資料2

感染症定期報告に関する今後の対応について

平成１６年度第５回
運営委員会確認事項
（平成１６年９月１７日）

1 基本的な方針

運営委員会に報告する資料においては、

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

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

2 具体的な方法

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

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

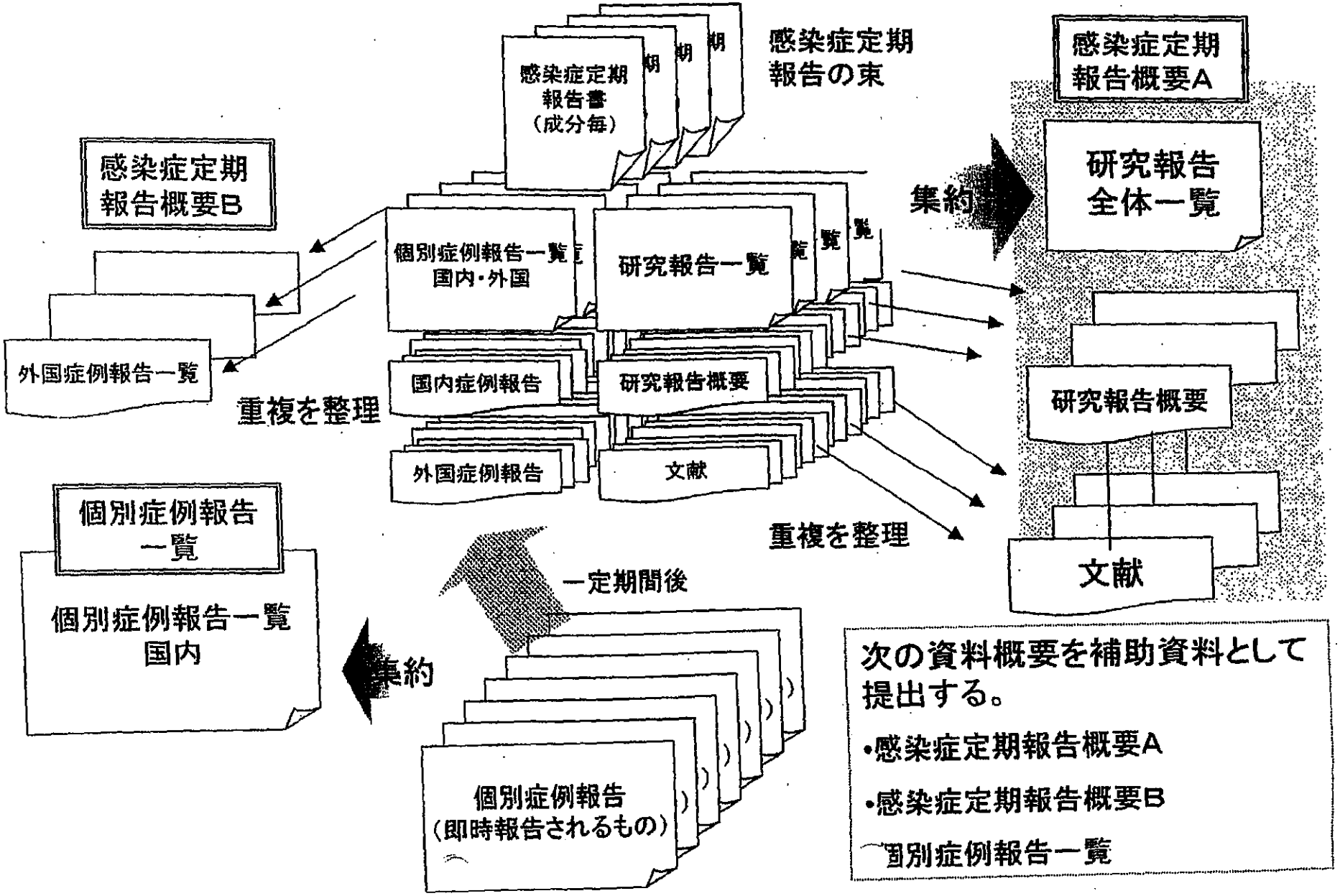
② 感染症発生症例報告のうち、発現国が「外国」の血漿分画製剤の使用による症例は、同一製品毎に報告期間を代表する感染症発生症例一覧（別紙様式第4）をまとめた**「資料概要B」**を事務局が作成し、送付する。

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

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

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

感染症定期報告・感染症個別症例報告の取り扱い



次の資料概要を補助資料として提出する。

- 感染症定期報告概要A
- 感染症定期報告概要B
- 個別症例報告一覧

感染症定期報告概要

（平成２４年９月２８日）

平成２４年４月１日～７月３１日受理分

Ａ　研究報告概要
Ｂ　個別症例報告概要

Ａ　研究報告概要

○　一覧表（感染症種類毎）
○　感染症毎の主要研究報告概要
○　研究報告写

研究報告のまとめ方について

１　平成２４年４月１日～７月３１日までに報告された感染症定期報告に含まれる研究報告（論文等）について、重複している分を除いた報告概要一覧表を作成した。

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

感染症定期報告の報告状況(2012/4/1～2012/7/31)

血対課ID	受理日	番号	感染症(PT)	出典	概要	新出文献番号
120011	29-May-12	120182	B型肝炎	Vaccine. 30(2012)2212-2219	B型肝炎ウイルス(HBV)感染の世界的疫学に関する報告。HBV感染症の疫学的調査を行うために関連する研究論文を体系的に再評価し、世界的なHBs抗原陽性率のデータを27年間分(1980年－2007年)収集した。また、1990 年と2005年における年齢別有病率及び流行性について推定した。その結果、1990年から2005年で多くの地域で慢性B型肝炎の有病率は減少していたが、HBs抗原陽性者の絶対数は増加した。年齢別の有病率はサハラ以南が最も高く、地理的地域によって変化した。アジア地域では、東アジアのHBs抗原陽性率は最高8.6%と高レベルであるが、南アジアでは低かった。北アメリカやヨーロッパでの有病率の減少は、B型肝炎ワクチンの普及や血液製剤検査の改善などが関連している。また、疾病負荷の大きい東南アジアや東アジアの地域でも、幼児へのB型肝炎ワクチン接種が広まった事に伴い有病率が減少している。	1
120011	01-Jun-12	120185	B型肝炎、C型肝炎、HIV	J Infect Dis. 205(2012)875-885	米国の供血者におけるヒト免疫不全ウイルス(HIV)、C型肝炎ウイルス(HCV)とB型肝炎ウイルス(HBV)の感染時期による遺伝型多様性に関する報告。HIV、HCV、HBVの遺伝的変化を観察するため、3,400万人の米国の供血者情報(2006－2009年)から感染者を特定し、最近感染した(incident)供血者とそれより以前に感染した(prevalent)供血者との間でウイルスゲノムを比較した。その結果、321例のHIV株(50%がincident)のうち、2.5%はサブタイプが非B型であった。プロテアーゼ阻害剤耐性変異は2%、逆転写酵素阻害剤耐性変異は11%の感染供血者に見られた。また、278例のHCV株(31%がincident)において、incident症例ではサブタイプ3aが高頻度で発見されたのに対し、prevalent症例では1bが多かった。193例のHBV株(26%がincident)サブジェノタイプは、incident症例でA2、prevalent症例ではA1、B2、B4がより高い頻度で見られた。また、HBVのエスケープミュータントはprevalent症例で高頻度で見られた。このような供血者のウイルスジェノタイプの変異分布は、米国の高リスク集団のジェノタイプ分布と類似していた。	2
120016	30-Apr-12	120060	パルボウイルス	Transfusion; published online. Feb 17, 2012-1	パルボウイルスB19(B19V)遺伝子型3の不活化に関する報告。北米・欧州では稀で他の遺伝子型と比較してデータが少ないB19V遺伝子型3の不活化について研究するために、人血清アルブミンの低温殺菌と低pHインキュベーションによるB19Vの不活化効率を細胞培養系への感染力の形で評価し、遺伝子型1、3で比較した。また、ウイルス中和試験を人免疫グロブリン製剤を用いて行った。その結果、アルブミンの低温殺菌と低pHインキュベーションは遺伝子型1、3のB19Vに対し同程度の不活化活性を示した。また、北米及び欧州由来のグロブリン製剤によって両遺伝子型のウイルスは速やかに中和された。これらの結果より、低温殺菌と低pHインキュベーションは遺伝子型3のB19Vに対しても不活化活性を持ち、また、人免疫グロブリン製剤が両遺伝子型に起因するB19V感染の治療に対して有効であることが示唆された。	3
120016	01-May-12	120061	チクングニヤウイルス感染	Transfusion; published online. Feb 17, 2012-2	血漿分画製剤製造工程におけるチクングニアウイルス(CHIKV)の不活化に関する報告。血漿分画製剤のウイルス不活化工程のCHIKVに対する影響を検討するために、低温殺菌、蒸気加熱、S/D処理及びpHインキュベーション法によるCHIKV及び類似ウイルスであるシンドビスウイルス(SINV)の不活化効率を調査した。その結果、処理後のウイルス力価からは評価した工程にCHIKV及びSINVに効果的な不活化効果があることが示された。これはモデルウイルスが用いられた初期のバリデーション研究の結果を裏付けた。	4
120020	05-Jun-12	120189	チクングニヤウイルス感染	Emerging Infectious Diseases. 18(2012)493-495	中国におけるチクングニヤ熱アウトブレイクの報告。2010年10月、中国広東省の2つの隣接した村でチクングニヤ熱アウトブレイクが発生し、173例の患者が報告された。最初の患者は9月1日に発症し、9月19日以降、患者数が急増した。死亡した患者はおらず、大部分の患者は1週間以内に回復した。12人の急性期患者及び3人の回復期患者から血清検体を採取し、このアウトブレイクを引き起こしているチクングニヤウイルスの系統発生解析を行ったところ、このチクングニヤウイルスは東/中央/南アフリカ(ECSA)サブグループのインド洋分岐群に属することが示された。前月、広東省の天気は雨が多く、これにより媒介蚊の個体数が増加したと考えられる。	5
120020	03-Jul-12	120234	ウイルス感染クロストリジウム感染	CDC/Press Release. Mar 14, 2012	米国における、胃腸炎による死亡例の増加の報告。米国疾病管理予防センター(CDC)は、胃腸炎により死亡した人数が1999年から2007年で2倍以上に増加したと発表した。65歳以上が83%を占めていた。Clostridium difficileとノロウイルスが最も多い感染原因であった。特にC. difficileは8年間で約5倍に増加し、死亡例の3分の2を占めており、これには高毒性株及び耐性株の出現と拡大が影響しているものと考えられた。ノロウイルスは新型株流行の有無によって死亡例数が増減するが、米国における胃腸炎流行の主要原因である。	6

120020	18-Apr-12	120044	バベシア症	Ann Intern Med. 155(2011)509-519	米国における輸血関連バベシア症に関する報告。1979年～2009年に米国で輸血を受けて2010年までにバベシア症と診断された患者を調査した。その結果、輸血関連バベシア症例は162例が特定され、B. microti感染が159例、B. duncani感染が3例であった。B. microti感染例ではほとんどの症例が赤血球製剤に関連した感染であったが、4例は全血由来血小板製剤に関連していた。87%が風土病とされる7州（マサチューセッツ、ニューヨーク、コネチカット、ミネソタ、ロードアイランド、ニュージャージー、ウィスコンシン）で発生していた。B. duncani感染例においては3例とも赤血球輸血関連で、ワシントン州で1例、カリフォルニア州で2例記録されていた。以上の結果から、輸血関連バベシア症感染のリスク軽減のため、ドナー検査の必要性が示唆された。	7
120020	04-May-12	120093	バベシア症	Pediatrics. 128(2011)e1019-e1024	早産新生児における輸血関連バベシア症に関する報告。米国北東部において、バベシアに感染していた2つの血液製剤の輸血により7例のバベシア症例が発生した。臨床症状はこの集団において大きなばらつきがあった。超低出生体重児（760g）は最も重症であった。抗生物質での治療は軽度及び無症候性の感染症に効果があったが、最も重症であった2症例は長期の多剤処置を伴う2倍量の交換輸血が必要であった。Babesia microti感染症のリスクは現在の血液銀行の業務手順では排除されない。流行地域の新生児科の医師は早産児における輸血関連バベシア症を考慮するべきである。	8
120051	05-May-12	120094	バベシア症	Transfusion; published online. Dec 13, 2011	米国におけるバベシア症の輸血感染の報告。カルフォルニア在住の59歳の鎌状赤血球症（HbSS）患者で、Babesia duncani（B.duncani）の感染症例が報告された。唯一のリスク因子は赤血球輸血を受けたことであった。患者は数カ月にわたり輸血量が増加した後、2008年9月に血液スメアにより赤血球内原虫が発見され、診断された。B.microti陰性、B.duncani陽性であり、原虫18SリボソームRNAが血液検体から増幅された。輸血血液の調査の結果、カリフォルニア在住67歳の関連供血者が陽性であり、スナネズミに血液検体を接種したところB.duncaniが分離された。当該患者は2008年5月に関連輸血を受けてから4カ月以上を経て診断された。本症例はB.duncaniに起因する輸血関連症例の3例目であり、B.microtiの検査では検出不可能なバベシアが疾病原因となり得ることを強調している。	9
120051	19-Apr-12	120045	マラリア	Transfusion. 51(2011)2398-2410	メキシコへの渡航者におけるマラリア感染リスクに関する報告。メキシコ渡航者における供血延期条件の妥当性を検討するため、2006年に米国血液センター6施設においてメキシコ渡航のために供血延期となった885例を対象にドナーの感染リスクを現地住民のマラリア感染率を用いて算出した。その結果、メキシコでのマラリア感染は75%がChiapas州及びOaxaca州において発生していたが、72%のドナーがQuintana Roo州を旅行しており、それらのドナーの感染リスクは0.0080例/年又は125年に1例であった。また、リスクの高いOaxaca州を除いた全ての地域の供血延期を解除すると、年間65000人の供血者を取り戻し、マラリア感染リスクは20年に1例となる。このことから、Quintana Roo州のようなリスクが低いと判断される地域に関しては供血延期条件を緩和するべきと考えられた。	10
120051	07-May-12	120096	マラリア	ECDC News. Oct 28,2011	ギリシャにおける三日熱マラリア感染の報告。2011年5月21日～10月26日の間に61症例の三日熱マラリア感染がギリシャで報告された。このうち33例は流行国への旅行歴がないギリシャ市民で、27症例が南ギリシャのLakonia県Evrotasから報告された。加えて、季節労働者の28症例もEvrotasから報告され、これらの移民の大部分（21例）はマラリア流行国の出身者であった。発症ピークは9月5～18日の週であり、それ以降報告数は減少した。全症例が三日熱マラリアと確認され、基礎疾患を持つ70歳代男性の1死亡例を除き、全員軽症であった。	11
120051	14-Jun-12	120198	トリパノソーマ症	Eurosurveillance. Mar 8, 2012	ケニアへのドイツ人旅行者におけるヒトアフリカトリパノソーマ症（HAT）の報告。2012年1月、ケニヤのマサイマラ地区から帰国したドイツ人旅行者においてHATが確認された。患者は62歳男性で、マサイマラ国立保護区を訪れ、大半の時間を半袖半ズボンで過ごし、昆虫忌避剤を使用していた。帰国後に発熱により入院し、ギムザ染色でTrypanosoma brucei rhodesienseが確認された。スラミンによる治療が行われ、回復した。1カ月後に、マサイマラ地区からの輸入HAT症例が他にも1例報告された。	12
120063	10-May-12	120099	その他	Transfusion. 51(2011)2367-2376	第2世代S-303病原体不活化処理後の保存赤血球（RBC）の生存率に関する報告。S-303はfrangible anchor-linker-effector（FRALE）複合体であり、アルキル化により強力に病原体を減少させる。第2世代S-303プロセス処理後のRBC生存能力を調査するために、27人の健康人より得られたRBCをS-303不活化群またはコントロール群振り分けて処理し、35日間保存した後に標識RBCを被験者に戻して生存能力を評価した。その結果、S-303処理RBCの輸血24時間後回収率はコントロールと同等であった。生存率、T50生存率の中央値はコントロールよりもそれぞれ13.7日、6..8日短かったが、RBC生存曲線下面積の差は1.38%にとどまった。また、臨床的に意味のある検査値の異常は認められなかった。S-303処理されたRBCは生理学的にも代謝的にも輸血に適していることが示唆された。	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
				2012. 3. 19	該当なし	

一般的名称	解凍人赤血球濃厚液	研究報告の公表状況		公表国
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)		Ott JJ, Stevens GA, Groeger J, Wiersma ST. Vaccine. 2012 Mar 9;30(12):2212-9. Epub 2012 Jan 24.	WHO

研究報告の概要

○B型肝炎ウイルス感染の世界的疫学:年齢別HBs抗原陽性率と流行性の推定

目的: 慢性B型肝炎は最も深刻な感染症のひとつであり、肝硬変や肝臓がんから死亡にいたる主要なリスク因子である。慢性HBV感染症患者の年齢、性別及び地域別有病率を推定し、慢性的な感染者の絶対数を計算することを目的として研究を行った。

方法: HBV感染症を報告している研究論文を体系的に再評価した。そして世界的なHBs抗原陽性率のデータを27年間分(1980年－2007年)収集した。観察データに基づき、経験ベイズ階層モデルを用いて1990 年と2005年における世界的レベル及び全世界地域の年齢別有病率及び流行性について推定した。

結果: 1990年から2005年に、多くの地域で慢性HBV感染症の有病率は減少し、これは特に中央サハラ以南のアフリカ、熱帯・中央ラテンアメリカ、東南アジア、中央ヨーロッパで明白であった。しかしながらHBs抗原陽性者の絶対数は1990年の2億2千3百万人から2005年の2億4千万人に増加した。年齢別の有病率はサハラ以南が最も高く、熱帯及び中央ラテンアメリカ、北アメリカ、西ヨーロッパのような地域における有病率は2%以下と、地理的地域によって変化した。アジア地域では、東アジアのHBs抗原陽性率は最高8.6%と高レベルであるが、南アジアでは中央値よりも低いという異なるパターンを示した。東南アジアの子供たちには有病率の顕著な減少が認められた。

北アメリカやヨーロッパでの有病率の減少は、B型肝炎ワクチンの普及や血液製剤検査の改善などが関連している。また、疾病負荷の大きい東南アジアや東アジアの地域でも、幼児へのB型肝炎ワクチン接種の範囲が劇的に広まった事に伴い有病率が減少した。

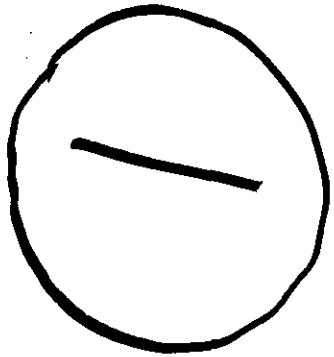
結論: HBV感染陽性率の減少は、予防接種の普及に関連がある可能性がある。慢性HBV感染症患者の絶対数の増加及び全世界のHBV流行の差異は、HBV関連死亡率及び疾病率に対するターゲットアプローチが必要であることを示している。HBV感染データは、疾病負荷の推定や健康対策及びワクチン接種の政策を立てるために、国家や準国家レベルで必要とされている。

報告企業の意見	今後の対応
1990年及び2005年における慢性HBV感染症患者の年齢、性別及び地域別有病率を推定し全世界での絶対数を算出したところ、多くの地域で有病率は減少したが地域によって大きな差があり、全世界での絶対数は増加したとの報告である。なお、現在日本では、多くの国で行われている全小児へのユニバーサルワクチン接種は行われていない。今後、日本のHBV感染予防対策を再検討する必要があるかも知れない。	HBV感染に関する新たな知見等について、今後も情報の収集に努める。

使用上の注意記載状況・その他参考事項等

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



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Global epidemiology of hepatitis B virus infection: New estimates of age-specific HBsAg seroprevalence and endemicity

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ABSTRACT

Objective: Chronic hepatitis B virus infection is one of the most serious infections and a major risk factor for deaths from cirrhosis and liver cancer. We estimate age-, sex- and region-specific prevalence of chronic HBV infection and calculate the absolute number of persons being chronically infected.

Methods: A systematic review of the literature for studies reporting HBV infection was conducted and worldwide HBsAg seroprevalence data was collected over a 27-year period (1980–2007). Based on observed data, age-specific prevalence and endemicity were estimated on a global level and for all world regions for 1990 and 2005 using an empirical Bayesian hierarchical model.

Findings: From 1990 to 2005, the prevalence of chronic HBV infection decreased in most regions. This was particularly evident in Central sub-Saharan Africa, Tropical and Central Latin America, South East Asia and Central Europe. Despite this decrease in prevalence, the absolute number of HBsAg positive persons increased from 223 million in 1990 to 240 million in 2005. Age-specific prevalence varied by geographical region with highest endemicity levels in sub-Saharan Africa and prevalence below 2% in regions such as Tropical and Central Latin America, North America and Western Europe. Asian regions showed distinct prevalence patterns with lower intermediate prevalence in South Asia, but up to 8.6% HBsAg prevalence in East Asia. Strong declines were seen in South East Asian children.

Conclusion: Declines in HBV infection prevalence may be related to expanded immunization. The increasing overall number of individuals being chronically infected with HBV, and the widespread global differences in HBV prevalence call for targeted approaches to tackle HBV-related mortality and morbidity. HBV infection prevalence data are needed at country and sub-national level to estimate disease burden and guide health and vaccine policy.

1. Introduction

Knowledge of region- and age-specific prevalence of hepatitis B infection is important for evaluating vaccination programs and national disease prevention and control efforts. Furthermore, any modeling and assessment of the disease burden associated with the hepatitis B virus (HBV) requires prevalence estimates. So far, global studies on HBV seroprevalence are limited and comprehensive data are not available for many countries. In addition, demographic changes and expanded vaccination can create new epidemiological patterns of the virus which impact on region-specific endemicity levels.

HBV is spread predominantly by percutaneous or mucosal exposure to infected blood and other body fluids with numerous forms of human transmission. The sequelae of HBV infection include acute and chronic infection, cirrhosis of the liver and primary liver cancer. The likelihood of progression to chronic infection is inversely related to age at the time of infection. Around 90% of infants infected perinatally become chronic carriers, unless vaccinated at birth. The risk for chronic HBV infection decreases to 30% of children infected between ages 1 and 4 years and to less than 5% of persons infected as adults [1,2].

Chronic HBV infection progresses nonlinearly through 3–4 phases, from the immune-tolerant phase to immune clearance or immunoactive phase, to nonreplicative inactive phase and possible reactivation [3,4]. After infection with HBV, most patients either develop immunity (87–90%) and clear the infection or become chronic carriers. A lower percentage will develop liver disease or chronic active hepatitis with an increased risk of developing cirrhosis, liver cancer or both [5]. The fatality of these diseases as well as their attribution to hepatitis infection is well known: 600,000 HBV-related deaths were estimated to occur annually [6] and 73%

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of all liver cancer deaths worldwide are due to hepatitis viruses, with much higher proportions in low and middle income countries [7].

The complex serology and natural history associated with HBV infection creates challenges for the assessment of HBV prevalence and the provision of comparable global estimates. This is due to the availability of multiple laboratory markers for hepatitis B infection. Antibodies and antigens associated with this infection include hepatitis B surface antigen (HBsAg), antibody to hepatitis surface antigen (anti-HBs), antibody to hepatitis B core antigen (anti-HBc), and IgM antibody subclass of anti-HBc (IgM anti-HBc). Some studies also report markers of high HBV replication such as hepatitis B "e" antigen (HBeAg), antibody to HBeAg (anti-HBe), and quantitative HBV-DNA.

HBsAg is the main clinical marker indicating acute or chronic infection and prevalence as well as endemicity of HBV infection is defined by the presence of HBsAg [8]. HBsAg testing is the primary way to identify persons with chronic HBV infection and several characteristics of this serological marker increase the precision of HBsAg estimates, including high specificity, long serum persistence, low possibility of chronic cases losing HBsAg [3,8,9]. However, routine population surveillance of chronic viral hepatitis is currently rare. Standardized monitoring would help not only in quantifying the disease burden on a population level but also in determining the characteristics of infected individuals, avoiding further transmission and allocating appropriate treatment. This is particularly important for populous countries that have been previously categorized as highly endemic for chronic hepatitis B infection such as China, Indonesia, Nigeria and parts of Africa and Asia, where an immense absolute number of people live with the virus [6,8]. However, up to date region-specific and globally comparable chronic HBV prevalence data are lacking and no relevant meta-analysis has been published on this topic. In addition, the absolute number of individuals being chronically infected with HBV is not known.

In the light of this gap, the objective of our study is to estimate age- and region specific HBsAg prevalence in 1990 and 2005 by conducting a systematic review and modeling HBsAg prevalence. This investigation is part of the Global Burden of Diseases, Injuries, and Risk Factors Study, which carries out complete systematic assessments of global data on disease and risk factors in order to produce comparable estimates for two time periods, 1990 and 2005.

We provide detailed HBsAg prevalence estimates that are categorized by the 21 regions as defined by the Global Burden of Disease Study and geographically mapped by endemicity level. The Global Burden of Disease regions are based on geographic regions or continents and are grouped based on child and adult mortality levels and major causes of death in each country (see Section 2).

2. Methods

2.1. Systematic review

A systematic review of published literature and data was conducted to identify articles reporting prevalence of hepatitis B, C, and D virus infection for all countries over a 27-year period, from 1980 to 2007. Articles published in this time frame were included regardless of when the data were collected. Within each database, medical subject heading terms and freetext search terms were included to identify article abstracts that contained (1) a term related to the hepatitis B, C or D virus or their markers of hepatitis infection and (2) a term related to either prevalence, incidence, or disease burden (search terms available by request from the author). Results were restricted to original research articles in English. Abstracts were screened and were required to report hepatitis B or C prevalence or incidence. Where abstracts were incomplete or missing, the full-text article was retrieved and reviewed manually to determine if it reported prevalence of hepatitis B or C virus infection. A total of 6064 English citations were found (3273 Medline, 2283 Embase, 508 Cinhal). Review articles, outbreak investigations and national infections disease notification reports were excluded since they provide information on incident or acute cases. Data reported in the article had to be reasonably representative of the general population rather than conducted among a special high-risk group (i.e. injecting drug users, HIV-positive individuals) or a population that was selected based on a risk factor for viral hepatitis or a condition associated with hepatitis infection (Fig. 1, exclusion criteria).

After applying manual de-duplication and the exclusion criteria on the abstract, 1233 articles were obtained (references are listed in Web Annex 1). These were further screened for the specified exclusion criteria and for HBsAg as the marker of interest in the full text before country-specific prevalence information was extracted. For one country, the United States (US), a representative primary national data source was available and data were included only from articles reporting prevalence from the National Health and Nutrition Examination Survey (NHANES) [10,11].[1]

Articles only reporting HCV marker (222) and those reporting summary or other markers of HBV (82) were excluded; 396 articles were determined to meet all eligibility criteria.

2.2. Analysis and modeling of data

Age-, sex- and country-specific HBsAg seroprevalence data were extracted. Additional information obtained was: primary author, year of publication, number of individuals tested for HBsAg, laboratory test/method, and study year. If the year was missing, two years prior to publication was assumed as the study year. In case age was not further specified, it was imputed based on contextual information such that pregnant women and those giving birth were assumed to be 15–49 years, army recruits and soldiers between 18 and 45 years, blood and organ donors between 17 and 65 years, and school children between 5 and 15 years. Extracted data were grouped according to 21 Global Burden of Disease Regions (Web Annex 2) and assigned a quality rating based on population size, sampling and representativeness of the general population. Using the extracted study seroprevalence data, prevalence of HBsAg was modeled using Dismod III v3.0, a generic disease modeling system [12]. DisMod III aims to synthesize data to generate estimates of the disease burden associated with more than 200 diseases and over 20 major risk factors for the Global Burden of Disease 2010 study [13]. DisMod III models multiple disease parameters, including incidence, prevalence, remission, and mortality, in order to ensure consistency among the parameters. Data on each of these parameters are synthesized using a hierarchical empirical Bayesian model to make estimates for 21 world regions based on observed data in each modeled region, data observed in other regions, and data from other time periods (by estimating a time trend). Briefly, Dismod III first fits an empirical prior estimate separately for each disease parameter (e.g., prevalence and incidence). The empirical prior has the following elements: geographic hierarchy, in which estimates for each region are informed by data from the same region and (to a lesser extent) data from other regions; a flexible age pattern; a linear time trend; and an offset for data on males. Second, for each time period (1980–1996 and 1997–present), sex, and region (of 21 world regions), Dismod fits a Bayesian model using all data in that

[1] The definition of chronic HBV infection and the respective marker used slightly varied by period of survey conduction. From 1988 on, HBV infection was defined as the presence of anti-HBc and HBsAg. We have included this estimate since all HBsAg positive individuals should be expected to have anti-HBc.

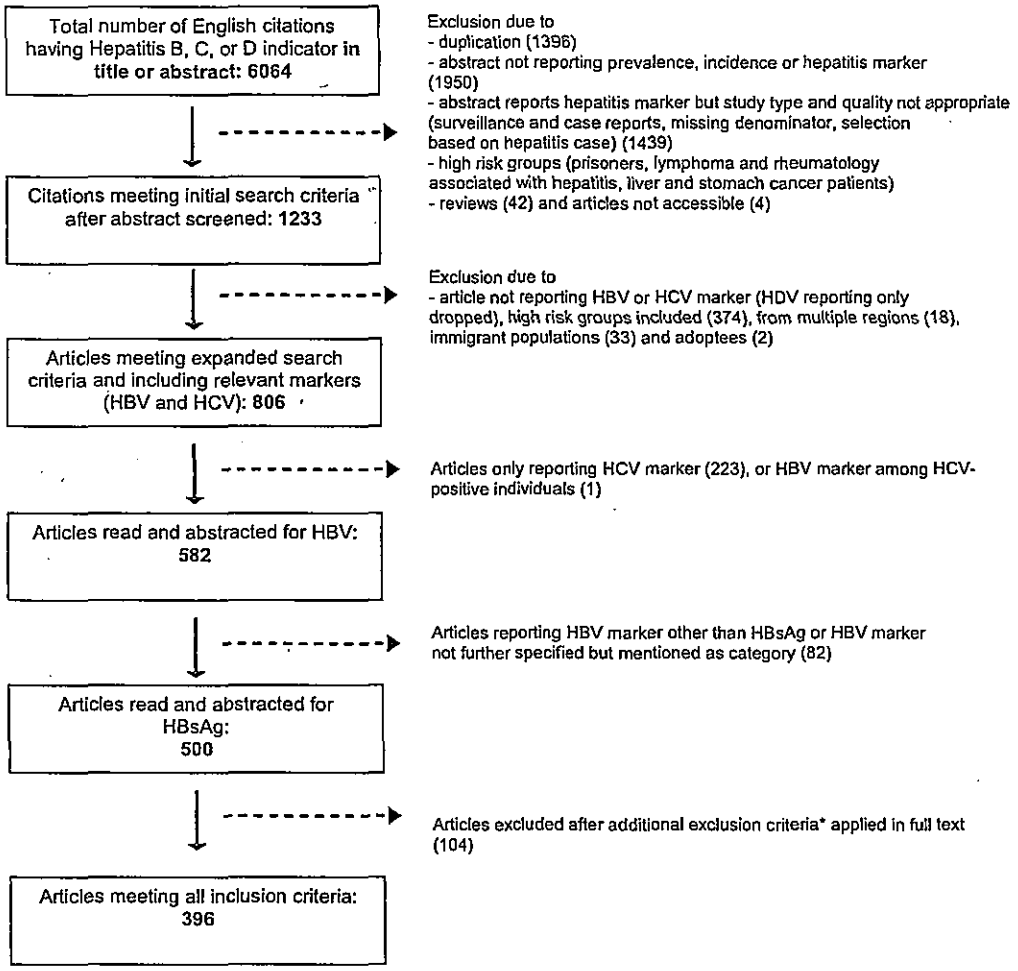


Fig. 1. Flowchart of article screening.
*Additional exclusion criteria applied in full text screening and not identified as such during first article review.
(a) Populations of persons at high risk for hepatitis including those with diseases related to HBV such as acute (viral) hepatitis cases, liver cancer, and cirrhosis; HIV-positives.
(b) Other high risk study populations and highly defined populations such as prisoners, HIV/STD clinic attendees indicating recently acquired sexually transmitted disease, sex workers, multi-transfused patients, drug addicted individuals and injecting drug users, liver transplant recipients, refugees, homeless people.
(c) Reports of acute diseases surveillance (reporting incident cases, acute cases or rates per 100,000 population).
(d) Reports and data that are incomplete (e.g. number tested not provided or below 20).
(e) Citations reporting HBsAg prevalence in the US other than NHANES prevalence.

time–sex–region group and empirical priors for all epidemiological parameters, generating posterior estimates of incidence, prevalence, remission, and mortality that are internally consistent. In our model, the empirical priors for incidence, remission, and mortality were uninformative; thus the posterior was informed only by prevalence data. Like the empirical prior, the posterior models also incorporate linear time trends, flexible age patterns, and offsets for data on males. Applying region-specific population figures for 1990 and 2005, age- and region-specific HBsAg prevalence was used to calculate the absolute number of individuals chronically infected with HBV.

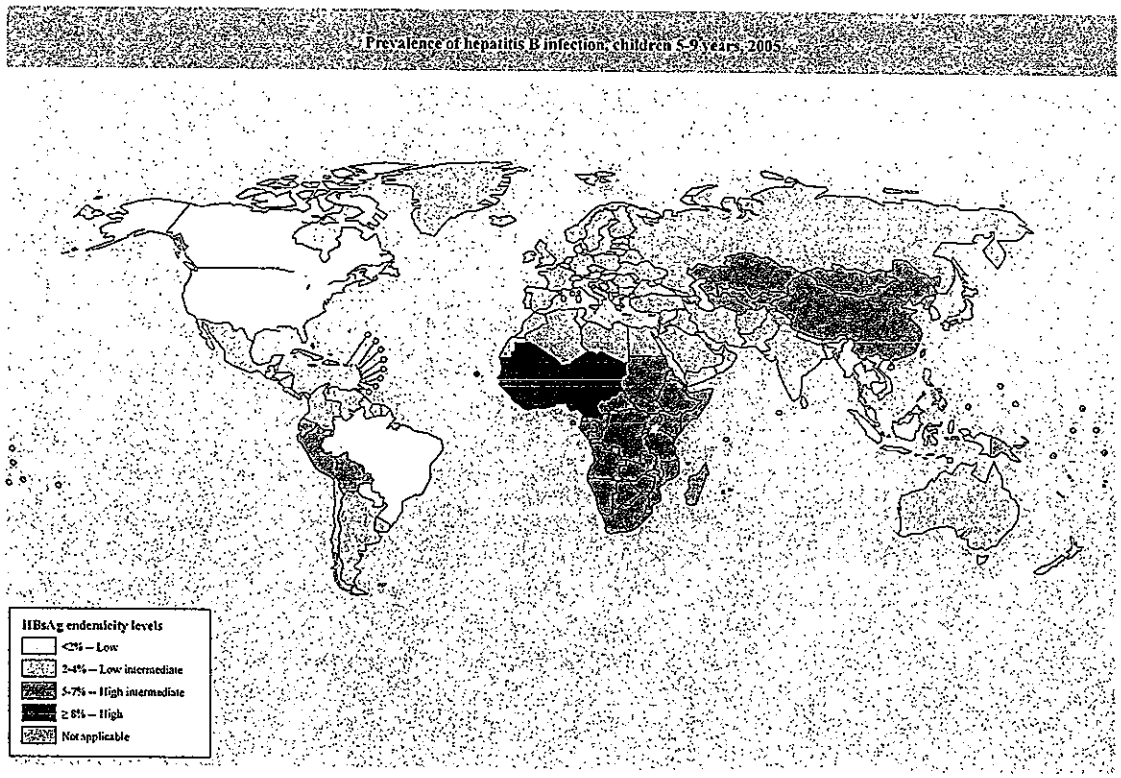
For the purpose of generating endemicity maps, endemicity levels of HBsAg were defined as low (<2%), lower intermediate (2–4%), higher intermediate (5–7%) and high (≥8%). HBsAg infection levels have traditionally been described according to three categories of endemicity indicating the proportion of the population being seropositive for HBsAg [8,14]. However, given that there are regions very close to low endemicity (<2%) (e.g. 2.0% prevalence among children in Western Europe and Central Latin America in 2005) and others rather close to high endemicity (≥8%) (e.g. 7.8% prevalence among adults in Eastern Asia in 2005) the split of the intermediate category better reflects regional differences and their implications.

3. Results

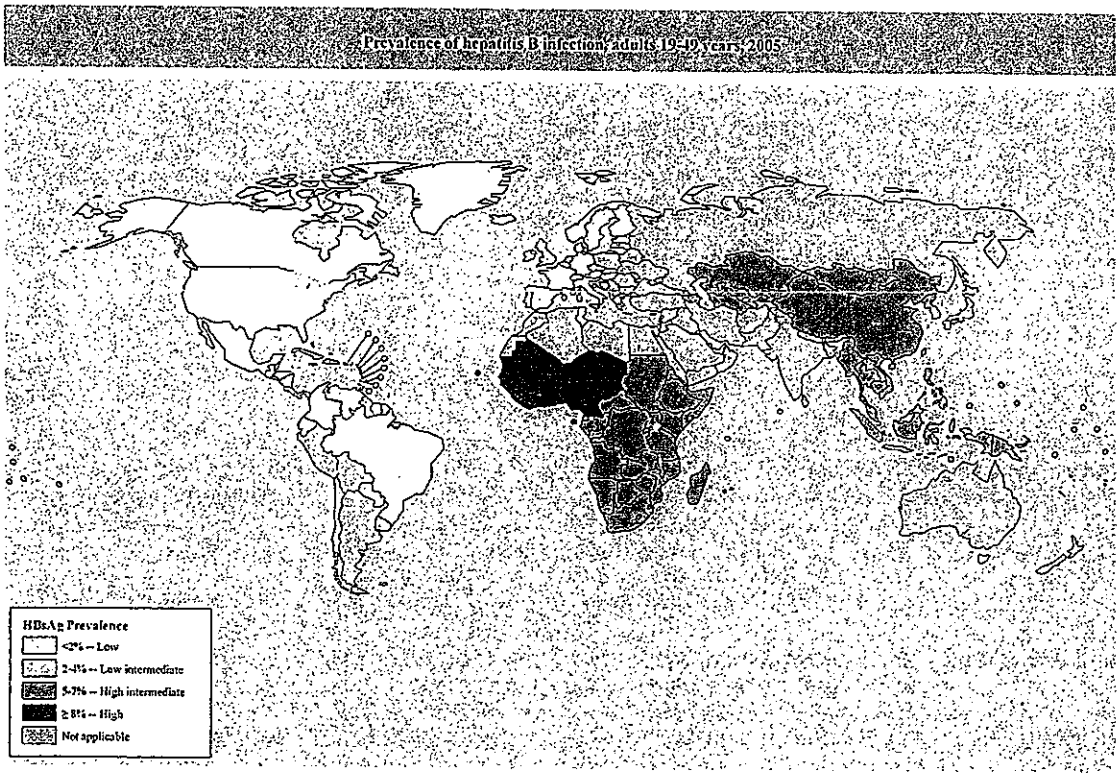
We identified 396 studies of HBsAg prevalence after applying all inclusion and exclusion criteria. To illustrate global endemicity, estimated and categorized HBsAg prevalence in 2005 is shown in Map 1 for children (5–9 years) and in Map 2 for adults (19–49 years) applying world population age weights.

The pattern of age-specific HBsAg prevalence varied greatly by region and the trend of a decreasing prevalence with age was more evident in 1990 as compared to 2005, where some regions, e.g. South East Asia showed an exceptional increase with age. For most regions, predominantly Tropical Latin America, West sub-Saharan Africa, Australasia, and North Africa, Figs. 2–5 indicate an overall decrease in HBsAg prevalence between 1990 and 2005. East Asia and Western Europe experienced some increase.

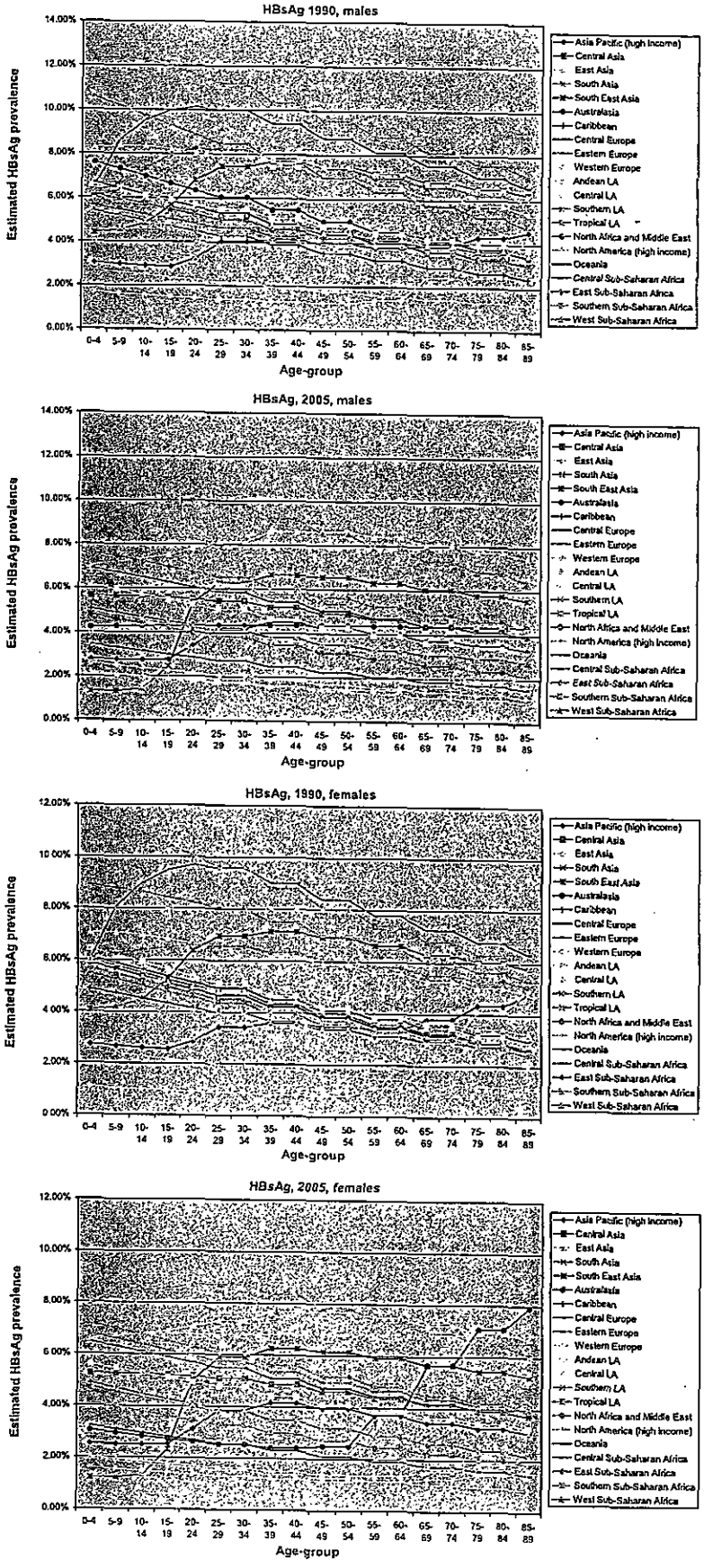
Global differences between males and females were small, although females had a lower overall HBsAg prevalence of 3.5% in



Map 1. Map for children.



Map 2. Map for adults.



Figs. 2–5. HBsAg seroprevalence by region and age-group for males and females separately, 1990 and 2005.

Table 1
Overview: Global HBsAg and people chronically infected.

Year	Males		Females		Both	
	Persons HBsAg positive	Prevalence	Persons HBsAg positive	Prevalence	Persons HBsAg positive	Prevalence
1990	118 million	4.4	105 million	4.0	223 million	4.2
2005	127 million	3.9	113 million	3.5	240 million	3.7

2005 compared to 3.9% in males. We estimated 240 million people chronically infected worldwide in 2005 (Table 1).

3.1. HBsAg prevalence by GBD world region (Tables 2–5: Web Annex 3)

HBV prevalence was most common in sub-Saharan regions of Africa. *Western sub-Saharan African* countries had some of the highest age-specific HBsAg prevalence in the world reaching up to 12% among children and adolescents in the age-groups up to 19 years in 1990. Although there was a decrease in 2005, the region continued to have high HBV endemicity, which is more pronounced among males.

An increase in chronic HBV infection among younger age-groups (0–14 years) occurred in *Southern sub-Saharan Africa* in 2005 compared to 1990 that resulted in age-specific prevalence of 8–9% among young females. Also *Eastern sub-Saharan African* countries faced an increase in the youngest ages and almost no change in other age-groups. In 2005, prevalence peaked at approximately 7% in 0–4 years aged boys and girls and declined with age in this region.

A decrease in prevalence was evident in *Central sub-Saharan Africa* which transitioned from high endemicity among younger individuals (age-groups up to 34 years) in 1990 into intermediate endemicity across all ages in 2005.

North Africa and the Middle Eastern region showed lower intermediate HBsAg endemicity across all age-groups in 2005. Prevalence decreased from 1990 to 2005, particularly among males up to 34 years.

The prevalence in high-income countries of *North America* (Canada and the United States) was low and declined among both sexes and across all ages between 1990 and 2005. Males had higher HBsAg positivity than females in both periods, peaking in the male 0–4 years age-group at 2.71% and 2.14% in 1990 and 2005, respectively. The oldest ages (65+ years) showed the lowest prevalence of approximately 1% in 2005.

Both *Tropical Latin America* and *Central Latin America* demonstrated a strong decrease in HBsAg prevalence between 1990 and 2005. Tropical Latin America changed from an intermediate into a low endemicity region. Where 0–9 year aged boys had a higher intermediate endemicity of over 5% in 1990, HBsAg prevalence was only 1.6% in 2005. Similarly, in Central Latin America prevalence has halved in this period and most adult age-groups shifted to a low endemicity level in 2005. Other Latin American regions such as *Andean Latin America* and *Southern Latin America* showed a decreasing prevalence by age but relatively constant intermediate endemicity levels. A slight decline in prevalence from 1990 to 2005 among Andean Latin Americans was paralleled by an increase in HBsAg prevalence in Southern Latin America.

HBV chronic infection rates in *Caribbean* children and adolescents aged 0–19 years ranged from 4.3% to 5.4% and was fairly constant over time. HBsAg prevalence decreased with age.

In the island nations of the Pacific and Indian Oceans (*Oceania*), HBsAg was highly endemic among men and women in 1990, peaking at approximately 10% in men aged 10–34. The decrease in prevalence up to 2005 led to a shift into a higher intermediate endemicity level among the age-groups up to age 54 and into a lower intermediate endemicity level in older adults (55+).

Among the European regions, seroprevalence of HBsAg showed consistently low prevalence in *Western Europe*. This was particularly true for females who had prevalence below 2% throughout the time periods. Nevertheless, between 1990 and 2005, an increase in both sexes was observed that led to a change from initially low endemicity in young males to a low-intermediate endemicity level in 2005; this was accompanied by a decrease in prevalence in the older individuals (65+ years). *Central and Eastern European* children had a higher intermediate HBsAg endemicity in 1990, which decreased in 2005. Older Central European females in particular demonstrated a strong HBsAg prevalence decrease up to 2005. Prevalence in infant and young girls declined from 6% in 1990 to 3% in 2005 in this region. In contrast, Eastern European countries did not experience as strong a reduction in HBsAg prevalence in the youngest age-groups. In both Central and Eastern Europe the age group 0–9 years remains the most affected by HBsAg infection.

Among all Asian regions, *East Asia* had the highest prevalence of HBV infection and there was not much of a change between 1990 and 2005 apart from a slight decrease in children and an increase in all age-groups above 25 years in 2005 as compared to 1990. In 1990, prevalence decreased in both sexes with age, but increased with age in 2005 and showed the highest prevalence of over 8% among males aged over 35 years. Generally, endemicity remains at high or close to a high endemicity in this region, which is particularly true for males. Intermediate HBsAg endemicity was also estimated for *Central Asia*, which includes the Caucasus and central Asian countries. A small decrease was observed between 1990 and 2005 but Central Asian children and younger adults had an HBsAg prevalence of around 5% in 2005. In *South Asia*, approximately 3% of the population up to age 45 was HBsAg positive with a decrease in older individuals who demonstrated a low prevalence in 2005.

Unlike other Asian regions, *South East Asia* experienced a strong reduction in HBsAg prevalence between 1990 and 2005, particularly in the young age groups of 0–14 years that had prevalence levels of 1.2–1.4% in 2005. In contrast, South East Asian adults appeared to continuously have higher-intermediate HBsAg prevalence of 5% to over 6% in 2005. The pattern of an increasing prevalence with age was very pronounced in 1990 and rather exceptional compared to the other regions.

Some reduction in HBsAg prevalence between 1990 and 2005 occurred also in high income *Asian Pacific* countries including Japan, the Republic of Korea, and Singapore. The middle ages (25–54) were the most affected age groups but overall, endemicity remained at an lower intermediate level of approximately 4% in 2005. Interestingly, in 1990 the oldest age-groups of over 75 years had the highest prevalence among all age-groups.

Australasian countries experienced a reduction in HBsAg prevalence and were categorized as a lower intermediate endemicity region in 2005. Males of all age-groups had prevalence in the range of 4% in 2005 whereas 2–3% of females up to age 55 were affected by chronic HBV infection with a sharp increase in the oldest age-groups.

4. Discussion

We found a very large burden of HBsAg infection in all sub-Saharan African regions, East Asia and, to a lesser extent, in Oceania

and Andean Latin America. Most other regions with high and middle income showed a mix of lower and higher intermediate HBV endemicity. Only a few regions demonstrated prevalence below 2% throughout most age-groups. Among these were Tropical Latin America, Central Latin America, North America and Western Europe. There was an overall decrease in HBsAg prevalence from 1990 to 2005 in younger age-groups, which may be closely related to widespread hepatitis B immunization, particularly in low income regions. Significant decreases in HBsAg prevalence due to immunization were reported from African countries such as the Gambia [15,16] and Senegal [17]. However, the infection remains extremely prevalent in sub-Saharan Africa and the attributable HBV-related disease burden can be expected to remain high. This is also reflected in a high mortality from primary liver cancer, one sequelae of chronic HBV infection and the most frequent cause of cancer deaths among men in this region [18].

The observed decreases in HBsAg prevalence in North America and Europe were temporally associated with increased hepatitis B vaccine coverage rates [19], improved screening of blood products and increased availability of safe injection materials [20,21]. The generally low HBsAg endemicity levels in these countries are paralleled by a steady decline in reported cases of acute hepatitis B [22,23]. On the other hand, the large number of individuals infected with HBV influences the number of liver cirrhosis and cancer cases in many world regions including high income countries [24] and highlights a need for screening and surveillance programs to identify chronically infected individuals and thereby prevent further transmission as well as to provide opportunities for secondary and tertiary prevention [3].

Asian countries, for example those in the GBD South East and East Asian regions, have also has experienced dramatic increases in coverage of routine infant hepatitis B vaccine that were accompanied by a reduction in HBsAg prevalence. The impact of this decrease in HBsAg prevalence was also measurable in a substantial reduction in the HBV-related disease burden in countries that were highly endemic in the past e.g. Taiwan [25,26] and China [27,28].

Strengths of this study include the extensive systematic literature review and the use of an empirical Bayesian hierarchical model to estimate region-specific HBsAg prevalence and endemicity and to subsequently calculate the absolute number of people being chronically infected with the HBV.

This study has a number of limitations. Observed HBsAg prevalence data are lacking in some regions and the quality of studies reporting these data is often low. Middle- and low income regions, e.g. Oceania, Central Asia, and Andean Latin America had a limited evidence base or studies were concentrated on one country as is the case with India as part of the South Asian region or Thailand located in the South East Asian region. Accordingly, simulations of prevalence may lead to potential underestimation of the true regional profile, particularly if studies were more likely to be conducted in countries with higher economic standards and better research infrastructure. To address issues of representativeness, grey and non-English literature should be considered in future studies and there is a need for generating more high quality data from low resource settings. Most high quality studies were conducted in high income countries, for example the Western European region. Nationally representative, population-based studies reporting HBsAg prevalence were only available from the US.

Another limitation is related to factors that were not considered in our analysis such as genotype information. It is known that viral genotypes vary between and within countries, depending on the populations at risk and their geographical origin. Very few HBV prevalence studies report on genotype and the genotype-specific HBV distribution by country has not been sufficiently studied. As a result, we did not adjust for this information, which might be crucial given the fact that some genotypes are associated with more severe disease or clinical response to treatment [29–31]. Similarly, the laboratory method used to detect HBsAg was only reported in a few studies and we did not consider this factor in the analysis, which could impact the comparability of HBsAg prevalence across studies.

Since the overall objective of this study is to provide a regional picture of HBV prevalence, the results do not capture the potential heterogeneity that exists between sub-populations within a country. It should be noted that some low endemicity areas in Western Europe and North America face great intra-country variation with higher prevalence and higher hepatitis-related mortality among migrants [32–35] and additional country-specific data would be crucial for comprehensively guiding national hepatitis B prevention and control programs and targeting most vulnerable population groups.

Prevalence data obtained from systematic reviews and modeling should be interpreted conservatively. Descriptive epidemiological research conducted in high income areas may generally focus more on marginalized and higher risk populations whereas studies from low income regions may focus on urban and higher educated populations that experience lower infection rates than those living in poorer areas. As a result, the prevalence of chronic HBV infection reported in this study could be overestimated for high income regions and underestimated for low income regions. This would, however, increase the estimated differences between these regions and support our findings.

There is a need for systematically collected and population-based HBV infection data. Data on other markers of HBV such as HBeAg and anti-HBc are also needed to describe current and future HBV-related disease burden.

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Contributors: JJO wrote the manuscript, modeled, analyzed, and interpreted the data. GAS provided guidance on data modeling and contributed to writing. JG designed and conducted the literature search and reviewed articles. STW initiated the study, supervised all components of the study and contributed to writing the manuscript.

Conflict of interest statement: None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.vaccine.2011.12.116.

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医薬品 研究報告 調査報告書

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販売名(企業名)	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)			米国

研究報告の概要

○米国の供血者におけるHIV、B型肝炎、C型肝炎の感染時期による遺伝型多様性

背景: ヒト免疫不全ウイルス(HIV)、C型肝炎ウイルス(HCV)とB型肝炎ウイルス(HBV)の変異は、診断検査法と治療に影響を及ぼす場合がある。サブタイプやジェノタイプ、薬剤や免疫耐性変異の最近の変化については、最近感染した(incident)供血者とそれより以前に感染した(prevalent)供血者を比較することによって特徴付けられる。

方法: 感染した供血者は、3,400万人の米国の供血者間(2006－2009年)から特定された。incidentは抗ウイルス抗体価がゼロまたは低いと定義した。ウイルスゲノムは部分的な配列決定を行った。

結果: 312のHIV株(50%がincident)のうち、2.5%はサブタイプが非B型であった。プロテアーゼ阻害剤耐性変異は2%、逆転写酵素(RT)阻害剤耐性変異は11%の感染供血者に見られた。

278のHCV株(31%がincident)のサブタイプの割合は1a>1b>3a>2b>2a>4a>6d, 6eであった。incident症例では3aが高頻度で発見されたのに対し、prevalent症例では1bが高頻度で発見された(P=.04)。

193のHBV株(26%がincident)の間の20のサブジェノタイプは、incident症例でA2、prevalent症例ではA1、B2、B4がより高い頻度で見られた(P=.007)。HBV株の薬剤耐性変異は検出されなかった。HBVのエスケープミュータントは、incident症例では6%であるのに対し、prevalent症例では26%に見られた(P=.01)。

結論: 供血者のウイルスジェノタイプの変異分布は、米国の高リスク集団のジェノタイプ分布と類似していた。大規模な通常の供血者スクリーニングを通じて血液媒介ウイルスを検出することで、高暴露人口の分子サーベイランス研究を補完することが出来る。

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
米国の供血者におけるHIV、HBV、HCVの遺伝的変異と流行を調べたところ、ウイルスジェノタイプの変異分布は米国の高リスク集団の分布と類似していたとの報告である。	日本赤十字社では、化学発光酵素免疫測定法(CLEIA)によりHIV抗体、HBs抗原、HBc抗体、HCV抗体検査を実施することに加えて、精度を向上させたNATシステムを導入し、20プールでスクリーニングNATを行い、陽性血液を排除している。HIV、HBV、HCV感染に関する新たな知見等について、今後も情報の収集に努める。

MAJOR ARTICLE

Genetic Diversity of Recently Acquired and Prevalent HIV, Hepatitis B Virus, and Hepatitis C Virus Infections in US Blood Donors

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(See the editorial commentary by Katz, on pages 867–9 and see the article by Stramer et al, on pages 886–94.)

Background. Genetic variations of human immunodeficiency virus (HIV), hepatitis C virus (HCV), and hepatitis B virus (HBV) can affect diagnostic assays and therapeutic interventions. Recent changes in prevalence of subtypes/genotypes and drug/immune-escape variants were characterized by comparing recently infected vs more remotely infected blood donors.

Methods. Infected donors were identified among approximately 34 million US blood donations, 2006–2009; incident infections were defined as having no or low antiviral antibody titers. Viral genomes were partially sequenced.

Results. Of 321 HIV strains (50% incident), 2.5% were non-B HIV subtypes. Protease and reverse transcriptase (RT) inhibitor resistance mutations were found in 2% and 11% of infected donors, respectively. Subtypes in 278 HCV strains (31% incident) yielded 1a>1b>3a>2b>2a>4a>6d, 6e: higher frequencies of 3a in incident cases vs higher frequencies of 1b in prevalent cases were found ($P = .04$). Twenty subgenotypes among 193 HBV strains (26% incident) yielded higher frequencies of A2 in incident cases and higher frequencies of A1, B2, and B4 in prevalent cases ($P = .007$). No HBV drug resistance mutations were detected. Six percent of incident vs 26% of prevalent HBV contained antibody neutralization escape mutations ($P = .01$).

Conclusions. Viral genetic variant distribution in blood donors was similar to that seen in high-risk US populations. Blood-borne viruses detected through large-scale routine screening of blood donors can complement molecular surveillance studies of highly exposed populations.

Volunteer (nonremunerated) blood donors provide whole blood and apheresis blood components used for transfusions as well as plasma for manufacture into therapeutic plasma derivatives. Past injection drug users (IDUs) and men who have had sex with men (MSM) since 1977 are currently excluded from donating. All donations in the United States are screened for antibodies to human immunodeficiency virus (HIV), hepatitis C virus (HCV), and hepatitis B virus (HBV), as well as for HIV and HCV RNA and HBV surface antigen (HBsAg); HBV DNA screening was widely implemented in 2009. Antibody and/or RNA/DNA/antigen-positive donors are notified, counseled, and excluded from further donation; all donations from viral marker-reactive donors are destroyed or used for research.

Blood donor screening can provide an efficient geographic and demographic sampling of individuals infected with these viruses that differ from the predominantly high-risk MSM and IDU populations that are the subjects of most viral molecular epidemiological studies. By routinely identifying recently acquired or incident infections, blood donation screening also

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provides an opportunity to analyze very recently transmitted viral strains at the forefront of currently active transmission chains. In this study, plasma samples from donors containing HIV, HBV, and HCV nucleic acids were classified as either recently acquired (incident) infections containing no or low concentrations of viral antibodies, or longer-term seropositive (prevalent) infections. Viral subtypes, drug resistance, and immune escape mutations were determined from representative incident and prevalent infections.

MATERIALS AND METHODS

Subjects, Specimens, and Case Definitions

This study included qualifying donations from 1 January 2006 through 31 December 31 2009 from 3 Retrovirus Epidemiology Donor Study-II (REDS-II) blood centers (Blood Centers of the Pacific, Blood Center of Wisconsin, and Hoxworth Blood Center/University of Cincinnati), all American Red Cross (ARC) Blood Services regions with data and samples provided through the Scientific Support Office in Gaithersburg, Maryland, and United Blood Services regions and the New York Blood Center (NYBC), with samples provided by Blood Systems Laboratory in Tempe, Arizona. Together, these centers account for approximately 70% of the US blood supply.

Data provided for all confirmed-positive donations included the date of donation, first-time/repeat donor status, date of birth, state of residency, race/ethnicity (if available), and sex. Screening and confirmatory test results for HIV and HCV nucleic acid testing (NAT), HIV and HCV antibody (Ab), HBsAg, and anti-HBV core antibody (anti-HBc) were also provided. Serologic and NAT screening and confirmatory testing were performed according to previously described algorithms using Food and Drug Administration (FDA)–licensed assays with documented performance characteristics including analytical sensitivities of NAT assays ([1–4]; see Appendix). Centers were requested to send residual test samples or samples from retrieved plasma units for all donors who qualified for study. Samples were stored at $\leq -20°C$ prior to testing.

Informed Consent and IRB Approvals

Institutional review board (IRB)–approved information sheets were provided to donors explaining that (1) surplus samples of their donations may be used for research purposes, (2) future research may be performed with the donor's blood without further consent if the IRB considers the research to be of negligible risk, (3) he or she will be contacted for additional consent depending on the nature of further research as determined by the IRB, and (4) the donor will be notified of any medically relevant information. IRBs representing each blood organization and the REDS-II Data Coordinating Center (Westat) approved the study protocol and determined that data related to HIV drug resistance mutations should be provided to donors as this may impact their treatment strategies; blood donors were not notified of other study results.

Sample Selection

The goal was to sequence samples from 150 incident and 150 prevalent cases for each virus over the 4-year study period. Samples with adequate volume from cases were consecutively selected from the contributing sites starting with donations made on January 1 2006 until the desired number of cases from a site was reached. Due to large numbers of HCV- and HBV-prevalent cases, site-specific sample numbers were based on proportions established by weighting the number of such donations detected by center in 2004 prior to study initiation. For donors selected for study with reported coinfections, each infecting virus was sequenced.

Viral Nucleic Acid Testing

Qualitative assays for HBV DNA (HBV Ampliscreen; Roche) were performed on HBsAg-positive/anti-HBc–negative donations to exclude possible incident cases that were likely due to false HBsAg positive results [1]. Viral load distributions were determined for all available samples that could not be amplified for sequence analysis, as well as a representative subset of successfully genotyped cases (Abbott RealTime HIV-1, HCV, and HBV Assays, Abbott Laboratories). All tests for this study, except for viral load determinations performed at Abbott Laboratories and routine donor screening and confirmation performed at blood center testing labs, were performed at the Blood Systems Research Institute.

Incidence Testing

Incident infections among HIV RNA-positive donors were defined as samples that were HIV-antibody negative or HIV-antibody positive with a less sensitive (LS) or "detuned" enzyme immunoassay (EIA: Vironostika HIV-1 MicroElisa; bioMérieux) with standardized-optical-density (SOD) ratio <1.0 using the serologic testing algorithm for recent HIV seroconversion, indicating that the infection was probably acquired <6 months prior to blood donation [5–7]. Conversely, HIV antibody–positive donations from first-time donors with LS-EIA SOD ratios ≥ 1.0 were defined as HIV-prevalent infections [8]. HCV RNA-positive and HBsAg-positive donations were classified as incident if anti-HCV and anti-HBc antibody tests, respectively, were nonreactive. HCV RNA and antibody positive donations were considered HCV-prevalent cases. HBsAg-positive/anti-HBc-reactive donations were considered HBV-prevalent infections. Starting with 2008 donations, HBV- and HCV-seropositive samples from repeat donors with a prior negative donation within the prior 2 years were also included as incident cases.

HIV, HCV, and HBV Sequencing

Total nucleic acid was extracted from 140 to 280 μL of plasma with QiaAMP Viral RNA Mini Kit or on a 96-well robotic

platform (QIAxtractor with Reagent Pack VX, Qiagen). Complementary DNA for HIV and HCV was synthesized using M-MLV reverse transcriptase and random primer (0.5 μg/μL) according to the manufacturer's instructions (Promega) and stored at −20°C.

Nested PCR was used to amplify an informative region of each virus. For HIV, a fragment of 1275 base pairs (bp) was amplified, including the protease and reverse transcriptase genes, using previously described PCR primers and conditions [9]. For HCV, a fragment of 363 bp in the core gene was amplified [10]. For HBV, a fragment of 2015 bp, including the envelope and polymerase genes, was amplified (Supplementary Methods in Appendix).

Sequence Analysis

Sequences were edited using Sequencher (version 4.9, Gene Codes Corporation). For HIV, the calibrated population resistance tool [11, 12] available through the Stanford University HIV Drug Resistance Database [13, 14] (http://cpr.stanford.edu/cpr.cgi) was used to determine subtype and identify transmitted drug resistance mutations in untreated persons. Mutations listed as causing or contributing to resistance are nonpolymorphic in untreated persons and apply to all HIV-1 subtypes in accordance with World Health Organization guidelines.

HCV sequences were subtyped using 2 online tools: the Oxford HCV subtyping tool (http://www.bioafrica.net/rega-genotype/html/subtypinghcv.html), a method based on phylogenetic analysis, and the NCBI viral genotyping tool, based on a sliding-window BLAST comparison (http://www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi).

HBV genotypes were determined using 2 online tools: Oxford HBV subtyping tool (http://www.bioafrica.net/rega-genotype/html/citetoolhbv.html), a method based on phylogenetic analysis [15, 16], and the STAR genotyping tool available online at the University College London Center for Infection and Immunity (http://www.vgb.ucl.ac.uk/starn.shtml). The STAR tool uses distances to reference genomes and a statistical model to assign genotypes [17]. The polymerase sequence was checked for drug resistance mutations using the mutation annotator tool available online at the HepSEQ-Research Database System website (http://www.hepseq.org/Public/Web_Front/main.php). To identify potential antibody neutralization escape mutations, a list of mutations from the literature was compiled [18, 19], and sequences were manually aligned and compared with the reference list. GenBank accession numbers are JN214594-JN215208 and JN604118-JN604319.

Statistical Methods

Fisher exact test was used to compare the variant (subtype, genotype, and drug resistance mutation) distribution among incident cases to that among prevalent cases for each virus. Because molecular characterization could not be performed for all submitted cases, logistic regression was used to assess the ability to successfully characterize viral strains as a function of viral load and donor type (ie, incident vs prevalent cases). Analyses were conducted using SAS 9.2 software (SAS Institute).

RESULTS

HIV, HCV, and HBV Infection Rates and Demographic Characteristics

From 1 January 2006 through 31 December 2009, the participating blood organizations screened 33 947 146 allogeneic donations, including 5 968 986 (17.6%) from first-time and 27 950 520 (82.3%) from repeat donors; prior donation status information was not provided for 27 640 (0.1%) donations. For each virus, frequencies were generated by donor status (first-time vs repeat) and donor demographic characteristics (sex, race/ethnicity, geographic region, and age) (Table 1). A disproportionate risk of infection by all 3 viruses was noted for first-time, male, black, and Hispanic donors, except for HBV where infection rates were the highest among Asians, consistent with prior findings (Table 1) [1, 2, 20–22].

HIV Subtypes and Drug Resistance Profiles

A total of 438 donation samples from HIV confirmed-positive donors were selected for sequencing from the 1056 submitted samples (Figure 1*A*). The 200 incident cases selected for viral sequencing included 34 RNA-positive, antibody-negative (NAT yield) donations and 166 of 210 donations classified as recent seroconvertors based on low antibody titers by LS-EIAtesting. A total of 238 of 320 prevalent HIV infections from first-time donors with high-titer antibody reactivity indicating long-standing infections were selected for further testing.

Of the 438 processed samples, 321 (73%) were successfully amplified and sequenced including 159 incident and 162 prevalent cases. Successful PCR amplification correlated with the viral load ($P < .0001$) Incident infections were 2.8-fold (95% confidence interval, 1.6–4.7) more likely to be genotyped than prevalent infections due to their higher viral loads (Figure 1*B*). Combining incident and prevalent cases, the success of obtaining sequences was approximately 10% if viral load was <1000 copies/mL, approximately 50% at 1000–10 000 copies/mL, and >90% if >10 000 copies/mL.

Of sequenced HIV strains, 97.5% (313) belonged to subtype B and 2.5% (n = 7) belonged to non-B subtypes: 4 subtype C (3 prevalent and 1 incident), 2 recombinant subtype CRF-02 (both incident) and 1 subtype D (incident) (Figure 1*C*). The number of non-B subtypes was too low to evaluate differences in frequency between incident and prevalent cases.

Four strains, all from incident cases, contained only protease inhibitor resistance mutations, and 33 strains (21 incident and 12 prevalent cases) contained only reverse transcriptase (RT) inhibitor resistance mutations (including 18 strains with only

Table 1. Numbers and Rates of Total Allogeneic Donations From Human Immunodeficiency Virus, Hepatitis C Virus, and Hepatitis B Virus Confirmed-Positive Donors by Donor Status (First-Time, Repeat) and Donor Demographic Characteristics Collected From 2 January 2006 Through 31 December 2009

Characteristics	Total Allogeneic Donations	All HIV Positive	Rate per 100 000 Donations	All HCV Positive	Rate per 100 000 Donations	All HBV Positive	Rate per 100 000 Donations
Total	33 947 146	1056	3.1	8015	23.6	3061	9.0
Donor status[a]							
First-time donors	5 968 986	633	10.6	6741	112.9	2561	42.9
Repeat donors	27 950 520	423	1.5	1274	4.6	500	1.8
Sex[a]							
Female	15 850 421	251	1.6	3047	19.2	1063	6.7
Male	18 054 540	805	4.5	4968	27.5	1995	11.0
Race or ethnicity[a]							
White	27 112 643	331	1.2	4399	16.2	651	2.4
Asian	510 633	24	4.7	81	15.9	848	166.1
Black	1 242 416	366	29.5	916	73.7	543	43.7
Hispanic	1 354 391	81	6.0	668	49.3	166	12.3
Other	628 302	37	5.9	211	33.6	189	30.1
Not available	3 071 121	217	7.1	1740	56.7	664	21.6
CDC regions[a]							
Midwest	10 523 749	172	1.6	900	8.6	292	2.8
Northeast	8 226 559	210	2.6	1957	23.8	913	11.1
South	8 911 822	474	5.3	3327	37.3	933	10.5
West	5 901 802	149	2.5	1760	29.8	900	15.2
Other	353 635	50	14.1	66	18.7	19	5.4
Age categories, years[a]							
<20	4 782 307	196	4.1	422	8.8	653	13.7
20–29	4 547 134	309	6.8	779	17.1	655	14.4
30–39	4 367 417	224	5.1	1023	23.4	576	13.2
40–49	7 080 519	201	2.8	2486	35.1	521	7.4
50–59	7 730 474	96	1.2	2696	34.9	428	5.5
60–69	3 927 177	26	0.7	478	12.2	163	4.2
≥70	1 482 979	4	0.3	131	8.8	65	4.4

Abbreviations: CDC, Centers for Disease Control and Prevention; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus.

[a] Donations that did not have information for donor status, sex, race/ethnicity, CDC region, or age were excluded.

non-nucleoside reverse transcriptase inhibitor resistance mutation K103N). Two prevalent infections contained both protease and RT inhibitor resistance mutations (pro-M46I,I84V,L90M and RT-M41L,D67N,Y181C,M184V,G190A,L210W,T215Y; and pro-M46I,L90M and RT-M41L,Y181C,T215D). Overall, 39 of 321 (12%) sequenced HIV strains showed the presence of a drug resistance mutation, including 6 (2%) directed to protease inhibitors and 35 (11%) to RT inhibitors; these were from 25 of 159 (15.7%) incident and 14 of 162 (8.6%) prevalent cases ($P = .06$).

HCV Subtypes

A total of 320 donation samples were selected for sequencing from 8015 HCV confirmed-positive donations (Figure 2*A*), including 112 of 153 HCV antibody-negative incident cases that had plasma aliquots available for testing. In addition, 12 incident cases were included based on antibody seroconversion within the previous 2 years, resulting in a total of 124 incident HCV cases. Of the 5446 HCV RNA and antibody-positive donations from first-time donors with prevalent infections, 196 representative samples were selected for molecular testing.

Of the 320 samples processed for PCR amplification, 278 (87%) were successfully amplified and sequenced including 85 of 112 (68.5%) incident and 193 of 196 (99%) prevalent cases. Two of the successfully amplified HCV prevalent cases were coinfections; one donor also had an incident HIV infection and the second had a prevalent HBV infection. The probability of successful PCR amplification and sequencing was associated with viral load in the donors' plasma for both incident and prevalent cases ($P < .0001$) but not with whether the donor was classified as a prevalent or incident case (Figure 2*B*). All samples with viral loads <100 copies/mL were negative for HCV core amplicons, approximately 50% of samples with viral loads of

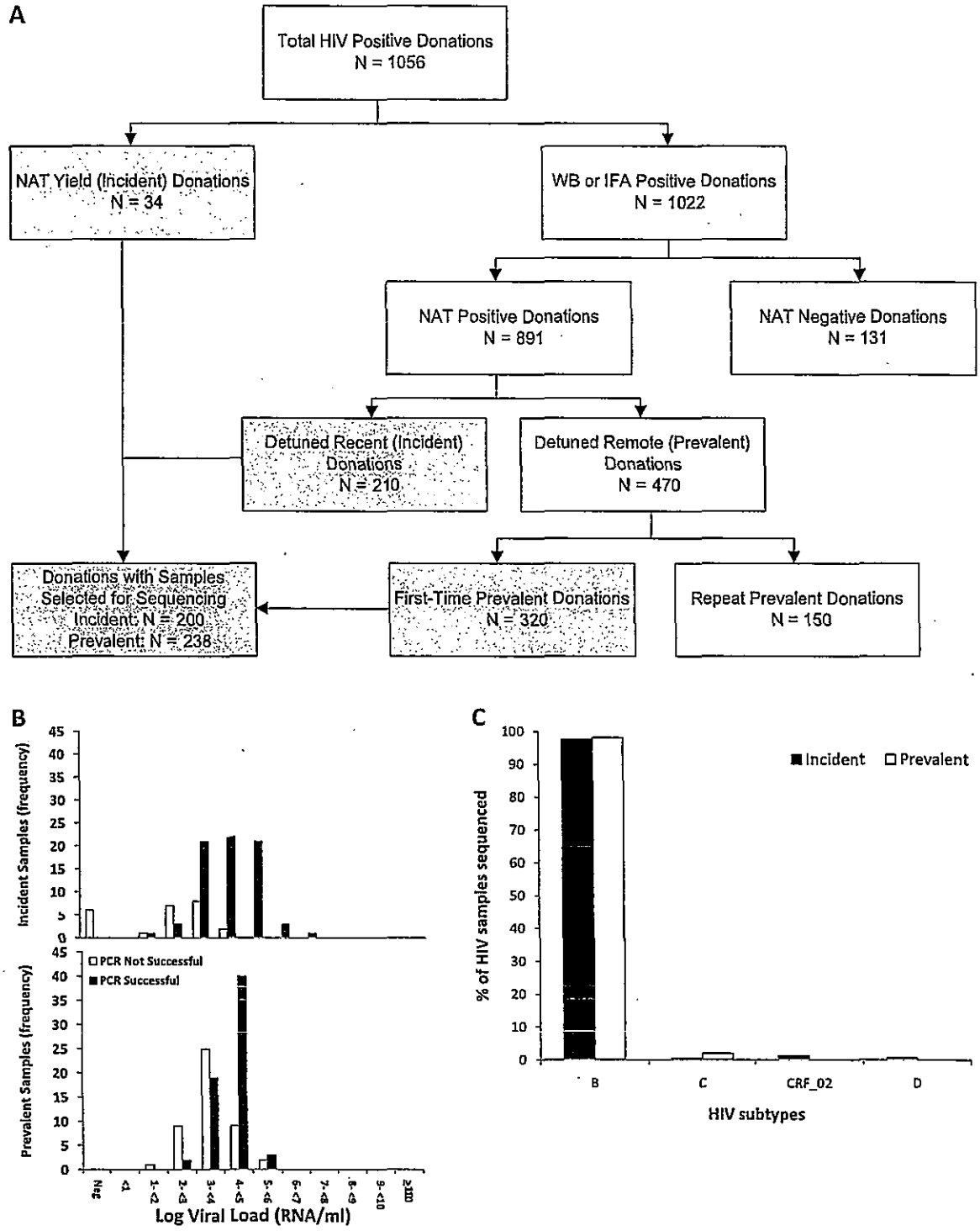

Figure 1. *A,* Algorithm for classification of human immunodeficiency virus (HIV)–positive donations as incident or prevalent infections for selection of cases for sequencing. Boxes in gray indicate the donations from which samples were selected for sequencing for this study. *B,* Histograms showing success of polymerase chain reaction (PCR) relative to viral loads; frequencies of donations from incident and prevalent cases are shown separately. *C,* HIV subtypes of sequenced samples by incident and prevalent case status. Abbreviations: IFA, immunofluorescence assay; NAT, nucleic acid testing; WB, Western blot.

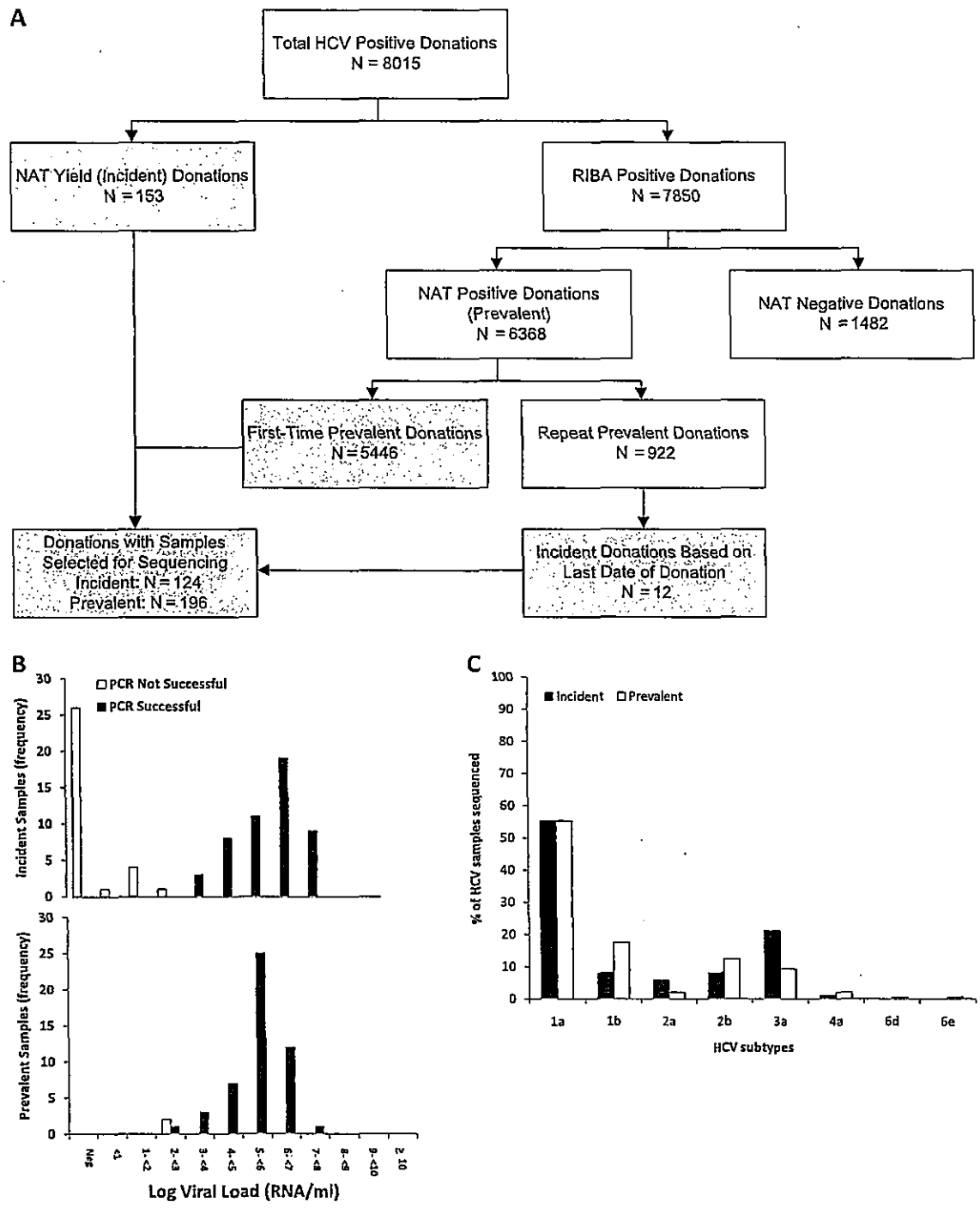

Figure 2. *A*, Algorithm for classification of hepatitis C virus (HCV)–positive donations as incident or prevalent infections for selection of cases for sequencing. Boxes in gray indicate the donations from which samples were selected for sequencing for this study. *B*, Histograms showing success of polymerase chain reaction (PCR) relative to viral loads; frequencies of donations from incident and prevalent cases are shown separately. *C*, HCV subtypes of sequenced samples by incident and prevalent case status. Abbreviations: NAT, nucleic acid testing; RIBA, recombinant immunoblot assay.

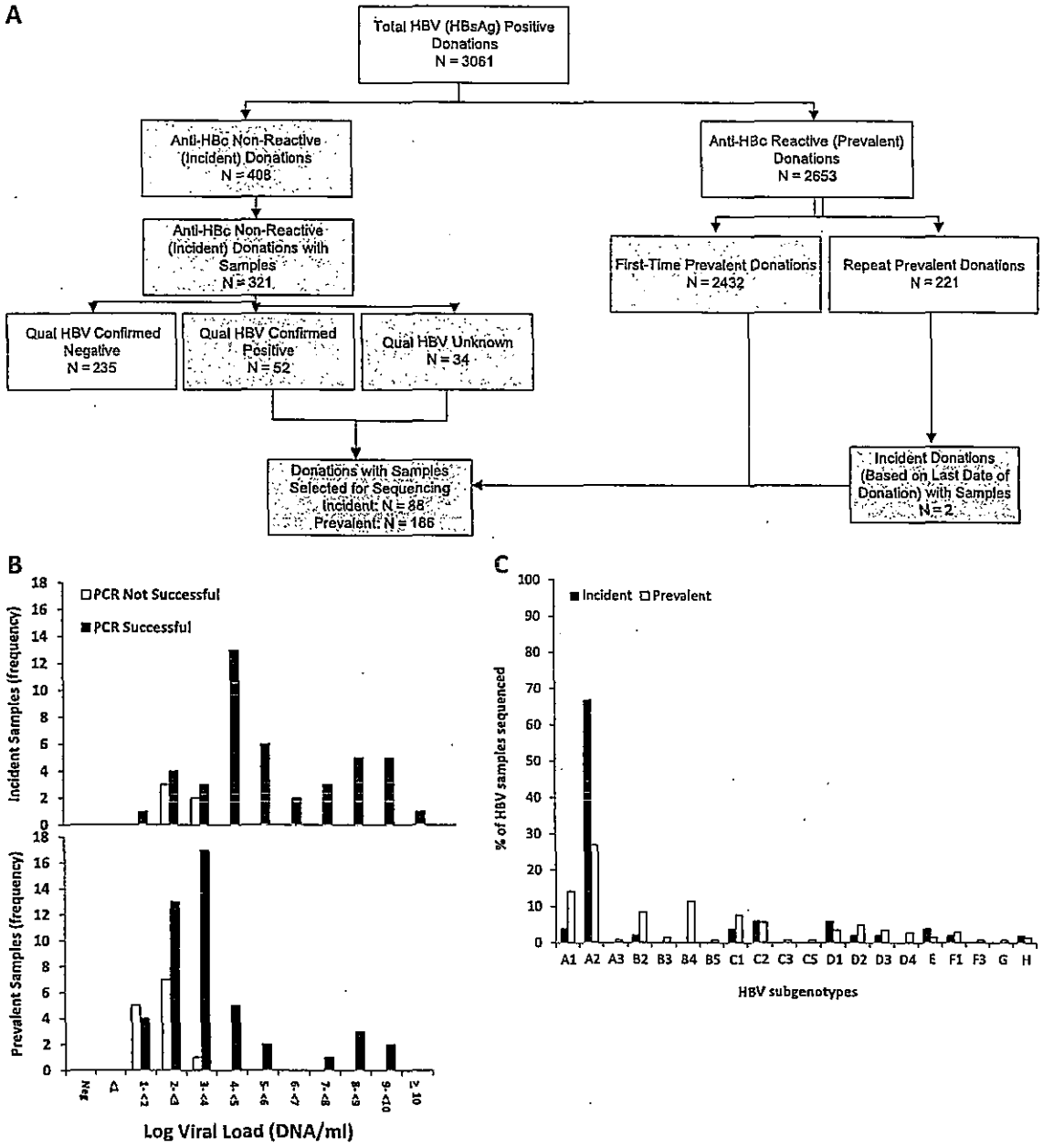

Figure 3. *A*, Algorithm for classification of hepatitis B virus (HBV)–positive donations as incident or prevalent infections for selection of cases for sequencing. Boxes in gray indicate the donations from which samples were selected for sequencing for this study. *B*, Histograms showing success of polymerase chain reaction (PCR) relative to viral loads; frequencies of donations from incident and prevalent cases are shown separately. *C*, HBV subgenotypes of sequenced samples by incident and prevalent case status. Abbreviations: HBc, anti-HBV core antibody; HBsAg, HBV surface antigen.

100–1000 copies/mL were successfully amplified and sequenced, and all samples with viral loads >1000 copies/mL yielded sequence data.

Eight subtypes of HCV were present with 1a being the dominant type in both incident and prevalent cases. The subtype distribution was 55% (n = 154) 1a, 15% (n = 41) 1b, 13% (n = 36) 3a, 11% (n = 31) 2b, 3% (n = 9) 2a, 2% (n = 5) 4a, and <1% 6d or 6e (n = 1 each) (Figure 2*C*). Incident donors had significantly more subtype 3a strains (21% vs 9%) but significantly fewer subtype 1b strains (8% vs 18%) than did prevalent donors ($P = .04$).

HBV Genotype, Drug Resistance, and Immune Escape Profiles

Of 3061 confirmed HBsAg-positive donations, 321 were anti-HBc-nonreactive and available (Figure 3A). However, only 52 of these possible incident infections were HBV DNA

positive, whereas 235 tested HBV DNA negative, indicating either false-positive HBsAg neutralization results or recent receipt of the HBV vaccine with detection vaccine-derived HBsAg [1]. The 52 DNA-positive donations, plus the remaining 34 that were not tested for HBV DNA, along with 2 additional cases where HBV seroconversion occurred within 2 years of the index donation, comprised the 88 incident cases. Of the 2432 HBsAg confirmed-positive, anti-HBc-reactive donations from first-time donors, 186 representative samples were selected as prevalent infections for molecular testing.

Of the 274 HBsAg-positive plasma samples selected, 193 (70%) had successful amplification and sequencing of the envelope and polymerase regions. As with HIV and HCV, the probability of successful PCR amplification and sequencing correlated with viral load ($P < .0001$) but not with incident/prevalent status. Approximately 50% of samples that had <100 copies/mL were negative for HBV PCR amplicons; in contrast, 70% of samples with 100–1000 copies/mL and 98% of those with >1000 copies/mL yielded informative sequence data.

The 193 donor strains successfully sequenced included 51 (26%) antibody-negative incident cases and 142 seropositive prevalent cases (Figure 3*B*). A total of 20 HBV subgenotypes were identified consisting of 72 A2, 22 A1, 16 B4, 13 B2, 13 C1, 11 C2, 8 D1, 8 D2, 6 D3, 5 F1, 4 D4, 4 F1, 3 H, 2 B3, and 1 each of the following subgenotypes: A3, B5, C3, C5, F3, and G (Figure 3*C*). Incident donors had significantly higher frequencies of subgenotype A2 (67%) vs those in prevalent donors (27%) who showed higher frequencies of subgenotypes A1, B2, C1, and D2 (8%–14% vs 0%–4%; $P = .007$).

Sequence analysis of the polymerase region of the 193 HBV strains did not detect any drug resistance–associated mutations. In the envelope sequences, 34 strains showed antibody neutralization escape–associated mutations, including 31 of 142 (22%) prevalent cases and a significantly lower proportion (3 of 52 [6%]) of incident cases ($P = .01$).

DISCUSSION

HIV subtype distribution varies in the United States depending on the population screened. Generally, the frequency of non-B subtypes has remained low in high-risk groups such as MSM and IDUs, as well as in non-IDU heterosexuals and blood donors [20–23]. The frequency of non-B subtype infections was greater in populations enriched for immigrants from nonclade B epidemic countries or military personnel who became infected overseas [24–28]. The frequency of non-B subtype in blood donors appears to be increasing at only a modest rate over the last 2 decades. Studies from the 1980s of seropositive donors and recipients of blood products found no nonclade B infections [23], whereas studies of seropositive donors from the 1990s identified approximately 1% nonclade B infections [7]; more recent studies of infected donors identified since 2000 reported rates of nonclade B infection in the 2%–5% range [3, 20, 22], similar to the 2.5% rate documented here.

Because the samples analyzed here were from asymptomatic blood donors who denied knowledge of their HIV infection, their drug resistance mutations are likely attributed to resistant virus acquired from their sources of infection who are presumed to have been on antiviral therapies. The frequency of HIV drug resistance mutations among blood donors trended but was not significantly higher in incident vs prevalent infections ($P = .06$). A stable frequency of drug resistance mutations also applied when resistance to the more recently introduced protease and the longer used RT inhibitors were analyzed separately. The frequency of transmitted drug resistance mutations appears to be stable among blood donors based on comparisons of rates among incident and prevalent infections in this study and in prior studies of HIV in US blood donors [3, 7, 20], an observation in keeping with reported rates of transmitted drug resistance mutations in high-risk untreated groups [29–32].

There are currently 7 HCV genotypes that are further subdivided into 83 subtypes (http://hcv.lanl.gov/content/sequence/HCV/classification/genotable.html) [33] that can vary widely in their geographic distribution (http://hcv.lanl.gov/components/sequence/HCV/geo/geo.comp). In high-risk groups in the United States, subtypes 1a and 1b predominate, whereas in most other countries the majority of HCV infections belong to other subtypes. Because HCV transmissions in the United States occur mainly among young IDUs [34] and reinfections can displace the original resident strain [35, 36], the distribution of HCV genotypes may rapidly change. Eight HCV subtypes were identified here with 1a (55%) and 1b (15%) predominating. The subtype distribution in prevalent cases was nearly identical to that reported for HCV-seropositive samples collected in 1988–1994 from a population reflecting that of the US [37], which supports the validity of our sampling strategy. In this study, we document a higher frequency of subtype 3a (21% vs 9%) and a lower frequency of 1b (8% vs 18%) in incident vs prevalent donors in keeping with a recent analysis showing decreasing genotype 1 frequencies in younger vs older IDUs [38].

HBV genotypes also vary greatly in their geographic distribution. Currently there are 8 genotypes that can be further subdivided into at least 24 subgenotypes defined as having >4% nucleotide difference [39]. Twenty of these 24 subgenotypes were identified among the 193 sequenced HBV strains. When the frequencies of the subgenotypes were compared, A2 occurred more frequently in incident cases (67% vs 27%) while A1, B2, and B4 frequencies were higher in prevalent cases (8%–14% vs 0%–4%).

No HBV antiviral drug resistance mutations were observed. Drug-resistant HBV variants may be inefficient at transmission and/or establishment of a chronic infection

or may be underrepresented in the pool of HBV being actively transmitted by sexual or parenteral routes. Neutralization escape mutations in the HBV envelope protein were heavily overrepresented in prevalent vs incident HBV infections (22% vs 6%). This observation is consistent with these mutations having been more strongly selected for in long-term infected donors in whom a strong antibody response develops than in very recent, anti-HBc–negative incident cases [40–43].

This study of viral diversity has several limitations. First, the analysis was restricted to infections detected as NAT or HBsAg positive by current blood supply screening assays, most of which were also confirmed antibody positive. Consequently, infections by highly divergent variants that would not be detected by these assays would not be identified. Given efforts of test manufacturers and regulators to ensure that blood donor screening and confirmatory tests are sensitive to viral variants, we believe that this issue has limited impact on our findings. Second, a moderate proportion of donations selected for molecular analysis were not able to be characterized due to failure of long-amplicon PCR. These results were largely explained by absence of detectable nucleic acid or lower viral load in the PCR-refractory samples. It is also well recognized that all donor screening assays have low but significant rates of false positivity, especially if the classification is made only upon the routine testing results and does not include further testing of an independent sample such as the retrieved frozen plasma unit or follow-up donor sample. This is a particular problem with possible NAT yield samples (ie, seronegative and reactive by a single NAT assay), as evidenced by the high rate of incident cases with negative PCR results in this study (Figure 2B), many of which are likely due to false- NAT results. Third, we performed bulk sequencing of PCR products and therefore may not have detected cases of dual infection or minor populations of drug resistance or immune escape variants represented in viral quasi-species.

Overall, our analysis indicates that the HIV epidemic is relatively stable in terms of subtypes and transmitted drug resistance mutations. The HCV data provide evidence of differences in subtypes between incident and prevalent cases that may be stochastically driven by random founder effects and/or result from immigration of infected individuals to the United States. The HBV subgenotypes also showed evidence of change, possibly driven by similar epidemiological factors. The relative frequencies of different viral genetic clades and resistance patterns observed in our study population showed general concordance with those in populations with admitted high-risk behavior [44, 45]. Molecular characterization of recently transmitted blood-borne viruses detected through the large-scale routine NAT and antibody screening of blood donors is therefore a good complement to studies in highly exposed populations. As predominant viral strains change over time, sequence data generated by such blood donor molecular surveillance studies may be of use to adjust primers used in nucleic acid detection methods [46, 47], as well as the specificities of antibodies and antigens used in serologic assays [48–50] in order to maintain the high sensitivity of blood donation screening assays.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://www.oxfordjournals.org/our_journals/jid/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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

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

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一般的名称	①② 乾燥抗 HBs 人免疫グロブリン ③ ポリエチレングリコール処理抗 HBs 人免疫グロブリン		研究報告の公表状況	TRANSFUSION 2012 ; Article first published online: 17 FEB	公表国 アメリカ	
販売名 （企業名）	① ヘブスブリン筋注用 200 単位　（ベネシス） ② ヘブスブリン筋注用 1000 単位　（ベネシス） ③ ヘブスブリン IH 静注 1000 単位　（ベネシス）					

研究報告の概要

パルボウイルス B19(B19V)は、ヒト血漿の一般的な感染性物質である。3 つの B19V 遺伝子型は、これらの DNA 配列に基づいて定義されている。予期外の配列変動性のため、遺伝子型 3 DNA の信頼性の高い検出には問題があることが判明した。
B19V 遺伝子型 3 は主に西アフリカで見つかるが、最近では北米の献血者からの血漿中に検出された。血漿由来医薬品の安全性は、B19V に関しては高力価献血の排除と特定の製造工程でのウイルスクリアランスとの組み合わせに依存している。B19V 不活化の研究は実施が難しく、遺伝子型 3 の不活化はまだ研究されていない。ヒト血清アルブミンの低温滅菌と低 pH でのインキュベーションによる B19V 遺伝子型 1 と 3 の不活化について、感染性ウイルス粒子を細胞培養法を用いて研究した。
低温滅菌と低 pH 処理は、B19V 遺伝子型 1 と 3 の不活化において同等の効果があった。中和実験では、北米や欧州起源のプールした免疫グロブリンは、両方の遺伝子に起因する疾患の治療に等しく効果的でありそうなことが示された。

使用上の注意記載状況・その他参考事項等

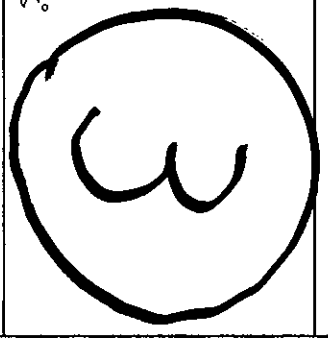
代表としてヘブスブリン IH 静注 1000 単位の記載を示す。
1. 慎重投与
(3)溶血性・失血性貧血の患者〔ヒトパルボウイルス B19 の感染を起こす可能性を否定できない。感染した場合には、発熱と急激な貧血を伴う重篤な全身症状を起こすことがある。〕
(4)免疫不全患者・免疫抑制状態の患者〔ヒトパルボウイルス B19 の感染を起こす可能性を否定できない。感染した場合には、持続性の貧血を起こすことがある。〕
2. 重要な基本的注意
(1)略
1)血漿分画製剤の現在の製造工程では、ヒトパルボウイルス B19 等のウイルスを完全に不活化・除去することが困難であるため、本剤の投与によりその感染の可能性を否定できないので、投与後の経過を十分に観察すること。
6. 妊婦、産婦、授乳婦等への投与
妊婦又は妊娠している可能性のある婦人には、治療上の有益性が危険性を上回ると判断される場合にのみ投与すること。〔妊娠中の投与に関する安全性は確立していない。本剤の投与によりヒトパルボウイルス B19 の感染の可能性を否定できない。感染した場合には胎児への障害（流産、胎児水腫、胎児死亡）が起こる可能性がある。〕

報告企業の意見

ヒトパルボウイルス B19（human parvovirus B19：B19）は、脂質エンベロープを持たない極めて小さな（約 20～26nm）DNA ウイルスで、輸血や血漿分画製剤による伝播が報告されている。他のウイルスに比べて、血漿分画製剤の製造工程での不活化・除去が困難であり、本ウイルスの伝播リスクを完全に否定することはできないため、1996 年 11 月より、使用上の注意に B19 についての記載を行い注意喚起を図ってきた。万一、原料血漿に B19 が混入したとしても、CPV をモデルウイルスとしたウイルスクリアランス試験成績及び B19 を用いた不活化・除去試験の結果から、本剤の製造工程において不活化・除去されると考えている。なお、原料への本ウイルス混入量低減のため、B19 ミニプール NAT が米国の原料供給元で行われている。

今後の対応

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。



ORIGINAL ARTICLE

Inactivation and neutralization of parvovirus B19 Genotype 3

Johannes Blümel, Lori A. Rinckel, Douglas C. Lee, Nathan J. Roth, and Sally A. Baylis

BACKGROUND: Parvovirus B19 (B19V) is a common contaminant of human plasma donations. Three B19V genotypes have been defined based on their DNA sequence. Reliable detection of Genotype 3 DNA has proved problematic because of unexpected sequence variability. B19V Genotype 3 is found primarily in West Africa, but was recently detected in plasma from a North American donor. The safety of plasma-derived medicinal products, with respect to B19V, relies on exclusion of high-titer donations, combined with virus clearance at specific manufacturing steps. Studies on inactivation of B19V are difficult to perform and inactivation of Genotype 3 has not yet been investigated.

STUDY DESIGN AND METHODS: Inactivation of B19V Genotypes 3 and 1 by pasteurization of human serum albumin and incubation at low pH was studied using a cell culture assay for infectious virus particles. Infected cells were detected by reverse transcription–polymerase chain reaction analysis of virus capsid mRNA. Neutralization of B19V Genotype 3 was investigated using human immunoglobulin preparations.

RESULTS: Genotypes 1 and 3 displayed comparable inactivation kinetics during pasteurization of albumin at 56°C, as well as by incubation at various low-pH conditions (pH 4.2 at 37°C and pH 4.5 at 23°C, respectively) used in immunoglobulin manufacturing. Both Genotypes were readily neutralized by pooled immunoglobulin preparations of North American or European origin.

CONCLUSION: Pasteurization and low-pH treatment were equally effective in inactivating B19V Genotypes 1 and 3. Neutralization experiments indicated that pooled immunoglobulin of North American or European origin is likely to be equally effective in treatment of disease induced by both genotypes.

Parvovirus B19 (B19V) is a frequent contaminant of human blood and plasma donations and has been transmitted by plasma-derived medicinal products.[1,2] In healthy immunocompetent individuals, B19V infections are mostly asymptomatic or cause erythema infectiosum. Infection occasionally leads to arthralgia or arthritis. B19V replication is strictly dependent on dividing erythroid progenitor cells. Therefore, B19V infection of individuals with underlying hematologic disorders may show transient aplastic crisis. Immunocompromised patients may be infected persistently with the manifestation of pure red blood cell aplasia and chronic anemia. Pregnant women are at special risk, since B19V infection may lead to hydrops fetalis or fetal death.

Three genotypes of B19V have been defined based on isolates having greater than 10% nucleotide divergence.[3] Genotype 1 is the most prevalent type currently circulating worldwide and is represented by the prototype strain Au (GenBank Accession Number M13178).[4] It has been estimated that Genotype 2 viruses circulated widely in Europe half a century ago, before they were largely replaced by Genotype 1.[5] Although rare, Genotype 2 B19V is found sporadically in plasma donations from both Europe and North America.[6-8] Genotype 3 has been found predominantly in West Africa (Ghana) and less frequently in France or other regions.[3,9] The high diversity of Genotype 3 viruses could be indicative of a longer evolutionary history, probably in Africa. Recent studies found Genotype 3 in samples from Europe and Asia or Brazil and indicate that Genotype 3 may be more widely distributed outside

ABBREVIATIONS: B19V = parvovirus B19; $CCID_{50}$ = 50% cell culture infectious dose.

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TRANSFUSION **;**:**-**.

Africa than previously believed.[10,11] Plasma donations containing high titers of a Subtype 3a B19V were recently identified from a single donor in the United States.[12] The pathogenic properties of B19V appear to be the same, regardless of the genotype.[3] However, reliable detection of Genotype 3 B19V by nucleic acid amplification techniques (NAT) using commercially available assays has proved difficult due to the sequence variability of this genotype.[13] The risk of plasma derivatives with respect to B19V is currently minimized by a combination of donor selection, NAT screening for highly viremic plasma donations, and manufacturing steps which result in B19V reduction such as virus filtration, heat treatments, or incubation at low pH—the latter employed during the manufacture of some immunoglobulin products.[14-21] Effective inactivation of B19 Genotype 1 by pasteurization of albumin has been demonstrated.[15] The B19V capsid is destroyed by heating or low-pH incubation, whereas the B19V DNA remains undamaged and is expelled from opened capsids.[15,21,22]

Recently, differential inactivation kinetics during pasteurization of human serum albumin (HSA) has been reported using different laboratory variants of hepatitis A virus (HAV).[23] Regarding the sequence variability of the B19V virus capsids, we wondered whether the phenotypic susceptibility of B19V Genotype 3 to virus inactivation was different from that of Genotype 1. In addition, we addressed the question whether the variability of Genotype 3 could result in escape from neutralization by immunoglobulin preparations from pooled plasma sourced from North America or Europe where this genotype is only very rarely detected. Using a cell culture system for detection of infectious B19V, we investigated the inactivation kinetics of B19V Genotypes 1 and 3, by pasteurization of albumin and by different low-pH treatments. Neutralization of B19V infectivity was tested using immunoglobulin preparations, manufactured by identical procedures, but prepared from plasma sourced from European or North American donors.

MATERIALS AND METHODS

Viruses and cells

Highly viremic plasma donations were used as sources of infectious B19V. A German blood donation, provided by W.K. Roth (German Red Cross, Hessen), was the source of the Genotype 1 B19V strain, termed DRK1, and has a DNA concentration of 11.6 log IU/mL. The nucleotide sequence of the DRK1 capsid-encoding region has been determined (GenBank Accession Number HQ664953). The Genotype 3 B19V strain, termed P1 (GenBank Accession Number FJ265736) was obtained from an American donor and sequence analysis confirmed that the virus belongs to Subtype 3a.[12] KU812Ep6 cells were provided by E. Miyagawa (Fujirebio, Inc., Tokyo, Japan).[24] The cells were propagated at 37°C, 5% CO_2 in RPMI supplemented with 10% fetal calf serum (Sigma, St Louis, MO) and 5 IU/mL recombinant erythropoietin (NeoRecormon, Roche, Welwyn Garden City, UK).

Pasteurization

Commercial 5% HSA was heated to 56.5°C in a thermoblock (Model 5436, Eppendorf, Hamburg, Germany). The temperature was measured throughout the experiment by monitoring an unspiked albumin sample incubated in parallel using a calibrated thermometer. One milliliter of heated albumin was spiked at a ratio of 1 in 20 with virus-containing plasma; 200-µL samples were withdrawn after 1, 5, 10, 30, and 60 minutes of heat treatment. Samples were immediately placed on ice and titrated on KU812Ep6 cells.

Low-pH treatment

Experiments were performed as described previously.[21] Virus was spiked at a ratio of 1 in 10 into phosphate-buffered saline (PBS). The pH was then adjusted with 1 mol/L HCl to the target pH and the sample incubated at the appropriate temperature for the specified period of time. Samples were withdrawn and immediately neutralized with 1 mol/L NaOH, buffered with phosphate buffer (100 mmol/L, pH 7.4), and subjected to virus titration.

Controls

Cytotoxicity controls

Cells were seeded into 24-well plates. One-hundred–microliter samples of a 10-fold dilution series of albumin were inoculated into each well and, after 7 days, cells were visually examined for cytotoxic effects (alteration of cell shape) using a light microscope.

Interference controls

A 10-fold dilution series of B19V in undiluted test matrix was prepared and 100 µL from each dilution was inoculated into cell cultures and examined for virus. Additionally, a 10-fold dilution series of B19V in 1-in-10–diluted test matrix was prepared and 100 µL from each dilution was inoculated into cell cultures and examined for virus.

Virus control

In each virus experiment, the titer of virus stock was verified by titration on KU812Ep6 cells.

Bench controls

In parallel to each inactivation experiment, the test matrix was spiked at room temperature (heat inactivation) or at neutral pH (low pH treatment). This material was held at

room temperature and titrated at the beginning and the end of the inactivation process.

Virus titration

A 10-fold dilution series was prepared using RPMI as diluent. One-hundred microliters from each dilution was inoculated onto 8×10^5 cells and incubated for 3 to 5 days. Cells were washed with PBS and analyzed for spliced mRNA by reverse transcription–polymerase chain reaction (RT-PCR). For titration using the mRNA assay, the infectious titer was determined by the endpoint dilution method and expressed as 50% cell culture infectious dose ($CCID_{50}$)/mL. Three mRNA assays were performed in parallel for each dilution step and the virus titer was calculated according to the maximum likelihood method. In samples where no virus was detected, the detection limit of the assay was calculated according to the Poisson distribution using the equation

$$c = -\ln p / v,$$

where 1–p is the 95% probability that the aliquot is free of infectious virus (p = 0.05), v is the test volume, and c is the virus concentration.[25] Messenger RNA was extracted using the mRNA capture kit (Roche, Mannheim, Germany). For detection of spliced mRNAs encoding for capsid proteins (VP), mRNA was dissolved in 50 µL of RT-PCR mix containing 600 nmol/L Primer XPP1 (5′-TTTCCTGGACT TTCTTGCTGT, Nucleotides 365-385), 600 nmol/L Primer TP2 (5′-TGGTCTGCCAAAGGTGTGTAG, Nucleotides 2171-2151), 200 nmol/L Probe "B19-Sonde-1" (FAM(6-carboxy-fluorescein)-CCGCGCTCTAGTACGCCCATCCT-TAMRA (6-carboxy-tetramethyl-rhodamine), Nucleotides 2050-2072), 1 mmol/L dNTPs, 2 µL of PCR enzymes, and 1× reaction buffer from a one-step RT-PCR kit (Qiagen, Hilden, Germany). For detection of spliced Genotype 3 mRNA, Primer XPP1 was replaced by Primer XPP5 (5′-CTTGCTGTTCTTTGCCTGCTA), Primer TP2 was replaced by Primer TP7 (5′-CTTCGGAGGAAACTGGGCTTC, Nucleotides 2123-2102), and Probe "B19 Sonde 2" was FAM(6-carboxy-fluorescein)-AACCCCGCGCTCTAGTAC-TAMRA (Nucleotides 2046 to 2063). All nucleotide position numbers of primers refer to strain Au.[4] RT was carried out for 30 minutes at 50°C and stopped by incubation at 95°C for 15 minutes in a PCR system cycler (GeneAmp 9700, Applied Biosystems, Weiterstadt, Germany). Thereafter, five PCR cycles (15 sec at 94°C, 15 sec at 60°C, 15 sec at 72°C) were performed followed by incubation for 10 minutes at 72°C. The reaction mixture was transferred to a sequence detection system (ABI Prism 7700, Applied Biosystems). After 2 minutes at 50°C and 10 minutes at 95°C, 45 cycles (15 sec at 95°C and 30 sec at 60°C) were performed. In each run, the infectivity (mRNA) from a sample of known titer was determined by the endpoint dilution method (two wells per dilution) and calculated as 50% mRNA-inducing dose ($mRNA_{50}$)/mL using the maximum likelihood method resulting in an absolute quantification of the $mRNA_{50}$/mL titer with a variation of ±0.8 log. Furthermore, this sample was used to generate a standard curve (two to three wells per dilution) for relative quantification of virus mRNA. One well per dilution was analyzed for mRNA and diluted samples with C_T values falling within the linear range of the standard curve were used for calculation of the titer using the sequence detection system software (ABI Prism 7700, Applied Biosystems).

Neutralization assay

Fifty microliters of virus-containing plasma was mixed with 50 µL of EU-licensed commercial normal immunoglobulin G (IgG) preparation from a twofold dilution series in PBS. The 100-µL mixture of virus and serum was incubated 2 hours at 37°C for neutralization. Thereafter, the whole sample was inoculated onto KU812Ep6 cells for titration of infectious virus by mRNA detection as described above.

RESULTS

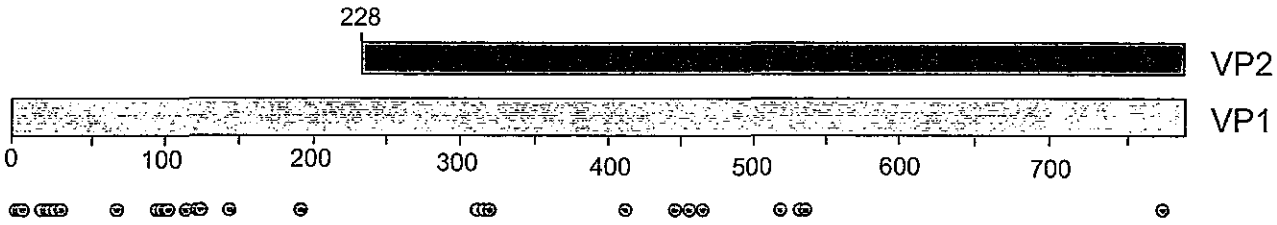
Characterization of virus isolates

Inactivation of B19V occurs by damaging the virus capsid shell. Therefore, we determined the amino acid sequence of the virus capsids. The nucleotide sequence of Genotype 1 strain termed "DRK-1" was determined by direct sequencing of DNA amplified by PCR (GenBank Accession Number HQ664953). The sequence of the Subtype 3a strain P1 (GenBank Accession Number FJ265736) has been described previously.[12] Figure 1 shows the different amino acids located in the virus capsid proteins. The capsid of B19V consists of two proteins VP1 and VP2, respectively, which are translated from overlapping open reading frames. VP2 forms 95% of the virus capsid. VP1 contains an N-terminal 228-amino-acid "unique region." Differences in 29 amino acids were identified between Genotype 1 and Genotype 3. These amino acid positions clustered preferentially (i.e., 18 of the 29 changes) in the VP1 unique region.

The plasma DNA concentrations of both Genotype samples were 11.9 log IU/mL. The infectious titers were 7.9 ± 0.4 and 8.5 ± 0.4 log $CCID_{50}$ for Genotypes 1 and 3, respectively, as determined by quadruplicate assays (SD indicated) using mRNA detection.

Inactivation kinetics during pasteurization

Heat treatment in aqueous solution of albumin and other plasma proteins is performed at 60°C for 10 hours (pasteurization). Immediate inactivation of B19V Genotype 1 has previously been demonstrated under these standard



Amino acid position	4	5	6	12	17	18	21	25	68	93	96	100	101	111	123
Genotype 1	K	S	G	D	K	A	Q	E	N	A	S	H	A	S	V
Genotype 3	T	T	D	S	Q	V	K	Q	S	S	P	S	T	Y	I

Amino acid position	125	144	192	308	313	319	418	445	454	463	521	531	533	772
Genotype 1	L	S	V	V	S	S	E	F	T	P	V	S	S	G
Genotype 3	Y	N	A	T	N	T	V	S	S	A	I	N	N	W

Fig. 1. Differences in amino acid composition of B19V capsid proteins. Differing amino acids of the virus capsid proteins of Genotype 1 (strain DRK1) or Genotype 3 (strain P1) are presented. The location of variant amino acid positions (indicated as dots) with respect to the VP1 and VP2 open reading frames (indicated as bars) is shown.

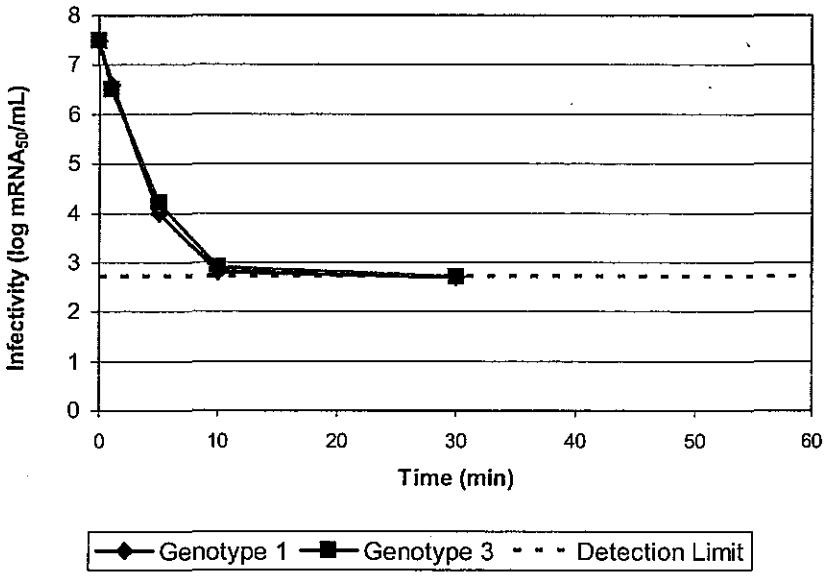


Fig. 2. Inactivation of B19V Genotypes 1 and 3 by heat treatment. Human albumin 5% was heated to 56.5°C and spiked 1 in 20 with B19V Genotype 1 or 3, respectively. Incubation at 56.5°C was continued and infectious titer was determined at various time points by titration of B19V on KU812Ep6 cells and analysis of B19V-specific mRNA. The starting point of inactivation kinetics (0 min) represents the infectious titer from a sample spiked at room temperature.

pasteurization conditions.[15] Inactivation studies of Genotypes 1 and 3, described herein, were carried out at lower temperature (56.5°C). Reducing the temperature of the heat treatment step slowed the rates of virus inactivation sufficiently to enable a more accurate and precise comparison of the inactivation kinetics of the different virus genotypes. As shown in Fig. 2, both genotypes were inactivated below the detection limit in less than 30 minutes. There was no significant difference in inactivation kinetics of the two virus genotypes.

Inactivation kinetics at low-pH treatment

During the manufacture of several immunoglobulin products, an enzymatic treatment may be performed at pH 4 and 37°C. Manufacture of other products includes incubation of the final product at pH 4 and room temperature for several weeks. Both procedures have been shown to be effective for inactivation of enveloped viruses and specific nonenveloped viruses. Inactivation of B19V at pH 4, 37°C was previously demonstrated.[21] In the studies described herein, we investigated inactivation kinetics of B19V Genotypes 1 and 3 under two different conditions (pH 4 at 37°C and pH 4.5 at 23°C). Ethanol (2% final concentration) was added to the samples incubated at pH 4 at 37°C to mimic typical conditions of enzymatic low-pH steps

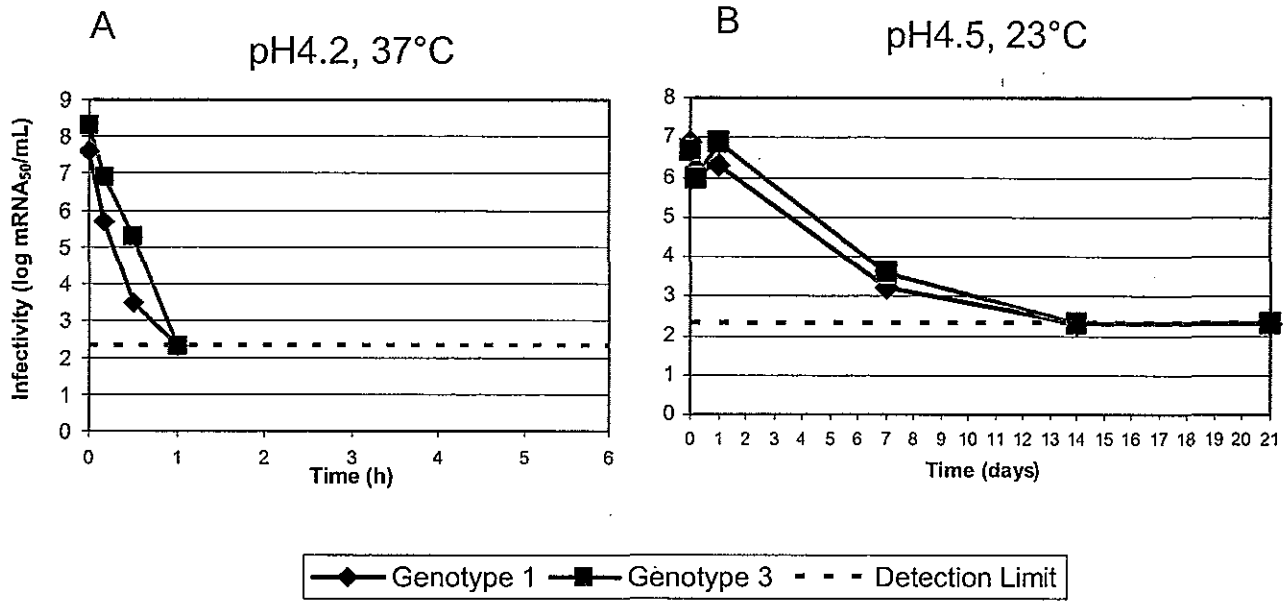


Fig. 3. Inactivation of B19V Genotypes 1 and 3 by low-pH treatment. B19V Genotypes 1 or 3 were spiked into test matrices (5% albumin, 2% ethanol, pH 4.2 [A] or PBS pH 4.5 [B]) and incubation was continued for 6 hours at 37°C (A) or 21 days at 23°C (B), respectively. Infectious titer was determined at several time points by titration on KU812Ep6 cells and analysis of B19V-specific mRNA. The starting point of inactivation kinetics represents the infectious titer tested immediately after spiking the low pH test matrix.

applied during immunoglobulin fractionation. Figures 3A and 3B show that both conditions for low-pH treatment were effective for inactivation of B19V. There was no significant difference in the inactivation kinetics of either genotype. Control samples, diluted in PBS (pH 7.4) and held at 37°C for a period of 6 hours, showed no loss of B19 infectivity. However, in the case of the samples maintained at 23°C for 3 weeks, it was observed that infectious titers of B19V controls diluted in PBS (pH 7.4) declined during the incubation period (4.2 log/mL for Genotype 1 and 3.0 log/mL Genotype 3, respectively). The pH of the controls remained stable throughout the incubation period.

Neutralization of B19V Genotypes 1 and 3

The amino acid variations of Genotype 3 virus capsids are located mainly in the N-terminal unique region of VP1 (Fig. 1). It has been reported that this more variable region contains antibody-binding epitopes and that the majority of virus-neutralizing antibodies that confer lifelong protection against reinfection with B19V are directed against the VP1-unique region.[26] So far, B19V Genotype 3 has only occasionally been described in regions outside Africa, and it is reasonable to assume that antibodies present in pools of plasma collected in Europe or the United States mainly represent antibodies from Genotype 1 infections.

Consequently, experiments were performed to determine whether B19V Genotype 3 would be neutralized by antibody preparations produced from plasma collected in either Europe (i.e., Austria, Germany, and the Czech Republic) or the United States. Two commercial normal immunoglobulin preparations for human use were used. These immunoglobulins were produced by the same manufacturing process including cold ethanol fractionation of plasma sourced either in Europe or in North America. As shown in Fig. 4, there was no difference in the neutralization curves of the two genotypes using the antibody preparations of either European or North American origin. Both genotypes were completely neutralized below the detection limit using immunoglobulin dilutions up to 1 in 80. Only marginally better neutralization of Genotype 1 was observed, that is, at a dilution point of 1 in 160.

DISCUSSION

The risk for B19V transmission via plasma-derived medicinal products is currently minimized by a combination of donor selection, plasma screening, and manufacturing steps that facilitate virus clearance. In individual plasma donations, virus titers may be extremely high (more than 10^{12} IU B19V DNA/mL). These high levels of virus can overload the virus clearance steps in the manufacturing process, resulting in transmission cases with, for example, coagulation factor concentrates.[27] B19V infections are ubiquitous and virus-containing donations, as

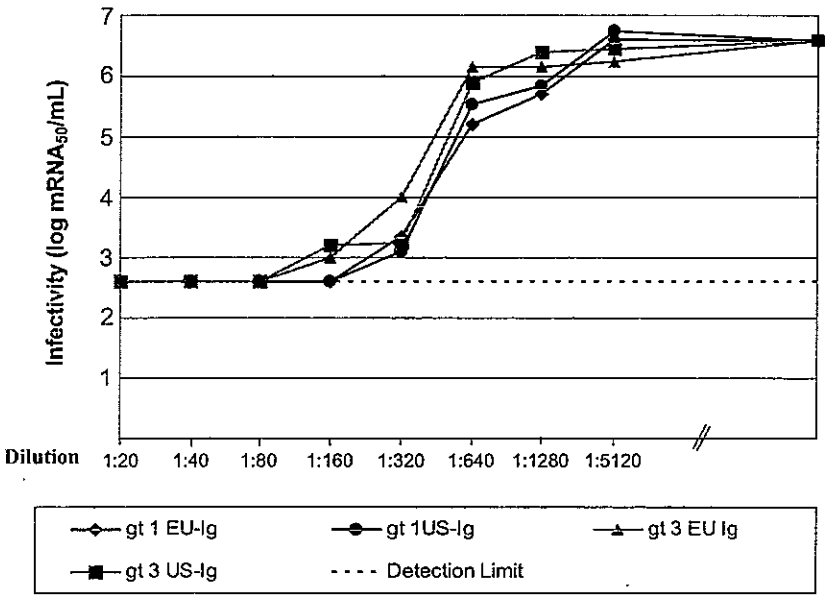


Fig. 4. Neutralization of B19V Genotypes (gt) 1 and 3. Neutralization reactions were set up by mixing 50 µL of dilutions of Genotype 1 or Genotype 3 at a DNA concentration of 9.9 log IU/mL with 50 µL of commercial IgG preparation from European plasma pools (EU-Ig) or American plasma pools (US-Ig): The mixture was incubated at 37°C for 2 hours. Thereafter, the samples were inoculated onto KU812Ep for titration of infectious virus by analysis of B19V-specific mRNA.

analyzed by NAT methods, have been reported at frequencies varying from 1 in 800 to 1 in 8000. It is currently not practical to exclude all B19V-containing plasma donations. However, it is feasible to identify and exclude highly viremic donations from the plasma manufacturing pools. The Plasma Therapeutic Protein Association introduced in 2002 a voluntary limit of not more than 10^5 genomes/mL in a production pool and FDA recommended a limit of not more than 10^4 IU B19 DNA/mL.[28] In Europe, the 10^4 IU/mL limit is prescribed in the European Pharmacopoeia for solvent/detergent plasma and for anti-D immunoglobulin preparations used prophylactically in pregnant women for the prevention of Rhesus disease. Genotype 3 has been predominantly found in West Africa.[9] Genotypes 2 and 3, however, may be occasionally found in Europe.[3,10,29-31] Highly viremic plasma donations have been identified in Europe and the United States.[6,8,12] Genotype 2 virus sequences have also been identified in coagulation factor concentrates.[32] The variant genotypes may not be reliably detected or correctly quantified by some of the current PCR assays.[7,33]

Manufacturing steps for virus inactivation and removal are important in ensuring the virus safety of plasma-derived medicinal products. The reduction capacity of such steps is validated by laboratory experiments where cell culture–adapted virus strains are spiked into process intermediates and these intermediates are further processed through a scaled-down version of the respective manufacturing process step. There has been always a theoretical concern as to what extent such model virus strains reflect actual virus particles from plasma donations. Recently, Farcet and coworkers[23] reported different inactivation kinetics of variants of cell culture–adapted HAV strains during pasteurization of HSA. These variants were derived from a single field isolate, indicating that mutations being selected during cell culture passage might affect the thermostability of virus particles. In this report, highly viremic plasma donations containing various strains of B19V were used as the source of the spiking agent, thereby excluding the possibility of such mutations. Similar inactivation kinetics of B19V from different plasma donations containing B19V Genotype 1 were demonstrated in earlier studies suggesting that natural variants do not contain mutations affecting thermal stability of virus particles.[15] In addition, inactivation of Genotype 2 strains was also similar to Genotype 1 isolates.[6,20]

Inactivation of virus particles at 60°C or pH 4 occurs by externalization of virus DNA from capsids as the result of a conformational change of capsids leading to externalization from capsids in the N-terminus of the VP1 protein.[22] Although many of the amino acid variations between B19V genotypes cluster in the N-terminal VP1 regions, such variations do not affect stability of virus capsids.[6,22] It has been speculated that the neutralizing immune response is mainly elicited by this region.[34]

Immunoglobulin preparations have been successfully used for treatment of persistent infections with B19V.[35] Considering the very rare detection of Genotypes 2 and 3 in Europe and the United States, it is reasonable to assume that most B19V-specific antibodies in immunoglobulin preparations from pooled plasma have been derived from infections with Genotype 1. Indeed, NAT analysis has shown that plasma fractionation pools, prepared from European and North American plasma, contain predominantly Genotype 1 B19V (L.A. Rinckel and T.M. Gierman, unpublished observations).[36] This has been confirmed by direct sequence analysis of B19V from numerous plasma pools (J. Blümel, unpublished observations). Therefore, we considered whether such preparations would be effective in neutralizing B19V Genotype 3 as well as Genotype 1. The results of these studies clearly show that immunoglobulins prepared from European or North American–sourced plasma were equally effective in

neutralizing Genotype 3. This indicated that such preparations should be effective in treatment of chronic infections caused by B19V Genotype 3.

In summary, the results from this study confirm the reliability of current virus inactivation steps and thus the safety of immunoglobulin preparations against the different B19V genotypes.

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CONFLICT OF INTEREST

DCL and NR are employees of Grifols Therapeutics, Inc., Research Triangle Park, NC. JB, SAB, and LR declare no conflicts of interest.

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一般的名称	①② 乾燥抗 HBs 人免疫グロブリン ③ ポリエチレングリコール処理抗 HBs 人免疫グロブリン	研究報告の公表状況	TRANSFUSION 2012 ; Article first published online : 17 FEB	公表国 オーストリア
販売名 （企業名）	① ヘブスブリン筋注用 200 単位　（ベネシス） ② ヘブスブリン筋注用 1000 単位　（ベネシス） ③ ヘブスブリン IH 静注 1000 単位　（ベネシス）			

研究報告の概要

チクングニアウイルス(CHIKV)流行は、以前アフリカ、インド洋諸島、南アジアと東南アジアの一部に限られていた。しかし、2007年に最初の土着のCHIKV伝播が欧州で報告された。高いレベルのウイルス血症、欧州や米国の大都市地域にも存在する媒介蚊、及び生理化学的な不活化工程に対するこのアルファウイルスの抵抗性についての不確実性は、血漿分画製剤の安全性についての関心を惹起した。
CHIKV に関する血漿製品の安全性の余裕を確認するために、普遍的に用いられるウイルス不活化工程（ヒト血清アルブミン（HSA）のための低温滅菌、活性を無視している第Ⅷ因子抑制剤のための蒸気加熱、静脈免疫グロブリン（IVIG）のための溶媒/界面活性剤（S/D）処理、IVIG のための低 pH インキュベーション）のこの新興ウイルス（CHIKV 及び密接に関連したシンドビスウイルス（SINV））に対する不活化効果を調べた。得られた結果は、西ナイルウイルスとよく使うモデルウイルス：ウシ下痢症ウイルスについて以前の研究と比較された。
生成されたデータは、評価した不活化工程が SINV と同様に CHIKV にも効果的な不活化効果があることを示し、それによってモデルウイルスが使われた初期のバリデーション研究からの結果を裏づけた。このことは血漿製品の安全に揺るがぬ安心を提供し、そしてこの結果は、新興ウイルスの物理化学的特性が良く特徴付けられている場合には、そのウイルスの不活化特性を予測するのにモデルウイルスの使用が適切であることを実証している。

報告企業の意見	今後の対応
チクングニアウイルス（chikungunya virus：CHIKV）は、トガウイルス科アルファウイルス属に分類される直径70nmのエンベロープを有する球状のRNAウイルスで、蚊（ヤブカ属のネッタイシマカやヒトスジシマカ）によって媒介される。万一、原料血漿にチクングニアウイルスが混入したとしても、BVDをモデルウイルスとしたウイルスクリアランス試験成績から、本剤の製造工程において不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

代表としてヘブスブリン IH 静注 1000 単位の記載を示す。
2. 重要な基本的注意
(1)本剤の原材料となる血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体陰性であることを確認している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した高力価の抗HBs抗体を含有する血漿を原料として、Cohnの低温エタノール分画で得た画分からポリエチレングリコール4000処理、DEAEセファデックス処理等により抗HBs人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及びウイルス除去膜によるろ過処理を施しているが、投与に際しては、次の点に十分注意すること。

ORIGINAL ARTICLE

Chikungunya virus and the safety of plasma products

Sandra M. Leydold, Maria R. Farcet,* Johanna Kindermann, Jens Modrof, Gerhard Pölsler, Andreas Berting, M. Keith Howard, P. Noel Barrett, and Thomas R. Kreil*

BACKGROUND: Chikungunya virus (CHIKV) outbreaks were previously restricted to parts of Africa, Indian Ocean Islands, South Asia, and Southeast Asia. In 2007, however, the first autochthonous CHIKV transmission was reported in Europe. High-level viremia, a mosquito vector that is also present in large urban areas of Europe and America, and uncertainty around the resistance of this Alphavirus toward physiochemical inactivation processes raised concerns about the safety of plasma derivatives. To verify the safety margins of plasma products with respect to CHIKV, commonly used virus inactivation steps were investigated for their effectiveness to inactivate this newly emerging virus.
STUDY DESIGN AND METHODS: Pasteurization for human serum albumin (HSA), vapor heating for Factor VIII inhibitor bypassing activity, solvent/detergent (S/D) treatment for intravenous immunoglobulin (IVIG), and incubation at low pH for IVIG were investigated for their capacity to inactivate CHIKV and the closely related Sindbis virus (SINV). The obtained results were compared to previous studies with West Nile virus and the commonly used model virus bovine viral diarrhea virus.
RESULTS: The data generated demonstrate the effective inactivation of CHIKV as well as SINV by the inactivation steps investigated and thereby support results from earlier validation studies in which model viruses were used.
CONCLUSION: High inactivation capacities with respect to CHIKV were demonstrated. This provides solid reassurance for the safety of plasma products and the results verify that the use of model viruses is appropriate to predict the inactivation characteristics of newly emerging viruses when their physicochemical properties are well characterized.

Chikungunya virus (CHIKV) was first isolated during an outbreak in Tanzania in the 1950s and has subsequently caused frequent outbreaks in Africa and in Asia.[1] In 2004, CHIKV spread from Kenya to the Western Indian Ocean Islands including the Comoros Islands, La Réunion, Mayotte, Mauritius, the Seychelles, and Madagascar and from there CHIKV emerged further in India and the Eastern Indian Ocean Islands, with millions of people infected.[2] Whereas the previous vector of CHIKV was *Aedes aegypti*, the primary vector in La Réunion and Mauritius was *Aedes albopictus*, the Asian tiger mosquito.[3] The adaptation of CHIKV to *A. albopictus* was due to a single amino acid substitution, which significantly increased CHIKV infectivity for this species,[4] that is widely endemic in urban areas of Europe and America.[5,6] Since 2006, CHIKV infections have been identified in an unprecedented number of travelers,[2] some of them returning with high-grade viremia to countries where competent vectors are present, which raises serious concerns with respect to a potentially global spread of the disease.[7] The first outbreak of autochthonously transmitted CHIKV in a temperate climate zone occurred in 2007 in the province of Ravenna

ABBREVIATIONS: BHK = baby hamster kidney; BVDV = bovine viral diarrhea virus; CHIKV = chikungunya virus; FEIBA = Factor VIII inhibitor bypassing activity; LOD = limit of detection; S/D = solvent/detergent; SINV = Sindbis virus; SSM = spiked starting material; $TCID_{50}$ = tissue culture infectious dose 50%; WNV = West Nile virus.

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TRANSFUSION **;**:**-**.

in northeastern Italy,[8] with four smaller clusters of local transmission detected up to 49 km away from the initial introduction by a presumably single individual.[9] Of 337 suspected CHIKV cases, 306 were examined and 217 were laboratory confirmed.[10] Later, autochthonous transmissions have also been reported from southeastern France.[11] As a consequence of these recent outbreaks, CHIKV fever is no longer considered a disease that is restricted to tropical countries, but has developed into a worldwide public health concern.[2]

While cases of CHIKV transmission via blood products or organ transplantation have not been reported so far, such transmission events are possible,[12] as during an epidemic the risk of viremic donations is substantial,[1] with approximately one in four CHIKV infections asymptomatic,[13] and the viral load during the acute phase of infection, which typically lasts for 2 to 4 days, as high as 10^9 copies/mL.[7] These facts have raised sufficient concerns about the safety of plasma products for the US Food and Drug Administration to include the subject in the agenda of a workshop.[14] Dedicated virus inactivation and removal steps are integrated into the manufacturing processes of all fractionated plasma products that are licensed by advanced regulatory authorities to ensure their safety.[15] These process steps have been validated for their effectiveness in inactivating a broad range of physicochemically diverse viruses, including relevant (e.g., human immunodeficiency virus, hepatitis A virus) and so-called model viruses.[16] One of these model viruses, bovine viral diarrhea virus (BVDV) of the *Pestivirus* genus (*Flaviviridae*) is like CHIKV, a lipid-enveloped RNA virus and similar in size. As BVDV as well as the closely related Flavivirus West Nile virus (WNV) have been demonstrated to be effectively inactivated by the virus reduction methods commonly used in the manufacture of plasma products,[17,18] high margins of safety are equally presumed for CHIKV. Experimental evidence for CHIKV itself was, however, not previously available. To provide adequate assurance of virus safety, particularly for the individuals who critically depend on plasma-derived therapies, as well as regulatory authorities who need to guarantee the safety of such products on the market, the present work investigated CHIKV inactivation experimentally. In addition, some of the inactivation methods were evaluated using Sindbis virus (SINV), a virus closely related to CHIKV, and also WNV, with the aim of generating further supportive evidence for the inactivation capacity of these dedicated virus inactivation steps with respect to CHIKV. The results confirmed the CHIKV inactivation behavior expected from earlier model virus data and thereby show that concerns for the safety of plasma products with respect to CHIKV are unwarranted.

MATERIALS AND METHODS

Viruses and cells

CHIKV strain "LR2006-OPY1" was originally isolated in 2006 from a 73-year-old patient returning from La Réunion, a French overseas department,[7] and was obtained from the Centre for Ecology and Hydrology (Oxford, UK). Virus identity was confirmed by partial sequencing and sequence alignment with the whole genome of strain "LR2006-OPY1" (GenBank Accession No. DQ443544), which showed greater than 99% sequence homology. CHIKV working stock was produced on Vero cells adapted to growth in serum-free medium and titrated on Vero cells (ECACC No. 84113001, European Collection of Cell Cultures, Salisbury, UK).

SINV, strain Ar-339 (ATCC VR-68), was propagated on Vero cells (ATCC CCL-81) or on baby hamster kidney (BHK-21 [C13]) cells (Lee Biomolecular, San Diego, CA). $TCID_{50}$ (tissue culture infectious dose 50%) titration of SINV was done on Vero cells, whereas BHK cells were used for plaque assay.

WNV was obtained from the liver of a snowy owl found dead in New York in 1999. The virus was isolated by filtration of the liver homogenate, passaged on Vero cells, and lyophilized ("Isolate 385-99"). The Isolate 385-99 was characterized by sequencing parts of the genome[19] and provided by Dr Robert E. Shope (University of Texas, Galveston, TX). WNV was propagated and titrated on Vero cells (ECACC No. 84113001).

Infectivity assays

Virus-containing samples were titrated on the respective indicator cells by $TCID_{50}$ assays (CHIKV, SINV, WNV) or by plaque assay (SINV) using serial 0.5 or 1 log dilutions of samples. The cytopathic effects induced by the viruses were evaluated after incubation of the cells with the samples for 3 days (WNV, SINV plaque assay), 5 to 6 days (SINV $TCID_{50}$ assay), or 7 days (CHIKV) at 36 ± 2°C. Virus concentrations were calculated according to the Poisson distribution (CHIKV, WNV) or according to Reed and Muench (SINV) and expressed as $\log(TCID_{50})$/mL. Virus concentrations determined with the plaque assay were expressed as plaque-forming units/mL. To lower the limit of detection (LOD) for the samples taken during CHIKV and WNV runs after 600 minutes of pasteurization, after 30 and 60 minutes of solvent/detergent (S/D) treatment as well as on Days 14 and 20 of low-pH treatment, large-volume titration (bulk) was performed.

Downscaled manufacturing processes for plasma derivatives

All virus inactivation steps investigated apply to manufacturing processes for Baxter Healthcare (Westlake

Village, CA) products. Downscaled models were established to mimic the manufacturing process steps as closely as possible. Except for SINV-spiked runs, process variables were adjusted to worst case conditions to investigate robustness of virus inactivation. The equivalence of the laboratory models to the respective large-scale processes was demonstrated by comparing critical process and selected product variables. Temperature as a critical process variable for virus inactivation was monitored continuously in all processes investigated. Process intermediates obtained from manufacture were used as starting material and spiked at a ratio between 1:10 to 1:20 with virus stock suspensions. From the spiked starting material (SSM) samples were taken and titrated to verify the amount of virus added. Further samples were collected during and at the end of the virus inactivation processes and titrated immediately. Corresponding unspiked or mock-spiked samples were taken from control runs without virus and tested for their potential cytotoxicity for indicator cell lines and for their potential interference with the detection of low virus titers. Virus reduction factors (log) were calculated in accordance with regulatory guidelines.[16]

Pasteurization of human serum albumin

During manufacture, human serum albumin (HSA) is heat treated as solution in the final container at 60°C for 600 to 660 minutes. The heat treatment is done for different concentrations of HSA, ranging from 5% to 25%. In the downscale, the virus inactivation capacity of the heat treatment step was investigated by incubation of the CHIKV- or WNV-spiked albumin solutions at 58 ± 1°C for 600 minutes. To show the robustness of the obtained virus inactivation, pasteurization of HSA was done at low and the highest protein concentration, that is, 5 and 25%. SINV-spiked HSA was heated at 60 ± 1°C for 600 minutes, at a protein concentration of 25% only. To show the equivalence of the downscale to the manufacturing process, selected biochemical variables such as aggregate concentration, purity, transferrin, and α-1 acidic glycoprotein levels were monitored and compared to sample results from manufacture.

Vapor heating of anti-inhibitor coagulant complex, that is, Factor VIII inhibitor bypassing activity

During the manufacture of Factor VIII inhibitor bypassing activity (FEIBA), a freeze-dried bulk intermediate with an adjusted residual moisture of 7% to 8% (wt/wt) is heat treated in a first phase at 60°C for a minimum of 510 minutes, followed by a second phase at 80°C for a minimum of 60 minutes. For the downscaled runs, process intermediate was spiked with virus and freeze-dried in 3-mL aliquots (2-mL aliquots for SINV-spiked intermediate). The residual moisture was adjusted before heat treatment by the addition of water for injection, using the method of Karl-Fischer. Heat treatment of CHIKV and WNV was done at 59.5 ± 0.5°C for 505 minutes followed by 79.5 ± 0.5°C for 55 minutes and a residual moisture content of 7% (wt/wt) and 8% (wt/wt), respectively. SINV-spiked intermediate was adjusted to a residual moisture of 7% to 8% (wt/wt) after freeze-drying and was heat treated at 60 ± 0.5°C for 600 minutes followed by 80 ± 0.5°C for 60 minutes. FEIBA activity (clotting assay), Factor (F)II (clotting assay), and FX activity (chromogenic assay) were determined for the downscale intermediate before and/or after the vapor-heating process, and the results were compared to the respective values for intermediates from manufacture, to confirm equivalence of the different scale processes.

S/D treatment of intravenous immunoglobulin

For S/D treatment of intravenous immunoglobulin (IVIG) products Gammagard S/D and Gammagard Liquid/KIOVIG (Baxter), a mixture of tri-*n*-butyl phosphate, Octoxynol-9 and Polysorbate 80 (Merck, Darmstadt, Germany) was added to target concentrations of 0.3% (vol/vol), 1% (vol/vol), and 0.3% (vol/vol), respectively, for at least 60 minutes at 18°C to 25°C and a pH value of 5.2. Downscale runs for CHIKV were done at 18 ± 1°C for 57 to 60 minutes. Concentrations of S/D components for CHIKV (Gammagard Liquid/KIOVIG) and SINV (Gammagard S/D) spiked runs were adjusted to 50% compared to the standard manufacturing conditions and the kinetics of virus inactivation further investigated using only 10% of the standard S/D concentrations. To prevent further inactivation of virus by the S/D reagents after sample drawing, S/D-containing samples were diluted immediately 1:100 or 1:20 (CHIKV, 50 or 10% of nominal S/D concentration) or 1:10 (SINV) in cold cell culture medium. The amount of S/D reagents added was measured by weighing and the concentration of each S/D reagent in solution was measured by specific assays in unspiked control runs. Protein concentration, conductivity, and immunoglobulin (Ig)G concentration of the respective intermediates of the two process scales were measured and compared to support the equivalence of the downscale to manufacture.

Low-pH treatment of IVIG (Gammagard Liquid/KIOVIG)

During the Gammagard Liquid/KIOVIG manufacturing process, the final product is incubated at a low pH of 4.4 to 4.9 and at a temperature of 30°C to 32°C for 21 to 24 days. To investigate virus inactivation of the low-pH incubation step at laboratory scale, CHIKV-spiked process intermediate was incubated at 29 ± 1°C for up to 21 days. The pH, adjusted to 4.4 and 4.9, was monitored and incubation temperature continuously recorded. To prevent further

inactivation of virus after sample drawing, samples were diluted immediately 1:3.16 with cold cell culture medium. Selected biochemical variables such as molecular size distribution, gammaglobulin purity, and functionally intact IgG were determined and compared to results of the large-scale process to verify the equivalence of the two process scales.

RESULTS

For each of the four dedicated virus inactivation steps as performed in downscaled versions in these verification studies for virus inactivation, the results for selected biochemical variables were always equivalent to those of the respective samples obtained from the manufacturing scale processes (data not shown), thereby confirming validity of the different scale processes. Generally, conditions least favorable for virus inactivation were chosen.

Pasteurization of HSA

As a dedicated virus inactivation step, the final HSA product is pasteurized. Investigation of the virus inactivation capacity in downscale runs was done below the lower limits of temperature and incubation time specified for manufacture, and robustness of the heat treatment step was further shown through the use of HSA with protein concentrations at the extremes of the specified manufacturing range. CHIKV was completely and rapidly inactivated to below the LOD within 30 minutes of incubation at 58 ± 1°C in both 5 and 25% HSA (Fig. 1, Table 1). SINV, which like CHIKV belongs to the Alphaviruses, showed very similar inactivation kinetics and was also completely inactivated to below the LOD within 30 minutes of pasteurization at 60 ± 1°C using 25% HSA (Fig. 1B, Table 1). Significant reduction factors (Table 1) and similar inactivation kinetics were seen for WNV, with complete inactivation achieved after 30 minutes in 25% HSA and after 60 or 180 minutes in 5% HSA (Fig. 1, Table 1). Comparing the results of the current study to earlier published BVDV pasteurization data,[18] this model virus for small enveloped RNA viruses was one of the most resistant against inactivation through pasteurization. Whereas CHIKV, SINV, and WNV infectivity was already significantly reduced during the heating phase to target temperature, BVDV was more resistant to heating (Fig. 1) and a longer pasteurization period was necessary to inactivate the virus to below the LOD (120 and 60 minutes in 5 and 25% HSA, respectively; Fig. 1, Table 1).

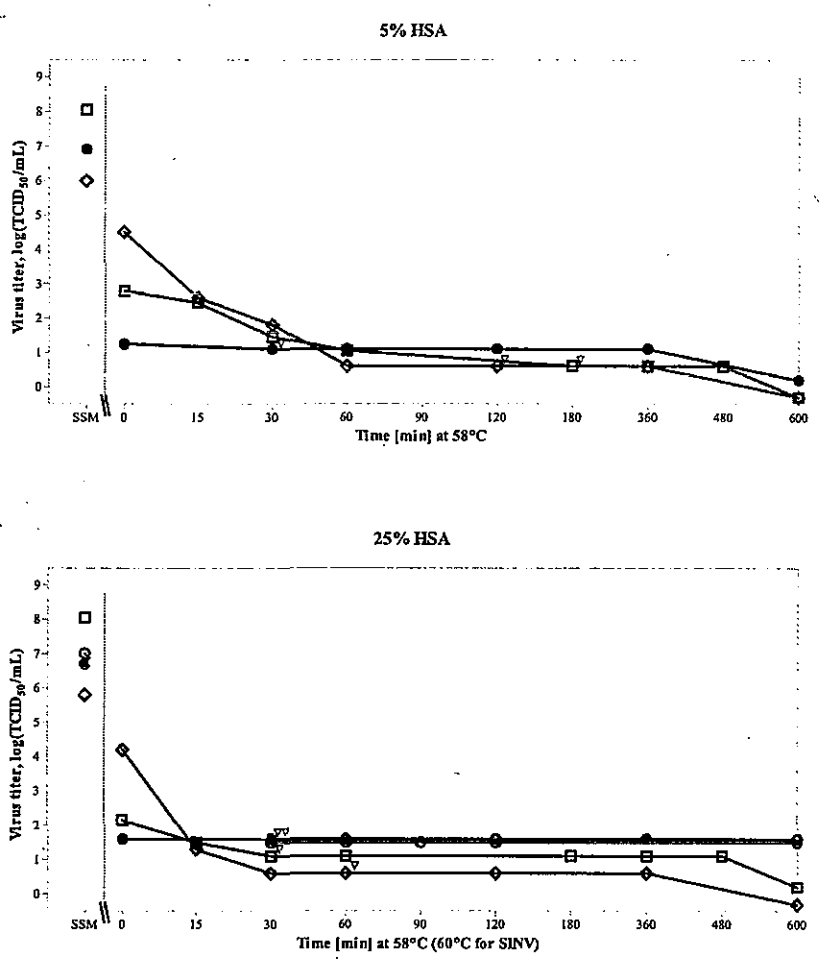

Fig. 1. Pasteurization of HSA. Virus inactivation kinetics for pasteurization at 58 ± 1°C (60 ± 1°C for SINV) of 5% HSA solution (A) and 25% HSA solution (B). Mean results for duplicate runs with CHIKV and WNV and single runs for BVDV and SINV are shown. SSM is virus-spiked HSA. For kinetic samples with no detected infectivity, the LOD is shown; (▽) first time point at which no infectivity was detected in all runs. Lower detection limits for virus titer toward the end of treatment is due to large-volume assays (bulk). (●) CHIKV; (◇) BVDV; (○) SINV; (□) WNV.

Vapor heating of FEIBA

The critical variables for the vapor heating process with respect to virus inactivation are temperature, time of heating, and residual moisture. FEIBA intermediate spiked with CHIKV or WNV was vapor heated at laboratory scale to the lower limit of temperature for just below the lower limit of the incubation time specified for the manufacturing process. To evaluate process robustness, the

TABLE 1. Reduction factors (log) and time points at which no remaining viral infectivity was detected (values in parentheses) in the current and previous inactivation studies

Process	CHIKV	SINV	WNV	BVDV*
Pasteurization of HSA,† 5%	>7.0/>6.9 (0-1/30 min)	ND	>8.3/>8.3 (60/180 min)	>6.4 (120 min)
25%	>5.9/>5.8 (0-1/30 min)	>5.5 (30 min)	>8.0/>7.7 (30/30 min)	>6.2 (60 min)
Freeze-drying/vapor heating of FEIBA,‡ 7%	>6.9 (360 min)	>5.3/>5.2/>4.7	>8.2 (360 min)	>5.6 (360 min)
8%	>7.3 (360 min)	(120/120/120 min)	>8.0 (360 min)	>5.6 (360 min)
S/D treatment of IVIG,§ 50%	>7.2/>6.7 (1-2/1-2 min)	>5.3 (1-2 min)	ND	>6.1/>6.2 (1-2/1-2 min)
10%	>7.2 (1-2 min)	>5.3 (1-2 min)	ND	ND
5%	ND	ND	>6.0‖/>5.9‖ (30/30 min)	>5.8 (1-2 min)
Low-pH incubation of IVIG,¶ pH 4.4	>7.9 (6 days)	ND	>5.5‖ (4 days)	>5.4 (14 days)
pH 4.9	>7.4 (14 days)	ND	>6.0/>6.1‖ (2/4 days)	>5.9/>5.6 (14/14 days)

* Data from Kreil et al.[18]
† Done using a solution with a concentration of 5 or 25% HSA.
‡ Done using product with residual moisture (wt/wt) of 7 or 8% except for SINV, where residual moisture was adjusted to 7% to 8%.
§ Done using 50, 10, or 5% of specified S/D chemicals.
‖ Data from Kreil et al.[17]
¶ Done using IVIG adjusted to a pH of 4.4 or 4.9, with the exception of WNV, for which solution adjusted to a pH of 4.5 was used.
ND = not done.

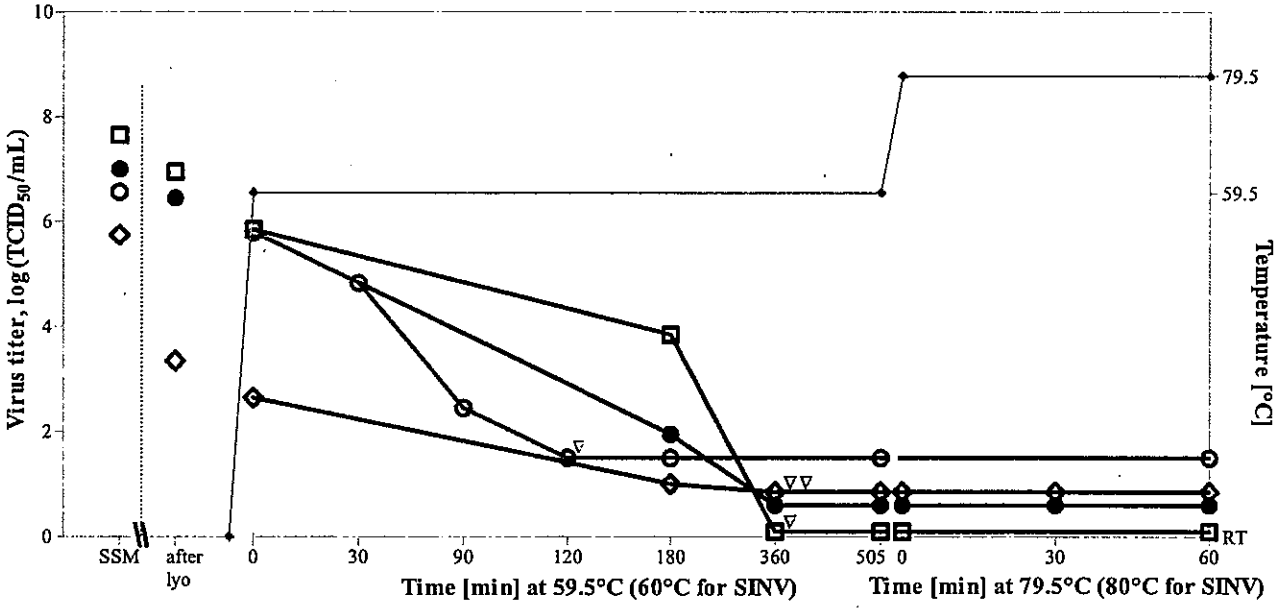

Fig. 2. Vapor heating of FEIBA. Virus inactivation kinetics by heating of virus-spiked, lyophilized, and moisture-adjusted (7%-8% [wt/wt]) FEIBA at 59.5 ± 0.5°C (60 ± 0.5°C for SINV), followed by 79.5 ± 0.5°C (80 ± 0.5°C for SINV) heating (temperature profile represented in graph by the gray line). Means of two samples per kinetic time point for all viruses except SINV (triplicates). The last SINV sample at 60 ± 0.5°C was taken after 600 minutes but shown in graph at 505 minutes for simplicity. SSM = virus-spiked FEIBA before lyophilization; "after lyo" = sample after lyophilization but before initiation of the heating process. For kinetic samples with no detected infectivity, the LOD is shown; (▽) first time point at which no infectivity was detected in all runs. RT = room temperature. (●) CHIKV; (◇) BVDV; (○) SINV; (□) WNV.

residual moisture was adjusted to the upper and lower limits of the manufacturing range. Using conditions least favorable for virus inactivation (temperature, time), the vapor heat treatment step in combination with the preceding freeze-drying inactivated CHIKV and WNV to below the LOD within 6 hours of the first heat treatment phase at a temperature of 59.5 ± 0.5°C (Fig. 2, Table 1). Heat treatment of SINV-spiked FEIBA intermediate at the same limits of temperature and residual moisture as specified during manufacture showed inactivation to below the LOD already after 2 hours in the first heat treatment phase (Fig. 2, Table 1). Comparing the results of the current study to earlier published BVDV vapor heating data,[18] efficient inactivation of the model virus was seen by this procedure, with no residual infectivity detected after 6 hours in the first phase of heat treatment, similar to

CHIKV and WNV (Fig. 2, Table 1). Interestingly, whereas the preceding lyophilization step had only a marginal effect on the infectivity of CHIKV, SINV, and WNV (Fig. 2), the infectivity of BVDV was more affected by this procedure, an observation that has been reported before.[20]

S/D treatment of IVIG (Gammagard Liquid/KIOVIG or Gammagard S/D)

At a final S/D concentration corresponding to only 50% of the manufacturing process, CHIKV and SINV were already inactivated to below the LOD after 1 to 2 minutes of S/D treatment (Fig. 3, Table 1). At 10% of the standard S/D concentration, CHIKV and SINV were also completely inactivated after 1 to 2 minutes of S/D treatment (Table 1), underlining the efficacy and robustness of the S/D treatment step. Comparing the results of the current study to earlier published BVDV S/D treatment data,[18] rapid and efficient inactivation of this model virus was seen at both 50 and 5% of the standard S/D concentration (Fig. 3, Table 1). In comparison, already available data for conditions deliberately chosen to be by far less stringent than the actual manufacturing process indicated that inactivation by S/D treatment was somewhat slower for WNV than for BVDV, as no infectious virus could be detected after 1 to 2 minutes of treatment with 5% of the standard S/D concentration for BVDV, but some low level of infectivity was detectable for WNV until 30 minutes of treatment (Fig. 3, Table 1).[17]

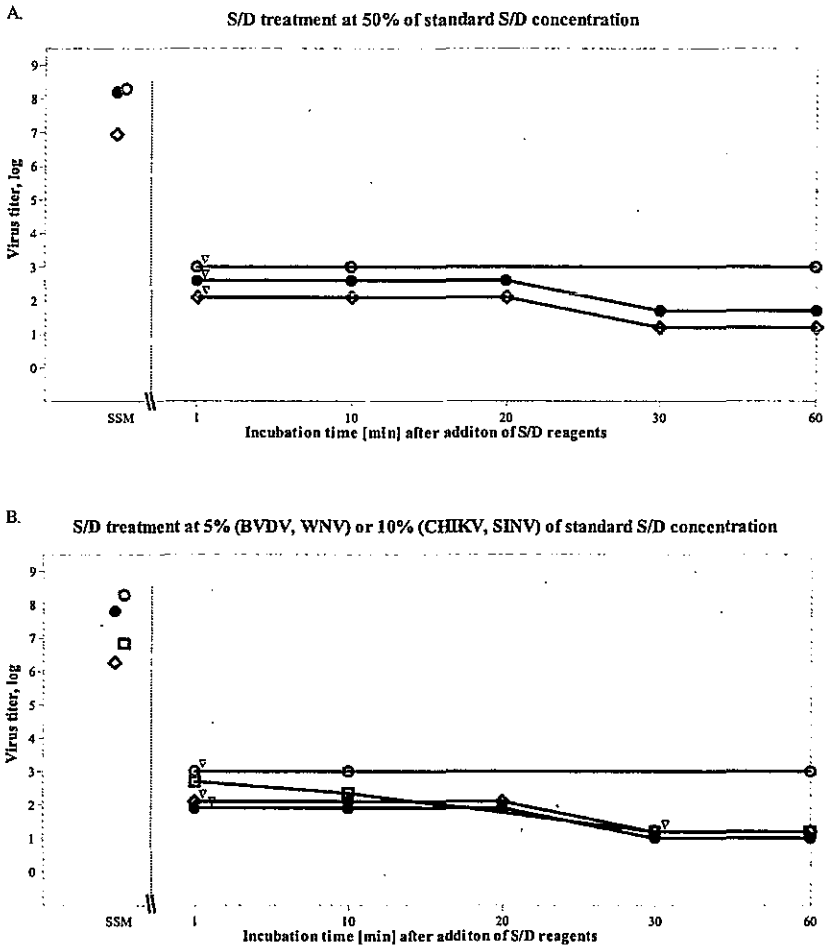

Fig. 3. S/D treatment of IVIG. Virus inactivation kinetics for 50% (A) and 5% or 10% (B) of standard S/D concentration for IVIG S/D treatment. Virus titers are log($TCID_{50}$/mL) for CHIKV, WNV, and BVDV and log(plaque-forming units/mL) for SINV. Mean results for duplicate runs with CHIKV and BVDV at 50% S/D concentration and WNV at 5% S/D concentration and single run results for all other experiments are shown. SSM = virus-spiked IVIG. For kinetic samples with no detected infectivity, the LOD is shown; (▽) first time point at which no infectivity was detected in all runs. Lower detection limits for virus titer toward the end of treatment is due to large-volume assays (bulk). (●) CHIKV; (◇) BVDV; (○) SINV; (□) WNV.

Low-pH treatment of IVIG (Gammagard Liquid/KIOVIG)

In the downscale investigation of the low-pH treatment step of the IVIG intermediate, CHIKV was inactivated to below the LOD on Day 6 for the run at pH 4.4 and on Day 14 for the run at pH 4.9 (Fig. 4, Table 1). Comparing the results of the current study to earlier published BVDV low-pH treatment data,[18] BVDV was similarly inactivated in the low-pH treatment, where by Day 14 complete inactivation was accomplished in all three experiments (Fig. 4, Table 1). In comparison, available data for WNV[17] showed that this Flavivirus was most rapidly inactivated by low-pH treatment. In one run at pH 4.9 WNV was already inactivated to below the LOD on Day 2 of incubation (Table 1). In the other two runs, one each at pH 4.5 and pH 4.9, no WNV could be detected in the first kinetic samples that were taken on Day 4 (Table 1). Complete and effective inactivation for all three viruses was therefore achieved at the two different extremes of the manufacturing specification for pH, demonstrating the robustness of the low-pH treatment step.

DISCUSSION

Over the past few years, the first autochthonously transmitted CHIKV infections have been reported in Europe,[8-11]

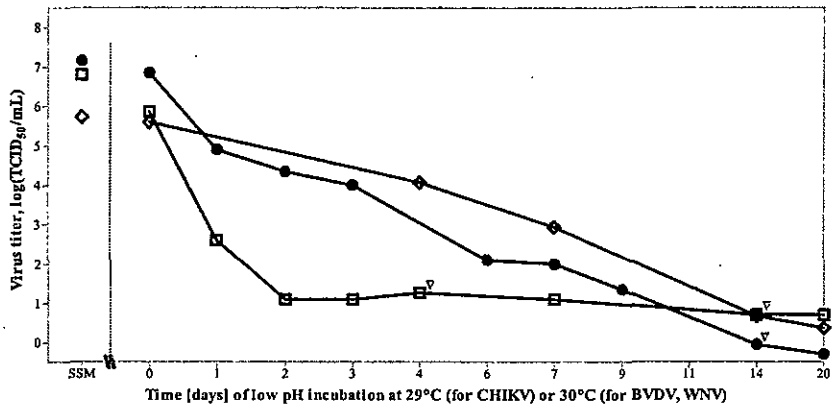

Fig. 4. Low-pH treatment of IVIG. Virus inactivation kinetics for incubation at pH 4.4 and pH 4.9 and 29 ± 1°C (CHIKV) or 30 ± 1°C (WNV, BVDV). Mean results of two runs (pH 4.4/pH 4.9) for CHIKV and three runs (pH 4.4/pH 4.9/pH 4.9) for BVDV and WNV (pH 4.5 instead of pH 4.4) are shown. SSM = virus-spiked IVIG. For kinetic samples with no detected infectivity, the LOD is shown; (▽) first time point at which no infectivity was detected in all runs. Lower detection limits for virus titer toward the end of treatment is due to large-volume assays (bulk). (●) CHIKV; (◇) BVDV; (□) WNV.

demonstrating that there is potential for the global spread of the virus[7] and that CHIKV has become a worldwide public health concern.[2] Similarly, spread into and subsequent autochthonous transmission of CHIKV would be entirely possible in the United States, where the mosquito vector *A. albopictus* is widely distributed in urban areas,[5,6] a scenario quite similar to the advent of WNV in the United States in 1999[21] and the epidemics since. Although reassuring data for the effective inactivation of small lipid-enveloped RNA viruses by the virus inactivation methods commonly used in the manufacture of plasma products exists,[17,18,22-25] this work sought to experimentally confirm the expected behavior for CHIKV, an Alphavirus and therefore a virus genus that is currently not typically used as a model virus.

CHIKV was subjected to virus inactivation methods commonly used in the manufacture of plasma products: pasteurization for HSA, vapor heating for FEIBA, S/D treatment for IVIG, and incubation at low pH for IVIG. In addition, some of the experiments were done with the closely related SINV and the relatively recent concern WNV, and the obtained results were compared to earlier published inactivation data for the model virus BVDV[18] and for WNV.[17]

Pasteurization of HSA resulted in effective inactivation of all the viruses tested, both at the low and at the upper protein concentration relevant in manufacture. It was shown that this method inactivated the Alphaviruses CHIKV and SINV more rapidly than BVDV and WNV.

During vapor heating of FEIBA, all the viruses investigated were already inactivated during the first heat treatment phase. Vapor heating was therefore confirmed as very effective, with the second heat treatment phase providing extra margins of safety. In addition, the results indicate that CHIKV, SINV, and WNV are more stable during lyophilization than BVDV (Fig. 2), confirming again the sensitivity of BVDV to this process step.[20] This is important to keep in mind should an investigation of virus inactivation for an isolated lyophilization step be desired, where BVDV as a model virus may not necessarily represent a worst-case choice of virus.

S/D treatment was again confirmed as being highly effective for the inactivation of all the viruses investigated.[25] Even only 5 or 10% of the S/D concentration specified for the manufacturing process was sufficient to rapidly and effectively inactivate all the viruses tested, and the results are thus in line with the original findings establishing this method as most effective in inactivating lipid-enveloped viruses.[26] Data from two different IVIG S/D treatment processes, Gammagard S/D and Gammagard Liquid/KIOVIG, are included as they use the same S/D reagents at identical concentrations. All available data show that there is no difference in efficacy of virus inactivation between these two IVIG S/D treatments when tested at process conditions as specified for manufacturing scale.

Complete and effective inactivation of all viruses investigated was obtained at the two different extremes of the manufacturing specifications for the low-pH treatment, where CHIKV and BVDV showed similar inactivation kinetics and WNV was most rapidly inactivated, an observation that is in line with the known acidic pH-dependent fusion machinery of Flaviviruses.[27] Comparing the resistance toward physicochemical inactivation across the different methods used in the manufacture of plasma products, CHIKV was very similar in susceptibility and virus inactivation kinetics to SINV, the other Alphavirus investigated, as well as to members of the Flaviviridae family, WNV and BVDV. Regarding the overall virus inactivation capacity, all the CHIKV spiked into experimental downscales was completely inactivated well before the end of these processes, indicating a great safety margin of these dedicated virus reduction techniques. Reassuringly, the inactivation methods tested were shown to completely inactivate all the virus that was spiked into the respective product intermediate and the data presented alleviate any CHIKV-associated concerns about the safety of plasma derivatives. Altogether, the CHIKV results obtained in this investigation provide solid reassurance of

the safety of plasma derivatives. In addition, our results verify that model viruses that are chosen according to suggestions of the relevant guidelines[16]—and if they are sufficiently well understood to be similar to transfusion-related viruses—are an adequate tool to predict the behavior of new viruses of interest.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

All authors are employees of Baxter BioScience: JM, GP, AB, MKH, PNB, and TRK have stock interests.

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医薬品 研究報告 調査報告書

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				2012. 3. 15	該当なし	

一般的名称	解凍人赤血球濃厚液	研究報告の公表状況	Emerging Infectious Disease Journal Vol.18 No.3; Available from: http://wwwnc.cdc.gov/eid/article/18/3/11-0034_article.htm	公表国 中国
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

○チクングニヤ熱アウトブレイク 中国広東省、2010年
2010年10月、中国広東省でチクングニヤ熱のアウトブレイクが報告された。
広東省東莞市の2つの隣接した村で173人の患者が確認された(2-93歳)。最初の患者は9月1日に発症し、9月19日以降、患者数が急増した。このアウトブレイクで死亡した患者はおらず、大部分の患者は発症してから1週間以内に回復した。患者やその家族の誰も、2010年7月以降に海外旅行をしていなかった。アウトブレイクの前月、広東省の天気は雨が多く、これにより媒介蚊の個体数が増加したと考えられる。
12人の急性患者及び3人の回復期患者から血清検体を採取し、このアウトブレイクを引き起こしているチクングニヤウイルスの系統発生解析を行ったところ、このチクングニヤウイルスは東/中央/南アフリカ(ECSA)サブグループのインド洋分岐群に属することが示された。
チクングニヤウイルスは2010年以前には中国の風土病ではなかったが、近年、東南アジアからのECSAジェノタイプに感染した患者から広東省に伝播したと考えられる。今回のアウトブレイクでは、症状がそれほど重篤でなかったこと及びデング熱との誤診により、ウイルス伝播が広範囲に及んだ。蚊の増加とチクングニヤウイルスの免疫保有者が少ないことが、このアウトブレイクに関連する要因となった。

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
2010年10月、中国広東省でチクングニヤ熱のアウトブレイクが起こり、東莞市の2つの隣接した村で173人の患者が確認されたとのことである。	日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

Chikungunya Outbreak in Guangdong Province, China, 2010

De Wu, Jie Wu, Qiaoli Zhang, Haojie Zhong, Changwen Ke, Xiaoling Deng, Dawei Guan, Hui Li, Yonghui Zhang, Huiqiong Zhou, Jianfeng He, Linghui Li, and Xingfen Yang

A disease outbreak with dengue-like symptoms was reported in Guangdong Province, China, in October 2010. Testing results confirmed that the pathogen causing the outbreak was chikungunya virus. Phylogenic analysis indicated that this virus was a member of the Indian Ocean clade of the East/Center/South African subgroup of chikungunya virus.

Chikungunya virus (CHIKV) is a mosquito-borne virus that causes fever, headache, rash, nausea, vomiting, myalgia, and arthralgia, and has had a major effect on human health (*1*,*2*). The first human infections caused by CHIKV were reported ≈60 years ago (1952–1953) in eastern Africa (*3*). CHIKV has now become a worldwide public health problem. Although this virus is indigenous to tropical Africa, outbreaks of CHIKV fever have been reported in countries in the Indian Ocean region and Southeast Asia (*4*–*6*). With an increase in global travel, the risk for spreading CHIKV to regions in which the virus is not endemic has increased (*7*).

Multiple sporadic cases of nonindigenous CHIKV infection have been reported in China. In 1987, CHIKV was isolated from the serum of a patient, and antibodies against CHIKV were detected in a second, convalescent-phase patient in Yunnan Province (*8*). Four imported case of CHIKV infection confirmed by reverse transcription PCR (RT-PCR) were detected in Guangzhou and Moming, Guangdong Province, in travelers returning from Sri Lanka and Malaysia in 2008 (*9*,*10*). Another imported case from India was confirmed by using RT-PCR in our laboratory in 2009. We report an outbreak of CHIKV fever that occurred in Guangdong Province, China, in 2010.

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The Study

Guangdong Province is located in a subtropical zone. It has a high relative humidity, an average yearly temperature of 19°C–24°C, and an average yearly rainfall of 1,300–2,500 mm. *Aedes albopictus* mosquitoes are abundant and widespread. However, *Ae. aegypti* mosquitoes are found only in western Guangdong Province and not in the region around the city of Dongguan. In the months before the outbreak, the weather in Guangdong Province was particularly rainy.

During September 2010, patients reporting an illness with dengue-like symptoms were recorded by local community clinics in the suburbs of Dongguan, Guangdong Province. For epidemiologic investigation, the Guangdong Center for Disease Control and Prevention defined a clinical case of CHIK fever as a case characterized by sudden onset of fever with arthralgia, maculopapular rash, or myalgia. We identified 173 patients (74 male and 99 female patients) 2–93 years of age in 2 adjacent villages who had similar symptoms. More than 85% of the patients were found in these 2 villages in 97 families (≥2 cases per family in 50 families).

The first patient became ill on September 1, and the number of CHIKV fever cases rapidly increased after September 19 (Figure 1), indicating an outbreak of CHIKV infections in the region. The outbreak spanned 2 months, and the peak occurred at the end of September/early October. None of the patients or any family members reported travel abroad since July 2010. No deaths were reported as a result of the outbreak, and most patients recovered within 1 week after onset of symptoms. No patients were hospitalized; however, several elderly patients reported joint pain after 2 weeks.

Densities of *Ae. albopictus* mosquitoes were investigated during the outbreak, and an especially high Breteau index of 77–180 was observed. The abundant rainfall likely resulted in an extremely high mosquito density. To control the outbreak, mosquito control measures were implemented and quarantine of patients with acute disease was enforced.

To identify the pathogen causing the outbreak, we collected 15 serum samples from 12 patients with acute disease and 3 patients with convalescent-phase disease who had dengue-like symptoms. Patient serum was assayed for CHIKV nucleic acid, antibody, and virus. DNA sequence analysis of amplified CHIKV envelope 1 (E1) was performed to infer possible source of transmission. Specimens were tested by real-time RT-PCR for CHIKV (*11*) and dengue virus.

Ten serum samples were positive for CHIKV. Virus-specific IgM and IgG were detected by IgM and IgG capture ELISAs (IBL, Hamburg, Germany). Seven samples were positive for IgM and 1 sample was positive for IgG (Table).

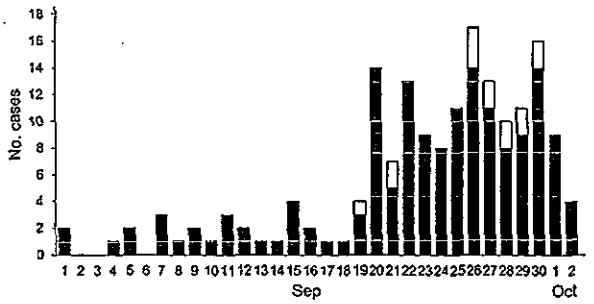

Figure 1. Cases of chikungunya infection in Guangdong, China, September 1–October 1, 2010. Black bar sections indicate clinical cases and white bar sections cases confirmed by molecular analysis.

There were 3 case-patients in whom CHIKV nucleic acid and antibody were found at the same time; 2 of these were in serum samples obtained 3–4 days after these samples were found to be positive for CHIKV IgM. We infer that high cross-reactivity in the ELISAs might contribute to these results.

For phylogenetic analysis, RT-PCR was performed as described (*12*), and 7 amplicons were sequenced. The 10 nucleic acid–positive specimens were placed on C6/36 and BHK-21 cell lines to isolate CHIKV. Serum samples were 2-fold serially diluted 6 times (1:50–1:1,600) in minimal essential medium, and 1 mL of diluted sample was added to each well of a 24-well culture plate. Specimens were incubated at 33°C in an atmosphere of 5% CO_2 and observed daily for ≤7 days for cytopathic effects (CPEs) (online Appendix Figure, wwwnc.cdc.gov/EID/article/18/3/11-0034-FA1.htm). After specimens were incubated for 4–7 days, 3 CPEs were observed on C6/36 and BHK-21 cells. Development of CPEs in C6/36 cells is unusual for CHIKV. However, we observed the effect of C6/36 cell fusion on 3 specimens. We speculate that a virus mutation causes an increase in virulence or changes effects on infected C6/36 cells.

Phylogenetic analysis was performed for partial E1 sequences (7 from this study and 24 from GenBank) by using MEGA5 (*13*). Nucleotide sequences were separated into 3 subgroups corresponding to the 3 globally circulating subgroups (Figure 2). Sequences of the 7 PCR products obtained in this study showed few differences from each other. Paired sequence identity ranged from 98% to 99% at the nucleotide level. Genetic analysis of the 325-nt fragment of E1 genes obtained in this study showed that all 7 sequences clustered in a unique branch within the Indian Ocean clade of the East/Central/South African (ECSA) genotype, and close to Thailand (GQ870312, FJ882911, GU301781), Malaysia (FJ998173), Taiwan (FJ807895), and China (GU199352, GU199353) isolates (98%–99%). The translated E1 gene fragment from 1 of the 7 isolates in this study (China/GD112/2010) had an expected 2-codon deletion. This deletion was also present in the ESCA clade but was not found in the other 6 isolates.

On the basis of sequence analysis, the highest degree of identity was observed with outbreak isolates and the E1 sequence from the Thailand strain (FJ882911) isolated in 2009. Paired identity values were 99% at the nucleotide level and 100% at the amino acid level. Nucleotide substitute analysis showed that a common nucleotide substitution was observed at partial E1 gene site 250 (T'C) in outbreak isolates and FJ882911. This substitution was not observed in other analyzed sequences from GenBank. These results suggested that the virus causing this outbreak was likely transmitted from a source in Southeast Asia and probably evolved from a strain that originated in Thailand.

Table. Characteristics of case-patients and serum sample detection for chikungunya virus, Guangdong, China, 2010*

Case-patient ID no.	Age, y/sex	Date of symptom onset, Sep 2010	Signs and symptoms						Test results		
			Fever	Red face	Headache	Arthralgia	Myalgia	MR	Virus isolation	Real-time RT-PCR/RT-PCR	IgM/IgG
D10112	33/F	27	+	–	–	+	+	+	–	+/+	–/–
D10113	7/M	29	+	+	–	+	+	+	+	+/+	–/–
D10114	62/M	30	+	+	+	+	–	–	+	+/+	–/–
D10115	48/F	30	+	–	–	+	–	+	+	+/+	–/–
D10116	60/M	28	+	–	–	+	+	–	–	+/–	–/–
D10117	39/M	27	+	+	–	+	+	+	–	+/–	+/–
D10118†	59/M	19	+	+	–	+	–	+	ND	–/ND	+/+
D10119	59/F	26	–	+	–	+	–	+	ND	–/ND	–/–
D10120	10/F	26	+	–	+	–	–	+	ND	–/ND	+/–
D10121†	56/F	21	+	+	–	+	–	+	ND	–/ND	+/–
D10122†	24/F	21	+	+	–	+	+	+	ND	–/ND	+/–
D10123	3/F	26	+	–	–	–	–	+	–	+/–	–/–
D10124	60/M	26	+	–	–	+	+	+	–	+/+	–/–
D10125	60/F	29	+	–	–	+	+	+	–	+/+	+/–
D10126	39/M	28	+	–	–	+	+	+	–	+/+	+/–

*All samples were obtained on October 1, 2010. ID, identification; MR, maculopapular rash; RT-PCR, reverse transcription PCR; +, positive; –, negative; ND, not done.
† Convalescent-phase case-patient.

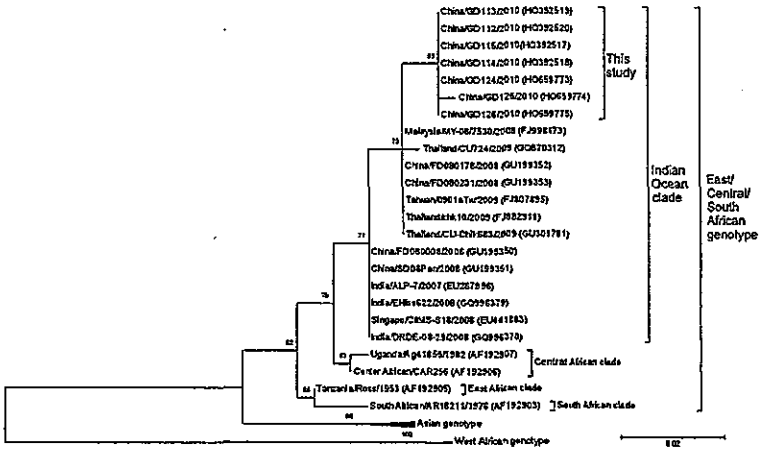

Figure 2. Phylogenetic analysis of partial envelope 1 gene sequences of chikungunya viruses, Guangdong, China, 2010. Numbers along branches indicate bootstrap values. GenBank accession numbers are indicated in parentheses. Scale bar indicates nucleotide substitutions per site.

Conclusions

CHIKV was not endemic to China before 2010. However, in recent years, CHIKV strains from Southeast Asia with the ECSA genotype have been transmitted by infected persons to Guangdong Province. We report an outbreak of CHIKV fever in China. The low severity of the disease and misdiagnosis of dengue fever has likely encouraged widespread transmission of the virus. High-density mosquito populations and an immunologically uninfected population were 2 contributing factors in this outbreak.

Acknowledgments

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医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2012 年 4 月 17 日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①②ポリエチレングリコール処理抗破傷風人免疫グロブリン ③乾燥抗破傷風人免疫グロブリン		研究報告の公表状況	CDC/Newsroom Home/Press Release/2012/03/14	公表国 アメリカ	
販売名 （企業名）	①テタノブリン IH 静注 250 単位　（ベネシス） ②テタノブリン IH 静注 1500 単位　（ベネシス） ③テタノブリン筋注用 250 単位　（ベネシス）					

研究報告の概要

胃腸炎による死亡が 2 倍
C. difficile とノロウイルスが主要な原因である

疾病管理予防センター（CDC）による検査によれば、胃腸炎（嘔吐と下痢を引き起こす胃と腸の炎症）で死亡した人の数は、1999 年から 2007 年までに 2 倍以上になった。調査結果は、本日、アトランタでの新興感染症に関する国際会議で発表される。
CDC の科学者は、米国における全年齢層の間で 1999 年から 2007 年までの胃腸炎関連の死亡を特定するために、全国健康統計センターからのデータを使用した。
「胃腸炎は、世界的に死亡の主たる原因である」と CDC のウイルス疾病部門の執筆者アロン・ホール氏は言った。「胃腸炎関連の死亡の原因及び危険に曝されていることを知ることによって、我々はより良好な治療を開発することができ、医療従事者が人々が病気になるのを防ぐのを手助けすることができる。」
8 年間の調査期間で、全ての原因による胃腸炎関連の死亡は年間ほぼ 7,000 から 17,000 以上まで増加した。65 歳以上の高齢者が死亡の 83%を占めた。クロストリジウム・ディフィシール（C. difficile）とノロウイルスが、胃腸炎関連の死亡の最も多い感染原因であった。保健医療の現場にしばしば関連した細菌の一種、C. difficile のために、年間死亡数は約 2,700 から 14,500 まで、5 倍増加した。下痢を引き起こす C. difficile は、死亡の 2/3 を占めた。C. difficile の発生率と死亡率の最近の増加の多くは、過剰毒性、耐性株の C. difficile の出現と広がりに起因していた。
ノロウイルスは毎年約 800 の死亡と関連し、流行がウイルスの新しい株に起因した年には 50%以上多い死亡数となった。ノロウイルスは高い接触感染性がある。それはヒトからヒトへの接触と汚染された食品、水及び体表面を介して広がる。人々は年間を通してノロウイルスの病気に罹患するが、症例は 12 月から 2 月の間にピークに達する。ノロウイルスは毎年 2000 万以上の病気を引き起こす、そしてそれは米国での胃腸炎流行の主要な原因である。
「C. difficile が胃腸炎関連の死亡の主要な一因であり続けると同時に、本研究はノロウイルスがおそらく第二の主要な感染原因であることを初めて示す」とホール氏は言った。「我々の調査研究は、特に高齢者の間の C. difficile とノロウイルスによる胃腸炎を防止、診断し、管理するための有効な手段の必要性を強調する。」

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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医薬品
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報告企業の意見	今後の対応	
クロストリジウム・ディフィシル（Clostridium difficile）は、大きさ0.5～1.9×3.0～16.9μmの偏性嫌気性のグラム陽性桿菌である。万一、原料血漿にC.difficileが混入したとしても、除菌ろ過等の製造工程において除去されると考えている。 また、ノロウイルス（Norovirus）は、カリシウイルス科（Caliciviridae）に属する直径：30～38nmの正二十面体（表面に32個のカップ状の窪みが見られる）のエンベロープを有しないプラス鎖の一本鎖RNAウイルスである。万一、原料血漿にノロウイルスが混入しても、EMC及びCPVをモデルウイルスとしたウイルスクリアランス試験成績から、本剤の製造工程において不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。	

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Press Release

For Immediate Release: March 14, 2012
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Deaths from gastroenteritis double

C. difficile and norovirus are the leading causes

The number of people who died from gastroenteritis (inflammation of the stomach and intestines that causes vomiting and diarrhea) more than doubled from 1999 to 2007, according to a study by the Centers for Disease Control and Prevention. The findings will be presented today at the International Conference on Emerging Infectious Diseases in Atlanta.

CDC scientists used data from the National Center for Health Statistics to identify gastroenteritis-associated deaths from 1999 to 2007 among all age groups in the United States.

"Gastroenteritis is a major cause of death worldwide," said lead author Aron Hall, D.V.M., M.S.P.H., of the CDC's Division of Viral Diseases. "By knowing the causes of gastroenteritis-associated deaths and who's at risk, we can develop better treatments and help health care providers prevent people from getting sick."

Over the eight-year study period, gastroenteritis-associated deaths from all causes increased from nearly 7,000 to more than 17,000 per year. Adults over 65 years old accounted for 83 percent of deaths. Clostridium difficile (C. difficile) and norovirus were the most common infectious causes of gastroenteritis-associated deaths.

There was a fivefold increase, from approximately 2,700 to 14,500 deaths per year, for C. difficile, a type of bacteria often associated with health care settings. C difficile, which causes diarrhea, accounted for two-thirds of the deaths. Much of the recent increase in the incidence and mortality of C. difficile is attributed to the emergence and spread of a hypervirulent, resistant strain of C. difficile.

Norovirus was associated with about 800 deaths annually, though there were 50 percent more deaths in years when epidemics were caused by new strains of the virus. Norovirus is highly contagious. It spreads through person-to-person contact and contaminated food, water, and surfaces. People can get norovirus illness throughout the year, but cases peaked between December-February. Norovirus causes more than 20 million illnesses annually, and it is the leading cause of gastroenteritis outbreaks in the United States.

"While C. difficile continues to be the leading contributor to gastroenteritis-associated deaths, this study shows for the first time that norovirus is likely the second leading infectious cause," said Hall. "Our findings highlight the need for effective measures to prevent, diagnose, and manage gastroenteritis, especially for C. difficile and norovirus among the elderly."

###

U.S. Department of Health and Human Services

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別紙様式第２

医薬品　研究報告　調査報告書

識別番号・報告回数		報告日	第一報入手日 2012年2月14日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①②フィブリノゲン加第XIII因子 ③④人血液凝固第XIII因子	研究報告の公表状況	Transfusion-associated Babesiosis in the United States: a description of cases Annals of Internal Medicine 2011:155(8)509-519	公表国 米国	
販売名（企業名）	①ベリプラストP コンビセット ②ベリプラストPコンビセット組織接着用 ③フィブロガミンP ④フィブロガミンP静注用 （CSLベーリング株式会社）				

研究報告の概要

【背景】バベシア症は赤血球内寄生虫による死に至る可能性がある疾病で、通常ダニ媒介により感染するが、輸血によっても感染する。米国では主に北東部および中西部の7州においてダニ媒介によるバベシア症が発生したが、血液ドナーに対するバベシア症検査は未認可である。【目的】本研究では、輸血によるバベシア症が初めて確認された1979年以降の米国における輸血関連バベシア症例データを検証した。【方法】1979年～2009年に輸血を受け、2010年までに輸血後感染バベシア症と診断され、輸血以外の感染経路のエビデンスが報告されておらず、ドナーの感染が検査で確認されている患者を対象とした。【結果】輸血関連バベシア症例162例（B. microti 159例、B. duncani 3例）が本研究の対象となった。B. microti 159例の年齢中央値は65歳であった。ほとんどの症例が赤血球関連であったが、4例は全血由来血小板関連であった。122例（77%）は2000年～2009年に発生し、138例（87%）は風土病とされる主な7州（マサチューセッツ、ニューヨーク、コネチカット、ミネソタ、ロードアイランド、ニュージャージー、ウィスコンシン）で発生した。B. duncani 3例は赤血球輸血関連で、ワシントン州で1例、カリフォルニア州で2例記録された。【結論】以上の結果から、輸血感染リスク軽減のためのドナー検査の対策を講じる必要性がある。バベシア症は、輸血後の原因不明の溶血性貧血や発熱を呈する疾患の鑑別診断に含まれるべきである。

使用上の注意記載状況・その他参考事項等

報告企業の意見	今後の対応
バベシア症は赤血球内にバベシア原虫が寄生するため発症するが、本剤は血漿を原材料にしているため感染はないと考えられる。	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。

Annals of Internal Medicine | ORIGINAL RESEARCH

Transfusion-Associated Babesiosis in the United States: A Description of Cases

Barbara L. Herwaldt, MD, MPH; Jeanne V. Linden, MD, MPH; Elizabeth Bosserman, MPH; Carolyn Young, MD; Danuta Olkowska, MD; and Marianna Wilson, MS

Background: Babesiosis is a potentially life-threatening disease caused by intraerythrocytic parasites, which usually are tickborne but also are transmissible by transfusion. Tickborne transmission of *Babesia microti* mainly occurs in 7 states in the Northeast and the upper Midwest of the United States. No *Babesia* test for screening blood donors has been licensed.

Objective: To ascertain and summarize data on U.S. transfusion-associated *Babesia* cases identified since the first described case in 1979.

Design: Case series.

Setting: United States.

Patients: Case patients were transfused during 1979–2009 and had posttransfusion *Babesia* infection diagnosed by 2010, without reported evidence that another transmission route was more likely than transfusion. Implicated donors had laboratory evidence of infection. Potential cases were excluded if all pertinent donors tested negative.

Measurements: Distributions of ascertained cases according to *Babesia* species and period and state of transfusion.

Results: 159 transfusion-associated *B. microti* cases were included; donors were implicated for 136 (86%). The case patients' median age was 65 years (range, <1 to 94 years). Most cases were associated with red blood cell components; 4 were linked to whole blood–derived platelets. Cases occurred in all 4 seasons and in 22 (of 31) years, but 77% (122 cases) occurred during 2000–2009. Cases occurred in 19 states, but 87% (138 cases) were in the 7 main *B. microti*–endemic states. In addition, 3 *B. duncani* cases were documented in western states.

Limitation: The extent to which cases were not diagnosed, investigated, reported, or ascertained is unknown.

Conclusion: Donor-screening strategies that mitigate the risk for transfusion transmission are needed. Babesiosis should be included in the differential diagnosis of unexplained posttransfusion hemolytic anemia or fever, regardless of the season or U.S. region.

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For author affiliations, see end of text.
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Babesiosis is caused by intraerythrocytic parasites, which usually are tickborne but also are transmissible by transfusion (1–9). In the United States, 2 species—*Babesia microti* and *B. duncani* (formerly, the WA1-type parasite [10, 11])—have been associated with both transmission routes. The predominant zoonotic agent is the rodent parasite *B. microti*, which is transmitted by *Ixodes scapularis* ticks in expanding foci in the Northeast and upper Midwest of the United States, particularly during spring and summer (1–3, 12). The first described tickborne and transfusion-associated *B. microti* cases occurred in Massachusetts in 1969 and 1979, respectively (13–15); the first such *B. duncani* cases were in Washington in 1991 and 1994 (10, 16).

Regardless of the transmission route, *Babesia* infection can range from asymptomatic to severe, in part depending on host factors (for example, asplenia and advanced age). Clinical infection is characterized by hemolytic anemia and nonspecific flu-like symptoms (such as fever, chills, and myalgia). Complications can include multiorgan dysfunction, disseminated intravascular coagulation, and death (1–3, 6, 7). Although a history of babesiosis is an exclusion criterion for blood donation (1), persons who meet all eligibility criteria (for example, they feel well, are afebrile, and are not anemic) can have low-level parasitemia and remain infective for months, even longer than a year (1–6, 16, 17). No *Babesia* assay for screening donors has been approved by the U.S. Food and Drug Administration (FDA) (1).

Posttransfusion babesiosis has been increasingly recognized (5–9, 18–29). However, national data and perspective about the U.S. burden of cases have been lacking. The Centers for Disease Control and Prevention (CDC) led a collaborative endeavor to ascertain and compile data on U.S. posttransfusion cases identified during the 3 decades since the first described case in 1979 (14). Here we summarize the transfusion-associated *Babesia* cases that we ascertained, including their distributions by species, time, and place.

METHODS

Data Sources

Since the 1960s, the CDC's Parasitic Diseases Laboratory has been a national reference laboratory for *Babesia* testing. The CDC is often contacted regarding diagnos-

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Conversion of graphics into slides

Context

Babesiosis, a parasitic infection transmitted through tick bites, can also be acquired via blood transfusion and may result in life-threatening disease. There is no U.S. Food and Drug Administration–licensed test to screen blood donors for *Babesia* infection.

Contribution

The risk for transfusion-associated *Babesia* infection may be increasing. Cases have occurred year-round and have been seen in states where *Babesia* species are not endemic.

Caution

Although the cases ascribed to transfusion undoubtedly represent a fraction of those that occurred, some tickborne cases inadvertently might have been included.

Implication

Improvements in the prevention and detection of transfusion-associated babesiosis are urgently needed.

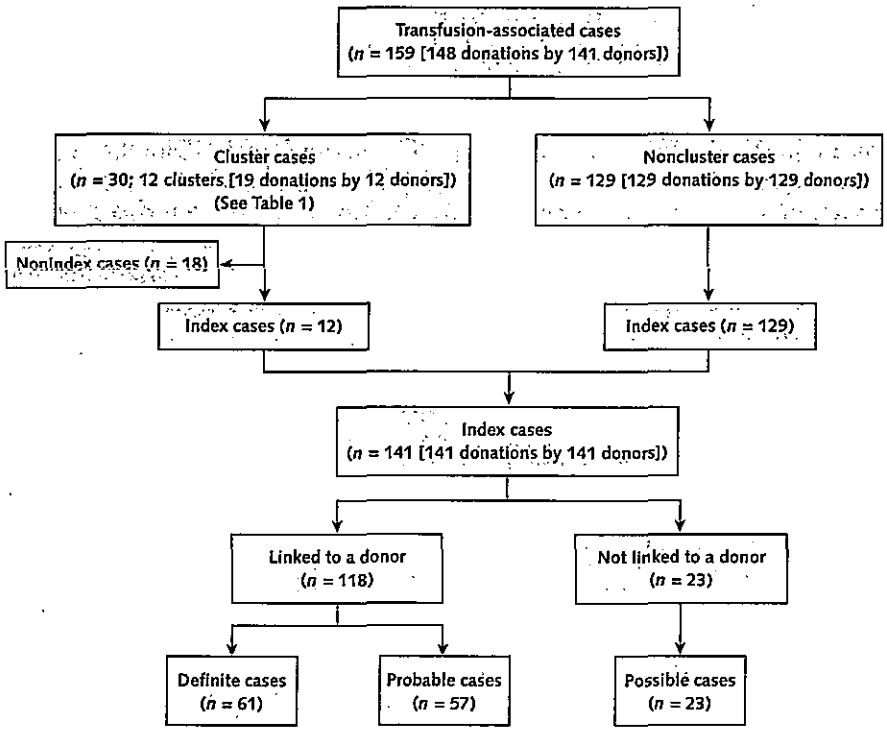
—The Editors

tic, clinical, and epidemiologic aspects of transfusion-associated and other *Babesia* cases. In addition to CDC records (such as records of test results, consultations, and case investigations), data sources for this endeavor included health departments, blood collection and transfusion services, other health professionals, and published materials and abstracts. The data available via health departments varied by jurisdiction and period; babesiosis was not a reportable disease in all states and was not nationally notifiable until January 2011. Although data were not systematically collected, some health departments, including those in babesiosis-endemic states, have routinely notified the CDC of potential transfusion cases and have submitted *Babesia* surveillance data to the CDC. Despite the inherent limitations of passive surveillance, collaborative relationships with public health and other pertinent agencies facilitated case ascertainment and data collection. We compiled and compared information obtained from multiple sources to maximize the quantity and quality of data and to minimize double counting.

Case Criteria and Classification

For these analyses, we established selection and classification criteria for transfusion-associated *Babesia* cases.

Figure 1. Stratification of 159 U.S. transfusion-associated *Babesia microti* cases, 1979–2009.



By type of case (cluster vs. not; index vs. not) and by class of index case (definite, probable, or possible). This figure, in conjunction with Table 1, provides perspective about the criteria for and the tallies of cases, donors, and donations. The 159 *B. microti* cases include 141 index cases and 18 nonindex, cluster cases. Each index case was associated with a different donor, whether implicated (n = 118) or virtual (n = 23; see Methods section). The 61 index cases classified as definite include the index cases for the 12 multicase clusters (Table 1), which encompass 18 additional cases, for a total of 79 cases. The 3 *B. duncani* cases are not included in the figure.

Table 1. Twelve Clusters of U.S. Transfusion-Associated *Babesia microti* Cases, 1979–2009*

Cluster	State (Year) of Transfusion	Case Type	Case Characteristics	Data on *Babesia* Case†	Comments About Recipients Other Than Case Patients‡
7 single-donation clusters§					
A	RI (2004)	Index	Preterm infant	Smear/PCR-positive	Another preterm corecipient of RBCs was treated empirically
		Corecipient	Preterm infant	Smear/PCR-positive	–
		Corecipient	Preterm infant	Smear/PCR-positive	–
B	RI (2006)	Index	Preterm infant	Smear-positive	No additional information
		Corecipient	Preterm infant	PCR-positive	–
		Corecipient	Preterm infant	PCR-positive	–
C	VA (2009)	Index	Preterm infant	Smear/PCR-positive	No other corecipients
		Corecipient	Preterm infant	Smear/PCR-positive	(Lookback: recipient of RBCs donated 3 mo earlier tested negative)
		Corecipient	Preterm infant	Smear/PCR-positive	–
D	NY (1997)	Index	Full-term infant	Smear/PCR-positive	Platelet corecipient (age 11 y) and 2 preterm corecipients of RBCs tested negative
		Corecipient	Preterm infant	Smear/PCR-positive	(Lookback >1 y earlier: RBC recipient tested negative; platelet recipient died ≤3 wk after transfusion)
		Corecipient	Age 70 y; GI bleeding	Smear/PCR-positive	–
E	NY (1999)	Index	Preterm infant	Smear/PCR-positive	Platelet corecipient reportedly was asymptomatic and was not tested
		Corecipient	Age 28 y; SCD	PCR-positive	(Lookback: "no adverse outcomes" reported for recipients associated with 2 previous donations)
F	CT (2006)	Index	Neonate	"Proven infection"	No additional information
		Corecipient	Age 32 y; SCD	"Proven infection"	–
G	MN (2008)	Index	Age 92 y; asplenic	Smear/PCR-positive	Double RBC donation: both recipients became infected and are listed here
		Corecipient	Age 36 y; surgery	PCR-positive	–
5 multidonation clusters‖					
H	MN (1999)	Lookback (July donation)	Age 78 y; GI bleeding	PCR-positive	Platelet corecipient (age 70 y) tested negative about 8 mo after transfusion
	MN (1999)	Lookback (September donation)	Age 80 y	PCR-positive	No corecipients
	MN (1999)	Index (November donation)	Age 68 y; surgery	Smear/PCR-positive	Platelet corecipient (age 81 y) tested negative about 6 mo after transfusion
	MN (2000)	Lookforward (January donation)	Age 67 y; surgery	Seropositive	RBC corecipient (age 73 y) died 2 d after transfusion
I	NY (2002)	Lookback (March donation)	Age 78 y; surgery	PCR-positive	(Further lookback: recipient associated with December 2001 donation tested negative)
	NY (2002)	Index (May donation)	Age 80 y; cirrhosis	Smear-positive	No corecipients
J	NY (2003)	Lookback (October donation)	Age 52 y; surgery	Seropositive	(Further lookback: no information about recipient of RBCs donated in August)
	MA (2004)	Index (December 2003 donation)	Age 74 y; carcinoma	Smear-positive	No additional information
K	WI (2007)	Lookback (August donation)	Age 83 y; surgery	Seropositive	(Status of other recipients of RBCs donated in 2007: 2 died; 1 tested negative; 1 lost to follow-up)
	FL (2008)	Index (February 2008 donation)	Age 83 y; GI bleeding	Smear/PCR-positive	No corecipients
L	MN (2008)	Index (August donation)	Age 61 y; leukemia	Smear/PCR-positive	No corecipients (lookback: RBC recipient associated with May donation tested negative)
	MN (2008)	Lookforward (October donation)	Age 53 y; surgery	Seropositive	No corecipients

CT = Connecticut; FL = Florida; GI = gastrointestinal; MA = Massachusetts; MN = Minnesota; NY = New York; PCR = polymerase chain reaction; RBC = red blood cell; RI = Rhode Island; SCD = sickle cell disease; VA = Virginia; WI = Wisconsin.

* The 12 identified clusters encompass 30 cases (1 per row) linked to 19 donations by the 12 implicated donors; the 30 cases include 12 index and 18 nonindex cases (11 in corecipients, 5 detected in lookback investigations, and 2 from lookforward investigations). One case was linked to whole blood–derived platelets (cluster H; fourth donation) (5); the other 29 were linked to RBC components. Among infants with available data, the smallest transfused volume was approximately 8 mL. In 2 multidonation clusters (J and K), case patients were identified in 2 states. In cluster J, both donations were in Maine, by a donor probably exposed in Massachusetts; in cluster K, a Wisconsin resident also donated in Florida. Five of 12 implicated donors had parasitologically confirmed infection, on the basis of testing an original unit segment (B, C, D, and G) or subsequent specimens (H); the donor linked to cluster H still had demonstrable parasitemia, by PCR analyses, 4 mo after the fourth donation, 10 mo after exposure (5). For cluster A's donor, a segment was available but results of PCR analyses were negative.

† "Seropositive" is noted only for the 4 nonindex cases that were not parasitologically confirmed: The reciprocal antibody titers ranged from 256 to 1024 in *B. microti* indirect fluorescent antibody testing.

‡ For recipients other than case patients, "tested negative" denotes seronegativity, at a minimum.

§ 18 cases (13 in infants and 5 in adults); 2–3 cases per cluster.

‖ 12 cases (all in adults); 1 case per donation; 2–4 donations per cluster.

Our minimal case criteria included receipt of 1 or more cellular blood components during 1979–2009, posttransfusion laboratory evidence of *Babesia* infection detected by 2010, and no reported evidence that another route of transmission (for example, tickborne or perinatal) was more likely than transfusion. We also required that linked (implicated) donors have laboratory evidence of infection. We excluded potential transfusion cases if all pertinent do-

Table 2. Characteristics of U.S. Transfusion-Associated *Babesia microti* Cases, Stratified by Type and Class (159 Total Cases, Including 141 Index Cases), 1979–2009*

Variable	All Cases (*n* = 159)	Stratification of All Cases, by Type (*n* = 159)	
		Index Cases (*n* = 141 [89%])	Nonindex Cases (*n* = 18 [11%])
Age at diagnosis, *n*	157	139	18
Median age (range; IQR), y†	65 (<1–94; 39–78)	66 (<1–94; 44–79)	34 (<1–83; <1–70)
Patients aged <1 y, *n (%)*†	18 (11)	11 (8)	7 (39)
Patients aged ≥1 y to <50 y, *n (%)*	33 (21)	30 (22)	3 (17)
Patients aged ≥50 y, *n (%)*	106 (68)	98 (71)	8 (44)
Male sex, *n/n (%)*	78/156 (50)	73/138 (53)	5/18 (28)
State of transfusion‡			
B. microti–endemic state, *subtotal n (%)*	138 (87)	122 (87)	16 (89)
Northeast (CT, MA, NJ, NY, or RI), *n*	118	108	10
Upper Midwest (MN or WI), *n*	20	14	6
Other state, *subtotal n (%)*	21 (13)	19 (13)	2 (11)
Eastern state, *n*	17	15	2
Not an eastern state, *n*	4	4	0
Year of transfusion			
Median (range)	2005 (1979–2009)	2005 (1979–2009)	2004 (1997–2009)
By period, *n (%)*			
1979–1984	4 (3)	4 (3)	0
1985–1989	3 (2)	3 (2)	0
1990–1994	6 (4)	6 (4)	0
1995–1999	24 (15)	19 (14)	5 (28)
2000–2004	31 (20)	26 (18)	5 (28)
2005–2009	91 (57)	83 (59)	8 (44)
Month of symptom onset or diagnosis, *n*§	–	128	–
Median (range)	–	Sep (Jan–Dec)	–
Interval from transfusion to diagnosis, *n*‖	–	114	–
Median (range; IQR), *d*	–	42 (14–230; 34–53)	–
Parasitologically confirmed infection, *n (%)*¶	153 (96)	139 (99)	14 (78)
Surgical splenectomy, *subtotal n***	32	32	0
History, *n*	17	17	0
Peritransfusion, *n*	12	12	0
≥1 mo after transfusion, *n*	3	3	0
Underlying condition or context for transfusion (1 per patient), *n*			
Hematologic disorder, *subtotal n*	39	37	2
Hematologic cancer	14	14	0
Sickle cell disease	11	9	2
Thalassemia major	7	7	0
Other hematologic disorder	7	7	0
Cardiovascular surgery or procedure	22	20	2
Gastrointestinal disease, bleeding, or surgery	19	17	2
Trauma with posttraumatic splenectomy**	8	8	0
Solid-organ transplantation††	5	5	0
Other surgery, procedure, or trauma	13	9	4
Newborn or complications of prematurity	16	9	7
Carcinoma	13	13	0
Other medical reason or diagnosis	14	14	0
Not specified	10	9	1
All-cause mortality, *n (%)*‡‡	28 (18)	27 (19)	1 (6)
Blood donor, *n (%)*§§	136 (86)	118 (84)	18 (100)
Parasitologically confirmed, *subtotal n*	24	24	–
PCR-positive unit segment, *n*	12	12	–

CT = Connecticut; IQR = interquartile range; MA = Massachusetts; MN = Minnesota; NJ = New Jersey; NY = New York; PCR = polymerase chain reaction; RI = Rhode Island; WI = Wisconsin.

* Data are number of cases/patients, unless otherwise noted. Diagnosis refers to babesiosis. Transfusion and blood donor refer to those associated with a case. Percentages might not total 100% because of rounding.

† Because a lower proportion of patients with index vs. nonindex cases were younger than 1 y ($P = 0.001$), the age distributions for index vs. nonindex patients were significantly different ($P = 0.009$), but not if the age comparison was limited to adults ($P = 0.3$).

‡ See Methods section and Figure 2. The "eastern state" category consists of Delaware, Florida, Indiana, Maryland, New Hampshire, North Carolina, Ohio, Pennsylvania, and Virginia. The "not an eastern state" category consists of California, Texas, and Washington.

§ If both were known and were different, the earlier month was specified. Data for the kidney donor (see text) were not included in analyses of month of diagnosis or interval to diagnosis.

‖ See Figure 3 regarding index patients. Among nonindex patients (Table 1), the interval to diagnosis depended on host factors, type of recipient (corecipient vs. other), and various aspects of the investigations. Although most of the ascertained nonindex patients who were adults reportedly were asymptomatic, clinical information in such regards typically was anecdotal or unspecified. In some investigations, other recipients could not be tested because they had already died.

Table 2—Continued

Stratification of Index Cases, by Class (n = 141)		
Definite Cases (n = 61 [43%])	Probable Cases (n = 57 [40%])	Possible Cases (n = 23 [16%])
60	56	23
69 (<1–94; 27–81)	65 (<1–92; 45–78)	67 (<1–87; 53–77)
9 (15)	1 (2)	1 (4)
11 (18)	16 (29)	3 (13)
40 (67)	39 (70)	19 (83)
25/60 (42)	33/55 (60)	15/23 (65)
44 (72)	57 (100)	21 (91)
–	–	–
–	–	–
17 (28)	0	2 (9)
–	–	–
–	–	–
2005 (1980–2009)	2006 (1979–2009)	2005 (1993–2009)
3 (5)	1 (2)	0
2 (3)	1 (2)	0
1 (2)	4 (7)	1 (4)
9 (15)	5 (9)	5 (22)
13 (21)	9 (16)	4 (17)
33 (54)	37 (65)	13 (57)
56	52	20
Aug (Jan–Dec)	Oct (Jan–Dec)	Sep (Jan–Dec)
53	50	11
43 (22–230; 35–52)	42 (14–225; 34–58)	42 (14–54; 21–52)
61 (100)	55 (96)	23 (100)
11	12	9
8	8	1
2	2	8
1	2	0
11	20	6
3	7	4
4	5	0
3	3	1
1	5	1
8	7	5
8	6	3
2	2	4
1	4	0
7	2	0
8	1	0
5	6	2
9	3	2
2	6	1
11 (18)	12 (21)	4 (17)
61 (100)	57 (100)	0
22	2	0
12	0	0

¶ Index cases were known or presumed to be parasitologically confirmed, with the exception of 2 cases classified as probable transfusion cases: the case in the kidney donor (see text) and a case diagnosed in retrospect, after recovery (30).

** The data constitute minimum numbers of case patients. Among the 12 known to have undergone splenectomy during the peritransfusion period, the contexts were trauma (n = 8) or abdominal surgery for other reasons (n = 4). The cases in the 3 patients known to have undergone posttransfusion splenectomy include 1 definite case (the index case of cluster L [25]; Table 1) and 2 probable cases, including the first described transfusion case (14).

†† Three received a kidney (living related [31], living unrelated, or cadaveric), 1 received a heart (29), and 1 underwent bilateral lung transplantation.

‡‡ Although outcome data were unavailable for some patients, we assumed that no other case patients died in the short term. The patients known to have died include 2 cluster-associated infants whose gestational ages were 23 and 24 wk, 2 (of 5) patients aged ≥90 y, and 6 (of 32) patients known to have undergone surgical splenectomy.

§§ In at least 4 case investigations, more than 1 donor had laboratory evidence of infection, typically 1 of whom was the most plausible on the basis of laboratory or epidemiologic data. However, the possibility of receipt of more than 1 contaminated unit could not be excluded.

nors tested negative. If multiple cases were linked to the same donor, we defined the interrelated cases as a cluster, the first identified case as the index case (1 per donor), and the other cases in the cluster as nonindex cases (Figure 1 and Table 1). To facilitate bookkeeping, we defined all cases that were not cluster-associated as index cases (1 per donor).

In general, index cases were parasitologically confirmed (Table 2) (30, 31); their detection prompted a transfusion investigation; and the linked donors and nonindex cases, if any, that were identified had parasitologic or serologic evidence of infection. We defined parasitologic evidence as detection of *Babesia* parasites (on blood smear or by animal inoculation) or *Babesia* DNA (by a molecular method). Serologic evidence of *B. microti* infection required positive results either by indirect fluorescent antibody (IFA) testing for total immunoglobulin or IgG or by immunoblot for IgG.

Index *Babesia* cases that fulfilled the selection criteria were classified as definite, probable, or possible transfusion-associated cases (Figure 1). If no donor was implicated among the subset of pertinent donors who could be tested, an index case was defined as a possible case, even if transfusion was the only known risk factor for infection. All index cases that were linked to a donor were classified as definite or probable cases. An index case was defined as a definite (vs. probable) transfusion case if at least 1 of the following additional criteria was fulfilled: 1) Transfusion was the only known or plausible risk factor for infection (for example, there was no history of residence or travel in babesiosis-endemic areas); 2) a multicase cluster was identified, with at least 1 nonindex case besides the index case (Table 1); 3) the linked donor's infection was parasitologically confirmed by testing an extant segment from the original blood unit; or 4) other donor evidence indicated active infection at the time of donation (for example, a polymerase chain reaction [PCR]–positive specimen that reflected the donor's status at donation).

Data Analysis

We conducted univariate analyses for descriptive purposes by using Epi Info, version 3.5.1 (CDC, Atlanta, Georgia), and SAS software, version 9.2 (SAS Institute, Cary, North Carolina). Proportions were compared by using the chi-square test, or if expected cell counts were less than 5, the Fisher exact test. The Wilcoxon 2-sample test was used to compare the ranked distributions of ordinal variables. Statistical significance was defined as a 2-sided P value less than 0.05.

Unless otherwise specified, we stratified cases by period and state of transfusion (Table 2 and Figure 2) (32). We refer to 7 states with well-established foci of zoonotic transmission as "*B. microti*–endemic states": 5 states in the Northeast (Connecticut, Massachusetts, New Jersey, New York, and Rhode Island) and 2 in the upper Midwest (Minnesota and Wisconsin) (1, 12). The distinction between these and other states (for example, in Figure 2) is not meant to imply that tickborne transmission occurs throughout these 7 states, that it occurred in all 7 states throughout 1979–2009, or that these are the only states in which it did or does occur. Of note, during case selection and classification, we considered the evolving focality of tickborne transmission within and among states.

Role of the Funding Source

The study received no external funding.

RESULTS

General Perspective and Summary

For the period of 1979–2009, we included 162 transfusion-associated cases: 159 *B. microti* cases and 3 *B. duncani* cases, which are described separately. The 159 *B. microti* cases include 12 multicase clusters encompassing 30 cases: 12 index cases (1 per cluster) and 18 nonindex cases (5, 8, 9, 20–25) (Figure 1 and Table 1). In total, 141 *B. microti* cases were defined as index cases: the 12 cluster-associated index cases and 129 additional cases (Table 2). Figure 2 shows their distribution by period and state of transfusion. During the initial 11 years (1979–1989), 7 index cases occurred in 5 states (14, 17, 32–36). In contrast, during the third decade (2000–2009), 109 index cases (77% of 141) and 122 total cases (77% of 159) occurred in 18 states (5–9, 18–21, 24–29, 37–42). The associated blood donations occurred in all 12 months (Appendix Figure, available at www.annals.org); 59% were during July–October.

Overall, 122 (87%) of the index cases (138 total cases [87%]) were associated with transfusions in the 7 main *B. microti*–endemic states (Figure 2 and Table 2), although not necessarily in areas of endemicity. The other 19 index cases (13%) generally were attributable to interstate movements of donors or blood components (Figure 2). Various scenarios are exemplified by the 4 cases not in eastern states (Table 2), 2 of which were attributable to donor travels: A Rhode Island resident donated while training in Washington (26), and a Texas resident donated in that state after spending the summer in Massachusetts (6). In contrast, the other case in Texas and the case in California were linked to donations in New Jersey and Maine (27), respectively. Local distributions of components collected in New Jersey also accounted for 2 cases in Pennsylvania (8, 37) and 1 in Delaware (18).

Case Characteristics

Table 2 summarizes selected characteristics of the cases, stratified by type of case (index vs. nonindex) and by class of index case (definite, probable, or possible). Table 1 provides additional perspective on the cluster-associated cases, which necessitated distinguishing between index and nonindex cases. Overall, the case patients had a median age of 65 years; 32% were either very old (33 were in the ninth or tenth decade of life) or very young (18 were infants, 13

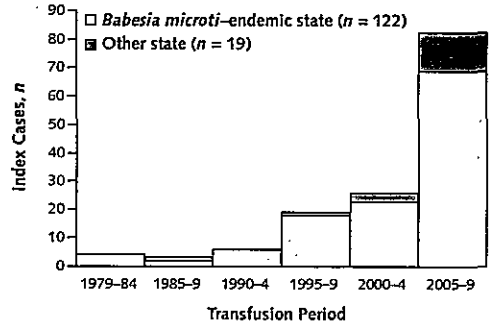
of whom were cluster-associated). The 19 patients with hereditary blood disorders account for 73% of the 26 patients in the age range of 4 to 43 years. These 19 patients include 11 with sickle cell disease (8, 9, 28), 7 with thalassemia major (35, 43), and 1 with Diamond–Blackfan anemia (18); they account for at least 9 of the 32 patients known to have undergone surgical splenectomy. Three elderly patients with hematologic disorders underwent posttransfusion splenectomy (32, 134, or 215 days later), and their *Babesia* cases were diagnosed thereafter (Figure 3 and Table 2). For 2 of these patients, parasites were noted during retrospective review of presplenectomy blood smears, a finding that refocused the investigations on earlier transfusions and donors than on those initially targeted.

Five patients with transfusion cases had been transplanted with solid organs within the previous 3 months (Table 2). In addition, indirect evidence suggests that a kidney donor who received multiple transfusions the day he died served as a conduit of *Babesia* parasites from 1 of his blood donors to both of his kidney recipients, who developed parasitologically confirmed infection (40). No *B. microti* antibodies were detected by IFA testing of archived pretransplantation serum from the kidney recipients or of pretransfusion serum from the kidney donor (Table 2). However, postdonation specimens from 1 of his blood donors were seropositive (24).

The median interval from transfusion to onset of clinical manifestations was 37 days (range, 11 to 176 days) among 84 index patients with available data (Figure 3). Although babesiosis generally is considered a febrile illness, 13 (of 105) index patients were afebrile (9, 26, 32), including at least 4 adults who had cancer or were receiving immunosuppressive therapy. The median interval from symptom onset to diagnosis of index cases was 6 days (range, 0 to 54 days; $n = 84$). Babesiosis often was diagnosed incidentally, in some instances during routine outpatient evaluations (6), during hospitalizations for unrelated reasons, or after the patient had recovered (30) or died (data not shown). Typically, *Babesia* parasites were an unexpected finding when a blood smear was examined, usually in the context of a complete blood count with a manual differential (9). When intraerythrocytic ring forms were noted, malaria was the first diagnostic consideration for more than 20 index patients, at least 14 of whom were initially treated for malaria.

The minimum all-cause mortality rate among index patients was 19% (6–9, 18, 19, 32–34, 40, 44) (Table 2); Figure 3 provides various intervals to death. Some patients had a bleak prognosis even without the potential compounding effects of babesiosis. The 27 index patients known to have died include the kidney donor described earlier, whose posttrauma death on the day of transfusion clearly was unrelated to babesiosis. For other patients with available data, there was a spectrum of likelihood that

Figure 2. Distribution of U.S. transfusion-associated *Babesia microti* index cases, 1979–2009.



Cases, *n*	1979–84	1985–9	1990–4	1995–9	2000–4	2005–9
Endemic states*						
Massachusetts	2				2	12
New York	2			7	10	26
Connecticut		1	4	7	1	6
Minnesota		1	1	1		8
Rhode Island			1	1	8	11
New Jersey				2	1	4
Wisconsin					1	2
Other states†						
New Hampshire‡		1				
Maryland§				1		2
Pennsylvania					1	2
Texas					1	1
Washington					1	
Ohio‖						2
Indiana‖						1
Delaware						1
North Carolina						1
California						1
Florida						2
Virginia§						1
Total index cases per period	4	3	6	19	26	83

By period and state of transfusion. The data are limited to the 141 *B. microti* index cases, 12 of which were associated with multicase clusters (Table 1). Data for the 3 *B. duncani* cases, which occurred in Washington (in 1994) and California (in 2000 and 2008), are not included. The *x*-axis includes one 6-year period (1979–1984), followed by five 5-year periods. See the Methods section for the distinction between the 7 main *B. microti*–endemic states and "other states"; within each category, for the tallies by state (by period), the states generally are listed in the order of their first identified case.

* Local and intraregional movements of donors and blood components were common both in the Northeast and in the upper Midwest (data not shown).

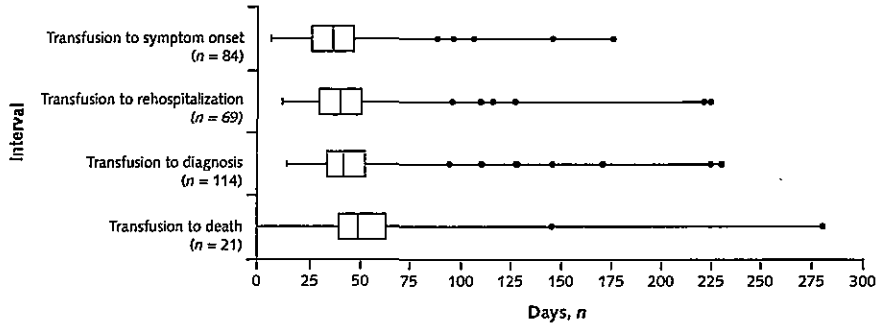
† Among the 19 index cases in 12 "other states," the North Carolina case and 1 Florida case were not linked to donors, the other Florida case was linked to a Wisconsin resident who donated blood while wintering in Florida (cluster K in Table 1), and 1 of 3 Pennsylvania cases was linked to a Pennsylvania donor who reportedly had not traveled to a known *B. microti*–endemic area in another state (8). Information on the donors linked to the other 15 index cases is provided in the text or the footnotes below for 7 and 8 cases, respectively.

‡ The donor was exposed in Massachusetts (32).

§ The 4 index cases in Maryland and Virginia were linked to donations in these states. The linked donors either were or could have been exposed in the Northeast.

‖ The cases in Ohio ($n = 2$) and Indiana ($n = 1$) were linked to donations in Indiana ($n = 2$) and Ohio ($n = 1$) by donors exposed in *B. microti*–endemic states.

Figure 3. Box-and-whisker plots of the distributions of time from transfusion to various events for U.S. transfusion-associated *Babesia microti* index cases, 1979–2009.



The data are limited to the subsets of the 141 index patients for whom particular intervals were relevant and were known or estimable (for example, the incubation period was unclear for some patients who had comorbid conditions or altered mental status). Each box represents the interquartile range (IQR), the internal vertical line indicates the median, the whiskers show the minimum and maximum, and the dots indicate the outliers with the longest intervals (≥75% quartile plus 1.5 × IQR). The 21 total dots—5 (6%), 6 (9%), 8 (7%), and 2 (10%) for the first, second, third, and fourth intervals from the top, respectively—are accounted for by 8 case patients, all of whom were linked to a donor. The farthest outliers include a patient with sickle cell disease who received hematopoietic progenitor cells from a sibling with sickle trait and became symptomatic approximately 6 months after the implicated peritransplantation transfusion (28) and 2 of 3 patients who underwent posttransfusion splenectomy (see text). The second interval from the top reflects the posttransfusion hospitalization during which babesiosis was explicitly diagnosed, for patients who had been discharged at least once in the interim or had been transfused as outpatients but were hospitalized thereafter. The fourth interval includes data for 21 of 27 index patients known to have died, including the kidney donor who died on the day he was transfused (see text). This interval was greater than 90 days for 2 immunocompromised patients whose intervals from diagnosis to death were less than 60 days. The patient who underwent splenectomy 215 days after transfusion died 280 days after transfusion; the patient's lymphoma also relapsed. For patients with available data, the median interval from symptom onset to death was 10 days (range, 2 to 51 days; $n = 18$) and the median interval from diagnosis to death was 7 days (range, 0 to 55 days; $n = 22$).

babesiosis had a causal or contributory role (6, 7); causes of death often were presumptive or unclear (data not shown).

Blood Donors and Components

A linked donor with laboratory evidence of *B. microti* infection was identified for 118 index cases (84%), which encompass 136 total cases (86%) (Figure 1). Among the 117 linked donors whose *B. microti* IFA test results were known, the median reciprocal antibody titer was 256 (range, 64 to 4096; interquartile range, 256 to 1024). Twenty-four donors (20%) had parasitologically confirmed infection (Table 2). The 20 donors with positive PCR results include 12 (71%) of 17 for whom blood retained from the original donation was tested compared with 8 (14%) of 56 for whom only postdonation specimens were available ($P < 0.001$). The median age of the 80 donors with available data was 49 years (range, 17 to 72 years); 18 donors (23%) were at least 60 years of age. Although clinical information typically was anecdotal or unspecified, some donors had pre- or postdonation symptoms or anemia of potential relevance (5, 24–27). For example, the donor who had 4 consecutive donations linked to transmission (cluster H in Table 1) had been temporarily deferred because he was anemic when he first attempted to donate after exposure (5).

Among the 151 cases for which the type of blood component was determined, 4 cases were linked to whole blood–derived platelets (4, 5, 14) and 147 were associated with red blood cells (RBCs). The median age of liquid-stored RBCs at the time of transfusion was 16 days (range, 4 to 40 days; $n = 106$); 4 case patients received RBCs that were 35 to 40 days old. At least 4 patients received frozen-deglycerolized (vs. liquid-stored) RBCs (18, 35, 43). Many patients received leukoreduced RBCs (data not shown); at least 10 received irradiated RBCs.

Babesia duncani Cases

The 3 documented *B. duncani* cases were linked to RBC transfusions in Washington (in 1994 [16]) and California (in 2000 [45] and 2008). In each instance, the case patient and implicated donor lived in the same state and had parasitologically confirmed infection. The case patients include a preterm infant (45), a 59-year-old man with a hemoglobinopathy (Bloch EM, Herwaldt BL, Leiby DA, et al. Unpublished data), and a 76-year-old man with a myelodysplastic syndrome who underwent cardiac surgery (16).

DISCUSSION

Babesiosis is an uncommon but potentially life-threatening complication of transfusion that has been increasingly recognized since the first described U.S. case in 1979. Donor-screening practices do not yet include routine testing for evidence of *Babesia* infection. In this context, prompt detection, treatment, investigation, and reporting of *Babesia* cases are essential. Babesiosis should be included in the differential diagnosis of unexplained post-transfusion hemolytic anemia, with or without fever, regardless of the season or U.S. region. To enhance the abil-

ity of public health authorities to detect, monitor, and prevent transfusion and tickborne cases, babesiosis has been designated a nationally notifiable condition, effective January 2011; as such, cases reported to health departments are notifiable to the CDC.

For the 31-year period of 1979–2009, we included 159 *B. microti* transfusion-associated cases, which were dispersed in time (all 4 seasons and 22 years) and place (19 states). Protracted parasitemia in some infected donors (5, 6, 16, 17), donor travels to and from areas of endemicity (6, 26), and distributions or shipments of blood components account for the potential for year-round transmission anywhere in the country. Donor travel also accounted for the 1 reported transfusion-associated case of babesiosis in Canada, which was linked to a Canadian donor infected during a camping trip in Massachusetts (46). The majority (87%) of the 159 identified U.S. cases occurred in the 7 main *B. microti*–endemic states, which probably reflects higher risk and greater awareness. The annual case counts fluctuated, both overall and by locale (data not shown); the limited available risk estimates for transfusion transmission also have varied in time and place (2, 3, 8, 9, 30, 39). Even so, that the majority (77%) of these 159 cases occurred during 2000–2009 is noteworthy, regardless of whether some of the aggregate increase reflects improved recognition and reporting. In comparison, for the period of 1979–2009, the CDC's National Malaria Surveillance System tallied 49 cases of transfusion-associated malaria, only 5 of which occurred during 2000–2009 (Arguin P. Personal communication). *Babesia microti* has become the most frequently reported transfusion-transmitted parasite in the United States (2, 3). In general, public health reports of tickborne *Babesia* cases also have increased in aggregate, with temporal and spatial fluctuations (CDC. Unpublished data); a national surveillance definition was first implemented in January 2011.

In addition to the 159 *B. microti* cases, we included 3 *B. duncani* cases in western states (16, 45), for a total of 162 transfusion-associated cases. The *B. duncani* cases, like those caused by *B. microti*, were in patients who ranged from preterm to elderly and who had comorbid conditions. That infection with *B. duncani*—and with other U.S. zoonotic *Babesia* agents described since the 1990s (47, 48)—is not detected by serologic or molecular assays for *B. microti* has implications for diagnostic testing, transfusion investigations, and potential future donor screening.

As expected, almost all cases for which the type of component was determined were associated with RBC transfusions. Red blood cell components of all storage ages, including greater than 5 weeks, were associated with transmission, as were components that had been leukoreduced, irradiated, or frozen. Although we did not conduct risk analyses, our findings underscore that *Babesia* parasites can survive blood bank procedures and storage conditions for RBC components. The 4 identified cases linked to whole blood–derived platelets span from 1979 (the first described transfusion case) to 2000 and presumably were attributable to residual RBCs or to extracellular parasites in the platelet units (4, 5, 14, 49). These 4 cases—and the cases in infants transfused with small RBC aliquots—underscore that small inocula can suffice to cause infection. However, even a segment from an implicated unit may test negative by PCR: The small volumes tested do not approximate the volumes transfused (1, 2).

Some of the demographic and other characteristics of the case patients reflect those of transfused patients in general (2, 4) but may have particular importance in the context of babesiosis. For example, advanced age is a risk factor for severe babesiosis, even in otherwise healthy persons; transfusion recipients often have comorbid conditions that can increase their vulnerability to the compounding effects of babesiosis and interrelated complications (such as multiorgan dysfunction and death) (6, 7, 18, 19, 33, 34). On the other hand, even some of the adult index patients were afebrile, including several patients receiving immunosuppressive therapies that may affect the host response to infection. Although most index cases with available data were diagnosed within 2 months of transfusion, a noteworthy minority of cases were diagnosed months later, such as in the context of posttransfusion splenectomy (Figure 3). These points not only have clinical relevance but also may affect transfusion investigations and case counts: The likelihood that transfusion transmission is considered and is investigated successfully may be lower for cases with longer intervals from the pertinent transfusion to symptom onset or diagnosis.

The 162 transfusion-associated cases we enumerated undoubtedly represent a fraction of those that occurred. The extent to which cases were not detected, investigated, or reported (to the CDC, to other public health authorities, or in publications) is unknown, both in general and with respect to periods, regions, and various case characteristics and outcomes. As underscored by the incidental diagnosis of *Babesia* infection, even severe cases in babesiosis-endemic regions can be missed or misdiagnosed, not just cases that are asymptomatic or mild or that occur in other U.S. regions. Even if a case is diagnosed, a transfusion investigation might not be considered, conducted, completed, or conclusive. The cases we included that were not linked to a donor (Figure 1 and Table 2) highlight the challenges associated with contacting all pertinent donors and obtaining posttransfusion specimens for testing; segments from the original donations typically are not still available. Our tallies probably constitute undercounts even of documented transfusion cases (for example, those that did not come to our attention or did not meet our selection criteria) but inadvertently might include some tickborne cases. As with all surveillance, case ascertainment, selection, and classification depended on the completeness and accuracy of the available data.

Our findings underscore the year-round vulnerability of the U.S. blood supply—especially, but not only, in and near babesiosis-endemic areas. They also highlight the importance of multiagency collaborative efforts to detect, investigate, and document transfusion cases; to assess the risks for transfusion transmission; and, thereby, to inform the scope of prevention measures. In 2009, the Transfusion-Transmitted Diseases Committee of AABB (formerly, the American Association of Blood Banks) categorized babesiosis in the highest risk level for blood safety to be prioritized for intervention (50). Donors with subclinical infection are not identified by existing measures (such as temporary deferral of persons with systemic symptoms, fever, or anemia), no *Babesia* assay for screening donors has been approved by the FDA, and pathogen reduction techniques for RBCs or platelets are not available in the United States (1, 2, 50). The FDA's Blood Products Advisory Committee that was convened on 26 July 2010 supported the concept of regional donor testing for *Babesia* (51). The increasing recognition of transfusion cases strengthens the impetus for screening strategies that mitigate the transmission risk (1–3, 50, 51), including testing approaches implemented under FDA-approved protocols (1, 3, 51) and longer-term strategies with development of a high-throughput *Babesia* screening assay.

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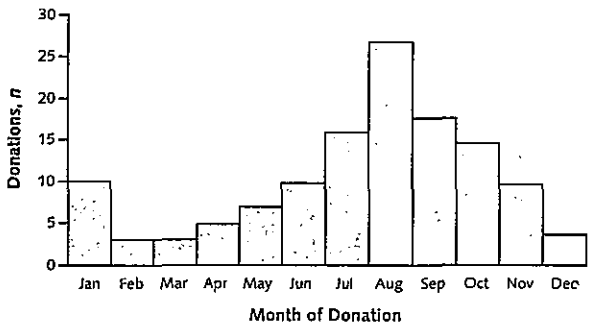
Appendix Figure. Distribution, by month of the blood donations associated with U.S. *Babesia microti* transfusion cases (n = 128 of 148 total donations), 1979–2009.



The month of donation was known or estimable for 128 of 148 donations (by 141 donors) associated with transmission (Figure 1). The 19 donations by the 12 donors linked to multicase clusters occurred in 10 different months. If applicable, the month of donation was approximated by subtracting 16 days (the median age of liquid-stored red blood cells at the time of transfusion; see text) from the transfusion date. The donations linked to the 3 *B. duncani* cases occurred in April (n = 2) and August (n = 1); these data are not included.

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
				2011. 10. 20	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社)		Simonsen KA, Harwell JI, Lainwala S. Pediatrics. 2011 Oct;128(4):e1019-24. Epub 2011 Sep 2.	米国

研究報告の概要

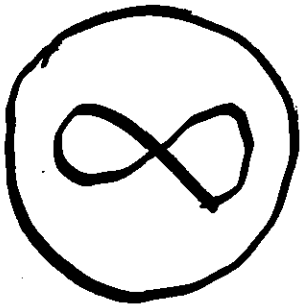
○早産新生児における輸血関連バベシア症の臨床症状と治療
米国北東部の新生児集中治療室で起きた、新生児輸血関連バベシア症の7症例を報告する。
バベシアに感染していた2つの血液製剤(赤血球)の輸血により7例のバベシア症例が発生した。臨床症状はこの集団において大きなばらつきがあった。超低出生体重児(760g)は最も重症であった。抗生物質での治療は軽度及び無症候性の感染症に効果があった。しかし最も重症であった2症例は長期の多剤処置を伴う2倍量の交換輸血が必要であった。
*Babesia microti*感染症のリスクは現在の血液銀行の業務手順では排除されない。流行地域の新生児科の医師は早産児における輸血関連バベシア症を考慮するべきである。

報告企業の意見	今後の対応
北米で早産新生児における輸血関連バベシア症が7例発生したとの報告である。	日本赤十字社では問診時にバベシア症の既往を確認し、該当する場合は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。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血

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



Clinical Presentation and Treatment of Transfusion-Associated Babesiosis in Premature Infants

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KEY WORDS
Babesia, babesiosis/diagnosis, babesiosis/transmission, babesiosis/parasitology, parasitemia/transmission, parasitemia/diagnosis, infant, premature/diseases, infant, premature/parasitology, blood transfusion/adverse effects

ABBREVIATIONS
PCR—polymerase chain reaction
pRBC—packed red blood cell
DOL—day of life
IFA—indirect fluorescent antibody
ELBW—extremely low birth weight

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abstract

We review here 7 cases of neonatal transfusion-associated babesiosis at a NICU in the northeast United States. Transfusion from 2 infected units of blood resulted in the 7 cases described. The clinical presentation was highly variable in this cohort; the extremely low birth weight neonates were the most severely affected. Antibiotic therapy was effective in neonates with mild and asymptomatic infection; however, double-volume exchange blood transfusion with prolonged multidrug treatment was required for the 2 most severe cases. The risk of *Babesia microti* infection is not eliminated through current blood-bank practices. Neonatologists in endemic areas should have a high index of suspicion for babesiosis in premature infants exposed to blood transfusions. *Pediatrics* 2011;128:e1019–e1024

Babesiosis is a zoonotic protozoal illness transmitted primarily by *Ixodes scapularis* ticks in North America. The majority of cases in the Northeast and the upper Midwestern regions are attributed to *Babesia microti*.[1] Transfusion-associated babesiosis is well documented in adults.[2–6] The clinical manifestations range from asymptomatic infection to fulminant disease and death.[3] Babesiosis in neonates occurs through blood transfusion or transplacental transmission.[8–12] Premature infants are at increased risk for babesiosis; they are immunologically compromised and may require multiple blood transfusions during their hospitalization.[13–15] We review here a series of neonatal transfusion-associated babesiosis cases and emphasize the clinical presentation and management strategies for severe disease.

METHODS

These cases occurred in 2 clusters. Index cases were identified clinically and diagnosed by using peripheral blood smears performed for manual white blood cell count and differential. Blood-bank tracing identified other exposed infants. All follow-up testing and quantifications of parasitemia were performed by using thick and thin blood smears. Blood samples for polymerase chain reaction (PCR) were obtained for confirmation of diagnosis on the day of initial positive smear result for the index patients and at the time of initial evaluation for other exposed infants. PCR testing was performed at a single reference laboratory (Mayo Medical Laboratories, Rochester, MN) using licensed methods (Roche Molecular Biochemicals, Indianapolis, IN). Infants in cluster 1 had additional confirmatory testing by PCR and blood smears performed at the Centers for Disease Control and Prevention (Atlanta, GA) using previously reported methods,[16] and all PCR results were concordant. Demographic and transfusion details of all but 1 infant (Table 1) have been included in other reports.[5,11]

Case Reports

Cluster 1

This cluster of cases included 4 very low birth weight (≤1500 g) infants with gestational ages that ranged from 24 to 27 weeks. The index case was a 25-week-gestational-age twin with a birth weight of 760 g. The infant's clinical course was significant for respiratory distress syndrome, chronic lung disease, intraventricular hemorrhage, and anemia of prematurity that required multiple packed red blood cell (pRBC) transfusions. The index transfusion occurred on day of life (DOL) 3.

On DOL 36, the infant's clinical status deteriorated with worsening respiratory status, poor perfusion, hyperthermia, generalized edema, and hepatosplenomegaly. Laboratory tests revealed anemia (hemoglobin: 10.4 g/dL), thrombocytopenia (17 000 cells per μL), and conjugated hyperbilirubinemia (14.4 mg/dL). The infant empirically received ampicillin, gentamicin, and amphotericin B while evaluation for sepsis was performed. All routine bacterial, viral, and fungal study results were negative. A peripheral blood smear performed on DOL 51 revealed that 17% of erythrocytes contained intraerythrocytic parasites consistent with *Babesia* sp. The infant began 20 mg/kg per day of clindamycin intravenously in 3 divided doses and 25 mg/kg per day of quinine orally in 3 divided doses. Double-volume exchange blood transfusion was performed, and the infant's parasitemia level decreased to 3.3%. By day 5 of therapy, parasitemia increased to 5.8% erythrocytes. The infant received a second exchange transfusion, and azithromycin (12 mg/kg per day intravenously) and atovaquone (40 mg/kg per day orally in 2 divided doses) were added to the antimicrobial regimen. The parasite load decreased significantly after the second exchange transfusion; however, low levels of detectable parasitemia persisted. Quinine was discontinued after 8 days of treatment, and all other antibiotics were discontinued on treatment day 28 after 2 peripheral blood smears obtained 3 days apart tested negative for *Babesia* sp (Table 1).

Three infants who received pRBC transfusions from the same donor blood as the index case were identified. Peripheral blood smears from all 3 infants revealed parasites, and the infants were treated with antibiotics (Table 1).[5,11] The peripheral blood smears from the mother and twin sibling of the index patient tested negative for intraerythrocytic parasites. An indirect fluorescent antibody (IFA) test on the plasma obtained from the donor pRBC sample was performed at the Centers for Disease Control and Prevention (CDC), and the results were positive for *B microti* (1:256). However, the results of PCR testing and peripheral blood smears from donor blood performed at the CDC were negative.

Cluster 2

The second cluster of babesiosis cases included 3 low birth weight (≤2500 g) infants who received pRBC transfusions from a single infected donor. The index case was born at 25 weeks' gestational age with a birth weight of 770 g. The infant's clinical course was significant for respiratory distress syndrome, chronic lung disease, intraventricular hemorrhage, and fungal sepsis. The infant received the infected pRBC transfusion on DOL 1. On DOL 33 the infant's clinical status acutely deteriorated with hypotension, respiratory distress, and splenomegaly. Laboratory evaluations revealed anemia (hemoglobin: 7.6 g/dL) and thrombocy-

TABLE 1 Summary of Cases of Transfusion-Associated Babesiosis

Case	Ref No.	Age at Transfusion, d	Gestational Age, wk	Birth Weight, g	Initial Parasitemia[a]	Signs and Symptoms at Time of Diagnosis	Time to Diagnosis, d[b]	Initial *Babesia* PCR Result[a]	Treatment (Duration of Each Antibiotic, d)	Time From First Positive to First Negative Smear Result, d
Cluster 1										
Index case 1	6	2	25	760	+ (17%)	Anemia, thrombocytopenia, hepatosplenomegaly, direct hyperbilirubinemia, respiratory distress	49	+	2 V2EBTs[c]: quinine (8); clindamycin (28); azithromycin (22); atovaquone (22)	22
2		41	24	520	+ (1 organism thick prep)	None	26	–	Clindamycin (9); quinine (9)	1
3	6	1	27	1220	+ (0.9%)	None	51	+	Clindamycin (14); quinine (14)	12
4	6 and 11	57	27	750	+ (1.5%)	Hepatosplenomegaly, anemia, hyperbilirubinemia	47	+	Clindamycin (14); quinine (14)	2
Cluster 2										
Index case 5	6	1	25	770	+ (17.2%)	Hypotension, anemia, thrombocytopenia, splenomegaly, respiratory distress	32	NA	1 V2EBT; clindamycin (16); quinine (6); quinidine (10)	13
6	6	2	27	960	–	Pallor, anemia, thrombocytopenia	35	+	Clindamycin (10); quinine (10)	—
7	6	98	32	1730	–	None	41	+	Clindamycin (10); quinine (10)	—

V2EBT indicates double-volume exchange blood transfusion; NA, not available.

[a] + indicates positive test result; –, negative test result (all PCR tests were performed on the day of first positive smear result or first evaluation for babesiosis).

[b] Days from the day of infected blood transfusion to the day that parasitemia was detected on blood smear or by PCR.

topenia (16 000 cells per μL), and on manual differential *Babesia* sp were suspected. Peripheral blood smear quantification revealed 17% parasitemia. The infant received a double-volume exchange blood transfusion and began clindamycin (30 mg/kg per day intravenously in 3 divided doses) and quinine (25 mg/kg per day orally in 3 divided doses). Five days later, quinine was discontinued and quinidine (0.02 mg/kg per minute intravenously) was started. Antibiotic therapy was discontinued after 3 peripheral blood smears (obtained daily) tested negative for intraerythrocytic *Babesia* sp (*Table 1*).

Two infants who received pRBC transfusions from the same donor blood were identified. Although blood smears tested negative, the results of PCR tests for *B microti* were positive for both infants. Their management is shown in Table 1. Donor blood from this cluster was not tested.

DISCUSSION

We present a series of transfusion-associated babesiosis cases in infants that encompass a wide spectrum of incubation periods and clinical presentations. We also report use of double-volume exchange blood transfusion as an adjuvant therapy for severe babesiosis in infants. Previous cases of transfusion-associated babesiosis in infants are summarized in Table 2.

Premature infants are at increased risk for transfusion-associated babesiosis. They are more susceptible to infections because of their immature immune systems and functional hyposplenia.[17,18] Also, extremely low birth weight (ELBW) (≤1000 g) infants are more likely to receive frequent transfusions during their first days of life.[13,14] In our report, the 2 most severely affected ELBW infants received infected blood transfusions on DOL 1 and 3. They had complicated neonatal

TABLE 2 Review of Reported Cases of Transfusion-Associated Babesiosis in Infants

Age at Transfusion	Ref No.	Diagnosis	Blood Smear Result	Signs and Symptoms at Time of Diagnosis	Time to Diagnosis	Test Results	Treatment (Duration, d)	Time to Negative Blood Smear Result, d
22 d	2 and 9	Hypoplastic lung	+	Fever	22 d	PCR, +; IFA, +; hamster inoculation, −	Quinine (12); clindamycin (12); atovaquone (8)	8
31 d	2 and 9	Prematurity	+	None	42 d	PCR, +; IFA, +; hamster inoculation, +	Azithromycin (7); atovaquone (7)	4
57 d	2 and 9	Prematurity	−	None	NA	PCR, −; IFA, −; hamster inoculation, −	Azithromycin (7); atovaquone (7)	NA
11 d	2 and 9	Prematurity, necrotizing enterocolitis	−	None	NA	PCR, −; IFA, −; hamster inoculation, −	Observation (NA)[a]	NA
42 d	25	Prematurity	+	Thrombocytopenia, anemia, hyperbilirubinemia, tachypnea, lethargy, jaundice, hepatosplenomegaly	6 wk	IFA, +	Chloroquine (2); clindamycin (7); quinine (7)	7
27 d	26 and 27	Prematurity	+	Apnea, bradycardia, desaturations, splenomegaly, anemia, thrombocytopenia, mildly elevated transaminases levels, respiratory distress	22 d	PCR, +; hamster inoculation, +; IFA, +	Quinine (15); clindamycin (15)	10
49 d	2	Prematurity	+	Respiratory distress, anemia	<40 d	IFA, +; PCR, +	Not available	Parasitemia resolved, day not reported
8 mo	28	Congenital heart disease, status post cardiac surgery	+	Fever, anemia, thrombocytopenia, elevated transaminase levels, pallor, cyanosis, hypoxia	1 mo	PCR, +; IFA, +	Azithromycin (10); atovaquone (10)	5 (parasitemia < 1%)
7 mo	28	Congenital heart disease, status post cardiac surgery	+	Fever, pancytopenia, tachypnea, hepatosplenomegaly, petechiae (eyelids), urinalysis positive for ketones, bilirubin, blood, and protein	25 d	PCR, +	Azithromycin (5); atovaquone (11)	NA

+ Indicates positive test result; −, negative test result; NA, not applicable.
[a] Not treated because of necrotizing enterocolitis.

clinical courses that included intraventricular hemorrhage, respiratory distress syndrome, and fungal infection and required prolonged antibiotic therapy and double-volume exchange blood transfusions to resolve the infections. A third ELBW infant received the infected blood on DOL 2 and presented with pallor, anemia, and thrombocytopenia. The incubation period in tick-acquired babesiosis is 1 to 6 weeks; however, in transfusion-associated cases it can be as long as 9 weeks.[19,20] In our series, the incubation period was 5 to 7 weeks. The clinical manifestations of babesiosis range from asymptomatic infection to fulminant disease including acute respiratory failure, congestive heart failure, disseminated intravascular coagulation, liver and renal failure, and splenic infarction.[19] A mortality rate of 5% to 21% has been reported in severe cases.[19] It is important to note that early signs and symptoms of infection were indistinguishable from other causes of sepsis in these premature infants. The diagnosis was made by using peripheral blood smears for the index cases. Exposed infants who were identified by using blood-bank records were considered to be infected if either the peripheral blood smear or PCR testing results were positive.

The recommended treatment for babesiosis includes a combination regimen of clindamycin and quinine or atovaquone and azithromycin for 7 to 10 days.[21,22] In our series, the 3 most severely affected infants were treated for 14, 16, and 28 days, respectively, which demonstrates that for severe illness prolonged treatment may be indicated. Once parasitemia was resolved (on the basis of 2 negative blood smear results obtained 3 days apart [index case 1] or 3 negative blood smear results obtained daily [index 2]), antibiotic therapy was discontinued (Table 1). In both index cases, enteral quinine

was discontinued when the patient remained symptomatic while on therapy. In critically ill premature neonates, enteral absorption through the immature gut may be unreliable, and intravenous quinidine should be considered an alternative. None of the infants in this series had evidence of recurrence of infection before discharge from the NICU.

Exchange transfusion is indicated for severe babesiosis associated with parasitemia of ≥10% or significant organ dysfunction.[19,22] Four adult patients with babesiosis treated with red blood cell exchange transfusion had a 50% to 90% reduction in parasitemia.[23] We report here successful use of exchange transfusion for the treatment of severe babesiosis in 2 ELBW infants who presented with initial parasitemia of 17%. After double-volume exchange blood transfusion, the parasite load in these infants decreased by 83% and 47%, respectively. Babesia parasites survive almost exclusively within erythrocytes; thus, exchange transfusion is beneficial by rapidly reducing the parasite burden and the circulating proinflammatory cytokines.[24]

In endemic areas such as Rhode Island in the northeast United States, an estimated 1 in 21 000 red blood cell units are infected with *Babesia*,[3] which places low birth weight infants at increased risk for transfusion-associated babesiosis. Splitting a single unit of pRBCs for transfusion to multiple neonates led to the clusters of infection described. ELBW infants can develop severe illness when exposed to infected blood in the first few days after birth.

Blood-banking organizations screen blood donors for babesiosis by history of infection alone. Currently, there is no US Food and Drug Administration–licensed screening assay for *B microti* in donated blood products, and no other reliable methods are available for eliminating the risk of transfusion-associated babesiosis. Removal of parasites by leukoreduction and γ irradiation are ineffective, pathogen-removal methods remain experimental, and geographic and seasonal blood-donation deferrals are impractical.[30] The American Association of Blood Banks is actively examining appropriate public health responses for effectively reducing the risk of transfusion-associated babesiosis.[3] Clinical awareness of the range of infections that result from transfusion-associated babesiosis is necessary to facilitate ongoing national discussions and participate in the development of strategies for specifically addressing this important pathogen. At present, selective screening of pRBC units for *Babesia* sp before transfusion to high-risk patients, including low birth weight infants, should be considered in endemic areas.[3]

CONCLUSIONS

Neonatologists, especially those in endemic areas, should consider babesiosis in blood transfusion-exposed infants with unexplained illness and request peripheral blood smear examination for *Babesia* sp. When transfusion-associated babesiosis is diagnosed, prompt notification to the blood bank and testing of all exposed infants is necessary. Review of our and other reported cases (Tables 1 and 2) revealed that 50% to 67% of transfusion-exposed infants develop symptomatic babesiosis. Antibiotic therapy for babesiosis in critically ill neonates may need to be prolonged beyond 7 to 10 days to ensure that parasitemia has resolved. Also, double-volume exchange blood transfusion effectively reduces the parasite load in premature infants.

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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

○3例目の輸血感染*Babesia duncani*(*B.duncani*)
背景: 米国で報告されたダニ媒介性及び輸血関連のバベシア症例はほとんどが*Babesia microti*(*B.microti*)に起因し、北東部や中西部で発生している。今回、カルフォルニア在住の59歳、鎌状赤血球症(HbSS)患者で、かつてはWA1型原虫と呼ばれた*B.duncani*による症例を調査した。唯一のリスク因子は赤血球輸血を受けたことであった。
症例報告: この症例は2008年9月に診断された。数カ月にわたり輸血量が増加した後、血液スメアにより赤血球内原虫が発見された。分子及び間接蛍光抗体(IFA)分析により*B.microti*陰性、*B.duncani*陽性(IFA価、1:1024)であった。原虫の完全な18Sリボソームrna遺伝子が血液検体から増幅された。DNA配列は1991年に分離された指標WA1型原虫の配列に一致した。すぐに輸血血液の調査が行われ、38人の関連供血者のうち34人が評価されたが*B.microti* IFAが陽性の者はいなかった。カリフォルニア在住67歳の関連供血者の*B.duncani* IFA価が1:4096であり、2009年3月にスナネズミに血液検体を接種し*B.duncani*が分離された。2008年4月の供血から10カ月以上が経っていた。当該患者は2008年5月に関連輸血を受けてから4カ月以上を経て診断された。
結論: この患者は*B.duncani*に起因する輸血関連症例の3例目である。この症例は*B.microti*のための検査では検出できないバベシアが疾病原因となり得ることを強調している。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血

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

10

報告企業の意見	今後の対応
輸血に関連した3例目の*Babesia duncani*感染症例の報告である。	日本赤十字社では問診時にバベシア症の既往を確認し、該当する場合は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。

ORIGINAL ARTICLE

The third described case of transfusion-transmitted *Babesia duncani*

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BACKGROUND: Almost all of the reported US tick-borne and transfusion-associated *Babesia* cases have been caused by *Babesia microti*, which is endemic in the Northeast and upper Midwest. We investigated a case caused by *B. duncani* (formerly, the WA1-type parasite), in a 59-year-old California resident with sickle cell disease (HbSS) whose only risk factor for infection was receipt of red blood cell transfusions.

CASE REPORT: The patient's case was diagnosed in September 2008: intraerythrocytic parasites were noted on a blood smear, after a several-month history of increasing transfusion requirements. Molecular and indirect fluorescent antibody (IFA) analyses were negative for *B. microti* but were positive for *B. duncani* (IFA titer, 1:1024). The complete 18S ribosomal RNA gene of the parasite was amplified from a blood specimen; the DNA sequence was identical to the sequence for the index WA1 parasite isolated in 1991. The patient's case prompted a transfusion investigation: 34 of 38 pertinent blood donors were evaluated, none of whom tested positive by *B. microti* IFA. The implicated donor—a 67-year-old California resident—had a *B. duncani* titer of 1:4096; *B. duncani* also was isolated by inoculating jirds (Mongolian gerbils) with a blood specimen from March 2009, more than 10 months after his index donation in April 2008. The patient's case was diagnosed more than 4 months after the implicated transfusion in May 2008.

CONCLUSIONS: This patient had the third documented transfusion case caused by *B. duncani*. His case underscores the fact that babesiosis can be caused by agents not detected by molecular or serologic analyses for *B. microti*.

Babesiosis is a tick-borne disease caused by intraerythrocytic parasites that also are transmissible by transfusion.[1-8] During the past three decades (1979-2009), more than 150 US cases of transfusion-associated babesiosis have been recognized,[2] most of which have been linked to red blood cell (RBC) components (liquid stored or frozen deglycerolized[9]); whole blood–derived platelets (PLTs) also have been implicated, presumably because of residual RBCs or extracellular parasites in PLT concentrates.[2,8,10] No test has been approved by the Food and Drug Administration (FDA) for

ABBREVIATIONS: ICU = intensive care unit; IFA = indirect fluorescent antibody.

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The findings and conclusions in this report are those of the authors and do not necessarily represent the policy of the Centers for Disease Control and Prevention.

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TRANSFUSION **;**:**-**.

screening US blood donors for evidence of *Babesia* infection.[2,3] Prevention of transfusion transmission currently relies on indefinite deferral of potential donors who have a history of babesiosis (those who answer "yes" to the question "have you ever had babesiosis?") and temporary deferral of donors who are febrile or anemic. However, persons who are otherwise healthy can meet the criteria for donating blood despite being infected; low-level parasitemia can persist for weeks to months, sometimes longer than a year.[1-3,8] *Babesia* infection also can be severe, even life-threatening,[1-4,9,11,12] particularly in persons who are asplenic, at either extreme of age, or immunocompromised, which are common characteristics of transfusion recipients.

Babesia microti, which is endemic in parts of the Northeast and upper Midwest, accounts for almost all of the reported US *Babesia* cases, including some transfusion cases documented in other US regions in the context of movement of donors or blood components.[2,6,9,11-14] The WA1- (for "Washington 1") type parasite, which was named *B. duncani* in 2006,[15] also has been associated with both routes of transmission, although its tick vector has not yet been identified. The index WA1 case occurred in 1991 in Washington State, in an immunocompetent 41-year-old man, who had a presumptive tick-borne case.[16] The next described case caused by the parasite now called *B. duncani* was transfusion associated: it occurred in 1994 in Washington, in a 76-year-old patient with myelodysplasia who underwent cardiac surgery.[17] The second documented transfusion case occurred in 2000 in California, in a preterm infant.[18] Both of these transfusion cases were linked to RBC components from healthy, 30-some-year-old donors who had presumptive tick-borne cases. Here we describe the third identified *B. duncani* transfusion case, which was diagnosed in 2008 in California, in a 59-year-old man with a transfusion-dependent hemoglobinopathy who had become refractory to RBC transfusions.

MATERIALS AND METHODS

The Centers for Disease Control and Prevention (CDC) conducted reference laboratory testing for *Babesia* infection. Giemsa-stained thin blood smears were examined by light microscopy for intraerythrocytic parasites; *B. duncani* is morphologically indistinguishable from *B. microti*.[15] Serum and plasma specimens were tested in serial fourfold dilutions, with indirect fluorescent antibody (IFA) assays for reactivity to *B. duncani* and *B. microti* antigens.[16,19]

Whole blood specimens were analyzed by molecular techniques. For *B. microti*, two-step nested polymerase chain reaction (PCR) was conducted.[8,20] The molecular approach used for *B. duncani* has been described previously, including the methods for DNA extraction, amplification, and sequencing.[21] In brief, the complete 18S ribosomal RNA (rRNA) gene was amplified by PCR, with primers that amplify DNA from parasites in the order Piroplasmida: the PCR primers were the forward primer CRYPTOFL (5′-AACCTGGTTG ATCCTGCCAG TAGTCAT-3′) and the reverse primer CRYPTORN (5′-GAATGATCCT TCCGCAGGTT CACCTAC-3′). For the organism referred to as the recipient's parasite, both strands of the PCR product were sequenced (BAB1615; GenBank Accession Number HQ289870); the sequence of the 18S rRNA coding region was compared with the sequence CDC obtained for the WA1 parasite isolated from the index case patient in 1991[16] (BAB2; Accession Number HQ285838).

Whole blood specimens (up to 1 mL) were inoculated intraperitoneally into jirds (Mongolian gerbils; *Meriones unguiculatus*), which are competent hosts for *B. duncani*.[16] The jirds were monitored weekly, up to 8 to 10 weeks or until positive, by examination of Giemsa-stained smears of blood obtained by tail snip. Animal use protocols were approved by CDC's Institutional Animal Care and Use Committee.

CASE REPORT

In late September 2008, a case of *B. duncani* infection was diagnosed in a 59-year-old California resident who had sickle cell disease, an autoinfarcted spleen, a 10-year history of transfusion-dependent anemia, and a several-month history of deteriorating health and increasing transfusion requirements. At baseline, RBC exchange transfusions at 3- to 4-week intervals sufficed to maintain a hemoglobin (Hb) level of 10 to 11 g/dL. Comorbidities included congestive heart failure and chronic renal insufficiency (baseline creatinine level of approx. 3 mg/dL), both of which had been controlled with medical therapy.

In early June 2008, several months before babesiosis was diagnosed, he was evaluated because of a febrile illness. He had a 1-week history of symptoms, which included anorexia, nausea, diarrhea, respiratory congestion, stiff neck, and 3 days of fever (37.2-38.9°C); his Hb level was 7.1 g/dL. The diagnoses included otitis media, viral infection, and dehydration; amoxicillin-clavulanate and fluids were prescribed.

During the summer of 2008, he remained afebrile. However, he had frequent outpatient and inpatient evaluations because of weakness, fatigue, and shortness of breath, which were attributed to anemia. In mid-July, his Hb level was 8.5 g/dL. After an exchange transfusion 1 week later, the interval between transfusions became progressively shorter than his 3- to 4-week norm. In mid-August, he noted darkening of his urine. He became refractory to transfusions, with Hb levels of not more than 6 g/dL, despite twice-weekly therapy with epoetin alfa. In addition, he received corticosteroid therapy from late August through mid-September for warm-antibody

autoimmune hemolytic anemia, which compounded his transfusion requirement but was considered insufficient to account for the severity of his anemia. The corticosteroid regimen included a 40-mg dose of methylprednisolone (accompanied by one dose of intravenous immune globulin), followed by tapering doses of prednisone. The patient received another 40-mg bolus of methylprednisolone in early September, with tapering doses of methylprednisolone thereafter. A Hb level of 6.9 g/dL in late August, after corticosteroid therapy had been initiated, prompted hospital admission, including a 10-day stay in the intensive care unit (ICU). On admission to the ICU, his Hb level was 4.9 g/dL. Diagnostic considerations included thrombotic thrombocytopenic purpura and a delayed-type transfusion reaction. Because of acute (superimposed on chronic) renal failure, hemodialysis was initiated and was continued thereafter.

He returned to the ICU later in September, with a 1-week history of nausea, vomiting, loose stools, anorexia, weakness, and pain (in the lower back and knees), in addition to persistent fatigue and weakness. On examination, his temperature was 37.3°C, his blood pressure was 76/45 mmHg, he was icteric and somnolent but oriented, and he had tremors in his tongue and upper extremities. Laboratory values included a Hb level of 5.8 g/dL, PLT count of 135×10^9/L, white blood cell count of 15.3×10^9/L (45% neutrophils, 20% lymphocytes, and 36% monocytes), reticulocyte count of 16.5%, total bilirubin of 9.6 mg/dL, aspartate aminotransferase of 464 U/L, and alanine aminotransferase of 117 U/L.

In late September, babesiosis was diagnosed, when intraerythrocytic parasites, including pathognomonic tetrads ("Maltese-Cross" forms), were noted on a blood smear (Fig. 1); according to the hospital laboratory, approximately 12% of the RBCs were infected. During retrospective examination of available smears, rare parasites were noted on a smear from mid-July 2008; none were found on a smear from November 2007. No smears between November and July were available.

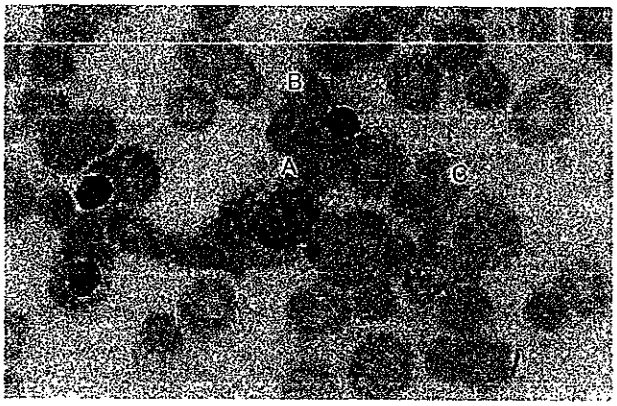

Fig. 1. Photograph of a Wright-Giemsa–stained peripheral smear of blood obtained from the patient in September 2008, showing typical *Babesia* forms: (A) a tetrad ("Maltese-Cross" dividing merozoite); (B) a piriform (tear drop); and (C) a ring-like trophozoite (magnification ×1000).

After babesiosis was diagnosed, combination therapy with clindamycin (600 mg every 6 hr by intravenous infusion) and quinine sulfate (648 mg loading dose per nasogastric tube, followed by 324 mg every 12 hr) was initiated. In addition to a several-week course of antimicrobial therapy, he received an 8-unit RBC exchange transfusion. Parasites were not demonstrable on several follow-up blood smears in October; his transfusion requirement also decreased. Because of persistent weakness and pain, which were attributed to polyneuropathy of critical illness, he was transferred to a rehabilitation facility. Over the next 6 months, his neuropathy and mentation gradually improved; he continued to receive hemodialysis.

Babesia laboratory testing

After a commercial laboratory obtained negative serologic results for *B. microti*, the CDC was consulted and conducted reference laboratory testing. CDC confirmed the diagnosis of babesiosis and determined that the etiologic agent was *B. duncani*. Results of molecular and IFA analyses of a blood specimen from late September 2008 were negative for *B. microti* but were positive by both modalities for *B. duncani* (IFA titer, 1:1024). PCR amplification of the complete 18S rRNA gene yielded a specific product of approximately 1700 bp. DNA sequencing analysis showed that the gene was 1768 bases long. The DNA sequence for the recipient's *Babesia* parasite was identical to the sequence for the index WA1 parasite that was isolated in 1991 and analyzed at CDC (see Materials and Methods for GenBank accession numbers). The CDC's 18S rRNA sequence data differ slightly (approx. 0.2%) from another laboratory's sequence for the index WA1 isolate (GenBank Accession Number AF158700), which may reflect different methods for sequencing and sequence editing. The recipient's *Babesia* parasite also was isolated by inoculating jirds with a blood specimen from the patient.

Transfusion investigation

The patient's only risk factor for *Babesia* infection was receipt of RBC transfusions: he lived in an urban area of a northern California county and did not have a history of rural outdoor activities during the previous 2 years. Diagnosis of his case of babesiosis prompted a multiagency investigation that encompassed the transfusions he received during September 2007 through June 2008, all of which were of leukoreduced, nonirradiated RBCs. Among 38 pertinent donors from the American Red Cross Northern California Region (Oakland, CA) and the Blood

Centers of the Pacific (San Francisco, CA), 34 provided specimens for testing; no segments from the original units were available.

None of the 34 tested donors had detectable antibodies to *B. microti*. Only one donor—Donor A (the implicated donor)—tested positive by *B. duncani* IFA (see below). The RBCs from Donor A were collected in April 2008, when his Hb value was 15.2 g/dL. The RBCs were transfused to the patient 25 days later, in May. The interval from this transfusion to the patient's diagnosis of babesiosis (in late September) was 130 days, and the interval from transfusion to the patient's first known positive smear (in mid-July) was 56 days. The case patient was the only recipient of a cellular component from the April donation.

Two specimens from Donor A were collected in November 2008 and March 2009 for *Babesia* testing. The *B. duncani* IFA titer was 1:4096 for both specimens, which were tested in parallel, on the same day. Molecular analyses and blood smears were negative. However, he had protracted, parasitologically confirmed infection: aliquots from the March 2009 specimen (>10 months after the index donation) were inoculated into two jirds, both of which were demonstrably parasitemic when examined on Day 21 postinoculation.

Donor A was a healthy 67-year-old resident of the San Francisco Bay Area, who was an avid hiker and mountain biker, including in the Bay Area, elsewhere in California (e.g., in the central Sierra Nevada foothills and in multiple regional and national parks), and in the Northwest. His interstate travel included hiking trips in Washington and British Columbia (the fall of 2004) and in Wyoming, Montana, and Idaho (the fall of 2007). Although he did not recall any tick bites, he reported having a possible tick bite reaction on his right shoulder after a hike in the rural Bay Area in April 2008, the month of the index blood donation. He recalled having a mild flu-like illness in October 2007, after a hike in the southern Bay Area. Although his wife had not accompanied him on that hike, she developed a similar illness; a blood specimen she provided in May 2009 did not react to *B. duncani* antigens in IFA testing at CDC.

Since 2007, Donor A had donated blood five times; the index donation in April 2008 was the third in the series of five. The two subsequent donations included one in early August 2008 (the recipient was lost to follow-up) and one in late September (the blood was discarded when the transfusion investigation was initiated). After Donor A was implicated, he was indefinitely deferred from donating blood. His two previous donations were in November 2007 (the blood was discarded during processing because of incomplete filtration) and in January 2008. RBCs from his January 2008 donation were transfused to an oncology patient in northern California, who reportedly was asymptomatic when evaluated in March of the following year. In April 2009 (415 days posttransfusion), a blood specimen from the recipient was collected for *Babesia* testing: the *B. duncani* IFA titer was 1:256, molecular analyses and a blood smear were negative, and two jirds inoculated with 22-day-old blood did not develop demonstrable parasitemia.

DISCUSSION

We investigated a case of *B. duncani* infection that was diagnosed in late September 2008, in a chronically transfused patient who had become refractory to transfusions. The patient had three reasons for hemolytic anemia: sickle cell disease, which previously had been well controlled; warm-antibody autoimmune hemolytic anemia, which was diagnosed in August 2008; and *B. duncani* infection, which was diagnosed more than 4 months after the May 2008 transfusion that was implicated in the multiagency investigation of the RBC transfusions and donors since September 2007. In retrospect, rare parasites were found on an extant blood smear from mid-July 2008, approximately 2 months after the implicated May transfusion. The interval from the implicated transfusion to onset of clinical manifestations is unclear. However, if the patient's only documented febrile illness, which occurred in early June 2008, was caused by babesiosis, the incubation period was approximately 2 weeks. His *Babesia* infection responded to therapy with clindamycin plus quinine, which remains the standard of care for severely ill patients.[1] However, his clinical course was complicated by prolonged morbidity from multifactorial renal failure and polyneuropathy.

Both the patient and the implicated donor (Donor A) were residents of northern California, were seropositive by *B. duncani* IFA (but seronegative by *B. microti* IFA), and had parasitologically confirmed infection. Donor A, who was otherwise healthy, had no overt manifestations of *Babesia* infection even though he was 67 years old (8 years older than the recipient). Although the duration of his infection is not known, his case underscores that *B. duncani*, like *B. microti*, can be associated with protracted, asymptomatic parasitemia: *B. duncani* was isolated from a blood specimen collected in March 2009, more than 10 months after his index donation in April 2008. Similarly, for the first documented *B. duncani* transfusion case, the parasite was isolated from a specimen obtained from the donor in March 1995, 7 months after the index donation in August 1994.[17] Although limited data suggest that *B. duncani* may be more pathogenic than *B. microti* in experimentally inoculated mice and hamsters,[15] the potential relevance of these animal data to human infection is unknown.

For Donor A, the possibility that recipients of cellular components from other donations became infected could not be proven or excluded. Although Donor A undoubtedly was infected when he donated again in August 2008, no information was available about the recipient. Donor

A's infection status back in January 2008 is unknown, and the *Babesia* test results more than 13 months posttransfusion for the recipient of RBCs from that donation (an oncology patient) are noteworthy but not definitive—in particular, the patient's *B. duncani* IFA titer of 1:256. The threshold for considering a *B. duncani* IFA result positive has not been well established: to our knowledge, fewer than 10 *B. duncani* cases have been parasitologically confirmed and monitored serologically. The oncology patient's possible case of *B. duncani* infection was not parasitologically confirmed, only one specimen was tested, and limited epidemiologic and clinical information was available. However, the negative results of PCR analyses and animal inoculation do not exclude the possibilities that the patient either had been or still was infected, regardless of the mode of transmission; these methods are not sufficiently sensitive for reliable detection of low-level parasitemia.

Much remains unknown about *B. duncani*, such as the interrelated issues of its geographic distribution, tick vector, and reservoir host(s),[15] as well as the incidence and prevalence of infection in humans, including blood donors and recipients. The case we described, the third documented transfusion case caused by *B. duncani*, underscores that the difficulties inherent to detecting and investigating *Babesia* cases are compounded in patients who have been chronically transfused, have multicause hemolytic anemia, and are infected with species other than *B. microti*. Human infection with *B. duncani* and other novel *Babesia* agents[21] is not detected by serologic or molecular assays for *B. microti*, which has ramifications not only for diagnostic testing and transfusion investigations but also for potential future screening of blood donors.[2] Effective measures for preventing transfusion transmission of *Babesia* parasites are needed. Although the highest near-term priority is the development and/or implementation of FDA-approved donor-screening test(s) for *B. microti*, there also is a need for *Babesia* genus- (vs. species-) level assays.

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CONFLICT OF INTEREST

None.

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別紙様式第２

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日 2012年2月14日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①②フィブリノゲン加第XIII因子 ③④人血液凝固第XIII因子		研究報告の公表状況	Deconstructing the risk for malaria in United States donors deferred for travel to Mexico Transfusion　2011:51(11)2398-2410	公表国 米国	
販売名（企業名）	①ベリプラストＰ コンビセット ②ベリプラストＰコンビセット組織接着用 ③フィブロガミンＰ ④フィブロガミンP静注用 （CSLベーリング株式会社）					

研究報告の概要

【背景】米国においてメキシコ旅行はマラリアによる供血延期の最も大きな割合を占めるが、メキシコ国内の大半の地域でマラリア感染リスクは極めて低く、輸血の安全性と有効性のバランスが不均衡であることが示唆される。本研究では、メキシコの一部地域で血液ドナーの供血延期要件を緩和すべきか検証した。【方法】2006年に血液センター6施設において、メキシコに旅行したため供血を延期された血液ドナー計885名を対象とし、ドナーの感染リスクを滞在先の現地住民のマラリア感染率から算出し、滞在期間により調整した。血液センターの供血延期数の比率をメキシコ旅行により供血延期されている推定66,554名/年に応用することで、米国全体における影響を推測した。【結果】メキシコにおけるマラリア感染は2000年に16州234郡で7,272例発生していたが、2009年では人口比3％以下の10州115郡で2,595例と減少していた。また、感染リスクは地域によって異なり、Chiapas州およびOaxaca州が全症例数の75％を占めていた。72％のドナー（推定47,939名/年）がユカタン半島にあるQuintana Roo州を旅行したことで供血を延期されているが、ドナーの感染リスクは0.0080例/年または125年に1例であった。感染リスクの高いOaxaca州に旅行して供血を延期された1,847名を除けば、残りの64,707名におけるマラリア感染リスクは20年に1例であった。【結論】Quintana Roo州のようなマラリア感染リスクが極めて低いと判断されるメキシコ国内の地域に関しては、供血延期要件を緩和すべきである。

使用上の注意記載状況・その他参考事項等

01

報告企業の意見	今後の対応
マラリアは赤血球内にマラリア原虫が寄生するため発症するが、本剤は血漿を原材料にしているため感染はないと考えられる。	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。

Deconstructing the risk for malaria in United States donors deferred for travel to Mexico

Bryan Spencer, Steven Kleinman, Brian Custer, Ritchard Cable, Susan L. Wilkinson, Whitney Steele, Patrick M. High, and David Wright for the NHLBI Retrovirus Epidemiology Donor Study-II (REDS-II)

BACKGROUND: More than 66,000 blood donors are deferred annually in the United States due to travel to malaria-endemic areas of Mexico. Mexico accounts for the largest share of malaria travel deferrals, yet it has extremely low risk for malaria transmission throughout most of its national territory, suggesting a suboptimal balance between blood safety and availability. This study sought to determine whether donor deferral requirements might be relaxed for parts of Mexico without compromising blood safety.

STUDY DESIGN AND METHODS: Travel destination was recorded from a representative sample of presenting blood donors deferred for malaria travel from six blood centers during 2006. We imputed to these donors reporting Mexican travel a risk for acquiring malaria equivalent to Mexican residents in the destination location, adjusted for length of stay. We extrapolated these results to the overall US blood donor population.

RESULTS: Risk for malaria in Mexico varies significantly across endemic areas and is greatest in areas infrequently visited by study donors. More than 70% of blood donor deferrals were triggered by travel to the state of Quintana Roo on the Yucatán Peninsula, an area of very low malaria transmission. Eliminating the travel deferral requirement for all areas except the state of Oaxaca might result in the recovery of almost 65,000 blood donors annually at risk of approximately one contaminated unit collected every 20 years.

CONCLUSION: Deferral requirements should be relaxed for presenting donors who traveled to areas within Mexico that confer exceptionally small risks for malaria, such as Quintana Roo.

Transfusion-transmitted malaria (TTM) is a potentially lethal outcome of blood transfusion, but one that is uncommon in the United States. On average, the past two decades have seen less than one case of TTM per year, representing a rate of less than 0.1 per 10^6 red blood cell transfusions.[1-3] TTM prevention currently relies on exclusion of donors who might present risk for malaria infection. Based on requirements and recommendations of the Food and Drug Administration (FDA)[4] and the AABB,[5] the deferral period is 3 years for those who report a history of malaria infection or of prior residence in a malaria-endemic country as defined by the Centers for Disease Control and Prevention (CDC),[6] whereas it is 1 year for donors with travel to parts of countries considered endemic for malaria by CDC.

The number of presenting or productive donors with nonzero risk for harboring malaria parasites is essentially

ABBREVIATIONS: API = annual parasitological index, number of cases reported per 1000 residents per year; BCP = Blood Centers of the Pacific; BCW = BloodCenter of Wisconsin; HOX = Hoxworth Blood Center, University of Cincinnati; ITxM = Institute for Transfusion Medicine; NEARC = American Red Cross, New England Region; SARC = American Red Cross, Southern Region; TTM = transfusion-transmitted malaria.

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unknown, as is the relative likelihood for infection across the different malaria deferral categories. However, empirical evidence in the United States[1,2,7] and other countries[8-11] clearly implicates donors from sub-Saharan Africa in cases of TTM over the past three decades. While many of these donors were improperly accepted due to an error during health history screening, others were rare biologic outliers whose semi-immune status allowed for *Plasmodium* parasite carriage for several years. This occurred most often with *Plasmodium falciparum* and also with *P. malariae*, a more benign species known to remain undetected for decades.[12]

In contrast to those with long-term residence in sub-Saharan Africa and a few other highly endemic areas, donors reporting routine travel to malarial areas appear to confer relatively low risk for transmitting malaria to recipients of blood products. In fact, over a 28-year period, only one donor out of 32 implicated in TTM cases was a native-born US resident on routine travel.[1,2,7] While denominator data that would allow for accurate risk comparisons across the different deferral categories are lacking, there can be no doubt that the 1-year deferral period for short-term travelers has a significant impact on blood availability. A recent analysis by our consortium estimates that annual deferrals by US blood centers might surpass 150,000,[13] and unpublished data suggest that several times that many might self-defer.[14]

To assess the risk that donors who report a travel history requiring malaria deferral might actually be infected with malaria parasites, our prior study detailed the travel destinations of a representative sample of blood donors with malaria travel deferral.[13] Using public data sources on imported malaria in the United States[15-20] and on numbers of travelers to different countries and regions,[21,22] we developed region-specific risks for malaria infection that suggested a 1000-fold greater risk for travelers to Africa versus Mexico. Traveler risk to Mexico was estimated as roughly 1 in 800,000 for those who visited an area triggering malaria deferral. We concluded that these data supported the relaxation or elimination of the year-long deferral period for travel to Mexico, with a negligible marginal increase in risk balanced by the recovery of tens of thousands of donors annually.[13]

Given the large number of deferrals after travel to Mexico, and the long-term trends in malaria incidence there, this article reports a follow-up analysis focusing only on this country. Mexico is a country of low and declining risk for malaria, one where the remaining areas of natural transmission are characterized as scattered foci in rural areas populated by indigenous inhabitants.[23] Although firm data are unavailable on how many US tourists visit any given location within Mexico, US malaria surveillance figures support the interpretation that most travelers visit areas with very low risk for malaria. For each year from 2000 to 2007, an average of approximately six and a maximum of 11 US residents have acquired malaria in Mexico that was diagnosed following return to the United States,[7,15-20,24] out of an average of roughly 20 million visitors annually with at least an overnight stay.[22] Furthermore, a disproportionate share of these malaria cases occurred in Mexican citizens now residing in the United States,[25] so the risk for routine, US-born travelers appears exquisitely low.

To develop risk estimates for malaria infection in blood donors that are independent of the travel destination within Mexico for overall US travelers, this article adopts an alternate method that derives donor risk from malaria risk figures for the local populations of the areas visited. Specifically, we recorded destination(s) of travel within Mexico for the same cohort of deferred donors described in the earlier analysis[13] and used malaria surveillance indicators developed by the Mexican Ministry of Health for the same areas during the same time frame as a measure of potential malaria risk faced by blood donors deferred for travel to Mexico. Because the Mexican morbidity data reflect risk for year-round residents, we adjust the donor risks for duration of exposure. To provide a basis for selective alterations to current deferral requirements for Mexico, we disaggregate the results by state, since distribution of risk across the country varies significantly.

MATERIALS AND METHODS

Source of data on presenting US blood donors deferred for travel to malaria-endemic areas in Mexico

Six blood centers participating in the Retrovirus Epidemiology Donor Study-II (REDS-II) program sponsored by the National Heart, Lung, and Blood Institute (NHLBI) provided data for this analysis. These centers represent geographically and demographically diverse populations and collectively account for more than 8% of annual allogeneic blood collections in the United States.[3] The REDS-II blood centers include the Blood Centers of the Pacific (BCP; San Francisco, CA); BloodCenter of Wisconsin (BCW; Milwaukee, WI); Hoxworth Blood Center, University of Cincinnati (HOX; Cincinnati, OH); the Institute for Transfusion Medicine (ITxM; Pittsburgh, PA); the American Red Cross, New England Region (NEARC; Dedham, MA); and the American Red Cross, Southern Region (SARC; Douglasville, GA). Each center retrieved blood donation records from the first 60 donors deferred for malaria travel in either the even-numbered (BCW, ITxM, SARC) or odd-numbered months (BCP, HOX, NEARC) throughout 2006 to ensure that seasonal patterns in travel were captured. Data were recorded on donor demographics, date of presentation, dates of travel in malaria-endemic regions, and the destination country or countries with malaria risk for up to five countries.

Countries were ranked by malaria risk,[26,27] and specific locations triggering malaria deferral were recorded verbatim from the blood donation records for the two countries with highest risk for malaria. This analysis reports only on the subset of donors for whom Mexico either was the only country visited with malaria risk or was the country with the highest risk. Deferral records lacking the destination country or the date that deferral began were excluded from the analysis.

Estimate of annual deferrals of US blood donors for malaria-risk travel to Mexico

The proportion of malaria travel deferrals that were allocated to Mexico for the six REDS-II centers was used to estimate the annual number of malaria travel deferrals associated with Mexico for US donors overall. Annual data on allogeneic donations and malaria travel deferrals were recorded and summed across all centers, and each center's contribution to national estimates was weighted by its share of the collective REDS-II malaria travel deferrals. Based on their aggregate contribution of 8.07% of US allogeneic donations,[3] the blood centers' estimates for malaria deferrals were multiplied by 12.4 to extrapolate to the US overall. These figures have been updated from the earlier article[13] to account for the availability of US donation data from 2006,[3] which increases the projections of US deferrals for malaria travel from 150,537 to 161,105. When a donor reported more than one trip with a visit to a malaria-endemic area, the most recent trip was chosen for analysis. When more than one geographic region within Mexico was visited, the donor's visit was allocated to the area of higher risk for malaria infection, based on Mexican public health data. Donor's travel destination was assigned to the smaller administrative unit possible between state- and county-level destinations. Donors for whom location within Mexico was not available were assumed to represent randomly missing data and were accounted for by proportional weighting for those donors who did report location, for each of the six blood centers.

Criteria for malaria travel deferral to different areas of Mexico

For US travelers, risk for malaria infection is described by the CDC on a country-by-country basis,[6] and by FDA requirement this information is used as the basis on which donor acceptability is determined.[4] For the time frame of this study–donor presentation during 2006 and donor travel during 2005 through 2006—the description of malaria risk in Mexico was as follows:

> Risk in rural areas, including resorts in rural areas of the following states: Campeche, Chiapas, Guerrero, Michoacán, Nayarit, Oaxaca, Quintana Roo, Sinaloa, and Tabasco. In addition, risk exists in the state of Jalisco (in its mountainous northern area only). Risk also exists in an area between 24° N and 28° N latitude and 106° W and 110° W longitude, which lies in parts of Sonora, Chihuahua, and Durango. No malaria risk exists along the United States-Mexico border. No malaria risk exists in the major resorts along the Pacific and Gulf coasts.[6]

The specific job aids or work instructions provided for health historians of each REDS-II blood center might have differed in detail during 2006, but the referent for each center would have been the foregoing text from CDC. Figure 1 shows a map of Mexico,[28] with those states named in the above excerpt shaded in gray. Depending on the specificity of information provided by donors with travel to Mexico, as well as on the detail of information available in reference materials for health historians, travel to one of the shaded states during 2005 through 2006 might not have necessarily triggered a deferral. It would, however, at a minimum require consultation with support documentation to exclude travel to a malaria risk area for the donor to be judged acceptable to donate.

Estimated risk for malaria infection in US travelers to different areas of Mexico

Population-adjusted risk measures for year-round Mexican residents at the state level are available from eight endemic states which together account for 98.5% of reported cases in 2005.[29] County-level risk measures were derived from malaria case counts at the county level for 2005[30] and the 2005 census population figures for each county.[31] State- and county-level risk measures for year-round Mexican residents were used as a proxy for potential malaria risk which US residents might face during travel to the same areas, appropriately adjusted for duration of exposure. Donors eligible for inclusion in this study—those deferred during calendar year 2006—reported travel that occurred during both 2005 and 2006. Mexican surveillance data from 2005 only are used to develop risk estimates due to unavailability of 2006 county-level malaria surveillance data. To evaluate longitudinal trends in malaria incidence in Mexico, and to establish any significant changes in the amount or distribution of malaria subsequent to the period used for our study, we referenced several years of publicly available Mexican government surveillance data.[32]

Duration of exposure was estimated by calculating donor-reported dates for beginning and ending of exposure in risk areas. This measure was available for donors from three of the six blood centers, and it was not possible to distinguish donors who simply reported the duration of their entire trip versus the proportion who correctly provided dates only for the portion of travel subject to deferral

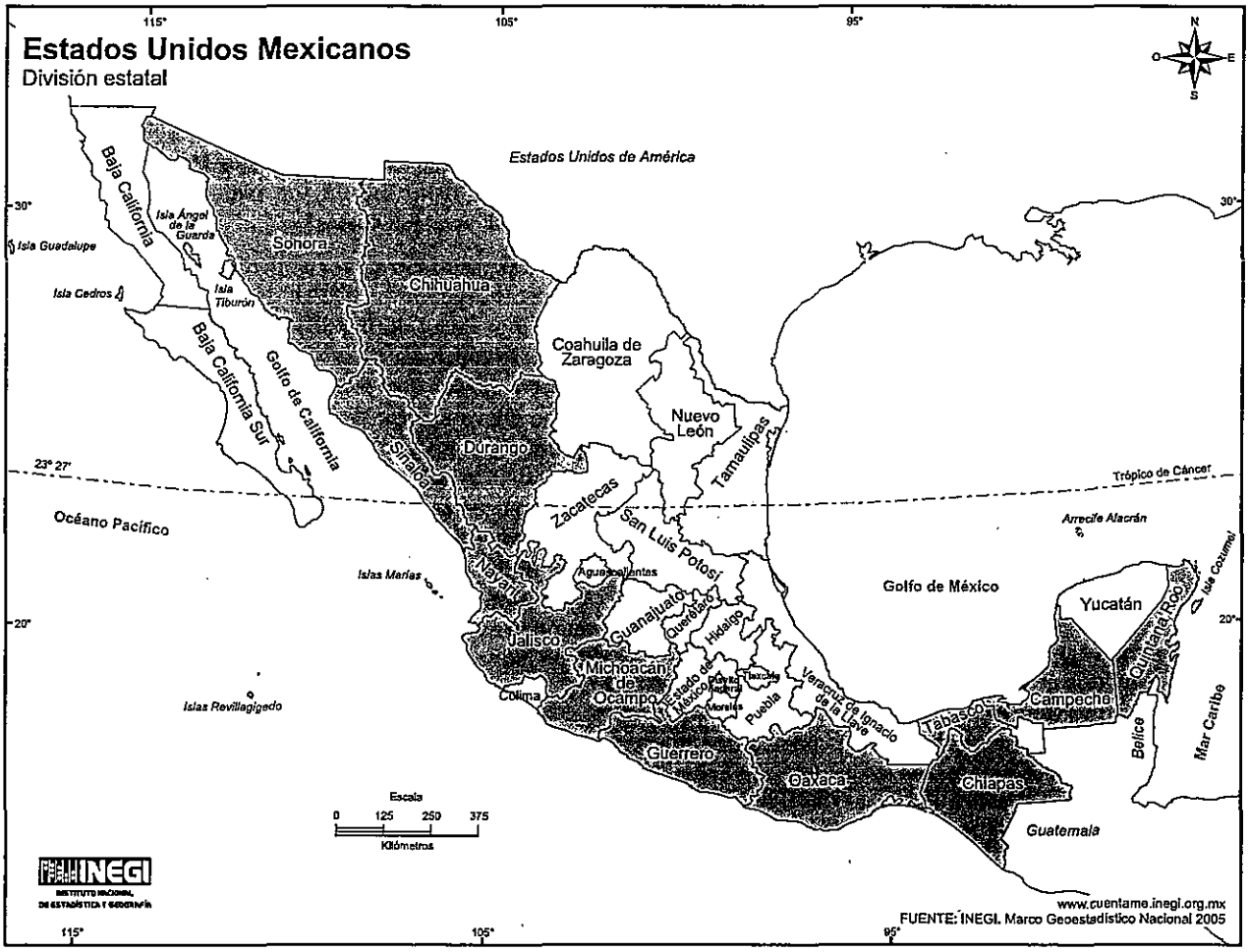

Fig. 1. States in Mexico where US CDC reports risk for malaria, 2005 through 2006. Map adapted from Instituto Nacional de Estatística e Geografía.[28]

due to presumed malaria risk. After reviewing the distribution of results, we chose a uniform duration of exposure (7 days) that encompasses over 70% of those donors with both start and end dates recorded.

Transfusion risk from blood donors with deferrable travel to Mexico

Transfusion risk is defined in this article as the risk that a donor might be accepted for donation while asymptomatically parasitemic, which we assume is equivalent to the risk that a donor might become infected with malaria while on travel in Mexico. Our risk estimates assume the absence of any deferral period for travel to Mexico, which means the donor could be found acceptable to donate the very day upon return from travel. Because the risk for asymptomatic malaria infection diminishes with increasing interval between return from travel and date of presentation—approximately 50% of *P. vivax* infections manifest within the first month and 70% within the first 3 months[13]—the actual risk from abolishing the deferral for Mexico might be even lower than we estimate.

RESULTS

Malaria travel deferrals to Mexico for REDS-II donors and extrapolation to the United States annually

The six REDS-II centers reported a total of 13,007 deferrals for travel to malaria-endemic areas by US residents in 2006 (Table 1), of which 2160 were sampled for further analysis. The required data were available for 2108 of these (97.6%), of which 885 donors were deferred for malaria travel to Mexico. Based on weighting that is proportional to each blood center's malaria deferral count, extrapolating to the US yields an estimated 66,554 deferrals annually for malaria travel to Mexico. The change from 870 donors deferred for travel to Mexico in the earlier analysis[13]

TABLE 1. Malaria travel deferrals to Mexico at six REDS-II Blood Centers, 2006

	BCP	BCW	HOX	ITxM	NEARC	SARC	Total	Weighted extrapolation to United States
Number of travel deferrals	2761	2128	1122	1622	3570	1804	13,007	161,105
Number of travel deferrals sampled	334	353	356	359	360	346	2,108	
Deferrals to Mexico	123	230	159	119	130	125	885	66,554
Travel deferrals due to travel to Mexico (%)	36.8	65.2	44.7	33.1	36.1	35.8	42.0	41.3
Mexico deferrals with identifiable location (%)	62	98	99	92	95	52	85	

TABLE 2. Mexican population at risk and reported malaria cases at the state level, 2005

State	Population, 2005	Population at risk, 2005	Number of counties reporting cases, 2005	Malaria cases, 2005	Population-adjusted risk, API (and 95% CI*), for malaria, 2005
Durango	1,554,948	14,126	3 of 39	114	8.07 (6.71-9.70)
Oaxaca	3,716,837	193,669	19 of 570	1432	7.39 (7.02-7.79)
Chihuahua	3,432,518	33,126	8 of 67	181	5.46 (4.72-6.32)
Sonora	2,487,066	5,777	4 of 72	29	5.02 (3.49-7.22)
Tabasco	2,069,522	119,497	9 of 17	97	0.81 (0.67-0.99)
Chiapas	4,417,084	1,062,455	65 of 119	852	0.80 (0.75-0.86)
Sinaloa	2,771,148	559,254	10 of 18	208	0.37 (0.32-0.43)
Quintana Roo	1,091,496	127,808	2 of 8	11	0.09 (0.05-0.16)
Nayarit and other states	24,466,964	684,288	13 of 667	42	0.06 (0.04-0.08)
Total	46,007,583	2,800,000	133 of 1,577	2966	1.06 (1.02-1.10)

* CIs do not account for temporal or geographic variation, and hence inference is to only the year 2005 and is specific to each state.

derives from changing from a macroregional to a country-based analysis, for which Mexico was the country of higher risk.

Risk for malaria infection in US travelers to Mexico

Population-adjusted risks for malaria at the state level are shown in Table 2, supporting the characterization of risk areas within Mexico as mostly scattered foci rather than large swaths of the national landscape and demonstrating that most residents of endemic areas face very low risk for malarial illness. First, of the 2.8 million Mexican residents living in risk areas, fewer than 10% reside in areas with an annual risk for malaria greater than 1 per 1000 (an annual parasitological index [API] of 1 means one case is reported per 1000 residents). Even in these regions—Durango, Chihuahua, and Sonora in the Northwest and Oaxaca in the South—the population at risk represents a small proportion of each state's population, and the number of counties with cases is low compared to each state's overall number of counties. Although the risk faced by residents of these four states is large relative to residents of the other endemic states, this elevated risk is confined to a very small fraction of the population. For Quintana Roo and four other states, which collectively account for 53 reported cases, the risk is less than 1 per 10,000 residents per annum.

Table 3 contextualizes the malaria risks across states in 2005 within a long-term trend of increasingly lower malaria risk in fewer and more circumscribed geographic areas. Compared to 1985, when more than 133,000 cases were reported from all but two Mexican states,[33] the number of cases declined by 95% by the year 2000, and within the past decade has been reduced by an additional 60%. Active transmission of *Plasmodium* parasites is now limited to 115 counties distributed across 10 states, down from 234 counties in 16 states in 2000, and applies to less than 3% of the population of more than 100 million. The country has not reported a malaria fatality in more than 10 years (data not shown), and the number of cases of *falciparum* malaria—the most malignant species and at constant risk for importation from neighboring countries—has been reduced to zero for 3 consecutive years. Across those areas with active transmission, risk is nonuniform, with two states—Chiapas and Oaxaca—accounting for more than 75% of all reported cases in each of the past 5 years.

Risk for malaria infection in US donors deferred for travel to Mexico

Table 4 combines the results for travel destination within Mexico reported by the REDS-II donors with the proxy risk attached to the identified locations. Overall, the information provided by a large majority of donors (85%) was sufficient to identify location of travel at least to the state level. For more than half of the remaining donors (n = 69, 7.8%), information on location was either altogether lacking or insufficiently specific ("went horseback riding, visited ruins, drove through rural area") to exclude

TABLE 3. Malaria cases at national and state level and population at risk in Mexico, 2000 through 2009

	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
Population at risk (million)	50.3	54.7	54.7	54.7	2.8	2.8	2.8	2.8	2.8	≈2.8
Number of states reporting ≥ 1 malaria case(s)	16	17	15	16	14	12	11	10	10	10
Number of counties reporting ≥ 1 malaria case(s)	234	202	189	177	148	133	NA	NA	115	NA
Number of *P. falciparum* cases	124	70	16	43	47	22	14	0	0	0
Individual states										
Campeche	36	57	54	21	4	1	0	0	0	0
Chiapas	3575	2522	2415	1757	1151	852	1349	1483	1136	1039
Chihuahua	695	404	420	259	184	181	122	148	185	438
Colima	0	1	0	0	0	0	0	0	0	0
Durango	171	136	104	79	52	114	121	46	38	42
Guerrero	161	88	24	7	3	0	0	0	0	0
Jalisco	50	29	11	6	5	2	2	2	10	8
Michoacán	135	52	21	2	7	0	0	0	0	0
Morelos	0	0	0	1	0	0	0	0	0	0
Nayarit	206	154	88	49	27	37	24	38	71	70
Oaxaca	654	285	260	699	1083	1432	575	369	804	896
Puebla	7	1	0	0	0	0	0	0	0	0
Quintana Roo	332	215	276	88	40	11	17	14	15	4
Sinaloa	790	616	664	377	513	208	98	108	76	72
Sonora	79	59	32	76	49	29	17	13	10	18
Tabasco	297	219	139	167	145	97	86	86	12	8
Veracruz	34	20	11	4	0	2	2	0	0	0
Yucatán	50	9	33	15	5	0	0	0	0	0
Total malaria cases	7272	4867	4552	3607	3268	2966	2413	2307	2357	2595

potential exposure to malaria, thus triggering deferral; records were no longer available for another 60 donors (6.8%). The adjusted distribution in Table 4 and the calculations based on them assume the distribution of travel by the 15% missing was random, and locations for these observations were reallocated according to the proportion missing for each blood center. Notably, more than nine of 10 donors in this analysis provided sufficient detail on their travel destination(s) within Mexico to identify both state and county visited.

Duration of exposure was estimable for 284 donors, and the distribution was bimodal. Nineteen percent of donors reported a duration of exposure of 0 days, which reflects day trips to risk areas. Another 24% reported 7-day stays in areas with malaria risk. The median and mode both were 7 days, and the mean was 7.4 days. Together, 73% of the 284 donors reported a duration of exposure of 7 or fewer days, and this duration was accordingly used to estimate the risks for malaria infection, discussed below and shown in Table 4.

A significant majority—72%—of REDS-II donors were deferred for travel to the state of Quintana Roo. Located on the Yucatán Peninsula, Quintana Roo draws heavy numbers of tourists arriving by both plane and cruise ship. As indicated in Table 3, the number of malaria cases reported in Quintana Roo has been reduced by more than 95% over the past decade, and in 2005 (Table 2) only two of the eight counties in the state had any cases. The information provided by travelers to Quintana Roo was sufficiently detailed to identify location at the county level for 98% of the donors deferred for travel to that state (Table 4). Risk for infection in donors with travel to Quintana Roo is derived from a weighted summation of location risk (county level for 98%, state level for 2%) multiplied by the number of US donors extrapolated from the REDS-II donors (Table 5). From these calculations, two figures stand out. First, 81% visited areas with zero risk for malaria in 2005 (Table 4 and Fig. 2[28]), mostly in the area of Cancún, Playa del Carmen, and other coastal areas of the northern third of the state. Second, after adjusting for duration of exposure (see above), we would expect the projected number of 47,939 donors deferred annually for travel to Quintana Roo to acquire malaria infection at a rate of 0.0080 per year, or one infection every 125 years. This estimate assumes no significant alteration in the degree or distribution of risk within the state, both of which seem reasonable inferences based on the number of cases reported statewide during 2006 through 2009 (Table 3).

After Quintana Roo, the states responsible for the second- and third-largest number of donor deferrals also represent very-low-risk travel. Both Guerrero and Nayarit account for more than 6% of travel deferrals to Mexico, but Guerrero reported no more than three cases of malaria (either zero or three, depending on the source) in 2005, and travelers to both states overwhelmingly reported travel destinations in counties with zero malaria cases throughout 2005 (see Fig. 3 for Nayarit[28]). Accordingly, the more than 8400 US donors projected to have been deferred for travel to these two states would be expected to acquire 0.0007 infections altogether, or one per 1400 years.

Continuing in like fashion across all the states responsible for donor deferrals, the results indicate that 76% of donors did not enter an area with even one malaria case

TABLE 4. Distribution of travel destination and estimated risk for acquiring malaria infection for 885 REDS-II donors deferred for malaria travel to Mexico and projections to US donors overall

	Travel destination (%)			Location risks		Projected US deferrals and infections in donors with malaria travel to Mexico		
State	Raw distribution	Adjusted distribution*	Percent assignable to county level*	Percent with travel to zero-risk area*	Estimated weighted risk, API	Number of projected US deferrals	Expected infections in number of deferred donors (and 95% CI†), 7-day exposure	Percent of total estimated risk
Quintana Roo	63.8 (n = 565)	72.0 (n = 637.5)	98	81	0.0086	47,939	0.0080 (0.0044-0.0145)	3.0
Nayarit	5.2 (n = 46)	6.5 (57.1)	89	89	0.0076	4,295	0.0006 (0.0004-0.0009)	0.2
Guerrero	4.9 (n = 43)	6.2 (55.0)	97	77	0.0010	4,140	0.0001 (0.0000-0.0006)	0.0
Oaxaca	2.3 (n = 20)	2.8 (24.6)	49	0	6.1106	1,847	0.2170 (0.1456-0.3234)	81.1
Sinaloa	1.8 (n = 17)	2.6 (22.9)	67	0	0.1253	1,751	0.0041 (0.0027-0.0064)	1.5
Jalisco	1.8 (n = 16)	2.5 (21.7)	53	53	0.0450	1,718	0.0014 (0.0002-0.0105)	0.5
Yucatán	1.8 (n = 16)	2.3 (20.3)	79	100	0.0000	1,524	0.0 (0.0-0.0)	0.0
Michoacán	1.5 (n = 13)	2.0 (17.8)	43	100	0.0000	1,342	0.0 (0.0-0.0)	0.0
11 other states and Mexico City	2.4 (n = 21)	3.2 (28.1)	78	51	0.8942	2,117	0.0364 (0.0226-0.0587)	13.6
Location unassigned	14.6 (n = 129)							
Total	885		92 (814/885)	76 (673/885)	0.2091†	66,554	0.2676 (0.2483-0.2885)	

* Adjusted distributions are reweighted according to the percent missing for each center, as shown in Table 1.
† CIs do not account for temporal or geographic variation of API, temporal variation in travel habits of US donors, or variation in donation habits of US donors.

TABLE 5. Detailed calculations for risk estimates for travel to state of Quintana Roo

County (raw/adjusted number)	Estimated resident risk, 2005	Percentage of REDS-II malaria deferrals with travel to Mexico	Number of projected US deferrals	Expected malaria infections, API (and 95% CI*), in donors (7-day exposure)
Cozumel (n = 8/8.136)	0.0000	0.92	612	0.0000 (0.0000-0.0000)
Felipe Carrillo Puerto (n = 1/1.938)	0.0306	0.22	146	0.0001 (0.0000-0.0004)
Isla Mujeres (n = 1/1.006)	0.0000	0.11	76	0.0000 (0.0000-0.0000)
Othón P. Blanco (n = 85/107.601)	0.0410	12.16	8,092	0.0064 (0.0034-0.0119)
Benito Juárez (n = 64/65.138)	0.0000	7.37	4,902	0.0000 (0.0000-0.0000)
José María Morelos (n = 0)	0.0000	0.0	0	0.0000 (0.0000-0.0000)
Lázaro Cárdenas (n = 0)	0.0000	0.0	0	0.0000 (0.0000-0.0000)
Solidaridad (n = 397/441.450)	0.0000	49.88	33,198	0.0000 (0.0000-0.0000)
Unspecified location (n = 9/12.149)	0.0861	1.37	914	0.0015 (0.0007-0.0034)
Totals		72.0.	47,939	0.0080 (0.0044-0.0145)

* CIs do not account for temporal or geographic variation of API, temporal variation in travel habits of US donors, or variation in donation habits of US donors.

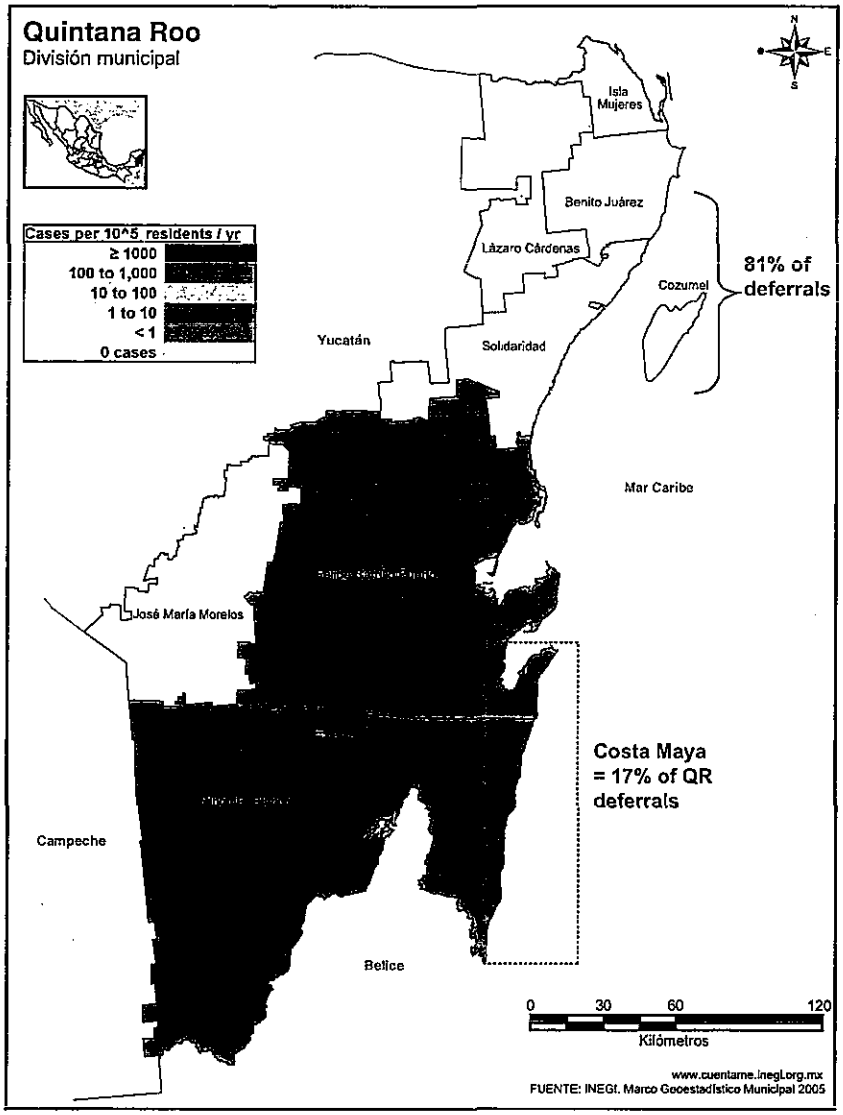

Fig. 2. Mexican State of Quintana Roo, with 2005 county-level malaria risk and general distribution of REDS-II malaria travel deferral destinations. Map adapted from Instituto Nacional de Estatística e Geografía.[28] [Correction added after online publication 12-May-2011: deferral numbers added.]

in 2005, and the weighted average location risk for all deferred donors is 0.2091 infections per 1000 year-round residents. Adjusted for duration of exposure, this estimate implies roughly a 1:250,000 malaria risk for US donors deferred for travel to Mexico. Given the 100-fold risk gradient across states shown for Mexican residents in Table 3, the donor risk categorized at the state level gives similar results. In fact, 81% of the risk attributed to donors deferred for travel to Mexico (0.2170 infections annually, or roughly one case per 4.6 years) is for travel to the state of Oaxaca, a risk associated with only 1847 donors. Omitting them from the analysis, the remaining 64,707 donors collectively incur risk for malaria infection of roughly one per 20 years. Inclusion of Oaxaca yields an estimated 0.2676 expected infections in 66,554 deferred donors per year, or roughly one infection every 3.7 years.

These frequencies can be understood as the maximum theoretical risk from fully repealing the donor deferral for travel to Mexico, which assumes that all donors present for donation immediately upon return. Given that 50% of vivax malaria infections in US travelers become symptomatic within 1 month and 70% within 3 months,[13] the actual risk under the expected distribution of intervals between return and presentation would likely be quite a bit lower. Moreover, as discussed below, imputing to US travelers a prorated risk for malaria infection equivalent to Mexican residents almost certainly overstates risk by a significant, albeit unquantifiable amount.

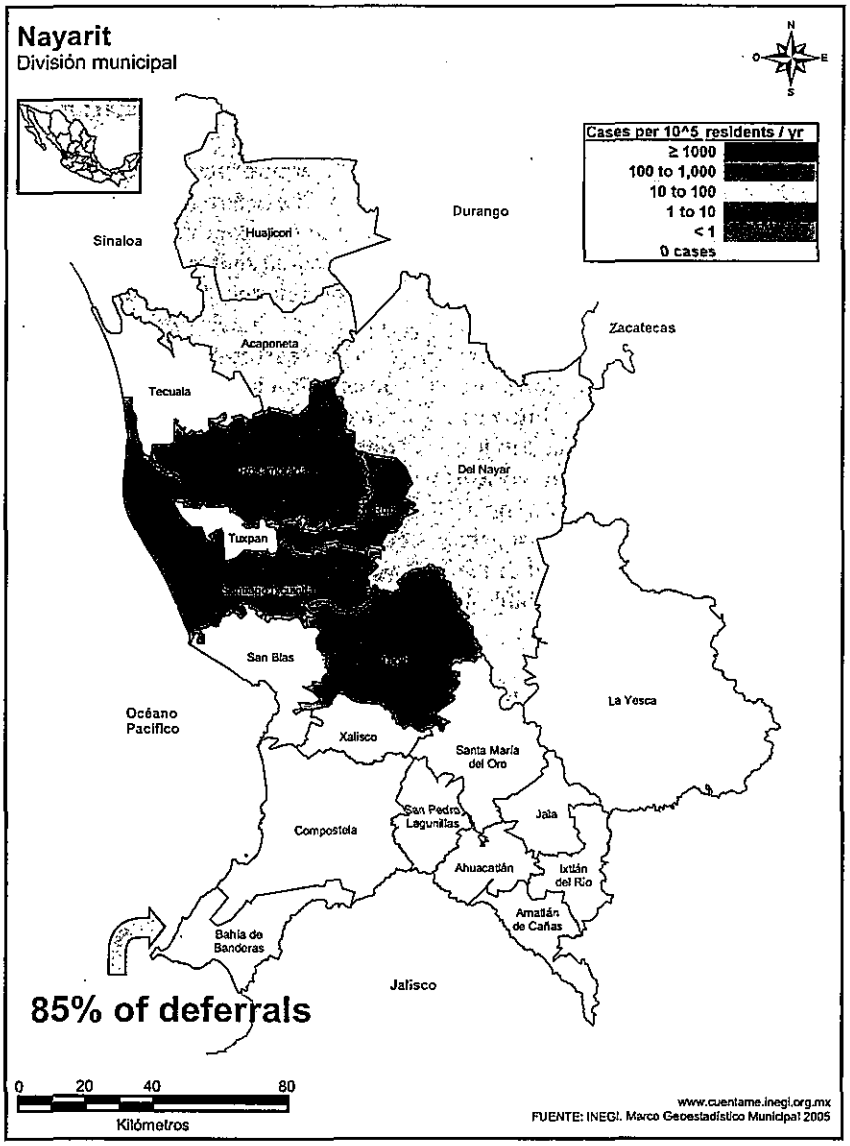

Fig. 3. Mexican State of Nayarit, with 2005 county-level malaria risk and destination location of large majority of REDS-II malaria travel deferrals. Map adapted from Instituto Nacional de Estatística e Geografía.[28] [Correction added after online publication 12-May-2011: deferral numbers added.]

It is noteworthy that donors were deferred for travel to 19 states, plus the Federal District (Mexico City), whereas only 13 states are listed by name in the Yellow Book. To be sure, these occurrences represent a small minority, but some of the states responsible for deferrals (Baja California Sur, Colima) had not reported a malaria case in several years.

DISCUSSION

While blood centers in the United States have previously expressed concern that the donor deferral for malaria risk in Mexico reflects a poor balance between risk averted and impact on blood availability,[34] no systematic effort had been undertaken to quantify either metric in a joint assessment of safety and availability. Our consortium's first analysis suggested that the risk for malaria infection associated with travel to Mexico might be low enough that the current 1-year deferral period could be scaled back with negligible impact on blood safety. Given the apparent size of the population of willing donors turned away after travel to Mexico, there are indeed significant potential gains in blood availability to be weighed in the assessment of risk and benefits that might follow any change in donor eligibility requirements. To address the potential concern that our estimates for malaria risk in US travelers to

Mexico might not have been based on accurate estimates of population at risk, we sought an alternate measure for malaria risk that was independent of US traveler patterns overall. The results presented herein support the conclusions from the original paper, while providing a more nuanced perspective on donor travel patterns and attendant malaria risk.

Based on the recognition that traveler risk and donor acceptance might be only loosely associated, this analysis purposefully did not restrict its focus only to the donor population that entered an area in Mexico with active malaria transmission. While a donor who reports having entered a risk area is clearly excluded from donation, other donors who did not enter such an area but who cannot provide sufficient detail to exclude the possibility of malaria exposure are also deferred. Thus, the critical population for the current analysis is the donor population who entered an area of Mexico that would trigger their exclusion from blood donation, independent of the level of risk they might have faced. As indicated in Table 4, a large majority (76%) of donors were deferred for travel to areas reporting zero malaria cases in 2005.

The discrepancy between risk areas described in the CDC Yellow Book and actual donor deferrals could have more than one explanation. First, the deferral of donors for travel to areas with no malaria could simply reflect the natural time lag between an area within Mexico having been determined to be free of active transmission, this information being published in the next version of the Yellow Book and the newest version of the Yellow Book being integrated into blood centers' documentation for donor screening for malaria risk. Indeed, the version of the Yellow Book subsequent to the one referenced for this study removed four states from the list of those considered risk areas: Campeche, Guerrero, Jalisco, and Michoacán. A second, perhaps more important factor, might be the limited amount of detail available in the Yellow Book coupled with limited information provided by the donor him- or herself. Consider, for example, the fact that the state of Nayarit has reported nearly all its malaria from the same six counties—Huajicori, Acaponeta, Del Nayar, Rosamorada, Ruiz, and Tepic—between 2002 and 2005[30] (see Fig. 3), but 85% of REDS-II donors were deferred for visiting Nuevo Vallarta and Sayulita in the county of Bahía de Banderas in the southern part of the state, where no malaria case has been reported since at least 1999.[30] A similar dynamic prevails in the states of Guerrero and Quintana Roo. The breadth of risk implied by the phrase "risk in rural areas" followed by a long list of states implies that risk is incurred by any traveler who leaves an urban locale in any of those states, and a high lack of specificity is the result. The issue of timeliness of risk information could in theory be partly remedied by the online malaria risk map CDC has developed,[35] if subsequent iterations of this application were to provide more detailed or more recent information than the print version of the Yellow Book. In sum, while the information provided by the CDC is valuable in assessing risk, it is often insufficient to exclude the need for deferral for many presenting donors.

The risk estimates presented herein should be evaluated in terms of how realistically they reflect the actual risk incurred by deferred donors. The intent of this analysis, as with the prior one, was to develop a conservative model that ensured that risk estimates were not artificially low. We are confident that the first model is successful in this sense, most notably by its prediction of one case of TTM every 6 years from a routine traveler, whereas empirical observation has identified only one such case in the past three decades. We believe that the low risk estimates for travel to each state in the current analysis also are conservative and provide a significant margin of safety. This is probably best illustrated by the data from the state of Oaxaca, which is responsible for the largest share of cases within Mexico and the second-highest population-adjusted risk in 2005. Notably, less than 5% of the state's population lives in risk areas, and only 19 (3.3%) of the 570 counties reported cases in 2005 (Table 2). Combining census and reported malaria data,[30,31] one sees that Oaxaca's 1432 cases are scattered across 265 different towns or hamlets (of 1249 in these 19 counties), which by simple arithmetic implies an average of five malaria cases per town or hamlet, each with mean population of 700 inhabitants. Altogether, the data suggest that the population-adjusted risk of 7.39 cases per thousand residents, or 1:135 residents annually, applies to a very small share of the population and the landscape of Oaxaca. If tourists to Oaxaca faced malaria risk equivalent to those of local residents, one should expect far greater numbers of US tourists to return with malaria. Oaxaca accounts for roughly 1% to 2% of air traffic from the United States to Mexico[25,36] and is associated with 3% of the deferrals in the REDS-II cohort. If even 1% of US overnight travelers to Mexico visit Oaxaca, stay 1 week, and face the malaria risk shown in Table 4, one would expect almost 24 malaria infections from this state alone, four times the average over the past 8 years for the whole country (200,000 travelers × 1:163 risk ÷ 52 for 1-week exposure = 23.6 infections). In sum, use of local resident risk as a proxy for traveler risk undoubtedly overstates the risk in our donor population.

Whether the predicted level of risk supports a complete or partial reversion of current deferral requirements for travel to certain parts of Mexico is a question that has been reviewed recently by policy makers.[37] In a discussion focused only on Quintana Roo, Mexico, the FDA Blood Products Advisory Committee recommended 17 to 1 in favor of allowing donation from donors with travel to that state. Our analysis reinforces this conclusion and supports extending a similar policy to other areas of Mexico with exquisitely low risk for malaria infection. Both within Quintana Roo and elsewhere, most donors reported travel

to areas with zero-reported malaria cases in 2005, and those that traveled to areas with theoretical risk generally incurred risks of very low magnitude. Specifically, three in four deferred donors were associated with areas that were malaria-free in 2005, and 85% of the remainder faced estimated 1-week risks of less than 1:640,000. Only the 3.5% of donors deferred for travel to Oaxaca, Chihuahua, and Sonora faced potential risks that could be considered nontrivial. This finding replicates that from our consortium's first analysis, albeit here on a subnational rather than global scale, the largest share of malaria travel deferrals is associated with travel to the area of lowest estimated risk. Just as Mexico reflects about a 1000-fold lower risk than Africa, donors who visited Quintana Roo appear to have faced an average 1 in 115,661 (annualized) risk, compared to travelers to Oaxaca, with its estimated risk of 1 in 164, a 700-fold difference. Adjusting each for a 1-week exposure, that leaves us with absolute risks on the order of 1 per 6 million for Quintana Roo versus 1 per 8510 for Oaxaca. Returning again to the earlier article, the absolute traveler risk for Quintana Roo is less than 8000 times that of the estimated traveler risk to Africa. We believe that at a minimum Quintana Roo should no longer be considered a deferrable travel destination, and strong consideration should be given to extending such a measure to other locations in Mexico with very low malaria risk.

This study is subject to a number of potential limitations. First, donors from the REDS-II blood centers might have travel patterns that are not representative of US donors overall. In fact, given the lack of REDS-II data from the southwestern United States, one might assume that at least that region's donors could have a different amount of travel to Mexico or undertake travel of a different nature compared to REDS-II donors. While such data are sparse, a parallel study to this one was presented to the FDA's Blood Products Advisory Committee. Using 2008 deferral data from blood centers in the border states of Texas, Arizona, and New Mexico, the study found a similar percentage of donors deferred for malaria travel as was found in REDS-II donors (0.9% vs. 1%), but found that a higher percentage were deferred for travel to Mexico (60% vs. 42%). The distribution across states differed from that in REDS-II donors, with more deferrals for travel to border states Chihuahua and Sonora within Mexico (15% vs. <1%); however, Quintana Roo still represented the state responsible for the largest share (37%) of deferrals. More broadly, the study suggested that a majority of border state donors are also deferred for visits to areas with very low risk (<1:250,000 assuming 1-week exposure).[38]

Another potential limitation is that location data were not available from 15% of the donors in this study who were deferred for travel to Mexico. To cause downward bias in our risk estimates, however, the missing data would have to be from donors with higher malaria risk while traveling to Mexico than other donors from the same blood center, an unlikely scenario. If anything, the missing data are likely to come from relatively low-risk travelers. Since by 2008, 100% of malaria in Mexico has been characterized as occurring in indigenous populations in scattered rural areas,[24] one should expect these areas to be visited by the more adventurous and/or sophisticated traveler, who almost certainly would be able to name at least the state they visited. In any case, because the missing data are adjusted for by reweighting the available data for each center, any difference in risk for donors across REDS-II centers is adequately accounted for.

A third concern might be that the reports of lower levels of malaria within Mexico could reflect diminished surveillance capacity rather than reduced malaria transmission. Available evidence, however, suggests this is not the case. Mexico accounts for less than 0.5% of malaria cases diagnosed across all endemic countries in the Americas, yet carries out more than 18% of all blood smears examined for diagnostic purposes.[39] A slide positivity rate that is a fraction of other countries' together with a higher proportion (36% vs. 21%)[40] of cases found through active case detection confirms that Mexico's malaria surveillance is both robust and committed to identifying all potential cases of infection.

One final possible limitation is that our estimates for risk and benefits associated with modifying the deferral guidelines to Mexico are based on risk estimates from 2005 only, and detailed county-level data are not available from 2006 onward. This could have implications for the reliability of the estimates presented herein as well as for inferences applied to subsequent time periods. While two-thirds of the donors in our analysis actually traveled in 2006, we have no a priori reason to assume that reliance on 2005 malaria surveillance data prejudices our risk estimates. First, between 2005 and 2006, the overall number of cases declined from 2966 to 2413, the API declined from 1.06 to 0.90,[26] and three of the four states with the highest API in 2005 all reported fewer cases in 2006: Chihuahua from 181 in 2005 to 122 in 2006, Oaxaca from 1432 to 575, and Sonora from 29 to 17; Durango, ranked highest in risk in 2005, was only slightly higher in 2006, increasing from 114 to 121 cases. Going forward, one might be concerned that the lack of county-level data beyond 2005 could mean that any relaxation of deferral guidelines might not have recent surveillance data of a high level of granularity to support it. While the ideal scenario would involve the availability of real-time, town- or hamlet-level data, the data presented here are part of a long, downward trend in risk for malaria in Mexico, and most of the areas shown to be malaria-free (or very low risk) in 2005 were also malaria-free or low-risk for several prior years. Further, malaria case reports at the state level are currently available on a weekly basis, with only a 2- to 3-week time lag,[31] so that any significant change in malaria transmission would likely become rapidly detectable. Unless the nature

of donor travel patterns to Mexico were to be reoriented toward risky travel in remote areas and away from beach resorts, the estimates of risk described in this analysis are likely to remain relatively stable.

Based on the preponderance of blood donor deferrals triggered by visits to areas of zero or very limited risk for malaria, we recommend that the deferral policies for donor travel to Mexico be revised in a way that balances risk averted with the significant donor loss. While multiple permutations of new regulations might reasonably be considered, we believe that the data from this analysis and available online are sufficient to inform these efforts.

APPENDIX

The Retrovirus Epidemiology Donor Study-II (REDS-II) is the responsibility of the following persons:

Blood centers:

American Red Cross Blood Services, New England Region: R. Cable, J. Rios, and R. Benjamin

American Red Cross Blood Services, Southern Region/ Department of Pathology and Laboratory Medicine, Emory University School of Medicine: J.D. Roback

Hoxworth Blood Center, University of Cincinnati Academic Health Center: R.A. Sacher, S.L. Wilkinson, and P.M. Carey

Blood Centers of the Pacific, University of California San Francisco, Blood Systems Research Institute: E.L. Murphy, B. Custer, and N. Hirschler

The Institute for Transfusion Medicine: D. Triulzi, R. Kakaiya, and J. Kiss

BloodCenter of Wisconsin: J. Gottschall and A. Mast

Coordinating center:

Westat, Inc.: J. Schulman and M. King

National Heart, Lung, and Blood Institute, NIH

G.J. Nemo

Central laboratory:

Blood Systems Research Institute: M.P. Busch and P. Norris

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CONFLICT OF INTEREST

The authors have no conflicts of interest or other financial involvement to declare.

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39. Pan American Health Organization. Malaria surveillance data. Table 4: Total blood slides examined and number of positive slides by level of malaria transmission, 2007. [cited 2011 Feb 25]. Available from: URL: http://www.paho.org/English/ad/dpc/cd/mal-americas-2007.pdf
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医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2011. 11. 14	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名（企業名）	新鮮凍結血漿-LR「日赤」（日本赤十字社） 新鮮凍結血漿-LR「日赤」成分採血（日本赤十字社）		European Centre for Disease Prevention and Control; News 28 October 2011	ヨーロッパ

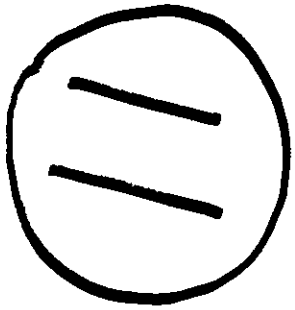
研究報告の概要

○ギリシャでのマラリア　2011年10月
2011年5月21日～10月26日の間に61症例の三日熱マラリア感染がギリシャで報告された。これらの症例のうち33例は流行国への旅行歴がないギリシャ市民で、そのうち27症例は南ギリシャのLakonia県にあるEvrotasから報告された。加えて、季節労働者の28症例もEvrotasから報告され、これらの移民の大部分（21人）はマラリア流行国の出身者であった。
Evrotasでの最初の報告症例は5月23～29日の週に発症し、最後の2症例は10月17～23日の週に発症した。報告された症例の発症ピークは9月5～18日の週の間であった。それ以降、報告数は減少した。全ての症例が三日熱マラリアと確認され、基礎疾患を持つ70歳代男性の1死亡例を除き、全員軽症であった。
10月上旬から当該地域の気温は20℃以下になり、媒介蚊数は急速に減少している。疫学的・昆虫学的な情報や、冬の季節の到来により、現在、当該地域におけるマラリア感染の勢力は非常に弱いと考えられ、まもなく終息すると予測される。この理由により、当該地域への訪問者に対するマラリアの予防服薬は必要ないと考えられる。しかし蚊の刺咬予防の為の標準的な方法の使用促進は継続される。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血

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
ギリシャで2011年5月～10月の間に61症例の三日熱マラリア感染が報告されたが、まもなく終息する見通しであるとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外滞在歴の有無を確認し、帰国（入国）後4週間は献血不適としている。また、マラリア流行地への旅行者または居住経験者の献血を一定期間延期している（1～3年の延期を行うとともに、帰国（入国）後マラリアを思わせる症状があった場合は、感染が否定されるまで、献血を見合わせる）。今後も引き続き、マラリア感染に関する新たな知見及び情報の収集、対応に努める。

JRC2011T-042

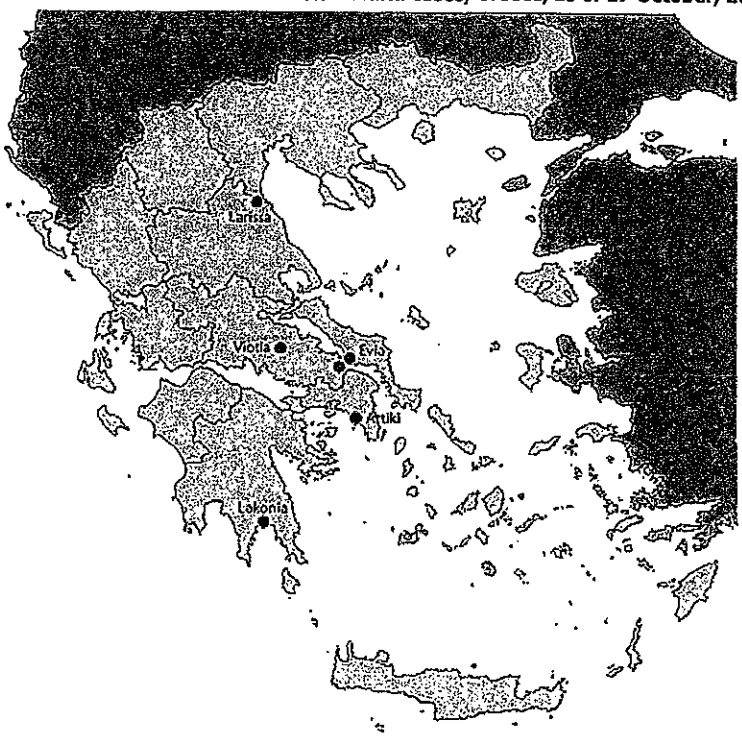
Epidemiological update: Malaria in Greece, October 2011

28 Oct 2011

According to the available epidemiological and entomological information, and the arriving winter season, the intensity of malaria transmission in Evrotas, Lakonia in Greece is believed to be very low and is expected to cease shortly. For this reason, chemoprophylaxis for malaria is not recommended for visitors to the area. The use of standard mosquito biting prevention measures continues to be encouraged.

Between 21 May and 26 October, 2011, 61 cases of *Plasmodium vivax* infection have been reported in Greece. Thirty-three of these cases were Greek citizens without travel history to an endemic country. Twenty seven cases are reported from the area of Evrotas, a 20 km2 river delta area, located in the district of Lakonia in Pelloponese, southern Greece (see map). The remaining six cases are from the municipalities of Attiki (n=2), Evoia (n=2), Viotia (n=1) and Larissa (n=1). In addition, 28 cases of *P. vivax* infection in migrant workers have been reported from the area of Evrotas. Of the 28 malaria cases identified in immigrants in Evrotas, Lakonia, the majority (n=21) originated from malaria endemic countries even though a clear importation status could not be determined; 19 originated from Pakistan and two from Afghanistan. For the remaining seven immigrant cases, five were from Romania (two were diagnosed in Romania), one from Morocco, and one from Poland. It should be noted that all reported cases in immigrants (including the two cases diagnosed in Romania) are in persons that work in the agricultural areas in this part of Greece.

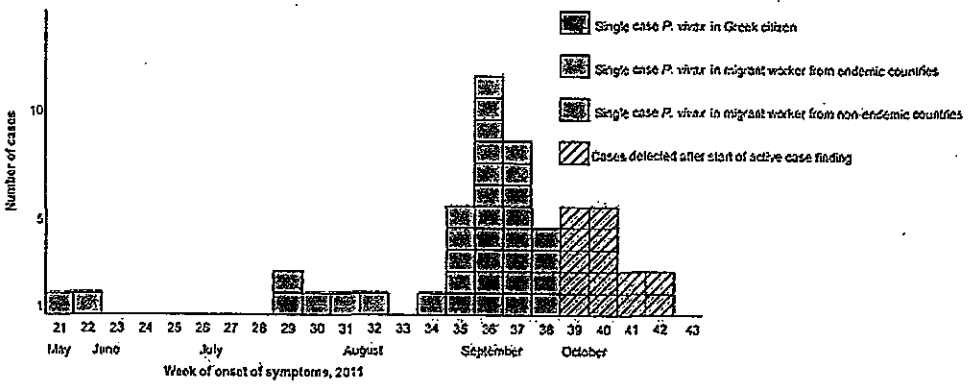
Map: Municipality of residence of *Plasmodium vivax* malaria cases, Greece, as of 17 October, 2011 (n=61)



The first reported cases in Evrotas had symptom onsets in epidemiological week 21 (23-29 May) and the last two cases had reported symptom onset during week 42 (17 – 23 October). A peak in reported symptom onset occurred during weeks 36 and 37 (5 – 18 September). Since then a steady decline in reported cases has been observed despite the ongoing active case finding in the area. All cases reported from other areas in Greece report symptom onset before September 2011. Cases reported in the last two weeks would have likely been infected during September or early October. All cases are confirmed *P. vivax* infections and all were mild, apart from one fatal case in a male aged over 70 years who had pulmonary co-infection and underlying medical conditions.

Data from entomological surveillance indicates that there have been very low larval densities detected in mosquito breeding sites and an absence of adult *Anopheles* mosquitoes captured in carbon dioxide light traps in Evrotas, Lakonia in recent weeks. Since early October, temperatures in the area have dropped below 20°C and the vector population is rapidly waning as expected for autumn and winter months.

Figure: Distribution of reported cases of P. vivax infection by date of onset and citizenship status, Lakonia, Greece, week 21-43, 2011 (n=53).



malaria cases in Romanian farm workers were diagnosed after their return to Romania are also not included. The last reported case was a migrant from Afghanistan who arrived in Greece during the previous 20 days.

Control measures implemented include enhancing surveillance in the affected areas to rapidly identify cases; active case finding in Evrotas, Lakonia (house to house visits since 1 October 2011) followed by microscopy diagnosis for malaria and supervised treatment according to the national protocol (chloroquine and primaquine for uncomplicated *P. vivax* infection); informing health care professionals on early malaria diagnosis and treatment; raising public awareness, and strengthening blood safety. A decree was issued by the Greek MOH to facilitate prompt diagnosis and treatment. Intensified mosquito control measures have also been carried out by local authorities. All *P. vivax* infections were sensitive to chloroquine.

Malaria is an infectious disease caused by the *Plasmodium* parasite, and transmitted by *Anopheles* mosquitoes. The incubation period varies between 7 and 15 days, but long incubation periods of several months (and years) have been observed for P. *vivax* malaria. Malaria is characterised by fever and influenza-like symptoms, including chills, headache, myalgia, and malaise; these symptoms can occur at intervals. More information can be found on the ECDC factsheet.

Following the two visits of ECDC and WHO experts to the affected areas, ECDC has not changed its risk assessment of this event since October 11, 2011, and considers the current risk for malaria infection in Greece to be to persons residing and/or working in the affected areas of Greece, particularly that of Evrotas, Lakonia. This is a geographically delimited area, having a small population and is not a touristic destination. According to the available epidemiological and entomological information, and the arriving winter season, the intensity of malaria transmission in Evrotas, Lakonia is believed to be very low and is expected to cease shortly. For this reason, chemoprophylaxis for malaria is not recommended for visitors to the area. The use of standard mosquito biting prevention measures continues to be encouraged.

Read more:
ECDC Rapid Risk Assessment malaria Greece 2011.
Eurosurveillance article describing the outbreak
More information is available on KEELPNO website
ECDC factsheet

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2012. 3. 15	該当なし	

一般的名称	解凍人赤血球濃厚液	研究報告の公表状況		公表国
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)		Eurosurveillance Edition 2012: Volume 17/ Issue 10; Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20114	ドイツ

研究報告の概要

○ケニヤのマサイマラ地区から帰国したドイツ人旅行者におけるローデシアトリパノソーマ感染

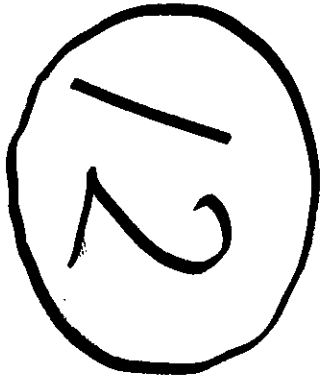
2012年1月、ケニヤのマサイマラ地区から帰国したドイツ人旅行者においてヒトアフリカトリパノソーマ症(HAT)が確認された。この患者は62歳男性で、1月18、19日にマサイマラ国立保護区を訪れ、キャンプ及びサファリ観光をした。大半の時間を半袖半ズボンで過ごし、昆虫忌避剤を使用していた。帰国後の1月28日に発熱により入院し、2月1日に厚層血液スメア標本のギムザ染色で*Trypanosoma brucei rhodesiense*が確認された。スラミンによる治療が行われ、回復した。この症例の1カ月後に、マサイマラ地区からの輸入HAT症例がもう一件報告された。過去10年にわたり、マサイマラ地区から報告されるHAT症例がなかったため、これは注目すべき報告である。この報告は、関連する地域からの旅行者を扱う臨床医に、HATへの認識を促すものとなるであろう。

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
ケニヤのマサイマラ地区から帰国したドイツ人旅行者にローデシアトリパノソーマ感染が確認されたとのことである。 なお、この情報に関して米国CDCからこの地区への旅行者に向けたトラベルアラートが発出されているほか、日本の厚生労働省検疫所のホームページでも情報提供がされ、注意が呼びかけられている。	日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、アメリカトリパノソーマ症の既往がある場合には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。



RAPID COMMUNICATIONS

Trypanosoma brucei rhodesiense infection in a German traveller returning from the Masai Mara area, Kenya, January 2012

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Article published on 8 March 2012

In January 2012, a case of Human African Trypanosomiasis (HAT) has been identified in Germany in a traveller returning from the Masai Mara area in Kenya. The 62-year-old man had travelled to the Masai Mara game park from 18 to 19 January 2012 and developed fever on 28 January. The infection with *Trypanosoma brucei rhodesiense* was confirmed by laboratory testing three days hereafter.

Case report

On 28 January 2012, a 62-year-old man was hospitalised after a sudden onset of fever with temperature up to 39°C in a local hospital near Frankfurt, Germany. The fever started after his return from a holiday trip to Kenya from 8 to 28 January. Upon arrival in Germany and admittance to a local hospital, the patient was suspected to have malaria and treatment with Atovaquon / Proguanil was administered for two consecutive days. The diagnosis was made on the basis of a thin smear, which was later re-evaluated after the patient's transfer to the Infectious Diseases Department of Frankfurt University Hospital and no Plasmodium parasites were detected.

He had travelled by airplane directly from Frankfurt to Mombasa and back and spent all the time at a beach resort south of Mombasa except for a trip to the Masai Mara area from 18 to 19 January. For this trip, he flew from Mombasa to the Ol Kiombo airstrip, stayed at a camp in the area, and then went on safari excursions within a radius of approximately 50 km from the camp. He wore shorts and short sleeved shirts most of the time and used insect repellents.

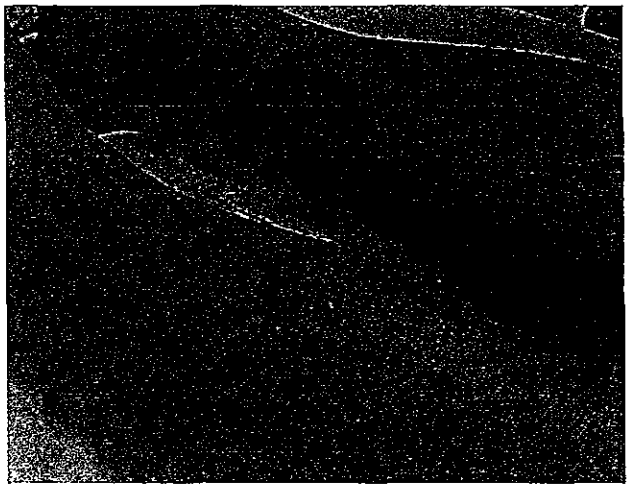
Despite anti-malarial treatment, the patient was still febrile on 31 January and was transferred to the Infectious Diseases Department of Frankfurt University Hospital. By then, the clinical symptoms had become more severe, with strong frontal headaches, vertigo, nausea and arthralgia. Fever was still high at 39.1°C. He had two distinct, painless skin lesions over both tibiae (Figure 1), but no localised or disseminated lymph node enlargement.

Malaria parasites were not confirmed in Quantitative Buffy Coat, Giemsa-stained thin or thick blood smears and the malaria antigen test (BinaxNow) was negative. However, *Trypanosoma brucei rhodesiense* was detected in thick blood smears stained with Giemsa (Figure 2) on 1 February.

Treatment was started three hours after diagnosis of trypanosomiasis with 1 g of suramin as a continuous infusion over one hour. As the substance was not readily available, it was brought to Frankfurt University Hospital from the "Missionsärztliche Klinik" Würzburg, Germany, where a regular stock of suramin is kept. In parallel, the patient was given prednisolone to prevent

FIGURE 1

Chancres due to infection with *Trypanosoma brucei rhodesiense* in a German traveller returning from the Masai Mara area, Kenya, January 2012



allergic reactions. The treatment was followed on day 1, 3, 7, 14 and 21 without complications.

A lumbar puncture performed on day 2 of therapy revealed a normal cerebrospinal fluid (CSF) pattern and a PCR with *Trypanosoma spp.* specific primers was negative from CSF as opposed to the peripheral blood, where it was found to be positive. The patient had leuko- and thrombopenia, an elevated complement regulatory protein (CRP) and aspartate and alanine transaminase levels two times the upper limit of normal. Electrocardiogram and echocardiography did not show any pathological findings.

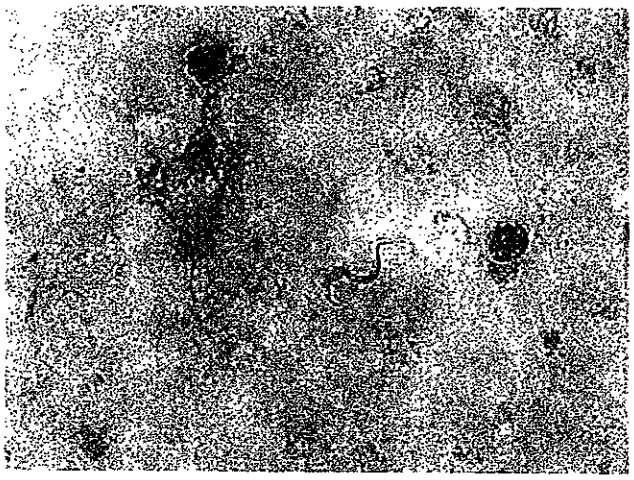
The fever subsided on day 2 of treatment and no parasites were detected from day 3 of the treatment onwards. *T. b. rhodesiense* antibodies were detected by immunofluorescence testing performed at the reference laboratory (Bernhard Nocht Institute, Hamburg, Germany) on day 8 of treatment, 12 days after the first symptoms whilst having been negative on day 1 of treatment. The patient concluded his treatment as planned on day 21 without any residual problems and left the hospital.

Discussion

Following the detection of a case of Human African Trypanosomiasis (HAT) we screened the literature for recent alerts of HAT in Kenya and only ProMED had previously published a report on the occurrence of HAT in Kenya. This however, was documented almost 11 years before the current case [1,2]. About a month after the occurrence of the case described here, there was a further case of HAT from the Masai Mara area described in this issue of *Eurosurveillance* [3].

FIGURE 2

Giemsa-stained *Trypanosoma brucei rhodesiense* in a thick blood smear from a German traveller returning from the Masai Mara area, Kenya, January 2012



64x magnification

A literature research on PubMed revealed two publications that reviewed the epidemiology of HAT in non-endemic countries. A review of HAT cases imported into Europe between 2005 and 2009 included 11 cases, five of which were infected with *T. b. rhodesiense*. There were no cases described from Kenya, but two infected patients had travelled to the Serengeti, which directly borders Masai Mara [4]. In another report, the bibliographic data were supplemented by the World Health Organization (WHO) data on requests of antitrypanosomal drugs from hospitals in non-endemic countries treating travellers. These data showed that 94 cases of HAT were identified between 2000 and 2010, 72% of which were caused by *T. b. rhodesiense*. Although 59% of the cases were identified in Tanzania, with the vast majority of cases being tracked back to the Serengeti, no cases have been reported from Kenya [5].

Trypanosomiasis is a disease that occurs in local clusters, and one such cluster was identified in 2002 through the TropNetEurop Sentinel Surveillance network when two index and seven consecutive cases were identified in non-disease endemic countries in Europe and South Africa [6]. These cases originated in the Serengeti and Tarangire National Parks in close proximity to Masai Mara, but with no documented case originating from the latter.

The above mentioned reports documented imported cases that were diagnosed in non-endemic countries. There are data on the cases diagnosed within the country however. The Kenyan reference hospital for sleeping sickness in Alupe, which is on the Ugandan border north of Lake Victoria, reported 31 patients with HAT caused by *T. b. rhodesiense* between 2000 and 2009. Twenty-two of the patients were diagnosed at the late stage of the diseases and coinfections and comorbidities were frequent [7]. Additionally, WHO extensively mapped the epidemiology of HAT in Africa between 2000 and 2009. For Kenya, sporadic cases were described in the very western provinces Bungoma, Teso and Busia, again on the Ugandan border, as well as in the Nyanza province. Epidemiological analysis of HAT in Kenya between 1950 and 2007 showed that infections occurred exclusively in these Western provinces, and the prevalence is altogether estimated to be low with only sporadic infections the 1990s onwards [8, 9].

Conclusion

We identified a case of HAT due to *T. b. rhodesiense* infection in a traveller who had returned from the Masai Mara area, Kenya. After this case, another report of an imported HAT infection from this area was diagnosed one month later and communicated worldwide [10]. This is noteworthy, as there were no cases described from Masai Mara in the last decade. Previously, there was documented disease activity in Kenya which was limited to the western provinces, as well as Serengeti which is essentially in direct vicinity to Masai Mara.
This report should alert clinicians to be aware of HAT when dealing with travellers from the area concerned.

We have been in contact with WHO in Geneva, Switzerland, who confirmed that the local authorities in Kenya have been informed and a WHO team of experts has been sent to the area to elucidate the situation.

Acknowledgments

We would specifically like to thank Prof. Dr. med. August Stich, affiliated with the "Missionsärztliche Klinik Würzburg, Germany" for his generous and swift support in providing us with suramin. The authors would also like to thank all the medical staff and the diagnostic team involved in the treatment and diagnosis. We would like to extend our gratitude to the patient for agreeing to this publication.

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医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
			2011. 11. 17	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況	Cancelas JA, Dumont LJ, Rugg N, Szczepiorkowski ZM, Herschel L, Siegel A, Pratt PG, Worsham DN, Erickson A, Propst M, North A, Sherman CD, Mufti NA, Reed WF, Corash L.. Transfusion. 2011 Nov;51(11):2367-76. doi: 10.1111/j.1537-2995.2011.03163.x.	公表国
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社)			米国

研究報告の概要

○第2世代S-303病原体不活化処理後の保存赤血球の生存率

背景: 輸血感染症は赤血球(RBC)輸血における懸念事項である。RBC製剤のための病原体不活化技術が輸血の安全性をより高めるために開発中である。S-303はfrangible anchor-linker-effector(FRALE)複合体であり、アルキル化により強力に病原体を減少させる。この研究において、第2世代S-303プロセス処理後35日間保存したRBCの生存能力を調査した。

研究デザイン及び方法: 27人の健康人より得られたRBCをS-303による不活化またはコントロールにランダム割り付けして処理し、35日間保存した。放射標識したRBCを被験者に戻し輸血し、24時間回収率、RBC生存期間を測定した。またin vitroでの代謝・生存能力の変化を分析した。

結果: S-303処理RBCの輸血24時間後回収率及びin vitroの溶血率はコントロールと同等で、FDAの基準を満たしていた。コントロールと比べて生存率は13.7日、T50生存率の中央値は6.8日短かったが、RBC生存曲線より下の領域の面積の差は1.38%にとどまった。不活化処理RBC輸血後、臨床的に意味のある検査値の異常は認められなかった。処理RBCの自己クロスマッチはすべて陰性であった。

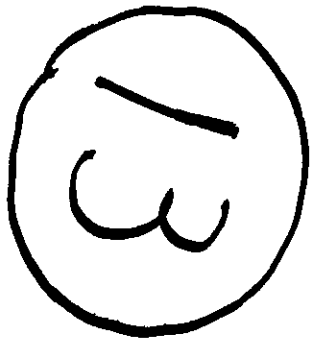
結論: S-303病原体不活化プロセスで処理されたRBCは35日間の保存後、生理学的にも代謝的にも輸血に適しており、24時間回収率もFDAガイダンスを満たし、抗体産生も誘発しなかった。

報告企業の意見	今後の対応
第2世代S-303病原体不活化処理した赤血球について保存35日後に調査したところ、生体内回収率は輸血に適する状態であったとの報告である。	今後も細菌やウイルスを不活化する方策について情報の収集に努める。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク



Stored red blood cell viability is maintained after treatment with a second-generation S-303 pathogen inactivation process

Jose A. Cancelas, Larry J. Dumont,* Neeta Rugg, Zbigniew M. Szczepiorkowski, Louis Herschel, Alan Siegel, P. Gayle Pratt, D. Nicole Worsham, Anne Erickson, Meisa Propst, Anne North, Claire D. Sherman, Nina A. Mufti, William F. Reed, and Laurence Corash*

BACKGROUND: Transfusion-transmitted infections and immunologic effects of viable residual lymphocytes remain a concern in red blood cell (RBC) transfusion. Pathogen reduction technologies for RBC components are under development to further improve transfusion safety. S-303 is a frangible anchor-linker-effector with labile alkylating activity and a robust pathogen reduction profile. This study characterized the viability of RBCs prepared with a second-generation S-303 process and stored for 35 days.

STUDY DESIGN AND METHODS: This was a two-center, single-blind randomized, controlled, crossover study in 27 healthy subjects. S-303 (test) or control RBCs were prepared in random sequence and stored for 35 days, at which time an aliquot of radiolabeled RBCs was transfused. The 24-hour recovery, RBC life span, and in vitro metabolic and viability variables were analyzed.

RESULTS: The mean 24-hour RBC recovery and hemolysis of test RBCs were similar to control RBCs and were consistent with the Food and Drug Administration (FDA) guidance for RBC viability. The mean differences in life span and median life span (T_{50}) of circulating test RBCs were 13.7 and 6.8 days, while the mean difference in the area under the curve of surviving RBCs was 1.38%, in favor of control RBCs. There were no clinically relevant abnormal laboratory values after the infusion of test RBCs. All crossmatch assays of autologous S-303 RBCs were nonreactive.

CONCLUSIONS: RBCs prepared using the S-303 pathogen inactivation process were physiologically and metabolically suitable for transfusion after 35 days of storage, met the FDA guidance criteria for 24-hour recovery, and did not induce antibody formation.

Transfusion-transmitted diseases (TTDs) persist despite implementation of screening tests and donor deferral policies. Blood donations within the window period of detection, containing a low pathogen copy number below the limit of detection of current screening tests, and emergence of new pathogens for which licensed tests are not available are the main causes of TTD persistence.[1-4] Inhibition of nucleic acid replication in cellular blood components inactivates pathogens and viable residual white blood cells (WBCs) associated with TTD and adverse immune-mediated reactions.[5,6] The current prevention strategy for immunologic effects, including transfusion-associated graft-versus-host disease prevention, is through leukoreduction and ionizing irradiation treatment of blood components.[7] However, this treatment is associated with a reduction in red blood cell (RBC) shelf life and the release of high levels of potassium into the extracellular medium[8] and requires two blood component inventories in hospitals that do not practice universal irradiation of all blood components.[9]

ABBREVIATIONS: AE(s) = adverse event(s); AUC(s) = area(s) under the curve; GSH = glutathione; MCHC = mean corpuscular hemoglobin concentration; TTD(s) = transfusion-transmitted disease(s).

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TRANSFUSION 2011;51:2367-2376.

Immunologic complications of transfusion, including transfusion-related alloimmunization and immunomodulation, have been associated with WBC antigens and WBC-derived compounds. While implementation of universal leukoreduction has resulted in a significant decrease in infectious complications,[10] residual WBCs are believed to be a major source of alloimmunization and immunosuppression after transfusion.[11-13] Some biologic data have suggested that use of pathogen inactivation methods may reduce alloimmunization associated to WBC antigen presentation.[14-17]

Treatment of RBCs with S-303 has been shown to effectively (≥4 log) reduce a wide range of blood-borne pathogens and inactivate residual WBCs.[18-22] S-303 contains an intercalator group that inserts into the helical region of the nucleic acid, an effector group that allows covalent modification of nucleic acid, and a central frangible bond that allows degradation of the compound. Activation and cross-linking occurs on transfer from a low pH environment to the neutral pH of blood. The process is completely light independent. The primary byproduct of S-303 degradation is S-300, a nonreactive species that is excreted. Glutathione (GSH), a naturally occurring substance present in most cells of the body at millimolar concentrations, is used in conjunction with the S-303 treatment to quench nonspecific reactions of S-303 such as surface modification of the RBC.

The first-generation S-303 treatment process for RBCs (0.2 mmol/L S-303 and 2 mmol/L GSH) was evaluated in a series of six clinical studies. Four of these studies were radiolabel recovery studies in healthy subjects,[23,24] and two were Phase III studies in patients requiring therapeutic RBC transfusion support.[25,26] The Phase III clinical study of chronic RBC transfusion was terminated before completion when antibodies to S-303–treated RBCs were detected in two patients requiring chronic RBC transfusion support.[27] To eliminate the immunoreactivity of the treated RBCs observed in the Phase III study, the S-303 treatment process was modified to reduce the amount of acridine bound to RBCs by increasing the concentration of the quencher, GSH, to 20 mmol/L and increasing the pH of the GSH by using a disodium salt form.

An initial in vivo kinetic study with RBCs treated under these modified conditions showed a reduced packed cell volume due to moderate dehydration and reduced 24-hour RBC recovery of S-303–treated RBC stored for 35 days compared to controls.[24] The S-303 treatment process was then optimized by replacing the hypertonic GSH containing supernatant after treatment with a standard, isotonic solution before storage. This report describes the in vitro characterization and viability of RBC prepared with this second-generation S-303 treatment process that incorporates the process modifications of treatment with higher pH and GSH concentration followed by a volume exchange step before storage.

MATERIALS AND METHODS

Study design and enrollment

This was a randomized, single-blind, controlled, two-period crossover study, designed to evaluate 24-hour post-transfusion recovery and life span in healthy adult subjects of autologous RBCs prepared using the second-generation S-303 treatment process and stored for 35 days. A total of 27 subjects were enrolled and consented using an informed consent document approved by the local institutional review board, at two study sites, the Hoxworth Blood Center, University of Cincinnati, and Dartmouth-Hitchcock Medical Center. The order in which each subject was transfused with S-303–treated (test) and control RBCs was randomly assigned at the time of enrollment. Each treatment period consisted of autologous blood donation, preparation of RBCs according to treatment assignment, infusion of radiolabeled autologous RBCs, and collection of blood samples for assessment of RBC recovery and life span (Fig. 1).

The primary endpoint was comparison of the 24-hour posttransfusion RBC recovery. Additional endpoints were mean life span, median life span (T_{50}), and area under the RBC clearance curve (AUC); the incidence of anti–S-303 development after transfusion of test RBCs (with or without S-303 RBC specificity) measured using a gel card crossmatch assay against S-303 RBCs; incidence of adverse events (AEs); and the in vitro characteristics of test and control RBCs.

Donor eligibility criteria included age 18 years or older, meeting AABB physical exam guidelines for allogeneic blood donation, no prior S-303 exposure, negative direct antiglobulin test, a predonation blood hemoglobin (Hb) level of 13.0 g/dL for females and 14.5 g/dL for males, and healthy by history and physical examination.

Sample size was determined to reach 80% power and detect a treatment difference of 4.4% (absolute difference) and 5.2 days in RBC recovery and T_{50} at the two-sided 0.05 significance level, assuming a standard deviation (SD) of 7.5% and 8.8 days for the paired difference in RBC recovery and T_{50}, respectively, based on a previous crossover study of S-303 RBC recovery and life span.[24]

Preparation of S-303 RBC and control components

On Day 0 of Treatment Periods 1 and 2, 1 unit of autologous whole blood (500 ± 50 mL) was drawn from the subject into a primary collection container containing citrate-phosphate-dextrose anticoagulant (Terumo Imuflex WB-RP blood bag system with in-line leukoreduction filter, Terumo Corp., Tokyo, Japan). Collected blood was leukoreduced and processed by centrifugation into RBCs by removing plasma and adding AS-5 (Optisol, Terumo Medical Corporation, Somerset, NJ) to achieve a final hematocrit (Hct) ranging from 50% to 65%. Both test

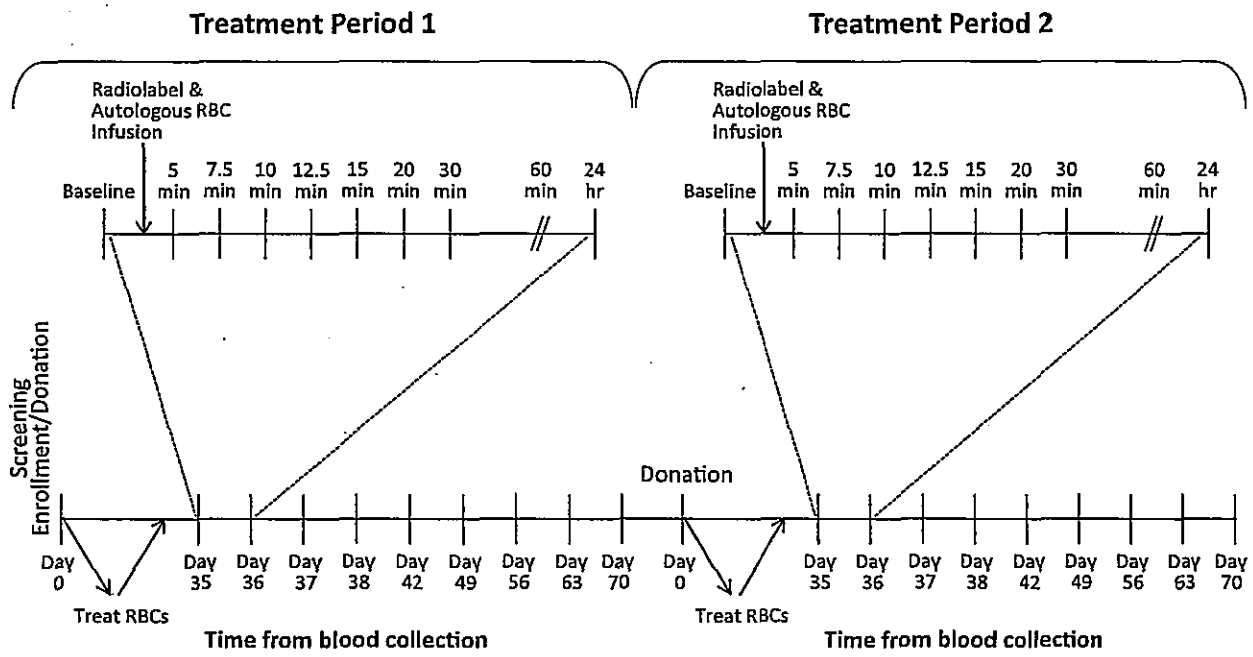

Fig. 1. Schema of the study design. Subjects were enrolled in two treatment periods (70 days each) where autologous control or test RBCs, randomly, were radiolabeled and infused.

and control RBCs were shipped to Cerus Corp. where the test RBCs were treated with the S-303 treatment process (Fig. 2) within 5 days after donation, and the control RBCs were untreated. All units were stored at 1 to 6°C in monitored refrigerators until return shipment to the study sites. All shipments were on wet ice, maintaining temperatures of 1 to 10°C.

Test RBCs were prepared by diluting RBCs in a solution containing 55 mmol/L dextrose, 1.3 mmol/L adenine, 55 mmol/L mannitol, and 20 mmol/L sodium citrate, to reach a Hct of approximately 40% in a closed processing set (Avail Medical, Irving, TX). GSH sodium salt (TAD 2500, BioMedica Foscama, Ferentino, Italy) and S-303 (AMRI, Albany, NY, and KP Pharmaceutical Technologies, Bloomingdale, IN) dissolved in 0.9% saline for injection was used. Approximately 15 mL of each was added to achieve final concentrations of 20 and 0.2 mmol/L, respectively. The RBCs were transferred to an incubation and storage container and stored at 20 to 25°C for approximately 18 hours. After being incubated, the RBC units were centrifuged for 6 minutes at 4100 × g, and approximately 250 mL of the supernatant was removed and replaced with 100 mL of AS-5, followed by storage at 1 to 6°C. Each RBC unit was returned to the respective study site within 14 days after S-303 treatment. Unlike the first generation of S-303 process,[23] the use of an exchange process avoided the need for a compound adsorption device.

Because the study RBCs had been out of their control when shipped off-site, each study site confirmed and documented that the study RBC units returned to them

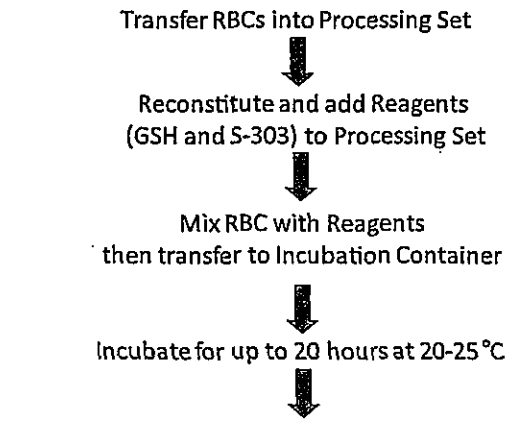

Fig. 2. Diagram of the treatment configuration used to prepare S-303 RBCs. The RBC unit and diluents solution are transferred to the mixing container of the processing set. The GSH and S-303 are reconstituted and then sterilely transferred to the mixing container of the processing set resulting in a final concentration of 0.2 mmol/L S-303 and 20 mmol/L GSH. After being mixed with GSH and S-303 with the RBC and diluent solution, the treated RBC unit is transferred to an incubation container. The RBC unit is incubated at room temperature (20-25°C) for up to 18 hours. At the end of the incubation period the RBC unit is centrifuged, the supernatant is expressed, and the RBC unit is stored in fresh additive solution.

were those donated by the subject recipient. The identity of each returned unit was confirmed by comparison, and verification of the RBC extended phenotype using a panel of RBC major and minor antigens comparing preshipment samples to postshipment samples.

Chromium-51–labeled RBC 24-hour recovery

Samples for bacterial culture were taken from each unit at least 5 days before the planned reinfusion date. On Day 35 of Treatment Periods 1 and 2, the day of reinfusion, each study RBC unit (either test or control) was inspected for signs of hemolysis, clotting, or discoloration, and the bacterial culture was confirmed negative. The unit was crossmatched against a fresh subject blood sample. The unit was mixed by hand and an aliquot of the RBCs was collected from the unit and labeled with 10 to 20 μCi of ^{51}Cr using standard techniques.[28] The labeling agent, sodium 51-chromate, was mixed aseptically with the aliquot of RBCs at room temperature for 30 minutes followed by a wash step to remove any unincorporated label. An aliquot of the final volume was reserved for assay as a standard. Also on Day 35 of Treatment Periods 1 and 2, a fresh sample of heparinized blood (approx. 10 mL) was collected from the subject and treated with a sterile tin pyrophosphate solution, containing approximately 2.0 μg of tin. After a 5-minute incubation at room temperature, the autologous RBCs were washed with 2 volumes of cold saline; 20 μCi of technetium-99m (^{99m}Tc) pertechnetate was then added and incubated at room temperature for 10 minutes. After an additional cold saline wash, approximately 4 mL of the labeled RBCs was drawn up into a syringe. The ^{99m}Tc-labeled RBCs were carefully added to the ^{51}Cr-labeled RBCs and the two populations of labeled RBCs were infused for assessment of subject blood volume and RBC recovery and survival, respectively. The amount of RBCs and the amount of radioactivity infused were determined based upon the methods of Moroff and colleagues[28] and the International Committee for Standardization in Hematology.[29]

The infusion of the radiolabeled aliquot and subsequent sampling was carried out according to study site standard operating procedures and as described by Moroff and colleagues.[28] Briefly, after infusion of approximately 10 mL of stored ^{51}Cr RBCs mixed with freshly collected ^{99m}Tc-labeled RBCs, seven blood samples were drawn over the first 25 to 30 minutes after completion of the infusions and one at 24 hours (Day 36). Additional blood samples for ^{51}Cr activity assessment to measure life span were collected at 48 hours (Day 37), 72 hours (Day 38), and 7 days (Day 42) postinfusion and then weekly through 35 days postinfusion (Days 49, 56, 63, and 70). The radioactivity of the samples was counted in a gamma counter to determine ^{51}Cr- and ^{99m}Tc-specific activity. The measurement of ^{51}Cr provided an assessment of RBC recovery, and the measurement of ^{99m}Tc was used to measure blood volume.[28] All ^{51}Cr specimens used for survival analysis were counted at the same time and corrected for decay.

RBC hematologic and chemical analysis

An aliquot was removed from the RBC units immediately after collection (Day 0 of Treatment Periods 1 and 2) and on the day of infusion (Day 35 of Treatment Periods 1 and 2) using closed-system sampling methods. These samples were assessed for RBC physiologic function measured by in vitro variables (pH at 37°C, free Hb, adenosine 5′-triphosphate [ATP], 2,3-diphosphoglycerate [2,3-DPG]), extracellular potassium, extracellular glucose, extracellular lactate, packed cell volume, and mean corpuscular Hb concentration (MCHC) as previously described.[30] In addition, a complete blood count with differential was obtained on all study units on Days 0 and 35.

Immunohematologic analysis

Sera from the subjects obtained before infusion on Day 35 of Treatment Periods 1 and 2 were tested for antibodies to autologous control and autologous test RBCs using a commercial anti-human immunoglobulin G gel card assay (Ortho Clinical Diagnostics, Raritan, NJ). In addition, test and control sera, collected and frozen on Days 49 and 70 of each period, were screened against three individual units of S-303–treated and untreated allogeneic RBCs to detect antibodies to S-303 RBCs. The allogeneic RBCs (with the phenotype O, Rh cde, K–) were prepared as conventional RBCs and as S-303–treated RBCs. Allogeneic RBCs were utilized to test the serum samples from Day 49 and Day 70. These assays were conducted at a central reference laboratory (American Red Cross Blood Services, Pomona, CA).

Calculations and statistical analysis

RBC life span was estimated by dividing the ^{51}Cr raw counts (adjusted for Hct) for time points greater than 24 hours (N_t) by the counts at time zero (N_0). The estimate of the percentage of surviving cells was corrected for the loss of ^{51}Cr label over time by using an elution multiplicative factor (emf).[31] Linear and exponential functions were used to fit the data distribution and a weighted mean approach was used to derive a single estimate of T_{50} and mean life span. The estimates of mean life span and T_{50} from each model (linear and exponential) were pooled as follows:

$$\text{Mean lifespan (weighted)} = \frac{(MSE_{exponential} \times \hat{\mu}_{linear} + MSE_{linear} \times \hat{\mu}_{exponential})}{(MSE_{exponential} + MSE_{linear})} \text{ and}$$

$$T_{50}\text{(weighted)} = \frac{(MSE_{exponential} \times \hat{m}_{linear} + MSE_{linear} \times \hat{m}_{exponential})}{(MSE_{exponential} + MSE_{linear})}$$

where MSE stands for the mean square error and $\hat{\mu}$ and $\hat{m}$ represent the means for each of the models (linear or exponential).

The AUC was determined by the trapezoid rule[32] where the survival curve is divided into a series of trapezoids (between time points), and the area of each trapezoid is calculated by the product of the width and mean height. The overall AUC is the sum of the trapezoidal areas.

For RBC recovery analysis, carryover effect was deemed insignificant ($p \geq 0.10$) so that a period-adjusted t test was used to test for treatment difference in mean recovery values. Unless otherwise stated, statistical analyses were performed using an analysis of variance (ANOVA) model with terms for sequence, period, treatment, and subject nested within sequence, where subject nested within sequence was a random effect, while the others were fixed effects, and except when stated otherwise, statistical tests were conducted using a two-sided alternative hypothesis at a significance level of 0.05. The one-sided 95% confidence interval (CI) for proportion of patients with a recovery of at least 75% was determined via the Clopper-Pearson method.

RESULTS

Demographics and baseline subject characteristics

Table 1 displays the demographic characteristics of the study subjects including ABO/Rh blood group. A total of 27 subjects, 11 males and 16 females, ranging in age from 25 to 66 years, met entry criteria; and 26 completed both phases of the in vivo recovery and survival studies. Direct antiglobulin tests and antibody screen were negative for all subjects enrolled at screening.

TABLE 1. Subject and immunohematologic demographics at screening (indirect antiglobulin test)*

Sex	
Male	11 (40.7)
Female	16 (59.3)
Blood group	
A	9 (33.3)
B	7 (25.9)
AB	1 (3.7)
O	10 (37.0)
Rh	
Negative	3 (11.1)
Positive	24 (88.9)

* Data are reported as number (%).

RBC content and prestorage hemolysis of S-303–treated units

S-303–treated RBC products, immediately after processing and before storage, had a net weight of 297 ± 10 g, with a Hct of 59 ± 1.8% and a Hb concentration of 18.8 ± 0.8 g/dL corresponding to a total Hb content of 53.1 ± 3.6 g. The mean prestorage hemolysis level was 0.1 ± 0.0%.

Twenty-four–hour posttransfusion recovery

The mean posttransfusion recoveries were 88.0 and 90.1% for test and control RBCs, respectively (Table 2). These means were greater than the Food and Drug Administration (FDA) guidance for a minimum mean recovery of 75%. The SDs for recovery in test and control were 8.50 and 6.88%, respectively, consistent with the FDA criteria of a maximum 9%. There was one subject in each treatment group with recovery less than 75%. The lower bound of the CI for the proportion of subjects with a recovery of 75% or greater exceeded the minimum of 70%. The lower confidence bounds for test and control were both 83.6%. The modest difference in mean 24-hour postinfusion recovery of test and control RBCs was not significant.

TABLE 2. 24-hour Cr/Tc posttransfusion recovery of RBCs (%)

	Test (n = 27)	Control (n = 27)	p value
Mean ± SD	88.0 ± 8.5	90.1 ± 6.9	0.31
Median	88.7	90.8	
Range	63.2-109.3	72.0-103.2	
Number of subjects with recovery < 75%	1	1	
95% LCB of one-sided CI for proportion of subjects with recovery ≥ 75%	83.6%	83.6%	
Mean difference (test – control)	−2.08		
Two-sided 95% CI of mean difference	−6.20 to 2.04		

LCB = lower confidence boundary.

RBC survival

The mean life span and T_{50} of RBCs was shorter compared to that of control RBCs (Table 3). The mean life spans (calculated by the weighted mean method) for test and control were 75 and 88 days, respectively. The mean difference in mean life span between test and control was −14 days ($p = 0.0008$). The mean T_{50} (calculated by the weighted mean method) for test and control were 33 and 40 days, respectively. Similar to the results obtained for mean life span, the mean median life span was significantly reduced in test compared to control for both sites combined ($p < 0.0001$). The mean AUCs for surviving cells, for test and control groups, were 23 and 24%, respectively ($p = 0.0124$).

Poststorage biochemical variables

In vitro variables indicative of RBC quality were analyzed for test and control RBCs (Table 4). On Day 35, mean hemolysis levels were 0.24 and

TABLE 3. Mean life span and T_{50} of RBCs

	Test (n = 26)	Control (n = 26)	Mean difference (test – control)	Two-sided 95% CI of mean difference	p value
Mean (days)	74.6	88.3	−13.73	−21.12 to −6.36	0.0008
T_{50} (days)	32.7	39.5	−6.77	−9.57 to −3.98	<0.0001
AUC (% × days)	22.5	23.9	−1.38	−2.44 to −0.33	0.0124

TABLE 4. RBC characteristics (mean ± SD)

Characteristic	Control (Day 35)	Test (Day 35)	p value
ATP (µmol/g Hb)	3.59 ± 0.808	3.34 ± 0.799	0.0028
Extracellular potassium (mmol/L)	52.7 ± 6.48	39.5 ± 3.56	<0.0001
Hemolysis (%)	0.22 ± 0.133	0.24 ± 0.132	0.44
Free Hb (mg/dL)	106.9 ± 64.95	104.43 ± 55.8	0.2628
Extracellular glucose (mmol/L)	14.6 ± 2.11	28.7 ± 5.45	<0.0001
Extracellular lactate (mmol/L)	28.8 ± 3.44	18.1 ± 2.72	<0.0001
pH at 37°C	6.483 ± 0.122	6.404 ± 0.039	0.0078
2,3-DPG (µmol/g Hb)	0.29 ± 0.280	0.12 ± 0.137	0.8202
MCHC (g/dL)	32.5 ± 1.96	33.9 ± 1.68	<0.0001
Spun Hct (%)	59.7 ± 2.4	62.4 ± 3.6	<0.0001

TABLE 5. Summary of AEs by subject

AE type	Test (n = 27)	Control (n = 27)	Overall (n = 27)	p value
Any AE	8	3	11	0.23
Mild (Grade 1)	5	3	8	
Moderate (Grade 2)	2	0	2	
Severe (Grade 3)	1	0	1	
Potentially life-threatening (Grade 4)	0	0	0	
Possibly or probably related AE	4	3	7	1.0
After donation and before infusion	1	1	2	
Postinfusion	3	2	5	
AE leading to study discontinuation	0	0	0	
Serious AE	1	0	1	

0.22% for test and control, respectively, with a maximum level of hemolysis at of 0.61% in test and 0.66% in control RBCs.

Biochemical variables measured on Day 35 showed glucose levels of 28.7 and 14.6 (mmol/L), for test and control, respectively, while mean lactate values increased to 18.1 and 28.8 (mmol/L) and pH dropped to 6.40 and 6.48 for test and control RBCs, respectively, suggesting increased glucose catabolism in S-303–treated RBCs. Mean potassium values on Day 35 were significantly lower in the test group as a consequence of the exchange protocol. The exchange protocol did not significantly modify the levels of 2,3-DPG or ATP immediately after processing. On Day 35, the mean levels of ATP were 3.34 and 3.6 µmol/g Hb for test and control RBCs, respectively, and, as expected, the intracellular concentration of 2,3-DPG was completely depleted by Day 35 of storage.

Immunohematologic analysis

In the context of a small-dose infusion, the results of the crossmatch assays on subjects' sera were negative for all subjects before infusion on Day 35. After the infusion, on Days 49 and 70 sera from all subjects were collected and were tested against allogeneic RBCs. All crossmatch analyses were negative, except for one subject with a weakly positive reaction (1+) for only one out of three test RBCs, on Day 49 after infusion of control RBCs in Period 2, suggesting that this reactivity was not likely to be specific for S-303–treated RBCs. The subject's Day 70 serum sample for the interval after infusion of S-303–treated RBCs was negative. Because there were no positive crossmatch tests to S-303 RBCs after RBC exposure, no further testing was done.

AEs

Overall, 12 AEs were recorded for 11 subjects. During the 24 hours after infusion, eight subjects experienced AEs after test RBCs and two subjects experienced AEs after the control RBCs (p = 0.2266, Table 5). One severe AE was due to hospitalization for severe knee inflammation 27 days after the infusion of test RBC infusion in Treatment Period 2. However, this latter event was consistent with an existing condition and was considered unlikely to be related to the study treatment. Three moderate AEs were reported by two subjects within 44 days after infusion of test RBCs. These events included nonhemorrhagic, papular rash in extremities of unknown etiology after receiving the RBC infusion in one subject and moderate stomach pain and diarrhea after receiving the RBC infusion in another subject. Both events were judged as unlikely related to study treatment by the local investigator: mild AEs within 24 hours after S-303–treated RBC infusion included mild, transient, decreased diastolic blood pressure, hyperhydrosis, iron deficiency, and dizziness. Mild AEs within 24 hours after control RBC infusion included local skin reaction at infusion site and increased blood lactate dehydrogenase. The overall AE incidence was not different between the test and control group infusions (p = 0.23, McNemar's test) and not different for AE possibly or probably associated with treatment (p = 1.0).

DISCUSSION

S-303 is derived from a new class of frangible anchor-linked effectors with labile alkylating activity.[25] The first generation of the S-303 RBC treatment system demonstrated inactivation of a large spectrum of pathogens and WBCs[33,34] and was evaluated in a series of six clinical studies. Four of these studies were radiolabel recovery studies in healthy subjects,[23,24] and two were Phase III studies in patients requiring therapeutic RBC transfusion support.[25,26] The Phase III clinical study of chronic RBC transfusion was terminated before completion when antibodies to S-303–treated RBCs were detected in two patients requiring chronic RBC transfusion support.[27] Low-titer antibodies against acridine, present on the membrane of the S-303–treated RBCs were detected by positive crossmatch reactions of the patient sera to S-303–treated RBCs. Antibody titers declined to undetectable levels in both patients during the follow-up period, and were shown to be unable to react against S-303–treated RBCs which were prepared with a modified protocol similar to the one presented in this article.[35] A second clinical trial of patients receiving transfusion of S-303–treated RBCs during cardiac surgery showed an identical frequency of crossmatch reactivity to S-303 RBCs (2 of 74 patients in test and control).[25]

A second generation of the S-303 treatment process, which was found to reduce the potential for immune reactions and maintain acceptable RBC in vitro quality throughout 35 days of storage, was tested in this Phase I clinical trial. The primary objective of this study was to evaluate the posttransfusion viability of autologous RBCs prepared using the second generation S-303 pathogen inactivation process and stored for 35 days. The study utilized a crossover design, which has the major advantage of allowing intrasubject comparison between treatments by minimizing the intersubject variability from the comparison. The primary endpoint was the posttransfusion recovery 24 hours after infusion of test and control RBCs. This primary endpoint was selected because 24-hour posttransfusion recovery represents the most commonly used criterion indicative of RBC transfusion efficacy. The 24-hour recovery was not significantly different between test and control RBCs. The 24-hour recovery for test RBCs was 88.0 ± 8.5% and that for control RBCs was 90.1 ± 6.88%. These results fall within the FDA guidance criteria defining the quality of RBC components based on 24-hour posttransfusion RBC recovery with respect to both the absolute proportional recovery and the maximum allowed variability in the 24-hour recovery measurements.

Mean life span, T_{50}, and AUC were evaluated as additional measures to characterize the viability of S-303–treated RBCs. These variables were assessed by ANOVA statistical analysis for a two-period crossover study design and by clinical relevance based on the criterion of bioequivalence applied to other biologic variables defined by a biologic variable within approximately 20% of its reference value.[36] Approximately 1% of RBCs (senescent RBCs) are normally cleared from the circulation each day and endogenous RBCs survive for approximately 120 days.[31] The model used to calculate the clearance of ^{51}Cr-transfused RBCs affects the measurement of RBC T_{50} and life span.[37] Linear clearance is thought to be most consistent with senescence, that is, the oldest RBCs preferentially leaving the circulation. Exponential clearance is thought to reflect clearance by a random process, perhaps due to physical or metabolic changes that affect viability.[38] Following the International Committee for Standardization in Hematology recommendations,[29] we modeled RBC survival through both a linear and an exponential mathematical function and used a weighted mean model when neither the exponential nor linear regressions demonstrated superior fit.[29] The mean test and control RBC T_{50} values were 32.7 and 39.5 days, respectively ($p < 0.0001$). For mean AUC, test RBCs were 22.5%, while control RBCs were 23.9% of cells surviving ($p = 0.0124$). The mean life span for test RBCs was 74.6 days, while that for control was 88.3 days ($p = 0.0008$). Of notice, the mean life span results are probably subject to a higher variation due to the intrinsic limitation of ^{51}Cr survival analysis which uses data extrapolation to calculate the axis intercept. For additional accuracy of RBC survival, we are planning further determinations of long-term RBC survival in the context of allogeneic transfusions of biotinylated RBCs. Despite these statistical differences, it is of note that all these indicators of RBC viability fall within the general guideline of 20% variation from the control consistent with the criterion of bioequivalence. The mean T_{50} falls within the published reference range of 28 to 35 days for conventional autologous RBC infusions.[39]

In addition to in vivo measures of viability, a series of in vitro variables were used to assess the quality of S-303 RBCs stored for 35 days. Minimal hemolysis, measured by plasma free Hb, is an index of RBC quality. On Day 35 all RBC units (both test and control) had hemolysis levels of less than 1%. The maximum hemolysis on Day 35 was 0.61% in test and 0.66% in control RBC components. Mean hemolysis on Day 35 was not statistically different between test and control RBCs (0.24% vs. 0.22%, $p = 0.44$).

ATP is an important reflection of RBC glycolytic metabolism and correlates with posttransfusion RBC recovery. By Day 35 of storage, the mean values of ATP for both test and control were well above the threshold value of 2 μmol/g Hb, which is considered acceptable for transfusion[40] and the marginal decrease in ATP concentration of test RBCs was not associated with diminished 24-hour recovery or increased hemolysis.

To assess RBC hydration, MCHC was measured using an automated hematology analyzer. This is an important

variable because RBCs with altered hydration status may exhibit diminished life span. For freshly drawn RBCs, the MCHC reference value is 34 g/dL, with 2 SDs lower being 31 g/dL.[39] For control RBCs, the Day 35 MCHC was 32.5 g/dL and that for test was 33.9 g/dL ($p < 0.0001$). Both of these values are within the reference range for fresh RBCs. Overall, these in vitro data indicate that RBCs prepared using the S-303 treatment process remain viable and metabolically suitable for transfusion through 35 days of storage. These data are also consistent with the 24-hour posttransfusion recovery results and with the three indicators of RBC life span indicating bioequivalence between test and control RBCs.

AEs were recorded for 24 hours after autologous blood donation and for 24 hours after each study transfusion. No AE led to study discontinuation and no significant AE was found to have a temporal or causal relationship to the infusion of test RBCs. There were eight AEs in the test group and three AEs in the control group ($p = 0.23$). Most AEs were mild to moderate with the exception of one severe AE in the test period that was considered not related to the infusion of S-303–treated RBCs. Because of the small-volume exposure to the test article and specific design to define the in vivo RBC recovery, this study had limited power to assess safety events. Despite that, we did not observe any significant AEs with a temporal or causal relationship to the infusion of S-303–treated RBCs.

Two patients in the RBC chronic transfusion trial with the first-generation S-303 process developed antibodies to S-303–treated RBCs. The immunoreactivity observed was due to the acridine moiety of the S-303 molecule and the lack of involvement of the RBC surface in this reactivity was confirmed by complete inhibition using acridine alone, consistent with the absence of neoantigens on the RBC surface. These antibodies did not induce detectable hemolysis in vivo in either of the two patients. In addition, a monocyte monolayer assay showed no increased RBC phagocytosis in the presence of sera of these two patients.[26,27,35] This second-generation process was designed to reduce the level of acridine bound to the RBC surface, thus reducing the potential for immune response. This has been supported by in vivo studies using a rabbit model of chronic RBC transfusion.[41] In these studies, chronic transfusion of RBCs with high levels of acridine (similar to the first-generation process) elicited an acridine-specific antibody response, whereas transfusion of RBCs with low levels of acridine (similar to the second generation process) did not.

In the context of infusion of small dose of S-303–treated RBCs, there was no evidence of immune response to 35-day-stored RBCs prepared using the S-303 exchange treatment process. S-303–treated RBC antibodies were not detected in any of the subjects' specimens when followed for up to 70 days postinfusion. Although the overall amount of infused RBCs was small, the absence of detectable antibody formation supports progression to studies of repeated transfusions of full units for recognized therapeutic indications.

In summary, in this Phase I two-period crossover study conducted in healthy volunteers, the 24-hour post-transfusion recovery of RBCs prepared using the S-303 pathogen inactivation process was not significantly different than the recovery of control RBCs and complied with the FDA guidance criteria for 24-hour RBC recovery and hemolysis at the end of the storage period, with no significant difference between test and control RBCs. RBC survival assessed through three different variables fell within 20% of control RBCs, indicating bioequivalence of RBC viability. There were no significant imbalances between test and control RBCs with respect to safety. Pathogen-inactivated RBCs produced using the S-303 treatment process show broad bioequivalence with control RBCs and the product appears suitable for advanced clinical development.

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CONFLICT OF INTEREST

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B　個別症例報告概要

○　総括一覧表
○　報告リスト

個別症例報告のまとめ方について

個別症例報告が添付されているもののうち、個別症例報告の重複を除いたものを一覧表の後に添付した（国内症例については、資料３において集積報告を行っているため、添付していない）。

別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発生国	性別	年齢	発現時期	転帰	出典	区分	備考
		器官別大分類	基本語								
第18回	1	感染症および寄生虫症	B型肝炎	米国	男	8歳	2011/12/12	不明	症例報告	外国製品	識別番号3-11000046 報告日:2011年12月27日
	1	臨床検査	血中免疫グロブリンM増加	米国	男	8歳	2011/12/12	不明	症例報告	外国製品	識別番号3-11000046 報告日:2011年12月27日

血対課ID	受理日	番号	報告者名	一般名	生物由来成分名	原材料名	原産国	含有区分	文献	症例	適正措置報告
120036	24-Apr-12	120108	CSLベーリング	乾燥pH4処理人免疫グロブリン	人免疫グロブリンG	ヒト血液	ドイツ	有効成分	あり	あり	なし
120037	24-Apr-12	120109	CSLベーリング	乾燥pH4処理人免疫グロブリン	ペプシン	ブタ胃粘膜	米国	製造工程	なし	あり	なし

別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢(歳)	発現時期(年/月/日)	転帰	出典	区分	備考			
		器官別大分類	基本語								識別番号	報告日	MedDRA(Ver.)	
第18回	18 -1	臨床検査	C型肝炎抗体陽性	米国	男性	57	2012/2/29	未回復	試験からの報告	外国製品	12000001	2012/6/4	15.0	2012年4月27日の初回報告後に追加報告をおこなったため、最新情報に更新した。報告回数：2回
第18回	18 -2	感染症および寄生虫症	C型肝炎	米国	男性	43	2011/12/26	未回復	自発報告	外国製品	11000048	2012/3/1	14.1	2012年1月26日の初回報告後に追加報告をおこなったため、最新情報に更新した。報告回数：2回
第18回	18 -3	臨床検査	B型肝炎コア抗体陽性	米国	女性	4	不明	不明	自発報告	外国製品	11000049	2012/2/6	14.1	
第18回	18 -3	臨床検査	B型肝炎e抗体陽性	米国	女性	4	不明	不明	自発報告	外国製品	11000049	2012/2/6	14.1	
第18回	18 -4	臨床検査	検査結果偽陽性	米国	男性	40	不明	不明	自発報告	外国製品	11000045	2011/12/27	14.1	

血対課ID	受理日	番号	報告者名	一般名	生物由来成分名	原材料名	原産国	含有区分	文献	症例	適正措置報告
120061	22-Jun-12	120216	バクスター	乾燥イオン交換樹脂処理人免疫グロブリン	人免疫グロブリンG	人血漿	米国	有効成分	なし	あり	なし
120062	22-Jun-12	120217	バクスター	乾燥イオン交換樹脂処理人免疫グロブリン	人血清アルブミン	人血漿	米国	添加物	なし	あり	なし