermore, during this study more than 300 negative controls set up for multiple PCR amplification assays targeting the MLV *gag* gene were performed, and all were negative.

Finally, a new set of blood samples was recently obtained from 8 of the original 25 patients followed in an academic medical center. Testing of the repeat blood samples, ~15 y after the index sample, showed that seven of eight patients examined had detectable MLV-*gag* gene sequences. Significant variations of MLV-like virus *gag* gene sequences amplified from the freshly obtained blood samples were identified as would be expected in retroviral infections, but not from contamination.

The ultimate proof of low-grade infection by MLV-related viruses in humans may rely on demonstrating the integration of the viral genes into the human genome (20). The identification of provirus integration sites will take more time and effort to investigate, given that we estimate only one virus gene copy in every 400–4,000 nucleated PBMCs. Also, previous work with XMRV indicates that integration sites are quite variable (21) and the same may be true for the polytropic mouse endogenous retroviruses, which are predominant in this study.

Why Have Other Studies Come to Different Conclusions? Although we find evidence of a broader group of MLV-related viruses, rather than just XMRV, in patients with CFS and healthy blood donors, our results clearly support the central argument by Lombardi et al. (3) that MLV-related viruses are associated with CFS and are present in some blood donors. However, four recent studies have failed to confirm the PCR results reported by Lombardi (7–10). There are various possible explanations for this disparity. As stated in the reports, there could be a difference in the prevalence of these infectious agents in CFS patient populations in different geographic areas. This argument is somewhat less plausible since the publication of a recent negative study with subjects from the United States (10). Nevertheless, the heterogeneity in *gag* gene sequences that we observed suggests that geographic differences in different MLV-related viruses may be considerable and could affect both the sensitivity and the specificity of molecular amplification using standard primer sets.

Indeed, it is possible that the PCR primers used in various studies may have different sensitivity in detecting the diverse group of MLV-related virus *gag* gene sequences that we found in the clinical samples. The 5′ *gag* leader sequence of previously described XMRVs represents the most divergent segment of the XMRV genome in comparison with the genomes of the other MLVs (4). In particular, there is evidently a unique 15-nt deletion in the 5′ *gag* leader region in all of the XMRVs previously identified in patients with prostate cancer and CFS (3, 4). To detect XMRVs in human samples with better sensitivity and specificity, some studies used a PCR primer spanning this unique deletion as the "XMRV-specific" primer (6). However, none of the viral *gag* gene sequences amplified from the blood samples of CFS patients and blood donors in our study has this particular deletion (Fig. S1). As a consequence, such primers might have been insensitive in detecting the MLV-related *gag* gene sequences that we have identified.

Finally, it is also quite possible that there is heterogeneity in the patients diagnosed with CFS in different studies. CFS is a syndrome defined exclusively by a group of noinspecific symptoms and thus has an ill-defined phenotype. Future studies should adhere to consensus case definitions such as that developed by the Centers for Disease Control and Prevention (CDC) (1). Conversely, putative "healthy" control subjects should explicitly deny the presence of those symptoms that constitute the case definition of CFS. Furthermore, even bona fide cases of CFS may have different viral or other etiologies.

Further Considerations. The finding of XMRV or MLV sequences in persons with CFS or other diseases does not constitute definitive proof of viral infection. However, in the study of Lombardi et al. (3) and studies reviewed subsequently by Silverman et al. (22) the evidence for XMRV infection in humans not only involved detection of viral nucleic acids using PCR, but also reported the detection of viral antigens, detection of anti-viral antibodies, the ability to culture the virus in a prostate cancer cell line, the detection of gamma retrovirus particles by electron microscopy, and transmission of infection to macaques. In sum, none of the four studies that have failed to confirm the PCR evidence reported by Lombardi et al. (3), nor our own study, has attempted to fully replicate that study.

It remains to be shown that the association that we have found, using the methods that we have described, can be generalized to a larger group of patients with CFS. Indeed, we suspect that the association will be lower in CFS cases identified through community-based surveys, as contrasted to cases seen at academic medical centers. Even if subsequent studies confirm an association between MLV-like viruses and CFS, that will not establish a causal role for these viruses in the pathogenesis of this illness. For example, such a high frequency of infections with MLV-related viruses in patients with CFS could reflect an increased susceptibility to viral infections due to an underlying CFS-related immune dysfunction, rather than a primary role for these viruses in the pathogenesis of CFS.

Finally, the finding of MLV-related virus *gag* gene sequences in nearly 7% of healthy volunteer blood donors in our study and of XMRV in 3.7% of healthy controls in the study of Lombardi et al. (3) raises additional issues. The possibility that these agents might be blood-transmitted and pathogenic in blood recipients warrants extensive research investigations of appropriately linked donor–recipient cohorts.

Materials and Methods

Samples from CFS Patients and Healthy Controls. Initially, we tested 41 whole-blood samples that had been obtained for culture isolation of mycoplasmal agents in the mid-1990s. We maintained whole-blood, PBMC, and plasma samples from CFS patients in frozen storage at −80 °C. Of the 41 patient samples, 29 were collected from 25 patients by one of us (A.L.K.) at the Chronic Fatigue Research Center, Brigham and Women's Hospital (Boston, MA). Four of the CFS patients also had blood obtained on a second occasion, ~2 y later. Most of the patients were from the New England area; none were related, and virtually none had any regular social contact. Each of the 25 patients was systematically evaluated with a standardized history (supplemented by a patient questionnaire), physical examination, and battery of laboratory tests. Each met the 1988 CDC criteria for CFS, and 21 also met the 1994 CDC criteria. The average age of the patients at the time of venepuncture was 44.4 y; 4 were male and 21 were female. All of the patients signed informed consent documents approved by the Institutional Review Board of Brigham and Women's Hospital. A new set of blood samples was obtained in 2010 from 8 of the original 25 patients followed in the academic medical center. The blood samples were processed for PCR study without first being frozen. The other 12 samples from CFS patients were sent by individual clinicians taking care of patients in the mid-1990s who were given the diagnosis of CFS. We do not have details regarding the methodology by which the referring clinicians established the diagnosis of CFS. The samples had also been sent in the mid-1990s and stored at −80 °C. Frozen PBMC samples from 44 normal blood donors from the Washington, DC, area were collected in 2003–2006 and stored at the Department of Transfusion Medicine, Clinical Center, National Institutes of Health. All patient and control samples were coded and tested in parallel. Details of the preparation of blood samples and DNA/RNA isolation are described in *SI Materials and Methods*.

XMRV/MLV *gag* Nested PCR. The nested PCR for the *gag* gene was performed according to the protocols described previously (3, 4) with minor modifications. Three primer sets used in the study are as follows: 419F and 1154R (3), GAG-I-F and GAG-I-R (4), NP116 forward, and NP117 reverse. The NP116/NP117 was an in-house–designed primer set based on the highly conserved sequences found in different MLV-like viruses and XMRVs (Fig. S1). Primer sequences and details of PCR sensitivity and specificity quality controls can be found in *SI Materials and Methods*.

Phylogenetic Analysis. To generate the neighbor-joining phylogenetic tree, the viral *gag* gene sequences obtained from blood samples of patients with CFS, normal blood donors, as well as all of the closely related MLV *gag* gene sequences selected from the National Center for Biotechnology Information (NCBI) database by BLAST querying with the *gag* gene sequences obtained in the PCR study (*SI Materials and Methods*) were aligned with ClustalW2 (http://www.ebi.ac.uk/tools/clustalw2) using default settings. The analysis produced the same phylogenetic trees with or without consideration of the sequence gaps in alignment.

Mouse Mitochondrial DNA Assay. The complete mtDNA sequences of humans and mice were downloaded from GenBank and aligned using ClustalW. Sequence alignment revealed the 439 bp of the 3′ end of mouse mtDNA (beyond 15,862 bp, according to the coordinates of BALB/c mouse; accession no. AJ512208) were not present in human mtDNA. Primer sets were designed for a semi-nested, mouse-specific mtDNA PCR based on the sequence in this region of mouse mtDNA using Primer-Blast from NCBI. The external PCR primers (*SI Materials and Methods*) were designated as mt15982F and mt16267R, which would produce a predicted amplicon of 286 bp. The internal primers of the semi-nested PCR were designated as mt16115F and mt16267R, which would produce a predicted amplicon of 153 bp from mouse mtDNA. PCR system and setup were the same as for the *gag* gene-nested PCR study. However, PCR conditions were slightly different: 4 min at 94 °C; (30 s at 94 °C, 30 s at 55 °C, 1 min at 72 °C) × 40–45 cycles; 10 min at 72 °C.

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ABC NEWSLETTER

CURRENT EVENTS AND TRENDS IN BLOOD SERVICES

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2010 #31 August 27, 2010

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Study Confirms Link Between MLV-Related Viruses and CFS

A federal research team has found a strong association between chronic fatigue syndrome (CFS) and a diverse group of murine leukemia virus (MLV)-related viruses, according to a study published online Monday by *Proceedings of the National Academy of Sciences (PNAS)*. The findings corroborate an earlier study and could have significant implications for blood centers, because the team detected MLV-like viral sequences in 6.8 percent of 44 healthy blood donors tested.

The research was carried out by Shyh-Ching Lo, MD, PhD, of the Food and Drug Administration; Harvey Alter, MD, of the National Institutes of Health Clinical Center; and five colleagues at FDA and Harvard Medical School. The results have been eagerly anticipated, because they confirm the results of a study published in *Science* in October but conflict with a number of more recent studies (see related story, below).

In the *Science* study, the research team found that the blood of 68 of 101 patients with CFS (67 percent) contained xenotropic murine leukemia virus-related virus (XMRV), a novel gamma retrovirus. It found the same virus in the blood of eight of 218 healthy people (3.7 percent). XMRV had been previously linked to prostate cancer, but not to CFS (see *ABC Newsletter*, 1/8/10). However, a number of follow-up studies in the US and around the world – the most recent was published online in *Retrovirology* on July 1 – have not been able to confirm those results. XMRV is a type of MLV-related virus.

(continued on page 3)

Conflicting Federal Study Sparked Scrutiny, Delay

Monday's release by *Proceedings of the National Academy of Sciences (PNAS)* of a study that found a correlation between chronic fatigue syndrome (CFS) and murine leukemia virus (MLV)-related viruses ends a months-long delay and a great deal of anticipation in the CFS community.

The study, led by Harvey Alter, MD, and Shyh-Ching Lo, MD, PhD, was accepted for publication in *PNAS* in May, but its publication was delayed when a team of scientists at the Centers for Disease Control and Prevention (CDC) completed a study in which they did not find XMRV in the blood of people with CFS. That team was led by William Switzer; its results were published in *Retrovirology* on July 1 (see *ABC Newsletter*, 7/2/10).

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別紙様式第2-1
番号8

医薬品
医薬部外品　研究報告　調査報告書
化粧品

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研究報告の概要

献血者からCSF患者を永久に延期（禁止）した英国
11月1日以後、慢性疲労症候群/筋痛性脳脊髄炎（CFS/ME）に罹ったことがある人の献血を永久に延期した。この決定は、2つの米国の基礎研究による調査結果に基づくものである。連邦当局の一人が、遺伝子的にマウス白血病ウイルスに類似したウイルス（xenotropic murine leukemia virus-related virus：XMRV）とCFSとの関連を裏付けた。
英国保健省の保健保護の責任者 Clara Swinson からの手紙によると、「この変更は、CFS/MEが再発状態の時、ドナーの安全を理由に行われた。それは、原因不明の他の再発状況、または神経病学的状況と結びついたCFS/MEの診療へ至る。」とするもの。
Swinson 氏によると、ドナー選択基準の変更は、（CFS/ME 専門諮問委員会と同様に）ドナーの維持と選択において、英国 BSSAC（Blood Services Standing Advisory Committee）による勧告に従った。これまで、保健省のカスタマーサービスセンターの Mary Heaton によると、CFS罹患者の体調が優れない期間のみ、献血を延期していた。

報告企業の意見	今後の対応
マウス白血病ウイルス（murine leukemia virus：MLV）は、レトロウイルス科ガンマレトロウイルスに属し、ビリオンは球形で直径約100nmの脂質エンベロープを有する比較的大きなRNAウイルスである。万一、MLVが原料血漿に混入したとしても、HIV-1をモデルウイルスとしたウイルスバリデーション試験結果から、本剤の製造工程において十分に不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

代表としてテタノブリンIH静注250単位の記載を示す。
2. 重要な基本的注意
(1)本剤の原材料となる血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体陰性であることを確認している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した高力価の破傷風抗毒素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破傷風人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及びウイルス除去膜によるろ過処理を施しているが、投与に際しては、次の点に十分注意すること。

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抗破傷風人免疫グロブリン

OUR SPACE

By ABC CEO Jim MacPherson

Scary Stuff

Earlier this month America's Blood Centers' summer meeting in Chicago provided a reality check to ABC member executives – as if they needed one – about the freight train headed their way. An executive forum was held that confirmed the huge challenges ahead and the opportunities they present.

The blood community has talked for 15 years about the pressures hospitals are under to cut costs. Yet the reality of Congress' recent healthcare reform legislation is extreme pressure – starting now – on hospitals to cut costs while they are improving patient outcomes, satisfaction, and quality measures. In exchange for millions of new "paying customers" (patients who are forced to buy insurance or who are otherwise covered under new federal programs), hospitals agreed to accept more than $150 billion in Medicare cuts over 10 years (Medicare pays for more than 55 percent of healthcare costs). Medicare only pays 88 cents for every dollar spent on covered patients, and hospitals already struggle to subsidize that loss. But should they agree that Medicare reimbursement is the new benchmark to hit?

Moreover, starting in 2012 Medicare will provide incentives for hospitals to integrate with physician groups and other providers to cut costs and improve quality. As noted in this column before, such accountable care organizations (ACOs) are poorly defined, but we do know that the targets for the most intense pressure will be big states like Florida and Texas, which have the highest costs per Medicare recipient and the lowest quality of care. Does this mean that states like Wisconsin and Minnesota, which have low cost and high quality, are to be emulated? And where do blood centers fit in?

While blood centers account for less than 1 percent of hospital costs, three factors make blood centers "low hanging fruit" for efforts to reduce costs: the new perception of blood as a commodity, blood being the number one budget item for hospital labs, "bloodless" advocates saying blood is being overused. At the same time, many blood providers are gearing up for a price war to keep or regain hospital marketshare.

In many ways today's scenario is reminiscent of the late 1990s, but many elements are new and blood centers are feeling their way on a day-to-day basis. Uncertainty is always scary – and a great teacher.

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ABC is an association of not-for-profit, independent community blood centers that helps its members provide excellence in transfusion medicine and related health services. ABC provides leadership in donor advocacy, education, national policy, quality, and safety; and in finding efficiencies for the benefit of donors, patients, and healthcare facilities by encouraging collaboration among blood organizations and by acting as a forum for sharing information and best practices.

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Study Links CFS and MLVs (continued from page 1)

Methodology. Dr. Lo, Dr. Alter, and their colleagues tested serum and whole-blood samples collected from 37 CFS patients in the mid-1990s. They also tested repeat blood samples collected two years later from four of the same patients, as well as eight repeat samples collected in 2010. As a control, they tested blood samples from 44 healthy volunteer blood donors.

The scientists used nested polymerase chain reaction (PCR) assays to determine whether MLV-like virus *gag* gene sequences were present in DNA from the samples. They also used reverse transcription (RT)-PCR assays to analyze the RNA from the patients' plasma samples. They confirmed positive results by sequencing.

> **Findings. The scientists found MLV-like virus *gag* gene sequences in blood samples from 32 of the 37 CFS patients (86.5 percent). They found MLVs in 3 of the 44 (6.8 percent) healthy blood donors. In addition, they found that seven of the eight patients who were tested both in the mid-1990s and in 2010 were still positive in 2010.**

Dr. Lo, Dr. Alter, and their colleagues also considered whether their results could have been due to contamination, but they found no evidence that mouse DNA had contaminated the PCR assay system or the clinical samples. Furthermore, the MLV-related viruses in these samples were genetically diverse, whereas contamination would have led to the same sequence in most or all of the samples.

In their discussion section, the authors pointed out that previous studies had found very similar nucleic acid sequences in the XMRV in the blood of CFS patients. In contrast, this research team found three different types of MLV-related virus *gag* gene sequences. In a conference call with reporters, Dr. Lo said the variability found in this study is consistent with typical MLV behavior. He also said that the researchers involved in the *Science* study are currently retesting their samples and finding more variability than they had originally discovered.

In their *PNAS* article, the researchers acknowledge that "the finding of XMRV or MLV sequences in persons with CFS or other diseases does not constitute definitive proof of viral infection," and they point out that "demonstrating the integration of the viral genes into the human genome" may be necessary to show viral infection. They also point out that their study reached a different conclusion about the

(continued on page 4)

UK to Permanently Defer CFS Patients from Donating Blood

As of Nov. 1, blood donors in the UK who report that they have had chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) will be permanently deferred from giving blood. The decision appears to be based on findings by two US-based studies, one of them federal, that corroborate a link between xenotropic murine leukemia virus-related virus (XMRV) and CFS (see stories, page 1). According to a letter to colleagues from Clara Swinson, director of Health Protection for the UK Department of Health, "This change is being made on the grounds of donor safety, as CFS/ME is a relapsing condition. It brings practice for CFS/ME into line with other relapsing conditions or neurological conditions of unknown origin." According to Ms. Swinson, the change to donor selection criteria was made following a recommendation by the UK Blood Services Standing Advisory Committee on the Care and Selection of Donors, as well as its Joint Professional Advisory Committee. Heretofore, the Department of Health had deferred only people with CFS while they felt unwell, according to Mary Heaton of the Department of Health's Customer Service Center. (Source: E-mail correspondence provided by K. Kimberly McCleary, president and CEO, The CFIDS Association of America)

Study Links CFS and MLVs (continued from page 3)

possible association between XMRV, MLVs, and CFS than a number of other studies. They posit that these varied results may be caused by geographic differences in MLV-related viruses, different sensitivities in the PCR primers used in various studies, or heterogeneity in patients with CFS.

The authors also call for further studies to determine whether their results can be generalized to other patients with CFS. They conclude by emphasizing that none of the published studies have demonstrated a causal role for MLVs in CFS, and that more research will be necessary to determine whether MLV-related viruses or XMRV "might be blood-transmitted and pathogenic in blood recipients."

In an accompanying editorial, Valerie Courgnaud, PhD, and three colleagues from the University of Montpellier in France and the University of Alberta in Canada warned that the discovery of XMRV and MLVs among blood donors could suggest "a more widespread source of infection." They also point out that there may be "a variety of xenotropic and polytropic MLVs in North America," both in people with CFS and in healthy people. They also point out that "it is likely that more than one environmental agent impacts on the development of both CFS and prostate cancer." If so, that could explain why some people have MLVs but do not develop CFS or prostate cancer.

Citations. Lo SC, *et al.* Detection of MLV-related virus gene sequences in blood of patients with chronic fatigue syndrome and healthy blood donors. PNAS. 2010 Aug. 23 (E-pub ahead of print). Courgnaud V, *et al.* Commentary: Mouse retroviruses and chronic fatigue syndrome: Does X (or P) mark the spot? PNAS. 2010 Aug. 23 (E-pub ahead of print). ♦

PNAS Publishes Its Study (continued from page 1)

FDA announced the release of the *PNAS* study in a press release on Monday, and NIH hosted a telebriefing during which Dr. Alter, Dr. Lo, and officials from FDA and CDC discussed the study with reporters. *PNAS* also published two editorials with the study results.

In an editorial in *PNAS* and in an interview with *The Scientist*, *PNAS* Editor-in-Chief Randy Schekman, PhD, said the request to hold the study had come from the authors, who wanted to review their findings in light of the study in *Retrovirology*. Although the article had already been through peer review with *PNAS*, Dr. Schekman solicited another review from "an established person in the field," who recommended that the paper not be published until the researchers "could demonstrate that the virus' genes were integrated into the human genome."

According to Dr. Schekman, Dr. Alter agreed that evidence of this process would be "the highest standard that would prove the case." However, as Dr. Alter told a *Wall Street Journal* health blogger, it would take months to collect the data that shows this integration. Dr. Schekman reported that Dr. Alter was concerned that delaying the publication for that long "would be a disservice to the CFS community." (The members of that community – particularly people with CFS – are eager for any information that sheds light on the disease, as its causes and treatments are still unknown.)

Dr. Alter told the *Wall Street Journal* that the research team instead did additional work to make sure their findings were not caused by laboratory contamination, rather than the viruses. He said that extra work strengthened their findings. Finally, the researchers concluded, and another retrovirus expert agreed, that the data were strong enough to publish the study results now. The authors did, however, add a comment to the paper, explaining the need for "proof of low-grade infection by MLV-related viruses in humans . . . by demonstrating the integration of the viral genes into the human genome."

(continued on page 5)

PNAS Publishes Its Study (continued from page 4)

In his editorial, Dr. Schekman asserted that the controversy over the association between MLVs and CFS "raises important issues regarding the release of research results to the public and the need for close collaboration with the authors and funding agencies when there is a direct link to public health."

What's Next. During the NIH's telebriefing, the CDC's Steve Monroe, PhD, said the results "raise as many questions as they answer," and he pointed out that the conflicting results from various laboratories "show there are a lot of things about the virus we don't know." For example, the officials pointed out that scientists still do not know how the people in the *PNAS* study were infected.

Importantly, none of the completed studies has been able to determine whether XMRV or related MLVs actually cause CFS or whether CFS may result in increased susceptibility to MLV infections. Another unknown is whether CFS might be transmissible through blood transfusions. Related to that is whether the viruses associated with CFS are present in the blood supply. Dr. Alter said a study of 1,000 blood donors that addresses that latter point is nearing completion.

Pending the results of that and other studies, AABB in June released an Association Bulletin recommending that its member blood collectors actively discourage potential donors who have been diagnosed with CFS from donating blood or blood components. The policy was recommended by AABB's Interorganizational Task Force on XMRV, which includes representatives from several government agencies, including the CDC, FDA, and the NIH, along with blood community representatives (see *ABC Newsletter*, 5/21/10 and 6/18/10). (Sources: www.the-scientist.com/blog/display/57628/, 8/23/10; http://blogs.wsj.com; 8/24/10; www.businessweek.com, 8/24/10; www.RGJ.com, 8/17/10)

Citations: Lo SC, *et al.* Detection of MLV-related virus gene sequences in blood of patients with chronic fatigue syndrome and healthy blood donors. PNAS. 2010 Aug. 23 (E-pub ahead of print). Schekman R. Patients, patience, and the publication process. PNAS. 2010 Aug. 23 (E-pub ahead of print). ♦

RESEARCH IN BRIEF

A new study identifies the unique mechanism that allowed pandemic H1N1 viruses originating from avian species to adapt so easily in humans. In a report in the Aug. 5 issue of *Public Library of Science Pathogens*, an international team of scientists provided new insights into the biologic processes of the influenza pandemic of 2009-2010 and revealed a genetic marker that public health officials can use to help fight future pandemics. "We have found why the pandemic H1N1 virus replicated so well in humans," co-author Yoshihiro Kawaoka, DVM, PhD, a professor of pathobiological sciences at the University of Wisconsin-Madison's School of Veterinary Medicine, told *Science Daily* (8/6/10). According to an author's summary of the paper, "Influenza viruses that originate from avian species likely have to acquire adapting amino acid changes to replicate efficiently in mammals." In most viruses of avian origin, the presence of two amino acids – lysine and asparagine – at specific sites on the polymerase PB2 protein enable the viruses to jump from the avian host and replicate easily in human cells. Though the pandemic H1N1 viruses possess an avian-like PB2 gene, they do not encode those same "human-type" amino acids – PB2-627K and PB2-701N. The lysine amino acid is present in the avian protein at a different position. The team found that a basic amino acid at position 591 of PB2 can compensate for the lack of PB2-627K and allows efficient replication of both highly pathogenic H5N1 and pandemic H1N1 viruses in mammalian species. The team also documented the structure of the C-terminal portion of a pandemic H1N1 PB2 protein. The structural data, said Dr. Kawaoka, shows how the virus

(continued on page 6)

別紙様式第2-1

No. 23

医薬品 研究報告 調査報告書

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				2010. 10. 7	該当なし	
一般的名称	人赤血球濃厚液		研究報告の公表状況	Yu MY, Alter HJ, Virata-Theimer ML, Geng Y, Ma L, Schechterly CA, Colvin CA, Luban NL.. Transfusion. 2010 Aug;50(8):1712-21. doi: 10.1111/j.1537-2995.2010.02591.x. Epub 2010 Feb 12.	公表国 米国	
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)					

研究報告の概要

○関連供血者と受血者のサンプル分析により確認された、赤血球輸血によるパルボウイルスB19(B19)感染

背景:B19の極めて高いウイルス血症レベルが、急性感染した無症候性の供血者に認められた。単一供血者からの血液成分の輸血による感染報告はまれであるが、前向き研究を行わない限り、そのような症例が検知されない可能性がある。そこで輸血による感染リスクを調査するため、供血者ー受血者サンプルを用いて前向き研究を行った。

方法:受血者のB19 DNAについてはPCR法、B19 IgG抗体についてはEIA法を用いて検査を行った。輸血に関連しない感染を除外するため、B19 DNA陽性受血者の輸血前サンプル及び関連供血者サンプルのB19 DNA、IgG、IgM抗体を検査した。感染の確認のためにDNA配列解析と系統発生解析を行った。

結果:受血者869人のうち14人(1.6%)がB19 DNA陽性であったが、そのうち1人(0.12%)が輸血による感染と確定された。この受血者は、急性感染した供血者からの赤血球(1単位中5×10^{10} IU のB19 DNAを含む)及び他の供血者3名からの赤血球(1,320 IUのB19 IgG抗体を含む)を輸血されていた。

結論:0.12%と感染率は低いが、米国において毎年数百～数千例の感染症が発症する可能性がある。ほとんどの場合無症候性であるが、新生児や免疫不全状態、溶血状態にある者の場合、重篤になることがある。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
パルボウイルスB19の、血液成分の輸血による感染のリスクを調査するため供血者ー受血者サンプルを用いて前向き研究を行ったところ、0.12%の感染率であったとの報告である。	今後も引き続き、ヒトパルボウイルスB19に関する新たな知見及び情報の収集に努める。日本赤十字社では、以前よりRHA法によるB19抗原検査を導入、ウイルス量の多い血液を排除してきた。また、2008年には検査法をより感度の高いCLEIA法に変更した。

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MedDRA/J Ver.13.1J

RESEARCH IN BRIEF (continued from page 5)

interacts with the host cell, and that could lead to antiviral agents for future flu viruses that use the same amino acid "trick" to infect humans. The structure was derived from an X-ray crystallographic study produced by the Seattle Structural Genomics Center for Infectious Disease, a consortium of Washington state-based organizations. The H1N1 virus, Dr. Kawaoka said, is actually a combination of four different avian and swine flu viruses that have emerged during the past 90 years, and it includes some genetic material from the 1918 pandemic virus, an influenza that killed as many as 20 million people. The H1N1 pandemic in 2009 and 2010 made as many as 34 million Americans ill and caused up to an estimated 6,000 deaths in the US. According to the World Health Organization, H1N1 had killed at least 18,398 people worldwide as of July 25. (Sources: *Science Daily*, 8/6/10)

Citation: Yamada S, *et al.* Biological and structural characterization of a host-adapting amino acid in influenza virus. PLoS Pathog. 2010 Aug 5;6(8):e1001034. ♦

Financial Realities a Focus at ABC's Interim Meeting

At America's Blood Centers' summer meeting this month, the message was clear: independent, community blood centers are operating in an increasingly competitive environment, and they will need to react nimbly in order to weather the changing conditions.

Blood centers are being pressured by hospitals and hospital chains – themselves facing healthcare-reform cost-cutting – to drop blood prices or lose market share to larger blood organizations. According to speakers at the Executive Leadership Forum on Aug. 9, the result of this state of affairs could be lower blood prices, fewer client hospitals, and further consolidation among blood centers. Though the situation is alarming, the speakers also suggested strategies that could help blood centers strengthen their relationships with their hospitals and deal with the challenges.

The Current Situation. During the morning session, Maureen Swan, of MedTrend Inc., focused on the financial implications of healthcare reform; Mindy Weinberg, a senior quality assurance analyst with the University of Pittsburgh Medical Center (UPMC), discussed how blood products fit into the supply chain at her hospital; and Robert Carden, PhD, president and CEO of Virginia Blood Services, focused on the economics of blood banking from blood centers' perspectives.

The key issue, which surfaced repeatedly, is the financial pressure being felt by hospitals and blood centers. As Ms. Swan pointed out, hospitals are facing pressure from two directions: government healthcare reform (which means less Medicare money for hospitals, as more people become eligible for Medicaid) and marketplace changes (which most likely will include new payment methods that place more emphasis on value and outcomes, rather than paying "per click," or per procedure).

Blood centers, too, are feeling financial pressure from multiple directions, as Dr. Carden made clear. On one hand, increased collections of red blood cells (RBCs) mean that supply is up. On the other hand, demand is down, in part because of better technologies (such as inventory management systems) and more conservative transfusion practices. The increase in the number of people with health insurance may drive demand for blood and blood products up, Ms. Swan said, but more and more people have high deductible plans, which encourage people to use less care and means that hospital volume drops. The net effect of these changes is therefore difficult to predict, she said.

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Parvovirus B19 infection transmitted by transfusion of red blood cells confirmed by molecular analysis of linked donor and recipient samples

Mei-ying W. Yu, Harvey J. Alter, Maria Luisa A. Virata-Theimer, Yansheng Geng, Li Ma, Cathy A. Schechterly, Camilla A. Colvin, and Naomi L.C. Luban

BACKGROUND: Extremely high viremic levels of parvovirus B19 (B19V) can be found in acutely infected, but asymptomatic donors. However, reports of transmission by single-donor blood components are rare. In this prospective study, paired donor-recipient samples were used to investigate the transfusion risk.

STUDY DESIGN AND METHODS: Posttransfusion plasma or blood samples from recipients were tested for B19V DNA by polymerase chain reaction, generally at 4 and 8 weeks, and for anti-B19V immunoglobulin (Ig)G by enzyme immunoassay at 12 and 24 weeks. To rule out infection unrelated to transfusion, pretransfusion samples and linked donor's samples for each B19V DNA–positive recipient were assayed for B19V DNA and anti-B19V IgG and IgM. To confirm transmission, sequencing and phylogenetic analysis were performed.

RESULTS: A total of 14 of 869 (1.6%) recipients were B19V DNA positive, but only 1 of 869 (0.12%; 95% confidence interval, 0.0029%-0.6409%) was negative for B19V DNA and anti-B19V IgG before transfusion and seroconverted posttransfusion. This newly infected patient received 5×10^{10} IU B19V DNA in one red blood cell (RBC) unit from an acutely infected anti-B19V–negative donor in addition to RBCs from three other donors that cumulatively contained 1320 IU of anti-B19V IgG. DNA sequencing and phylogenetic analysis showed that sequences from the linked donor and recipient were identical (Genotype 1), thus establishing transfusion transmission.

CONCLUSIONS: The 0.12% transmission rate documented here, although low, could nonetheless result in hundreds or thousands of infections annually in the United States based on calculated confidence limits. Although most would be asymptomatic, some could have severe clinical outcomes, especially in neonates and those with immunocompromised or hemolytic states.

Parvovirus B19 (B19V) is a small, nonenveloped, DNA virus of the *Erythrovirus* genus in the Parvoviridae family. It resists viral inactivation procedures commonly used in the manufacture of pooled plasma products and is the only parvovirus shown to be pathogenic in humans.

When B19V infects erythropoietic progenitors, transient but significant red blood cell (RBC) hypoplasia or aplasia ensues. This is especially common in children, but it also occurs in seronaive adults who have coexisting hemolytic anemias, such as sickle cell disease, hereditary spherocytosis, or RBC enzymopathies (see review[1]). Furthermore, B19V-induced RBC aplasia is seen in patients

ABBREVIATIONS: B19V = parvovirus B19; CBER = Center for Biologics Evaluation and Research; EOS = end of study; TRIPS = Transfusion-Related Infections Prospectively Studied; WB = whole blood.

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with congenital and acquired immunodeficiency because high-titer viremia in the absence of an effective humoral immune response can result in prolonged hypoplastic anemia. When B19V infection occurs during pregnancy, hydrops fetalis and fetal loss can result. Arthropathy, neutropenia, and thrombocytopenia have been reported in both children and adults.[1]

B19V transmission occurs through the respiratory route, vertically from mother to fetus, and through transfusion or transplantation—most often during the viremia that precedes clinical presentation. Household, day care, and school transmissions are common. Viral levels as high as 10^{13} genome equivalents (geq)/mL are often found in the blood of asymptomatic individuals during the early phase of acute infection.[2] There are numerous reports of transmission by pooled plasma-derived products, including clotting factor concentrates despite solvent/detergent (S/D) treatment, heat treatment, and/or other viral inactivation methods.[3-6] Infectivity has been correlated with a high concentration of B19V prompting plasma fractionators to implement screening for B19V DNA by nucleic acid testing (NAT) to exclude high-titer donations from entering manufacturing pools.[8-11]

The presence of anti-B19V IgG in the recipient or product may play a role in attenuating transmission. The prevalence of anti-B19V IgG exceeds 50% in some donor populations.[12,13] These antibodies are considered to be neutralizing[14,15] and to confer lifelong immunity.[1] The prevalence of B19 viremia in blood and plasma donors ranges from 0.003% to 0.88%[16-20] depending on the sensitivity of the NAT method employed and whether the testing is performed at the time of an epidemic. Low levels of B19V DNA, ranging from 10 to 10^3 IU or geq/mL, coexist with anti-B19V IgG and may persist for 3 to 5 years in immunocompetent blood donors.[21-24] Infectivity is presumably dependent on the relative balance between viral and neutralizing antibody titers, albeit the minimum infectious dose of B19V DNA, with or without coexisting anti-B19V IgG, is unknown. The IgG antibodies from multiple plasma donors are generally sufficient to render pooled plasma products noninfectious if no donor in the pool has high-level viremia. This provides the rationale for excluding only those plasma donations for further manufacturing that have viral titers exceeding approximately 10^6 IU/mL.[11,25] To date, universal blood donor screening for B19V NAT is not performed in the United States. Case reports of transmission by single-donor blood components are rare. Only four cases of transfusion-transmitted B19V-induced anemia have been documented.[16,26-28] The rarity of such case reports may reflect the fact that most infected individuals are asymptomatic and hence undetected unless enrolled in a prospective study. In this article, we describe a case of transfusion-transmitted B19V infection identified in a prospective study designated TRIPS (Transfusion-Related Infections Prospectively Studied). Pre- and serial posttransfusion samples from recipients and linked donor specimens were collected and placed in frozen storage. This permitted an estimate of the frequency of B19V infection by blood components and the infectious dose relative to the titer of antibody. Further, linked donor-recipient samples allowed for confirmation of causality by DNA sequencing and phylogenetic analysis.

MATERIALS AND METHODS

Patient population and study specimens

The TRIPS repository was initiated in November 2001 and is composed of linked donor-recipient specimens from transfusion recipients enrolled at the NIH Clinical Center (Bethesda, MD) and the Children's National Medical Center (Washington, DC) and from Suburban Hospital (Bethesda, MD). Informed consent was obtained from all donors and recipients in accordance with the Declaration of Helsinki for participation in NIH-sponsored and institutional review board–approved protocols (NIH Protocol 01-CC-0231; Children's National Medical Center, Protocol 2540). Human subjects were assigned a code number, and samples for testing were identified only by that code; the testing laboratories, including the parvovirus testing laboratory at the Center for Biologics Evaluation and Research (CBER), FDA, had no capability of linking the code number to the study participant's name. Thus far, pretransfusion plasma and/or whole blood (WB) samples from 869 enrolled recipients have been collected, generally at 4, 8, 12, and 24 weeks posttransfusion (and/or at the end of study [EOS] for repeatedly transfused subjects followed longer than 24 weeks). WB and/or plasma samples from donors and recipients were stored in 1- to 2-mL aliquots at −80°C in a central repository (SeraCare BioServices, Gaithersburg, MD). Medical records review was performed to obtain details of underlying diseases and clinical circumstances surrounding the transfusion.

Detection and quantitation of B19V DNA by polymerase chain reaction

B19V DNA levels were determined on the first available samples collected after transfusion from each recipient; usually at 4 and 8 weeks, but occasionally also at 2 weeks. DNA was extracted from 0.2 mL of plasma or WB, and B19V DNA was detected and semiquantified by an in-house nested polymerase chain reaction (PCR) method as described previously.[6] A final amplified product of 243 bp was obtained extending from Nucleotide 2951 to Nucleotide 3193, thus covering the junction of the VP1/VP2 region based on the nucleotide numbering of the published B19 Au sequence[29] (GenBank M13178). The first WHO International Standard for B19V DNA[30] (NIBSC

99/800, 10^6 IU/mL when reconstituted) was diluted 10^3-fold and used as a positive control for extraction and quantification. The level of B19V DNA, expressed as IU/mL, was determined by limiting dilution analysis since the conversion ratio from a geq (or a copy) to IU detected by PCR was 1:1 based on our PCR method. The sensitivity of the PCR assay with an original sample volume of 0.2 mL was 20 IU/mL, as previously described.[8] This B19V PCR procedure detects both Genotypes 1 and 2 of B19V, but not the Genotype 3 variant (see Discussion).

A posttransfusion sample was considered B19V DNA positive only when a separate 0.2-mL sample aliquot was also positive. Only if B19V DNA was positive after transfusion was the pretransfusion sample from that recipient requested from the repository, and a 0.2-mL aliquot was similarly tested by PCR. If the recipient's pretransfusion sample tested B19V DNA negative, suggesting the possibility of transfusion transmission, samples from the linked donor(s) plasma or WB were tested for B19V DNA.

Anti-B19V antibody assays

Anti-B19V IgG testing was performed qualitatively according to the manufacturer's instructions on 12- and 24-week (or EOS) samples with an FDA-cleared enzyme immunoassay (EIA) kit (Biotrin International Ltd., Dublin, Ireland) consisting of wells coated with recombinant B19 capsid protein (VP2). Index values of less than 0.9 are deemed negative and more than 1.1 are positive, while those between 0.9 and 1.1 are equivocal. The index value is obtained from the mean absorbance value for the test sample divided by the cutoff value, which is, as instructed by the kit, computed by multiplying the mean absorbance of the calibrator by the lot-specific constant. Further, we developed a semiquantitative assay to measure anti-B19V IgG levels that permitted calculating an infectious dose when B19V DNA and anti-B19V IgG were both present. This assay made use of a working standard solution consisting of 1 IU/mL anti-B19V IgG prepared by diluting the first WHO anti-B19 serum IgG standard[31] (NIBSC 93/724, 100 IU of anti-B19V IgG/mL when reconstituted) with sample diluent from the kit. A six-point standard curve was set up by twofold serial dilutions of the WHO working solution, providing a range from 0.031 to 1 IU/mL. Each plasma sample was diluted 30-fold or more, and then twofold serial dilutions were made. Aliquots of 100-µL of diluted standard or plasma solutions were incubated with the coated wells according to the manufacturer's instructions. Anti-B19V IgG levels in IU/mL were calculated by using a parallel-line model in statistical analysis software (CombiStats, Version 4.0) provided by the European Directorate for the Quality of Medicines & HealthCare (EDQM), Council of Europe (Strasbourg, France).

If recipients were confirmed B19V DNA positive (i.e., ≥20 IU/mL) from the first available sample collected after transfusion, anti-B19V IgM assays were performed on all plasma samples collected from the recipient, along with the associated donor samples. A B19V IgM EIA kit (Biotrin) was used to detect the presence of captured antibodies in human serum or plasma by means of biotinylated B19V VP2 protein according to the kit's instructions with similar computation of index values mentioned above for anti-B19V IgG testing. When a sample was scored as anti-B19V IgM equivocal, the same sample was retested for confirmation since plasma collected within 1 to 2 weeks of the initial reactive result was not available for retesting as recommended by the kit manufacturer.

DNA sequencing and phylogenetic analysis

Extracted DNA samples were amplified by a seminested B19V PCR procedure described previously[8] so that a longer final amplified product of 786 bp (Nucleotides 2408-3193), covering the entire VP1-unique region and a portion of the VP2 region, could be obtained. The amplified product was further purified by a PCR purification kit (QIAquick, Qiagen, Inc., Valencia, CA) and directly sequenced without the need for cloning since the samples sequenced contained relatively high levels of B19 DNA. The sequencing primers were the same as those used for the seminested PCR procedure. The WHO B19V DNA standard was similarly extracted and amplified, and the amplified product was sequenced for comparison. Sequences, each 710 nucleotides in length corresponding to Nucleotides 2465 to 3124 of the VP1-unique region plus Nucleotides 3125 to 3174 of the VP2 region, were aligned against other corresponding published sequences from GenBank by using ClustalW2, a general-purpose multiple alignment program. Phylogenetic analysis was performed with a neighbor-joining algorithm in computer software (*Molecular Evolutionary Genetics Analysis, v.4, MEGA4*; Center of Evolutionary Functional Genomics, Arizona State University, Tempe, AZ).

Statistical analysis

Statistical software (StatXact, v.8, Cytel, Inc., Cambridge, MA) was used to calculate 95% confidence interval (CI) based on one observed B19V infection in this study.

RESULTS

Fourteen of the 869 (1.6%) recipients were found to be B19V DNA positive in their early posttransfusion specimens; of these, seven were children and seven (Recipients 3-6, 8, 12, and 14 in Table 1) were adults. Pretransfusion and serial posttransfusion plasma samples from these 14 B19V DNA–positive recipients were tested for viral levels and IgM/IgG anti-B19V antibodies, and the results are shown in Table 1. Six

TABLE 1. Analysis of 14 recipients positive for B19V DNA after transfusion*

	Before transfusion			After transfusion		
	B19V DNA	B19V antibodies		B19V DNA†	B19V antibodies†	
Recipient	(IU/mL)	IgG	IgM	(IU/mL)	IgG	IgM
1	63	Positive	Negative	20	Positive	Negative
2	20	Positive	Negative	40	Positive	Negative
3	20	Positive	Negative	58	Positive	Negative
4	60	Positive	Negative	40	Positive	Negative
5	20	Positive	Negative	630	Positive	Negative
6	40	Positive	Negative	63	Positive	Negative
7	Negative	Positive	Negative	20	Positive	Negative
8	632	Positive	Positive	1.4×10^3	Positive	Negative
9	6.3×10^6	Positive	Positive	6.3×10^5	Positive	Positive
10	2×10^7	Positive	Positive	630	Positive	Positive
11	$>2 \times 10^{10}$‡	Negative	Positive	1.4×10^4	Positive	Positive
12	63	Negative	Negative	200	Positive	Negative
13	Negative	Negative	Negative	200	Negative	Negative
14	Negative	Negative	Negative	6×10^8	Positive	Positive (12 week)

* Posttransfusion samples were tested first. Only 14 recipients tested positive and hence their pretransfusion samples were obtained from the repository and tested for all B19V markers. All other recipients whose posttransfusion samples were negative for B19V DNA (i.e., <20 IU/mL) were not further investigated.

† B19V DNA levels listed were determined from the first available samples collected after transfusion, mostly at 4 weeks, except at 8 weeks for Recipients 5, 11, and 12 and at 2 weeks for Recipients 6 and 14.

‡ For each sample, the qualitative method for detecting anti-B19V (either IgG or IgM) has been described in detail under Materials and Methods. However, a designation of positive or negative in this table refers to the results obtained for testing both 12- and 24-week (or EOS) samples with the following exceptions: anti-B19V IgM was positive only in the 12-week sample for Recipient 14; anti-B19V testing was performed only on the 12-week sample for Recipients 5, 10, and 12; only on the 24-week (or EOS) sample for Recipients 1, 6, and 13; and only on the 4-week sample for Recipient 9 because of sample availability.

recipients (Recipients 1-6) had low-level viremia and anti-B19V IgG in their pretransfusion sample, indicating prior chronic infection unrelated to the index transfusion. One recipient (Recipient 7) was anti-B19V IgG positive and B19V DNA negative before transfusion and then, in the 4-week posttransfusion sample, displayed very low-level, transient viremia that coexisted with anti-B19V IgG. This case was considered to have existing B19V infection with fluctuating low-level viremia. Three recipients (Recipients 8-10) were found positive for both B19V DNA ($\leq 2 \times 10^7$ IU/mL) and anti-B19V (both IgM and IgG) before transfusion, two (Recipients 9-10) showing diminishing titers after transfusion and one (Recipient 8) showing a slight increase in titer. These three patients appear to have had an acute B19V infection that predated the transfusion. Recipient 11 clearly had an acute B19 infection that predated the transfusion since the pretransfusion sample had a very high B19V DNA level ($>2 \times 10^{10}$ IU/mL) associated with IgM antibody in the absence of IgG; after transfusion the viral level diminished and the patient seroconverted for anti-B19V IgG. Recipient 12 appeared to be in the seronegative window period of infection before transfusion since only low-level B19V DNA was detected before transfusion, and seroconversion for anti-B19V IgG was demonstrated after transfusion. Recipient 13 was negative for all B19V markers before transfusion; a single posttransfusion sample at 4 weeks had a B19V DNA level of 200 IU/mL, but later samples were negative and there was no evidence of antibody seroconversion. We considered this more likely a false-positive DNA determination than a transfusion-associated infection. Thus, only Recipient 14 fulfilled the criterion for a transfusion-related infection in that the recipient was negative for all B19V markers before transfusion and then developed high-level B19V DNA (6×10^8 IU/mL) and underwent seroconversion for IgM and IgG antibodies after transfusion. This patient is the subject of the following case report.

CASE REPORT

The single recipient infected with B19V through transfusion during the course of this study was a 35-year-old white female with a primary diagnosis of low-grade adenocarcinoma of the appendix with peritoneal carcinomatosis. She was married, had no children, and had no prior history of receiving a blood transfusion. As part of her surgical protocol, the patient received irradiated, leukoreduced RBCs from a total of six donors, consisting of two different units given every other day over a period of 5 days. A pretransfusion sample was collected on May 23, 2005, before her surgery.

As shown in Table 2, this seronegative recipient became strongly B19V DNA positive 2 weeks posttransfusion and remained positive at 4, 8, and 12 weeks, but was negative by Week 24. Anti-B19V (IgM and IgG) were absent before transfusion and then detected in both the 8- and the 12-week posttransfusion samples. IgG antibody persisted through the 24-week sample, but IgM antibody was no longer detectable at that time.

TABLE 2. B19V DNA and anti-B19V in a recipient*

Plasma sample	B19V DNA (IU/mL)	Anti-B19V IgG	Anti-B19V IgM
Before transfusion	Negative†	Negative	Negative
After transfusion			
2 weeks	6×10^8	Negative	Equivocal‡
4 weeks	20	Negative	Negative
8 weeks	630	Positive	Positive
12 weeks	140	Positive	Positive
24 weeks/EOS	Negative	Positive	Negative

* Recipient 14 in Table 1.
† Less than 20 IU/mL B19V DNA.
‡ Retested sample was also scored as "equivocal" according to the kit's instructions.

TABLE 3. B19V marker testing of donations received by the B19V-infected recipient

Donor number	Transfusion day	B19V DNA (IU/mL)	Anti-B19V IgG (IU/mL)	Anti-B19V IgM
1	0*	NT†	NT	NT
2	0	Negative	Negative	Negative
3	2	Negative	Positive (22‡)	Negative
4	2	5×10^6‡	Negative	Negative
5	4	Negative	Positive (77‡)	Negative
6	4	Negative	Positive (33‡)	Negative

* RBC units from two donors were transfused every other day starting on Day 0, the date of surgery, for a total of 6 RBC units.
† NT = not tested; sample was not available for testing.
‡ Geometric mean titer from three independent assays.

In the complex setting of surgical blood loss and transfusion replacement, measurement of hematocrit and hemoglobin could not discern whether the parvovirus infection had a suppressive effect on RBC production. Because the infection was not recognized until stored samples were retrospectively tested, reticulocyte counts were not performed. Platelet (PLT) and white blood cell counts remained normal and hospital chart review showed no record of the temporal occurrence of fever, rash, arthritis, or cardiopulmonary dysfunction.

The recipient was transfused with 6 RBC units: 2 units during exploratory surgery and 2 units on Postoperative Days 2 and 4, respectively. Of the six donors, one had no stored sample available for testing; one was negative for all B19V markers; three donors were positive for anti-B19V IgG (22, 77, and 33 IU/mL, respectively), but negative for IgM antibody and B19V DNA; and one donor (Donor 4) was acutely infected and had high-level B19V DNA (5×10^8 IU/mL) without any detectable anti-B19V (Table 3).

We calculated the total infectious dose by making two assumptions, namely, that there was 10 mL of residual plasma per RBC unit and that the unit unavailable for testing was negative for all B19V markers. With these assumptions, we estimate that the recipient received 5×10^{10} IU of B19V DNA from the acutely infected donor's RBC unit and 1320 IU of anti-B19 IgG from three donors' units (220, 770, and 330 IU).

To confirm that the B19V-contaminated RBC unit was the source of infection, direct sequencing of PCR-amplified products from the linked, implicated donor and the recipient was performed. We chose to sequence primarily the VP1-unique region, which exhibits the most variation in both DNA sequences and protein expression.[6,32] Plasma and WB from the implicated B19V DNA-positive donor (Donor 4 in Table 3) and plasma from the 2- and 8-week posttransfusion recipient samples were extracted for DNA and amplified by PCR using primers encompassing the entire VP1-unique region and a portion of the VP2 region. The amplified regions were sequenced and subjected to sequence alignment analysis. For comparison, a positive control (B19V DNA present in the WHO B19V DNA standard) was similarly PCR amplified and the purified amplified product was sequenced. Identical 710 nucleotides covering the 650-nucleotide VP1-unique region and the 60-nucleotide VP2 region were obtained from the recipient's 2- and 8-week plasma samples and the donor's plasma and WB samples (Fig. 1A). In contrast, sequences similarly obtained from the WHO International Standard for B19V DNA had four nucleotide differences (C2531G, C2578T, A2736G, and T2786C) within the VP1-unique region while the published sequences of a well-known B19V strain, designated as Au,[29] differed by three nucleotide substitutions (the first three). These nucleotide differences would yield only two amino acid residue changes between the infecting strain in our patient and the WHO standard or the Au strain. By phylogenetic analysis, both the recipient and the donor were closely related on the same branch of the Genotype 1 phylogenetic tree and were distinct from the WHO standard and other known B19 isolates (Fig. 1B). Thus, in this prospective study, although 14 of 869 (1.6%) recipients were found to be B19V DNA positive, the observed transmission rate by transfusion was 1 in 869 (0.12%) with a 95% CI of 0.0029% to 0.6409%.

DISCUSSION

Of four previously reported cases of B19V transmission by single-donor blood components, three[16,26,27] were by RBC transfusions and one[28] was by PLTs. All these cases were identified by retrospective studies triggered by clinical symptoms or unexplained anemia observed in recipients who were immunocompromised and negative for all B19V markers before transfusion (pretransfusion sample not available in one case[16]). In two studies,[26,28] the implicated donors were positive for both B19V DNA and IgM antibody, while in the third study,[16] the implicated RBC unit was B19V DNA positive in the absence of antibody. In the fourth reported case,[27] donor samples were not available. In only one study[28] was DNA sequencing and phylogenetic analysis performed to confirm that the donor was the source of infection. Furthermore, in all these case reports, detection of B19V DNA by PCR was qualitative, and hence the amount of B19V DNA infused is unknown.

Two other studies[33,34] assessed the safety of single-donor blood products containing a known amount of B19V DNA. In one,[33] 200 mL of a WB unit containing a low level (5.8×10^2 IU/mL) of B19V DNA was transfused to a seronegative (hence susceptible) pediatric recipient. The other study[34] retrospectively evaluated several adult hematologic patients who received blood components with B19V DNA levels ranging from less than 600 to 2.2×10^6 geq/mL. However, neither of the recipient populations had any clinical or laboratory evidence of B19V infection, possibly because anti-B19V IgG was present in either the transfused blood components or the recipients. Although quantitative titers of B19V IgG were not provided, the antibodies likely played a protective role in attenuating transmission.[14,15]

One recent study[35] evaluated retrospectively the rate of transmission in susceptible recipients, that is, those B19V IgG-negative individuals who received B19V DNA–positive blood components, by using linked donor and recipient repository samples established in 2000 to 2003 within the United States. A B19V DNA prevalence of 0.84% (105/12,529) was detected in those linked donations, consistent with the 0.88% prevalence[20] found earlier in unlinked donations from the same repository. Of the 105 recipients of B19V DNA–positive donations, 78% had anti-B19V IgG in their pretransfusion samples leaving only 24 susceptible recipients eligible for evaluation. No susceptible recipient was infected by transfusion; however, all received blood products containing only low-level (<10^4 IU/mL) B19V DNA that coexisted with anti-B19V IgG. In addition, the study design did not include early posttransfusion samples to detect transient viremia or the appearance of anti-B19V IgM. Three blood components derived from donations with higher titers of B19V DNA (>10^5 IU/mL) and devoid of anti-B19 IgG were transfused but their infectivity could not be

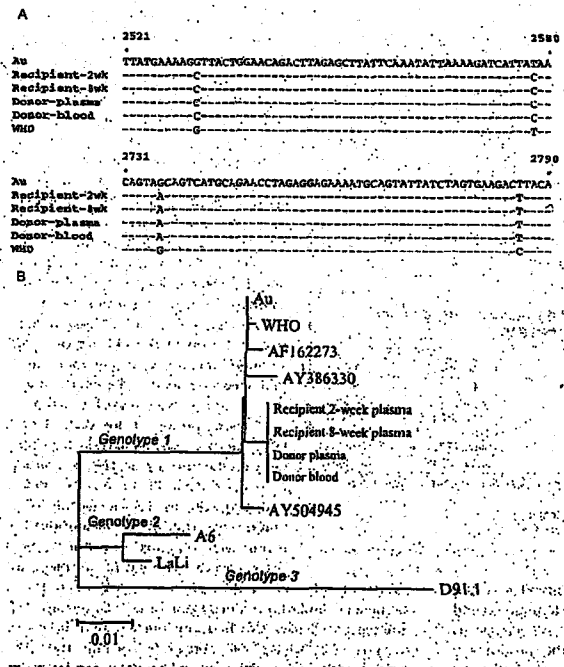

Fig. 1. (A) B19 nucleotide sequence alignments within the VP1-unique region (Nucleotides 2465-3124) and a portion of the N-terminal VP2 region (Nucleotides 3125-3174). All sequences obtained for Nucleotides 2465 to 2520, 2581 to 2730, and 2791 to 3174 were identical and therefore are not shown. Nucleotide numbering of the sequence is based on the published B19 Au strain (M13178). Sequences were determined directly from purified PCR-amplified products derived from the patient's 2- and 8-week plasma samples and from plasma and WB samples from Donor 4 (Table 3). As the positive control, the WHO B19V DNA standard was similarly sequenced. (B) Phylogenetic comparison of the above-mentioned B19 sequences (Nucleotides 2465-3174; 710 nucleotides) along with other corresponding published B19 sequences in the GenBank, that is, Genotype 1 = M13178 (Au), AF162273, AY386330, and AY504945; Genotype 2 = AY064476 (A6) and AY044266 (LaLi); and a Genotype 3 = AY083234 (D91.1). Evolutionary distances are in units of the number of base substitutions per site (0.01 unit shown).

evaluated since each was infused into a nonsusceptible *recipient*.

The TRIPS study described herein is the first to investigate prospectively B19V transmission associated with transfusion of blood and blood components to susceptible immunocompromised or immunocompetent recipients. After the initial demonstration that 1.6% (14/869) of recipients had detectable B19V DNA after transfusion, the availability of pretransfusion samples allowed identification of those who were already infected with B19V. The majority had clear evidence of B19V infection existing *before entry into the study and, in one case, evidence of a* very recent infection that might have been attributed to transfusion if the appropriate pre- and posttransfusion samples had not been available.

Of the 14 recipients who were viremic after transfusion, only one was seronegative before transfusion and subsequently exhibited a seroconversion profile indicative of B19V transmission. This occurred after receiving a unit of RBC that had a minimum volume of plasma associated with it. By assuming that approximately 10 mL of plasma was present, we estimated that the patient received 5×10^{10} IU of B19V DNA. In reports of seronegative persons with hemophilia infused with contaminated coagulation concentrates[1] or seronegative volunteers experimentally exposed to B19V,[36] viral DNA was usually detected within 1 week after exposure. In the latter study,[36] peak viremia and seroconversion to anti-B19V IgM occurred within 2 weeks. The recipient infected in our study exhibited maximal viremia (6×10^8 IU/mL) 2 weeks after transfusion, but anti-B19V IgM was not detected until Week 8, simultaneous with the appearance of anti-B19V IgG. This delay might have been due to the modulating effect of B19V IgG–neutralizing antibodies present in three other RBC units transfused in close proximity to the implicated unit.[1,14,15]

Sequencing/phylogenetic analysis established that both the donor and the recipient were infected with B19V Genotype 1. Although B19V strains are genetically more diverse than previously thought and have been classified into three genotypes,[37] Genotype 1 is still the most prevalent in western countries while Genotype 2, though less common, has been detected in plasma and coagulation factor concentrates.[38-40] Genotype 3 is found predominantly in West Africa[41] and rarely in other areas of the world. Because some B19V NAT assays detect only Genotype 1,[40,42] some earlier investigations may have underestimated the diversity of B19V in the specimens examined. *Recently a source plasma donor infected with a Genotype* 3 strain was identified in the United States by a plasma fractionator performing a B19V NAT screening procedure capable of detecting all three genotypes in a minipool format.[43] The NAT procedure used in our study detects both Genotypes 1 and 2, but not Genotype 3.[11] Interestingly, B19V genotype variants have been shown to be very similar in functional and immunologic studies, and *current data suggest that only one serotype exists for* B19V.[38,41,44]

In our study, the infectious dose received by the susceptible (seronegative) recipient was 5×10^{10} IU of B19V DNA from 1 RBC unit (derived from an acutely infected donor) given in temporal proximity to a total of 1320 IU of anti-B19V IgG antibodies from 3 other RBC units. In the B19V transmission incident associated with pooled plasma, S/D treated, a pooled plasma product known to contain anti-B19V IgG,[9,45] the infectious dose received by *susceptible immunocompetent volunteers was more than* 2×10^9 geq or IU of B19V DNA, that is, 200 mL per implicated lot containing more than 10^7 geq/mL. In contrast, those same susceptible individuals were not infected when each received a dose of less than 2×10^8 IU of B19V DNA from product lots containing less than 10^3 IU/mL, indicating that infectivity is dependent on the balance between viral and neutralizing antibody titers. Moreover, when recipients were seropositive before transfusion, with anti-B19V IgG levels ranging from 19 to 39 IU/mL, the high-titer implicated product (i.e., that with $>10^7$ IU of B19V DNA/mL) was not infectious. Thus, there must be a specific level of anti-B19V IgG that confers protection against infection.[45] The lowest infectious dose reported was 2×10^4 IU of B19V DNA administered to a susceptible immunocompetent individual who received a Factor (F)VIII coagulation product devoid of any detectable anti-B19 IgG.[8]

The case found in our study represents the fifth documented B19V infection transmitted by cellular blood products and the fourth transmitted by RBCs. Although the observed incidence rate of 0.12% (1/869) is relatively low, it could translate to 4800 transfusion-transmitted B19V infections annually among the approximately 4 million blood recipients in the United States. However, because only one definite B19V transmission was observed in this population, the 95% CI for the incidence *rate is very broad (0.0029%-0.6409%) and the number of* projected cases could be as low as 116 or as high as 25,636. The proportion of such infections that would have serious clinical consequences is unknown, but clearly neonates, persons with congenital and acquired hemolytic anemias, and immunocompromised patients would be at increased risk of untoward clinical events.

Currently, most plasma fractionators in the United States perform minipool B19V NAT screening as an in-process control to detect and exclude donations with *B19V DNA levels of approximately* 10^6 *IU/mL or higher so* that the level of B19V DNA in manufacturing plasma pools destined for the production of plasma derivatives will not exceed the limit of 10^4 IU/mL.[11,46] In Europe, a similar limit has been in place for plasma pools used for manufacturing anti-D immunoglobulins and plasma treated for virus inactivation.[40,47-50] It is recommended that all B19V NAT procedures detect all three B19V genotypes.[46,50] In addition to screening, viral inactivation and removal procedures have been incorporated into the manufacture of plasma derivatives. The net effect of screening and virus inactivation when combined with complexing and/or neutralizing anti-B19 IgG antibodies that are invariably present in large plasma pools is that the final products contain little or no infectious virus. *This has been confirmed in a recent survey of FVIII concentrates.*[51]

In contrast to pooled plasma products, WB donations are not tested for B19V DNA, and no viral inactivation procedure is in place for blood components in the United States. The fact that screening for B19V DNA is not performed is due both to the lack of licensed commercial assays and to the absence of compelling evidence for significant clinical risk. Nonetheless, it is legitimate to ask whether the small, but finite risk could be significantly reduced by testing WB donations and withholding units that exceed a threshold level of B19V DNA (e.g., that applied to units of plasma used for manufacturing pooled products) without compromising the supply of blood components. Some blood centers in Germany and Austria have screened WB units by a B19V minipool real-time NAT procedure for several years. Blood components associated with donations having 10^5 IU/mL or more, regardless of *whether they contain anti-B19V IgG antibodies, are dis*carded to protect at-risk individuals, whereas units with less than 10^5 IU/mL are released because of the apparently universal coexistence of anti-B19V IgG.[19] In a subsequent retrospective, linked donor-recipient infectivity study,[51] preliminary data indicate that nearly 50% (7/15) of recipients transfused with RBC units from WB donations having more than 10^5 IU/mL of B19V DNA were B19V infected, and the link between the B19V donor and recipient was demonstrated by sequence analysis. In contrast, none of *16 recipients transfused with RBC units containing less* than 10^5 IU/mL B19V DNA was infected. Thus, in that study, WB screening with a threshold level of 10^5 IU/mL seems to have been warranted.

In conclusion, this study revealed a new B19V infection related to single-donor blood products, only the fifth such case in the literature. In the absence of an obvious clinical syndrome, detection of B19V infection depended on the availability of pre- and posttransfusion samples to differentiate new from existing infection. The transmission was confirmed by sequencing and phylogenetic analysis of linked donor-recipient samples and demonstrated an identical Genotype 1 sequence over the 710-nucleotide skein analyzed. Despite the low rate of transmission documented in this study, this incidence of new transfusion-transmitted B19V infections could result in numerous infections annually, some of which would have *clinical consequences in susceptible populations.* The introduction of B19V NAT screening of WB with a threshold level of 10^5 or more or 10^6 IU/mL or more would have interdicted transfusion of the product from this donor acutely infected with B19V and avoided this proven transmission. However, the decision to introduce universal B19V donor testing is complex and needs to be guided by additional prospective studies or further retrospective analyses of repository samples from prior studies with appropriate donor-recipient linkage.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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MAJOR ARTICLE

West Nile Fever Characteristics among Viremic Persons Identified through Blood Donor Screening

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Nucleic acid testing (NAT) of blood donors provides opportunities for identifying West Nile virus (WNV)–infected persons before symptoms develop and for characterizing subsequent illness. From June 2003 through 2008, the American Red Cross performed follow-up interviews with and additional laboratory testing for 1436 donors whose donations had initial test results that were reactive for WNV RNA; 821 of the donors were subsequently confirmed to have WNV infection, and the remainder were unconfirmed or determined to have false-positive results. Symptoms attributed to WNV infection were determined by comparing symptom frequency among 576 donors identified with early WNV infection (immunoglobulin M antibody negative) and those with unconfirmed infection. We estimate that 26% of WNV-infected persons become symptomatic, defined by the presence of at least 3 of 8 indicator symptoms. Nearly one-half of symptomatic persons sought medical care; only 5% received a diagnosis of WNV infection. Female subjects and persons with higher viral loads detected in the index donations were more likely than other subjects to develop symptoms.

The constellation of symptoms referred to as West Nile fever (WNF) is by far the most commonly recognized clinical manifestation of West Nile virus (WNV) infection [1–3]. Although studies indicate that <1% of persons infected develop neuroinvasive disease [4–6], a serological survey conducted after the 1999 New York City outbreak indicated that 21% develop febrile illness after WNV infection [6]. However, this estimate was based on the identification of only 6 (32%) of 19 seropositive persons reporting recent febrile illness, compared with 70 (11%) of 648 seronegative participants. The subsequent implementation of WNV blood donation screening by nucleic acid test (NAT) in the United States and Canada began in June 2003 and provides a unique opportunity to identify many WNV-infected persons very soon after infection and usually before symptom onset, thus eliminating the sample size limitations of serological surveys and the biases of clinical case detection [4, 7–12]. Three follow-up studies involving NAT-positive blood donors have studied the frequency, symptoms, and risk factors for WNF [11–13]. Brown et al. [12] estimated that 30% of infected persons became symptomatic, but the study lacked a control group. Orton et al. [11] estimated that 41% became symptomatic (61% minus 20% among controls); Custer et al. [13] found that 34% of infected persons and 20% of controls had multiple symptoms that were compatible with WNF. These figures varied in part because of different definitions of WNF and different time periods studied surrounding the index donation. One study found that lower age and higher viral load independently correlated with symptomatic infection [12]; however, another study failed to identify a relationship between age and symptomatic infection [13].

The study reported here extends the work of Orton et al. [11] at the American Red Cross (ARC) to further define the frequency of WNF-related symptoms, to define demographic and virologic factors associated with

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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. 10. 7	該当なし	
一般的名称	人赤血球濃厚液			公表国	
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)	研究報告の公表状況	Zou S, Foster GA, Dodd RY, Petersen LR, Stramer SL. J Infect Dis. 2010 Nov 1;202(9):1354-61.	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
○供血者スクリーニングによりウイルス血症と特定された者におけるウエストナイル熱の特徴 供血者のNAT検査は、症状が発現する以前のウエストナイルウイルス(WNV)感染者の特定や症状の特徴付けを可能とした。2003年6月から2008年にかけて米国赤十字は、初回検査でWNV RNA陽性となった血液供血者1,436名について、経過観察と追跡調査を行ったところ、821名の供血者がWNV感染症であると確認され、残りの者は未確定または偽陽性であった。WNV感染症の症状は576名の初期WNV感染者と、未感染者の間で比較された。それによるとWNV感染者の26%に、8つの症状(新しい発疹、全身の虚脱感、頭痛、重篤な筋肉痛、関節痛、発熱、悪寒、眼痛)のうち少なくとも3つが存在すると推定された。 症状を有する患者の半数近くが治療を求めたが、医療機関の認識不足のためWNV感染の診断を受けた者はわずか5%であった。また、女性および高いウイルス量の者は他の被験者より症状が発現する可能性が高かった。	赤血球濃厚液-LR「日赤」 照射赤血球濃厚液-LR「日赤」 血液を介するウイルス、細菌、原虫等の感染 vCJD等の伝播のリスク

報告企業の意見	今後の対応
米国赤十字が2003年6月から2008年にかけて、ウエストナイルウイルスRNA陽性になった供血者の経過観察及び追加検査を行い、症状の特徴が明らかになったとの報告である。	日本赤十字社では、輸血感染症対策として受付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く緊急対応(献血制限、NAT検査)のほか、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。

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symptom development, and to examine health care seeking behavior of WNV-infected persons. This study represents, to our knowledge, the largest collection of WNV infections among otherwise healthy adults across different demographic groups and geographic areas that has ever been studied.

METHODS

General approach. The study population was drawn from WNV RNA–reactive blood donors identified during routine blood donation screening from June 2003 through 2008, including those who were previously reported by Orton et al [11]. RNA-reactive donors were asked to return for a follow-up blood sample and interview. Based on subsequent laboratory testing, these donors were classified as WNV-confirmed (true positive) or unconfirmed (ie, donors with samples that had false-reactive test results during routine blood donation screening). Interviews using standardized questionnaires were conducted before completion of laboratory testing and prior to receipt of confirmatory results by donors, thus allowing a relatively unbiased assessment of symptoms, because donors did not know of their WNV confirmatory status at the time of interview. Symptoms were then compared among donors with confirmed WNV infection and donors without confirmed infection. Risk factors for symptom development among the donors with confirmed WNV infection, including viral load of the index donation, were analyzed.

Donor identification and laboratory testing. Since 2003, ARC blood donors have undergone WNV NAT screening using transcription-mediated amplification (TMA; Gen-Probe and Novartis) in minipools (MPs) of 16 donations [7, 11]. Reactive pools were resolved by individually testing each donation sample comprising the pool. Routine individual donation (ID) NAT was implemented in place of MP NAT in areas where reactive donations, defined as likely to confirm, exceeded an established trigger [7]. The triggers used to convert from MP to ID NAT in response to ongoing WNV activity progressively became more sensitive, such that currently 1 reactive donation having a high signal in a reactive MP is used to convert a defined geographic location to ID NAT [14–17]. All TMA-reactive index samples identified by ID or MP NAT were also tested for WNV RNA by research-based qualitative and quantitative polymerase chain reaction (PCR) assays (National Genetics Institute). Frozen plasma components from all TMA-reactive donations were retrieved, aliquots were prepared, and samples were tested for RNA (by PCR and TMA in replicates of up to 10) and for WNV-specific antibodies. WNV antibody testing was performed with a research immunoglobulin (Ig) M assay (Abbott Laboratories) in 2003 and a combination of US Food and Drug Administration–cleared tests for IgM and IgG starting in 2004 (Focus Technologies) according to methods described elsewhere [7]. Follow-up samples collected from consenting TMA-reactive donors were also tested for RNA (by TMA and PCR) and WNV antibodies.

Donors whose index donation samples were reactive on initial screening for WNV RNA by TMA were confirmed to have WNV infection (confirmed or true positive) through replicate RNA testing by TMA and PCR of the index donation or through observed seroconversion in follow-up testing [7, 11]. False-positive donors lacked RNA reactivity upon replicate testing of samples from the index donation using 2 different methods (TMA and PCR) and did not have IgM antibodies detected in the index donation or did not seroconvert when followed. The sensitivity of the confirmatory testing algorithm, based on index donation results by TMA, PCR, or IgM testing and verified by subsequent seroconversion (IgM or IgM with IgG), was 99% [18]. Follow-up samples were collected and questionnaires were completed within 120 days after the index donation.

Epidemiological investigation. TMA-reactive donors were notified of their initial test results by letter and were contacted at the same time to schedule a follow-up visit. The follow-up visit consisted of collection of a blood sample and a face-to-face interview conducted by trained donor counselors or donor center physicians with use of standardized questionnaires. The questionnaires gathered information regarding demographic characteristics and the presence of 14 symptoms consistent with WNV infection on the day of and/or 2 weeks after the index donation. The interviewers, using a standardized 25-question survey, also queried donors about medical care seeking, including visiting a doctor as a result of WNV-related symptoms, hospitalization because of those symptoms, and whether a WNV infection was specifically diagnosed.

Donors did not know their WNV confirmatory status at the time of interview. The final study population considered for analysis consisted of donors with completed questionnaires who either did not have confirmed infection or had confirmed WNV infection and tested IgM-antibody negative at the index donation. Because donors with confirmed WNV infection who lacked IgM antibody at the index donation donated during the earliest phase of infection and were most likely to have donated before symptoms would have developed [19], including only these donors in the study minimized potential bias attributed to symptomatic individuals who failed to donate. Preliminary analysis did, in fact, demonstrate lower post-donation symptom frequency among IgM-positive (15% reported ≥1 symptom), compared with IgM-negative (53% reported ≥1 symptom), donors with confirmed WNV RNA in their samples.

Data analysis was performed with SAS software (SAS Institute) [20]. Comparison of categorical variables was assessed using the χ^2 test. Viral load was compared with use of analysis of variance. Multivariate analysis was performed by logistic regression [21].

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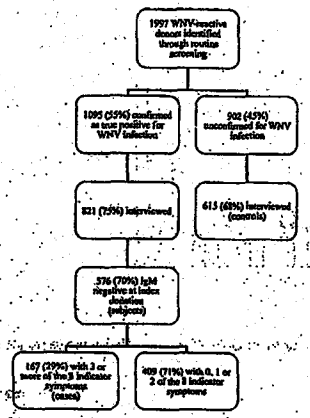

Figure 1. Study flow chart. IgM, immunoglobulin M; WNV, West Nile virus.

The ARC Institutional Review Board reviewed and approved all aspects of the study, including donor notification and consent forms for follow-up sampling and administration of the questionnaire.

RESULTS

Study population. From June 2003 through 2008, 1997 blood donors had samples that were initially reactive for WNV RNA by TMA; subsequent laboratory testing confirmed 1095 (55%) to have WNV infection, and 902 (45%) did not have infection confirmed or were found to have false-positive test results (Figure 1). A total of 966 (88%) of the 1095 donors had infection confirmed through replicate RNA testing by TMA and PCR at the time of the index donation, 116 (11%) had infection confirmed through IgM testing at the time of the index donation, and 13 (1%) had infection confirmed through IgM testing of a follow-up donation up to 63 days after the index donation (Table 1). The 1095 donors with confirmed WNV infection represented approximately one-half of the >2000 WNV-infected donors reported by all blood centers in the United States during the study period.

Completed surveys were received from 1436 (72%) of the 1997 donors. Donors confirmed as WNV RNA positive were more likely to have completed the survey (821 donors; 75%) than were donors who were not confirmed as being WNV RNA positive (615 donors; 68%; odds ratio [OR], 1.4; 95% confidence interval [CI], 1.2–1.7) (Figure 1). Seventy-eight percent of the interviews occurred within 90 days after the date of the index blood donation. There was no systematic difference in the distribution of intervals from index donation to interview between donors who were confirmed as being WNV RNA positive and those who were not confirmed as being positive (Figure 2). The frequencies of reported symptoms did not vary by duration of the follow-up interval. The final study population consisted of 576 donors who were confirmed to be positive for WNV infection and who were IgM-negative at the index donation (subjects) and 615 donors without confirmed infection (controls) (Figure 1). The remaining 245 of 821 donors with confirmed WNV infection had index donation samples that were positive for IgM. Subjects were more likely to be male (332 [58%] of 576) than were controls (292 [47%] of 615; $P < .01$).

Symptom frequency and association with WNV infection. The frequency of each symptom attributable to WNV infection was calculated by subtracting the frequency with which each symptom was reported among the 615 control donors from the frequency with which the symptom was reported among the 576 subject donors. Eight reported symptoms (new rash, generalized weakness, headache, severe muscle pain, joint pain, fever, chills and painful eyes) had an attributable frequency of >10% and had an odds of being reported by subjects that was at least 5 times the odds of being reported by controls (Table 2). Of the 576 subject donors, 270 (47%) reported none of the 8 indicator symptoms, with 85 (15%), 54 (9%), 75 (13%), 39 (7%), 23 (4%), 18 (3%), 7 (1%), and 5 (1%) reporting 1 to 8 symptoms, respectively. The symptoms were not independent from each other, judged by χ^2 analyses of each 2-symptom pair or through multivariate logistic regression analyses among subject donors, although some symptoms were more closely associated with each other (such as fever and chills or generalized weakness and severe muscle pain) than were others.

Symptomatic donors (cases) were defined as having ≥3 of

Table 1. Breakdown of Confirmatory Criteria for 1095 West Nile Virus RNA–Infected Donors from the American Red Cross, June 2003 through 2008

	No. (%) of subjects, by replicate RNA test result (TMA and PCR) at index donation	
IgM at index donation	Positive	Negative
Positive	241 (22.0)	116 (10.6)
Negative	725 (66.2)	13 (1.2)[a]

NOTE. IgM, immunoglobulin M; PCR, polymerase chain reaction; TMA, transcription-mediated amplification.

[a] Confirmed by IgM testing at follow-up 7–63 days after index donation.

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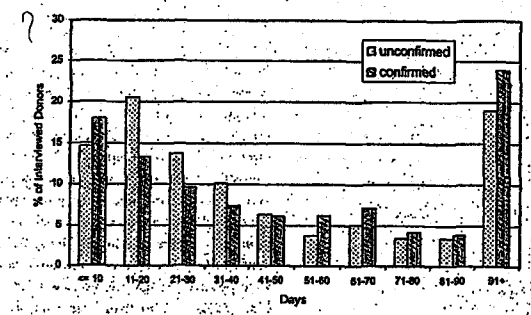

Figure 2. Distribution of intervals between index donation and interview for 821 donors confirmed as West Nile virus RNA-positive versus 615 donors without confirmed infection, American Red Cross, June 2003 through 2008.

the 8 symptoms; 167 (29%) of the 576 subjects and 20 (3%) of the 615 controls met this definition, of which the most commonly observed triad among subjects was headache, generalized weakness, and fever (55; 9.5%). Among the 167 cases, only 94 (56%) reported fever (Table 3). We estimate that 26% (29% minus 3%) of persons infected with WNV develop symptoms that meet the case definition of ≥3 of the 8 indicator symptoms due to the infection. However, it is noteworthy that 53% (306) of the subjects developed ≥1 of the 8 symptoms, compared with 11% (69) of the controls (OR, 9.0), which suggests that up to 42% of infections may result in symptoms.

Demographic characteristics and viral load. Among the 576 subjects, females (85 [35%] of 244) were more likely than were males (82 [25%] of 332) to have met the case definition for symptomatic infection ($P < .01$; OR, 1.6; 95% CI, 1.1–2.2). There was no consistent relationship between donor age and

Table 2. Frequency of Symptoms on the Day of Donation or during the 14 Days after Donation among 576 West Nile Virus–Infected Subjects and 615 Controls Identified by the American Red Cross, June 2003 through 2008

Symptom	No. (%) of donors with symptom: Subjects[a] (*n* = 576)	Controls (*n* = 615)	Odds ratio (95% CI)	Adjusted percentage of donors reporting symptoms, % (95% CI)
New rash	155 (27)	6 (1)	37.4 (16.4–85.3)	26 (22–30)
Severe muscle pain	95 (16)	11 (2)	10.8 (5.7–20.5)	15 (12–18)
Painful eyes	75 (13)	9 (1)	10.1 (5.0–20.3)	12 (9–16)
Joint pain	94 (16)	11 (2)	10.7 (5.7–20.2)	15 (11–18)
Generalized weakness	159 (28)	22 (4)	10.3 (6.5–16.3)	24 (20–28)
Chills	86 (15)	13 (2)	8.1 (4.5–14.7)	13 (10–16)
Bone pain	34 (6)	5 (1)	7.7 (3.0–19.7)	5 (3–7)
Fever	109 (19)	22 (4)	6.3 (3.9–10.1)	15 (12–19)
Swollen glands	50 (9)	11 (2)	5.2 (2.7–10.1)	7 (4–9)
Headache	178 (31)	45 (7)	5.7 (4.0–8.0)	24 (19–28)
Tremor	5 (1)	1 (0)	5.4 (0.6–46.2)	1 (0–2)
New difficulty thinking	32 (6)	8 (1)	4.5 (2.0–9.8)	4 (2–6)
Vomiting or diarrhea	62 (11)	17 (3)	4.2 (2.4–7.3)	8 (5–11)
Abdominal pain	42 (7)	13 (2)	3.6 (1.9–6.9)	5 (3–8)

NOTE. CI, confidence interval.

[a] West Nile virus RNA–positive, immunoglobulin M–negative donors.

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Table 3. Frequency of Symptoms on the Day of Donation or during the 14 Days after Donation among 167 West Nile Virus–Infected Subjects Meeting the Clinical Case Definition (≥3 Indicator Symptoms) Identified by the American Red Cross, June 2003 through 2008

Symptom	No. (%) of donors with symptom
Headache[a]	125 (75)
Generalized weakness[a]	125 (75)
New rash[a]	97 (58)
Fever[a]	94 (56)
Severe muscle pain[a]	90 (54)
Joint pain[a]	81 (49)
Chills[a]	79 (47)
Painful eyes[a]	67 (40)
Vomiting or diarrhea	45 (27)
Swollen glands	36 (22)
Abdominal pain	31 (19)
New difficulty thinking	29 (17)
Bone pain	27 (16)
Tremor	4 (2)

NOTE. Subjects were West Nile virus RNA–positive, immunoglobulin M–negative donors.

[a] Indicator symptom.

the development of symptoms ($P > .05$ among both males and females), and the females appeared to be more likely than males to develop symptoms regardless of age (Table 4).

Subjects meeting the case definition had higher viral loads detected in the index donation sample, compared with those not meeting the case definition ($P < .01$; Table 5). Viral loads were similar among males and females but differed among age groups ($P = .02$); donors 20–29 years of age had the lowest viral load (median viral load, 795 copies/mL; maximum viral load, 220,000 copies/mL). There was no linear trend of viral load with age for all other age groups (median viral load, 2035–7750 copies/mL with a maximum of 650,000 copies/mL). Logistic regression analysis of the relationship between viral load detected in the index donation, sex, and age and meeting the case definition showed that the odds of meeting the case definition were again significantly higher in women (OR, 1.7; 95% CI, 1.2–2.4) and increased ~1.5 times for every 10-fold increase in viral load (OR, 1.5; 95% CI, 1.2–1.8); age was not statistically significant ($P = .43$).

Medical care seeking among donors with confirmed WNV infection. Of the 167 subject donors who met the case definition during the 2 weeks after blood donation, 73 (44%) reported having visited a doctor because of their WNV-related symptoms, and 5 (3%) were hospitalized. Among the 615 controls, 38 (6%) reported having visited a doctor because of their symptoms, and 6 (1%) were hospitalized; none received a diagnosis of WNV infection. These results suggest that 38% (44% minus 6%) of donors who met the case definition had a doctor visit attributable to WNV infection, and similarly, 2% (3% minus 1%) were hospitalized as a result of their symptoms. Among the 73 patients who reported seeking medical care, only 4 (5%) of the 5 hospitalized patients received a diagnosis of WNV infection, and none received a diagnosis of neuroinvasive disease. Because interviews were conducted before final confirmatory notification (that is, donors did not know their final WNV test results at the time of the interview), all donors who sought medical care should not have been influenced by a definite diagnosis of WNV infection. Therefore, comparing subject donors who met the case definition with control donors, as was done in this study, provides an estimate of the proportion of WNV-infected individuals who seek medical care.

DISCUSSION

This study suggests that 26 percent of persons infected with WNV develop symptoms that meet our case definition and are attributable to the infection. Our case definition, which is based

Table 4. Analysis of Relationship of Donor Age to Case Definition Stratified by Sex among 576 West Nile Virus–Infected Subjects Identified by the American Red Cross, June 2003 through 2008

Age, years	Female sex: No. of subjects	Female sex: No. (%) of cases	Male sex: No. of subjects	Male sex: No. (%) of cases	OR (95% CI)
<20	15	5 (33)	14	4 (29)	1.3 (0.3–6.1)
20–29	18	7 (39)	21	4 (19)	2.7 (0.8–11.5)
30–39	26	13 (50)	33	8 (24)	3.1 (1.0–9.5)
40–49	83	30 (36)	96	30 (31)	1.2 (0.7–2.3)
50–59	63	16 (24)	98	28 (29)	0.8 (0.4–1.6)
≥60	39	15 (38)	70	8 (11)	4.8 (1.8–12.9)

NOTE. Subjects were West Nile virus RNA–positive, immunoglobulin M–negative donors.

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Table 5. Viral Load among 576 West Nile Virus–Infected Subjects Identified by the American Red Cross, June 2003 through 2008

Variable	No. of subjects[a]	Viral load, geometric mean $\log_{10}$ copies/mL[b] (± SD)	Viral load			P
			Median copies/mL	Minimum copies/mL	Maximum copies/mL	
Met the case definition[c]						
No	400	3.39 ± 1.11	1900	5	650,000	<.01
Yes	163	3.82 ± 0.99	7900	50	580,000	
Sex						
Female	241	3.53 ± 1.07	3500	5	650,000	.87
Male	322	3.51 ± 1.11	3400	5	530,000	
Age						
<20	29	3.29 ± 1.04	2035	100	230,000	.02
20–29	37	3.19 ± 1.07	795	5	220,000	
30–39	57	3.88 ± 0.79	7750	50	580,000	
40–49	175	3.61 ± 1.16	4750	5	650,000	
50–59	158	3.42 ± 1.09	2550	5	470,000	
≥60	107	3.49 ± 1.10	2850	5	530,000	

NOTE. Subjects were West Nile virus RNA–positive, immunoglobulin M–negative donors.

[a] Viral load data were only available for 563 donors.

[b] The case definition was ≥3 of the following 8 symptoms: new rash, severe muscle pain, painful eyes, joint pain, generalized weakness, headache, fever, and chills.

on the presence of at least 3 of 8 indicator symptoms (new rash, severe muscle pain, painful eyes, joint pain, generalized weakness, headache, fever, and chills), was highly correlated with WNV infection, having been met by 29% of the subjects and only 3% of the controls. Nevertheless, the estimate that 26% of WNV-infected persons become symptomatic may be low, because the presence of even 1 of the 8 indicator symptoms was highly predictive of WNV infection; ≥1 symptom was present in 53% of subjects versus 11% of controls (attributable percentage, 42%).

Our results can be compared with results from several other blood donor follow-up studies. Brown et al [12], who used a case definition of the presence of both fever and headache, estimated that 30% of infected persons become symptomatic; however, no WNV-negative control group was present for adjustment. In a study by Custer et al [13] that used a definition of symptomatic infection of ≥3 symptoms, 34% of WNV-infected donors and 20% of false-positive donors were symptomatic, which suggests that only 14% had symptoms that were attributable to WNV. It is unknown why the Custer et al [13] study had a relatively small difference observed between the percentage of subjects and the percentage of controls, which yielded a substantially lower rate of adjusted symptom reporting. This may have been related to a less rigorous laboratory-based definition of subjects and controls [13]. However, even with only 14% of individuals having symptoms attributable to WNV infection, Custer et al [13] reported a remarkably similar distribution of individual symptoms, with headache being the most common.

One notable finding of our study was the absence of reported fever among a substantial proportion of the symptomatic persons (44%). This finding was corroborated by a study involving 534 persons with WNF identified by surveillance in California, in which 31% of subjects did not report fever [22]. These findings suggest that many symptomatic WNV infections are without recognized fever.

Our data indicate that many WNV-related illnesses are clinically significant but are nonspecific and remain undiagnosed. Of the 26% of persons infected with WNV who developed symptoms meeting our case definition that were attributable to infection, we observed that 38% had visited a doctor for their symptoms, and 2% were hospitalized as a result of the infection. However, only 5% of those who sought medical care received a diagnosis of WNV infection. These results are higher than those reported in the study by Custer et al [13], which found that an adjusted 4% of individuals sought medical care (ie, 12% of all confirmed-positive donors, regardless of the number of symptoms, compared with 8% of false-positive donors). Similarly, only 2 (6%) of the 35 confirmed-positive donors who sought medical care received a diagnosis of WNV infection [13]. Only 12 (9%) of 135 viremic donors, or 29% of those meeting the study case definition for WNF, sought medical care in Colorado [12]. This low rate of recognition of WNF is consistent with surveillance data that shows that the

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15,800 WNF cases reported through 2007 in the United States were a small fraction of the >300,000 infections that are estimated to have occurred [23].

Similar to the findings of Brown et al [12], we found that a higher plasma viral load at the time of index donation predicted the development of symptoms. However, there were inconsistencies with other studies regarding other possible factors associated with reported symptoms. Although Brown demonstrated decreasing proportions of infected persons developing symptoms as donor age increased, particularly among men [12], no such relationship was observed in our study or in the study of Custer et al [13]. In our study, women were more likely to have reported symptoms (OR, 1.6), similar to the report by Custer et al [13] (OR, 1.4). Nevertheless, Brown et al [12] found no relationship between sex and symptom development. We observed similar plasma viremia levels between men and women, which suggests that plasma viremia level is not responsible for increased reporting of symptoms among women.

Several limitations to our study exist. Approximately one-quarter to one-third of NAT-reactive donors did not consent to interview. If donors who developed symptoms were more likely to participate, this would bias the study towards an increased frequency of symptoms. The initial notification of preliminary WNV screening test results could have increased nonspecific symptom reporting, although there should not be a differential effect between subjects and controls. Blood donors are healthier than the general population and thus may not be representative of the entire population. However, underlying illnesses or conditions that may influence the development of WNF have not been identified in the general population. In addition, plasma viremia levels were measured only on the day of index donation and thus may not reflect peak levels or the extent of viral replication and dissemination throughout the acute infection period. Nevertheless, the time of donation in relationship to each donor's plasma viremia curve should be randomly distributed among persons within each age and sex subgroup, and thus, comparisons among these groups should reflect true population differences in viremia levels.

In summary, our data provide several insights into the clinical characteristics of WNV infection. Our data demonstrate that demographic risk factors for neuroinvasive disease strikingly contrast with those for WNF. Although surveillance data indicate that men are at greater risk than are women for neuroinvasive disease [24], our data suggest that women may be at higher risk for developing WNF. The substantial increase in the risk of neuroinvasive disease with advancing age [24] was not observed for WNF in our study. Although 26% of our WNF-confirmed study population developed symptoms that met our case definition and were attributable to infection, our analysis suggested that as many as 42% had ≥1 indicator symptom that was attributed to the infection. The fact that nearly one-half of the patients who met our symptomatic case definition sought medical care yet few received a diagnosis of WNV infection suggests that WNF has significant but largely unrecognized clinical impact [25].

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研究報告の概要

○ボリビアとペルーにおけるヒトへのGuaroaウイルス感染

コロンビアでは1959年に初めてGuaroaウイルス(GROV)がヒトから分離された。その後、ブラジル、コロンビア、パナマの発熱患者および蚊からウイルス分離株が採取されたが、ヒトの疾患とウイルスの関連性は不明であった。ボリビアとペルーの発熱疾患患者からGROV14株が分離され、また3症例でIgMセロコンバージョンが確認された。ペルーの都市、イキトス居住者の抗GROV抗体陽性率は13%であり、林業、漁業、油田労働等の職に就く者がもっとも陽性率が高かった。代表的なGROV分離株の遺伝子学的特性からは、ボリビアとペルーの株が、以前にブラジルとコロンビアで分離された株とは異なる可能性のある単一系統グループを形成していることが示された。本試験で、GROVが中南米の熱帯地域における発熱疾患の原因であることが確認された。

使用上の注意記載状況・その他参考事項等

解凍赤血球濃厚液「日赤」
照射解凍赤血球濃厚液「日赤」
解凍赤血球-LR「日赤」
照射解凍赤血球-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
今までヒトの疾患とGuaroaウイルス(GROV)の関連は不明であったが、調査の結果、GROVが中南米の熱帯地域における発熱疾患の原因であることが確認されたとの報告である。	日本赤十字社では、輸血感染症対策として受付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。

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Guaroa Virus Infection among Humans in Bolivia and Peru

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Abstract. Guaroa virus (GROV) was first isolated from humans in Colombia in 1959. Subsequent isolates of the virus have been recovered from febrile patients and mosquitoes in Brazil, Colombia, and Panama; however, association of the virus with human disease has been unclear. As part of a study on the etiology of febrile illnesses in Peru and Bolivia, 14 GROV strains were isolated from patients with febrile illnesses, and 3 additional cases were confirmed by IgM seroconversion. The prevalence rate of GROV antibodies among Iquitos residents was 13%; the highest rates were among persons with occupations such as woodcutters, fisherman, and oil-field workers. Genetic characterization of representative GROV isolates indicated that strains from Peru and Bolivia form a monophyletic group that can be distinguished from strains isolated earlier in Brazil and Colombia. This study confirms GROV as a cause of febrile illness in tropical regions of Central and South America.

INTRODUCTION

Guaroa virus (GROV) was first isolated in Guaroa, Meta Department, Colombia, in 1959 from people without overt illness.[1] Subsequently, isolates of GROV have been made from febrile persons in Brazil and mosquitoes in Colombia, Panama, and Brazil.[2–4] Epidemiological investigations conducted in Colombia from 1956–1961 revealed that a significant number of people living in the Middle Magdalena Valley (especially adults) had GROV antibodies.[5] Ecological investigations conducted thereafter have repeatedly isolated GROV from *Anopheles (Kerteszia) neivai*; consequently, this species has been implicated as the putative mosquito vector of GROV, which constitutes a rare event for arboviruses.[4,6] Other arboviruses potentially transmitted by *Anopheles* mosquitoes include Breu Branco, Kadipiro, and Getah.[7,8]

Follow-up serologic studies in the town of Guaroa (Colombia) in 1956 indicated that 49 of 69 (75%) residents of the community had neutralizing antibodies to GROV.[1] Another serosurvey in northern Brazil (Para state) found that 18% of residents had hemagglutination-inhibition (HI) antibodies to GROV.[2] Low titers of HI antibodies to GROV have also been reported in sera of residents of Argentina, Sao Paulo state in Brazil, Peru, and Guatemala.[9] Collectively, the results of these studies suggest that GROV is widely distributed in Central and South America; however, its association with a specific human illness or disease syndrome remains unclear.

The virus is a member of the family *Bunyaviridae*, genus *Orthobunyavirus*; it contains a segmented negative-strand RNA genome of three segments (S, M, and L). The L segment encodes the L protein (RNA polymerase); the M segment encodes the polyprotein precursor of the virion glycoproteins, G1 and G2, and the nonstructural protein NSm, and the S segment encodes for the N and NSs proteins.[10–12] The taxonomic status of GROV has been controversial. Initially, GROV was considered to be a member of the California antigenic group (family *Bunyaviridae*) based on the results of hemagglutination-inhibition HI tests.[13,14] Subsequently, Whitman and Shope[14] showed that GROV was antigenically related to viruses in both the California and Bunyamwera serogroups. Based on the results of complement-fixation (CF) tests, GROV could be placed in the Bunyamwera group, but based on neutralization tests, it was more closely related to the California group. Because the two tests measure different gene products (nucleocapsid and glycoproteins, respectively), Bishop[15] suggested that GROV represented a reassortant virus that possesses RNA segments originally derived from Bunyamwera and California virus groups. Subsequent sequence data for the S RNA of GROV indicated that it should be classified in the Bunyamwera serogroup rather than the California serogroup.[16] In the Eighth Report of the International Committee on Taxonomy of Viruses,[10] GROV is currently classified as a unique species within the genus *Orthobunyavirus*, and it is considered distinct from viruses included in the Bunyamwera and California species complexes.

In 2000, the United States Naval Medical Research Center Detachment (NMRCD) in Lima, in collaboration with the Ministries of Health of Bolivia and Peru, initiated a passive surveillance study to investigate etiology of febrile illnesses. As part of the surveillance program in Peru and Bolivia, 17 confirmed cases of GROV infection were diagnosed in patients with acute, self-limited febrile illnesses. Nine of seventeen confirmed cases were reported in 2007 in Peru (between January and November), and two cases were also confirmed in 2007 and 2009 in febrile patients from Bolivia, providing the first evidence that GROV also circulates in that country.

In the present study, we investigated the epidemiology of GROV infection, the prevalence of GROV antibodies and risk factors for infection among residents of the Amazonian city of Iquitos, Peru, and the phylogenetic relationship among the GROV strains isolated in Peru, Bolivia, and other South American countries.

MATERIALS AND METHODS

Study sites. The confirmed GROV human cases reported in this study lived in several distinct areas of Peru, including the cities of Iquitos in the Department of Loreto, Puerto Maldonado in the Department of Madre de Dios, La Merced in the Department of Junin, and Tumbes on the coast. Iquitos is a city of about 380,000 inhabitants located 120 m above sea level in the Amazon Basin in northeastern Peru. Puerto Maldonado is a city of approximately 56,000 habitants located in southeastern Peru about 256 m above sea level on the banks of the Madre de Dios river near the border with Brazil and Bolivia. La Merced is a city of about 50,000 persons located 751 m above sea level in the Department of Junin. Tumbes is a Pacific coastal city located in the north near the border with Ecuador; according to the 2007 census, it had a population of 139,811 inhabitants. Two GROV isolates were also obtained from febrile patients living in Cochabamba Department in Bolivia. Figure 1 shows the approximate geographic locations of the study sites where GROV infections were confirmed.

Passive febrile surveillance study population. The study protocols were approved by the Ministries of Health of Peru and Bolivia and the Naval Medical Research Center Institutional Review Board (protocols NMRCD.2000.0006, NMRCD.2000.0008, and NMRCD.2008.0002). The study subjects were patients (> 5 years of age) who presented with a diagnosis of an acute, febrile undifferentiated illness in their home or at military or civilian outpatient clinics at the study sites. Demographic and clinical information was obtained from each patient at the time of voluntary enrollment, and a signed consent form was obtained from each subject. The criteria for inclusion in the program were fever ≥ 38°C of no more than 5 days in duration, headache, myalgia, and other nonspecific symptoms. Two paired blood samples were collected, one during the acute phase of illness and the second sample 2–4 weeks after onset of symptoms. Acute samples were tested for virus by cell culture, and both acute and convalescent samples were assayed for IgM antibodies to a variety of arboviruses (including GROV) by an enzyme-linked immunosorbent assay (ELISA), as described previously.[17] Diagnosis of a confirmed GROV infection (case) was based on isolation of the virus and/or a 4-fold or greater increase in IgM antibody titer between the acute and convalescent serum samples. A case was considered as presumptive when IgM antibodies were detected in a single acute sample or in both acute and convalescent samples without a 4-fold increase in titer.

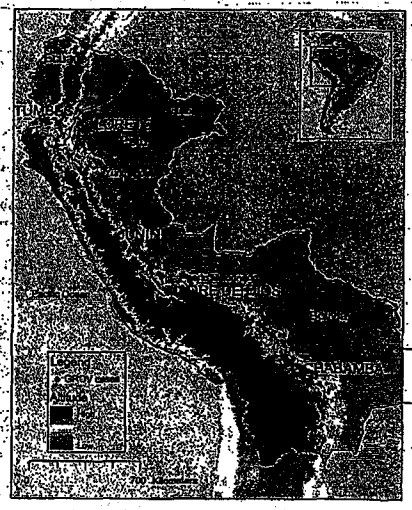

FIGURE 1. Geographic distribution of the Guaroa virus human cases identified as part of the febrile disease surveillance program. This figure appears in color at www.ajtmh.org.

Antibody prevalence studies. The antibody prevalence of GROV was determined in Iquitos by testing a total of 1,124 human serum samples for IgG antibodies to GROV by an ELISA, as previously described.[17] The samples were collected in 2006 as part of a cross-sectional antibody prevalence study carried out in Iquitos after an outbreak of febrile illness associated with Venezuelan equine encephalitis virus (VEEV) infection. Samples were collected in three Iquitos neighborhoods where Venezuelan equine encephalitis (VEE) cases were reported as well as in a control neighborhood where VEE cases were not reported.[18] Thus, the selected population represented a suitable population to test the prevalence of GROV and other arboviral diseases. Serum samples from a subset of the original study participants, who agreed to the future use of their samples, were tested. All ELISA IgG antibody-positive samples were further evaluated using an 80% plaque-reduction neutralization assay (PRNT) for GROV. Briefly, sera were heat-inactivated at 56°C for 30 minutes, and two 2-fold serum dilutions were prepared, mixed with 100 plaque forming units (PFUs) of GROV (prototype Peruvian strain OBS 0069), and incubated at 4°C overnight. The virus–serum dilutions mixtures were inoculated onto confluent monolayer of Vero cells propagated in microplates and incubated at 37°C for 1 hour before adding an overlay of 0.4% of agarose in Eagle's minimum essential medium (EMEM). After 72 hours of incubation at 37°C, the plates were stained with 0.25% crystal violet in 20% methanol, and plaques were counted. All IgG-positive samples were tested at an initial concentration of 1:20, and all positive sera were further titrated to the endpoint. Neutralization titers were considered as the highest serum dilution that reduced plaque formation by ≥ 80%.

Virus isolation. Patient's serum specimens were diluted 1:5 in EMEM, supplemented with 2% fetal bovine serum, 200 μg streptomycin, and 200 U/mL penicillin. Two hundred microliters of diluted samples were then inoculated into flasks with confluent monolayers of African green monkey kidney cells (Vero) and *Aedes albopictus* mosquito (C6/36) cells. Vero cell cultures were examined daily for evidence of viral cytopathic effect (CPE). Spot slides of C6/36 and Vero cells were subsequently prepared, and an immunofluorescence assay (IFA) was done using polyclonal antibodies against arboviruses endemic in Peru.[17,19–22] A variety of arboviruses were isolated from these samples and will be reported elsewhere. The 14 GROV isolates are listed in Table 1.

Extraction of RNA, reverse transcription, and PCR amplification of S, M, and L segments. Viral RNA was extracted using the QIAamp viral RNA mini kit (Qiagen, Valencia, CA) or Trizol reagent (Invitrogen, Carlsbad, CA) following the manufacturer's protocol. The reverse transcription reaction (RT) was done using 1× RT buffer, 0.2 mM deoxyribonucleotide triphosphate (dNTPs), 1 μM primers, 80 units RNAsin ribonuclease inhibitor (Promega, Madison, WI), 1 mM dithiothreitol, 200 U SuperScript reverse transcriptase (Invitrogen), and 5 μL RNA. The reactions were incubated at 42°C for 1 hour. The PCR

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TABLE 1

Guaroa confirmed cases included in the study

Strain	Study	Location	Year	Host	Age	Sex	Occupation	Laboratory diagnostic
CoH 352111	NA	Colombia	1956	Human	NA	NA	NA	Virus isolation*
CoAr 2526	NA	Colombia	1964	Mosquito	NA	NA	NA	*
BeH 22063	NA	Para, Brazil	1960	Human	NA	NA	NA	Virus isolation*
31498	NA	NA	NA	NA	NA	NA	NA	*
FVB 0546	Febrile surveillance	Cochabamba, Bolivia	2007	Human	17	Female	Student	Virus isolation
FVB 2032	Febrile surveillance	Cochabamba, Bolivia	2009	Human	24	Male	Agricultural worker	Virus isolation
OBS 0069	Outbreak investigations	Iquitos, Loreto, Peru	1995	Human	38	Male	Agricultural worker	Virus isolation
IQU 1091	Febrile surveillance	Iquitos, Loreto, Peru	1999	Human	30	Male	Air Force	Virus isolation
IQD 8537	Febrile surveillance	Iquitos, Loreto, Peru	2004	Human	31	Male	Agricultural worker	Virus isolation
FSJ 1266	Febrile surveillance	La Merced, Junin, Peru	2006	Human	32	Male	Agricultural worker	Seroconversion
FSJ 1318	Febrile surveillance	La Merced, Junin, Peru	2007	Human	30	Male	Driver	Virus isolation
FSJ 1335	Febrile surveillance	La Merced, Junin, Peru	2007	Human	35	Male	Agricultural worker	Virus isolation
FSJ 1340	Febrile surveillance	La Merced, Junin, Peru	2007	Human	43	Male	Driver	Virus isolation
FST 1122	Febrile surveillance	Zarumilla, Tumbes, Peru	2007	Human	27	Female	Seller	Seroconversion
FMD 1553	Febrile surveillance	Iberia, Madre de Dios, Peru	2007	Human	17	Female	House wife	Virus isolation
OBT 5637	Outbreak investigations	Puerto Maldonado, Madre de Dios, Peru	2007	Human	21	Female	House wife	Virus isolation
OBT 5655	Outbreak investigations	Puerto Maldonado, Madre de Dios, Peru	2007	Human	30	Male	Local healer	Virus isolation
OBT 5667	Outbreak investigations	Puerto Maldonado, Madre de Dios, Peru	2007	Human	27	Male	Miner	Virus isolation
FMD 1720	Febrile surveillance	Madre de Dios, Peru	2007	Human	17	Female	Miner	Seroconversion
FMD 1806	Febrile surveillance	Tambopata, Madre de Dios, Peru	2008	Human	37	Male	Health worker	Virus isolation
MIS 0239	Other	Puerto Maldonado, Madre de Dios, Peru	2008	Human	NA	Male	NA	Virus isolation

NA = not applicable.
*Samples provided by the World Health Organization Reference Collection, University of Texas Medical Branch (UTMB).

included 1× PCR buffer, 0.25 mM dNTPs, 1 μM primers, 3 mM $MgCl_2$, 2.5 U *GoTaq DNA polymerase (Promega)*, and 5 μL cDNA. The conditions for the PCRs included incubation at 94°C for 2 minutes, 35 cycles of 94°C for 30 seconds, 50°C for 1 minute, 72°C for 1.5 minutes, and a final extension of 72°C for 10 minutes to ensure complete double-stranded DNA synthesis. The primers used for the PCR amplification have been previously described and included Bunya 1 (GTCACA GTAGTGTACTCCAC) and Bunya 2 (CTGACAGTAGTGT GCTCCAC), which amplifies the S segment, M14C (CGGA ATTCAGTAGTGTACTACC) and M619R (GACATATG(CT) TGATTGAAGCAAGCATG) that amplifies the M segment, and M13CBunL1C (TGTAAAACGACGGCCAGTAGTGT ACTCCT) and BunL605R (AGTGAAGTCICCATGTGC), which amplifies the L segment.[24]

Sequencing and phylogenetic analyses. To genetically characterize the GROV strains isolated in Peru, partial sequences of the S, M, and L segments were obtained and compared with those of GROV isolates from Brazil, Colombia, and Bolivia using a previously described methodology.[24] Purified PCR products were sequenced directly, and sequencing analyses of the PCR products were performed using an Applied Biosystems (Foster City, CA) Prism automated DNA sequencing kit according to the manufacturer's protocol. Sequences were aligned using the Clustal program in the MacVector (MacVector Inc., Cary, NC) software package, and phylogenetic analyses were performed using the maximum parsimony, neighbor-joining, and maximum likelihood methods implemented in the phylogenetic analysis using parsimony (PAUP) software (Sinauer Associates, Sunderland, MA).[25,26] For the neighbor-joining analyses, the HKY85 distance was used. Bootstrap values to place confidence values on groupings within trees were calculated based on 1,000 replicates.

Statistical analyses. Proportions were compared using a χ^2 test using the FREQ procedure in SAS (SAS version 8; SAS Institute Inc., Cary, NC). Risk factors for infection with GROV were evaluated by logistic regression using LOGISTIC in SAS. Models were constructed with the dichotomous dependent variable: PRNT positive for GROV antibody at a titer of ≥ 20 and the following independent variables: gender, age (adult or child), occupation, type of house, travel history, and neighborhoods.

RESULTS

Description of GROV cases detected through febrile surveillance. In 1995, GROV was isolated for the first time in Peru from a patient presenting with an undifferentiated febrile illness. Between 2000 and 2009, 16 additional confirmed GROV cases and 30 presumptive cases were identified in patients presenting with febrile illness. The most common symptoms among these patients were headache, chills, malaise, myalgia, arthralgia, and bone pain (Table 2). The majority of patients with confirmed GROV infection were males (12 of 17, 70.6%) with a mean age of 28.5 years (range = 17–43) (Table 1). These patients were mainly miners, wood cutters, agricultural workers, and students living in areas with high levels of arbovirus circulation.[17,19–23,27–30] Fifteen of the cases were detected in Peru, whereas two GROV cases were confirmed in Cochabamba, Bolivia, providing the first evidence of circulation in that country. The Bolivian patients were a 17-year-old female student and a 24-year-old male agricultural worker. Year-round GROV activity was observed in Peru.

The largest number of GROV cases were detected in 2007 and 2008 from samples collected in La Merced, Junín, and Puerto Maldonado, Madre de Dios. One GROV case was detected in the coastal city of Tumbes in Peru in 2007 (Tables 1 and 3). A slight increase in GROV activity was observed in 2007 in La Merced and in 2008 in Madre de Dios (Table 3). The majority of patients with GROV infection (44% of the cases) in Puerto Maldonado reported having recently visited or worked in Bajo Puquiri, a gold-prospecting and extraction area in Madre de Dios Department that is currently undergoing intensive environmental modification.

Risk factors for GROV infection in the city of Iquitos, Peru. Antibody prevalence and risk-factor data were obtained from blood samples collected as part of a cross-sectional antibody prevalence study carried out in four neighborhoods in Iquitos after an outbreak of febrile illness associated with VEEV infection.[18] Iquitos was selected for study, because GROV was first detected in this city in 1995 and thus, there was evidence that the virus had been circulating in the area for more than 10 years.

In Iquitos, the overall antibody prevalence of GROV was 15% (184/1,124). The prevalence of GROV antibodies in the Iquitos population increased with age after adulthood (> 19 years of age), suggesting endemic circulation of the virus in this Amazon region of Peru (4.4% in 5- to 9-year-olds to 35.9% in 60- to 69-year-olds) (Figure 2). The antibody prevalence in adults was 16% compared with 5.6% in children [odds ratio (OR) = 3.3; 95% confidence interval (CI) = 1.992–5.467]. Persons who reported overnight travel had higher antibody prevalence rates than those who did not ($P < 0.05$).

Persons with high-risk occupations were fishermen, wood cutters, and oil workers; these groups had a higher prevalence of GROV antibodies than people with other occupations [7/23 (30%) versus 137/1,101 (12%)]. In addition, persons living in concrete/brick houses had significantly lower antibody prevalence rates than those living in wood houses (OR = 0.312; 95% CI = 0.179–0.546), and persons living in neighborhoods closer to the rivers surrounding the city (Belen, Bellavista, and San Juan) also had a higher antibody prevalence than those in the north-central parts of Iquitos where socio-economic conditions are higher (Figure 3). The univariate logistic regression analysis did not detect an association between gender and GROV antibody prevalence.

Genetic characterization of the GROV isolates from Peru and other regions of South America. Phylogenetic analyses using maximum parsimony, neighbor-joining, and maximum likelihood methods all generated similar tree topologies. Only the neighbor-joining phylogenetic trees are shown for simplicity reasons. The neighbor-joining tree based on the partial S and L segment sequences revealed a single genotype within the isolates from Peru and Bolivia. In contrast, strains from Colombia and Brazil (isolated between 1956 and 1964) differed by 4% at the amino acid level compared with the more recent (1995–2008) Peruvian and Bolivian isolates, and thus, they group within different genotypes in the phylogenetic tree (Figures 4 and 5). The neighbor-joining tree based on the

TABLE 2

Principal clinical manifestations for 13 patients with confirmed Guaroa virus infection

Sign and symptom	Patients (%)
Headache	92
Malaise	92
Chills	85
Myalgia	77
Arthralgia	69
Bone pain	62
Retro-orbital pain	54
Nausea	46
Asthenia	46
Abdominal pain	23
Conjunctival injection	23
Vomiting	23
Rash	15
Rhinorrhea	15
Cough	15
Weight Loss	8
Petechiae	8
Ear pain	8
Arthritis	8
Expectoration	8

TABLE 3

Cases of Guaroa virus infection among febrile patients residing in Peru and Bolivia

	Madre de Dios			Junín				Iquitos, Loreto							Tumbes		Cusco	Yurimaguas		Bolivia	
Guaroa virus cases	2007	2008	2009	2006	2007	2008	2009	1995	1999	2004	2005	2007	2008	2009	2007	2008	2008	2007	2009	2007	2009	Total
Virus isolations	4	2	0	0	3	0	0	1	1	1	0	0	0	0	0	0	0	0	0	1	1	14
Seroconversion	1	0	0	1	0	0	0	ND	ND	ND	0	0	0	0	0	1	0	0	0	0	0	3
Presumptive cases	3	8	2	0	3	1	0	ND	ND	ND	1	2	3	2	0	0	3	1	1	0	0	30
Total confirmed and presumptive GROV cases	8	10	2	1	6	1	0	1	1	1	1	2	3	2	1	0	3	1	1	1	2	50
Total number febrile cases seen at site	593	301	355	120	105	133	111	867	810	2,308	1,459	1,197	1,885	1,104	193	264	121	309	280	645	537	13,697
Percentage GROV cases/ total febrile cases	1.3	3.3	0.6	0.8	5.7	0.8	0	0.1	0.1	0.04	0.07	0.2	0.2	0.2	0.5	0	2.5	0.3	0.4	0.2	0.4	0.37

ND = not done.

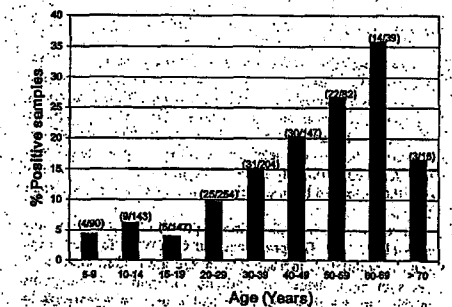

FIGURE 2. Guaroa virus antibody prevalence among residents by age groups in Iquitos.

M segment produced a similar tree topology as the S and L phylogenetic tree; however, the strains isolated from Junín, Perú grouped within a distinct genotype from the other isolates from different geographical regions in Peru (Figure 6).

DISCUSSION

GROV was initially isolated in Colombia from asymptomatic individuals as well as from persons exhibiting mild fever, raising the question of whether the virus consistently causes disease.[1] Later, GROV cases identified in Brazil included individuals with fever and other symptoms, such as headache, myalgia, and prostration; however, several of these subjects also had *Plasmodium falciparum* infections.[31,32] GROV was also isolated from the liver biopsy of a Brazilian patient with paralysis.[2] In the present study of febrile illnesses in Peru and Bolivia, additional evidence for the disease potential of GROV was obtained. Seventeen patients who presented with undifferentiated febrile illnesses were diagnosed with GROV infection.

The most common clinical symptoms in the patients with confirmed GROV infection were chills, malaise, bone pain, headache, retro-orbital pain, myalgia, and arthralgia. Given the non-specific clinical manifestations in these cases, it would be very difficult to differentiate GROV infection clinically from other endemic arboviral illnesses. Because our surveillance activities included only eight sites in Peru and three in Bolivia (Figure 1), it is very likely that GROV infections occur in other areas of these countries but remain undiagnosed. Additional studies are needed to determine the ratio of apparent to unapparent cases and to fully measure the burden and the public-health impact of GROV infection in Peru, Bolivia, and other regions of South America.

Previous epidemiologic studies conducted in South America revealed a high antibody prevalence to GROV in selected

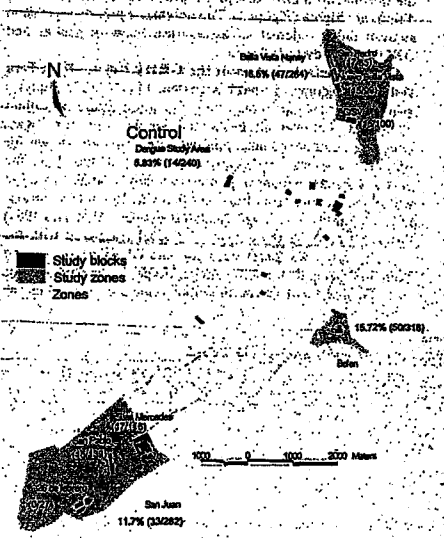

FIGURE 3. Guaroa virus antibody prevalence among residents by neighborhood in Iquitos.

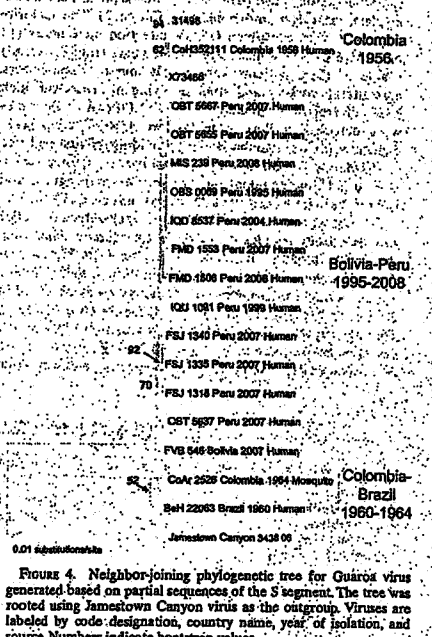

FIGURE 4. Neighbor-joining phylogenetic tree for Guaroa virus generated based on partial sequences of the S segment. The tree was rooted using Jamestown Canyon virus as the outgroup. Viruses are labeled by code designation, country name, year of isolation, and source. Numbers indicate bootstrap values.

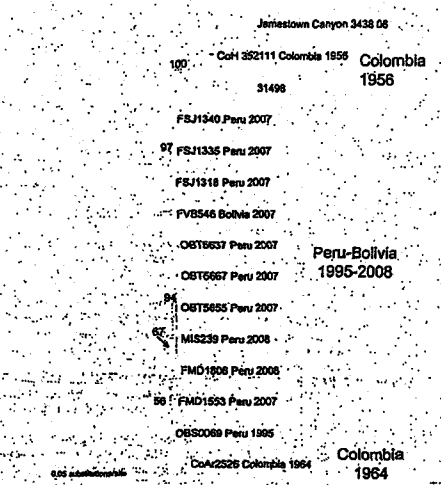

FIGURE 5. Neighbor-joining phylogenetic tree for Guaroa virus generated based on partial sequences of the M segment. The tree was rooted using Jamestown Canyon virus as the outgroup. Viruses are labeled by code designation, country name, and year of isolation. Numbers indicate bootstrap values.

populations in Colombia (43%) and Brazil (18%).[1,31] Low HI antibody titers to GROV antigen were also found in sera of residents from Argentina, Peru, and Guatemala.[3] However, further testing of the positive samples, using a confirmatory PRNT to GROV, was not performed. Because of the cross-reactivity of HI antibodies among orthobunyaviruses, it is uncertain whether those antibodies were specific to GROV or to other related members of this genus that are known to circulate in the area.[31,33]

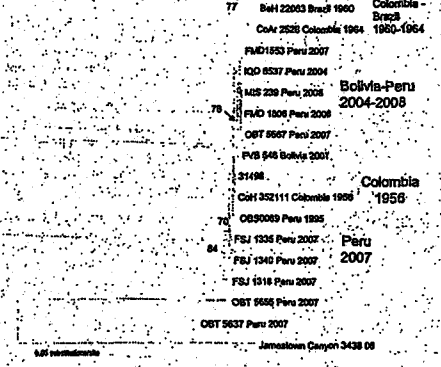

FIGURE 6. Neighbor-joining phylogenetic tree for Guaroa virus generated based on partial sequences of the L segment. The tree was rooted using Jamestown Canyon virus as the outgroup. Numbers indicate bootstrap values.

To further investigate the frequency of GROV infection in Peru and the risk factors associated with infection in an endemic area of transmission, human serum samples collected in Iquitos in 2006 were assayed for GROV antibodies. An overall antibody prevalence rate of 13% was found among the Iquitos population. These results are consistent with previous serological studies done in Peru in 1965.[29] Factors associated with infection in Iquitos included living in neighborhoods located near surrounding rivers that are seasonally flooded and occupations such as agriculture, fishing, and mineral prospecting and extraction. Results from our study also suggested that GROV transmission probably occurs in the forest or away from home.

Although GROV was first detected in Iquitos in 1995, the number of confirmed infections identified in our current surveillance activity was relatively low (~0.04–0.2%), despite the 13% overall antibody prevalence rate. In contrast, the presence of GROV in Madre de Dios was not detected until 2007, when evidence of GROV infection was observed in about 1% of the febrile cases in the region. By 2008, 3% of the febrile cases at the site had evidence of recent GROV infection. Eight of eighteen patients with evidence of GROV infection (2007–2008) reported recent work or travel in the area. It seems likely that environmental and land-use changes as well as human migration have played an important role in the emergence of this human pathogen in Madre de Dios. Improper and unregulated mining activities have been implicated as a factor responsible for the emergence of other vector-borne diseases such as malaria and yellow fever in Brazil.[34] Further research is needed to determine more precisely the impact of these activities on the emergence of GROV and other arboviral diseases in Madre de Dios.

Despite our current knowledge of GROV as a human pathogen, little is known about its reservoir hosts and vectors, especially in Peru. Earlier investigations in Colombia, Panama, and Brazil detected the virus in *Anopheles* mosquitoes[4,31]; however, studies to evaluate the susceptibility and transmission potential of this mosquito genus have not been done. During previous ecological studies conducted in the Amazon region of Peru, many arboviruses were isolated from other mosquito genera,[29] but GROV was never isolated from these other mosquitoes, suggesting that they are not involved in GROV transmission. Additional ecological studies are needed in Peru and Bolivia to identify the mosquito vectors and reservoir hosts involved in GROV transmission. Likewise, previous serological investigations in Brazil suggested that birds may act as reservoir hosts for GROV; however, these results remain to be confirmed.[3]

In summary, results of this study further confirm that GROV is a cause of febrile illness among humans in tropical regions of Central and South America.

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別紙様式第2-1

No. 13

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2010. 10. 8	該当なし	
一般的名称	人赤血球濃厚液		研究報告の公表状況	Stone R. Science. 2010 Oct 1;330(6000):20-1.	公表国	
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)				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
○感染症:中国中央部における新型致死性ウイルスの特定 中国中央部でこの3年間、夏になると数百人が高熱と胃腸障害をきたし、多くの患者が多量出血し、ある地域では患者の30%程度が死亡した。ヒト顆粒球アナプラズマ症が疑われたが、テキサス大学医学部のダニ媒介性疾患の専門家が新型のブニヤウイルスを特定した。その後の研究によりこのウイルスは重症発熱性血小板減少症候群(SFTS)ウイルスと命名され、ブニヤウイルス科フレボウイルス属に分類された。しかしこのウイルスの感染による致死率や、媒介生物はまだ分かっていない。	赤血球濃厚液-LR「日赤」 照射赤血球濃厚液-LR「日赤」 血液を介するウイルス、細菌、原虫等の感染 vCJD等の伝播のリスク

報告企業の意見	今後の対応
中国で新型で致死性のブニヤウイルスが特定されたとの報告である。	日本赤十字社では、輸血感染症対策として受付時に海外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。

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NEWS OF THE WEEK

INFECTIOUS DISEASES:

Rival Teams Identify a Virus Behind Deaths in Central China

Richard Stone

BEIJING—When Xue-jie Yu came to China last year to probe a lethal fever outbreak, everyone—Yu included—assumed he would provide damning testimony against a known suspect. Every summer for 3 years, hundreds of people in central China came down with an illness characterized by high fever and gastrointestinal (GI) distress. Many victims bled profusely, and an alarming number of the sick—rough estimates are as high as 30% in some areas—died. By early 2007, scientists at the Chinese Center for Disease Control and Prevention (CDC) here fingered the killer as human granulocytic anaplasmosis (HGA), an emerging bacterial infection from tick bites. But to Yu, an expert on tick-borne diseases at the University of Texas Medical Branch in Galveston, things didn't add up.

"The fatality rate was too high," Yu says, and in his experience it was "rare" for HGA patients to have GI symptoms. Working at the Chinese CDC's National Institute of Communicable Disease Control and Prevention (NICDC) here, Yu tested blood samples for *Anaplasma phagocytophilum*, the HGA bacterium—and came up empty. Last December, his team identified a new kind of bunyavirus, a family that includes infamous members such as hantavirus and Rift Valley fever virus. The finding, in a paper submitted to *The New England Journal of Medicine* (*NEJM*), unmasks a dangerous new emerging virus—not a bacterial outbreak—and explains why antibiotics failed to stop it.

Behind the scenes, however, a fierce argument has broken out over who discovered the virus. This summer, a Chinese CDC team led by hantavirus expert Li Dexin, director of the agency's Institute of Virology, also uncovered a bunyavirus—possibly the same one Yu's group identified. They have submitted a deeper analysis of the pathogen, including complete RNA sequences of 11 strains, to *The Lancet*. Yu charges Li's group with trying to rob him of the discovery; Li says Yu's viral sequence is incomplete and that his team identified the virus as the killer.

Several key questions are disputed or unanswered. For starters, researchers do not know how lethal the virus is. The mortality rate may be high in China in part because clinics often prescribe the steroid dexamethasone to bring down high fevers; steroids suppress the immune system, which usually worsens infections. Scientists also differ on whether the virus should be handled in a biosafety level 3 facility—reserved for dangerous pathogens—or in less secure laboratories. And although the infection shows a seasonal pattern associated with tick-borne diseases—cases begin in early spring and peak in midsummer before tapering off by autumn—the vector is still a mystery.

Into the hot zone. Xue-jie Yu (*left*, in blue shirt) looks on as farmers check a dog for ticks; forest-hugging farms (*right*) were hard hit by the emerging virus (*inset*).

CREDITS: COURTESY OF XUE-JIE YU/ UNIVERSITY OF TEXAS MEDICAL BRANCH; (INSET) COURTESY OF LI DEXIN/CHINESE CDC

[Larger version of this image]

One indisputable fact is that the emerging disease has claimed scores of lives—mostly of farmers—in China's heartland. The first documented outbreak was in 2006, in Anhui Province. At that time, a team led by NICDC Director Xu Jianguo, Chinese CDC's chief bacteriologist, rushed to Anhui. Using PCR they found *A. phagocytophilum* DNA in the blood of one patient who died and in family members and hospital staff who became infected. HGA had been recognized in the United States in 1990 and in Europe in 1997; Xu's group reported China's first cases in the 19 November 2008 issue of *The Journal of the American Medical Association*.

Curiously, however, none of the patients recalled having been bitten by ticks. And when outbreaks recurred in 2007 and 2008, the disease did not respond to antibiotics. Thinking it might have an unusual *Anaplasma* variant on its hands, NICDC in 2009 invited Yu as a short-term visiting researcher under the 1000 Talents Program, which brings overseas scientists to China.

That summer, the pathogen surfaced in Henan and Hubei provinces. In June, Yu went to Hubei's capital, Wuhan, to collect blood samples from patients. "They did not look like typical HGA cases," he says. After he failed to detect *A. phagocytophilum*, Yu says he urged Chinese CDC scientists to consider a viral pathogen—but researchers there flatly rejected the idea. Yu persisted and spotted virus particles that December in cell culture using electron microscopy. Then in February, he says, a member of his Texas lab, Yan Liu, "cracked the code of the viral genome." Two days after he informed scientists at the Chinese CDC about his findings, he says, his 1000 Talents affiliation with NICDC was terminated.

Chinese CDC Director Wang Yu was intrigued by Xue-jie Yu's findings and invited him to share them at a 15 April meeting at CDC headquarters to plot strategy for studying the disease. Among the attendees were Li and CDC virologist Liang Mifang; they found Yu's presentation unconvincing. "He said he isolated a bunyavirus, but he had gotten just fragments," says Liang. Yu confirms that the virologists were dismissive: "Li tried to deny the importance of my work," Yu says. Yu and his colleagues have named the virus Dabie Mountain virus after the range that straddles the borders of Hubei, Anhui, and Henan provinces where they collected samples. But Yu was not invited back to China this summer

to continue his research. "I am the first scientist to discover the viral pathogen for an emerging infectious disease who has no access to study the virus and the disease anymore," he says.

In May, the Chinese CDC set up surveillance for the pathogen in Henan and Hubei provinces. The disease flared up in four other provinces as well, and Li's team collected blood and serum from all six affected provinces. They amplified viral RNA sequences and from more than 500 clones linked 14 to bunyavirus. They also isolated bunyavirus in cell culture and sequenced 11 strains. They have named it severe febrile and thrombocytopenic syndrome (SFTS) virus and have classified it in the phlebovirus genus of bunyavirus. Li's group also detected the virus in 36 patients from three provinces. "It's solid work. They clearly show that a new virus is causing disease," says a U.S. scientist who has seen the data and asked to remain anonymous.

But the rival claim did not sit well with Xue-jie Yu. When he heard that Li's team had submitted its findings to *The Lancet*, he sent an e-mail to the journal accusing Li of poaching his discovery. Liang says that's not true: "All data in our manuscript belong to us, not anyone else," she says. On 17 September, *The Lancet* asked Li's group to withdraw the paper and resubmit it after settling the author's dispute.

The squabbling has put Wang, the Chinese CDC's director, in an awkward position. There is no doubt, she says, that Xue-jie Yu "discovered the novel bunavirus." While noting that Yu's results are not as "rich" as Li's team's, Wang says, "everyone knows what a scientific breakthrough is, and what is accumulating work." After the *NEJM* paper is published, he hopes, "other papers can go smoothly." But it may take Wang's best diplomatic skills to get any collaboration on the emerging virus to go smoothly.

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Human T-cell leukemia virus type I (HTLV-1) proviral load and disease progression in asymptomatic HTLV-1 carriers: a nationwide prospective study in Japan

Masako Iwanaga, Toshiki Watanabe, Atae Utsunomiya, Akihiko Okayama, Kaoru Uchimaru, Ki-Ryang Koh, Masao Ogata, Hiroshi Kikuchi, Yasuko Sagara, Kimiharu Uozumi, Manabu Mochizuki, Kunihiro Tsukasaki, Yoshio Saburi, Masaomi Yamamura, Junji Tanaka, Yukiyoshi Moriuchi, Shigeo Hino, Shimeru Kamihira, Kazunari Yamaguchi and for the Joint Study on Predisposing Factors of ATL Development Investigators

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No. 16

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 2010. 9. 15	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	解凍人赤血球濃厚液		研究報告の公表状況	Iwanaga M, Watanabe T, Utsunomiya A, Okayama A, Uchimaru K, Koh KR, Ogata M, Kikuchi H, Sagara Y, Uozumi K, Mochizuki M, Tsukasaki K, Saburi Y, Yamamura M, Tanaka J, Moriuchi Y, Hino S, Kamihira S, Yamaguchi K; Joint Study on Predisposing Factors of ATL Development Investigators. Blood. 2010 Aug 26;116(8):1211-9. Epub 2010 May 6.	公表国 日本	
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(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
○無症候性ヒトT細胞白血病ウイルスⅠ型(HTLV-1)キャリアにおける日本国内の前向き調査:プロウイルス量と疾患発症との関連 無症候性HTLV-1キャリアにおける成人T細胞白血病(ATL)発症の確実なリスク因子は、現在も不明である。近年、HTLV-1プロウイルス量は、ATLの重要な予測因子として評価されているが、少数の小規模前向き試験が実施されているだけである。2002年～2008年に、登録された無症候性HTLV-1キャリア1,218名(男性426名、女性792名)を前向きに評価した。登録時のプロウイルス量は、女性と比べ男性(末梢血単核細胞[PBMCs]100あたりの中央値1.39 vs 2.10 ; P<0.001)、40歳以下の集団に対し年齢40～49歳および50～59歳の集団(それぞれP = 0.02 , 0.007)、ATL家族歴を有さない集団に対し有する集団(PBMCs 100あたりの中央値1.33 vs 2.32 ; P=0.005)の方が、有意に多かった。14名の登録者が顕在性ATLへ進行した。この登録者の登録時のプロウイルス量は多かった(範囲:PBMCs 100あたり4.17～28.58)。登録時のプロウイルス量が4コピー以下の登録者は、ATLを発症しなかった。また多変量Cox解析では、プロウイルス量だけでなく、高年齢、ATLの家族歴、他疾患治療時における初回HTLV-1検査が、ATLの進行の独立リスク因子であることが示された。	解凍赤血球濃厚液「日赤」 照射解凍赤血球濃厚液「日赤」 解凍赤血球-LR「日赤」 照射解凍赤血球-LR「日赤」 血液を介するウイルス、細菌、原虫等の感染 vCJD等の伝播のリスク

報告企業の意見	今後の対応
日本国内で無症候性ヒトT細胞白血病ウイルスⅠ型キャリアのプロウイルス量と疾患進行の前向き調査を行ったところ、プロウイルス量は男性や40歳以上、成人T細胞白血病(ATL)の家族歴を有する集団で高く、登録時のプロウイルス量がATLの発症に影響を与えたとの報告である。	日本赤十字社では、献血時のスクリーニング法として、化学発光酵素免疫測定法(CLEIA)によるHTLV-1抗体のスクリーニング検査を行っている。今後も引き続き情報の収集に努める。

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Human T-cell leukemia virus type I (HTLV-1) proviral load and disease progression in asymptomatic HTLV-1 carriers: a nationwide prospective study in Japan

Masako Iwanaga,[1,2] Toshiki Watanabe,[3] Atae Utsunomiya,[4] Akihiko Okayama,[5] Kaoru Uchimaru,[6] Ki-Ryang Koh,[7] Masao Ogata,[8] Hiroshi Kikuchi,[9] Yasuko Sagara,[10] Kimiharu Uozumi,[11] Manabu Mochizuki,[12] Kunihiko Tsukasaki,[13] Yoshio Saburi,[13] Masaomi Yamamura,[14] Junji Tanaka,[15] Yukiyoshi Moriuchi,[16] Shigeo Hino,[17] Shimeru Kamihira,[18] and Kazunari Yamaguchi,[19] for the Joint Study on Predisposing Factors of ATL Development investigators

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Definitive risk factors for the development of adult T-cell leukemia (ATL) among asymptomatic human T-cell leukemia virus type I (HTLV-1) carriers remain unclear. Recently, HTLV-1 proviral loads have been evaluated as important predictors of ATL, but a few small prospective studies have been conducted. We prospectively evaluated 1218 asymptomatic HTLV-1 carriers (426 males and 792 females) who were enrolled during 2002 to 2008. The proviral load at enrollment was significantly higher in males than females (median, 2.10 vs 1.39 copies/100 peripheral blood mononuclear cells [PBMCs]; $P < .001$), in those 40 to 49 and 50 to 59 years of age than that of those 40 years of age and younger ($P = .02$ and .007, respectively), and in those with a family history of ATL than those without the history (median, 2.32 vs 1.33 copies/100 PBMCs; $P = .005$). During follow-up, 14 participants progressed to overt ATL. Their baseline proviral load was high (range, 4.17-28.58 copies/100 PBMCs). None developed ATL among those with a baseline proviral load lower than approximately 4 copies. Multivariate Cox analyses indicated that not only a higher proviral load, advanced age, family history of ATL, and first opportunity for HTLV-1 testing during treatment for other diseases were independent risk factors for progression of ATL. (*Blood*. 2010;116(8):1211-1219)

Introduction

Human T-cell leukemia virus type I (HTLV-1), the first human retrovirus to be identified, is etiologically associated with adult T-cell leukemia (ATL), HTLV-I–associated myelopathy/tropical spastic paraparesis (HAM/TSP), and HTLV-1 uveitis/HTLV-I–associated uveitis (HU/HAU).[1-3] Worldwide, endemic areas for the virus are unevenly distributed, which include southwest Japan, the Caribbean islands, South America, and a part of Central Africa.[4] In Japan, the number of HTLV-I carriers was estimated to be approximately 1.2 million people during the late 1980s.[5] The majority of HTLV-1 carriers remain asymptomatic throughout their lives. The lifetime risks of developing ATL and HAM/TSP are estimated to be approximately 2.5% to 5%[6,7] and 0.3% to 2%,[8,9] respectively.

Several molecular biologic studies have reported that various cellular dysfunctions induced by viral genes (eg, *tax* and *HBZ*), genetic and epigenetic alterations, and the host immune system may be involved in the leukemogenesis of ATL.[10-12] Clinical and epidemiologic studies have also reported a variety of possible risk factors for ATL, including vertical transmission of HTLV-1 infection, male gender, a long latent period, increased leukocyte counts or abnormal lymphocyte counts, and higher levels of anti–HTLV-1 antibody titers and soluble interleukin-2 receptor-α.[13-19] However, there are no clear determinants that separate those who develop ATL from those who remain healthy carriers.

Recently, HTLV-1 proviral load levels have been evaluated as important predictors of development of ATL and HAM/TSP. Some cross-sectional studies showed that HTLV-1 proviral load levels were higher in ATL and HAM/TSP compared with asymptomatic HTLV-1 carriers.[20,21] However, the proviral load levels of asymptomatic HTLV-1 carriers exhibited a very wide range,[20,22,23] and these levels may vary by sex, race, habitats, and comorbidities.[24] The proviral load levels of asymptomatic HTLV-1 carriers were also examined serially in some prospective studies; however, the number of reported cases was very small.[25-28] Although these previous studies suggest a possible important role for HTLV-1 proviral load in the development of ATL and HAM/TSP, the association between HTLV-1 proviral load and diseases development remains unclear.

The identification of risk factors for developing ATL among virus carriers is necessary to prevent these diseases in HTLV-1 endemic areas. To investigate detailed viral- and host-specific determinants of disease development, larger and longer prospective studies are warranted. In 2002, we established a nationwide cohort study for asymptomatic HTLV-1 carriers in Japan named the Joint Study on Predisposing Factors of ATL Development (JSPFAD).[29] The main objective of this project is to establish reliable predisposing factors for developing ATL by prospectively following a large number of asymptomatic HTLV-1 carriers. Here, for the first time, we report the study method, baseline demographic characteristics, and distribution characteristics of baseline HTLV-1 proviral load of asymptomatic HTLV-1 carriers. We have also evaluated progression to ATL and its risk predictors.

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Methods

Participants and study design

The JSPFAD is a nationwide prospective study of HTLV-1 carriers, which was approved by the Ministry of Education, Culture, Sports, Science and Technology of Japan. The project was established in August 2002 by Japanese clinicians and basic researchers of 41 institutions composed of 14 university hospitals and 27 educational hospitals located in various areas of Japan (supplemental Appendix, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Objectives of the project are to establish reliable predisposing factors for development of ATL by prospectively following a large number of asymptomatic HTLV-1 carriers. This includes performing clinical examinations and biomarker assays, as well as establishing a biomaterial resource bank of plasma, viable peripheral blood mononuclear cells (PBMCs), frozen PBMCs pellets, and genomic DNA from PBMCs of HTLV-1–infected persons for the future evaluations with new molecular biology techniques.

Hematologists at the collaborating institutions were responsible for enrolling participants after receiving approval from their Institutional Review Boards. The study protocol was approved by the Ministry of Education, Culture, Sports, Science and Technology of Japan. Eligible participants were those who had known of their HTLV-1 infection and had confirmed the HTLV-1–positive serology at any of the medical institutions. Potential participants visited any of the collaborating institutions directly or via the website of the JSPFAD (www.htlv1.org/). They received adequate explanations for the enrollment procedure from the hematologists at the collaborating institutions. Enrollment was conditional on participants giving written informed consent in accordance with the Declaration of Helsinki. The primary participants were asymptomatic HTLV-1 carriers. A small number of patients with definite ATL, HAM/TSP, and HU/HAU were also enrolled as controls.

Data collection and sample storage

After providing written informed consent, participants were expected to fill out a questionnaire regarding demographic information, to provide peripheral blood samples, and to periodically visit the institution for follow-up. After reconfirming the asymptomatic HTLV-1 carrier status of the participants, hematologists at the collaborating institutions assigned a unique identification number to each participant and subsequently sent all materials (individual questionnaire sheets, clinical data, and blood samples drawn into ethylenediaminetetraacetic acid and heparin tubes) to the JSPFAD office (Department of Medical Genome Sciences, Laboratory of Tumor Cell Biology, Graduate School of Frontier Sciences, University of Tokyo, Japan).

The self-administered questionnaire included items on demographic characteristics, birthplaces of the participants and their mothers, family history regarding HTLV-1 status and HTLV-I–associated diseases, length of marriage, partner's HTLV-I status, first opportunity for HTLV-I testing, and histories of disease manifestations other than HTLV-I–associated diseases. Additional questionnaire items, information on prior blood transfusion, and smoking habits (present, past, or nonsmoking) were also included after April 2008.

Clinical data included information on the date of visit, complete blood cell count, differential cell counts (including abnormal lymphocytes per 100 leukocytes), lactate dehydrogenase, HTLV-1 serologic test, comorbidities other than HTLV-I–associated diseases, and the development of any HTLV-I–associated diseases during follow-up. Blood samples were collected at enrollment, annually thereafter (in principal), and as needed. Blood samples sent to the study office at the University of Tokyo were separated into plasma, PBMCs, and genomic DNA and then used for viral marker assays at the University of Tokyo or stored for the biomaterial bank at the Japanese Red Cross Fukuoka Blood Center.

Viral marker assays

HTLV-1 proviral load of PBMC samples was measured by real-time polymerase chain reaction (PCR) using the ABI PRISM 7000 Sequence Detection System (Applied Biosystems Japan), as previously described with minor modifications.[30,31] Genomic DNA from PBMCs was isolated using a QIAGEN Blood Kit (QIAGEN). Quantitative real-time PCR was performed using multiplex PCR with 2 sets of primers specific for the HTLV-1 provirus and the human gene encoding the RNase P enzyme. The primers and the probe for the gene encoding RNase P were purchased from Applied Biosystems; those for the pX region of the HTLV-1 provirus were described previously.[30,31] Genomic DNA of normal control PBMCs mixed with a plasmid DNA, which contained almost the whole genome of the HTLV-1 provirus (*SacI* site of 5′-LTR to *SacI* site of 3′-LTR), was used as control template. The copy number of the plasmid DNA was calculated based on the size and weight of the plasmid DNA, as measured by spectrophotometry. The proviral loads were expressed as copy numbers per 100 PBMCs, based on the assumption that infected cells harbored 1 copy of the integrated HTLV-1 provirus per cell. Samples with a higher proviral load (> 20 copies/100 PBMCs) were subjected to Southern blot analysis to examine the clonality of the infected cells. Assays to detect the integrated band of HTLV-1 provirus genome were described previously.[32] Genomic DNA samples (10 mg) were digested with *PstI* or *Eco*RI restriction enzymes and were size-fractionated on 0.7% agarose gels. They were then transferred onto a nylon membrane by the Southern blot technique. Hybridization to randomly primed ^{32}P-labeled DNA probes for the whole proviral genome (*SacI* to *SacI* fragment of the HTLV-1 proviral genome) was performed, followed by appropriate stringency washing steps and autoradiography. Soluble interleukin-2 receptor was measured by a commercial laboratory (SRL Inc) using an enzyme-linked immunosorbent assay (Endogen) and reported as units per milliliter.

Statistical analysis

Analyses were performed for participants who enrolled as of December 2008. Age at enrollment was categorized into 5 groups: younger than 40, 40 to 49, 50 to 59, 60 to 69, and 70 years or older. Geographic location was divided into 4 areas: northern (Hokkaido and Tohoku), metropolitan (Tokyo, Osaka, and Nagoya), southern (Kyushu and Okinawa), and others (supplemental Figure 1). First opportunity for HTLV-1 testing was divided into 3 categories: by screening for HTLV-I (regional-mass, multiphasic, blood donor, and maternal screenings), by the presence of HTLV-I–infected family members (including spouse), and by the patient status under treatment for diseases unrelated to HTLV-I. A positive family history was considered to be present when participants had information on first-degree relatives (parents, siblings, or offspring) who were HTLV-I carriers or had HTLV-I–associated diseases (ie, ATL, HAM/TSP, and HU/HAU). Any leukemia and/or lymphoma other than ATL were also taken into consideration. A positive comorbidity at enrollment was considered to be present when any information on diseases other than HTLV-I–associated diseases

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was available at enrollment. HTLV-1 proviral loads (copy numbers/100 PBMCs) were used as a continuous variable (raw and the power-transformed data) or by categorizing them into quartiles. We applied a square-root transformation to the raw data of proviral loads to reduce the skewness. Continuous data were presented as median (range) values and compared using a Mann-Whitney test. Categorical data were compared using a χ^2 test or Fisher exact test. We calculated person-years of follow-up for each participant from the date of enrollment to the date of ATL diagnosis, the date of last follow-up, or September 30, 2009, whichever came first. Cumulative progression to ATL was estimated using Kaplan-Meier curves. To estimate the effect of baseline HTLV-1 proviral load and selected demographic factors on ATL development, we performed Cox proportional hazards analyses, and expressed as hazard ratios (HR) and 95% confidence intervals (CI), which were calculated by robust sandwich variance estimates. To check for possible incompleteness in the multivariate model, we also performed analyses using sub-datasets. All statistical analyses were performed using SAS Version 9.1 (SAS Institute Japan) with a 2-tailed significance level of .05.

Results

Baseline demographic characteristics

From August 2002 to December 2008, 1259 participants of asymptomatic HTLV-1 carriers were enrolled in this study. However, HTLV-1 proviral load was not measured for 41 participants. Thus, a total of 1218 participants (426 males and 792 females) were included in this analysis. Demographic characteristics of the participants at enrollment are shown in Table 1. The median ages at enrollment in the cohort were 59.6 years (range, 6.9-92.8 years) for males or 58.3 years (range, 17.8-90.3 years) for females. The largest percentage of study participants was from the southern area, which is a well-known HTLV-1 endemic area in Japan, followed by the metropolitan area. The southern area also had the largest percentage for birthplaces for most participants and their mothers.

One-half of the participants came to know of their HTLV-1 infections through screening for HTLV-1, and one-fourth was informed of their infections while receiving treatments for diseases other than HTLV-1–associated diseases. More than half of the participants did not know their family status of HTLV-1 infection. Only 119 female participants knew about the HTLV-1 infection status of their husbands, of whom 53 (45%) of the husbands were positive for HTLV-1 (data not shown). However, we were not able to obtain reliable information on male-to-female transmission for the female participants. We obtained information on comorbidities at enrollment from 257 participants, of which 45 had comorbid infectious diseases (eg, strongyloidiasis, chronic bronchitis, hepatitis C virus infection, lymphadenitis), 29 had autoimmune diseases (rheumatoid arthritis, chronic thyroiditis, Sjögren syndrome, and other autoimmune or chronic inflammatory diseases), 80 had a variety of definite malignant diseases other than ATL (non-Hodgkin lymphoma, acute myeloid leukemia, gastric cancer, lung cancer, or other malignancies), 16 had skin diseases, and 87 had other common diseases (eg, hypertension, diabetes).

Distributions of baseline HTLV-1 proviral load

Figure 1 shows distribution of baseline HTLV-1 proviral load in 1218 participants. There was a wide range of skewness in the raw data, with a median of 1.60 copies/100 PBMCs (range, 0-55.8 copies/100 PBMCs; 25th-75th percentile, 0.29-4.54 copies/100 PBMCs; Figure 1A). The square-root transformation reduced the skew in the raw data, with a median of 1.26 copies/100

Table 1. Baseline demographic characteristics of asymptomatic HTLV-1 carriers

Variable	Male, no. (%)	Female, no. (%)
Total	426	792
Age, y		
Younger than 40	48 (11.3)	119 (15.0)
40-49	70 (16.4)	130 (16.4)
50-59	99 (23.2)	174 (22.0)
60-69	88 (20.7)	172 (21.7)
70 or older	121 (28.4)	197 (24.9)
Place of enrollment		
Northern area	10 (2.3)	32 (4.0)
Metropolitan area	75 (17.6)	144 (18.1)
Southern area	[illegible]	[illegible]
Other areas	8 (1.9)	19 (2.4)
Birthplace of participants		
Northern area	18 (4.2)	33 (4.2)
Metropolitan area	[illegible]	[illegible]
Southern area	240 (56.3)	400 (50.5)
Other areas	[illegible]	[illegible]
Unknown	118 (27.7)	225 (28.4)
Birthplace of participants' mothers		
Northern area	16 (3.8)	32 (4.0)
Metropolitan area	[illegible]	[illegible]
Southern area	247 (58.0)	426 (53.8)
Other areas	[illegible]	[illegible]
Unknown	122 (28.6)	231 (29.2)
First opportunity for HTLV-1 testing		
Screening for HTLV-1	209 (49.1)	452 (57.1)
Regional mass screening	[illegible]	[illegible]
Multiphasic screening	24	44
Blood donor screening	[illegible]	[illegible]
Maternal screening	0	116
Relatives of HTLV-1-positive family	[illegible]	[illegible]
During treatment of other diseases	117 (27.5)	148 (18.7)
Unknown	[illegible]	[illegible]
Family history of HTLV-1–associated diseases*		
Absent	[illegible]	[illegible]
Absent for a first-degree relative but having an infected spouse	6 (1.4)	23 (2.9)
Carrier only	[illegible]	[illegible]
HU/HAU only	2 (0.5)	1 (0.1)
HAM	[illegible]	[illegible]
ATL	34 (8.0)	74 (9.3)
Leukemia or lymphoma	[illegible]	[illegible]
Unknown family history	248 (58.2)	433 (54.7)
Comorbidity†		
Absent	331 (77.7)	630 (79.5)
Present	[illegible]	[illegible]
Infectious diseases	20	25
Autoimmune diseases	[illegible]	[illegible]
Malignant diseases	36	44
Skin diseases	[illegible]	[illegible]
Other disease	28	59

HTLV-1 indicates human T-cell leukemia virus type 1; HU, HTLV-1 uveitis; HAU, HTLV-1–associated uveitis; HAM, HTLV-1 myelopathy; and ATL, adult T-cell leukemia.

*Family history was restricted to a first-degree relative. "Present" indicates that participants have a parent, sibling, or offspring diagnosed with HTLV-1-associated diseases. Family members with HAM and HU/HAU were included into the category of "HAM." Family members with ATL and HAM and/or HU/HAU were included into the category of "ATL."

†Comorbidity indicates that participants have any diseases other than HTLV-1–associated diseases at enrollment.

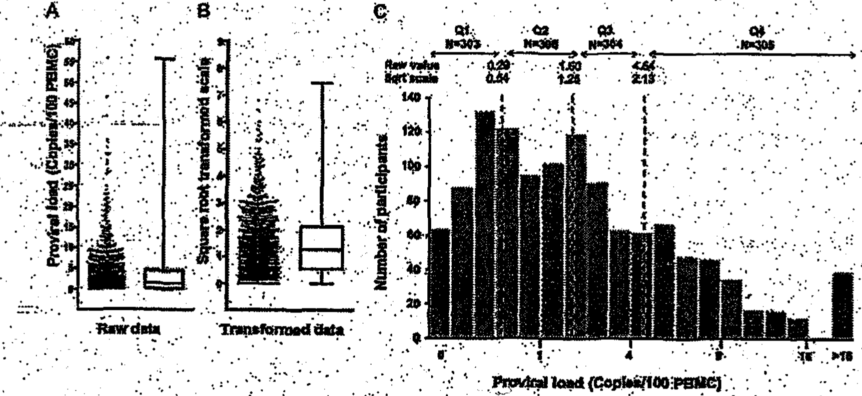

Figure 1. Distribution of baseline HTLV-1 proviral load levels among 1218 asymptomatic HTLV-1 carriers. (A) Scatter plot of raw data of proviral load (left) and the vertical box and whiskers plot (right): the box delineates 25th percentile (0.29 copies/100 peripheral blood mononuclear cells [PBMCs]), median (1.60 copies/100 PBMCs), and 75th percentiles (4.54 copies/100 PBMCs), and the whiskers delineate the minimum (0 copies/100 PBMCs) and maximum (55.8 copies/100 PBMCs). (B) Scatter plot of square-root transformed values of the raw proviral load (left) and the vertical box and whiskers plot (right): the box delineates 25th percentile (0.54 copies/100 PBMCs), median (1.26 copies/100 PBMCs), and 75th percentiles (2.13 copies/100 PBMCs), and the whiskers delineate the minimum (0 copies/100 PBMCs) and maximum (7.47 copies/100 PBMCs). (C) The frequency of participants in the quartile distributions of proviral load. Q1 indicates quartile 1 (< 25th percentile); Q2, quartile 2 (25th percentile to median); Q3, quartile 3 (median to 75th percentile); Q4: quartile 4 (> 75th percentile); Sqrt, square-root transformation; and N, number of participants.

PBMCs (range, 0-7.47 copies/100 PBMCs; 25th-75th percentile, 0.54-2.13 copies/100 PBMCs; Figure 1B). Figure 1C shows the frequency of participants in each quartile of proviral load.

The median proviral load and a frequency of subjects in each quartile of proviral load by demographic characteristics are shown in Table 2. Males and females were significantly different in proviral load levels, with a median value of 2.10 copies/100 PBMCs (range, 0-46.6 copies/100 PBMCs) for males and that of 1.39 copies/100 PBMCs (range, 0-55.8 copies/100 PBMCs) for females ($P < .001$). Males were probably distributed in the highest quartile of proviral load level than females.

Among age groups, the median proviral load of those 40 to 49 and 50 to 59 years of age was significantly higher than that of those less than or equal to 40 years ($P = .02$ and $P = .007$, respectively). Both age groups were probably distributed in the highest quartile of proviral load levels. Because we found a significantly different median proviral load by sex, we additionally evaluated the proviral load level by age group in each sex. The highest median value was found in those 50 to 59 years of age (2.89 copies/100 PBMCs) in males, but in 40 to 49 years of age (1.49 copies/100 PBMCs) in females, although there were no statistical differences by age group for both sexes (data not shown).

Among the categories for the first opportunity for HTLV-1 testing, the proviral load level was significantly higher ($P = .002$) in participants informed of their infection during treatment for diseases unrelated to HTLV-1 compared with those who came to know of their infection by screenings (Table 2). Participants informed of their infection during treatment for diseases unrelated to HTLV-1 were probably distributed in the highest quartile of proviral load levels. There was no difference in the proviral load level between those who came to know of their infection by the presence of HTLV-1–positive family members and those who came to know of their infection by screenings.

When we evaluated the proviral load level by family history status, participants who had no family history of HTLV-1 infection, who had only HTLV-1 carriers in the family, who had only an HTLV-1 carrier husband, and who had only HU/HAU in the family were grouped together as a reference category. The proviral load levels of those with a family history of HAM/TSP (median 3.85 copies/100 PBMCs) and ATL (median 2.32 copies/100 PBMCs) were significantly higher ($P = .01$ and $P = .005$, respectively) compared with those of the reference group (Table 2). Indeed, those with a family history of HAM/TSP and ATL were probably distributed in the third and fourth quartiles of proviral load levels. Of interest, the median proviral load level of those with a family history of leukemia or lymphoma was also significantly higher ($P = .009$) compared with those of the reference group.

Among the categories for comorbidity, there was no statistical difference in the proviral load levels when we simply compared between those with and without comorbidity at enrollment (data not shown). However, when we compared those without comorbidity and those with infectious diseases at enrollment, the median proviral load of the latter was significantly higher than that of the former ($P = .05$; Table 2).

Prognosis

During a median follow-up period of 1.0 year (range, 0-6.6 years) and a total of 1981.2 person-years, 14 (1.1%) participants (4 males and 10 females) progressed to overt ATL (2 acute, 2 lymphoma, and 10 smoldering types; Table 3). The incidence rate of ATL was 7.1 per 1000 person-years for all types of ATL and 2.0 per 1000 person-years for the aggressive types (acute and lymphoma) of ATL. The median duration from date of enrollment to date of diagnosis of ATL was 13.8 months (range, 2.8-64.4 months). The cumulative probability of progression to ATL was reached 4.8% (95% CI, 1.9%-11.8%) at 5.4 years (Figure 2).

Table 2. HTLV-1 VL levels by demographic characteristics

Demographic characteristics	No.	Median VL (range) (copies/100 PBMCs)	Frequency of subjects by VL level, n (% of row): Quartile 1 (VL: < 0.29)‡	Quartile 2 (VL: 0.29-1.60)	Quartile 3 (VL: 1.60-4.54)	Quartile 4 (VL: ≥ 4.54)
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Sex						
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Female	792	1.39 (0-55.8)†	219 (27.7)	206 (26.0)	211 (26.6)	156 (19.7)
[illegible]						
Younger than 40	167	1.37 (0-16.4)†	49 (29.3)	43 (25.8)	50 (29.9)	25 (15.0)
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
50-59	273	1.84 (0-38.1)*	64 (23.4)	64 (23.4)	63 (23.1)	82 (30.4)
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
70 or older	318	1.52 (0-55.8)	81 (25.5)	81 (25.5)	79 (24.8)	77 (24.2)
[illegible]						
Screening	661	1.46 (0-55.8)†	182 (27.5)	160 (24.2)	175 (26.5)	144 (21.8)
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
During treatment for other diseases	265	1.93 (0-41.7)*	56 (21.1)	66 (24.9)	57 (21.5)	86 (32.5)
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Family history of HTLV-1–related diseases						
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
HAM/TSP	9	3.85 (1.2-9.4)*	0	1 (11.1)	5 (55.6)	3 (33.3)
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Leukemia or lymphoma	35	2.47 (0-12.8)*	3 (8.6)	9 (25.7)	11 (31.4)	12 (34.3)
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Comorbidity						
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Infectious diseases	45	2.75 (0-28.6)*	7 (15.6)	8 (17.8)	13 (28.9)	17 (37.8)
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Malignant diseases	80	1.57 (0-19.4)	19 (23.8)	21 (26.3)	23 (28.8)	17 (21.3)
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Other disease	87	1.17 (0-22.0)	20 (23.0)	31 (35.6)	17 (19.5)	19 (21.8)

HTLV-1 indicates human T-cell leukemia virus type 1; VL, HTLV-1 proviral load; PBMCs, peripheral blood mononuclear cells; HU, HTLV-1 uveitis; HAU, HTLV-1–associated uveitis; HAM, HTLV-1 myelopathy; TSP, tropical spastic paraparesis; and ATL, adult T-cell leukemia.

*Mann-Whitney test revealed a statistically significant difference in the VL level compared with the reference group.

†Reference group.

‡The VL was categorized based on quartile cutoff points (the 25th, 50th, and 75th percentiles of the VL distribution) in 1218 HTLV-1 carriers. The unit of VL was copies/100 PBMCs.

The median proviral load at enrollment for these 14 participants was 10.3 copies/100 PBMCs (range, 4.17-28.58 copies/100 PBMCs), which was significantly higher than those who did not develop ATL (1.56 copies/100 PBMCs; range, 0-55.8 copies/100 PBMCs; $P < .001$). Of interest, the median proviral load level at enrollment was significantly higher for those who developed smoldering types of ATL than for those who developed aggressive types of ATL (11.4 and 5.1 copies/100 PBMCs, respectively, $P = .02$), whereas the median entry age was significantly younger for the former than for the latter (59.8 and 73.9 years, respectively, $P = .02$). Distribution of the 14 participants who developed ATL by demographic characteristics and by quartile of proviral load levels is shown in Table 4. Among 14 ATLs, 13 occurred in the highest quartile of baseline proviral load (> 4.54 copies/100 PBMCs) and 1 occurred in the third quartile (1.60-4.54 copies/100 PBMCs), whereas no ATL developed in quartiles 1 and 2 (< 1.60 copies/100 PBMCs). A high frequency of ATL was also seen in older age group, those with first opportunity for HTLV-1 testing during treatment of other diseases and those with a family history of ATL. Therefore, we decided to include the baseline HTLV-1 proviral load (the square-root transformed continuous value), age, first opportunity for HTLV-1 testing, and family history into Cox hazard analyses as covariates to test the effects on the development of ATL.

We identified that baseline proviral load was strongly associated with the risk of progression to ATL on both univariate and multivariate Cox analyses. In the multivariate analysis, the adjusted HR for the square-root transformed proviral load per unit increase was 3.57 (95% CI, 2.25-5.68; Table 5). We also found that advanced age, family history of ATL, and first opportunity to learn of HTLV-1 infection during treatment of other diseases were independently associated with the development of ATL, after adjusting the effect of proviral load. The adjusted HR for developing ATL per 5-year increase of age from 40 years was 1.67 (95% CI, 1.12-2.50). HTLV-1 carriers having a family history of ATL had 12 times higher risk of developing ATL compared with those not having the history (adjusted HR = 12.1; 95% CI, 2.26-64.7), and those who came to know their HTLV-1 infection during treatment for other diseases had 4 times higher risk of developing ATL compared with references (adjusted HR = 4.16; 95% CI, 1.37-12.6), although the CIs were wide because of the smaller group sizes (Table 5). Of interest, male gender was not a significant risk factor for developing ATL, even though the median proviral load was significantly higher in males than in females (Table 2).

Because the distribution of proviral load was skewed even after the value was square-root transformed, it was possible that ATL events in subjects with skewed high proviral loads contributed to results. To check the possibility, we performed a multivariate analysis using a sub-dataset that excluded subjects with skewed proviral load (> 16 copies in Figure 1C; n = 39, including 3 who developed ATL). Nevertheless, we observed similar results as the original dataset, although age factor was no longer statistically

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Table 3. Cases who developed ATL from HTLV-I carrier status

Case no.	Sex	Age, y	Place of birth	First opportunity for HTLV-1 testing	Family history of HTLV-1–related disease	Comorbidity at enrollment	HTLV-1 VL, copies/100 PBMCs	sIL-2R, U/mL	Abnormal lymphocytes, percentage	LDH, IU/L	WBC, ×10³/mm²	Clinical type	Duration from enrollment, mo
	Demographic characteristics						Baseline clinical and biologic values					ATL development	
[illegible]													
1	M	79.9	Southern	ATL family	ATL	None	5.47	479	2	157	4200	Acute	7.4
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
3	M	71.9	Southern	Other disease	None	Skin disease	4.17	1450	0	351	5140	Lymph	4.6
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Progression to indolent type of ATL													
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
6	F	71.9	Southern	Multiphasic screening	None	None	10.60	1320	2	199	4000	Sm	29.8
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
8	F	74.0	Southern	Other disease	Unknown	Gallbladder cancer	10.11	1110	2	240	2700	Sm	26.8
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
10	F	43.3	Southern	Pregnancy	ATL	None	13.90	372	1	ND	5400	Sm	64.4
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
12	M	57.6	Southern	Other disease	Unknown	None	7.67	ND	2	234	5500	Sm	15.4
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
14	M	65.1	Southern	Other disease	None	Prostatitis	28.58	2660	0	158	5500	Sm	2.8

ATL indicates adult T-cell leukemia; HTLV-1, human T-cell leukemia virus type 1; VL, HTLV-1 proviral load; PBMCs, peripheral blood mononuclear cells; sIL-2R, soluble interleukin-2 receptor; LDH, lactate dehydrogenase; WBC, white blood cell count; Sm, smoldering type; and ND, not done.

significant ($P = .07$; supplemental Table 1). It is also possible that effects of some of the risk factors are weighted because of only 1 patient with an event because only 14 were analyzed as events in the multivariate analyses. To check the possibility, we performed 14 leave-one-out analyses, omitting 1 of 14 cases at a time from the original dataset. The Jackknifed coefficient of each parameter revealed the stability, which indicated that none of 14 cases affected the original model (data not shown).

Discussion

Previous studies reported no significant differences in the HTLV-1 proviral load by sex and age in asymptomatic HTLV-1 carriers.[21,22,24,33] In the present study, however, we found that there were significant differences in the proviral load by sex and age (Table 2). The median HTLV-1 proviral load was significantly higher in males than females. The median HTLV-1 proviral load for those 40 to 49 and 50 to 59 years of age was significantly higher than for those less than or equal to 40 years. The discrepancy between results of previous studies and those of the present study may be primarily explained by the differences in study population characteristics. We also found sex differences in age distributions of HTLV-1 proviral load; in male subjects, the median proviral load level was the highest at 50 to 59 years of age, whereas in female subjects it was highest at 40 to 49 years of age, although there were no statistical differences. These distribution characteristics of HTLV-1 proviral load are of interest when we consider the differences in sex and age at onset between ATL and HAM/TSP. ATL occurs predominantly in older males (~ 60 years), whereas HAM/TSP occurs predominantly in middle-aged females (~ 45-55 years). Thus, the proviral load levels of asymptomatic HTLV-1 carriers might be the highest in the age groups approximately 5 to 10 years before the average age at onset of ATL and HAM/TSP. These distribution characteristics may be related to differences in host immune responses to HTLV-1 and other unknown host factors.[34]

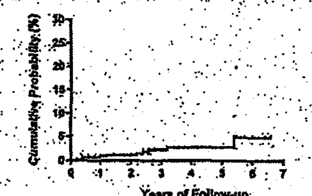

Figure 2. Probability of progression to ATL among 1218 asymptomatic HTLV-1 carriers.

The present study revealed that the median proviral load level of those with a family history of ATL or HAM/TSP was significantly higher than for those with no family history (Table 2). These results support previous studies indicating that HTLV-1–infected blood donors and asymptomatic carriers with familial HAM/TSP or ATL tend to have a higher HTLV-1 proviral load than those without family history.[21,33] In the present study, the proviral loads were also higher in those with a family history of leukemia or lymphoma than those without such history. We assume that the family history of leukemia or lymphoma may have included some ATL cases because some participants provided a diagnosis as just unknown leukemia or lymphoma. Although the present study was a large cohort, data collection regarding family history of HTLV-1–associated diseases was insufficient because one-half of the participants did not know their family HTLV-1 status. Further detailed data collection is needed to confirm the characteristics of HTLV-1 proviral load levels by family histories among asymptomatic HTLV-1 carriers, as this is necessary to determine genetic determinants of HTLV-1–associated diseases.

HTLV-1 carriers have various comorbidities, such as infectious, autoimmune, and malignant diseases.[4,25,35-38] In the present study, 45 participants had various infectious diseases at enrollment (Table

Table 4. Frequency of subjects who developed ATL by demographic characteristics and by VL level

Demographic characteristics	No. of subjects	No. of ATLs (% of subjects)	Frequency of ATL by VL level, n (% of subjects in each quartile in Table 2): Quartiles 1 and 2 (VL: < 1.60)*	Quartile 3 (VL: 1.60-4.54)	Quartile 4 (VL: ≥ 4.54)
Total	1218	14 (1.1)	0	1 (0.3)	13 (4.3)
Sex					
Male	[illegible]	[illegible]	0	[illegible]	[illegible]
Female	792	10 (1.3)	0	0	10 (6.4)
Age, y					
Younger than 40	167	0	—	—	—
40-49	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
50-59	273	3 (1.1)	0	0	3 (3.7)
60-69	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
70 or older	318	6 (1.9)	0	1 (1.3)	5 (6.5)
First opportunity for HTLV-1 testing					
Screening	661	4 (0.6)	0	0	4 (2.8)
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
During treatment for other diseases	265	7 (2.6)	0	1 (1.8)	6 (7.0)
Unknown	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Family history of HTLV-1–related diseases					
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
HAM/TSP	9	0	—	—	—
ATL	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Leukemia or lymphoma	35	0	0	0	0
Unknown family history	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Comorbidity					
Absence	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Infectious diseases	45	1 (2.2)	0	0	1 (5.9)
Autoimmune diseases	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Malignant diseases	80	1 (1.3)	0	0	1 (5.9)
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Other disease	87	1 (1.1)	0	0	1 (5.3)

ATL indicates adult T-cell leukemia; HTLV-1, human T-cell leukemia virus type 1; VL, HTLV-1 proviral load; HU, HTLV-1 uveitis; HAU, HTLV-1–associated uveitis; HAM, HTLV-1 myelopathy; TSP, tropical spastic paraparesis; and —, not applicable.

*The VL was categorized based on quartile cutoff points (the 25th, 50th, and 75th percentiles of the VL distribution) in 1218 HTLV-1 carriers. The unit of VL was copies/100 PBMCs.

1). We found that the median proviral load of these participants was significantly higher than that of those with no comorbidity (Table 2). The results of the present study support previous reports indicating higher HTLV-1 proviral loads in HTLV-1 carriers with comorbid *Strongyloides stercoralis* or bladder and kidney infections than those without such infections.[25,35,36] HTLV-1 carriers with rheumatoid arthritis or connective tissue disease and those with myelodysplastic syndromes carrying HLA-A26 were also reported to have higher HTLV-1 proviral loads compared with the median proviral load of those without such diseases.[37,38] In the present study, however, the median proviral load was not significantly high in those with autoimmune and malignant diseases. Further studies are required to find other predisposing factors affecting the proviral load level in each person.

A high HTLV-1 proviral load is currently considered as one of the main indicators for the progression to ATL.[20,28] In the present study, 14 participants of asymptomatic HTLV-1 carriers progressed to overt ATL as of 2009, all of whose baseline proviral load levels were high (range, 4.17-28.58 copies/100 PBMCs; Table 3). Therefore, we suggest that those with a high proviral load level (~ > 4 copies/100 PBMCs) are in a high-risk group for developing ATL (this group accounted for ~ 29% of the cohort). Multivariate Cox analyses confirmed that a higher proviral load level was a strong factor in the development of ATL (Table 5). This result strongly supports previous small-scale studies.[20,28] However, the role of the high proviral load level still remains unclear because the majority of asymptomatic carriers with a high HTLV-1 proviral load level in the present study remain carrier status. In the present study, male gender was not a significant risk factor for ATL, even though the median proviral load was significantly higher in males than in females. A high HTLV-1 proviral load is also reported to be associated with HAM/TSP.[20,21,27] These findings suggest that a high

Table 5. Cox proportional hazards modeling of risk factors for ATL development

	Univariate analysis: HR (95% CI)	P	Multivariate analysis: HR (95% CI)	P
Male sex (vs female)	[illegible]	[illegible]	[illegible]	[illegible]
Square-root transformed VL per unit increase	2.55(1.91-3.41)	< .001	3.57(2.25-5.68)	< .001
Age (per 5-year increase from 30 y)	[illegible]	[illegible]	[illegible]	[illegible]
Family history of ATL (vs others)	2.68(0.80-8.98)	.11	12.1 (2.28-64.7)	.004
First opportunity for HTLV-1 testing during treatment of other diseases (vs others)	[illegible]	[illegible]	[illegible]	[illegible]

Analyses were performed using robust sandwich variance estimates.

ATL indicates adult T-cell leukemia; HTLV-1, human T-cell leukemia virus type 1; VL, HTLV-1 proviral load; HR, hazard ratio; and CI, confidence interval.

proviral load alone is not a unique predictive marker for ATL. In addition, the present study showed that the median proviral load level at enrollment was lower in those who developed aggressive types of ATL (5.1 copies/100 PBMCs) than that in those who developed smoldering types of ATL (11.4 copies/100 PBMCs; $P = .02$). This also suggests that a high proviral load alone is not a predictive marker for aggressive types of ATL.

In the present study, multivariate Cox analysis indicated that increased age, family history of ATL, and first opportunity to learn of HTLV-1 infection during treatment of other diseases were also independent risk factors for the development of ATL, after adjusting for proviral load (Table 5). This suggests that multiple risk factors (including unknown factors) are related to the progression from HTLV-1 carrier status to ATL. The reason why "opportunity to learn of HTLV-1 infection during treatment of other diseases" was an independent risk factor is unknown. The findings that more advanced states of HTLV-1 carriers (ie, an intermediate state[6] and a preleukemic state[13]) tend to be complicated by various comorbid diseases and that HTLV-1 carriers with various comorbid diseases had higher HTLV-1 proviral loads[23,35-38] could in part explain the reason.

Some prospective studies serially evaluated HTLV-1 proviral loads in HTLV-1 carriers and reported that their proviral load level was relatively stable over time with a certain level of fluctuations for persons.[25,26,28] Taylor et al reported that proviral loads of 20 HTLV-1 carriers were stable over a mean of 27 months, even though 9 carriers with various comorbidities showed high proviral load levels.[25] Meanwhile, an increasing proviral load was observed before progression to HAM/TSP and ATL.[27,28] However, there remain more questions how much of the fluctuations in proviral load over time could predict disease progression over the natural fluctuations within persons. Factors other than the proviral load level might be influencing the development of HTLV-1–associated diseases. Future studies should perform serial evaluations of HTLV-1 proviral loads by considering risk factors that have been confirmed in the present study.

The present study has several limitations. The number of ATL events was very small to obtain a conclusive result. However, we have a confidence for our results because we used a robust variance estimate in the multivariate analysis and because 2 validity analyses confirmed the original results. Data collection was insufficient for some items in the questionnaire. To resolve this issue, we will need to administer the questionnaire repeatedly. Our study design did not include enough information for evaluating the development of HAM/TSP. The follow-up duration is too short with regard to the natural history of ATL that has a long latency. Further follow-up of this cohort and similar prospective investigations should provide data needed to support more detailed conclusions. We did not compare the proviral loads by place of enrollment because we realized that many HTLV-1 carriers have migrated from the southern area to the metropolitan area.[39] The migration of HTLV-1 carriers has raised some public health issues in Japan. Screening for HTLV-1 in pregnant women and prevention programs for mother-to-child transmission of HTLV-1 are conducted in endemic areas[40,41] but not in metropolitan areas, which could introduce a higher chance of new HTLV-1 infections in the metropolitan area. To date, there is no nationwide program for preventing new HTLV-1 infections in Japan. Further nationwide studies are needed to determine the precise numbers of HTLV-1 carriers and to prevent HTLV-1 infection.

In conclusion, the present cohort study of 1218 asymptomatic HTLV-1 carriers provided detailed distributions for HTLV-1 proviral loads regarding the host-specific characteristics and the associations with the development of ATL. We confirmed that a higher proviral load levels (especially ~ > 4 copies/100 PBMCs), advanced age, family history of ATL, and having the first opportunity to learn of HTLV-1 infection during treatment of other diseases were independent risk factors for progression from carrier status to ATL. Further large-scale epidemiologic studies are needed to clearly identify the determinants of ATL for early detection and rapid cure for HTLV-1–associated diseases.

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Authorship

Contribution: M.I. managed the study database, analyzed data, and wrote the manuscript; T.W. organized the study and managed processing of the samples and measurement of proviral loads; A.U., A.O., K. Uchimaru, K.-R.K., M.O., H.K., K. Uozumi, M.M., K.T., Y. Saburi, M.Y., I.T., and Y.M. were responsible for participant enrollment and data collection; Y. Sagara managed the biomaterial bank; S.H. organized the study and managed the database; S.K. and K.Y. established the study; and all authors critically reviewed the article and approved the final version.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

A complete list of JSPFAD participants is available online in the supplemental Appendix.

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研究報告の概要

○日本の蚊で伝播する新種ウイルス感染症 チクングニヤ熱が4類感染症に指定へ

2010年10月1日に開催された厚生科学審議会感染症分科会感染症部会は、アジア・アフリカ諸国での流行が問題となっているチクングニヤ熱を感染症法における4類感染症に追加することに合意した。4類感染症に指定されることで、患者を診断した医師には速やかな届け出が義務づけられる。さらに今回の部会では、チクングニヤ熱を検疫の対象となる感染症(検疫感染症)に追加することも合意された。

チクングニヤ熱は日本国内に広く生息するヒトスジシマカの媒介で伝播しうる。急性期の患者における血中のウイルス量は多く、そのような患者を刺した蚊を介してさらに感染者が広まる危険性がある。そのため感染症部会は、日本に持ち込まれたチクングニヤ熱が、国内で広まる可能性は十分高いと危惧している。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
厚生科学審議会感染症分科会感染症部会は、アジア・アフリカ諸国での流行が問題となっているチクングニヤ熱を感染症法における4類感染症に追加することに合意したとの報告である。	日本赤十字社では、輸血感染症対策として受付時に海外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。

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NEWS

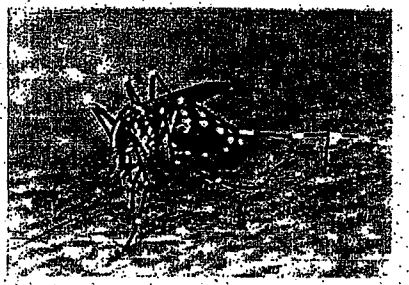
2010. 10. 1

日本の蚊で伝播する新種ウイルス感染症

チクングニア熱が4類感染症に指定へ

小坂橋佳子＝日経メディカル

10月1日に開催された厚生科学審議会感染症分科会感染症部会は、アジア・アフリカ諸国での流行が問題となっているチクングニア熱を感染症法における4類感染症に追加することを合意した。チクングニア熱は日本に広く生息するヒトスジシマカ（写真）を介して伝播する。



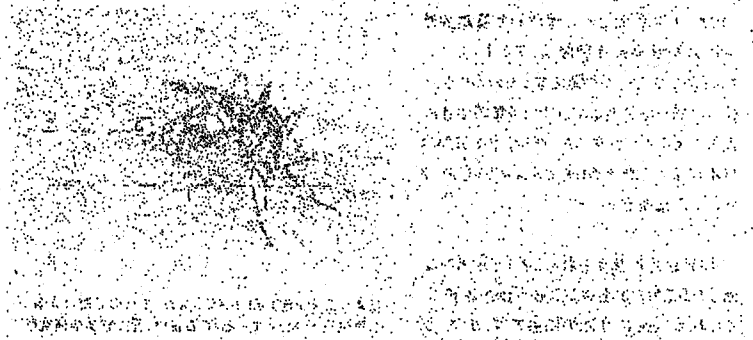
日本に広く分布するヒトスジシマカ。生息の北限は青森八戸付近といわれている（写真提供：国立感染症研究所昆虫医科学部）

4類感染症に指定されることで、患者を診断した医師には速やかな届け出が義務づけられる。さらに今回の部会では、チクングニア熱を検疫の対象となる感染症（検疫感染症）に追加することも合意された。

チクングニア熱は、チクングニアウイルスにより発症する急性熱性疾患で、発熱、全身倦怠、リンパ節腫脹、頭痛、筋肉痛に加え、手関節などに関節炎を生じる。インドやスリランカなどのアジア諸国で流行しており、日本においても、18人のチクングニア熱が報告されており、その全員が東南アジアなどへの渡航歴がある。

これまでチクングニア熱は死亡例の報告がほとんどなかったが、近年流行している株による感染では、呼吸不全や心不全、髄膜脳炎、劇症肝炎、腎不全などによる死亡例が報告されている。突然変異によって病原性の高いウイルス株に変異している可能性を指摘する専門家もいる。

チクングニア熱は、日本国内に広く生息するヒトスジシマカの媒介で伝播する。急性期の患者における血中のウイルス量は多く、そのような患者を刺した蚊を介してさらに感染者が広まる危険性がある。そのため感染症部会は、日本に持ち込まれたチクングニア熱が、国内で広まる可能性は十分に高いと危惧している。実際、2007年にはイタリアで、インドへの渡航歴がある1人の患者から感染が広まり、約250人を超える感染患者が出ている。今年9月末にはフランスで、海外への渡航歴がないチクングニア熱患者が報告された。



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Inactivation and removal of influenza A virus H1N1 during the manufacture of plasma derivatives

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ABSTRACT

Although transmission of pandemic influenza A virus H1N1 2009 is still occurring globally, little has been reported about how this outbreak has affected the safety of plasma derivatives. To evaluate the safety of plasma derivatives, dedicated virus clearance processes used during their production were investigated for their effectiveness in eliminating this virus of recent concern. In this study, influenza A virus H1N1 strain A/NWS/33 (H1N1) was chosen as a surrogate. H1N1 was completely inactivated by fraction IV fractionation as well as pasteurization during the manufacture of albumin. H1N1 was also effectively removed into the precipitate by fraction III fractionation and completely inactivated by low pH incubation as well as pasteurization during the manufacture of intravenous immunoglobulin. H1N1 was completely inactivated within 1 min of solvent/detergent treatment using 0.3% tri (n-butyl) phosphate and 1.0% Triton X-100 and also completely inactivated within 10 min of dry-heat treatment at 98 °C during the manufacture of factor VIII. H1N1 was completely removed by virus filtration process using Viresolve NFP filter and also completely inactivated by pasteurization during the manufacture of antithrombin III. These results indicate that all the virus clearance processes commonly used have sufficient H1N1 reducing capacity to achieve a high margin of safety.

1. Introduction

The 2009 flu pandemic is a global outbreak of a recently emerged novel influenza virus. The virus was identified to be a swine-origin influenza A virus H1N1 [1–3]. According to World Health Organization monitoring report of July 2, 2010, more than 214 countries have reported laboratory confirmed cases of pandemic influenza A virus H1N1 2009, including over 18,239 deaths [4]. This virus has a hemagglutinin (HA) gene that is derived from the 1918 swine influenza virus and other genes from human, avian, and Eurasian swine influenza viruses.

H1N1, H1N2 and H3N2 subtype swine influenza viruses and highly pathogenic avian influenza virus H5N1 have occasionally infected humans before but such zoonotic transmission events did not lead to sustained human-to-human transmission in the manner that the swine-origin influenza virus has done [5]. Transmission of pandemic influenza A virus H1N1 2009 is still occurring globally. There are concerns that the virus may reassort with existing human influenza virus giving 'rise' to more transmissible or more pathogenic viruses. The virus appears to retain the potential to transmit back to swine and thus continued reassortment with swine viruses is a cause for concern.

Although the occurrence of influenza virus in the blood has been reported in some previous studies [6–9], no cases of transfusion transmission of influenza have been reported to date [10]. However, the confirmed human cases of influenza viremia have raised concerns about the safety of plasma derivatives as well as labile blood products for transfusion [10,11]. The potential for transmission of pandemic influenza H1N1 through blood transfusion remains unknown because there is limited information available on pandemic (H1N1) 2009 virus viremia, especially during the asymptomatic period. At this time, the pandemic (H1N1) 2009

Abbreviations: H1N1, Influenza A virus H1N1 strain A/NWS/33; HA, Hemagglutinin; NA, Neuraminidase; MDCK, Madin–Darby canine kidney; CPE, Cytopathic effect; CPMP, Committee for Proprietary Medicinal Products; IVIG, Intravenous Immunoglobulin; S/D, Solvent/detergent; Factor VIII, Antihemophilic factor VIII; TNBP, Tri (n-butyl) phosphate; ATIII, Anti-thrombin III.

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研究報告の概要

2009年のインフルエンザAウイルスH1N1の世界的流行が未だ猛威をふるっている。これまで、輸血による感染症例は報告されていないが、インフルエンザのウイルス血症症例が確認されていることで、血液製剤同様血漿分画製剤の安全性への懸念が高まっている。血漿分画製剤の安全性を評価するため、このウイルスの除去効果について、製造で使用される特定のウイルスクリアランス工程が調査された。

本研究では、インフルエンザAウイルスH1N1株A/NWS/33(H1N1)がモデルウイルスとして選択された。アルブミンの製造工程において、パスツリゼーションおよびフラクションIV分画により H1N1 は不活性化された（対数減少係数 ≧5.34 および ≧2.53）。また、静注用免疫グロブリンの製造工程においては、フラクションIII分画によって効果的に除去され（対数減少係数 ≧4.66）、低 pH インキュベーションおよびパスツリゼーションによって不活性化された（対数減少係数 ≧5.41 および ≧4.65）。第Ⅷ因子の製造工程では、0.3%リン酸トリ-n-ブチルと 1.0%トリトン X-100 を用いた S/D 処理で 1 分以内に不活化され（対数減少係数 ≧3.54）、また 98℃乾燥加熱でも 10 分以内に不活化された（対数減少係数 ≧4.36）。アンチトロンビンIIIの製造工程においても、Viresolve NFP フィルターで除去され（対数減少係数 ≧4.92）、パスツリゼーションで不活化された（対数減少係数 ≧3.75）。

この研究を通じて、H1N1は、一般に用いられるウイルスクリアランス工程（低温エタノール分画、パスツリゼーション、低pH処理、S/D処理、乾燥過熱処理、ウイルスろ過工程）により、効果的に不活化・除去されることがわかった。これは、様々なモデルウイルスや関連ウイルスを用いて種々の血漿分画製剤で試験されたときに得られる通常の結果と一致している。また、限られた種類のモデルウイルスによる特定の工程を検証するために用いられる一般的な方法を支持するものであった。これらの結果より、ウイルスバリデーション試験を含む血漿分画製剤の製造工程は、高い安全性をもたらす十分なH1N1除去能力を持っていることが示された。

使用上の注意記載状況・その他参考事項等

報告企業の意見	今後の対応
今回の研究で、インフルエンザAウイルスH1N1は、通常の血漿分画製剤の製造工程において、不活化・除去されていることがわかった。よって、本剤の製造工程においてもインフルエンザAウイルスH1N1は不活化・除去されると考えられる。	今後とも新しい感染症に関する情報収集に努める所存である。

virus has not been isolated from blood or serum of asymptomatic, infected individuals.

The manufacturing processes of plasma derivatives must include virus inactivation and/or removal processes to ensure viral safety, because plasma derivatives are manufactured from pooled human plasma. Specific viral inactivation processes such as solvent/detergent (S/D) treatment, pasteurization, dry-heat treatment, and low pH incubation are the cornerstone in ensuring a sufficient margin of safety of plasma products. Fractionation of plasma by ethanol precipitation and protein purification by chromatography contribute to the removal of viruses by partitioning. Nanofiltration is a specific approach to eliminate viruses [12,13]. Validation of the process for viral inactivation and/or removal plays an essential and important role in establishing the safety of plasma derivatives [14,15].

To date, there is no information about the inactivation and removal of influenza A virus H1N1 during the manufacture of plasma derivatives. To evaluate the safety of plasma derivatives against influenza A virus H1N1, dedicated virus clearance processes used during the production of plasma derivatives were investigated for their effectiveness in inactivating and/or removing this virus of recent concern. In this study, influenza A virus H1N1 strain A/NWS/33 (H1N1) was chosen as a model for pandemic influenza A virus H1N1 2009.

2. Materials and methods

2.1. Preparation and titration of influenza A virus H1N1

In this study, H1N1 strain A/NWS/33 (ATCC VR-219) was chosen as a surrogate of pandemic influenza A virus H1N1 2009 [16]. For the propagation and titration of H1N1, Madin–Darby canine kidney (MDCK) cells (KCLB 10034) were used. MDCK cells were grown in a high glucose Dulbecco's modified Eagle's medium (Hyclone, Thermo Fisher Scientific Inc., Logan, USA) containing 10% fetal bovine serum.

An aliquot from each sample from the virus inactivation studies and an appropriate control were titrated immediately after being collected in 7-fold serial dilutions to the end point using a quantal 50% tissue culture infectious dose ($TCID_{50}$) assay [16,17]. When no infectious virus was detected, the virus titer was calculated using a theoretical minimum detectable level of infectious virus, with a 95% upper confidence level. As a part of the virus validation protocol, cytotoxicity, interference, and load titer tests were performed. The load titer assays were performed to determine precisely the point at which spiking the virus into the starting material resulted in a loss in the virus titer. The virus log reduction factor was defined as the $\log_{10}$ of the ratio of the virus loads in the spiked starting and post process materials, in accordance with Committee for Proprietary Medicinal Products (CPMP) guidance [15]. All the virus inactivation experiments were carried out in duplicate and mean values are given.

2.2. Downscaled manufacturing processes for plasma derivatives

To ensure that the performance of the scale-down process was representative of the production situation, critical process parameters and selected product parameters were compared. To ensure that the performance of the scale-down processes of fraction IV precipitation and fraction III fractionation was representative of the production scale, the physico-chemical properties, such as the recovery yield of protein, pH, temperature, and ethanol concentration, were compared. To ensure that the scale-down pasteurization procedures were equivalent to those used in the manufacture of 20% albumin and immunoglobulin in production facilities, the physico-chemical properties, such as the pH, concentrations of proteins, and aggregate contents, were compared. For the validation of the scale-down process of S/D treatment, the concentration of inactivating agents was evaluated. To ensure that the scale-down dry-heat procedure was equivalent to that used in the manufacture of factor VIII in production facility, the physico-chemical properties, such as factor VIII activity, moisture content, and clottable proteins, were compared. To ensure that the scale-down nanofiltration and pasteurization procedures were equivalent to those used in the manufacture of ATIII in production facility, the physico-chemical properties, such as ATIII activity, pH, and protein content, were compared. Temperature as a critical process parameter for virus inactivation was monitored throughout all the processes investigated. Process intermediates were obtained from the manufacturing scale and used as starting materials.

2.3. Fraction IV fractionation and pasteurization process for albumin

The partitioning profile of the virus after the fraction IV fractionation was assessed in order to evaluate the efficacy of the fraction IV fractionation process in eliminating H1N1. Fraction IV4–2 was prepared by adding 34.7 ml of 95% ethanol to 80 ml of fraction IV4-1, while maintaining the temperature at −5.5 °C, over a 7 h period. The final ethanol concentration reached was 40%. An 80 ml aliquot of the fraction IV4–2 suspension, at −5.5 °C, was then spiked with 8 ml of virus. An 8 ml aliquot of the sample was then immediately removed and titrated. The remaining material was maintained at −5.5 °C over a period of 8 h and then filtered at a pressure of 1.0–1.5 kg/cm^2 using 0.2 μm Supra-80 membranes (Seitz-Schenk, Germany). The resulting filtrate fraction was collected and the retained fraction IV paste was resuspended in cell culture medium with an equivalent volume to that of the filtrate. All samples were neutralized to pH 6.5–7.5 on collection where required and an aliquot from each sample was immediately titrated.

The effectiveness of pasteurization (heat treatment at 60 °C for 10 h) in inactivating H1N1 was determined. Virus inactivation was investigated at 58 °C as the worst-case process temperature, that is just below the temperature specified for manufacturing. A 60 ml aliquot of the final 20% albumin solution containing a stabilizer (13.3 mg sodium caprylacid and 19.7 mg acetyl tryptophane per 1 g albumin) was spiked with 6 ml of virus stock solution and then a 6 ml load sample was removed for titration. The remaining material was heated in a water bath and equilibrated to 58 ± 0.5 °C. Samples were removed at different times over 10 h. An aliquot of each sample was immediately titrated.

2.4. Fraction III fractionation, pasteurization, and low pH incubation for intravenous immunoglobulin (IVIG)

The efficacy and mechanism of fraction III fractionation in the clearance of H1N1 was evaluated. A 96 ml aliquot of the fraction I+II + III-w suspension was spiked with 9 ml of the virus. An 8 ml aliquot of the sample was then immediately removed to determine the viral titer in the spiked starting material. The remaining material was continuously added with 95% ethanol, at a temperature of −20 °C, to a final concentration of 18%, while maintaining the temperature at −5.5 °C, over a 20 h period. The material was then filtered at a pressure of 1.0–1.5 kg/cm^2 using 0.2 μm Supra-80 membranes (Seitz-Schenk, Germany). The resulting filtrate was collected and the retained paste I+III fraction was resuspended in cell culture medium with an equivalent volume to that of the supernatant. An aliquot from each sample was immediately titrated.

The effectiveness of pasteurization (heat treatment at 60 °C for 10 h) in inactivating H1N1 was determined. Virus inactivation was investigated at 58 °C as the worst-case process temperature. The dissolved fraction II solution was dialyzed against water to remove the residual ethanol, and 35 ml of the aliquot solution was then spiked with 3.5 ml of the virus. A 10 ml of the aliquot sample was then immediately removed to determine the viral titer in the spiked starting material. Sorbitol was added to the remaining solution as much as 33% (w/v) and the resulting solution was pasteurized at 58 ± 0.5 °C for 10 h. Samples were removed at different times and then immediately titrated. Protein concentration and pH of IVIG solution during pasteurization were 3.3% and 5.0, respectively.

The efficacy of low pH treatment in inactivating H1N1 was determined. A 54 ml aliquot of IVIG solution was spiked with 6 ml of the virus. A 10 ml of the aliquot sample was then immediately removed to determine the viral titer in the spiked starting material. pH of the remaining solution was adjusted to 3.9 and then the solution was incubated at 25 °C for 14 days. Samples were removed at various times and then immediately titrated. Protein concentration during low pH treatment was 5%.

2.5. S/D and dry-heat treatments for antihemophilic factor VIII (factor VIII)

To validate the effectiveness and robustness of S/D treatment in inactivating H1N1, 5 ml of the virus stock was spiked into 45 ml of pre-S/D solution (supernatants of solubilized cryoprecipitate) equilibrated at 22 °C. A 10 ml aliquot sample was then immediately removed to determine the viral titer in the spiked starting material. The remaining solution was treated with tri (n-butyl) phosphate (TnBP) and Triton X-100, with the final concentration of 0.3% (v/v) and 1.0% (v/v), respectively and then incubated at 22 °C. Samples were taken at indicated time periods, diluted 128-folds with cell culture medium upon being collected to stop the cytotoxic and interfering effects of the S/D, and then titrated immediately. To assess further the robustness of S/D treatment, the virus-spiked solution was treated with the concentration of 50% and 25% of the standard S/D concentrations. Protein concentration and pH of the cryoprecipitate solution during S/D treatment were 13.6 mg/ml and 7.1, respectively.

The effectiveness of dry-heat treatment at 100 °C for 30 min in inactivating H1N1 was evaluated. As the worst-case condition for dry-heat treatment, 98 ± 0.5 °C was adopted. The virus stock was spiked to the final factor VIII complex solution as 10% (v/v) of the total volume of the material. The virus-spiked samples were distributed in final containers at 10 ml/vial. As reference samples for monitoring the temperature, factor VIII activity, and residual moisture content, the factor VIII solution, without the addition of virus, was filled into the vials. After lyophilization, the residual moisture content of the freeze–dried factor VIII was determined by a loss on drying method. Titers of viruses were measured before and after lyophilization. The lyophilized samples were loaded into a convection-drying oven (Korea Science, Seoul, Korea), equilibrated at 30 °C. The dry-heat treatment was then processed for 30 min at 98 ± 1 °C. As soon as the temperature reached 98 °C, the incubation period of dry heating was initiated. Samples were removed at different times, and the titers of the viruses were then measured.

2.6. Pasteurization and nanofiltration for anti-thrombin III (ATIII)

The effectiveness of pasteurization (heat treatment at 60 °C for 10 h) in inactivating H1N1 was determined. Virus inactivation was investigated at 58 °C as the worst-case process temperature. A 5 ml of the virus stock was spiked to 45 ml of purified bulk solution. A 10 ml of the aliquot sample was then immediately removed to determine the viral titer in the spiked starting material. 40 ml of tri-sodium citrate (1 M, pH 7.0) was added to the remaining solution as the stabilizer and the resulting solution were pasteurized at 58 ± 0.5 °C for 10 h. Samples were removed at different times and then immediately titrated. Protein concentration and pH of ATIII during pasteurization were 4.5 mg/ml and 7.2, respectively.

To evaluate the effectiveness and robustness of the Viresolve NFP (Millipore, Jaffrey, USA) filtration process in eliminating H1N1, a Viresolve NFP disk membrane (active filtration area of 3.5 cm^2) was used to simulate the process performance of the production scale cartridges at 20 °C. ATIII solution was pre-filtered using a 0.1-μm membrane (Millex-VV, Millipore, Cork, Ireland). This pre-filtration was performed within 5 min prior to spiking the test solution with the virus. 47.5 ml of the pre-filtered ATIII solution was spiked with 2.5 ml of the virus stock solution. A 10 ml of the aliquot sample was then immediately removed to determine the viral titer in the spiked starting material. The virus-spiked ATIII solution was then filtered using a 0.22 μm membrane (Millex-GV, Millipore, Cork, Ireland) to remove any viral aggregates, particulates, host cells, or viruses bound to proteins. A 10 ml of the aliquot sample was then immediately taken to determine the viral titer in the pre-filtered ATIII solution. Subsequently, 30 ml of the remaining solution was filtered through a Viresolve NFP disk membrane at a constant pressure of 2.0 bar and then the filtrate was immediately titrated. Thereafter, the post-run integrity of the filter was tested by submerging the assembled membrane holder in water for 1 min at 2.7 bar and checking for any leaks as seen by the formation of bubbles. Sterile pressurized air was used during the filtration and the virus filtration process was conducted in a cold chamber at 4–8 °C. Protein concentration and pH of ATIII during nanofiltration were 10 mg/ml and 7.0, respectively.

3. Results

3.1. Inactivation of H1N1 by fraction IV fractionation and pasteurization during the manufacture of albumin

After the separation of the precipitates, the titers of the virus in the resulting filtrate and the fraction IV paste were analyzed (Table 1). No infectious virus was detected in the filtrate, thereby indicating the complete elimination of the virus during fraction IV fractionation. The log reduction factor achieved was ≥2.53. Furthermore no infectious virus was found to be present in the paste. These results indicate that all the spiked viruses were inactivated during fraction IV fractionation by the addition of ethanol, thus suggesting that the mechanism of reduction was inactivation rather than partitioning

Table 1
Inactivation of H1N1 by fraction IV fractionation and pasteurization during the manufacture of albumin.

Process	Sample	Total virus titer ($TCID_{50}$)
Fraction IV fractionation	Starting material spiked with virus	7.67
	Fraction IV supernatant	5.15
	Fraction IV paste	5.17
	LRF reduction factor	≥2.53
Pasteurization at 58 °C	Starting material spiked with virus	7.67
	0.5 h after pasteurization	5.00
	1 h after pasteurization	2.15
	2 h after pasteurization	2.14
	5 h after pasteurization	2.12
	10 h after pasteurization	2.12
	LRF reduction factor	≥5.55

under these experimental conditions. To confirm the mechanism of H1N1 elimination during fraction IV fractionation, the inactivation kinetics of H1N1 under the conditions of fraction IV fractionation (ethanol 40%, pH 6.0, −5.5 °C) were studied. H1N1 was inactivated from an initial titer of 7.90 $\log_{10}$ $TCID_{50}$ to undetectable levels within 30 min of treatment (data not shown).

The effectiveness of heat treatment at 58 °C as the worst-case process temperature for pasteurization in inactivating H1N1 was determined (Table 1). H1N1 was inactivated from an initial titer of 7.47 $\log_{10}$ $TCID_{50}$ to 3.00 $\log_{10}$ $TCID_{50}$ after 0.5 h of treatment and then completely inactivated to undetectable levels within 1 h of treatment. The log reduction factor obtained was ≥5.34.

3.2. Partitioning and inactivation of H1N1 by fraction III fractionation, pasteurization, and low pH incubation processes during the manufacture of IVIG

H1N1 was effectively removed during the fraction III fractionation process (Table 2). After the separation of the precipitates, no infectious H1N1 was detected in the filtrate but a major quantity of virus was recovered in the waste fraction III paste. The log reduction factor achieved was ≥4.66.

The effectiveness of heat treatment at 58 °C as the worst-case process temperature for pasteurization in inactivating H1N1 was determined (Table 2). H1N1 was completely inactivated from an initial titer of 7.21 $\log_{10}$ $TCID_{50}$ to undetectable levels within 0.5 h of incubation. The log reduction factor obtained was ≥4.65.

The efficacy of low pH incubation at pH 3.9 and 25 °C for 14 days in inactivating H1N1 was determined. H1N1 was inactivated instantaneously from an initial titer of 7.54 $\log_{10}$ $TCID_{50}$ to undetectable levels within 1 min of incubation (Table 2). The log reduction factor obtained was ≥5.41.

3.3. Inactivation of H1N1 by S/D treatment and dry-heat treatment during the manufacture of factor VIII

H1N1 was inactivated to undetectable levels within 1 min of S/D treatment (Table 3). The log reduction factor achieved was ≥3.54. To further assess the robustness of S/D treatment, the kinetics of inactivation of H1N1 was studied at decreased concentrations of TnBP and Triton X-100. Virus solutions were treated with the concentration of 50% and 25% of the standard manufacturing conditions and then the results were compared with that obtained with the standard S/D concentration (data not shown). S/D treatment with the reduced concentrations was also very effective in inactivating H1N1. H1N1 inactivation was complete within 1 min even when the concentrations of TnBP and Triton X-100 were reduced to 25% of the standard concentrations.

The effect of dry-heat treatment at 98 °C after lyophilization on the inactivation of H1N1 was measured (Table 3). H1N1 was highly resistant to the lyophilization process. The titer of H1N1 was slightly reduced from 6.96 $\log_{10}$ $TCID_{50}$ to 6.35 $\log_{10}$ $TCID_{50}$ during the lyophilization, with an average log reduction factor of 0.61. The remaining H1N1 after lyophilization was completely inactivated to undetectable levels within 10 min of dry-heat treatment. The log reduction factor obtained was ≥4.36. The mean residual moisture values of factor VIII were 0.70% after lyophilization and 0.69% after dry-heat treatment.

Table 2
Removal and inactivation of H1N1 by fraction III fractionation, pasteurization, and low pH incubation during the manufacture of IVIG.

Process	Sample	Total virus titer ($\log_{10}$ $TCID_{50}$)
Fraction III fractionation	Starting material spiked with virus	8.82
	Fraction III supernatant	≤4.16
	Fraction III paste	7.51
	Log reduction factor	≥4.66
Pasteurization at 58 °C	Starting material spiked with virus	7.21
	0.5 h after pasteurization	≤2.56
	1 h after pasteurization	≤2.56
	2 h after pasteurization	≤2.56
	5 h after pasteurization	≤2.56
	10 h after pasteurization	≤2.56
	Log reduction factor	≥4.65
Low pH treatment at pH 3.9 and 25 °C	Starting material spiked with virus	7.54
	1 min after treatment	≤2.13
	1 day after treatment	≤2.13
	5 day after treatment	≤2.13
	9 day after treatment	≤2.13
	14 day after treatment	≤2.13
	Log reduction factor	≥5.41

Table 3
Inactivation of H1N1 by S/D treatment and dry-heat treatment processes during the manufacture of factor VIII.

Process	Sample	Total virus titer ($\log_{10}$ $TCID_{50}$)
0.3% TNBP + 1% Triton X-100 treatment	Starting material spiked with virus	7.50
	1 min after S/D treatment	≤3.96
	5 min after S/D treatment	≤3.96
	10 min after S/D treatment	≤3.96
	30 min after S/D treatment	≤3.96
	Log reduction factor	≥3.54
Dry-heat treatment at 98 °C	Starting material spiked with virus	6.96
	After lyophilization	6.35
	10 min after dry-heat treatment	≤2.60
	20 min after dry-heat treatment	≤2.60
	30 min after dry-heat treatment	≤2.60
	Log reduction factor	≥4.36

3.4. Removal and inactivation of H1N1 by nanofiltration and pasteurization during the manufacture of ATIII

To evaluate the effectiveness and robustness of the Viresolve NFP filtration process in eliminating H1N1, three different lots of Viresolve NFP disk membranes were challenged with H1N1 (Table 4). None of the infectious virus was detected in the filtrate of any of the three filter lots tested, indicating that H1N1 was completely removed to undetectable levels. The log reduction factor achieved was ≥4.92.

The effectiveness of heat treatment at 58 °C as the worst-case process temperature for pasteurization in inactivating H1N1 was determined (Table 4). H1N1 was completely inactivated from an initial titer of 6.65 $\log_{10}$ $TCID_{50}$ to undetectable levels within 0.5 h of incubation. The log reduction factor obtained was ≥3.75.

4. Discussion

Ethanol is known to be both bactericidal and virucidal and is the principal precipitation agent in cold ethanol fractionation used in the manufacture of plasma products. The highest concentration of ethanol in the fractionation is 40% and in some steps only 8–25%. In addition to the virucidal effect of ethanol, the partitioning of viruses during fractionation is also important in determining the effectiveness of this process in the production of safe plasma products [18]. The mechanism of H1N1 clearance during fraction IV fractionation, which involved the use of a higher ethanol concentration of 40%, was found to be inactivation rather than partitioning. Previous reports have also shown that the mechanism of reduction for the enveloped viruses such as human immunodeficiency viruses (HIV), bovine viral diarrhea virus (BVDV), and bovine herpes virus (BHV) during fraction IV fractionation was inactivation rather than partitioning, however, it was partitioning in the case of the resistant non-enveloped viruses such as hepatitis A virus (HAV), murine encephalomyocarditis virus (EMCV), and porcine parvovirus (PPV) [19–21]. H1N1 was also completely removed during fraction III fractionation. However, the major mechanism of H1N1-reduction during fraction III fractionation was found to be partitioning. Partial inactivation of H1N1 was also observed during fraction III fractionation. Although no infectious viruses were detected in the filtrate, the recovery of H1N1 in the fraction III paste was lower than the amount of spiked virus. The difference of total virus titer before and after the fraction III fractionation was about 1.31, which showed that infectivity of H1N1 was also reduced, to a limited extent, by the added ethanol during the fraction III fractionation. This result is consistent with the previous report that the mechanism of reduction in eliminating enveloped viruses such as BHV and BVDV was principle removal but with also some inactivation [22].

A commonly used method for the inactivation of virus contaminants in plasma products is heating in a liquid for at least 10 h at 60 °C [19–21,23]. The inactivation kinetics of H1N1 showed that H1N1 was completely inactivated during the pasteurization process, with no residual infectivity being detected on completion of the pasteurization process resulting in a high level of virus inactivation. The times needed for achieving a complete inactivation of H1N1 in these experimental conditions were 1 h for albumin, 0.5 h for IVIG, and 0.5 h for ATIII. According to these results, it can be concluded that pasteurization process is a robust and effective step in eliminating H1N1. It has been also reported that H1N1 was inactivated to undetectable levels within 5 min, 2.5 min, and 1 min of heat treatment at 70, 80, and 90 °C, respectively [16]. Avian influenza A virus H5N1 was also very sensitive to pasteurization, being completely inactivated within 30 min of pasteurization during the manufacture of albumin [24].

Low pH incubation of IVIG has been reported by a number of investigators to effectively inactivate enveloped viruses such as HIV, BVDV, BHV, herpes simplex virus, cytomegalovirus, vesicular stomatitis virus, and Semliki Forest virus [22,25,26]. H1N1 was extremely sensitive to low pH treatment. H1N1 was inactivated in 1 min of treatment at pH 3.9. Previous report has also shown that avian influenza A virus H5N1 was inactivated instantaneously by the treatment at pH 4.4 or 4.9 [24]. This extreme sensitivity of influenza viruses to low pH was probably due to the low pH-triggered conformational change in influenza virus HA [27]. During the infection cycle of influenza viruses a low pH-induced fusion event is needed for the virus to escape from the late endosome into the cytosol, and if this event occurs outside the susceptible cell, the virus would be rendered noninfectious [24].

One of the most widespread methods for inactivating enveloped virus contaminants in plasma products is the S/D treatment [28,29]. The solvent frequently used in the S/D method is TnBP and the detergent is either Tween 80, Triton X-100, sodium cholate, Tween-20, or Triton X-45. Organic S/D mixtures disrupt the membranes of viruses which have lipid envelopes. The results of inactivation kinetics obtained from the present study showed that S/D treatment is a robust and effective step in eliminating H1N1. This virus was completely inactivated to undetectable levels within 1 min of the 60 min process time by treatment with 0.3% TNBP and 1% Triton X-100. In addition, H1N1 was completely inactivated within 1 min when a concentration of 25% of the standard manufacturing concentration was used. These conditions were chosen as the worst-case that could be encountered in the manufacturing process.

Dry-heat treatment is another of the choices generally recommended for virus inactivation [30–32]. It is known that virus infectivity substantially diminishes after being subjected to lyophilization as well as dry-heat treatment. Therefore, the effects of both of these processes on the inactivation of H1N1 were measured. H1N1 was highly resistant to the lyophilization process, with an average log reduction factor of 0.61. However H1N1 remaining after lyophilization was completely inactivated to undetectable levels within 10 min of dry-heat treatment at 98 °C.

Another potential way to increase the safety of therapeutic biological products is the use of virus-retentive filters [33,34]. H1N1 is an 80–120 nm size pleiomorphic virus [35]. In this experiment, H1N1 was completely removed to levels below the detection limit during the Viresolve NFP (pore-size 20 nm/molecular weight cut off 160 kDa) filtration process for ATIII. This result indicates that the filtration process was a robust and effective method for removal of H1N1 from plasma derivatives. H1N1 would, based on its size, also be expected to be removed by the now widely used virus filters with nominal pore sizes in the 15- to 75-nm range.

Through this study it was found that H1N1 is effectively inactivated or removed by all the commonly used virus clearance procedures, such as cold ethanol fractionation, pasteurization, low pH treatment, S/D treatment, dry-heat treatment, and virus filtration process. This is in agreement with the general results obtained using a range of model and relevant viruses when tested on various plasma products. Also this lends further support to the general strategy used for validating specific processes with a small range of model viruses. These results indicate that the production processes for plasma derivatives, containing validated virus clearance procedures, have a high H1N1-reduction capacity to achieve a high margin of safety.

Table 4
Removal and inactivation of H1N1 by Viresolve NFP filtration and pasteurization during the manufacture of ATIII.

Process	Sample	Total virus titer ($\log_{10}$ $TCID_{50}$)
Viresolve NFP filtration	Starting material spiked with virus	7.35
	Pre-filtered solution	7.20
	Filtrate after Viresolve NFP filtration	≤2.43
	Log reduction factor	≥4.92
Pasteurization at 58 °C	Starting material spiked with virus	6.65
	0.5 h after pasteurization	≤2.90
	1 h after pasteurization	≤2.90
	2 h after pasteurization	≤2.90
	6 h after pasteurization	≤2.90
	10 h after pasteurization	≤2.90
	Log reduction factor	≥3.75

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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	○中国のデング熱 デング熱は蚊が媒介する急性感染症であり、深刻な世界的公衆衛生問題となっている。 中国では大規模アウトブレイクが1978年に既に報告されている。1978～2008年の30年間に合計655,324症例が報告され、610名が死亡した。 1990年代以降、デング熱の流行は、南部沿岸地域から北部や西部にまで広がりつつある。 中国本土において、デング熱ウイルスの主要な伝播媒介動物であるヤブカ属の生物学的行動および媒介能が大きく変化してきており、これはおそらく都市化の加速や地球温暖化によるものである。また、人口増加や頻繁な国際旅行もデング熱流行の増加要因となる。デング熱制御への方法は、媒介蚊のコントロール、流行を予測する迅速ウイルス発見システムの設立、地域に密着した教育、そして安全かつ有効なワクチンを開発することである。	使用上の注意記載状況・その他参考事項等 解凍赤血球濃厚液「日赤」 照射解凍赤血球濃厚液「日赤」 解凍赤血球-LR「日赤」 照射解凍赤血球-LR「日赤」 血液を介するウイルス、細菌、原虫等の感染 vCJD等の伝播のリスク

報告企業の意見	今後の対応
急速な都市化や地球温暖化の影響により、中国におけるデング熱の流行は南部沿岸地域から北部や西部に広がりつつあり、デング熱制御への対策が必要であるとの報告である。	日本赤十字社では、輸血感染症対策として受付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。

MedDRA/J Ver.13.1J

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Review: Dengue Fever in Mainland China

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Abstract. Dengue is an acute emerging infectious disease transmitted by *Aedes* mosquitoes and has become a serious global public health problem. In mainland China, a number of large dengue outbreaks with serious consequences have been reported as early as 1978. In the three decades from 1978 to 2008, a total of 655,324 cases were reported, resulting in 610 deaths. Since the 1990s, dengue epidemics have spread gradually from Guangdong, Hainan, and Guangxi provinces in the southern coastal regions to the relatively northern and western regions including Fujian, Zhejiang, and Yunnan provinces. As the major transmission vectors of dengue viruses, the biological behavior and vectorial capacity of *Aedes* mosquitoes have undergone significant changes in the last two decades in mainland China, most likely the result of urbanization and global climate changes. In this review, we summarize the geographic and temporal distributions, the serotype and genotype distributions of dengue viruses in mainland China, and analyze the current status of surveillance and control of vectors for dengue transmission.

INTRODUCTION

Dengue fever (DF) is an acute infectious disease caused by dengue viruses and transmitted by *Aedes* mosquitoes. This disease is endemic or epidemic in more than 100 countries and regions in Asia, Oceania, America, and Africa, and it is especially prevalent in Southeast Asia, the west Pacific Ocean regions, and southern Africa. The rapid increase of DF incidence in recent years has become a serious public health threat to nearly half of the world's population.[1] The World Health Organization (WHO) estimates that approximately 2.5 billion people worldwide are at risk and 50 million people are infected by dengue viruses each year. Half a million people suffer from dengue hemorrhagic fever (DHF), which results in more than 25,000 deaths (http://www.who.int/tdr/publications/publications/dengue). Dengue is the second-most serious vector-borne disease in the world, only behind malaria in terms of morbidity and mortality.

The first outbreak of DF in mainland China occurred in Guangdong province in 1978. Since then, dengue outbreaks were recorded sequentially in Hainan, Guangxi, Fujian, and Zhejiang provinces.[2] These epidemics have had significant negative impact on the affected population, the society in general, and the development of the economy. The rapid urbanization in China changed the characteristics of DF epidemics. Dengue epidemics have spread from Guangdong, Hainan, and Guangxi provinces in the southern coastal regions to the relatively northern and western regions including Fujian, Zhejiang, and Yunnan provinces, with shorter epidemic intervals as compared with those experienced before the 1990s.

PREVALENCE

Geographic distribution. Dengue fever in mainland China is still characterized as an imported epidemic disease, and so far has not been confirmed to be an endemic.[3] Sporadic cases and outbreaks of DF in the southeast coast region, the middle and lower reaches of the Yangzi River were documented in the early 1940s,[4,5] but since then, no cases were reported. In May, 1978, a sudden outbreak of DF occurred in Foshan, Guangdong province, and it was spread to seven adjacent counties and cities where a total of 22,122 cases, including 16 fatalities were reported.[6] In the past 30 years (1978 to 2008), DF outbreaks in varying scales have occurred in China, and a total of 655,324 cases were documented, resulting in 610 deaths. Figure 1 presents the annual cases of DF in the mainland from 1978 to 2008. In Hainan province, the two most severe outbreaks of DF and DHF occurred in 1980 and 1986, resulting in > 600,000 cases with 475 deaths overall. The 1980 outbreak alone caused 454,205 cases.[7] However, no additional DF outbreaks have been reported from Hainan province since 1991.[7] In recent years, Guangdong province has the highest incidence of DF epidemics (Figure 2), with cases reported every year since 1997.[8,9]

Before the 1990s, large-scale epidemics of DF were characterized by a sudden outbreak, fast progression, quick transmission, and were under control within 2 or 3 years of onset (Figure 1). After the major outbreaks, the following DF epidemics would usually become weak either in the incidence or in transmission speed, but the affected area was more extensive. The DF epidemic now spreads gradually from Guangdong, Hainan, and Guangxi provinces in the Southern coastal regions to the relatively Northern regions, including Fujian, Zhejiang, and Yunnan provinces (Figure 3). In Fujian province in particular, a major outbreak with 1,549 cases was reported in 1999 in Fuzhou.[10] In 2004, a DF outbreak with 83 cases reported in Zhejiang province.[11] An outbreak with 56 cases was reported in 2008 in Yunnan province (State Ministry of Public Heath in October 2008, http://www.moh.gov.cn/publicfiles/business/htmlfiles/zwgkzt/pyq/index.htm), which was the first outbreak of the disease in this region since 1949.

Currently, no evidence is available to support the presence of epidemic foci in China, and most of the researchers attributed the prevalence of DF to the imported cases.[12–17] However, with the rapid growth of the Chinese economy, international exchanges are more frequent than ever. In the meantime, more than 10% of China's population has moved away from their original residences, mainly from poor rural areas to urban centers, searching for better working and living opportunities. This migration promotes the transmission of infectious diseases and creates major challenges to prevent and control them.[18] It is possible that DF is transforming from an imported to an endemic infectious disease, especially in Guangdong province where the climate favors the survival and transmission of Dengue virus. Although DF in mainland China has not resulted in large epidemics since 1990 (> 10,000 cases), frequent small outbreaks of DF (1,000–7,000 cases) ensure long-term viral circulation in local regions, which has the potential of making DF endemic if no effective intervention is implemented.

Temporal distribution. In mainland China, DF is prevalent mostly in the tropical and subtropical regions (south of 29° north latitude), where the mosquito vectors of dengue viruses breed throughout the year. Figure 4 shows the total number of DF cases reported monthly from 2002 to 2008 (http://www.moh.gov.cn/publicfiles//business/htmlfiles/zwgkzt/pyq/index.htm). January to May is identified as the period of sporadic occurrence, while June to December is recognized as the prevalent period of DF. In general, July is the early stage of the epidemic, which then increases from August to October with 14,487 cases, accounting for 92.34% of the total cases reported within these years. It is documented that the prevalence of virus is associated highly with the breeding activity of the *Aedes* mosquitoes. *Aedes albopictus* is the predominant species in South China. It can breed in various small containers or plants that hold accumulated water (such as tree holes, bamboo

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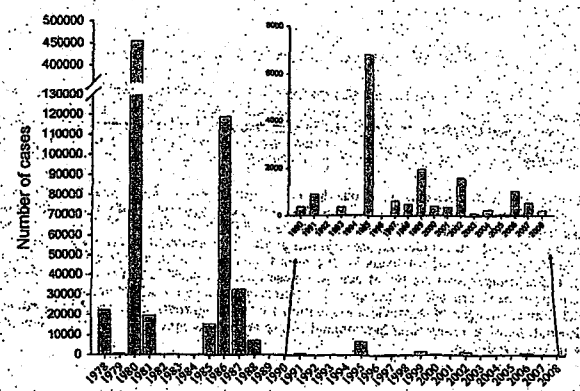

FIGURE 1. Annual number of dengue fever cases in mainland China from 1978 to 2008. Data for 1978–2002 are summarized in Refs. 7–10. Data for 2003–2008 are derived from reports of the State Ministry of Public Heath (http://www.moh.gov.cn/publicfiles/business/htmlfiles/zwgkzt/pyq/index.htm).

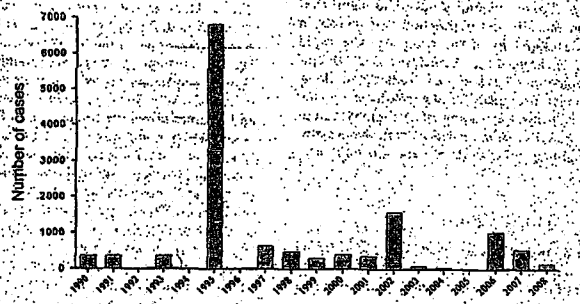

FIGURE 2. Annual number of dengue fever cases in Guangdong Province from 1990 to 2008. The 1990–2000 and 2001–2006 data are from Refs. 8 and 9. The 2007–2008 data are from reports of the State Ministry of Public Health (http://www.moh.gov.cn/publicfiles /business/htmlfiles/zwgkzt/pyq/index.htm).

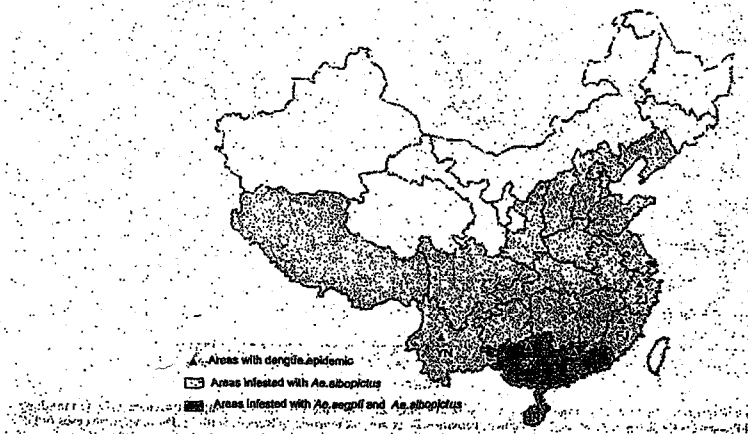

FIGURE 3. Approximate distribution of dengue and *Aedes* mosquitoes in mainland China. ZJ: Zhejiang Province; FJ: Fujian Province; GD: Guangdong Province; GX: Guangxi Province; HN: Hainan Province, YN: Yunnan Province. Data are summarized in Refs. 7, 8, 10–13.

stems, or leaf axils) that are found in gardens or backyards. The large amount of rainfall from July to September increases the breeding places of the mosquitoes. The emergence of large numbers of larvae causes drastic expansion of the mosquito populations and greatly increases the probability of DF epidemic.[7] Yi and others[8] analyzed DF cases in Guangdong province from 1990 to 2001 and *Aedes* surveillance and climate from 1995 to 2001. They showed a clear seasonal pattern of DF occurrence, with a high frequency occurring mostly in hot and humid seasons. Geographic distribution of dengue epidemic has spread, gradually from southern (Guangdong, Guangxi, Hainan) to relatively northern and western regions including Fujian, Zhejiang, and Yunnan provinces (Figure 3), which may be associated with the intensifying global warming.[10,12] However, the impact of global warming on the spread of vector-borne diseases in tropical and sub-tropical regions in Asia is a subject of debate.[23] Countries or regions experiencing increasing DF should establish as part of their disease control

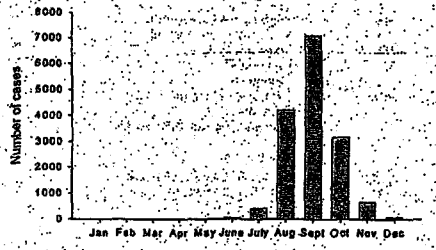

FIGURE 4. Total number of dengue fever cases reported monthly from 2002 to 2008. Data are summarized from (http://www.moh.gov.cn/publicfiles/business/htmlfiles/zwgkzt/pyq/index.htm).

efforts surveillance that allows an evaluation of the variation in DF incidence and prevalence as the climate in the regions change.[24]

Serotype and genotype distribution of dengue viruses. Four serotypes (DV1, DV2, DV3, and DV4) of dengue viruses have been identified and they all are capable of causing DHF and dengue shock syndrome.[11,25] Although the association between the serotype and the severity of the disease is not clear, the DV2 serotype, particularly in the event of superinfection following infection of another serotype, has been shown to correlate with dengue shock syndrome.[26] All four serotypes have caused epidemics in the mainland. Figure 5 shows the geographic distribution of dengue epidemics reflecting a gradual spread from southern to northern regions. Interestingly, no DF epidemics outbreaks have been reported in Guangxi and Hainan province since 1986 and 1991, respectively. The outbreaks in Guangdong province, where the latitude and weather conditions are similar to Guangxi and Hainan, have been reported more frequently since 1990. In the mainland, DV3 was the principal serotype of the viruses reported early (1978), and then DV1 and DV4 were recorded. However, DV1 has become the main serotype since the 1990s. The outbreak of DV4 DF was first reported in Foshan, Guangdong province in 1978[12] and it reappeared here in 1990.[8] The epidemic of DV3 DF was first reported in north Shanbei County of Hainan province in October, 1979; the epidemic then spread north along the west coast to compromise Zhanjiang, Foshan, Guangzhou, Shantou, and Shaoguan of Guangdong province, and then to Beihai and Hepu of Guangxi province, lasting for 3 years.[12] The epidemic caused by DV2 was reported in north Shanbei County, Hainan province in September, 1985, and by the following year it had compromised the entire Island (e.g., Hainan province) and spread further to Guangzhou of Guangdong province and Beihai of Guangxi province, and persisted until 1988.[7] The DV2 caused DF epidemics in

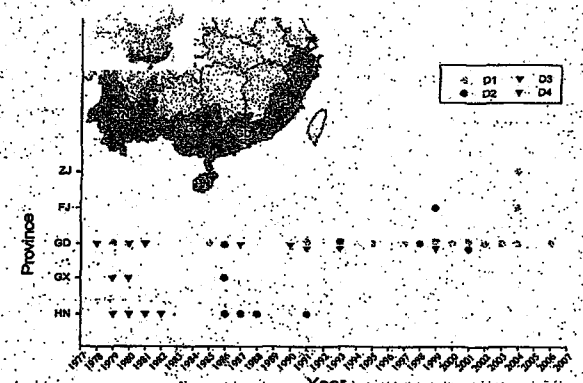

FIGURE 5. The epidemics of four serotypes of dengue virus in mainland China. ZJ: Zhejiang Province; FJ: Fujian Province; GD: Guangdong Province; GX: Guangxi Province; HN: Hainan Province, YN: Yunnan Province. Data are summarized in Refs. 7, 8, 10–13. There were 56 cases of DF in Yunnan Province (2008) according to the report from the Department of Health [http://www.moh.gov.cn/publicfiles/business/htmlfiles/zwgkzt/pyq/index.htm], but no publication regarding the serotype of this virus caused this outbreak was found. This figure appears in color at www.ajtmh.org.

Guangdong province in 1993, 1998, 2001, and in the Fujian province in 1999.[13] In the 1993 epidemic, several cases were found to consist of both DV2 and DV4, which represented the rare cases of concurrent infections in mainland China.[7] The DV1 was responsible for the outbreaks of DF in Guangdong province in 1979 and 1985, respectively,[12] and then several outbreaks caused by the same virus were reported in 1991, and from 1995 to 2006.[8]

According to the full-length sequence of the E/NS1 gene, the four serotypes of the virus can be divided into different genotypes. The DV1 virus has five genotypes,[27] DV2 has six,[27] DV3 has four,[28] and DV4 has two genotypes, I and II.[29] Genotyping of the virus strains isolated from different outbreaks has provided some insight into the variation and transmission of the prevalent strains.[26] Analysis of the genotypes of the virus strains in mainland China show complex origins consistent with the interpretation that the viruses might be imported from more than one country (Table 1). A molecular epidemiological analysis comparing the viral gene sequences of the DV1 strains isolated from four DF outbreaks (1991, 1995, 1997, and 1999) in Guangdong province revealed extensive nucleotide variation over the whole genome. The strain GD03/91, isolated in 1991, shows 97% nucleotide identity with the strains GD25/95 isolated in 1995, 93% identity with GD14/97 isolated in 1997, and only 93% identity was identified between strains GD25/95 and GD14/97, indicating that GD03/91 has a closer genetic

TABLE 1
Genotype of dengue virus recognized in mainland China

Serotype	Year	Region	Genotype	Origin (homology)	Reference
D1	1985	Guangzhou, Guangdong Province	V	Thailand	33
	1991	Guangzhou, Guangdong Province	IV	Southeast Asia, Australia	13,32
	1995	Guangzhou, Guangdong Province	IV	Southeast Asia, Australia	32
		Chaozhou, Guangdong Province	IV	Southeast Asia	13,26
	1997	Chaozhou, Guangdong Province	I	Cambodia	13,26
	1999	Zhongshan, Guangdong Province	I	Cambodia	26
	2002	Guangzhou, Guangdong Province	IV	Australia TI4 strain	32
	2003	Guangzhou, Guangdong Province	I	Cambodia	34
	2006	Guangzhou, Guangdong Province	V	Thailand, Taiwan ThD1 strain	35
	2004	Fujian Province	I	Thailand	36
	2004	Zhengjiang Province	I	Thailand	12
	1989	Hainan Province	III	Jamaican or Brazil 90 strain	37
	1998	Nanhai, Guangdong Province	III	Thailand ThNH81/93 strain	13,38
D2	1993	Fanshan, Guangdong Province	I	Australia TSV01 strain	26,39
	1999	Fujian Province	IV	Indonesia, Sri Lanka	40
	2001	Jiangmen, Guangdong Province	I	Australia TSV01 strain	26
D4	1978	Fanshan, Guangdong Province	II	Indonesia	41
	1990	Guangdong Province	I	Philippines	31

relationship to GD23/95 than GD14/97. The phylogenetic tree is consistent with the hypothesis that strains GD03/91 and GD23/95 derive from Southeast Asia or the Pacific islands.[15] Strains GD14/97 and GD05/99 isolated in 1999 had sequences similar to the Cambodia strain, showing nucleotide and amino acid identities of 98% and 99%, respectively. Phylogenetic tree analysis indicated that the strain GD14/97, GD05/99, and GD23/95 belong to two different genotypes.[20] Fang and others[38] compared the nucleotide sequences of a number of genes between the DV2 strains GD06/93 and GD01/98 isolated from the outbreak in 1993 and 1998, respectively, in Guangdong province and found that these two strains and the strain (04) isolated in Hainan province in 1985 belong to different genotypes. However, GD01/98 shows high similarity to the strain ThNH2P28/93 from Thailand with nucleotide and amino acid sequences at 98% and 100% identity, respectively. Comparison of the sequences among the strain GD01/98, GD06/93, and HN04 was consistent with the interpretation that these three strains originate from different regions. Yao and others[31] analyzed the NS2 gene in DV4 virus isolated in 1978 and 1990 in mainland China, and they found a 96% identity between the 1990 strain and the Philippine strain, and a 96% identity between the strain 7856B2 (isolated in 1978 in mainland China) and the Caribbean strain 814669. Therefore, they presumed that these two strains isolated in mainland China might come from these two regions. These results are consistent with the conclusion that DF epidemics in Guangdong province may be primarily the result of the virus imported from multiple regions. Molecular epidemiological analysis of DF in the last three decades did not identify new variants of dengue viruses in mainland China (Table 1). Therefore, we propose that the rapid expansion of the urban population and the great migration of citizens as well as frequent international travel may result in an increase in the frequency of DF epidemics.

VECTORS

Behaviors. *Aedes aegypti* and *Ae. albopictus* are the two most important mosquito species for transmission of dengue viruses in mainland China. *Aedes aegypti*, a domestic mosquito, can breed inside and outside houses, especially in water-containing vessels in the kitchen or in jars with accumulated rain water. This species is found mostly in the regions south of the 22° north latitude, including the coastal areas in West Guangdong province and the Beibu Gulf of Guangxi Province (Figure 3). *Aedes albopictus* breeds mostly in the wild and depends on accumulated water in various utensils or plants. It is found in nearly one-third of China ranging from Shenyang in the North, Longxian County and Baoji in the Northwest, South Tibet in the Southwest to the South Changjiang regions, where this mosquito is most common (Figure 3).[42] However, the environment and ecology of these mosquitoes has changed greatly because of the rapid urbanization, which may cause significant alteration in the number and types of vector breeding sites. Su and others[42] showed following close monitoring that the Breteau index of *Ae. aegypti* in Haiko city, Hainan province, dropped from 32.81 to 1.73, and the number of *Ae. aegypti*-positive communities reduced from 23 to 2, from 1987 to 2002. Conversely, the Breteau index of *Ae. albopictus* increased gradually from 13.15 to 21.84, and the number of positive communities increased from 19 in 1987 to 23 in 2002. *Aedes albopictus* is considered to be only semi-domestic, but according to a survey by Lin and others,[44] the density of this mosquito inside houses was higher than outside in all of the four monitoring sites. They proposed that the indoor environment provides a suitable breeding place for this mosquito and it has adapted to being a domestic mosquito like *Ae. aegypti*.

Efficiency for virus transmission. The transmission cycles of arboviruses result from horizontal (by adult mosquitoes) and vertical (transovarial) transmission of the mosquito vectors with the significance of each of these components varying for the specific virus. Furthermore, the transmission dynamics of the viruses depend largely on the efficiency of the vector in terms of the susceptibility of mosquitoes to the virus, its subsequent transmissibility, and the transovarial transmission ability of the virus. All of these factors may be affecting *Aedes* transmission of dengue viruses. It is clear that the susceptibility and transmission efficiency of mosquitoes to dengue viruses is affected by the species and strains of mosquito[45–48] and the virus serotypes,[47,48] in addition to the other factors, such as temperature of the environment and the nutrition status of the vector.[49,50]

Temperature may cause alterations in the length of the extrinsic incubation period by affecting viral replication *in vivo*. Within the optimal range (26–35°C), relatively higher temperature (35°C) can promote viral replication *in vivo*, shorten the extrinsic incubation period, and enhance the transmissibility of the virus. Exposure to a relatively lower temperature (≤10°C) alone may reduce the physiological activities of the mosquito, decrease the viral replication rate, thus prolong the extrinsic incubation period and attenuate the transmissibility of the virus.[51] The question then arises over the preservation of the virus in winter and dry seasons when the mosquito hosts are present in low abundance. A possible answer is that the virus can survive in a vector in functional diapause, or has been transmitted to the next generation through the eggs. Lin and others[52] orally infected *Ae. albopictus* with DV1 and DV2 and homogenates prepared from parental and F1–F3 generations. Dengue viruses in these samples were identified following inoculation of C6/36 cell cultures and specific viral antigen was detected by indirect immunofluoresence assay (IFA). These results provide compelling evidence that *Ae. albopictus* could transmit virus to the F2 generation by the eggs. Zhao and others[53] used the same method to infect three geographic strains of *Ae. albopictus* with dengue viruses and the viruses in following generations were detected by IFA and reverse transcription-polymerase chain reaction (RT-PCR). Their result also confirmed that four serotypes of dengue virus are capable of vertical transmission by *Ae. albopictus*. However, the virus can be seldom detected in the vector mosquitoes collected from the field. Fang and others[54] performed RT-PCR of over 6,000 *Ae. albopictus* specimens collected between 1996 and 1998 from three monitoring sites in the suburbs of Guangzhou, Guangdong province, and found none positive for dengue viruses. Similarly, Duan and others[55] collected > 300 *Ae. albopictus* from three monitoring sites in Jieyang, Foshan, and Guangzhou, and they also failed to detect the presence of virus using a TaqMan MGB real-time PCR method. In contrast, many studies from other countries have reported a high infective rate of dengue virus in wild-derived *Aedes* mosquitoes. For example, the minimum infective rate (MIR) in *Ae. albopictus* and *Ae. aegypti* collected in Singapore, from April 1995 to July 1996, was 50 and 57.6, respectively.[56] Kow

and others[57] reported that they used a single-step RT-PCR to assay the male vector (adults) collected from the field of Singapore and found that 1.33% and 2.15% of *Ae. aegypti* and *Ae. albopictus*, respectively, were positive for dengue viruses. The serotypes detected in male *Ae. aegypti* were DV1 (44%), followed by DV2 (22.2%) and DV3 (22.2%) and DV4 (11.1%). In *Ae. albopictus* males, the serotypes were DV4 (38.9%), followed by DV2 (33.3%), DV3 (16.7%), and DV1 (11.1%). These results emphasize the need for sensitive methods for the identification of the viruses that would allow us to address questions about the presence and absence of infectious virus, the origins of the strains in specific outbreaks, and whether viral genotypes in one epidemic are related to those causing previous or subsequent epidemic.

PREVENTION AND CONTROL

In mainland China, the primary goals of DF control should be timely epidemic detection, prevention, and control of new cases to prevent large-scale outbreaks and to minimize the damage of the epidemic. On the basis of the global DF/DHF prevention and control strategies drafted by WHO in 1995,[2] and considering the actual domestic condition, China has instituted its DF control strategy with close monitoring as the key, accompanied by control of mosquito vector density, high vigilance for imported cases, improvement of diagnosis and treatment, enhancing the cooperation among the related different public health departments, and mobilizing the social effort to combat the epidemic.

Mosquito vector monitoring. The DF is characterized by periodical and sudden occurrence with rapid transmission, often resulting in an unexpected large-scale epidemic.[7] A sensitive monitoring system that allows prediction and early detection of an epidemic is critical to the reduction and arresting of the epidemic. This monitoring system involves examination of vector species, their distribution, breeding places, density, sensitivity to pesticides, and viral subtype identification. China currently monitors the major vector mosquitoes of the genus *Aedes*, primarily by examining mosquito larvae using the Breteau index, house index, and container index. This approach has produced encouraging results in the high-risk regions for DF (such as Hainan and Guangxi).[9,58,59] However, the larval and pupal density of mosquitoes in a defined environment may not represent the exact density of the adult mosquitoes, therefore such an approach can be laborious and the results subjected to influences by various factors. Because *Ae. albopictus* is generally not domestic, Lin and others[60] designed a mosquito trap based on the U.S. Centers for Disease Control and Prevention (CDC, Atlanta, GA) trap. This new trap allows close observation of the seasonal variation in the number of adult *Ae. albopictus*, and complete data sets on mosquito densities in the larval, pupal, and adult stages. Adult mosquitoes trapped also are useful as specimens for virus detection, which can be of great significance in the prediction and control of an DF outbreak.[61–63] The preliminary testing results of this new mosquito trap showed its greater advantages over the Breteau index for *Ae. albopictus* monitoring in the urban areas.[64]

Vector control. Because no DF vaccine is currently available, the only effective means for DF prevention is mosquito vector control. The key to this control relies on the maintenance of environmental hygiene and elimination of the breeding places of the mosquitoes.[65] Pesticide application remains the conventional means for eliminating the vectors in the breeding sites. However, these activities are associated with the emergence of pesticide resistance and this is evident already in some regions. The use of pesticides is by no means an environmentally friendly solution to the mosquito problem. More environmentally friendly methods therefore are urged for controlling the breeding places of the mosquitoes. Some cost-effective biocontrol agents such as *Bacillus thuringiensis* var. *israelensis* and *predatory cyclops* that do not cause environmental damage or produce resistance in the mosquitoes after long-term use are promising.[66,67] However, these slow-acting agents may not seem appropriate in emergency settings,[66] and pesticides are still necessary for mosquito control during an DF or DHF outbreak. The WHO[68] recommends the ultra-low volume (ULV) spray or thermospray of malathion and sumithion to eliminate the mosquitoes around breeding sites. However, because of its poor effect against female mosquitoes and the larvae,[69,70] UVL aerosol is seldom used for outdoor mosquito control during the epidemic of DF or DHF.[68]

FUTURE CHALLENGE

Accelerated urbanization, expanding urban populations, frequent international travel, and perhaps global warming, all may contribute to increasing the frequencies of DF/DHF epidemics. The absence of effective vaccines and robust measures for the vector management make DF and DHF control difficult, and they will remain a major public health issue in the tropical and subtropical regions. The challenges in DF control depend on the following four aspects: 1) vector control: much effort is needed to establish a monitoring system for early prediction of the occurrence and distribution of the mosquitoes on the basis of their chemical, biological and environmental control. Chinese researchers[71–73] have made preliminary but meaningful attempts at DF control using a geographic information system (GIS),[74] which allows prediction of the occurrence and distribution of the vectors as well as estimation of the probability of DF epidemic. 2) The establishment of a rapid virus detection system to provide early prediction of the epidemic, which necessitates effective international communication and cooperation. At the same time, the deciphering of the genome of *Aedes* mosquito[75] and the application of transgenic technology[76,77] have provided many platforms for dengue prevention and control. 3) Community-based education also is critical. The full knowledge of DF and dengue viruses among the community residents may help greatly to achieve the goal of controlling the monitoring indices within the safe limits, and the participation by the community can be of great importance in winning the battle against vector mosquitoes. 4) Elimination of the technical obstacles to develop safe and effective vaccines. Rapid progress is being made in research of the immune and pathogenic mechanisms of dengue fever, and better understanding of these mechanisms will facilitate the development of the safe and effective vaccines against dengue virus.

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

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[1] Philippines (Iloilo)
Date: Wed 22 Sep 2010
Source: The Star [edited]
<http://www.thenewstoday.info/2010/09/22/iloilo.dengue.cases.hit.4.825.with.27.

Dengue cases in Iloilo province have now reached 4825 with 27 deaths as reported by the Iloilo Provincial Health Office from 1 Jan - 18 Sep 2010. Of the total dengue cases, the number of patients confined in the 12 provincial and district hospitals in Iloilo for this month [September 2010] alone starting 19 Aug [2010] is 32.

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[The numbers of cases and deaths in affected municipalities are given in the above URL.

The 25 Sep 2010 edition of the Macau DAily Times
(<http://www.macaudailytimes.com.mo/asia-pacific/17224-Philippines-Pools-the-ri
[2009].

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別紙様式第2-1

医薬品 研究報告 調査報告書

No. 20

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	人赤血球濃厚液		2010.10.4	該当なし	
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)	研究報告の公表状況	ProMED 20100927.3506 27-SEP-2010	公表国 フィリピンほか	使用上の注意記載状況・その他参考事項等 赤血球濃厚液-LR「日赤」照射赤血球濃厚液-LR「日赤」

研究報告の概要

○デング熱更新情報
・フィリピン
2010年1月1日から9月18日までのIloilo市における症例数は4,825例に達し、死亡は27例であった。全症例のうちIloilo市の12病院に限定した患者数は8月19日から9月までで32名であった。
・タイ
2010年1月から9月11日までの患者数は75,852例で、死亡は87例であった。ほとんどの患者は北東部で認められ、ついで中部、南部及び北部地域で認められた。国内の関連機関は各地域内での疾患の発症を管理するため、デング熱に対する予防措置に関して住民に指導するよう命じられた。
・マレーシア
死亡者数が昨年の同時期の70名に比べ今年は107名に増加し、死亡率は53%に上昇した。副首相は、疾患を一掃するために遺伝子組換えを行った蚊を放つ計画について、冷静な姿勢を示した。
・インド・デリー
首都圏で患者が急増しており、2010年9月26日には5例以上の症例が報告された。
・プエルトリコ
2010年の死亡例は23例である。2010年9月25日に発表された。8月27日から9月2日の週に新たに835例の症例が報告された。
・パラグアイ
2010年の疑い症例数が21,443例であり、確定症例数が13,678例であることを明らかにした。保健省は国民に対してネッタイシマカの発生源を排除するよう呼びかけている。

報告企業の意見

世界各地におけるデングウイルス感染状況の最新情報である。

今後の対応

日本赤十字社では、輸血感染症対策として受付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に関する情報の収集に努める。

(1)

A map showing the provinces in the Philippines can be accessed at <http://en.wikipedia.org/wiki/Provinces_of_the_Philippines>. A HealthMap/ProMED-mail interactive map of the Philippines can be accessed at <http://healthmap.org/r/00bk>. - Mod.TY]

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[2] Taiwan (Kaohsiung City)
Date: Sun 26 Sep 2010
Source: RTI [edited]
<http://english.rti.org.tw/Content/GetSingleNews.aspx?ContentID=110467&BlockID=

Concern grows over the worsening dengue situation in Kaohsiung City. That was the word from Health Minister Yaung Chih-liang on Sunday [26 Sep 2010]. Dengue fever is raging in Kaohsiung, with 227 cases reported so far this year [2010]. Yaung is visiting the southern city to review the epidemic prevention work there. Yaung said that in addition to the indigenous cases in the city, there has been an increase in the number of imported cases of dengue.

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[A map of Taiwan can be accessed at <http://www.lib.utexas.edu/maps/middle_east_and_asia/taiwan_pol92.jpg>. A HealthMap/ProMED-mail interactive map showing the location of the island of Taiwan can be accessed at <http://healthmap.org/r/00tB>. - Mod.TY]

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[3] Viet Nam
Date: Tue 21 Sep 2010
Source: Vietnam Plus [edited]
<http://en.vietnamplus.vn/Home/Prime-Minister-calls-for-action-on-dengue-fever/

Prime Minister Nguyen Tan Dung has instructed health officials to take firm measures to combat dengue fever, which has claimed 42 lives so far this year [2010] out of 55 400 cases reported nationwide. He urged the Ministry of Education and Training to mobilise pupils nationwide to join campaigns to kill mosquitoes -- the main carrier of the disease [virus] -- in their houses and public places.

Ha Noi reported that the disease had hit 27 out of the city's 29 districts. In August 2010 alone, the whole country recorded 19 577 patients and 11 deaths in 54 cities and provinces, according to reports from the Preventive Medicine Department.

The number of patients hospitalised in HCM City in the 1st 2 weeks of this month [September 2010] increased 30 percent, in comparison with the same period in previous months. Children's Hospital No 2 reported that it had received 555 dengue fever patients since the start of this month, and on average was receiving between 15-30 patients a day.

The preventive medicine department said it was working with relevant agencies to take preventive measures in key localities since early this year [2010], focusing on cleaning the environment and killing mosquitoes.

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[A map of Viet Nam with provinces can be accessed at <http://upload.wikimedia.org/wikipedia/commons/f/f0/VietnameseProvincesMap.png> Mod.TY]

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[4] Thailand
Date: Sat 18 Sep 2010
Source: National News Bureau of Thailand (NNT), Public Relations Department [edited]
<http://thainews.prd.go.th/en/news.php?id=255309180018>

Public Health Minister Jurin Laksanawisit stated that from January - 11 Sep 2010, the numbers of patients infected by dengue fever were at 75 852 with 87 deaths. Most patients were found in Northeastern provinces, followed by the Central, Southern and Northern regions.

However, the Minister said the number of dengue fever patients in the South was already reduced, but the North and Northeast still needed to be closely monitored. The Ministry has also instructed all related agencies to report the progress of problem-solving continuously.

Relevant agencies nationwide are ordered to educate the people on preventive measures against dengue fever to control disease outbreaks in each area.

[Byline: Panita Norasing]

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[According to the Thai Ministry of Public Health, Bureau of Epidemiology's (BOE) report of the situation of dengue infection in Thailand, between 1 Jan 2010 and 10 Sep 2010, available in Thai at <http://epid.moph.go.th/dhf/situation/y53/Dengue_10Sep2010.pdf>, a total of 75 852 cases and 87 deaths were reported nationwide. The attack rate was 119.53 per 100 000 population. The case fatality rate was 0.11 percent. The highest percentage of cases was reported in the 15-24 years old age group (27.5 percent), followed by the 10-14 years old age group (25.1 percent), 5-9 years old age group (16.7 percent) and 25-34 years old age group (11.8 percent).

In the last 4 weeks, the 10 provinces with the highest attack rates of dengue infection (per 100 000 population) were Phayao (65.9), Chiang Mai (51.0), Songkhla (31.6), Narathiwat (31.5), Phatthalung (31.3), Petchabun (27.6), Rayong (27.1), Tak (25.9), Lopburi (25.3) and Surin (24.8).

Maps of Thailand showing the distribution of cases in the last 4 weeks are available on the last page of the report. In the map, the red color represents provinces that reported cases with an attack rate of 10.01 per 100 000 population and above; yellow represents provinces that reported cases with an attack rate between 5.01 and 10.00 per 100 000 population, and green, provinces that reported cases with attack rates between 0.01 and 5.00 per 100 000 population.

For maps showing Thailand's provinces, see <http://www.thailand-map.net/thailand_provinces/> and regions, see <http://thailandforvisitors.com/general/regions.html>. For the interactive HealthMap/ProMED-mail map of Thailand with links to other recent ProMED-mail and PRO/MBDS postings, see <http://healthmap.org/r/00cC>. - Mod.SCM]

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[5] Indonesia (Jakarta)
Date: Sun 26 Sep 2010
Source: Berita Jakarta [edited]
<http://www.beritajakarta.com/2008/en/newsview.aspx?idwil=0&id=16741>

It seems that dengue fever is still a serious threat for Central Jakarta residents, especially with the transition weather happening in the last few days. Evidently, the number of dengue cases in Central Jakarta area from January-September this year [2010] reached 1565 cases ... and from 1-20 Sep 2010, 260 dengue cases have been recorded.

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[Dengue cases in specific sub-districts can be seen at the above URL.

A map of Indonesia showing the location of Jakarta in Banten province on Java Island can be accessed at <http://www.lib.utexas.edu/maps/middle_east_and_asia/indonesia_pol_2002.jpg>. A HealthMap/ProMED-mail interactive map of Indonesia can be accessed at <http://healthmap.org/promed/en?v=-2.6,120.9,5>. - Mod.TY]

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[6] Malaysia
Date: Wed 22 Sep 2010
Source: Arab Times [edited]
<http://www.arabtimesonline.com/NewsDetails/tabid/96/smid/414/ArticleID/159843/

Malaysia said Monday [20 Sep 2010] its dengue fever death rate spiraled 53 percent this year [2010], but backed away from a controversial trial of releasing genetically modified mosquitoes to wipe out the disease.

"There was a major rise in deaths due to dengue fever, with 107 deaths so far this year [2010] compared to 70 deaths for the same period last year," said deputy premier Muhyiddin Yassin.

Muhyiddin said the majority of the deaths could have been avoided, and urged the public to take action to eradicate the _Aedes aegypti_ mosquitoes -- which spread dengue [viruses] -- from their homes and workplaces. "We have identified 19 hotspots throughout the country where the disease is prevalent, and action is being taken to tackle the situation in these areas," he said.

However, Muhyiddin was cool on a plan to release genetically modified male mosquitoes designed to combat dengue fever, in a proposed landmark field trial that has come in for criticism from environmentalists. In the 1st experiment of its kind in Asia, 2000-3000 male _Aedes aegypti_ mosquitoes were to be released in 2 Malaysian states in October or November 2010 if the plan had won government support. The insects in the study have been engineered so that their offspring quickly die, curbing the growth of the population in a technique researchers hope could eventually eradicate the dengue mosquito altogether. Muhyiddin said the project would not be implemented "at the moment." The field trial for the GM anti-dengue mosquitoes was developed by a British-based insect bio-tech company, Oxitec, and was to be undertaken by Malaysia's Institute for Medical Research, an agency under the health ministry.

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[It appears that Oxitech is applying the sex-specific alternative splicing in insects to engineer female-specific autocidal genetic systems developed in the Mediterranean fruit fly, _Ceratitis capitata_ to _Aedes aegypti_. It will be interesting to see what the results are of this approach to dengue vector control if and when field trials are carried out. Success would likely depend on the released, genetically modified male mosquitoes being able to survive in nature and compete with wild males for mating with wild females.

Reference:
Fu G, Condon KC, Epton MJ, Gong P, Jin L, Condon GC, Morrison NI, Dafa'alla TH, Alphey L. 2007. Female-specific insect lethality engineered using alternative splicing. Nat Biotechnol 25(3):353-357.

A HealthMap/ProMED-mail interactive map of Malaysia can be accessed at <http://healthmap.org/r/009L>. - Mod.TY]

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[7] China (Hong Kong)
Date: Thu 23 Sep 2010
Source: MB.com.ph [edited]
<http://www.mb.com.ph/articles/278536/hong-kong-reports-first-local-case-denque

A case of locally-acquired dengue fever, involving a 46-year-old man, was confirmed in Hong Kong, which was the 1st local [locally acquired] case in 7 years, the Center for Health Protection under the city's Department of Health said Wednesday [22 Sep 2010]. A spokesman with the Center for Health Protection said the Department of Health was highly concerned about this case of locally-acquired dengue fever infection since the last local dengue fever outbreak in Hong Kong occurred in 2002. "We are working with the Food and Environmental Hygiene Department closely to make an all-out effort to assess if there is any spread of the infection, contain the infection, and prevent the spread," he said.

A total of 61 cases of dengue fever [60 imported] has been reported to the Center for Health Protection in Hong Kong so far this year [2010]. There were 43 cases in 2009, and all of them were classified as imported.

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A map showing the location of Hong Kong China's east coast can be accessed at
<http://www.lib.utexas.edu/maps/middle_east_and_asia/china_pol01.jpg>.
A HealthMap/ProMED-mail interactive map of China can be accessed at <http://healthmap.org/r/00cv>. - Mod.TY]

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[8] Nepal
Date: Mon 20 Sep 2010
Source: Xinhua Net [edited]
<http://news.xinhuanet.com/english2010/health/2010-09/20/c_13521157.htm>

At least 19 persons have died in a month, and more than 7000 have been afflicted with dengue and viral fever in Chitwan in central Nepal, The Himalayan Times daily reported on Monday [20 Sep 2010]. According to Mahendra Prasad Shrestha, District Public Health Officer, 9 persons have died of dengue and viral fever in Chitwan Medical College (CMC), 6 in Bharatpur Medical College and 4 in Bharatpur Hospital since the spread of the diseases in the district in the last week of August 2010.

According to Dr Shital Adhikari, head of the Medical Department of CMC, 40 patients with dengue fever were visiting the hospital daily, and at least 12 of them had tested positive for dengue.

[Editor: Deng Shasha]

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[A HealthMap/ProMED-mail interactive map of Nepal showing the location of Kathmandu can be accessed at
<http://healthmap.org/promed/en?v=28.3,83.9,5>. - Mod.TY]

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[9] India (Delhi)
Date: Sun 26 Sep 2010
Source: The Times of India [edited]
<http://timesofindia.indiatimes.com/city/delhi/95-more-dengue-cases-in-Delhi-to

Dengue continues to surge in the national capital, with 95 more cases being reported on Sunday [26 Sep 2010], taking the total number of people infected with the mosquito-borne disease to 2916, an official said. This year, 5 people have succumbed to dengue in the capital, including one from outside Delhi, a Municipal Corporation of Delhi (MCD) official said.

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[Since patients coming to private clinics and hospitals may not be reported officially, this number is probably a significant under-estimate of the actual number of cases.

A HealthMap/ProMED-mail interactive map of India showing the location of Delhi can be accessed at <http://healthmap.org/r/01Jf>. - Mod.TY]

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[10] India (Bihar)
Date: Wed 22 Sep 2010
Source: Data News & Analysis [edited]

<http://www.dnaindia.com/india/report_dengue-death-toll-in-bihar-at-6-518-other

In the Munger district of Bihar, 6 people have died so far due to the outbreak of dengue, while 518 others have tested positive, official sources said today [22 Sep 2010]. Unofficial sources have, however, claimed that the number of casualties due to suspected mosquito-bred [transmitted] fever [virus] stands at 27. As many as 518 out of 1361 people diagnosed for symptoms of the dengue fever have tested positive, officials said.

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[A map of India showing the location of Bihar state in the far northeast can be accessed at
<http://www.lib.utexas.edu/maps/middle_east_and_asia/india_pol01.jpg>. - Mod.TY]

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[11] Pakistan (Karachi)
Date: Thu 23 Sep 2030
Source: Daily Times [edited]
<http://www.dailytimes.com.pk/default.asp?page=2010\09\23\story_23-9-2010_pg12_

Officials said the number of citizens affected with dengue virus has also increased in the province, as 13 new cases were reported during the last 24 hours; 9 of them were admitted [to hospital], while 4 were sent home, they added. According to the Provincial Dengue Surveillance Cell, the total number of dengue cases reported this year [2010] was 356, of which 216 people were tested positive; while one person, who was taken to a private hospital on 10 Sep [2010], died on 21 Sep [2010].

[Byline: Irfan Aligi]

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[The 26 Sep 2010 edition of The Business Recorder
(<http://beta.brecorder.com/section/37/1/1106118:many-suffering-from-dengue-fev
city.

A map showing the locations of Sindh province and Karachi can be accessed at
<http://www.lib.utexas.edu/maps/middle_east_and_asia/pakistan_pol_2002.jpg>. A HealthMap/ProMED-mail interactive map of Pakistan can be accessed at
<http://healthmap.org/r/05Pd>. - Mod.TY]

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[12] Australia (North Queensland)
Date: Fri 24 Sep 2010
Source: Sydney Morning Herald [edited]
<http://news.smh.com.au/breaking-news-national/dengue-outbreak-in-cairns-201009

An outbreak of dengue fever in Cairns has doctors concerned it could foreshadow a bad season for the mosquito-borne disease. 4 people from the suburb of Parramatta Park have tested positive to dengue, and another 8 are awaiting test results, Queensland Health medical director Dr Jeffrey Hanna said. More confirmed cases of dengue type 2 [virus infection] are expected, he said. And there are concerns the disease will be spread by travellers setting out from the far north Queensland city.

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[The 27 Sep 2010 edition of Couriermail.com.au
(<http://www.couriermail.com.au/news/queensland/cairns-records-its-fifth-case-c
virus.

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Maps showing the location of Cairns in Queensland can be accessed at
<http://www.lib.utexas.edu/maps/australia/australia_pol99.jpg> and the HealthMap/ProMED-mail interactive map of Australia at
<http://healthmap.org/r/03Bp>. - Mod.TY]

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[13] Monaco ex Caribbean
Date: Fri 24 Sep 2010
Source: Maville.com [in French, trans. Corr.SB. summ., edited]
<http://www.monaco.maprincipaute.com/actu/actudet_--Monaco-Premier-cas-de-dengu

A young resident, aged 18, returned from the Caribbean with the disease. Since early September 2010, the government has been strengthening mosquito control.

"Monaco does not have any indigenous dengue cases," said Stephane Valeri, Government Counsellor for Social Affairs and Health. "However, we have identified a case of imported dengue fever in early September [2010]. There is nothing to worry about for this young 18 year old resident of Monaco, who returned from the Caribbean with the disease. He is now in perfect health," said Stephane Valeri.

However, with the announcement of the 1st indigenous dengue fever cases in Nice, mosquito control, already assiduous in gardens and public spaces, has been strengthened. The 1st objective is to kill the tiger mosquito larvae. "The tiger mosquito [_Aedes albopictus_. - JW] has been located in our area for 3 years now, says Philip Porcu, Territory Chief Technician, Directorate of Planning and Urban Development.

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[All it takes to initiate a dengue outbreak is the presence of a viremic individual in an area where there is a significant population of _Aedes_ mosquito vectors, as has been the case in nearby Nice, France this month (September 2010). The concern and vigorous preventive actions by Monaco health authorities are justified.

Although ProMED does not normally report imported dengue cases with no subsequent local transmission, the risk of transmission elicited this report.

A HealthMap/ProMED-mail interactive map of Monaco can be accessed at
<http://healthmap.org/promed/en?v=43.7,7.4,5>. - Mod.TY]

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[14] France (Corsica)
Date: Tue 21 Sep 2010
Source: 24 Ore [in French trans. Corr.SB, edited]
<http://24ore.club-corsica.com/il-nous-rend-dengue_15043_2183.html#7>

Since the beginning of the season [2010], 3 people with symptoms of dengue have been reported on the island: 2 in Corsica, San Gavinu di Fiumorbu and Biguglia last week, and a 3rd in Corse-du-Sud, in a tourist. [These] 3 cases, called "imported," arose from a trip to Asia. As a precaution, the Vector and Mosquito Control services of the General Council of Haute-Corse conducted a chemical treatment around residential areas of the sufferers.

On the mainland, Paca (Province, Alpes, Cote d'Azur), 120 cases (non-indigenous) have been detected in 4.5 months. On Sunday [19 Sep 2010], the Ministry of Health confirmed the presence of a 2nd "indigenous" case in Nice . Hopefully, the tiger mosquito [_Aedes albopictus_. - JW] carrying the disease [virus] does not reach us [Corsica].

[Byline: Emmanuelle Peretti]

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[This is another location having a significant risk of local dengue

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