資料２－２

Ａ　研究報告（詳細版）

平成２７年２月２５日
（平成２６年１１月～平成２７年１月受理分）

別紙様式第２－１

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
一般的名称	―		研究報告の公表状況	Transfusion 54.8 (Aug 2014): 2134-5.	公表国 仏国	
販売名（企業名）	―					

研究報告の概要

輸血によるＥ型肝炎ウイルスの伝播を予防するための安全対策
Ｅ型肝炎ウイルスの伝播は、血漿分画製剤による伝播が証明されていないのに対し、不活化処理した血漿を含む不安定な血液製剤を介して、世界中の複数の国で報告されている。
HEVは主に腸管から伝播し、環境要因に関連するリスクの方が輸血によるリスクよりも高い。
先進国における HEV は人獣共通感染と食品媒介病原体として循環しており、感染例の大部分は自己限定の急性感染であることからリスクのある供血者の選別は効果がない。
また、IgGおよびIgM検査によるスクリーニングは、無症候性の血清検査陰性でRNA陽性ドナーからの輸血汚染を排除できないだろう(最近の研究では、RNA陽性ドナーでIgM陽性であったのはわずか23%)。
したがって、予防安全対策は HEV NAT のみで行うことができるが、すべての供血で行うのか、リスクのある受血者のみを対象とすべきかとの問題が生じる。
高感度の血液スクリーニング検査は輸血による伝播を防ぐ利点があるが、プールによる検査が最も手頃な価格で実現可能な方法である。
供血者において広範囲なHEV RNAレベル(<25～100,000UI/mL)が報告されており、リスクのある受血者へ輸血される血液製剤に限定されたスクリーニングはプールによる感受性低下を避けるため個別検査が勧められる。
意志決定をする前に、HEV NATを行う根拠について、曝露患者における輸血リスクと費用対効果の分析により評価されるべきである。

使用上の注意記載状況・その他参考事項等

前文
本剤は、貴重なヒト血液を原料として製剤化したものである。原料となった血液を採取する際には、問診、感染症関連の検査を実施するとともに、製造工程において一定の不活化・除去処理などを実施し、感染症に対する安全対策を講じているが、ヒト血液を原料としていることによる感染症伝播のリスクを完全に排除することはできないため、疾病の治療上の必要性を十分に検討の上、必要最小限の使用にとどめること。

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

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5. Gurusamy KS, Li J, Vaughan J, et al. Cardiopulmonary interventions to decrease blood loss and blood transfusion requirements for liver resection. Cochrane Database Syst Rev 2012;(5):CD007338.

Coagulation values in extreme premature infants

We read with interest the recent publication by Christensen and colleagues.[1] Standard coagulation test results vary with gestational age. Consequently, gestation-specific reference ranges for these variables are required. The paucity of published reference values for coagulation variables in extremely premature neonates represents a challenge for neonatologists managing infants at greatest risk of intraventricular hemorrhage and other bleeding complications. The data presented by Christensen and coworkers[1] pertaining to infants of less than 28 weeks' gestation are helpful, but were based on a small sample size of 24 infants. Moreover, tests were performed on cord blood. An equivalency comparison of samples obtained from cord and peripheral blood was limited to full blood count variables.[2]

Fetal plasma clotting times vary with maturation[3] and do not necessarily correlate with published reference ranges for delivered premature infants at equivalent gestations.[4] Physiologic changes and medical interventions (including vitamin K administration) after delivery appear to influence these variables. We have recently published data pertaining to the extremely premature neonatal population (n = 183) born at less than 27 weeks' gestation.[5] In this large patient cohort, we observed longer clotting times than those reported by Christensen and coworkers in a similar but smaller patient population. In our cohort, median (range 5th-95th percentile) prothrombin time (PT) and activated partial thromboplastin time (APTT) values were 20.2 (14.8-32.6) and 67.4 (43.3-130.2) seconds, respectively. In our population the coagulation values were obtained using combination of PT-S (2004-2008) and PT-Recombinant PlasTin 2G reagent (2008-2010) for PT values and APTT-SP reagent (2004-2008) and lupus-sensitive silica-based APTT reagent SytnASIL (2008-2010) for APTT values compared with Diagnostica Stago kits used by Christensen and colleagues. Of particular note, the blood source differed between the two studies. Christensen and colleagues report results obtained from plasma prepared from cord blood. In contrast, blood was obtained from peripheral catheters in our study, before instillation of heparinized saline. The degree to which coagulation variables derived from cord blood correlate with those obtained from peripheral blood in newly delivered extremely premature infants remains unclear. Coagulation values are dynamic and vary with gestational and postnatal age and other environmental stressors and therefore the different sources of blood may account for some of the differences in values observed.

We aimed to pragmatically evaluate coagulation test results in a large cohort of infants born at less than 27 weeks in a retrospective study design. Until recently, interpretation of coagulation test results in this patient population was virtually impossible. We hope that the data described herein and future large prospective studies will further define reference ranges for coagulation variables in this high-risk patient cohort and ultimately guide clinical decision making in extremely premature infants.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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REFERENCES

1. Christensen RD, Baer VL, Lambert DK, et al. Reference intervals for common coagulation tests of preterm infants. Transfusion 2013;54:627-32:quiz 626.
2. Carroll PD, Nankervis CA, Iams J, et al. Umbilical cord blood as a replacement source for admission complete blood count in premature infants. J Perinatol 2012;32:97-102.
3. Reverdiau-Moalic P, Delahousse B, Body G, et al. Evolution of blood coagulation activators and inhibitors in the healthy human fetus. Blood 1996;88:900-6.
4. Andrew M, Paes B, Milner R, et al. Development of the human coagulation system in the healthy premature infant. Blood 1988;72:1651-7.
5. Neary E, Okafor I, Al-Awaysheh F, et al. Laboratory coagulation parameters in extremely premature infants born earlier than 27 gestational weeks upon admission to a neonatal intensive care unit. Neonatology 2013;104:222-7.

Safety measures to prevent hepatitis E virus transmission by blood transfusion

The transmission of hepatitis E virus (HEV) by transfusion has been reported in several countries over the world

through labile blood products, including Intercept-treated plasma as recently published,[1] whereas no transmission by plasma-derived products has been evidenced. The severe consequences of infection observed in immunocompromised individuals (such as transplant patients), in patients with chronic liver disease, and in pregnant women have prompted some practitioners to advocate the systematic screening of blood donations for HEV RNA in countries where hepatitis E is endemic.[2] However, HEV being mainly enterically transmitted, the risk related to environmental factors is certainly higher than that linked to transfusion. This is supported by the small number of severe posttransfusion HEV infections reported by the Hemovigilance Network[3] contrasting with an observed HEV RNA prevalence in European blood donors up to one in 2000.[4,5] As HEV circulates as a zoonotic and food-borne pathogen in developed countries and because the majority of infected individuals have a self-limiting acute infection, the selection of at-risk blood donors is ineffective. Moreover, a screening of blood donations based on immunoglobulin (Ig)G and IgM testing would not preclude a transfusion contamination from asymptomatic seronegative and RNA-positive donors (in a recent study, only 23% of RNA-positive donors were also positive for IgM[5]). Thus, preventive safety measures can only be based on HEV nucleic acid testing (NAT). However, such a strategy raises questions: should it be done in all blood donations or only targeted for at-risk recipients? Universal screening has the advantage of preventing transfusion transmission, provided that the assay is adapted to the blood screening throughput with the highest sensitivity level. Even though the pooled testing could be considered as the most affordable and feasible option, the wide range of HEV RNA levels (<25 IU/mL to more than 100,000 IU/mL) reported in blood donors[5] would encourage the use of NAT in single testing to avoid a loss of sensitivity due to a sample pooling A targeted screening limited to blood products transfused to at-risk recipients would make individual testing more feasible. However, this latter choice would require to define precisely at-risk transfused patients, of whom characteristics are still unclear and probably exceed those currently selected to prevent transfusion cytomegalovirus infection. Before making any decision, the rationale of HEV NAT should be investigated by fully assessing the transfusion risk in exposed patients (in progress in different countries) and by analyzing the cost-effectiveness of each strategy. In addition, because of the high environmental infection pressure, the introduction of any donor screening for HEV will not preclude the necessity to check each vulnerable patient with elevated liver enzymes for presence of HEV.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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REFERENCES

1. Hauser L, Roque-Afonso AM, Beyloune A, et al. Hepatitis E transmission by transfusion of Intercept blood system-treated plasma. Blood 2014;123:796-7.
2. Feray C, Pawlotsky JM, Roque-Afonso AM, et al. Should we screen blood products for hepatitis E virus RNA? Lancet 2014;383:218.
3. Agence nationale de sécurité du médicament et des produits de santé. Rapport d'activité d'hémovilance 2012 (France). 2012 [cited 2014 May 22]. Available from: http://ansm.sante.fr/var/ansm_site/storage/original/application/b893629101bd8fdb10d446fabf34768b.pdf
4. Slot E, Hogema BM, Riezebos-Brilman A, et al. Silent hepatitis E virus infection in Dutch blood donors, 2011 to 2012. Euro Surveill 2013;18:pii: 20550.
5. Vollmer T, Diekmann J, Johne R, et al. Novel approach for detection of hepatitis E virus infection in German blood donors. J Clin Microbiol 2012;50:2708-13.

別紙様式第2-1

No. 6

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
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販売名(企業名)	解凍赤血球液-LR「日赤」(日本赤十字社) 照射解凍赤血球液-LR「日赤」(日本赤十字社)					

研究報告の概要

○20プール核酸増幅検査(NAT)陰性血液の輸血による本邦初のHIV感染事例の報告
日本赤十字社の血液センターでは血清学的検査及びNATを用いて感染性病原体の献血血液検査を行っている。NATスクリーニング強化のため、2004年に20プールNATを導入して以来、輸血によるHIV感染は報告されていなかった。
2013年11月、複数回献血者である40歳代男性の献血検体からHIV抗体が検出され、その後HIV-1感染が確認された。血液製剤の遡及調査ガイドラインに従い、2013年2月の献血の保管検体を3法の個別NATで検査(いずれも1回測定)したところ、1法のみHIV-1 RNAを検出した。
当該献血からは赤血球製剤(RBC)と新鮮凍結血漿(FFP)が製造され、2人の受血者に輸血されていた。RBCは献血10日後に80歳代女性患者に輸血されたが、輸血前と輸血9カ月後の血液におけるHIV検査は全て陰性であった。FFPは献血8カ月後に60歳代男性患者に輸血され、輸血前血液検体はHIV抗体陰性であったが、輸血34日後の血液検体における血清学的検査及びNATで、HIV-1感染が確認された(血漿中のHIV-1ウイルス量は1.1×10^{6}コピー/mL)。献血者とFFP受血者の血液検体のHIV-1ウイルス相同性を解析した結果、env領域は99.7%、pol領域は99.9%一致し、この高い相同性により、献血者からFFP受血者にHIVが伝播したことが示された。両者からのHIV-1分離株は、日本のHIV-1感染者に最も一般的なサブタイプBであった。当該献血者の陽転後血漿を用いた限界希釈法により、輸血感染を引き起こした血液のHIV-1ウイルス量を推定したところ、2法の高感度NATの検出限界(約10コピー/mL)以下であり、当該FFP中のHIV-1ウイルス量は2,400コピー以下と推測された。当該献血血液中のウイルス量は非常に低く、感染初期のウィンドウ期に献血されたと思われる。

使用上の注意記載状況・その他参考事項等

解凍赤血球液-LR「日赤」
照射解凍赤血球液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
献血血液検査に20プールNATが導入されて以来、本邦初となる輸血HIV感染が発生したとの報告である。	日本赤十字社では、化学発光酵素免疫測定法(CLEIA)による抗体検査を行い、陰性の検体についてHIV-1/2及びHIVグループOの検出が可能な20プールNATを実施していたが、更なる安全性の向上を目的に、2014年8月1日よりNATシステムを変更し、全検体に対し個別検体によるNAT(個別NAT)スクリーニングを開始している。HIV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。

LETTERS TO THE EDITOR

First report of human immunodeficiency virus transmission via a blood donation that tested negative by 20-minipool nucleic acid amplification in Japan

The Japanese Red Cross (JRC) blood centers screen donated blood for infectious agents using serologic assays and nucleic acid amplification testing (NAT). A multiplex NAT for hepatitis B virus, hepatitis C virus, and human immunodeficiency virus Type 1 (HIV-1) with a minipool (MP) format comprising 50 seronegative samples was started in 2000.[1] During the implementation of the 50-MP-NAT in 2003, HIV-1 was transmitted through fresh-frozen plasma (FFP) from one blood donor during the window period. To reinforce NAT screening, the pool size was decreased to 20 in 2004. Since 20-MP-NAT implementation in 2004, we have found 19 donations that were seronegative but positive for HIV in the 20-MP-NAT. The rate of HIV-infected donations that were positive only in the NAT was approximately 1 in 2.7 million. No transfusion-transmitted HIV infection (TT-HIV) has been reported in Japan since the 20-MP-NAT was introduced.

In November 2013, anti-HIV was detected in a blood sample from a repeat male blood donor aged in his 40s. Western blotting (New LAV Blot 1, Bio-Rad, Hercules, CA), real-time reverse transcription–polymerase chain reaction assay (Cobas TaqScreen HIV, Roche, Basel, Switzerland), and transcription-mediated amplification assay using a kit (Procleix Ultrio ABD, Novartis Diagnostics, Emeryville, CA) confirmed HIV-1 infection. A qualitative NAT for HIV-1 (Cobas TaqMan, Roche) detected a plasma HIV-1 viral load of 4.7×10^4 copies/mL. A cryopreserved sample of plasma from his previous donation in February 2013 was retested in accordance with the Japanese guidelines for lookback studies on blood products. Using individual donation (ID-) NAT, the Cobas TaqScreen HIV (plasma input volume, 850 µL; 95% limit of detection [LOD], 24.3 IU/mL) detected HIV-1 RNA in an archived blood sample from his previous donation, whereas the Procleix (plasma input volume, 500 µL; 95% LOD, 19.6 IU/mL) did not. Each of these NAT assays was performed as a single test. The low plasma volume in the archival sample did not allow for repeat analysis.

Red blood cell (RBC) and FFP components were prepared from the previous donation and transfused into two recipients. The RBCs were transfused to a female patient in her 80s. A pretransfusion sample and a posttransfusion sample collected 9 months after transfusion were HIV seronegative. The latter sample was also negative for HIV RNA. The FFP was transfused 8 months after donation to a male patient in his 60s, from whom a pretransfusion sample was seronegative for HIV. Serologic tests and NAT assay identified HIV-1 infection in this recipient at 34 days after transfusion, and the plasma HIV-1 viral load was 1.1×10^6 copies/mL (Fig. 1).

The viral sequences determined in blood samples from both the donor (postseroconversion donation) and the FFP recipient differed by only one among 341 nucleotides in the *env* region (99.7% identity) and by four of 2800 nucleotides in the *pol* region (99.9% identity). Such high genetic similarity among the sequences supported the notion that HIV had been transferred from the donor to the FFP recipient. Isolates of HIV-1 from the donor and recipient were Subtype B, which is the most common among individuals infected with HIV-1 in Japan. Major antiretroviral drug-resistant mutations were not detected in either the donor or the recipient. Sequencing the HIV-1 5′-long terminal repeat, which was the target region of our NAT screen, did not detect HIV-1 mutations that caused false-negative NAT results.[2]

To estimate the HIV-1 viral load in the implicated blood, the sensitivity of both the Cobas TaqScreen HIV and the Procleix was reassessed by probit analysis using serial threefold dilutions (four replicates per dilution) of postseroconverted plasma (4.7×10^4 copies/mL) from the donor, which revealed that the 95% LOD of both NAT screens was 10 copies/mL. The archived blood sample from the implicated donation was reactive in the Cobas, but not in the Procleix screen; therefore, we speculated that the viral load in the donor plasma was approximately at the detection limit of the two NATs. Thus, the estimated total amount of HIV-1 in the FFP (containing

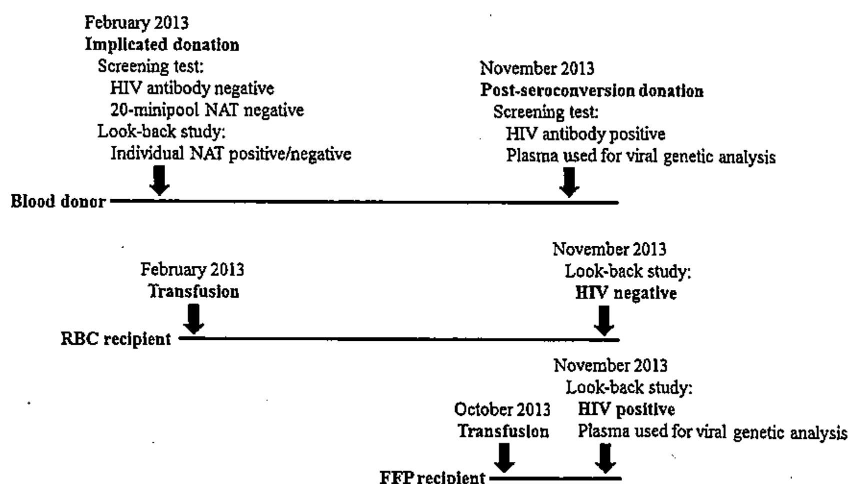

Fig. 1. Timeline of blood donations, transfusions, and infections.

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approximately 240 mL of plasma) that caused TT-HIV was fewer than 2400 copies.

The prevalence of HIV infections detected by NAT or antibody screening among blood donors during 2012 was 1.3 per 100,000 donations, whereas the prevalence among first-time blood donors has recently been five to six per 100,000 donations. This figure exceeded the frequency of individuals who were newly diagnosed with HIV infection among the general population aged 15 to 64 years in 2012 (1.2 per 100,000).[3] The donor described herein was probably in the very early stage of HIV-1 infection at the time of the implicated donation. That blood donation is being used for testing individuals with high-risk behaviors for HIV transmission is a concern. Questions given to blood donors about behaviors that confer risk for HIV are probably not being answered precisely. Methods of confirming whether or not donors understand the questions and the need to answer them precisely need to be improved.

This report describes the first known case of TT-HIV through a 20-MP-NAT–negative blood component in Japan. Our results indicate that ID-NAT using even the most sensitive methods available today might not detect HIV-1 in window period donations. Transfusion with FFP, but not RBC, components resulted in HIV-1 transmission in the TT-HIV case described herein. Similar cases with differential HIV transmission via blood components have been reported in other countries.[4] However, TT-HIV arising via an ID-NAT–negative blood component has not been identified in Japan or in any other country where donated blood is screened by NAT for HIV. We estimated that the risk of collecting a unit during the ID-NAT–negative HIV-1 window period is 2.75 per 5.5 million donations in Japan.[5] The JRC will introduce ID-NAT screening in August 2014, which should further reduce window period transmissions. However, even ID-NAT might not completely eliminate the window period of HIV-1 infection.

In the era of very high-sensitive ID-NAT or MP-NAT, TT-HIV will arise mainly in patients transfused with components with larger plasma volumes such as FFP and apheresis platelets, because these components contain a larger amount of HIV. Although pathogen inactivation technologies might help to reduce residual risk, the introduction of pathogen inactivation technology should be determined after carefully considering the balance among benefit, risk, and cost.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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REFERENCES

1. Mine H, Emura H, Miyamoto M, et al.; Japanese Red Cross NAT Research Group. High throughput screening of 16 million serologically negative blood donors for hepatitis B virus, hepatitis C virus and human immunodeficiency virus type-1 by nucleic acid amplification testing with specific and sensitive multiplex reagent in Japan. J Virol Methods 2003;112:145-51.
2. Müller B, Nübling CM, Kress J, et al. How safe is safe: new human immunodeficiency virus Type 1 variants missed by nucleic acid testing. Transfusion 2013;53:2422-30.
3. National Institute of Infectious Diseases, Tokyo, Japan. HIV/AIDS in Japan, 2012. Infect Agents Surveill Rep 2013;34: 251-2.
4. Ferreira MC, Niel TJ. Differential transmission of human immunodeficiency virus (HIV) via blood components from an HIV-infected donor. Transfusion 2006;46:156-7.
5. Satake M. Infectious risks associated with the transfusion of blood components and pathogen inactivation in Japan. Int J Hematol 2004;80:306-10.

N-Acetylcysteine for thrombotic thrombocytopenic purpura: is a von Willebrand factor–inhibitory dose feasible in vivo?

We read with interest the recent report by Li and colleagues[1] describing the first apparent successful use of *N*-acetylcysteine (NAC) in refractory thrombotic thrombocytopenic purpura (TTP). As we had previously described a case of NAC failure in a refractory TTP case that subsequently responded to bortezomib,[2] we sought to clarify whether our NAC dosing protocol differed from that utilized by Li and coworkers.[1]

As the only precedent for NAC dosing in TTP was preclinical,[3] we too adapted a clinical acetaminophen overdose protocol.[4] Our patient received 150 mg/kg NAC as a 1-hour bolus, followed by 50 mg/kg over 4 hours and then 100 mg/kg over the next 16 hours for the first day of dosing (with a cumulative exposure of 15 g in the first 24 hr). Twice-daily plasma exchange could not be interrupted

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識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	厚生労働省処理欄
				2014 年 09 月 30 日	該当なし	
一般的名称	乾燥濃縮人アンチトロンビンⅢ		研究報告の公表状況	日本輸血細胞治療学会誌 2014; 60(5): 561-564	公表国 日本	
販売名（企業名）	①ノイアート静注用 500 単位　（日本血液製剤機構） ②ノイアート静注用 1500 単位（日本血液製剤機構）					

研究報告の概要	使用上の注意記載状況・その他参考事項等
日本赤十字社血液センターでは、1997 年 9 月より献血血液の B19 抗原スクリーニング検査を RHA 法より開始した。2008 年から検査方法を CLEIA 法に変更して検出感度の向上を図ったことにより、輸血後副作用調査による B19 感染疑い例の副作用発生頻度は概ね 1 回/1 年から 1 回/4～5 年に改善した。 本来、B19V はその殆どが不顕性感染であり、これは輸血においても同様であると考えられるが、輸血後副作用調査の解析により B19V 感染と疑われる副作用報告が過去 15 年余の間に 10 例発生している。これらの受血者のうち 7 例は血液疾患関連、2 例は妊娠出産関連で、その殆どが B19V 感染後に副作用が発生し易い状況であった。また、輸血された血液はすべて B19 抗原検出感度以下、B19DNA 陽性かつ B19IgM 抗体陽性の感染初期状態のものであった。 現在輸血用血液について B19 抗原検査のさらなる検出感度の向上にむけた検討を行っているが、併せて B19IgM 抗体検査の有用性についても今後の検討課題である。	1. 慎重投与 (1)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。〕 (2)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	今後の対応
ヒトパルボウイルス B19（Human parvovirus B19：B19）は、パルボウイルス科(Parvoviridae)パルボウイルス亜科(Parvovirinae)エリスロウイルス属(Erythrovirus)に属するエンベロープを持たない極めて小さな（約 18～24nm）1 本鎖 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。また、原料血漿への B19 混入量低減のため、日本赤十字社により CLEIA 法を用いたドナースクリーニングが行われている。原料血漿に B19 が混入しても、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。	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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—【総　　説】— Review —

本邦におけるヒトパルボウイルスB19の輸血副作用発生状況と安全対策

松倉　晴道

キーワード：ヒトパルボウイルスB19，RHA，CLEIA，輸血後感染，安全対策

はじめに

ヒトパルボウイルスB19(B19V)は，1975年Cossartらによってヒト血清中から発見され，この時の検体番号「B19」からウイルス名が命名された[1]．本邦では献血血液中にNAKATANI抗原として見いだされていたものがB19Vと同一であることが1984年大河内らによって報告された[2]．

B19Vは赤血球系前駆細胞～赤芽球に主に感染し，そのレセプターは赤血球型抗原のP抗原(グロボシド)である[3]．グロボシドは赤血球系のみならず巨核球，内皮細胞，胎盤，胎児肝，胎児心筋等にも存在する[4]．P抗原陰性者はB19Vの感染に抵抗性を示し，P抗原陰性血液は稀な血液型として頻度が極めて低い．また，B19Vにはエンベロープが無いため有機溶剤や界面活性剤による不活化が困難であるが，5%または25%ヒト血清アルブミンやリン酸緩衝液下での60℃熱処理に対しては動物パルボウイルスと異なりin vitroでの感染能失活が認められている[5]．

主な感染経路は飛沫による経気道感染であり，まれに経胎盤による感染がある．B19 DNAは感染細胞の核内にエピゾームとして存在し，ウイルスの複製に伴って細胞障害が亢進し宿主細胞の破壊とともに増殖したウイルスが放出される．

B19Vによる代表的な疾患として幼児期では飛沫感染による伝染性紅斑（リンゴ病）がよく知られている．成人では，多発性関節炎，急性赤芽球癆，慢性骨髄不全，胎児水腫等がある．健常者の感染では軽度の感冒様症状のみで殆どが不顕性であるが，B19抗体陰性の妊婦やその胎児，および臓器移植，白血病や悪性リンパ腫等への化学療法，AIDSに伴う免疫不全，溶血性貧血等で造血機能が亢進している患者は上記疾患が発症し易いと考えられる[6][7]．

日本赤十字社血液センターでは，1997年より献血血液のB19抗原スクリーニング検査を開始し，輸血用血液および血漿分画製剤の原料血漿からB19ウイルス血症期と想定される血液を除外している．

本稿では，輸血後副作用調査によるB19V輸血感染疑い例の解析と血液センター研究グループによる献血者のB19V関連マーカーの分布調査結果等をもとに，本邦におけるB19V輸血感染による副作用の発生状況とその安全対策について述べる．

献血血液のB19V検査

日本赤十字社血液センターでは1997年9月からRHA法(Receptor mediated Hemagglutination Assay)により全献血血液のB19抗原スクリーニング検査を開始した[8]．これにより，輸血用血液および血漿分画製剤の原料血漿から，B19ウイルス血症期と想定される血液を除外することが可能となった．2008年より検査方法をCLEIA法（Chemiluminescent Enzyme Immuno Assay）に変更し，検出感度はそれまでの約10^{10}IU/mlから約10^{6}IU/mlに向上して現在に至っている[9]．

なお，欧米ではミニプールNATによるB19 DNAのスクリーニング検査(検出感度：$10^{5\sim6}$IU/ml)が実施されている[10][11]．

献血者におけるB19V関連マーカーの分布は，血液センターの研究グループにより調査が行われ，それによるB19抗原陽性率は，2001年～2007年の全国調査の結果，0.009%（RHA法）と推定されている．年齢別陽性率は，18～19歳：0.0055%，20歳代：0.0061%，30歳代：0.016%，40歳代：0.012%，50歳代：0.0035%，60歳代：0.0028%で，30歳代と40歳代が高値で男女差は認められていない[12]．また，2008年～2009年に行われた調査では，献血者の年代，性別，献血時期を均等化して抽出し，B19抗体とB19DNAを測定した結果，B19 IgG抗体陽性率は50～60%で年代や男女差は認められていない．RHA法でB19抗原陰性群のB19 DNA陽性率は15%前後で，この全例がウイルス量10^{3}IU/ml以下かつB19 IgG抗体が陽性であった[12]．一方，一般健常者のB19 DNA陽性率は0.2%という報告もある[13]．

日本赤十字社近畿ブロック血液センター

Table 1 Japanese Red Cross (JRC) to analyzed and evaluated suspected transfusion-transmitted B19 viral infectious cases reported voluntarily by medical institutions to JRC blood centers. With the case between 1997 and 2012, ten cases of B19 infection were confirmed by a positive viral nucleic acid test on a repository sample of the donation involved.

Case	Year	Patient profile	Before transfusion	After transfusion	Transfused components (repository samples)
1	2000	10 s teens, male ALL	DNA(−)	DNA(+)	DNA(+), IgM(+), IgG(+)
2	2002	60 s teens, male AML, After chemotherapy	DNA(−)	DNA(+)	DNA(+), IgM(+), IgG(+)
3	2002	70 s teens, male Malignant lymphoma	?	DNA(+), IgM(+)	DNA(+), IgM(+), IgG(+)
4	2002	20 s teens, female Hemolytic anemia	DNA(−)	DNA(+)	DNA(+), IgM(+), IgG(+)
5	2003	50 s teens, Female Paroxysmal nocturnal hemoglobinuria	?	DNA(+), IgM(+)	DNA(+) 6.8×10^{3}cp/m*l*, IgM(+), IgG(+)
6	2005	40 s teens, male Hairy cell leukemia, After chemotherapy	?	DNA(+)	DNA(+) 1.8×10^{5}cp/m*l*, IgM(+), IgG(+)
7	2005	50 s teens, male AML, After chemotherapy	DNA(−) IgM(−), IgG(−)	DNA(+)	DNA(+) 9.7×10^{8}cp/m*l*, IgM(+), IgG(−)
8	2005	30 s teens, female Placenta previa	DNA(−) IgM(−), IgG(−)	DNA(+)	DNA(+) 3.2×10^{5}cp/m*l*, IgM(+), IgG(+)
9	2006	50 s teens, male Rectal cancer	DNA(−)	DNA(+) IgM(−)	DNA(+) 5.1×10^{3}cp/m*l*, IgM(+), IgG(+)
10	2011	30 s teens, female Cesarean section	DNA(−) IgM(−), IgG(−)	DNA(+)	DNA(+), IgM(+), IgG(+)

Case	Symptoms and laboratory findings[15]
2	High fever, Disseminated erythema, Pure RBC aplasia (7 weeks), Reticulocytopenia
6	RBC aplasia (3 months), Reticulocytopenia (1 months), Viremia level of B19DNA: 1×10^{12}cp/m*l*
7	Pure RBC aplasia (approx. 2 months)
8	Fever, Systemic eruption (3 weeks)
9	Sustaind high fever (5 days)

この原因の一つとして，B19V のアポトーシス誘導による感染細胞 DNA の断片化とともに，B19 DNA にも何らかの変化が生じた結果，B19DNA 測定時の増幅長の違いにより検出感度が異なる結果となったものと想定して，現在日本赤十字社血液センターで検討を進めている．

B19V 感染による輸血副作用の発生状況

1997 年 9 月より献血血液の B19 抗原スクリーニング検査を開始して以来，輸血後副作用調査の結果，2012 年までの 15 年余の間に 10 例の輸血後 B19V 感染疑い例が医療機関より報告された[14]．これら 10 例のうち 7 例の受血者が輸血前の B19 DNA が陰性，3 例の受血者が不明であった．受血者の原疾患は 7 例が血液疾患関連，2 例が妊娠出産関連，1 例が悪性腫瘍であった．佐竹らは 10 例中 5 例の受血者（Table 1：Case 2，6〜9）について輸血後の症状を調査した結果，いずれの例も輸血後に発熱，貧血，網赤血球減少等が認められたが，これらの症状はその後回復している[15]．

日本赤十字社では B19V のみならず輸血後感染症を含めた輸血副作用の原因究明のために，1996 年 9 月より全献血者の検体を 11 年間保管しており，これら 10 例についても輸血血液に対応した保管検体の検査を実施した結果，全例に B19 DNA および B19 IgM 抗体が認められている（Table 1）．

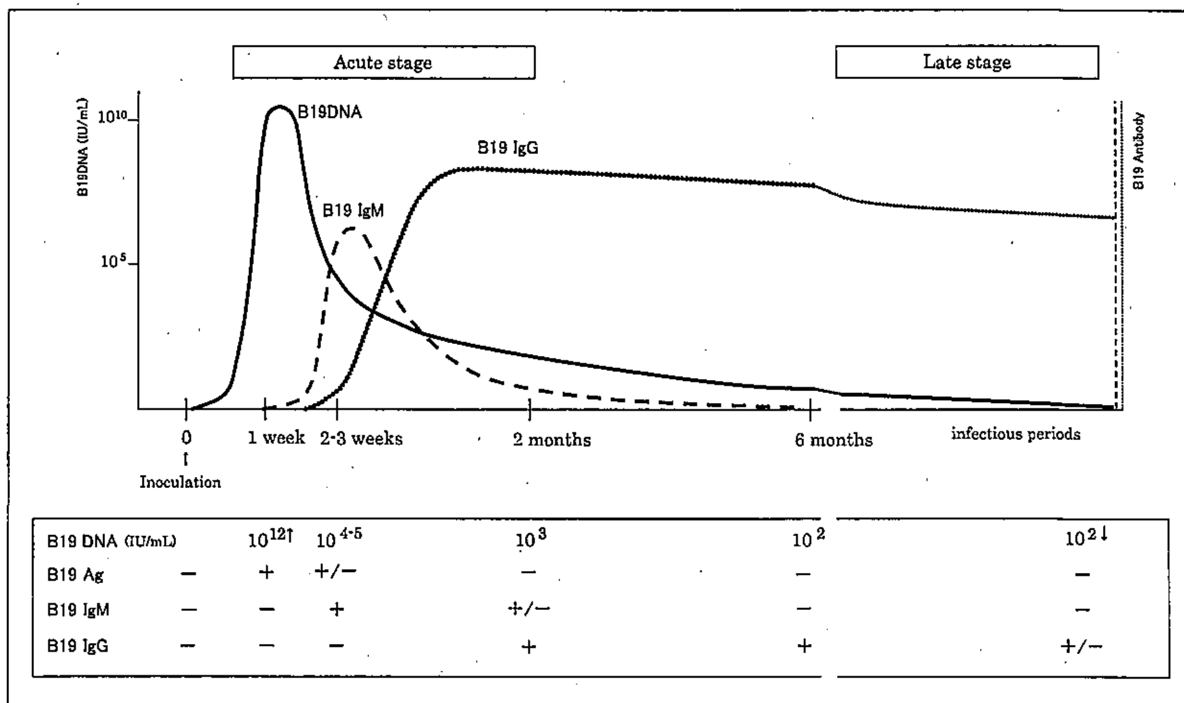
B19V 輸血感染による副作用例の検証と今後の課題

過去 15 年余の間に輸血後 B19V 感染と疑われる副作用が 10 例発生し，それらの受血者の原疾患は 10 例中 7 例が血液疾患関連で他の 3 例も妊娠，悪性腫瘍であり，B19V 感染による副作用が発症し易い状態であったことが考えられる．

輸血された血液はすべて B19 DNA かつ B19 IgM 抗体陽性であり，献血時には B19V 感染の初期状態であったと考えられる．また，B19 DNA 陽性で B19 IgM，IgG 抗体が共に陰性の，感染の極初期状態と考えられる血液が輸血された事例は確認できなかった．

一方，B19 抗原陰性献血者群に存在する B19 DNA 陽性で IgM 抗体陰性かつ IgG 抗体陽性の血液による感染疑い例は認められていない．中和抗体については実際に免疫不全状態の B19V 感染患者に対して B19 抗体含有免疫グロブリン製剤による治療症例が報告されている[16]．

また，B19V は 5 年前後の間隔で感染流行期が存在し，



B19 DNA (IU/mL)		10^{12}↑	10^{4-5}	10^{3}	10^{2}	10^{2}↓
B19 Ag	−	+	+/−	−	−	−
B19 IgM	−	−	+	+/−	−	−
B19 IgG	−	−	−	+	+	+/−

Fig. 1　A composite diagram of immune response to B19 DNA, B19 antigen, and B19 IgM, IgG during the acute and convalescence periods.

国立感染症研究所による伝染性紅斑発症の定点サーベイランス調査からも推測できる．B19V 感染流行期と 10 例の輸血後 B19V 感染疑い例の発生時期とを比較した結果，その関連性は認められなかった．

以上から，現在のスクリーニング体制における本邦の B19V 輸血感染による副作用の発生は，血液疾患等による免疫抑制下の状態にある患者に，B19 IgM 抗体陽性で B19 抗原が検出感度以下の感染初期の血液が輸血された場合が多いといえる．また，感染極初期の B19 IgM 抗体陰性期の輸血については，感染疑い事例の報告はないが念のため考慮する必要がある．

輸血後副作用調査による B19V 輸血感染と考えられる副作用の発生頻度は，RHA 法によるスクリーニング検査を実施した 1997 年～2007 年の約 10 年間で 9 例であったことから，当時は 1 年に 1 例程度の発生であったことがうかがえる．現在の頻度は，2008 年前半から B19 抗原検査の検出感度を向上して以来 2012 年までの間に，その疑い事例が 1 例確認された（Table 1，Case 10）ことから，概ね 4～5 年に 1 例であることが推測される．

一方，血漿分画製剤は，原料プール血漿中に含まれる B19 DNA 量が 10^4IU/ml 以下であれば，最終製品による B19V 感染リスクが大幅に低減できることが 1999 年に米国 FDA から提言され[17]，現在，本邦も含めた諸国でこの提言に沿った原料血漿のスクリーニング検査が実施されている．加えて，製造工程中でのウイルス除去膜による処理やこれらの検証としてウイルスプロセスバリデーションが行われ，最終製剤中の B19 DNA が検出感度以下となっていることが確認されており[18]，これらの製剤からの B19V 感染は認められていない．

B19V の輸血感染動態の概念を Fig. 1 に示した．B19V は高ウイルス量を呈するウイルス血症の期間が短いため感染初期における検出感度以下のウインドウピリオドも他のウイルスに比べて短いと考えられる．血液センターではこの期間をできる限り短縮するため B19 抗原検査のさらなる検出感度向上にむけた検査試薬の改良を行っている．また，引き続き詳細な輸血副作用調査や B19V 感染動態の解析をふまえて B19 IgM 抗体検査の有用性についても検討の余地があると考える．

ま と め

日本赤十字社血液センターでは，1997 年 9 月より献血血液の B19 抗原スクリーニング検査を RHA 法により開始した．2008 年から検査方法を CLEIA 法に変更して検出感度の向上を図ったことにより，輸血後副作用調査による B19V 感染疑い例の副作用発生頻度は概ね 1 回/1 年から 1 回/4～5 年に改善した．

本来，B19V はその殆どが不顕性感染であり，これは輸血においても同様であると考えられるが，輸血後副作用調査の解析により B19V 感染と疑われる副作用報告が過去 15 年余の間に 10 例発生している．これらの受

血者のうち７例は血液疾患関連，２例は妊娠出産関連で，その殆どがB19V感染後に副作用が発生し易い状況であった．また，輸血された血液はすべてB19抗原検出感度以下，B19DNA陽性かつB19 IgM抗体陽性の感染初期状態のものであった．

現在輸血用血液についてB19抗原検査のさらなる検出感度の向上にむけた検討を行っているが，併せてB19 IgM抗体検査の有用性についても今後の検討課題である．

著者のCOI開示：本論文発表内容に関連して特に申告なし

文　献

1) Cossart YE, Cant B, Field AM, et al: Parvovirus-like particles in human serum. Lancet, i: 72—73, 1975.
2) Okochi K, Mori R, Miyazaki MB, et al: Nakatani antigen and human parvovirus (B19). Lancet, i: 664—665, 1981.
3) Brown KE, Anderson SM, Young NS: Erythrocyte P antigen: cellular receptor for B19 parvovirus. Science, 262: 114—117, 1993.
4) Brown KE, Hibbs JR, Gallinella G, et al: Resistance to parvovirus B19 infection due to lack of virus receptor (erythrocyte P antigen). N Engl J Med, 330: 1192—1196, 1994.
5) Yunoki M, Tsujikawa M, Urayama T, et al: Heat sensitivity of human parvovirus B19. Vox Sang, 84: 164—169, 2003.
6) Kutzman G, Ozawa K, Cohen B, et al: Chronic bone marrow failure due to persistent B19 parvovirus infection. N Engl J Med, 317: 287—294, 1987.
7) Young NS: B19 parvovirus. In: Young NS, ed, Viruses and Bone Marrow: Basic Research and Clinical Practice, Marcel Dekker, New York, 1993, 75—117.
8) Sato H, Takakura F, Kojima E, et al: Screening of blood domors for parvovirus B19. Lancet, 346: 1237—1238, 1995.
9) Sakata H, Matsubayashi K, Ihara H, et al: Impact of chemiluminescent enzyme immunoassay screening for human parvovirus B19 antigen in Japanese blood donors. Transfusion, 53: 2556—2566, 2013.
10) Schmit M, Themann A, Drexler C, et al: Blood donor screening for parvovirus B19 in Germany and Austria. Transfusion, 47: 1775—1782, 2007.
11) Steven H, Kleinman, Simone A, et al: A linked donor-recipient study to evaluate parvovirus B19 transmission by blood component transfusion. Blood, 114: 3677—3683, 2009.
12) 日本赤十字社ヒトパルボウイルス陰性血液の確保に関する研究グループ：献血者におけるヒトパルボウイルスB19の分布状況．血液事業，35：232—238, 2012.
13) Ihara T, Furusyo N, Hayashi T, et al: A Population-based epidemiological survey of human parvovirus B19 infection: a project of the Kyushu and Okinawa Population Study (KOPS). Arch Virol, 158: 1465—1472, 2013.
14) Japanese Red Cross Haemovigilance Information: Transfusion Transmitted Infectious cases. 1997-2012.
15) Satake M, Hoshi Y, Taira R, et al: Symptomatic parvovirus B19 infection caused by blood component transfusion. Transfusion, 51: 1887—1895, 2011.
16) Kurtzman G, Frickhofen N, Kimball J, et al: Pure red-cell aplasia of 10 years duration due to persistent parvovirus B19 infection and its cure with immunoglobulin therapy. N Engl J Med, 321: 519—523, 1989.
17) Kevin E, Neal S, Barbara M, et al: Parvovirus B19: implicaions for transfusion medicine. Summary of a workshop. Transfusion, 41: 130—135, 2001.
18) Japanese Red Cross Haemovigilance Information: Transfusion information No.51, 1999.

POSTTRANSFUSIONAL SIDE EFFECT CAUSED BY HUMAN PARVOVIRUS B19 AND SAFETY MEASURES IN JAPAN

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Japanese Red Cross Kinki Block Blood Center

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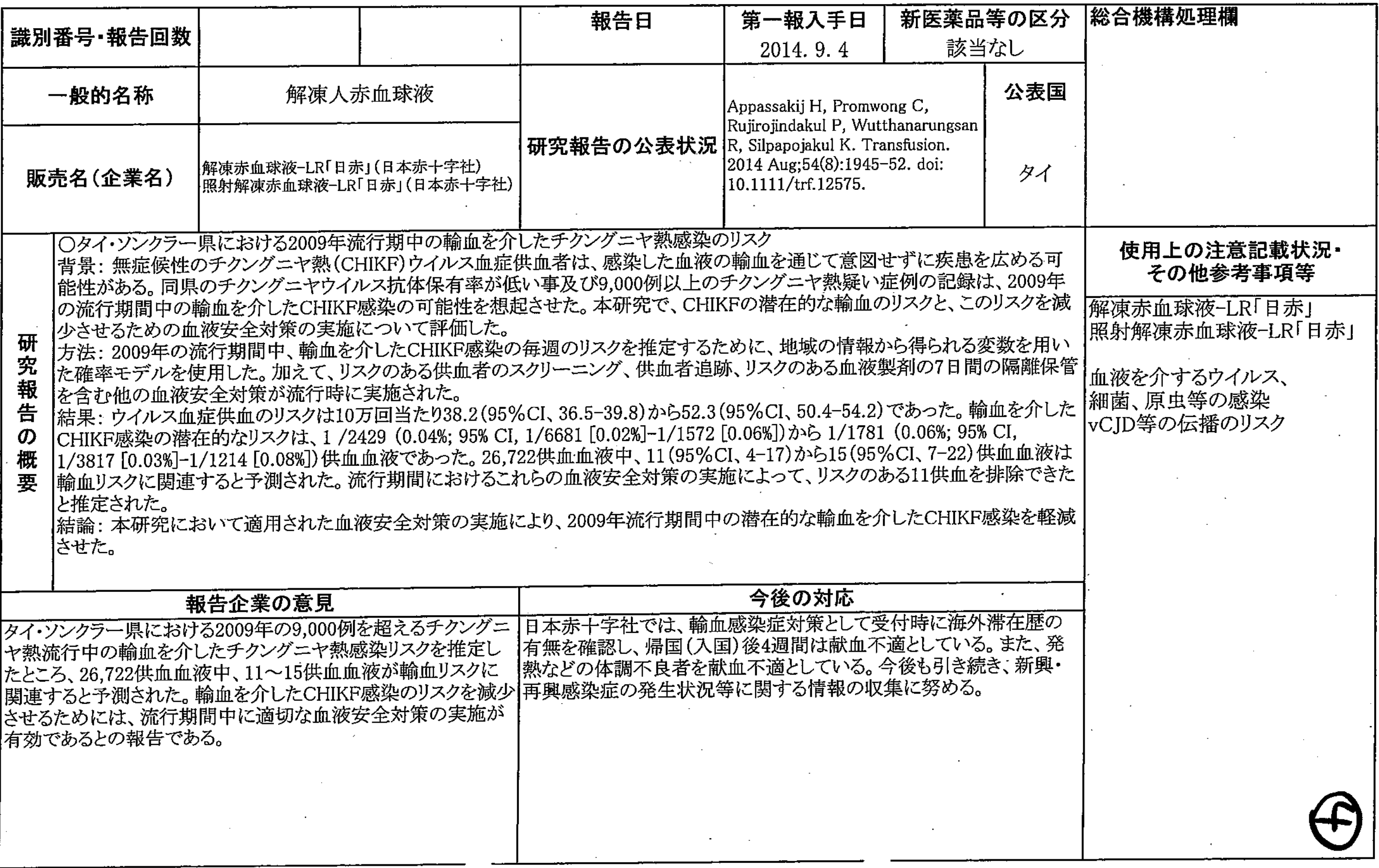

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研究報告の概要

○タイ・ソンクラー県における2009年流行期中の輸血を介したチクングニヤ熱感染のリスク

背景：無症候性のチクングニヤ熱（CHIKF）ウイルス血症供血者は、感染した血液の輸血を通じて意図せずに疾患を広める可能性がある。同県のチクングニヤウイルス抗体保有率が低い事及び9,000例以上のチクングニヤ熱疑い症例の記録は、2009年の流行期間中の輸血を介したCHIKF感染の可能性を想起させた。本研究で、CHIKFの潜在的な輸血のリスクと、このリスクを減少させるための血液安全対策の実施について評価した。

方法：2009年の流行期間中、輸血を介したCHIKF感染の毎週のリスクを推定するために、地域の情報から得られる変数を用いた確率モデルを使用した。加えて、リスクのある供血者のスクリーニング、供血者追跡、リスクのある血液製剤の7日間の隔離保管を含む他の血液安全対策が流行時に実施された。

結果：ウイルス血症供血のリスクは10万回当たり38.2（95%CI、36.5-39.8）から52.3（95%CI、50.4-54.2）であった。輸血を介したCHIKF感染の潜在的なリスクは、1 /2429 (0.04%; 95% CI, 1/6681 [0.02%]-1/1572 [0.06%])から 1/1781 (0.06%; 95% CI, 1/3817 [0.03%]-1/1214 [0.08%])供血血液であった。26,722供血血液中、11（95%CI、4-17）から15（95%CI、7-22）供血血液は輸血リスクに関連すると予測された。流行期間におけるこれらの血液安全対策の実施によって、リスクのある11供血を排除できたと推定された。

結論：本研究において適用された血液安全対策の実施により、2009年流行期間中の潜在的な輸血を介したCHIKF感染を軽減させた。

使用上の注意記載状況・その他参考事項等

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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
タイ・ソンクラー県における2009年の9,000例を超えるチクングニヤ熱流行中の輸血を介したチクングニヤ熱感染リスクを推定したところ、26,722供血血液中、11～15供血血液が輸血リスクに関連すると予測された。輸血を介したCHIKF感染のリスクを減少させるためには、流行期間中に適切な血液安全対策の実施が有効であるとの報告である。	日本赤十字社では、輸血感染症対策として受付時に海外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。

④

The risk of blood transfusion–associated Chikungunya fever during the 2009 epidemic in Songkhla Province, Thailand

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BACKGROUND: Asymptomatic Chikungunya fever (CHIKF)-viremic blood donors could be a potential threat of spreading the disease unwittingly through contaminated blood transfusions. The relatively low prevalence of Chikungunya virus antibodies in the population and the records of more than 9000 suspected CHIKF cases raised concern about the potential transfusion-associated CHIKF during the 2009 epidemic. This study assessed the potential transfusion risk for CHIKF and the implementation of blood safety measures to mitigate this risk.

STUDY DESIGN AND METHODS: A probabilistic model using key variables obtained from local information was used to estimate the weekly risk of transfusion-associated CHIKF during the 2009 epidemic. In addition, other blood safety measure–based strategies involving screening for donors at risk, donor tracing, and a 7-day quarantine of blood components at risk were implemented at the time of the epidemic.

RESULTS: The risk of viremic donations per 100,000 ranged from 38.2 (95% confidence interval [CI], 36.5-39.8) to 52.3 (95% CI, 50.4-54.2). The potential risk of transfusion-associated CHIKF per 100,000 was estimated to be 1 in 2429 (0.04%; 95% CI, 1 in 6681 [0.02%]-1 in 1572 [0.06%]) to 1 in 1781 (0.06%; 95% CI, 1 in 3817 [0.03%]-1 in 1214 (0.08%]) donations. Among 26,722 donations, 11 (95% CI, 4-17) to 15 (95% CI, 7-22) donations were predicted to associate with transfusion risk. The implementation of blood safety measure–based strategies for this epidemic period suggested to deter 11 blood donations of transfusion risk.

CONCLUSION: The interventions for blood safety measures applied in this study had mitigated the potential transfusion-associated CHIKF during the 2009 epidemic.

The presence of high viremic loads in Chikungunya virus (CHIKV)-infected individuals[1] and an abundance of *Aedes* mosquitoes[2] were important factors facilitating the widespread Chikungunya fever (CHIKF) epidemic in southern Thailand, where at least 49,089 persons were finally affected by the end of 2009.[3]

Although no cases of transfusion-associated CHIKF have yet been established,[4-6] an increasing concern is being recognized that this disease might be transferred via transfusions, particularly during an outbreak,[4-6] because CHIKV produces a high attack rate[1,7] and has a rapid replication rate,[8] high viremic levels,[1,8-10] and a significant proportion of asymptomatic infections.[1,4-6] Moreover, although CHIKV acquired via blood transfusion may constitute only a small proportion of all CHIKF cases during an outbreak, the CHIKV genome was identified in 1 of 250 donated platelet (PLT) units screened by nucleic acid amplification testing during the massive 2006 epidemic in Reunion Island.[5] Recently, we documented that viremic

ABBREVIATIONS: CHIKF = Chikungunya fever; CHIKV = Chikungunya virus; DENV = dengue virus.

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asymptomatic CHIKV-infected cases could have a high potential as disseminators of transfusion-associated CHIKF, since CHIKV levels capable of inducing CHIKF were found in the blood of these asymptomatic cases.[1] In addition, the finding of high viremic levels in the first few days of symptomatic CHIKV-infected cases led to the suggestion that high viremic levels could also be present during the presymptomatic viremic period.[1] If presymptomatic individuals donate blood, obviously there is a threat of passing on the infection through such donations. These groups of potential infected donors are unlikely to self-defer or be excluded from donation by the predonation medical screening.

Before 2009, an outbreak of CHIKF had never been reported in Songkhla Province in southern Thailand.[11-13] The seroprevalence of CHIKV antibodies in the adult population of Songkhla Province before 2009 was 1.8% to 9.8%, which was the lowest seroprevalence in Thailand.[14-16] The finding had been considered as a likely low herd immunity area to CHIKV infection.[14-16] In 2009 there was an epidemic of CHIKF involving more than 9000 cases in Songkhla Province.[12,13] These situations raised transfusion safety concerns.

In this report, we used key variables with values specified using local information in the probabilistic model to estimate the potential transfusion risk of CHIKF, aiming to aid decisions to implement safety measures. This study also assessed the implementation of blood safety measures to mitigate this potential risk.

MATERIALS AND METHODS

A probabilistic model for transfusion risk

A probabilistic model,[17] as shown in the formula below, was used to estimate the transfusion-associated risk during the 2009 epidemic. The modeling assumptions were mainly dependent on the proportion of asymptomatic viremic donors, the duration of viremia, and the prevalence of infected donors.

$$\text{Estimated transfusion-associated risk} \approx [(Pa \times Da)+(Ps \times Ds)]/L \times Pr,$$

where Pa is the proportion of asymptomatic CHIKV-viremic donors, Da is the duration of viremia for asymptomatic donors, Ps is the proportion of presymptomatic CHIKV donors, Ds is the duration of viremia for presymptomatic CHIKV donors, Pr is the prevalence of CHIKV-viremic donations, and L is the length of the outbreak.

The modeling assumptions used in this study were, first, symptomatic CHIKF cases would either self-defer owing to their being too sick to donate or be excluded from donation by the predonation screening.[4] However, these assumptions would not exclude infected individuals who remained asymptomatic or became symptomatic after their blood donations. Second, the blood of any viremic asymptomatic CHIKF donor could potentially transmit the disease due to the presence of relatively high viremic levels in asymptomatic CHIKV-infected cases[1] and the relatively low herd immunity to CHIKV in the recipients.[14-16] Third, the incidence of viremic blood donors with respect to mosquito exposure and infection risk was assumed to be similar to the population at large.[17]

The confidence intervals (CIs) of mean and maximal risk estimates[18] as well as key variables with values specified using local information[1] (Table 1) were used to encapsulate uncertainty in the variable assumptions. The CIs were computed using the methods described by Petersen and colleagues.[18]

Risk model variables

The number of clinically suspected CHIKF patients

Clinically suspected CHIKF cases were defined as patients who developed acute fever of up to 7 days' duration, arthralgia, and/or rash.[12,13] The numbers of suspected CHIKF cases were determined from the records of the two main offices dealing with such things in this part of southern Thailand, the Office of Disease Prevention and Control 12 Songkhla (ODPC 12) and the Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand (Fig. 1).[12,13]

The epidemic was first reported in August 2008 from Narathiwat Province in southern Thailand and

TABLE 1. Key variables for modeling assumptions

Variable	Modeling assumption: Mean risk	Modeling assumption: Maximal risk
Proportion of asymptomatic cases (Pa)	10%	10%
Proportion of presymptomatic cases (Ps)	90%	90%
Total duration of viremia (Da)	9.5 days	18.5 days
Duration of presymptomatic viremia (Ds)	1.5 days	1.5 days
Estimated number of symptomatic CHIKF cases	Number of suspected CHIKF cases × 0.938* × 8.03†	Number of suspected CHIKF cases × 0.938 × 8.03

* 0.938 = factor for adjusting exclusive CHIKF cases (see Materials and Methods).
† 8.03 = factor for adjusting active CHIKF cases (see Materials and Methods).

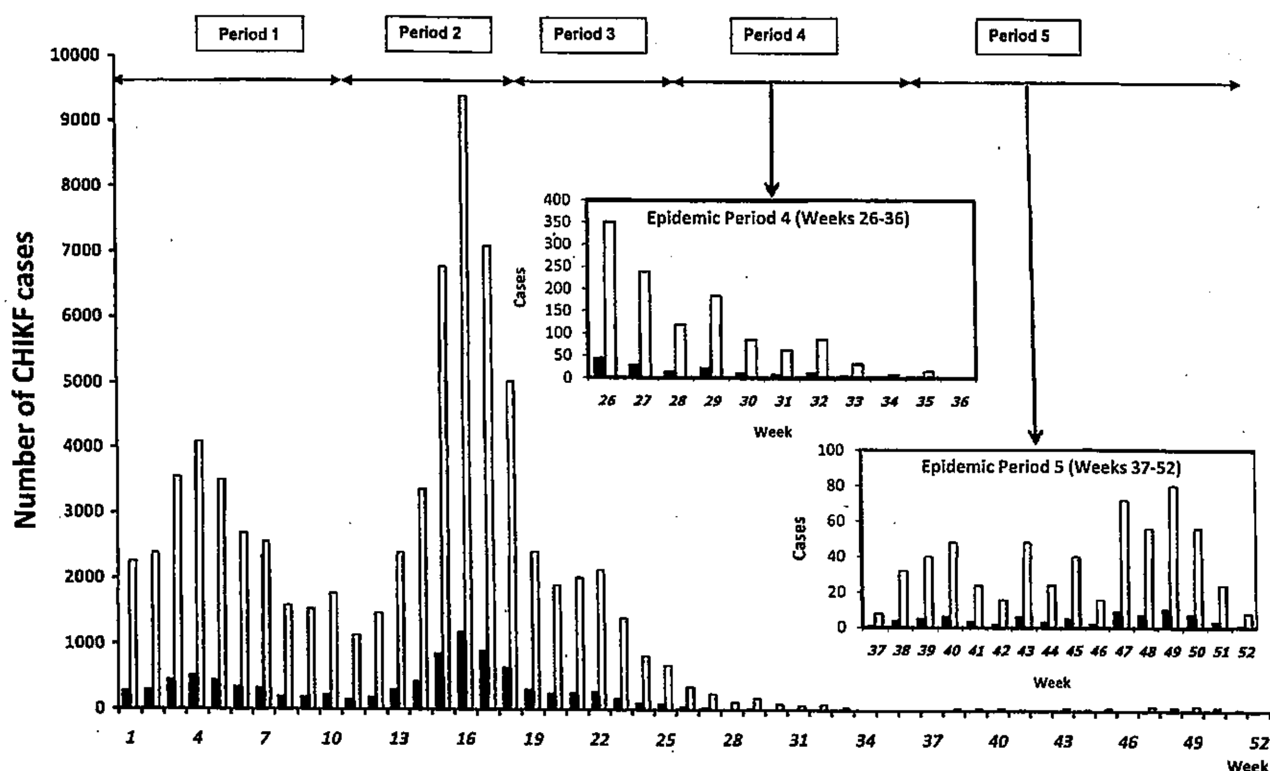

Fig. 1. Number of clinically suspected CHIKF cases (■) and estimated number of CHIKF cases (□) by week of onset reported during the 2009 epidemic periods in Songkhla Province, southern Thailand. *Epidemic periods: Period 1, Weeks 1 to 11 (January 4-March 21, 2009); Period 2, Weeks 12 to 18 (March 22-May 9, 2009); Period 3, Weeks 19 to 25 (May 10-June 27, 2009); Period 4, Weeks 26 to 36 (June 28-September 12, 2009); and Period 5, Weeks 37 to 52 (September 13, 2009-January 2, 2010).

subsequently spread to neighboring provinces including Pattani, Yala, and Songkhla. We chose to model the CHIKF epidemic period in Songkhla Province from January to December 2009. The estimated population size of Songkhla Province in 2009 was 1,343,954.[12]

Prevalence of CHIKV infection

Our previous report performed at the time of this epidemic found that 6.2% of patients with clinically suspected CHIKF were actually affected by other febrile illnesses.[1] In this study, we thus readjusted the incidence of exclusive symptomatic CHIKF patients by multiplying the number of clinically suspected CHIKF patients by a factor of 0.938.

It had to be noted that, during the 2009 epidemic, the records of suspected CHIKF cases were underestimated[12,13] as evidenced by a comparative study between an active community-based surveillance and passive notification records in the late epidemic period (June 28-November 4, 2009): an actual 8.03 suspected CHIKF cases were identified by this active case finding for every suspected case identified by the passively noticed (http://www.chikungunya.org/, access date November 18, 2009). Thus, the final estimated number of symptomatic CHIKF cases used in the calculation was the number of clinically suspected CHIKF cases multiplied by 8.03.

The proportion and the duration of viremia in presymptomatic CHIKF cases

The proportion of presymptomatic CHIKV-infected cases in modeling assumptions was assumed to be similar to the symptomatic CHIKF patients at 90%.[1] The duration of the presymptomatic viremic period (Ds) was approximated based on previous published studies to be 1.5 days (range, 1-2 days).[6,19]

The proportion and the duration of viremia in asymptomatic CHIKF blood donors

The proportion of viremic asymptomatic CHIKF cases obtained from a case-control study carried out in CHIKV-affected areas between March and April 2009 in Songkhla Province was 10%.[1] There have been no published data about the kinetics of CHIKV viremia in asymptomatic cases. We, however, assumed that the total duration of viremia in asymptomatic CHIKV infection was similar to those in symptomatic cases, which was up to 8 days after the onset of symptoms as revealed by viral isolation or clinical symptoms, or up to 17 days by detection of CHIKV

RNA.[1] Therefore, we assumed that the duration of transfusion risk in asymptomatic CHIKV infection was 9.5 days (1.5 + 8) on average with a maximum of 18.5 (1.5 + 17) days, respectively (Table 1).

Numbers of blood donations

From January to December 2009, a total of 26,722 blood donations were collected at Songklanagarind University Hospital in Songkhla Province.

Accuracy of the risk model

The accuracy of the risk model[17] was assessed by CHIKV RNA assay of 2000 donated blood units collected near the final weeks of the epidemic (Week 36 to Week 41, September 12-October 18, 2009). Two-hundred pooled serum samples from every 10 donors were first screened for the presence of CHIKV RNA. If any pooled serum sample was positive, each of the 10 individual serum samples was then individually tested for CHIKV RNA.[20]

Transfusion-associated CHIKF management

Measures to ensure blood safety were applied from April to October 2009, by using specific predonation questions about CHIKF-related symptoms (acute fever, arthralgia, and/or rash)[9,10,14] and an enhanced postdonation report by donors to call back if CHIKF-related symptoms developed. Donated blood components at risk were quarantined for 7 days after donation and all donors at risk were interviewed by telephone to confirm the status of the donors on Day 7 after their donation. Blood units from donors at risk who developed CHIKF-related symptoms within 7 days of donations were discarded.

Ethical approval

This study was approved by the Ethics Committee of the Faculty of Medicine, Prince of Songkla University.

RESULTS

The number of clinically suspected CHIKF cases

From January to December 2009, a total of 9440 clinically suspected CHIKF cases were reported. The number of suspected CHIKF cases dramatically increased from the first week of 2009 and fluctuated between 0 (Week 36) and 1170 cases (Week 16) per week with three peaks appearing at Week 4 (510 cases), Week 16 (1170 cases), and Week 22 (266 cases; Fig. 1).

The epidemic can be divided into five periods: Period 1 (Weeks 1-11), Period 2 (Weeks 12-18), Period 3 (Weeks 19-25), Period 4 (Weeks 26-36), and Period 5 (Weeks 37-52). The numbers of suspected CHIKF cases were 251.2, 329.1, 105.4, 10.7, and 5.9 per 100,000 in Periods 1, 2, 3, 4, and 5, respectively, with three peaks: Week 4 (37.9 per 100,000), Week 16 (87.0 per 100,000), and Week 22 (19.8 per 100,000; Fig. 1).

Estimated prevalence of CHIKV infection

The mean weekly prevalence of CHIKV infections (symptomatic and asymptomatic cases) among the population at large during the entire epidemic period was estimated to be 111.9 (95% CI, 109.1-114.7), varying from 0 to 721.2 (95% CI, 668.8-773.7) per 100,000 (Fig. 2).

Estimated risk of viremic donations

A probabilistic model was used to estimate the weekly risk of viremic donations during the 2009 epidemic under the indicated assumptions of a potential estimated mean and maximal risk (Table 1). The mean weekly risk of viremic donations during the entire epidemic period was estimated to be 38.2 (95% CI, 36.5-39.8), varying from 0 to 237.0 (95% CI; 206.9-267.1) per 100,000 (Fig. 2). Similarly, the maximum weekly risk of viremic donations during the entire epidemic period was estimated to be 52.3 (95% CI, 50.4-54.2), varying from 0 to 329.6 (95% CI; 294.1-365.1) per 100,000 (Fig. 2). Overall, the maximal risk estimates were 1.4-fold greater than the mean risk estimates.

The potential risk of blood transfusion–associated CHIKF

Among the 26,722 blood donations collected from January to December 2009, on mean potential risk of transfusion-associated CHIKF, 11 (95% CI, 4-17) CHIKF-contaminated blood donations would be expected to be received, with a risk of transfusion-associated CHIKF being 1 in 2429 (0.04%; 95% CI, 1 in 6681 [0.02%]-1 in 1572 [0.06%]). The highest peak of the epidemic (Week 16) was 1 in 422 (0.24%; 95% CI, 1 in 382 [0.26%]-1 in 462 [0.22%]).

Similarly, on maximal potential risk of transfusion-associated CHIKF during the entire epidemic period, 15 (95% CI, 7-22) CHIKF-contaminated blood donations would be received, with a risk of transfusion-associated CHIKF being 1 in 1781 (0.06%; 95% CI, 1 in 3817 [0.03%]-1 in 1214 [0.08%]). The highest peak of the epidemic was 1 in 303 (0.33%; 95% CI, 1 in 273 [0.37%]-1 in 330 [0.30%]).

To assess the accuracy of the risk model, the following facts were noted: 0.10% of the 2000 blood units collected during Weeks 36 to 41 (September 12 to October 18, 2009) were found to be positive for CHIKV RNA whereas 0.20% (95% CI, 0.19-0.20) to 0.33% (95% CI, 0.32-0.33) were predicted by this model. Overall, 11 (95% CI, 4-7) to 15 (95% CI, 7-22), or 0.04% (95% CI, 0.02%-0.06%) to 0.06% (95%

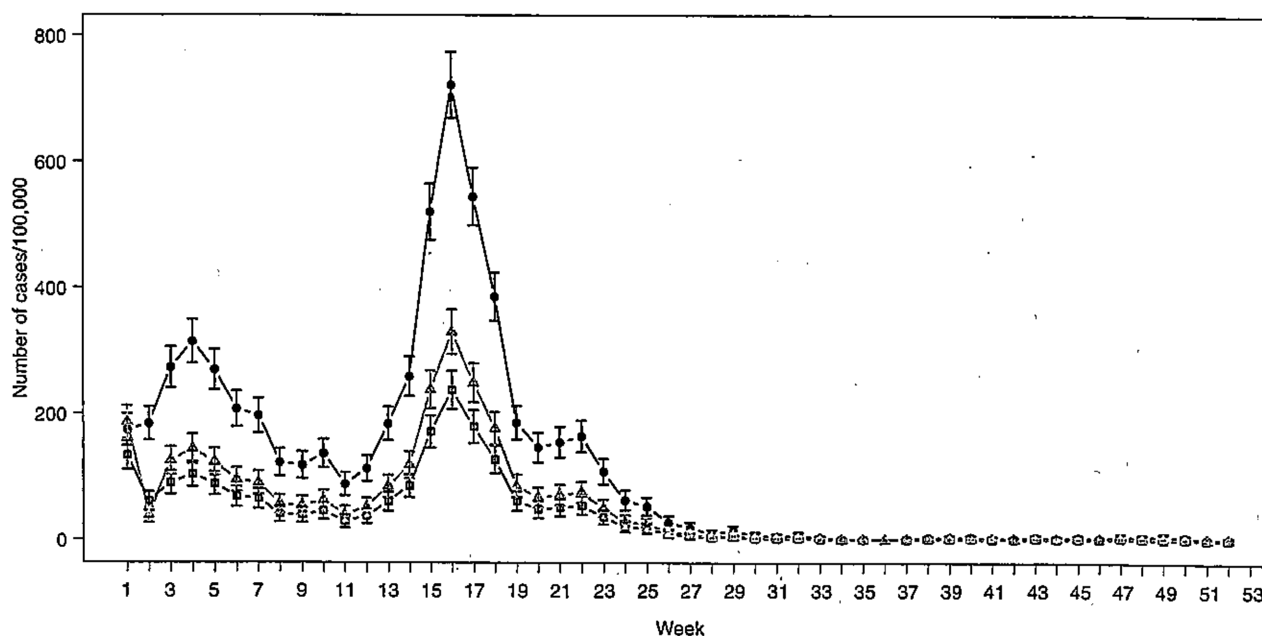

Fig. 2. Estimated weekly prevalence of CHIKV infection (●) and estimated weekly risk of viremic donations (□, mean; Δ, maximal) during the 2009 epidemic in Songkhla Province, Thailand. Simultaneous 95th percentile-t CIs are shown as vertical bars.

CI, 0.03%-0.08%), viremic asymptomatic CHIKF blood donors were predicted to associate with transfusion-induced CHIKF.

Transfusion-associated CHIKF management

Blood safety measure–based strategies involving screening of donors at risk using specific predonation questions and a 7-day quarantine of blood components as well as donor tracing were implemented from Week 13 to Week 43 (April to October 2009). Among the 15,513 donations collected from April to October 2009, seven (95% CI, 4-12) to nine (95% CI, 6-22) donations were predicted to associate with a transfusion risk. The implementation of blood safety measured–based strategies for this period suggested that 299 of the 13,202 (2.3%) were donations at risk. Of those 299 donations at risk, 271 (90.6%) were normal individual donations, 17 (5.7%) could not be contacted on Day 7 after their donations, and 11 (3.7%) were suggestive CHIKF-contaminated blood donations. Only one available serum sample of those 11 suggestive CHIKF-contaminated blood donations was confirmed for positive CHIKV viremia at the levels of 8.7×10^6 plaque-forming units per milliliter. However, these 11 blood units were deterred transfusion-associated risk by removing from the blood bank.

DISCUSSION

This study applied a probabilistic model using key variables obtained from local information to estimate the weekly CHIKV-viremic donations during the 2009 CHIKV epidemic in Songkhla Province, Thailand. This type of prediction is useful in helping policy makers to implement safety measures.[4-6,17] The interventions for blood safety measures applied at the time of the epidemic had mitigated this potential risk.

It was found that the mean and maximal risks of viremic donations were 38 to 52 per 100,000 donations or 1 in 2429 (0.04%) to 1 in 1781 (0.06%) donations, respectively. In a risk modeling performed by Brouard and colleagues[5] during the 2005 to 2007 epidemic on Reunion Island, where 38% of the 766,000 subjects of the study were infected, the mean risk was estimated to be 32 per 100,000 donations or 1 in 758 (0.13%) donations, whereas the highest peak of the outbreak was 1500 per 100,000 donations or 1 in 67 (1.49%) donations. Similarly, at Cervia where the largest outbreak in Italy occurred, the highest weekly risk was estimated at 1 in 3801 (0.03%) donations.[6] [Correction statement added after online publication 17-Feb-2014: mean risk changed to highest weekly risk.] The estimated CHIKV transfusion risk found in this study and in the previous publications were of the same magnitude. In addition, the estimates of CHIKV-viremic donations were also comparable to that of dengue virus (DENV).[18,21]

The model variables used in this study (Table 1) were compared to other reports. There were fewer variables and more certainty in the duration of presymptomatic viremia (1.5 days[6,19] vs. 1.5-2 days[5,6]) and the duration of viremia in asymptomatic CHIKF by means of viral isolation or

clinical symptoms (8.0 days[1] vs. 6.0 days of illness[4-6]) while the prevalence of asymptomatic CHIKF (10%[1] vs. 15%[4-6]) was more variable. Theoretically, the ratio of asymptomatic CHIKF to symptomatic CHIKF may not be constant over time. Since Songkhla Province had been considered as a likely low herd immunity area to CHIKV infection,[11,14-16] both of the current circulating CHIKV strains (A226 and 226V strains) displayed not prominent distinct antigenic structure,[15,22,23] CHIKV infection has low infective dose,[24,25] and CHIKV produces a high rate of prominent symptomatic CHIKF;[1,7,9,11] therefore, the authors hypothesized that the uncertainty of low asymptomatic rate would have narrow range and hence the variation of viremic asymptomatic rate could be neglected.[1,4-7,9,10] This is in contrast to a DENV transfusion risk model because the DENV viremic donations varied considerably by season and year.[18] Several factors may be responsible for this involving population including herd immunity to DENV,[11,14-16,26] geography,[18,26] age of cases,[14,15] virus circulating strains,[14,26] prevalence of infection,[18,26] and human genetic-associated factors.[27] Previously, the authors have suggested that the duration of detectable viable CHIKV (up to 8 days of illness) is less than the duration of CHIKV RNA positivity (up to 17 days of illness).[1] Nevertheless, from the viewpoint of recipient safety and to get more information on decision-making policies, all CHIKV RNA–positive units should conservatively be considered potentially infectious donations. In this study, the authors used the total duration of viremia in asymptomatic CHIKF by means of RNA detection (up to 18.5 days instead of 9.5 days of illness) as the maximum estimated transfusion risk. Generally, the maximal risk estimate was 1.4-fold greater than the mean risk estimates. Another difference was the use of the prevalence of exclusive CHIKV-infected individuals as mentioned earlier under Materials and Methods.

A limitation of this study on accuracy of the risk model is the absence of blood testing at the appropriate times (e.g., March to July 2009). However, the estimated potential risk (0.20%-0.33%) and the observed potential risk (0.10%) of blood transfusion–associated CHIKF that were evaluated near the end of the epidemic period (September 12 to October 18, 2009) were in the same magnitude. In addition, this risk model was previously validated to be reliable for the prediction of transfusion-associated CHIKV,[5] DENV,[18,20] and West Nile virus modeling.[17] For example, Brouard and colleagues[5] estimated the mean risk of having viremic donations at 0.7%, which agreed closely with the observed rate of 0.4% CHIKV RNA–positive testing of PLT donations. Another limitation of this study was the inference of actual CHIKV infection prevalence from the underestimated sentinel records as mentioned earlier.

As an estimated transfusion risk model provides only an estimated risk of viremic donations, the actual risk of disease after transfusion may differ from the risk modeling estimates. In the scenario of Songkhla Province modeled in this study, although not proven, the transfused CHIKV from the potentially viremic donors was supposed to be a high-risk transfusion transmission because of the plausible relatively high viremic levels in asymptomatic CHIKV-infected cases[1] and the existence of relatively low herd immunity to CHIKV.[15,16] In a contrary situation, transfused DENV from potentially viremic donors was considered as likely to be unpredictable, in part, due to the existence of high herd immunity to DENV[15,16,18] and the existence of various circulating DENV serotypes.[14-16,26] However, to date, the actual rates of each transfusion-transmitted disease are not known. Future studies are needed to determine the rates of transfusion-associated diseases and their clinical consequences in recipients.

The risk model is a pragmatic and cost-effective tool for risk assessment compared with a screening test. However, the threshold numbers of the viremic donors targeted for blood safety measures and CHIKF management should err on the side of caution. According to the published reports, managing the risk of transfusion-associated CHIKF varies among countries. Liumbruno and colleagues[6] described the use of blood safety measure–based strategies implemented during the entire period of the outbreak in Italy where blood collection in the affected areas was interrupted in the early period of the outbreak. Later on, new precautionary measures were applied that included predicted viremic donation modeling, 21-day deferral for blood donors who had visited the affected areas, quarantine of blood components for 5 days (subsequently reduced to 2 days), and pathogen inactivation of PLT concentrates, ultimately resulting in the loss of 5130 units of red blood cells and 2871 L of fresh-frozen plasma and an economic loss exceeding £1.3 million. In another experience from Reunion Island, Brouard and colleagues[5] also used the policy of stopping local blood donations before the peak of the outbreak to minimize transfusion-associated CHIKF and a return to regular blood donation services 18 months after the epidemic had subsided. That study reported that blood donations were interrupted from January 2006 to April 2007 and concluded that at least 40 potentially viremic donations were avoided.[5] Although the economic loss was not reported, it was thought to be high. During the 2009 epidemic in southern Thailand, including Songkhla Province the focus of this study, the implementation of blood donor testing for CHIKV RNA was conducted on a cost–benefit analysis of the effectiveness of screening and further limited due to a paucity of resources. Since CHIKV features prominent clinical symptoms along with a relatively low rate of asymptomatic-infected individuals and a relatively short viremic period,[1] the authors suggest that the affordable intervention of blood safety measures to mitigate the risk of transfusion-spread disease, involving the use of

predictive risk modeling, screening for donors at risk, donor tracing, and quarantine of blood components at risk, was suitably applied. Only 17 (5.7%) of 299 donors at risk were lost to contact 7 days after blood collection and of these 11 (3.7%) were suggestive CHIKF-contaminated blood donation donors. At least one of those suggestive CHIKF blood donations was confirmed for CHIKV-viremic donor. This approach was apparently effective as 11 suggestive viremic donations avoided transfusion-associated CHIKF.

A screening blood test and/or a deferral-based strategy should be considered for donors at risk (e.g., residents living in affected areas or having visited affected areas, tourists at risk returning to their home country and/or to nonendemic areas). Alternatively, specific CHIKF-related questions in predonation examinations coupled with deferral of at least 4 weeks (CHIKV RNA is detectable up to 17 days of illness) after each stay or visit to an affected area would cover various mosquito-borne viruses including CHIKV.[1,20]

Based on this study and previous reports, it is suggested that interventions of blood safety measures during an outbreak need to be applied. Nevertheless, it appears that currently no guideline is available.

In conclusion, the CHIKV transfusion risk in Songkhla Province donors during the 2009 epidemic was predicted by a probabilistic model using local risk model variables. [Correction added after online publication 17-Feb-2014: sentence updated for clarity.] The estimated number of viremic donors was in the same magnitude as previous reports. To mitigate the transfusion risk of CHIKF, appropriate interventions for blood safety measures need to be applied during an outbreak.

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CONFLICT OF INTEREST

The authors report no conflicts of interest or funding sources.

REFERENCES

1. Appassakij H, Khuntikij P, Kemapunmanus M, et al. Viremic profiles in asymptomatic and symptomatic chikungunya fever: a blood transfusion threat? Transfusion 2013;53:2567-74.
2. Thavara U, Tawatsin A, Pengsakul T, et al. Outbreak of Chikungunya fever in Thailand and virus detection in field population of vector mosquito *Aedes aegypti* and *Aedes albopictus*. Southeast Asian J Trop Med Public Health 2009;40:951-62.
3. Sawongtho P, Thavara U. Situation of chikungunya. Weekly Epidemiological Surveillance Report (Thailand) 2009;40:878.
4. Peterson LR, Busch MP. Transfusion-transmitted arboviruses. Vox Sang 2010;98:495-503.
5. Brouard C, Bernillon P, Quatresous I, et al. Estimated risk of Chikungunya viremic blood donation during an epidemic on Reunion Island in the Indian Ocean, 2005 to 2007. Transfusion 2008;48:1333-41.
6. Liumbruno GM, Calteri D, Petropulacos K, et al. The Chikungunya epidemic in Italy and its repercussion on the blood system. Blood Transfus 2008;6:199-210.
7. Palaux G, Gaüzere BA, Jaureguiberry S, et al. Chikungunya, an epidemic arbovirosis. Lancet Infect Dis 2007;7:319-27.
8. Solignat M, Gay B, Higgs S, et al. Replication cycle of chikungunya: a re-emerging arbovirus. Virology 2009;393: 183-97.
9. Parola P, de Lamballerie X, Jourdan J, et al. Novel chikungunya virus variant in travelers returning from Indian Ocean islands. Emerg Infect Dis 2006;12: 1493-9.
10. Panning M, Grywna K, van Esbroeck M, et al. Chikungunya fever in travelers returning to Europe from the Indian Ocean region, 2006. Emerg Infect Dis 2008;14: 416-22.
11. Wutthanarungsan R, Sornsrivichai V, Thammapalo S, et al. Risk factors for chikungunya fever in Thepha and Chana Districts, Songkhla Province, March–April 2009. Weekly Epidemiological Surveillance Report (Thailand) 2010;41:36-41.
12. Office of Disease Prevention and Control, 12 Songkhla (ODPC 12), Department of Disease Control, Ministry of Public Health, Thailand. Chikungunya, 2009. [cited 2010 Jan 30]. Available from: http://www.dpc12.ddc.moph.go.th/dpc_12/
13. Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand. Chikungunya, 2008 to 2009. [cited 2010 Jan 30]. Available from: http://www.boe.moph.go.th/
14. Nimmannitya S, Halstead SB, Cohen SN, et al. Dengue and chikungunya virus infection in man in Thailand, 1962-1964. I. Observations on hospitalized patients with hemorrhagic fever. Am J Trop Med Hyg 1969;18: 954-71.
15. Halstead SB, Udomsakdi S, Scanlon JE, et al. Dengue and chikungunya virus infection in man in Thailand, 1962-1964. V. Epidemiologic observations outside Bangkok. Am J Trop Med Hyg 1969;8:1022-33.
16. Fukunaga T, Rojanasuphot S, Pisuthipornkul S, et al. Seroepidemiologic study of arbovirus infections in the north-east and south of Thailand. Biken J 1974;17: 169-82.

17. Biggerstaff BJ, Peterson LR. Estimated risk of transmission of the West Nile virus through blood transfusion in the US, 2002. Transfusion 2003;42:1007-17.
18. Petersen LR, Tomashek KM, Biggerstaff BJ. Estimated prevalence of dengue viremia in Puerto Rican blood donations, 1995 through 2010. Transfusion 2012;52:1647-51.
19. Ng LC, Tan LK, Tan CH, et al. Entomologic and virologic investigation of Chikungunya, Singapore. Emerg Infect Dis 2009;15:1243-8.
20. Dash PB, Parida M, Santhosa SR, et al. Development and evaluation of a 1-step duplex reverse transcription polymerase chain reaction for differential diagnosis of a chikungunya and dengue infection. Diagn Microbiol Infect Dis 2008;62:52-7.
21. Stramer SL, Linnen JM, Carrick JM, et al. Dengue viremia in blood donors identified by RNA and detection of dengue transfusion transmission during the 2007 dengue outbreak in Puerto Rico. Transfusion 2012;52: 1657-66.
22. Suwannakarn K, Theamboonlers A, Poovorawan Y. Molecular genome tracking of East, Central and South African genotype of Chikungunya virus in Southeast Asia between 2006 and 2009. Asian Pac J Trop Med 2011;4:535-40.
23. Yap G, Pok KY, Lai YL, et al. Evaluation of chikungunya diagnostic assays: differences in sensitivity of serology assays in two independent outbreaks. PLoS Negl Trop Dis 2010;4:e753.
24. Paul SD, Singh KR. Experimental infection of *Macaca radiate* with chikungunya virus and transmission of virus by mosquitoes. Indian J Med Res 1968;56: 802-11.
25. Labadie K, Larcher T, Joubert C, et al. Chikungunya disease in nonhuman primates involves long-term viral persistence in macrophages. J Clin Invest 2010;120: 894-906.
26. Endy TP, Anderson KB, Nisalak A, et al. Determinants of inapparent and symptomatic dengue infection in a prospective study of primary school children in Kamphaeng Phet, Thailand. PLoS Negl Trop Dis 2011;5:e975.
27. Stephens HA. HLA and other gene associations with dengue disease severity. Curr Top Microbiol Immunol 2010;338:99-114.

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 2014. 10. 4	新医薬品等の区分 該当なし	総合機構処理欄
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販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社) 赤血球液-LR「日赤」(日本赤十字社) 照射赤血球液-LR「日赤」(日本赤十字社)					

研究報告の概要

○中国の供血者における重症熱性血小板減少症候群ウイルス(SFTSV)の抗体陽性率と無症候性ウイルス血症の頻度

背景：新興のダニ媒介性病原体であるSFTSVは2009年に中国で初めて同定され、血液伝播する可能性も示唆される。中国の流行地1地域(信陽市)及び非流行地2地域(綿陽市、洛陽市)の血液センターからの供血サンプルにおいて、SFTSVの抗体陽性率とウイルス血症の頻度を調査した。

方法：2012年5月1日～10月31日、信陽市で供血を行った全供血者(n=14,764)及び綿陽市と洛陽市の血液センターから選択した供血者(それぞれn=1,130、1,326)から血漿サンプルを収集し、EIA法を用いてSFTSV抗体検査を行った。また、信陽市の9,960サンプルから4サンプルずつのプールを作り、リアルタイムRT-PCR検査を行い、陽性となったプールは個別サンプルのRT-PCR検査を行った。

結果：統計学的な有意差はなかったが、流行地域からの供血者における抗体陽性率(0.59%)は、非流行地域よりも高かった(それぞれ0.27%、0.28%)。流行地域における抗体陽性供血者と陰性供血者間の人口統計学的特徴に有意差はなかった。流行地域の2,490プールのうち、RNA検査に反応したのは63プールであった。その中で個別サンプルにおいて、2例が反応性を示し、ウイルス量は10pfu/mL以下と推定された。信陽市の供血者におけるSFTSV RNA陽性率は0.02%(2/9,960)であった。

結論：中国の3地域におけるSFTSV抗体陽性率は0.27～0.59%であった。供血者におけるウイルス血症の低い頻度は、これらの地域における血液安全性へのSFTSVの影響が限られていることを示唆するが、特に流行地域の供血者における更なるSFTSV調査は血液安全性のために必要である。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」
赤血球液-LR「日赤」
照射赤血球液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
中国のSFTSV流行地域及び非流行地域の供血者において抗体検査を行ったところ、抗体陽性率は0.27～0.59%であった。また流行地域の供血者におけるSFTSV RNA陽性率は0.02%と低く、これらの地域における血液安全性への同ウイルスの影響が限られていることを示唆するとの報告である。	今後も引き続き情報の収集に努める。

⑤

conducted in 2011-2013 at blood centers collecting ~50% of the US supply. Cases were defined as donors whose donations were confirmed positive on serology-only (HTLV) or were serology/NAT or NAT-only confirmed (HIV, HBV, HCV). Controls had repeat reactive-unconfirmed, NAT-negative donations. Cases and controls were asked to complete an interviewer-administered telephone or in-person questionnaire inquiring about risk factors potentially associated with each infection. Factors associated with each infection compared to controls were independently assessed by using multivariable logistic regression. Odds ratios adjusted for gender, age, race, income, and/or education are reported; p values ≤0.001 were considered highly significant. **Results/Findings:** Behavioral factors associated with each infection are presented in the Table. For HIV, an HIV+ sex partner and MSM behavior were highly associated with infection. For HBV, an IVDU sex partner, born outside the United States, and a family history of hepatitis were highly significant. For HCV, potential donor's IVDU, an IVDU sex partner, spending 3+ nights in detention/jail, and a family history of hepatitis were highly significant. For HTLV, an IVDU sex partner, history of sex work/trade, and born outside the United States were highly significant. Few recent infections based on NAT-only positivity were included: 3 HIV and 10 HCV. Overall, infections for which no putative risk factor was reported were uncommon (<2% of cases). **Conclusion:** This study identified several factors associated with transfusion-transmissible infections that follow epidemiological patterns in the general population and that have implications for general donor eligibility policies. Strategies to increase disclosure of specific deferrable behaviors, including IVDU, MSM, and sexual partner risks, are needed.

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TABLE.

Odds ratios of association with each infection compared to 1587 control donors*

Behaviors	HIV n = 196	HBV n = 292	HCV n = 316	HTLV n = 198
Men who have sex with men (MSM)	**65.3†**	—	—	—
HIV+ sex partner	**142.0†**	—	—	—
Intravenous drug use (IVDU)	**3.4**	**4.4**	**48.3†**	—
IVDU sex partner	—	**9.3†**	**9.9†**	**22.3†**
Multiple sex partners	**2.2**	—	—	**3.7†**
Sex work/trade	—	—	—	**10.0†**
Born outside the US	—	**8.9†**	**3.8†**	**6.7†**
Tattoo/piercing	**2.6†**	—	**3.4†**	
3+ nights in detention/jail	**2.9**	**3.0†**	**9.9†**	—
Hepatitis history in family	—	**7.5†**	**11.1†**	—
Transfusion	—	—	**4.9†**	—

* Separate models for each infection. All factors not presented.
† Highly significant, p ≤ 0.001, others p ≤ 0.05.

SP385

First Report of Human Immunodeficiency Virus Transmission through 20-Minipool Nucleic Acid Amplification Test-Negative Blood Donation in Japan

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Background/Case Studies: The Japanese Red Cross blood centers implement a 20-minipool (MP) nucleic acid amplification test (NAT) for hepatitis B virus, hepatitis C virus, and human immunodeficiency virus Type 1 (HIV-1). No transfusion-transmitted HIV infection (TT-HIV) has been reported in Japan since 20-MP-NAT was introduced. However, in 2013, we found a case of TT-HIV through a look-back study. Case description: In November 2013, HIV-1 infection was confirmed in a male repeat blood donor in his 40 s. The Cobas TaqScreen HIV (Roche) detected HIV-1 RNA in an archived blood sample from his previous donation in February 2013, whereas the Procleix UlTrio ABD (Novartis) did not. Two recipients of blood products from the previous donation were tested for HIV-1. A female recipient of red blood cells in her 80 s was negative, as determined by serological tests and NAT for HIV-1 9 mo after the transfusion. On the other hand, HIV-1 infection was identified in a male recipient of fresh-frozen plasma (FFP) in his 60 s 34 d after transfusion. **Study Design/Methods:** To investigate the homology of the HIV-1 genome from the donor and FFP recipient, nested PCR and direct sequencing analysis of *env* and *pol* were performed. In addition, sequence analysis of the HIV-1 long terminal repeat (LTR), which is the target region of our NAT screen, was performed. To estimate the HIV-1 viral load in the implicated blood, the sensitivities of the Cobas and the Procleix were assessed by probit analysis using post-seroconverted plasma from the donor. **Results/Findings:** The viral sequences determined in blood samples from the donor (post-seroconversion donation) and the FFP recipient differed by only 1 among 341 nucleotides in the *env* (99.7% identity) and by 4 of 2,800 nucleotides in the *pol* (99.9% identity). Such high genetic similarities among the sequences supported the notion that HIV was transferred from the donor to the FFP recipient. HIV-1 isolates from the donor and recipient were Subtype B, which is the most common subtype among HIV-1-infected individuals in Japan. Mutations in the LTR that could cause false-negative NAT results were not detected. The HIV-1 viral load in the implicated blood was estimated to be below the detection limit of the two NATs (10 copies/mL). The estimated total load of HIV-1 in the FFP that caused TT-HIV was <2,400 copies. **Conclusion:** This is the first reported case of TT-HIV through a 20-MP-NAT-negative blood component in Japan. The donor was probably in the very early stage of HIV-1 infection at the time of the implicated donation. Our results indicate that individual-donation (ID)-NAT using even the most sensitive methods available today might not detect HIV-1 in window-period donations, and that FFP with a viral load below the limits of ID-NAT sensitivity can be infectious.

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SP386

Seroprevalence and Rates of Asymptomatic Viremia of Severe Fever with Thrombocytopenia Syndrome Virus (SFTSV) in Chinese Blood Donors

P Zeng[1], J Wang[1], X Huang[2], R Cao[3], L Zhu[4], T Lee[5], J Liu[6], M Yuan[7], P M Ness[6], H Shan[6], F the International Component of the NHLBI Recipient Epidemiology and Donor Evaluation Study-III (REDS-III)[8]. [1]*Institute of Blood Transfusion, CAMS, Chengdu, China;* [2]*Xinyang Blood Center, Xinyang, China;* [3]*Mianyang Blood Center, Mianyang, China;* [4]*Luoyang Blood Center, Luoyang, China;* [5]*Molecular Biology, Blood System Research Institute, San Francisco, MD, United States;* [6]*Transfusion Medicine, Johns Hopkins Hospital, Baltimore, MD, United States;* [7]*RTI International, Rockville, MD, United States;* [8]*NIH, Bethesda, MD, United States*

Background/Case Studies: SFTSV, an emergent tick-borne pathogen that can cause fatal severe fever with thrombocytopenia syndrome (SFTS), was

first identified in China in 2009. There is evidence suggesting that SFTSV can be transmitted via contact with blood. We conducted a study on donor samples from three Chinese blood centers in one epidemic region (Xinyang) and two nonepidemic regions (Mianyang and Luoyang) to investigate the seroprevalence and viremic status of SFTSV among Chinese blood donors. **Study Design/Methods:** Plasma samples were collected from May 1 to October 31, 2012, from all donors donating at Xinyang (sample size, n = 14,764) and systematically selected donors from Mianyang (n = 1130) and Luoyang blood centers (n = 1326). All samples were tested for anti-SFTSV total antibody (IgG and IgM) with a commercial EIA assay (Xin-Lian-Xin, Inc. Wuxi) following manufacturer's instructions. Reverse transcription real-time PCR testing on pools of 4 samples from 9960 samples from Xinyang was conducted with an in-house PCR assay based on the Taqman probe method at the one-sample/two-wells level. Samples from RT-PCR-positive pools were then tested again in RT-PCR at a single-sample level. **Results/Findings:** The seropositive rate in donors from the endemic region was higher (0.59%) than in those from the nonendemic regions, 0.27% and 0.28 %, respectively, although these differences were not statistically significant. No significant differences were found in donor demographic characteristics between seropositive and negative donors in the endemic region. We tested 2490 pools of endemic-region samples for SFTSV RNA; 63 pools were reactive. Individual-sample RT-PCR testing of the 63 reactive pools found 2 reactive donor samples. The amplification cycle numbers (Cqs) for the reactive donor samples were 39.7 and 39.3, both with estimated viral load <10 pfu/ml. The RNA prevalence rate for SFTSV among donors in Xinyang was 0.02% (2/9960). Results of RT-PCR single-sample testing of all seropositive samples were negative, which suggests that these donors may have cleared the SFTSV after the initial exposure. **Conclusion:** We conducted the first multi-region SFTSV seroprevalence and virus-prevalence study in Chinese blood donors to assess the potential threat of SFTSV transmission through transfusion. The seroprevalence of SFTSV in donors from three Chinese regions ranged from 0.27% to 0.59%. No significant association between seropositive status and demographic characteristics was identified. The low rate of viremia among donors may suggest a limited impact of SFTSV on blood safety in these regions. However, continuing investigation on SFTSV among larger numbers of donors, especially those from epidemic regions, is needed to further monitor the potential threats of SFTSV to blood safety.

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SP387

Outcome Assessment with Implementation of Second-Generation Hepatitis B Virus (HBV) NAT in Blood Donation

J Dunn Williams[2], G F Robertson[2], M Dixon[2], N Haubert[2], P C Williamson[2], G F Leparc[2,3], S Caglioti[2], R Spizman[2], M D Bravo[4], R C Williams[1]. [1]*National Institutes of Health, Epidemiology and Clinical Research Branch, Phoenix, AZ, United States;* [2]*Creative Testing Solutions, Tempe, AZ, United States;* [3]*OneBlood, St. Petersburg, FL, United States;* [4]*Blood Systems, Inc, Scottsdale, AZ, United States*

Background/Case Studies: Early blood donor screening for hepatitis B virus (HBV) infection was based on NAT for HBV DNA detection and/or serological screening for hepatitis B surface antigen (HBsAg). Confirmation was by neutralization tests, and by antibody to HBV core antigen (anti-HBc) tests due to the lack of NAT assay sensitivity. In October 2012, the FDA released a guidance document recommending that all units of whole blood and blood components used for transfusion be tested by an FDA-licensed HBV NAT with a sensitivity standard of 100 IU/mL. A second-generation HBV NAT test was implemented to meet this standard. **Study Design/Methods:** We evaluated the detection of HBV NAT by comparing the Procleix Ultrio (ULT) first-generation screening test to the Procleix Ultrio Plus second-generation assay (ULTP). Procleix discriminatory HBV (dHBV) assays as well as HBsAg results were used in the analyses. **Results/Findings:** A total of 5,690,572 blood donations were analyzed by NAT assay performance: ULT 2,728,621 (July 2012-April 2013) and ULTP 2,961,951 (May 2013-February 2014). HBV NAT-discriminated donations were 545 (0.02%) ULT and 581 (0.02%) ULTP; Chi-square p = 0.762. NAT-nondiscriminated donations were 84 (0.003%) ULT and 70 (0.002%) ULTP; Chi-square p = 0.101. There was however, statistical significance when evaluating NAT ULT and ULTP with HBsAg, p =< 0.0001 (Table). There were fewer NAT-nonreactive [NR]/HBsAg-reactive [R] donations (NR/R) with ULTP (662, 0.022%) than with ULT (1644, 0.060%),; and a higher number of NAT-R/HBsAg-NR (R-R/NR) donations were identified with ULTP (57, 0.002%) than ULT (32, 0.001%). The immunoassays changed from Ortho Diagnostics EIA testing to Abbott Prism ChLIA in July 2013. **Conclusion:** Second- generation NAT, the Procleix Ultrio Plus assay, showed greater detection of early-infected HBV donations (those reactive and nonreactive for HBsAg) than did Procleix Ultrio. The occurrence of HBV NAT discrimination and nondiscrimination was similar for both assays.

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TABLE. NAT and HbsAg Serology

Test Platform								
NAT-dHBV/EIA or ChLIA	NR-R/R	NR/NR	NR/R	R-NR/NR	R-NR/R	R-R/NR	R-R/R	Total
Ultrio	69	2,725,200	1644	1255	10	32	563	2,728,773
Ultrio Plus	59	2,961,632	662	1304	9	57	574	2,964,297
Total	128	5,686,832	2306	2559	19	89	1137	5,693,070

SP388

Risk of Transfusion-Transmitted Viral Infections: Contribution of Hepatitis B Vaccination of Newborns in an Endemic Country

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Background/Case Studies: With the implementation of strict donor selection and sensitive laboratory screening procedures such as nucleic acid testing (NAT), the risks of transfusion-transmitted viral infections are low at present. In this study, we analyzed the residual risks of HIV-, HCV-, and HBV-infectious donations, highlighting the contribution of a nationwide hepatitis B vaccination program that has been in place since 1985. **Study Design/Methods:** Data for the 2 million donations between February 2013 and March 2014 were collected. Of these donations, 92% were provided by repeat-tested (RT) donors and were analyzed for determination of risk by using the window-period model. The window periods used throughout the analysis were 9.1 d for HIV-1, 7.4 d for HCV, and 30 d for HBV. The RT donors with a confirmed-seropositive result and those with a NAT-positive result were considered as infected and were used for determination of risk, that is, the risk caused by their prior donations. The risk of HBV was determined as that of confirmed infections multiplied by the adjustment factor 2.38 (Korelitz et al, Transfusion 1997), in considering the risk introduced through the prior donations from donors recovered from their infection as well as from donors positive at the time of donation. **Results/Findings:**

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研究報告の概要

○シャーガス病既往歴の自己申告のために採血延期となった供血者：リスクは減少するか？
背景：現行のFDAガイダンスには、全ての供血者に対してシャーガス病の既往歴を尋ねるよう明記されている。
方法：2000年1月から2011年8月の間、米国赤十字社の全ての供血者のうちシャーガス病既往歴の自己申告のために供血を延期した人を特定した。これらの供血者に連絡し、*Trypanosoma cruzi*（*T.cruzi*）抗体検査のための来訪を依頼した。
2007年1月以降については、受け入れた全ての供血者（シャーガス病歴なし）に*T.cruzi*抗体検査を実施している。
結果：12年間で約8,800万人の供血者中、34人がシャーガス病歴の自己申告のために採血を延期した。連絡した際、7人はリスク（流行地への旅行や居住歴、媒介昆虫曝露など）を回答し、うち6人が*T.cruzi*抗体検査を受け、1人が確認のための放射性免疫沈降アッセイ（RIPA）で陽性となった。他の6人は質問に誤って答えていた。残りの21人とは連絡が取れなかったが、供血者記録から、13人はヒスパニック系民族またはスペイン語を話し、シャーガス病リスクや疾患の詳細情報があることを確認した。
結論：12年間で、シャーガス病既往歴に関する質問を用いて感染の可能性が特定されたのは28人のみであった。今回の検査データは、これらの少数にしか感染既往の所見がなかったことを示す。対照的に、約5年間の*T.cruzi*抗体スクリーニングで488人のRIPA陽性供血者を確認し、これらのうちシャーガス病既往歴の質問に「はい」と答えた者は誰もいなかった。この研究における推定によると、シャーガス病既往歴の質問を維持することは10億供血当たり0.4人の感染供血者からの採血を防ぐことと言い換えられる。このように、*T.cruzi*抗体検査の実施下では、シャーガス病既往歴の質問は意味がないと考えられる。

使用上の注意記載状況・その他参考事項等

解凍赤血球液-LR「日赤」
照射解凍赤血球液-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見

12年間でシャーガス病既往歴に関する質問を用いて*Trypanosoma.cruzi*感染の可能性が特定された供血者は28人のみであり、*T.cruzi*抗体のルーチンスクリーニングを実施している米国では、シャーガス病既往歴の質問は意味がないと考えられるとの報告である。

今後の対応

日本赤十字社では、輸血感染症対策としてシャーガス病の既往がある場合には献血不適としている。また、中南米出身者（母親が出身を含む）、通算4週間以上の中南米滞在歴を有する献血者からの血液は、血漿分画製剤の原料のみ使用する対策を実施している。今後も引き続き情報の収集に努める。

Donors deferred for self-reported Chagas disease history: does it reduce risk?

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BACKGROUND: Current Food and Drug Administration guidance specifies that all blood donors must be asked about a history of Chagas disease.

STUDY DESIGN AND METHODS: We identified all American Red Cross donors deferred for Chagas disease history from January 2000 to August 2011. Attempts were made to contact all deferred donors and invite them back for anti-*Trypanosoma cruzi* testing. After January 2007, all accepted donors (no Chagas history) were anti-*T. cruzi* tested.

RESULTS: Over the 12-year period (approx. 88 million donor presentations), 34 donors had a Chagas deferral. When contacted, seven reported risk (e.g., travel or residence in an endemic area, vector exposure) and six were anti-*T. cruzi* tested with one radioimmunoprecipitation assay (RIPA) positive. Six others had answered the question incorrectly. The remaining 21 could not be contacted but from the donor record it could be determined that 13 were Hispanic ethnicity or Spanish speaking and/or provided specific details of Chagas risk or disease.

CONCLUSIONS: In 12 years, only 28 potentially infected donors were identified using the Chagas question. Limited testing data suggest that few of these would have had serologic evidence of prior infection. In contrast, nearly 5 years of anti-*T. cruzi* screening identified 488 RIPA-positive donors, none of whom answered "yes" to the Chagas question. According to estimates in this study, the value of retaining the questionnaire in addition to testing translates to preventing the collection of 0.4 infected donors per billion. Thus, the Chagas history question has no meaningful value.

Trypanosoma cruzi is the etiologic agent of Chagas disease, which can be transfusion transmitted. Vector-borne transmission occurs only in the Americas and the greatest risk of transmission is limited to the rural, less-developed regions of Mexico, Central America, and South America. While there have been some autochthonous transmissions in the United States,[1] other risks in the United States are from human-to-human transmissions by transfusion, organ transplant, or vertically from mother to child, although all are relatively rare.[2,3] Due to the influx of immigrants from Mexico and Central and South America, there are an estimated 300,000 individuals with Chagas disease living in the United States currently.[4] Because Chagas disease can have a long asymptomatic phase, a person may not realize he or she has a chronic infection and present to give blood. Because so many people are unaware of their infection status, to protect the blood supply, blood donors in the United States are now tested at least once by a licensed screening assay as well as being asked about a history of Chagas disease at every donation.[5]

Obtaining an accurate health history to assure donor suitability before blood donation is a key step to ensuring blood safety. This is accomplished in the United States

ABBREVIATIONS: ARC = American Red Cross; BDR = blood donor record; DHQ = donor history questionnaire; RR = repeatedly reactive.

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with the donor history questionnaire (DHQ). An effective question on the health history questionnaire is especially important when there are no available screening tests to detect an agent of concern.[6] Questions are also helpful when possible window-period infections might need to be intercepted or as a layer of safety against inventory control errors.[7] Before the availability of a Food and Drug Administration (FDA)-licensed antibody test, the only intervention to reduce the risk of transfusion transmission of *T. cruzi* was to ask donors about a history of Chagas disease. A deferral for a history of Chagas disease was added to the AABB Standards in 1991 and a question regarding Chagas disease was added as early as 1992 to the AABB DHQ.

In 2010, following the licensure of anti-*T. cruzi* screening tests (first licensed in December 2006), the FDA issued a Guidance for Industry recommending that all presenting donors should continue to be asked about a history of Chagas disease in addition to being tested at least once with a licensed screening test.[5] A "yes" response to the Chagas history question or reactive antibody screen results in indefinite deferral of the donor. All donors to the American Red Cross (ARC) are now tested for anti-*T. cruzi* at least once as well as being asked the Chagas question every time they donate. In view of the routine testing for anti-*T. cruzi*, we sought to evaluate the residual value of the Chagas history question.

MATERIALS AND METHODS

The ARC national data warehouse was queried to identify all donors deferred for a self-reported previous history of Chagas disease from January 2000 to August 2011. All Chagas disease–deferred donors at the ARC are assigned a specific assertion code to identify them as being permanently deferred for risk for Chagas disease. The donors assigned this code were originally identified either by answering "yes" to the specific question on the DHQ or because they had a note in their deferral record saying that the donor had provided additional information resulting in a Chagas disease risk deferral.

The DHQ question currently reads "Have you ever had Chagas' disease?", but before 2005 at the ARC it was part of a compound question that included babesiosis and sometimes leishmania. Therefore, donors who had said yes to the babesia or leishmania part of the question were excluded and documented as such using the notes in the blood donor record (BDR). The additional information that the donor provided that would have resulted in a Chagas disease deferral includes reporting having lived in a household with someone who had Chagas disease, being unsure about their answer to the Chagas question, or being unsure about having had Chagas. Deferrals that occurred at the time of presentation or because of postdonation information were included.

In the fall of 2011, trained donor counselors attempted to contact all Chagas history–deferred donors by phone and by letter to conduct interviews to clarify responses and, if applicable, to bring donors back for anti-*T. cruzi* testing. Multiple attempts were made to reach each donor using the address and phone numbers in the donor record. Donors that were reached were asked about their history of Chagas disease and asked to provide details to further explain why they had said yes to the question. Donors who provided information that substantiated a risk for Chagas disease were asked to provide a follow-up sample for anti-*T. cruzi* testing.

If a donor could not be reached after multiple attempts, his or her BDR was reviewed to determine if he or she were of Hispanic ethnicity or Spanish-speaking and for possible evidence of Chagas disease, diagnosis, or risk factors that may have been provided at the time of deferral. This information was evaluated to determine the likelihood of their being truly infected.

In January 2007, the ARC began testing all accepted donors using an FDA-licensed *T. cruzi* test system (Ortho Clinical Diagnostics, Raritan, NJ) having 99.88% sensitivity (95% confidence interval, 99.35%-100%; package insert). Donors who had a signal to cutoff on the Ortho enzyme-linked immunosorbent assay (ELISA) of 1.00 or greater initially and then following duplicate repeat testing (if reactive in one or both duplicate retests) were considered repeatedly reactive (RR). All RR donations were further tested using a laboratory-developed test, a radioimmunoprecipitation assay (RIPA), available through Quest Diagnostics (Chantilly, VA). Accepted donors were also screened by the current donor health history questionnaire and had answered "no" to the question regarding having had Chagas disease. Test result information on donors who were accepted and tested using the Ortho ELISA between January 2007 and August 2011 are provided as a comparison group. Donors who were both screen and RIPA positive during this time period, but answered "no" to the Chagas question, represent failures of the screening question to detect a true positive. All contacted Chagas-deferred donors that returned were tested for anti-*T. cruzi* using the Ortho ELISA described, and those that screened RR were tested by RIPA for confirmation.

RESULTS

Database searches identified 34 donors deferred for having a self-reported Chagas disease history over the period January 2000 to August 2011 at ARC. During this same time period there were more than 76 million donations to the ARC plus more than 11 million presenting donors who were deferred from donation for any reason.

Of the 34 donors identified, 13 could be reached and were interviewed by the donor counselors. During the

follow-up interviews conducted in the fall of 2011, seven of the 13 contacted donors provided information to the counselor that supported the possibility that the donor had Chagas disease (Table 1). Four of the donors reported living in an endemic area for a year or more. All four of these donors provided information about additional risk factors such as living in a rural area of that endemic country and/or being bitten by the vector insect. Three donors reported having tested positive for Chagas disease in the past, one who lived in Chile (Donor 1), one who had been exposed by a needlestick in 1993 (Donor 5), and one who had tested "positive" at another blood bank (Donor 6). Donor 7 did not provide any additional supporting information that he may have had Chagas disease, but did reconfirm that he may have had the disease in the past. All seven of these donors were invited to return for testing and six returned for testing. Only one, a Hispanic female who lived in Chile in housing with dirt floors and a thatched roof, was both screen and RIPA positive for anti-*T. cruzi*.

Of the 13 donors contacted, it was determined that the remaining six did not have a history of Chagas disease or any other reason why they should have been deferred for Chagas. All of these donors who denied a Chagas disease history in 2011 had provided a history at the time of deferral that had caused them to be deferred for Chagas disease. The comments from the BDR at the time of deferral are reported in Table 1 (Donors 8-13). At the time they had been deferred, four of the six had provided specific dates of their presumed Chagas disease recorded in their BDR, one said she had an illness that possibly could have been Chagas but did not provide an exact date, and the final donor had a vague indefinite deferral classified as Chagas. When questioned by the donor counselors in 2011, three of the donors denied a history of Chagas but said they had previously had Lyme disease (Table 1, Donors 8-10). One of them said she had mentioned having Lyme disease at the time of deferral, and the date recorded in the BDR regarding the occurrence of Chagas disease was the same as the date of the Lyme disease reported in 2011 (Table 1, Donor 9). One donor had said at the time of deferral in 2005 that she had an undiagnosed illness after a trip to Nigeria that was possible Chagas disease, but when questioned in 2011 said she had never had Chagas disease (Table 1, Donor 11). Since *T. cruzi* is not present in Africa, this was clearly not Chagas disease. Donor 12 stated no history of Chagas disease but instead a history of yellow jaundice before age 11, but also denied any history of hepatitis. The final donor's record did not mention Chagas disease, but was deferred with a code of Chagas deferral anyway (Table 1, Donor 13). All of these donors were non-Hispanic, were not primarily Spanish speaking, and did not report living or visiting areas endemic for Chagas. None of these donors was invited for testing based on their follow-up interview. All six donors that were found to have answered the question incorrectly had the permanent deferral code for Chagas removed from their records.

The remaining 21 donors associated with Chagas disease history deferrals could not be reached in 2011. For the purposes of the study, their BDR from the time of the original deferral was reexamined to determine how likely the deferral was actually due to Chagas disease history. Critical elements that were considered included specific details about when and where the diagnosis of Chagas disease had been made, if they self-reported as Hispanic and/or had a preferred contact language of Spanish, if they self-reported as living in or traveling to an endemic country, and if they gave details about a family member having Chagas. Living in the same household as someone with Chagas meets the criteria for an ARC deferral.

Thirteen of the 21 remaining donors had information in their blood donor deferral records that were suggestive of Chagas disease or living with a family member with Chagas disease (Table 2). Five donors who reported having a diagnosis of Chagas disease and gave a date of their diagnosis were also Hispanic or Spanish speaking (Table 2, Donors 3-4 and 9-11). Three donors reported living in or traveling to an endemic country and also having symptoms of or a diagnosis of Chagas disease (Table 2, Donors 12 and 14-15). One donor tested "positive" at another blood center for Chagas disease and had the diagnosis confirmed by a physician (Table 2, Donor 13). Three donors lived in a household where others had Chagas, but did not have it themselves and one of them was Hispanic and Spanish speaking (Table 2, Donors 16-18). Finally, one donor was unsure of his answer but self-reported Hispanic ethnicity (Table 2, Donor 21). The remaining donors did not have enough evidence in their record to be suggestive that their Chagas disease history was likely.

Starting in January 2007, all ARC donors were tested one or more times for anti-*T. cruzi*. During the period of January 2007 to August 2011, approximately 21 million donations were screened of which 2506 (0.012%) were RR on the Ortho anti-*T. cruzi* ELISA and 488 (20% or approx. 1:43,600 donations) were RIPA positive. Since they were accepted donors, all of these donors had denied a Chagas disease history during the predonation screening process.

DISCUSSION

The length and complexity of the current donor health history questionnaire lead to donor complaints about both the time it takes to complete and the ambiguity of some questions.[7] If no apparent, quantifiable value is added by a question, then the question should be removed. While the removal of one question from the donor health history questionnaire may not seem significant, consider that it was asked more than 80 million

TABLE 1. ARC donors deferred for Chagas disease history who were contacted in 2011 and provided additional information about their deferral

Donor number	State where donor was deferred	Age in 2011 (years)	Sex	Race/ Ethnicity	Preferred contact language	Original year of deferral	Comment information at the time of deferral	Information from recent follow-up call with donor	Tested by the ARC in 2011*
Donors successfully contacted in 2011 and provided substantive evidence that they may have had Chagas disease. These donors were invited back for testing.									
1	WA	42	F	Hispanic	English	2003	Donor tested positive in 2003.	Donor lived in Chile in housing that had dirt floors and thatched roof. Donor had been tested in Chile and was told she had Chagas.	Yes, screen and RIPA positive
2	TN	32	F	Mixed race	English	2010	Date of first occurrence or symptoms of Chagas was in 1998.	Donor lived in South America in 1997 through 1998. She reported being bitten on the face by the triatomine bug in July 1998 and developing the typical Chagas swelling at the bite. Donor was told she had Chagas but no blood test was done to confirm.	Yes, nonreactive
3	ID	57	M	Caucasian	English	2008	Date of first occurrence or symptoms of Chagas was in 1993.	Lived in Costa Rica for 1 year and was bitten by the triatomine bug. Donor was tested in Costa Rica and told he was positive, but then retested and told he was negative.	Yes, nonreactive
4	AR	51	F	Caucasian	English	2003	Donor had Chagas in 1981.	Donor lived in rural area of Paraguay from 1986 through 1988 and was bitten by a benchuca bug.	Yes, nonreactive
5	CA	61	F	Not given	English	2006	Chagas disease exposure in 1993 after a needlestick injury caring for squirrel monkeys infected with Chagas (*T. cruzi*).	Donor did have a subsequent blood test that was positive for Chagas. However, no information is available on additional confirmatory testing.	Yes, nonreactive
6	GA	30	M	Caucasian	English	2008 (PDI)	Tested Chagas positive (RIPA positive) at another blood bank in 2008.	Per donor, CDC confirmed that he did not have Chagas.	Yes, nonreactive
7	VA	58	M	Caucasian	English	2006	Date of first occurrence or symptoms of Chagas in 1988.	Donor said he may have had Chagas but was not interested in being tested.	No
Donors that were successfully contacted in 2011 but found to have been erroneously deferred for Chagas. These donors were not invited for testing.									
8	GA	55	F	Caucasian	English	2007	Date of first occurrence or symptoms of Chagas was June 1, 1997	Donor denied history of Chagas and babesiosis. Donor had Lyme disease in 2001, treated successfully with antibiotics. Donor denied having babesiosis.	No
9	GA	65	F	Caucasian	English	2007	Date of first occurrence or symptoms of Chagas was 2006	Donor denied history of Chagas disease. Donor said she had mentioned to the health historian at the time of deferral that she had Lyme disease in 2006 and was treated and fully recovered.	No
10	IL	53	M	Caucasian	English	2011	Donor thinks that he may have had Chagas disease in March 2010.	Donor denied history of Chagas. Did say he had a history of Lyme disease.	No
11	MO	60	F	Caucasian	English	2005	Donor had undiagnosed illness after trip to Nigeria, possible Chagas.	Donor denied ever having Chagas or babesiosis. Reported having an illness after a trip to Nigeria.	No
12	PA	26	M	Asian	English	2007	Date of first occurrence or symptoms of Chagas was 1986.	Donor denied history of Chagas. Did have history of yellow jaundice before age 11; denied history of hepatitis.	No
13	MO	72	M	Caucasian	English	2010	Autologous BDR "indefinite deferral for homologous donation."	Donor denied history of Chagas disease.	No

* Includes test results for those donors invited back for testing.
PDI = postdonation information; CDC = Centers for Diseas[illegible]ntrol and Prevention.

TABLE 2. ARC donors deferred for Chagas disease history who could not be contacted and the BDR information used to determine the likelihood of their being infected*

Donor number	State where donor was deferred	Age in 2011 (years)	Sex	Race/Ethnicity	Preferred contact language	Original year of deferral	Comment information at the time of deferral
Donors who gave specific details at the time of deferral regarding when they reportedly had Chagas or symptoms of Chagas or when they were told they may have had Chagas.							
1	CA	26	F	Not given	English	2004	Donor reports having had Chagas or symptoms of Chagas in 2001.
2	OH	41	M	Caucasian	English	2009	Donor reports having had Chagas or symptoms of Chagas in 1994.
3*	NJ	49	M	Not given	Spanish	2007	Donor reports having had Chagas or symptoms of Chagas in 2003.
4*	NJ	38	F	Not given	Spanish	2002	Donor reports having had Chagas or symptoms of Chagas in 1996.
5	NJ	28	M	Not given	English	2003	Donor reports having had Chagas or symptoms of Chagas in 2002.
6	MD	34	M	Not given	English	2011	Donor reports having had Chagas or symptoms of Chagas in 1995.
7	MD	27	F	Asian	English	2004	Donor reports having had Chagas or symptoms of Chagas in 1985.
8	VA	40	M	Not given	English	2008	Donor reports having had Chagas or symptoms of Chagas in 1992.
9*	TX	33	F	Hispanic	Spanish	2001	Donor reports having had Chagas disease at age 11.
10*	TX	41	M	Hispanic	English	2004	Donor reports having had Chagas disease.
11*	CA	52	M	Hispanic	Spanish	2002	In 1998, the donor was told by his doctor in El Salvador that he had Chagas.
12*	MO	63	M	Other	English	2003	In 1999, the donor was told that he may have Chagas disease. The donor was in Peru and Columbia in the late 1970s.
13*	OR	46	F	Not given	English	2008 (PDI)	Reports testing positive for Chagas at another blood center in 2008; says confirmed by her physician.
14*	CT	66	F	Not given	English	2003	Had a parasitic disease in 1997 following trip to Belize.
15*	NC	32	M	Caucasian	English	2000	In 1998, donor traveled to Costa Rica for 3 months, while there showed symptoms of Chagas.
Donors reporting living in a household with someone who has Chagas. Living in the same household as someone with Chagas meets the criteria for an ARC deferral.							
16*	VA	34	F	Caucasian	Unknown	2003 (PDI)	Donor reports that her mother had Chagas disease many years ago, before donor was born.
17*	CA	39	M	Hispanic	Spanish	2002	Father has Chagas disease; donor lived in same household as father.
18*	UT	40	M	Caucasian	English	2001	Donor tested negative for Chagas disease, but has lived in the same household with people who may have Chagas.
Donors reporting testing false positive at another blood bank.							
19	CA	66	M	Caucasian	English	2009	Donor had false positive test result for Chagas at another blood bank
Donors that were unsure of their answer to the Chagas question at the time of their deferral and had to be deferred.							
20	NY	28	F	African American	English	2001	Unsure if diagnosed with Chagas.
21*	CA	36	M	Hispanic	English	2002	Donor unsure of answer to question "Have you ever had Chagas disease or babesiosis?"

* Donors considered to have some risk for Chagas are identified.
PDI = postdonation information.

times during the 12 years reviewed by this publication. It is unknown how often the health historian was asked about what Chagas disease was and the length of time each of these inquiries added to the donation experience.

In 12 years in this study, there were only 34 Chagas disease deferrals from over 88 million donation presentations. Since six of the contacted donors were determined to have answered the question incorrectly, that means that there were only a maximum of 28 potentially *T. cruzi*–infected donors that were detected using the Chagas disease history question (seven contacted with risk determined plus 21 not contacted). However, given that five of the six (83%) tested donors were negative, it is unlikely that the remaining 21 not contacted deferred donors were each positive for anti-*T. cruzi*. It is also unlikely that all 21 were positive given that only 13 of the 21 had any evidence that they were likely to have Chagas disease according to their BDRs.

In the absence of testing, and if all 28 donors deferred based on the Chagas disease question were considered at risk, the rate of detection of infected donors by the questionnaire would be 28 per 76 million donations or 0.368 per million. In contrast, anti-*T. cruzi* screening at the ARC detected 488 RIPA-positive donors from approximately 21 million donations screened for a rate of 23.24 per million, or 63-fold more effective than the Chagas question. Stated differently, we would expect the detection of one at-risk donor by questioning versus 63 detected by testing. The current Ortho screening test has a claimed sensitivity of 99.88%; this then implies that ARC screening should have detected an additional 0.59 anti-*T. cruzi*–positive donors (i.e., 0.12% above the 488 already detected per approx. 21 million donations screened, [0.0012×488] per 21 million), or an undetected rate of 0.028 per million. However, applying the calculated detection rate of the questionnaire versus testing, as we estimated above (at 1/63), only one per 63 of the 0.59 infected donors who were potentially undetected by testing would be detected by the continued use of the questionnaire. Thus, the estimated maximum value of retaining the questionnaire is in preventing the collection of approximately 0.4 infected donors per billion (i.e., 0.028/63 per million, or 0.00044 per million).

While there were potentially 28 infected donors detected by questioning during the 12-year period of observation, there were 488 RIPA-positive donors definitively identified during almost 5 years of anti-*T. cruzi* screening at the ARC. It is important to note that none of these anti-*T. cruzi*–positive donors answered "yes" to the Chagas disease history question, meaning either they were unaware of their Chagas disease status or they chose not to disclose it. Given that many people with chronic Chagas disease are unaware of it, the former is more likely than the latter. We found that testing donors (5-year experience) was far more sensitive than donor questioning (12-year experience) and, thus, in the presence of testing, we consider that the Chagas disease history question has no meaningful value.

CONFLICT OF INTEREST

The authors report no conflicts of interest or funding sources.

REFERENCES

1. Cantey PT, Stramer SL, Townsend RL, et al. The United States *Trypanosoma cruzi* Infection Study: evidence for vector-borne transmission of the parasite that causes Chagas disease among United States blood donors. Transfusion 2012;52:1922-30.
2. Bern C, Kjos S, Yabsley MJ, et al. *Trypanosoma cruzi* and Chagas' disease in the United States. Clin Microbiol Rev 2011;24:655-81.
3. Rassi A Jr, Rassi A, Marin-Neto JA. Chagas disease. Lancet 2010;375:1388-402.
4. Bern C, Montgomery SP. An estimate of the burden of Chagas disease in the United States. Clin Infect Dis 2009; 49:e52-4.
5. Food and Drug Administration. Guidance for industry: use of serological tests to reduce the risk of transmission of Trypanosoma cruzi infection in whole blood and blood components intended for transfusion. 2010 [cited 2013 Oct 22]. Available from: http://www.fda.gov/BiologicsBloodVaccines/GuidanceCompliance RegulatoryInformation/Guidances/Blood/ucm235855.htm
6. Germain M, Goldman M. Blood donor selection and screening: strategies to reduce recipient risk. Am J Ther 2002;9:406-10.
7. Fridey JL, Townsend MJ, Kessler DA, et al. A question of clarity: redesigning the American Association of Blood Banks blood donor history questionnaire—a chronology and model for donor screening. Transfus Med Rev 2007;21:181-204.

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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

○米国の*Babesia microti*（*B.microti*）非流行地域と流行地域の供血者保管検体に対する臨床研究中の*B.microti*スクリーニング

背景：赤血球内寄生原虫であるB.*microti*は米国で輸血伝播の頻度が増加しているが、FDAに承認された供血者スクリーニングアッセイはない。本研究で、供血者における*B.microti*抗体及びDNAを検出するために臨床研究中のarrayed fluorescence immunoassay (AFIA)及びPCRを用いて調査を行った。

方法：AFIAとリアルタイムPCRによる検査は、2010年及び2011年の5～9月に、米国の非流行地域（アリゾナ州、オクラホマ州）、中程度の流行地域（ミネソタ州、ウィスコンシン州）、高流行地域（コネチカット州、マサチューセッツ州）において採取した凍結ペア血漿(AFIA)と全血(PCR)に対して行われた。スクリーニングプロトコールの特異度を計算するために非流行地域からのデータが用いられた。AFIAまたはPCRに陽性または判定保留となった全ての供血者は供血延期とし、再検査と人口統計学及び危険因子に関するアンケートを含む追加研究への協力を求められた。受血者追跡は当該及び以降の供血、または12カ月前までに輸血された血液に対して行われた。

結果：非流行地域からの4,022例、中流高地域Iからの4,167例、高流行地域からの5,080例を含む13,269例のペアサンプルが検査され、*B.microti*抗体及び/またはDNA陽性率はそれぞれ0.025%（95%CI、0.00－0.14%）、0.12%（95%CI、0.04－0.28%）、0.75%（95%CI、0.53－1.03%）であった。64倍希釈をAFIAカットオフにした場合、特異度は99.95%（95%CI、99.82－99.99%）、128倍以上では99.8%（95%CI、99.86－100.00%）であった。

結論：*B.microti*抗体陽性率は予想通り地理的なパターンに従った。スクリーニングは他の感染症スクリーニングアッセイに相当または勝る性能を有する。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」
赤血球液-LR「日赤」
照射赤血球液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
臨床研究中の*Babesia microti*スクリーニング（AFIA及びPCR）を用いて、米国の非～高流行地域における供血者保管検体の検査を行ったところ、*B.microti*抗体陽性率は予想された地理的なパターンに従い、本スクリーニングは他の感染症供血者スクリーニングアッセイに相当するか勝る性能を有することが分かったとの報告である。	日本赤十字社では問診時にバベシア症の既往歴を確認し、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。

⑦

Investigational screening for *Babesia microti* in a large repository of blood donor samples from nonendemic and endemic areas of the United States

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BACKGROUND: *Babesia microti*, a transfusion-transmissible intraerythrocytic parasite, is increasing in frequency in the United States with no available FDA-licensed donor screening assay. We utilized investigational arrayed fluorescence immunoassay (AFIA) and polymerase chain reaction (PCR) to detect *B. microti* antibodies and DNA in blood donors.

STUDY DESIGN AND METHODS: AFIA and real-time PCR were performed on frozen paired EDTA plasma (AFIA) and EDTA whole blood (PCR) samples collected from May to September 2010 to 2011 in nonendemic (Arizona [AZ] and Oklahoma [OK]), moderately endemic (Minnesota [MN] and Wisconsin [WI]), and highly endemic (Connecticut [CT] and Massachusetts [MA]) areas of the United States. AFIA utilized *B. microti* piroplasm as an antigen substrate; PCR primers and probes targeted the *B. microti* 18S ribosomal RNA gene. Data from AZ and OK were used to calculate specificity. All AFIA- or PCR-positive or -inconclusive donors were deferred, notified, and invited to participate in a follow-up study involving repeat testing and a demographic and risk-factor questionnaire. Recipient tracing was performed for any cellular component transfused at index, at subsequent donation, or within the prior 12 months.

RESULTS: Testing of 13,269 paired samples included 4022 from AZ and OK, 4167 from MN and WI, and 5080 from CT and MA. *B. microti* antibody and/or DNA prevalences were 0.025% (95% confidence interval [CI], 0.00%-0.14%), 0.12% (95% CI, 0.04%-0.28%), and 0.75% (95% CI, 0.53%-1.03%) in the nonendemic, mid-endemic, and high-endemic regions, respectively. Specificities were 99.95% (95% CI, 99.82%-99.99%) at a 1-in-64 AFIA cutoff and 99.98% (95% CI, 99.86%-100.00%) at a 1-in-128 cutoff.

CONCLUSIONS: *B. microti* prevalence followed expected geographical patterns. Screening was feasible with a performance comparable or superior to other infectious disease blood donor screening assays.

Babesia microti is an intraerythrocytic parasite that can cause the disease babesiosis.[1] The severity of *Babesia* infection ranges from asymptomatic, most commonly in healthy adults, to fatal, which most often occurs in the elderly; the very young; individuals with compromised immune systems, sickle cell diseases, or other hemoglobinopathies; and those lacking a spleen.[2-5] In the United States, vector-borne *B. microti* is primarily transmitted to humans through the bite of *Ixodes scapularis* (also called the blacklegged or deer tick).[6] Blood-borne transmission from infected blood donors to recipients, via contaminated red blood cell (RBC) units or platelets (PLTs) contaminated with RBCs resulting in transfusion-transmitted babesiosis (TTB), is of increasing concern.[7-12] Between 1979 and 2009, a total of 162 cases of TTB were reported in the United States, 159 of which were caused by *B. microti*. These numbers likely underestimate the true incidence of TTB, as infections may be overlooked or misdiagnosed and transfusion investigations may not be considered.[13] There are no Food and Drug Administration

ABBREVIATIONS: AFIA = arrayed fluorescence immunoassay; ARC = American Red Cross; Ct = cycle threshold; ePCR = enhanced polymerase chain reaction; IFA = indirect immunofluorescence antibody assay; NSF = nonspecific fluorescence; TTB = transfusion-transmitted babesiosis; WB(s) = Western blot(s).

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(FDA)-licensed blood donation screening tests; the only intervention is a question asking donors at presentation if they have ever had babesiosis followed by their indefinite deferral for those responding "yes." This method is ineffective.[5,11,12]

National surveillance for babesiosis began in January 2011 within 18 states and one metropolitan area using a standard case definition developed by the Centers for Disease Control and Prevention for the Council of State and Territorial Epidemiologists.[14] During the first year, 1124 confirmed and probable babesiosis cases were reported, of which 1092 (97%) occurred in seven *B. microti*-endemic states: Connecticut (CT), Massachusetts (MA), Minnesota (MN), New Jersey, New York (including New York City), Rhode Island, and Wisconsin (WI).[13,14] Ten known TTB cases in blood recipients were among those reported within this first year of babesiosis national surveillance.[14] More recently, the total number of babesiosis cases reported in 2011 was adjusted to 1128 cases with an additional 937 cases reported in 2012 (http://wonder.cdc.gov)[15] and another 1446 cases reported during 2013 (to December 28 2013; http://www.cdc.gov/mmwr/pdf/wk/mm6252md.pdf).

Traditionally, the diagnosis of babesiosis has been based on clinical presentation coupled with visual examination of blood smears and detection of antibodies using indirect immunofluorescence antibody assays (IFA)[16,17] and more recently by molecular techniques such as real-time polymerase chain reaction (PCR).[5] Some cases have only been recognized after observation of the parasites on routine blood smears examined for other purposes.[18,19] Blood donation screening has been ranked as a priority, particularly in endemic areas of the United States, because: 1) *B. microti* can be transmitted via blood transfusion, 2) infection results in clinically significant and often fatal disease (approx. 20% cases in recipients are fatal), and 3) most infected blood donors will be asymptomatic when they donate.[5,8,11,12] Preliminary results of investigational and research testing[20-23] have been reported in small studies from localized areas of the United States, but larger studies across multiple geographies using standardized methods are lacking. The aims of this study were to: 1) estimate *B. microti* DNA and antibody prevalence in blood donors in nonendemic and endemic areas of the United States using investigational tests, 2) characterize the duration of marker positivity, 3) determine the specificity of testing using the combination of investigational antibody and DNA tests, and 4) determine the feasibility of blood donation screening for *B. microti*.

MATERIALS AND METHODS

Sample collection

Linked, surplus, paired EDTA-plasma retention samples collected in plasma preparation tubes (Becton Dickinson, Franklin Lakes, NJ), and EDTA-anticoagulated whole blood samples with 3 mL or more per tube were retained frozen (–80°C) in a repository. Repository samples were selected at random for testing from donations collected from May through September 2010 and again in 2011 at American Red Cross (ARC) blood drives and collection sites in Arizona (AZ), Oklahoma (OK), MN, WI, MA, and two highly endemic counties in CT. Results for samples collected from "nonendemic" regions of the United States (AZ and OK) were used to calculate the specificity of the screening protocol. MN and WI were considered "moderately endemic" areas and CT and MA were considered "highly endemic" areas. Frozen samples were shipped to IMUGEN, Inc. (Norwood, MA), where they remained frozen (–20°C) until tested.

Screening protocol

Linked studies were performed using investigational tests developed, validated, manufactured, and with all testing performed by IMUGEN, the sponsor of the investigational new drug studies. The screening protocol consisted of two assays: an arrayed fluorescence immunoassay (AFIA) and PCR, requiring a minimum of 3.0 mL of EDTA whole blood or 2.5 mL of whole blood and 0.5 mL of plasma or serum, to complete the testing algorithm (including repeat and supplemental testing).

The AFIA is a second-generation IFA designed to facilitate high-throughput screening.[23] The format and antigen source were selected in response to the performance limitations of available recombinant and peptide antigens used in enzyme-linked immunosorbent assay (ELISAs) where the number of unconfirmed reactive samples by ELISA was three- to 21-fold higher than the IFA comparator. The range reflects different tested populations (high vs. low endemicity) and either recombinants or peptides as the antigen source.[24-26] The AFIA detects immunoglobulin (Ig)G H+L-specific antibodies to *B. microti* cytoplasmic antigens in plasma or serum samples. Briefly, samples are diluted, added to wells containing calibrated numbers of fixed intact *B. microti*-infected RBCs, and incubated (37°C for 30 min). Unbound sample is removed by washing, a preparation of antibody to human IgG conjugated to a fluorophore and a nonbabesia and non–immunoglobulin-specific flourophore of distinct spectral emissions are added, incubated (37°C for 30 min), and unbound conjugate is removed by washing. Test results from immunoglobulin and non–immunoglobulin-specific emissions are evaluated utilizing an Epi-fluorescence microscope incorporating optical, electrooptical, and electromechanical technologies and components (Nikon Instruments, Inc., Melville NY; Andor Technologies PIC, Belfast, UK; Prior Scientific, Cambridge, UK; Chroma Corporation, McHenry, IL; Microvideo Instruments, Inc., Avon, MA; Lumencor, Inc.,

TABLE 1. Testing algorithm for AFIA for screening blood donors for IgG antibodies to *B. microti*

B. microti AFIA results and interpretations				
Initial result	Retest result (×2)	Retest result	Endpoint dilution*	Final interpretation
Negative	NP	NP	NP	Negative
Positive	Negative	Negative	NP	Negative
Positive	**Positive**	**Positive**	**≥1:64**	**Positive**
Positive	**Positive**	Negative	**≥1:64**	**Positive**
Positive	**Positive**	NSF	**≥1:64**	**Positive**
Positive	NSF	Negative	NP	Inconclusive
Positive	NSF	NSF	NP	Inconclusive
NSF	Negative	Negative	NP	Negative
NSF	**Positive**	**Positive**	**≥1:64**	**Positive**
NSF	**Positive**	Negative	NP	Inconclusive
NSF	NSF	Negative	NP	Inconclusive
NSF	NSF	NSF	NP	Inconclusive

* Endpoint dilution range is 1: 64 to 1: 1024.
NP = not performed.

Beaverton, OR; and Lenovo, Morrisville, NC). Negative and positive controls on each plate must react as expected for the results to be considered valid.[21] *B. microti* antibody titers of 64 or greater were considered initially reactive and retested in duplicate, Table 1 presents the AFIA testing algorithm and result interpretations, and the Supporting Information (available in the online version of this paper) provides data for AFIA correlations with PCR and blood smears. Specificity was calculated at cutoff dilutions of 1 in 64 and 1 in 128, the latter used for ongoing prospective investigational new drug screening.[23,27] The 1-in-64 cutoff was used in this study to assess the appropriate cutoff for future prospective investigational screening studies.

The qualitative real-time PCR (95% limit of detection, 66 piroplasms/mL) is a Taqman-based multiplex assay employing primers and probes that target the *B. microti* 18S ribosomal RNA and a conserved human genome-specific 18S locus which serves as an endogenous internal control (Young et al. with modifications).[23] DNA was extracted and concentrated from EDTA-whole blood using automated membrane-based isolation and purification technology (Taigen Bioscience, Taipei City, Taiwan; IMUGEN, Inc.). Four controls for each PCR plate were included (a template-negative, high-template, low-template, and reagent control).[23] Positive results were defined as a threshold value obtained in 44 or fewer thermodynamic cycles (cycle threshold [Ct]) utilizing thermocyclers (ABI 7500, Applied Biosystems, Life Technologies, Grand Island, NY). Internal data (IMUGEN) demonstrated that the PCR assay has an amplification efficiency of 101%. Figures S1-S3 (available as supporting information in the online version of this paper) provide data in support of the PCR lower limit of detection, assay linearity, and correlations with blood smears and AFIA. The PCR testing algorithm, validity criteria, and assay interpretations are presented in Table 2.

Samples screening positive or inconclusive by AFIA or PCR were considered the index donation for the donor in terms of donor follow-up (see below); however, if multiple samples from the same donor were available from the repository and tested as part of this study, then the earliest donation was considered the index.

Supplemental testing

Additional testing at IMUGEN was performed on positive and inconclusive samples to assess the validity of the screening result. Enhanced sensitivity digital PCR (ePCR; 95% limit of detection, 10 piroplasms/mL), employing a larger volume of sample and a higher number of assay replicates than the standard screening protocol, was performed on all samples that were AFIA positive or inconclusive and PCR negative. In-house Western blot (WB) assays were used to detect *B. microti* IgM and IgG for all samples that were positive or inconclusive by AFIA. Samples were tested using standard WB methods with enzyme conjugates. Bands were read against molecular weight standards; each run included positive and negative controls required to react as expected for a run to be considered valid. Table 3 provides the use of each supplemental assay as determined by the AFIA and PCR screening results; the supporting information gives additional details about the ePCR and WB assays.

For the specificity calculation in nonendemic areas, initially reactive samples that were unconfirmed by IgM and IgG WBs and ePCR were considered false positives. To estimate parasite load, samples that screened PCR positive were submitted for quantitative PCR (supporting information).

Follow-up testing

All donor and product actions taken as part of this linked study were consistent with those used for other infectious disease screening protocols. Donors of positive or inconclusive samples were indefinitely deferred from donating blood and invited to participate in follow-up studies. Consenting donors participating in follow-up returned to

TABLE 2. Testing algorithm for PCR for screening blood donors for *B. microti* DNA

B. microti PCR results and interpretations

Initial result		Reextraction result*		Reextraction retest result (×3)		
B. microti	IC†	*B. microti*	IC	*B. microti*	IC	Final interpretation
Negative	Valid	NP	NP	NP	NP	Negative
Negative	Invalid	Negative	Valid	NP	NP	Negative
Negative	Invalid	Negative	Invalid	NP	NP	Specimen unsuitable
Negative	Invalid	Positive	NA	Negative (×3)	Valid	Inconclusive
Negative	Invalid	**Positive**	NA	Negative (×3)	Invalid	Inconclusive
Negative	Invalid	**Positive**	NA	**Positive** (≥1 replicate)	NA	**Positive**
Positive	NA	NP	NP	**Positive** (≥1 replicate)	NA	Positive
Positive	NA	NP	NP	Negative (×3)	Valid	Inconclusive
Positive	NA	NP	NP	Negative (×3)	Invalid	Inconclusive

* Reextraction results: *B. microti*–negative samples with an invalid internal control result are reextracted and retested.
† IC = internal control. The PCR is a multiplex assay that in addition to *B. microti*–specific primers and probes include an internal control consisting of primers and probes that amplify and detect a conserved region of the human genome (the human 18S ribosomal DNA analog of the 16S ribosomal DNA of *B. microti*). This control enables the evaluation of all variables associated with specimen addition; DNA extraction; and the PCR reagents, conditions, and instrumentation. If the Ct value of the control does not fall within a predefined range, then the specimen results are considered "invalid." When *B. microti* is present, amplified, and detected, the Ct value of the IC is irrelevant.
NA = not applicable; NP = not performed.

TABLE 3. Supplemental testing algorithm as determined by the AFIA and PCR screening results

Screening result		Supplemental testing	
AFIA	PCR	IgM and IgG WB	ePCR*
Negative	Negative	NP	NP
Positive	Negative	Performed	Performed
Negative	**Positive**	Performed	NP
Positive	**Positive**	Performed	NP
Positive	Inconclusive	Performed	Performed
Negative	Inconclusive	Performed	Performed
Inconclusive	Negative	Performed	Performed
Inconclusive	**Positive**	Performed	NP
Inconclusive	Inconclusive	Performed	Performed

* Described in the text and supporting information.
NP = not performed.

an ARC fixed site to provide two EDTA and two whole blood tubes without anticoagulant for testing. Samples were held at 2 to 8°C following collection (EDTA-whole blood and serum separated from whole blood) and sent to IMUGEN for testing within 5 days.

Component tracing and retrieval were performed on cellular components from the index donation, distributed cellular components from that donor up to 1 year prior to the positive or inconclusive donation, and on any unit collected after the positive or inconclusive donation. Health care facilities were notified that their patient may have received a suspect product; recipients were eligible to participate in follow-up. All follow-up testing was performed at IMUGEN using protocols analogous to those for donor screening.

Human subjects approval

The study protocol and all study materials were approved by the ARC and New England Institutional Review Boards.

Mapping and statistical analysis

The reported residential zip codes of tested and positive or inconclusive donors were plotted using mapping software (ArcGIS Desktop 10.0, Environmental Systems Research Institute, Inc., Redlands, CA). The prevalence of *B. microti* antibody and/or DNA in nonendemic, moderately endemic, and highly endemic regions were compared using the chi-square or Fisher's exact test. Exact binomial confidence intervals (CI) were determined for the specificity calculations. CI calculations and prevalence comparisons were made using statistical software (SAS 9.2, SAS Institute, Cary, NC).

RESULTS

A total of 13,269 paired samples from donations collected from May through September 2010 and 2011 was tested (Fig. 1). In AZ and OK, testing was conducted on 4022 paired samples; 4167 and 5080 paired samples were tested in MN and WI and MA and CT, respectively. Forty-nine donors were positive or inconclusive for *B. microti* antibodies, DNA, or both (Table 4). After false-positive samples (n = 5, AFIA titers 64-128) or those with nonspecific fluorescence (NSF) negative by supplemental tests were removed, the prevalence of *B. microti* antibody, DNA, or both in the six regions combined based on 44 confirmed-positive donors was 0.33% (95% CI, 0.24%-0.45%). The percentages of positive donations in nonendemic, moderately endemic, and highly endemic areas were 0.025%, 0.12% (including two PCR-positive donors with AFIA titers of 256 to ≥1024), and 0.75% (including five PCR-positive donors with AFIA titers of 256 to ≥1024), respectively (Table 4, Figs. 2 and 3). The *B. microti* prevalence in highly endemic areas was significantly higher than that in moderately and nonendemic areas ($p < 0.0001$ for both comparisons). The prevalence

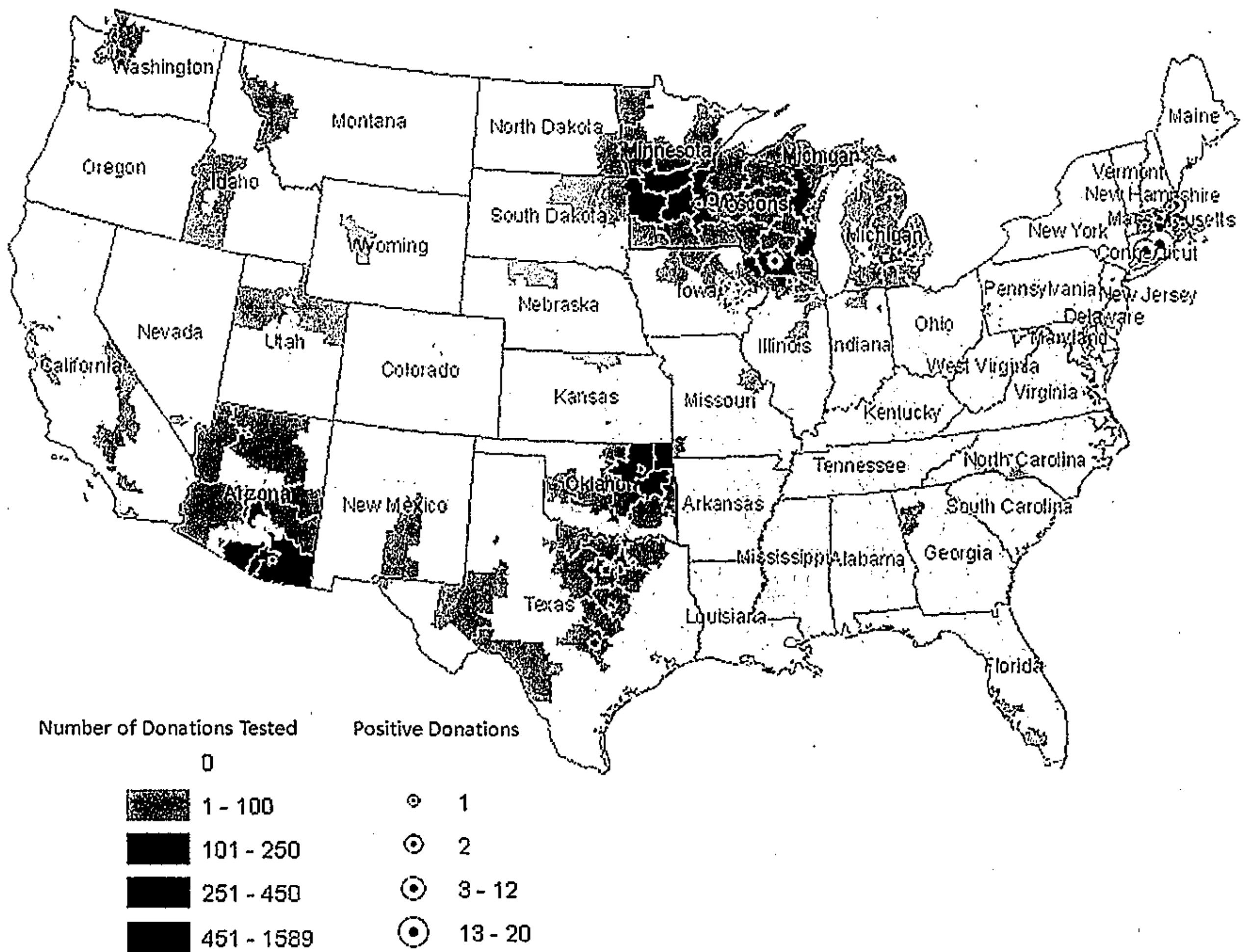


Fig. 1. US map depicting reported residential zip codes of donors collected from six regions who were tested with an investigational screening protocol to detect evidence of antibodies to and DNA from *B. microti* (blue). Yellow circled dots indicate the residential zip codes of donors who were positive by AFIA with or without PCR positivity.

in moderately endemic areas was higher than the prevalence in nonendemic areas; however, this difference was not significant (p = 0.22).

Antibody-positive donors with elevated titers (≥256) with or without PCR positivity were considered more likely to have an active or recent infection than PCR-negative donors with low-titer antibodies. No such donor occurred in the nonendemic states. All seven PCR-positive donors had an AFIA titer of 256 or greater. Parasite loads for the seven PCR-positive and AFIA-positive donors ranged from 40 to 13,000 piroplasms/mL. Fourteen (38%) donors of the remaining 37 confirmed-positive donors had AFIA titers of 256 or higher, 12 had a 64 titer, and 11 had a 128 titer. No donor was PCR positive, antibody negative. All antibody-positive, PCR-negative donors were confirmed by additional tests at index; none was ePCR positive.

Seventy-four follow-up samples were collected from 27 of 49 (55%) positive or inconclusive donors. Seven of these individuals provided one follow-up sample, while 20 donors provided two to six follow-up samples. The mean time between index and first follow-up was 552 days (range, 223-863 days), while the mean time between the index and last follow-up collection was 676 days (range, 223-996 days). At last follow-up, 20 of 27 (74.1%; 95% CI, 53.7%-88.9%) donors retained antibodies to *B. microti* at a 1-in-64 cutoff; five had titers of 256 or greater. Of the seven PCR-positive donors, five were followed; none remained PCR positive at first follow-up but all remained antibody positive. Risk information, from a questionnaire administered by trained counselors, was obtained for each of these PCR-positive donors; the most frequently reported risks were time spent outdoors in the past 2 years in wooded, grassy, or brush-covered areas; e.g., hiking, camping, jogging, hunting, fishing, outdoor sports, or clearing land (n = 5, 100%) and/or finding a tick on their body in the past 2 years (n = 3, 60%). For the remaining 22 antibody-positive donors, who were considered confirmed positive and participated in follow-up, risk

TABLE 4. Numbers tested and results for donors screened through repository testing of blood donation samples collected in nonendemic (AZ, OK), moderately endemic (MN, WI), and endemic (CT, MA) areas of the United States

AFIA result	PCR result: Reactive	Negative	Inconclusive	Total	Numbers for prevalence calculations	Prevalence (95% CI), %
AZ and OK						
Reactive	0	3*	0	3	1	0.025 (0.00-0.14)
Negative	0	4,019	0	4,019	4,021	
Inconclusive	0	0	0	0		
MN and WI						
Reactive	2	3	0	5	5	0.12 (0.04-0.28)
Negative	0	4,160	1†	4,161	4,162	
Inconclusive	0	1‡	0	1		
CT and MA						
Reactive	5	33	0	38	38	0.75 (0.53-1.03)
Negative	0	5,041	0	5,041	5,042	
Inconclusive	0	1§	0	1		
Total	7	13,261	1	13,269	13,269	

* Two samples (one AFIA positive at 1 in 64 and one positive at 1 in 128 on initial screening) were negative for *B. microti* IgM and IgG antibody in additional testing using WBs and considered to be false positive. One sample (AFIA titer of 64 on initial screening) was positive for IgG antibody on WB; the donor also reported several risk factors on his questionnaire. This sample was considered a true remote infection. All three samples were IgM WB negative and ePCR negative.

† Sample was negative for IgM and IgG in additional testing using WBs and ePCR; donor follow-up sample provided at 358 days postindex was PCR and AFIA negative; index donation considered a false positive.

‡ Sample was negative for IgM and IgG in additional testing using WBs and ePCR; donor follow-up sample provided at 320 days postindex was PCR negative and AFIA NSF; donor follow-up sample provided at 412 days postindex was PCR and AFIA negative; index donation considered to be false positive.

§ Sample was negative for IgM and IgG in additional testing using WBs and ePCR; donor follow-up samples provided at 317, 422, 520, and 598 days postindex were PCR negative and AFIA NSF; index donation considered to be false positive.

information was obtained for 16; the most frequently reported risks were the same as in PCR-positive donors, with 16 (100%) reporting time spent outdoors and 11 (69%) reporting finding a tick on their body.

As an example of the long duration of *B. microti* antibody, one AFIA-positive donor was identified with an August 13, 2012, collection date during a subsequent study involving prospective *B. microti* donor screening in CT and MA (Table 5).[27] This 55-year-old male, repeat donor (10 donations September 2009-August 2012) had samples coincidently tested from the repository since the two studies were running concurrently. The first positive result was a high-titer, IgG- and IgM-antibody and PCR-positive repository sample collected on August 30, 2010 (AFIA titer ≥1024; estimated parasite load, 3000 piroplasms/mL); the donor's antibody reactivity then persisted from June 7, 2011, through August 5, 2013 (128-256 AFIA titer; IgG WB positive). One can infer that infection was present in the donor before August 30, 2010, with subsequent duration of detectable antibody for at least 3 years.

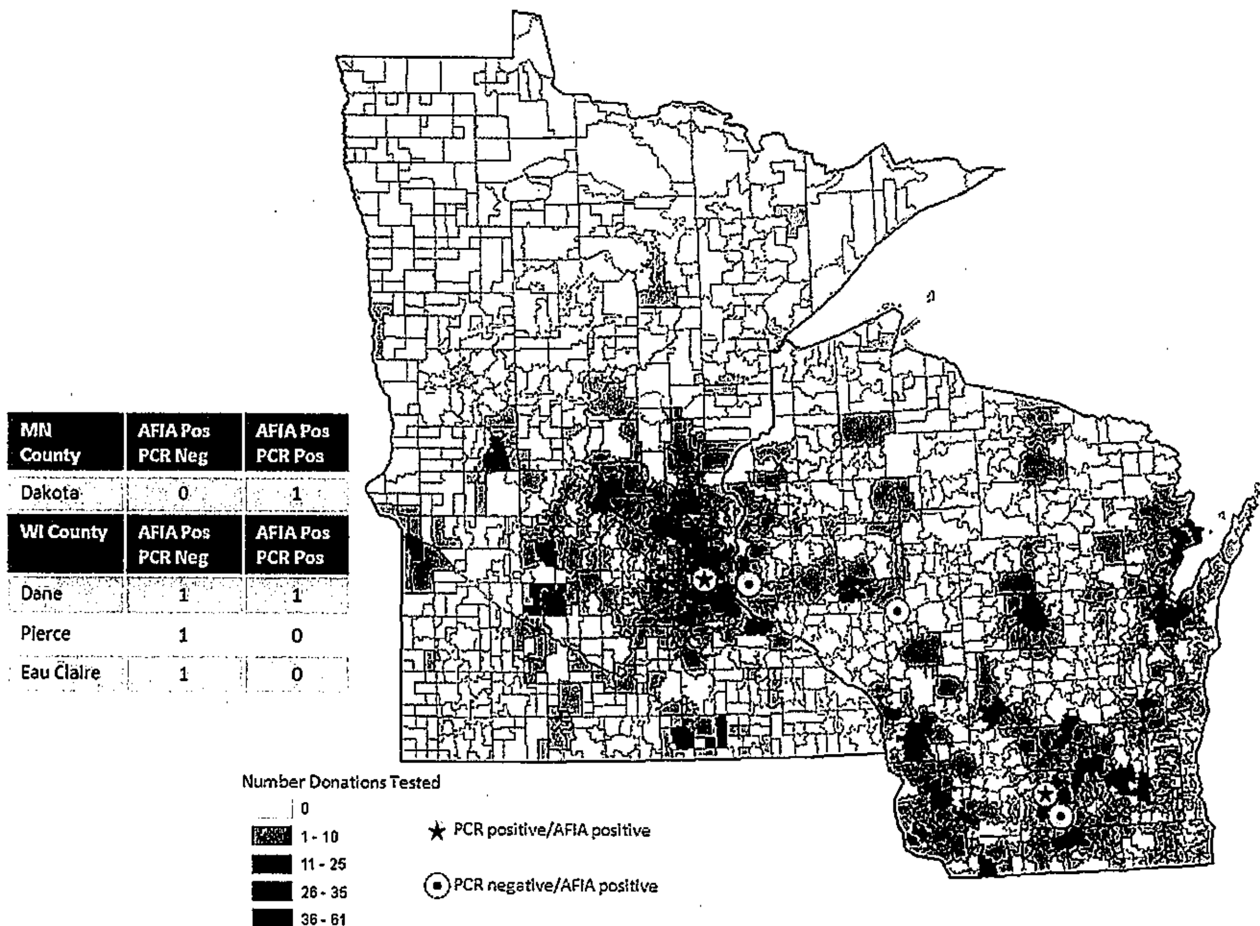
Three recipients who received RBCs or PLTs from antibody-positive donors and were transfused between November 2010 and February 2012 provided one follow-up sample each; none was reactive for *B. microti* antibodies or DNA (Table 6). Two of the 3 index units were PCR positive, with estimated parasite loads of 800 and 13,000 piroplasms/mL, but each transfused component from these PCR-positive donors was from a subsequent donation (collected 80-161 days later) at which time the marker status of the donor was unknown.

Specificity was calculated using data from the 4022 paired samples collected from areas assumed to be nonendemic for *B. microti* (AZ and OK). Three donors were AFIA positive, PCR negative, with AFIA titers of 64 for two and 128 for the third (Table 4). One male donor (Donor 007GV) from AZ was classified as having had a "true remote infection." This was based on an index AFIA titer of 64 (ePCR negative, IgM WB negative but IgG WB positive; Fig. 4). The donor's IgG reactivity resolved when followed more than 1 year later; no recipients from this donor's prior donations consented for testing. Specificities were 99.95% (4019/4021; 95% CI, 99.82%-99.99%) at a cutoff of 1 in 64 and 99.98% (4020/4021; 95% CI, 99.86%-100.00%) at a cutoff of 1 in 128.

All final results from IMUGEN were reported electronically to the ARC. Results for individually tested AFIA and PCR batches were reported within 10 hours of testing and within 24 hours for those samples requiring repeat testing. The reporting times for initial and repeat testing in this retrospective study demonstrate the feasibility of future blood donation screening.

DISCUSSION

From 2000 to 2009, a total of 159 TTB cases attributable to *B. microti* were described in the United States; cases in the series by Herwaldt and colleagues[13] were reported during



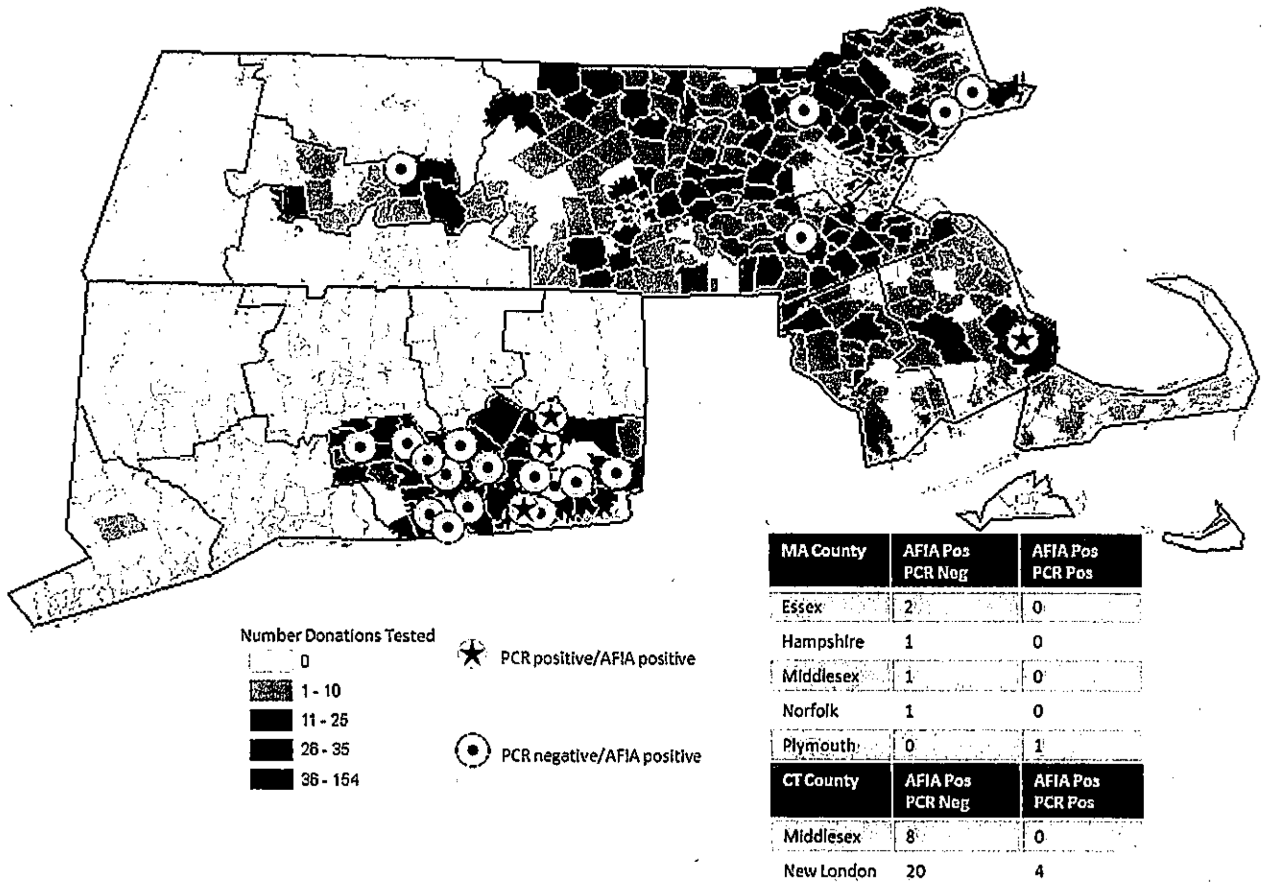
MN County	AFIA Pos PCR Neg	AFIA Pos PCR Pos
Dakota	0	1
WI County	**AFIA Pos PCR Neg**	**AFIA Pos PCR Pos**
Dane	1	1
Pierce	1	0
Eau Claire	1	0

Fig. 2. Map of MN and WI depicting the residential zip codes of donors who were tested with an investigational screening protocol to detect evidence of antibodies to and DNA from *B. microti* (blue). Yellow circled dots indicate the residential zip codes of donors who were positive by AFIA; red stars indicate AFIA-positive donations that were also PCR positive. Neg = negative; Pos = positive.

each month of the year with peak numbers reported from July through October, roughly equivalent to the date range for collected samples in our repository. Since 2000, reporting of TTB cases is on the rise, likely due to increased frequency and/or increased patient recognition and reporting.[8,13] The majority (87%) of the 159 TTB cases were reported from the seven *B. microti*–endemic states, four of which were included in our study.

In this study, the prevalence of antibodies to and DNA from *B. microti* followed expected patterns in a geographically diverse repository of blood donor samples (i.e., the lowest prevalence was found in nonendemic areas and the highest prevalence was found in highly endemic areas). The specificity of this investigational *B. microti* screening protocol including AFIA and PCR of 99.95 to 99.98% (cutoff dependent) is comparable to or better than current blood donor screening assays used for other infectious agents (ARC internal data). Testing was conducted on a large and geographically diverse sample of blood donors. Repository samples were collected from six targeted regions in the United States, roughly corresponding to six states. However, reported residential zip codes from donors of tested samples suggest that the geographic distribution of sampled donors (Fig. 1) is broader (including 26 states) than the locations of the six regional blood centers included in this study. Moreover, the large sample size contributes a sizable amount of data to those already available in the literature.[20-24] This study also includes donor data from nonendemic areas, which have not previously been reported.

Prior studies of *B. microti* testing in endemic areas using predominantly research IFAs yielded seroprevalence rates of 1.2% to 1.8% in the highly endemic counties of Middlesex and New London, CT, which were also evaluated in this study, and that those rates were stable over 8 years of study.[20] Higher rates (2%) were recently documented in endemic counties of MN using a similar research IFA, with a single PCR-positive sample identified of 2150 donors tested.[22] The same investigators reported a seroprevalence rate of 2.5% in the two highly



MA County	AFIA Pos PCR Neg	AFIA Pos PCR Pos
Essex	2	0
Hampshire	1	0
Middlesex	1	0
Norfolk	1	0
Plymouth	0	1

CT County	AFIA Pos PCR Neg	AFIA Pos PCR Pos
Middlesex	8	0
New London	20	4

Fig. 3. Map of MA and CT (two selected counties) depicting the residential zip codes of donors who were tested with an investigational screening protocol to detect evidence of antibodies to and DNA from *B. microti* (blue). Yellow circled dots indicate the residential zip codes of donors who were positive by AFIA; red stars indicate AFIA-positive donations that were also PCR positive with two PCR-positive donors from one zip code in New London county in CT. Neg = negative; Pos = positive.

TABLE 5. Testing results for a donor identified both through the retrospective study and through prospective testing

Collect date	Study	Sample type	Test result	Supplemental testing
August 5, 2013	Prospective	Follow-up	**AFIA 1:128**/PCR Neg	IgM Neg, **IgG Pos**
April 29, 2013	Prospective	Follow-up	**AFIA 1:128**/PCR Neg	IgM Neg, **IgG Pos**
January 14, 2013	Prospective	Follow-up	**AFIA 1:128**/PCR Neg	IgM Neg, **IgG Pos**
October 11, 2012	Prospective	Follow-up	**AFIA 1:128**/PCR Neg	IgM Neg, **IgG Pos**
August 13, 2012	Prospective	Prospective donation	**AFIA 1:128**/PCR Neg	IgM Neg, **IgG Pos**, ePCR Neg
May 30, 2012	Prospective	Retrieved plasma	**AFIA 1:128**/PCR not done	IgM Neg, **IgG Pos**
December 15, 2011	None			
August 31, 2011	None			
June 7, 2011	Retrospective	Repository	**AFIA 1:256**/PCR Neg	IgM Neg, **IgG Pos**, ePCR Neg
December 29, 2010	None			
August 30, 2010	Retrospective	Repository-"index"	**AFIA ≥ 1:1024/PCR Pos**	**IgM Pos, IgG Pos, 3000 piroplasms/mL**
May 3, 2010	None			
January 5, 2010	None			
September 30, 2009	None			

Neg = negative; Pos = positive.

endemic counties of CT.[21] In that study, 1002 donors were tested by IFA and PCR, with 25 IFA-positive donors identified, two of which were PCR positive. A third donor was identified from a September donation who was PCR positive but IFA negative (<1:64), suggesting a window period collection and the possible value of PCR in prospective donor screening algorithms. Rhode Island Blood Center has been screening donors for *B. microti* with

TABLE 6. Results of follow-up testing for three recipients of units donated by antibody-positive donors

State	Recipient condition	Date transfused	Product transfused/unit chronology/collect date	Index donor results/collect date	Recipient follow-up results
CT	Unknown	November 23, 2010	RBCs/subsequent*/November 2, 2010	AFIA ≥ 1:1024 PCR Pos 13,000 piroplasms/mL/August 13, 2010	AFIA < 1:64 PCR Neg
CT	Unknown	February 23, 2012	RBCs/subsequent/January 27, 2012	AFIA 1:256 PCR Neg/August 27, 2010	AFIA < 1:64 PCR Neg
MN	Leukemia	February 5, 2012	PLTs†/subsequent/February 1, 2012	AFIA 1:256 PCR Pos 800 piroplasms/mL/August 24, 2011	AFIA < 1:64 PCR Neg

* "Subsequent unit" refers to transfused units that were collected from a positive/inconclusive donor after that donor's index result.
† Apheresis donor.
Neg = negative; Pos = positive.

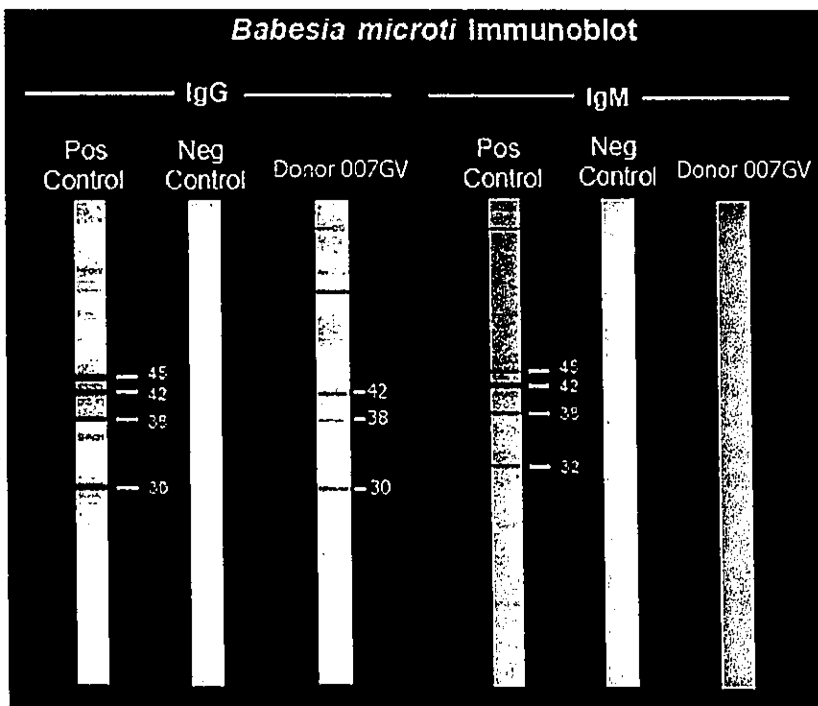


Fig. 4. IgG and IgM WBs of positive and negative controls and the index sample from Donor 007GV. To be considered positive on WB, reactivity to the 30-kDa protein was required in addition to reactivity to two additional proteins of 38, 42, or 45 kDa. The origin of this donor's reactivity is unclear: he lived in New York from 1955 to 1970 and spent time in Pennsylvania and New Jersey in 1968, but did not recall spending time in wooded or grassy areas or finding a tick on his body. He was stationed in Germany from 1975 to 1976, where he reported tick bites. Currently, he reports spending substantial amounts of time outdoors in AZ and owning three "outdoor" dogs and reported flulike symptoms in the past 2 years. Neg = negative; Pos = positive.

investigational IFA and PCR using earlier versions of the tests used in this study[23] and employing an algorithm in which labeled *B. microti* test–negative units are available on request for select at-risk patients (e.g., neonates and pediatric patients with hemoglobinopathies). Of 2113 units screened using a 1-in-128 IFA cutoff, a seroprevalence rate of 1.23% (including 26 IFA-positive samples, of which one donor was PCR inconclusive) was reported. No TTB was reported from any screened unit.

In contrast, in our study the rates of seropositivity in moderately endemic (MN and WI) and highly endemic (CT and MA) areas were lower: 0.12 and 0.75%, respectively. Different time periods, assay specificities, and county or blood drive selection may have been responsible for these differences. However, our study found a higher number of antibody-positive, PCR-positive donors in moderately and highly endemic areas (2/4167 and 5/5080, respectively; Table 4). Preliminary studies of prospective *B. microti* donor screening in endemic areas have identified PCR-positive donor units (both antibody positive and negative) that were infectious in animal models, suggesting that PCR is an important component of donor screening.[27]

This study has several potential limitations, some of which may result in underestimating the true prevalence of *B. microti* in blood donors. Testing was conducted on frozen samples, possibly resulting in some loss of sample quality. However, unpublished data from IMUGEN suggest that AFIA-positive serum and PCR-positive whole blood samples from blood donors remain stable for up to 19 months after freezing and storage at −16°C or less; additional ARC data demonstrate antibody frozen stability up to 5 months (Tables S1-S3, available as supporting information in the online version of this paper), and

AFIA-positive serum samples from clinical cases of babesiosis retain their titer for 12 years after freezing and storage at −65°C or less (data not shown; IMUGEN personal communication). A smaller proportion of samples were collected in May and June than in July, August, and September, which is a possible reason as to why window period units were not detected. It is likely that incident cases of *B. microti* occur in higher numbers in the late spring and early summer when our study undersampled collections and thus may have missed donors in the early stages of infection. The majority of samples collected for this study were not selected based on county, despite the fact that the incidence of *B. microti* infection has been shown to vary within states and that certain counties are hyperendemic.[20,28,29] Thus, we may have underestimated prevalence since some highly endemic counties may have been underrepresented or excluded from the repository.

More than 50% of *B. microti*–positive or -inconclusive donors participated in our follow-up study; however, the rate of follow-up from recipients of transfused units from positive donors was disappointing. Johnson and coworkers[10] found that nearly 13% of recipients who received a seropositive index or prior component from a seropositive donor subsequently tested *B. microti* antibody and/or PCR positive. Seropositive index units transmitted more frequently (50%) than prior donations from seropositive donors (7.3%), and transmission more often occurred if the donor was also PCR positive (33.3%) versus PCR negative (2.9%).[10] Traced components from positive donors included RBC units (7-42 days old at transfusion) and 1 PLT unit (5 days old at transfusion).[10] Although our study tested only three recipients of cellular components from antibody-positive donors, none was AFIA or PCR positive. This included two recipients (one of RBCs, one of PLTs) of subsequent units donated by PCR-positive donors 80 to 161 days after their PCR-positive donation (Table 6). The likely explanations for the absence of transmission include resolution of active infection by the time the donor donated the transfused unit or a parasite load in the subsequent donation that was too low to transmit; it also should be noted that TTB has never been documented by an apheresis PLT unit, only those prepared from whole blood collections.[13] However, it is noteworthy that the PCR-positive and AFIA-positive donors (800-13,000 piroplasms/mL and AFIA titers of 256 to ≥1024) were not associated with transmissions in donations more than 2 months later. Until more data are collected on recipients, it will be difficult to empirically estimate the risk of antibody- and/or PCR-positive blood to those being transfused with such units.

While donor follow-up data collection is ongoing, available data from this study suggest that donors maintain antibody reactivity for a substantial period of time. The mean time between initial reactivity at the index donation and last follow-up collection was 676 days. The majority (74.1%) of donors retained antibodies at last follow-up, suggesting that antibodies are often present for at least 2 years, consistent with prior observations.[26] Such data will be a key consideration for setting policies on donor reentry. Follow-up and supplemental test data also indicate that an AFIA cutoff of 1 in 64 is unnecessarily low for blood donation screening when coupled with PCR screening. Further studies from prospective screening using both AFIA and PCR could indicate that a 1-in-128 AFIA cutoff may also be too low considering the absence of infectivity[27] coupled with unnecessary donor loss.

The incidence of human exposure to *B. microti* is increasing in the United States and is a threat to recipients of blood products due to the absence of an FDA-licensed intervention.[12,13] This study contributes important data to our understanding of *B. microti* in blood donors, including the prevalence of *B. microti* in nonendemic and endemic areas of the United States, the duration of antibody detected in positive donors, and the specificity of an investigational screening protocol, demonstrating the feasibility of *B. microti* blood donation screening in the United States. Deploying sensitive and specific laboratory assays for donor screening is a worthy goal for mitigating this existing and expanding threat to the blood supply.

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CONFLICT OF INTEREST

EDM, CSW, STJ, DEK, RLT, GAF, and SLS have disclosed no conflicts of interest. PD, PM, EB, and VPB are employees of IMUGEN, Inc.

REFERENCES

1. Western KA, Benson GD, Gleason NN, et al. Babesiosis in a Massachusetts resident. N Engl J Med 1970;283:854-6.
2. Ruebush TK 2nd, Juranek DD, Chisholm ES, et al. Human babesiosis on Nantucket Island: evidence for self-limited and subclinical infections. N Engl J Med 1977;297:825-7.
3. Gorenflot A, Moubri K, Precigout E, et al. Human babesiosis. Ann Trop Med Parasitol 1998;92:489-501.
4. Hunfeld KP, Hildebrandt A, Gray JS. Babesiosis: recent insights into an ancient disease. Int J Parasitol 2008;38:1219-37.

5. Vannier E, Krause PJ. Human babesiosis. N Engl J Med 2012;366:2397-407.
6. Homer MJ, Aguilar-Delfin I, Telford SR, et al. Babesiosis. Clin Microbiol Rev 2000;13:451-69.
7. Jacoby GA, Hunt JV, Kosinski KS, et al. Treatments of transfusion-transmitted babesiosis by exchange transfusion. N Engl J Med 1980;303:1098-100.
8. Tonnetti L, Eder AF, Dy B, et al. Transfusion-transmitted *Babesia microti* identified through hemovigilance. Transfusion 2009;49:2557-63.
9. Asad S, Sweeney J, Mermel LA. Tranfusion-transmitted babesiosis in Rhode Island. Transfusion 2009;49:2564-73.
10. Johnson ST, Cable RG, Leiby DA. Lookback investigations of *Babesia microti*-seropositive blood donors: seven-year experience in a Babesia-endemic area. Transfusion 2012; 52:1509-16.
11. Leiby DA. Transfusion-transmitted babesia spp.: bull's eye on *Babesia microti*. Clin Microbiol Rev 2011;155:509-19.
12. Stramer SL, Hollinger FB, Katz LM, et al. Emerging infectious disease agents and their potential threat to transfusion safety. Transfusion 2009;49(Suppl 2):1S-29S.
13. Herwaldt BL, Linden JV, Bosserman E, et al. Transfusion-associated babesiosis in the United States: a description of cases. Ann Intern Med 2011;155:509-19.
14. Centers for Disease Control and Prevention. Babesiosis surveillance—18 states, 2011. MMWR Morb Mortal Wkly Rep 2012;61:505-9.
15. Centers for Disease Control and Prevention. Final 2012 reports of nationally notifiable infectious diseases. MMWR Morb Mortal Wkly Rep 2013;62:669-82.
16. Healy GR, Ruebush TK. Morphology of *Babesia microti* in human blood smears. Am J Clin Pathol 1980;73:107-9.
17. Chisholm ES, Ruebush TK, Sulzer AJ, et al. *Babesia microti* infection in man: evaluation of an indirect immunofluorescent antibody test. Am J Trop Med Hyg 1978;27:14-9.
18. Senanayake SN, Paparini A, Latimer M, et al. First report of human babesiosis in Australia. Med J Aust 2012;196: 350-2.
19. Perez Acosta ME, Ender PT, Smith EM, et al. *Babesia microti* infection, eastern Pennsylvania, USA. Emerg Infect Dis 2013;19:1105-7.
20. Johnson ST, Cable RG, Tonnetti L, et al. Seroprevalence of *Babesia microti* in blood donors from *Babesia*-endemic areas of the northeastern United States: 2000 through 2007. Transfusion 2009;49:2574-82.
21. Johnson ST, Van Tassell ER, Tonnetti L, et al. *Babesia microti* real-time polymerase chain reaction testing of Connecticut blood donors: potential implications for screening algorithms. Transfusion 2013;53:2644-9.
22. Tonnetti L, Thorp AM, Deisting B, et al. *Babesia microti* seroprevalence in Minnesota blood donors. Transfusion 2013;53:1698-705.
23. Young C, Chawla A, Berardi V, et al., Babesia Testing Investigational Containment Study Group. Preventing transfusion-transmitted babesiosis: preliminary experience of the first laboratory-based blood donor screening program. Transfusion 2012;52:1523-9.
24. Leiby DA, Chung AP, Gill JE, et al. Demonstrable parasitemia among Connecticut blood donors with antibodies to *Babesia microti*. Transfusion 2005;45:1804-10.
25. Levin AE, Williamson PC, Erwin JL, et al. Determination of seroprevalence of *Babesia microti* (Bm) in endemic and non-endemic blood donor populations using an investigational ELISA [abstract]. Transfusion 2013;53(Suppl): 42-3A.
26. Gubernot DM, Nakhasi HL, Mied PA, et al. Transfusion-transmitted babesiosis in the United States: summary of a workshop. Transfusion 2009;49:2759-71.
27. Moritz E, Johnson S, Winton C, et al. Prospective investigational blood donation screening for *Babesia microti* [abstract]. Transfusion 2013;53(Suppl):13A.
28. Krause PJ, Telford SR, Ryan R, et al. Geographical and temporal distribution of babesial infection in Connecticut. J Clin Microbiol 1991;29:1-4.
29. Anderson JF, Mintz ED, Gadbaw JJ, et al. *Babesia microti*, human babesiosis, and *Borrelia burgdorferi* in Connecticut. J Clin Microbiol 1991;29:2779-83.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's Web site:

Fig. S1. *Babesia microti* PCR assay detection frequency vs. piroplasms/mL concentration.
Fig. S2. *Babesia microti* PCR linearity: PCR threshold cycles (Ct values) vs. total piroplasms/mL (all data points).
Fig. S3. *Babesia microti* PCR correlation with smear from 221 PCR and smear positive patients: PCR threshold cycles (Ct values) vs. percent total piroplasms/mL.
Table S1. *Babesia microti* AFIA correlation between 208 index blood donation screening results from donors tested AFIA reactive, inconclusive, or negative (unfrozen) and frozen plasma (n=194) or frozen index retention samples (n=14) from the index donated unit (≤-16°C). Data are from ongoing prospective investigational screening; samples were frozen for a mean of 55 days (range 11-189) prior to testing
Table S2. *Babesia microti* antibody stability data from 18 EDTA-plasma blood donor samples frozen (≤-16°C) for 12 to 18 months
Table S3. *Babesia microti* DNA stability data from frozen whole-blood samples from blood donors (-20°C) for 15 to 24 months

別紙様式第2-1

No. 20

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
			2014. 10. 4	該当なし	
一般的名称	人赤血球液	研究報告の公表状況	Leiby DA, Johnson ST, Won KY, Nace EK, Slemenda SB, Pieniazek NJ, Cable RG, Herwaldt BL. Transfusion. 2014 Sep;54(9):2217-25. doi: 10.1111/trf.12622. Epub 2014 Mar 28.	公表国 米国	
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社) 赤血球液-LR「日赤」(日本赤十字社) 照射赤血球液-LR「日赤」(日本赤十字社)				

研究報告の概要

○*Babesia microti*(*B.microti*)抗体陽性供血者における追跡調査

背景: バベシア症はダニ媒介性の赤血球内寄生原虫により引き起こされ、輸血感染症例の報告が増加している。米国ではこれまで供血者スクリーニングのために承認されたバベシア症検査はなかった。本研究では、血清学的研究で抗体陽性が確認された供血者におけるバベシア感染の経過やマーカーを調査した。

方法: IFA法で*B.microti*抗体価が64倍以上の供血者を本研究の対象とした。被験者にはIFA法に加えnested PCR、寄生虫学的検査(ハムスターへの接種、血液スメア)を行い、3年間追跡した。

結果: 研究対象供血者115人のうち84人(73%)が参加した。9カ月にわたり採取された検体に各種検査を行い、18人(21%)からの30検体(181検体中の17%)に原虫血症のエビデンスが見られた。14人は、2カ所の検査施設の双方で1検体以上がPCR陽性(12人)及び/または寄生虫学的検査で感染が確認された(8人)。1検体以上の原虫血症エビデンスを示した9人中3人の検体は、断続的に陽性となった。数人は、最後の陽性検体が採取された時には少なくとも1年感染が持続していた。7人の供血者の最後の3検体は、IFAを含む本研究で用いた全検査で陰性であった。

結論: *B.microti*抗体陽性供血者は、低レベルの原虫血症が長期化し、寄生虫学的検査や分子生物学的検査で断続的に検出される。従って、供血者スクリーニングアルゴリズムには、分子生物学的検査のみではなく血清学的検査も行うべきである。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」
赤血球液-LR「日赤」
照射赤血球液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見

IFA法による*Babesia microti*抗体価が64倍以上の供血者において3年間の追跡調査を行ったところ、これらの供血者は低レベルの原虫血症が長期化し、寄生虫学的検査や分子生物学的検査で断続的に陽性となる供血者が確認されたとの報告である。

今後の対応

日本赤十字社では問診時にバベシア症の既往歴を確認し、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。

A longitudinal study of *Babesia microti* infection in seropositive blood donors

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BACKGROUND: *Babesia* infection is caused by intraerythrocytic tick-borne parasites. Cases of transfusion-transmitted babesiosis have been increasingly recognized. To date, no *Babesia* test has been licensed for screening US blood donors. We conducted a longitudinal study to assess the course and markers of *Babesia* infection among seropositive donors identified in a seroprevalence study.

STUDY DESIGN AND METHODS: Eligible donors had *B. microti* indirect fluorescent antibody (IFA) titers of 64 or greater. Enrollees were monitored up to 3 years, by IFA and three methods for evidence of parasitemia: *B. microti* nested polymerase chain reaction (PCR) analysis (at two laboratories), hamster inoculation, and blood-smear examination.

RESULTS: Among 115 eligible donors, 84 (73%) enrolled. Eighteen enrollees (21%) had evidence of parasitemia for 30 total specimens (17% of 181), which were collected in 9 different months and tested positive by various approaches: PCR (25 specimens/16 persons), hamster inoculation (13 specimens/8 persons), and blood smear (one specimen positive by all three approaches). Overall, 14 persons had one or more specimen with positive PCR results at both laboratories (12 persons) and/or had parasitologically confirmed infection (eight persons). Three of nine persons who had more than one specimen with evidence of parasitemia had nonconsecutive positives. Several enrollees likely had been infected at least 1 year when their last positive specimen was collected. The final three specimens for seven persons tested negative by all study methods, including IFA.

CONCLUSION: Seropositive blood donors can have protracted low-level parasitemia that is variably and intermittently detected by parasitologic and molecular methods. Donor-screening algorithms should include serologic testing and not solely rely on molecular testing.

Human babesiosis is caused by intraerythrocytic protozoan parasites, which are tick-borne in nature but also are transmissible via blood transfusion.[1-11] Most of the documented US cases of babesiosis have been caused by *Babesia microti*, which is transmitted by *Ixodes scapularis* ticks in the Northeast and upper Midwest, primarily during the spring and summer.[1-3] *B. microti* infection can range from asymptomatic to severe. Persons, such as transfusion recipients, who are asplenic, elderly, premature, or immunocompromised, are at increased risk for clinically manifest and life-threatening infection.

More than 160 US cases of transfusion-transmitted babesiosis (TTB) have been identified during the three decades since the first described TTB case in 1979,[12] most (>75%) of which occurred during the past decade.[1] To date, no *Babesia* test has been licensed by the US

ABBREVIATIONS: ARC = American Red Cross; IFA = indirect fluorescent antibody; TTB = transfusion-transmitted babesiosis.

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Food and Drug Administration (FDA) for screening blood donors;[1,4-6] donor-screening algorithms do not routinely include testing for evidence of *Babesia* infection.[1] Although donors routinely are asked if they have a "history of babesiosis,"[6,7] persons with undiagnosed asymptomatic infection can fulfill all criteria for donating blood despite having low levels of potentially transmissible bloodstream parasites, which can suffice to cause infection in transfusion recipients.[1]

Relatively few *B. microti*–infected persons have been monitored systematically for extended periods,[13-15] most of whom initially had symptomatic acute cases of babesiosis. We assessed the course and laboratory markers of *B. microti* infection in settings relevant to transfusion medicine by conducting a longitudinal study among seropositive blood donors, who were evaluated up to 3 years, by serologic, parasitologic, and molecular methods as well as structured questionnaires. Although the study was not designed to evaluate the performance of particular methods as diagnostic or donor-screening assays, our findings pertain to the development and implementation of donor-testing and management strategies.

MATERIALS AND METHODS

Study design and enrollment

Seropositive donors whose *B. microti* indirect fluorescent antibody (IFA) titer was at least 64 on initial testing during May 2000 through April 2004 in a previously described seroprevalence study[16] were eligible to enroll in the longitudinal study, which began in June 2000; the last study specimen was collected in July 2006. In the seroprevalence study, donors in southeastern Connecticut (Middlesex and New London Counties) were targeted initially; the catchment area gradually expanded within Connecticut, and donors in Massachusetts (Dukes and Nantucket Counties) were added in 2003.

The protocol for the longitudinal study was approved by the institutional review boards of the American Red Cross (ARC) and the Centers for Disease Control and Prevention (CDC). On enrollment, participants provided written informed consent and their first study specimen, referred to as their enrollment specimen. Each study specimen comprised three tubes of blood, which were collected by regional ARC staff and shipped at 4°C on wet ice to the ARC's Holland Laboratory (one serum-separator tube and one EDTA tube) and to CDC (one EDTA tube). The specimens were tested by IFA (at the ARC) and by three methods for evidence of parasitemia: two parasitologic methods (blood-smear examination and animal inoculation at CDC) and one molecular method (nested polymerase chain reaction [PCR] analysis at both laboratories). In the data analyses, positive results by any of these three methods, at either laboratory, constituted evidence of parasitemia. Unless otherwise specified, positive and tested positive refer to evidence of parasitemia rather than to seropositivity. Participants who had positive results were encouraged to share them with their physician and were given contact information for a clinical babesiosis expert. Study subjects were asked to provide a specimen every 2 to 3 months (monthly, if they had evidence of parasitemia) until they had three consecutive specimens with negative results by all methods, including IFA, or 3 years had elapsed.

Laboratory methods

The ARC conducted the serologic testing using a nonautomated IFA assay for immunoglobulin G antibodies to *B. microti* antigens; IFA slides and reagents were purchased from Focus Technologies, Inc. (Cypress, CA). If seroreactivity was noted at the lowest dilution of serum tested (64), the specimen was defined as IFA positive (in accordance with the manufacturer's instructions and the protocol for the seroprevalence study in which eligible subjects were identified) and was tested to endpoint in serial twofold dilutions.[17] Of note, the study was not designed to evaluate this particular IFA assay or cutoff (64) for donor-testing purposes. Positive and negative controls were used. The same positive control serum specimen was used throughout the study; when *B. microti* antigen lots changed, the positive control was used to certify the new lot and was observed to perform consistently. Because of the subjectivity inherent to determining the endpoint titer in this nonautomated assay, only highly trained, designated staff conducted the testing. After completion of the study, serial specimens from multiple subjects were retested in parallel, on the same day.

CDC conducted the parasitologic testing: two thick and two thin Giemsa-stained blood smears (10 µL/smear) were examined for *Babesia* parasites by light microscopy, under oil immersion. In addition, two golden Syrian hamsters (*Mesocricetus auratus*) were inoculated intraperitoneally with 1-mL aliquots of whole blood and were monitored weekly, by examination of Giemsa-stained thin blood smears, until parasites were noted or 8 weeks had elapsed. Hamsters are competent (amplifying) hosts of *B. microti*, which is not cultivable in vitro. CDC's Institutional Animal Care and Use Committee approved animal experiments and procedures.

The ARC and CDC independently analyzed blood specimens by PCR, using primers designed to amplify *B. microti* DNA from the 18S ribosomal RNA gene[18] and a previously described two-step nested PCR protocol.[9,16,17] Total DNA was extracted from 200 µL of whole blood (i.e., a fivefold lower volume than was inoculated into each hamster), by using a DNA blood mini kit (QIAamp; Qiagen, Inc., Valencia, CA). An aliquot of extracted DNA was amplified with primers Bab1 and Bab4, the product was amplified further with internal primers Bab2 and

Bab3, and the final product was visualized in a 2% agarose gel stained with ethidium bromide. Positive, negative, and extraction controls were included. DNA extraction, amplification, and electrophoretic analysis were conducted in physically separate work areas; other standard measures (e.g., irradiation with ultraviolet light) were used to prevent contamination.

Questionnaires

Epidemiologic and clinical data were obtained via structured questionnaires. An extensive "long" questionnaire, which focused on the previous 24 months, was included in the enrollment packet and was completed on site or submitted later. It addressed demographic factors, places of residence and travel, outdoor activities, tick exposures, and clinical data (e.g., flu-like symptoms, anti-*Babesia* therapy, surgical splenectomy). Persons with tick exposures were asked if the ticks were attached (difficult to pull off) and if they were small versus large, in comparison with unlabeled photographs of *I. scapularis* versus *Dermacentor variabilis* ticks, respectively; duration of attachment and tick engorgement were not assessed. During all study visits, participants were asked to complete a "short" questionnaire, which addressed interim activities, exposures, symptoms, and treatment.

Statistical analysis

Univariate analyses were conducted for descriptive purposes. Proportions were compared by using the chi-square test or, if expected cell counts were less than five, the Fisher's exact test. The Wilcoxon two-sample test was used to compare the ranked distributions of ordinal variables. The serologic results obtained using a non-automated IFA assay are provided and analyzed for illustrative purposes, even though the absolute magnitude of the titers might not always be reproducible or generalizable to other laboratories. In analyses of the distributions of the serologic data, $\log_2$ values were used, from 5 (for an IFA result of <64) to 10 (for a titer of 1024, the highest documented in the study). Significance was defined as a two-tailed p value of less than 0.05.

RESULTS

Eighty-four (73%) of 115 eligible *B. microti*-seropositive donors enrolled in the longitudinal study. Demographic and serologic data for the 84 who enrolled and the 31 who declined to participate were not significantly different (data not shown). The 84 enrollees had a median age of 50 years and 54 (64%) were men. On enrollment, 60 persons (71%) still had an IFA titer of at least 64, whereas 24 (29%) were seronegative; the median interval between collection of the initial and the enrollment specimens was 51 days (Table 1). In aggregate, the 84 enrollees provided 540 study specimens over a 6-year period.

Eighteen enrollees (21%)—referred to as Subjects A though R (Fig. 1)—tested positive for evidence of parasitemia, for a total of 30 specimens (17% of 181; Table 1). The epidemiologic profiles of these 18 persons with evidence of parasitemia and the 66 enrollees without demonstrable parasitemia were comparable in univariate analyses. However, in aggregate, these 18 persons had higher IFA titers on initial testing and on enrollment (Table 1). The median IFA result for the 30 specimens that had evidence of parasitemia was 256 (range, <64 to 1024).

Serial laboratory data for these 18 enrollees are depicted in timelines (Fig. 1; see right bottom for summary data). Their 30 positive specimens had evidence of parasitemia by various permutations and combinations of methods and laboratories—i.e., by PCR analysis at either laboratory (25 specimens), hamster inoculation (13 specimens), and blood-smear examination (one specimen). In the study as a whole, including all 84 participants, the PCR results were concordant for 521 (98%) of the 533 specimens that were tested by both laboratories (Table 1 and Fig. 1). Of the 25 PCR-positive specimens, 12 (48%) had positive results at only one laboratory: these 12 specimens, which account for the overall discordance rate of 2%, were from nine persons, five of whom (A, D, E, H, and O) had other specimens that tested positive by PCR at both laboratories or by hamster inoculation. Overall, 14 (of 18) persons had positive PCR results at both laboratories and/or had parasitologically confirmed infection, four of whom (A, C, D, and P) also had positive lookback investigations—i.e., a *B. microti* PCR-positive recipient of red blood cells (RBCs) they had donated was identified.[17]

Overall, nine of the 18 persons had more than one specimen with evidence of parasitemia: six persons had consecutive positive specimens but not necessarily by the same methods or laboratories, and three persons had positive results for nonconsecutive specimens. For example, Subject P had two nonconsecutive hamster-positive specimens. Of interest, he did not have demonstrable parasitemia until his fourth study specimen (in April), even though all of his specimens had IFA titers of at least 512 and a lookback investigation of his blood donation the previous July was positive; more than 1 year after that July donation, his second hamster-positive specimen (his sixth study specimen) was collected (Fig. 1).

Six of the 18 persons reported receipt of anti-*Babesia* therapy, four of whom had posttreatment positive PCR results, including Subjects N and Q, who were treated before enrollment, and Subjects A and E, who were treated after they enrolled (Fig. 1). Subjects N, A, and E are particularly illustrative. Subject N, the only asplenic participant, became acutely ill, was hospitalized, and started a several-week course of anti-*Babesia* therapy 10 days after his initial IFA testing in August. His enrollment specimen

TABLE 1. Characteristics of the 84 *B. microti*–seropositive study participants, stratified by evidence of parasitemia*

Variable	All study subjects (n = 84)	Ever tested positive: Yes (n = 18)	Ever tested positive: No (n = 66)	p value	Comments
Age (years)	50 (19-79; 41-61)	52 (26-79; 39-62)	49 (19-79; 41-61)	0.7	
Male	54 (64)	12 (67)	42 (64)	0.8	
Donated blood in Connecticut†	77 (92)	17 (94)	60 (91)	1.0	
Completed the "long" questionnaire‡,§	80 (95)	16 (89)	64 (97)	0.2	
Lived in a rural area‡	49/80 (61)	11/16 (69)	38/64 (59)	0.5	
Had ≥1 acre of undeveloped land	56/79 (71)	13/16 (81)	43/63 (68)	0.4	
Saw deer on property or nearby	73/80 (91)	14/16 (88)	59/64 (92)	0.4	
Year of initial IFA testing	2001 (2001-2003)	2000 (2000-2002)	2002 (2001-2003)	0.005‖	
By year					
2000¶	18 (21)	10 (56)‖	8 (12)	<0.001	Comparison of 2000 vs. later¶
2001	25 (30)	2 (11)	23 (35)		Subjects K and N
2002	14 (17)	3 (17)	11 (17)		Subjects H, O, and P
2003	25 (30)	3 (17)	22 (33)		Subjects E, Q, and R
2004	2 (2)	0	2 (3)		
Month of initial IFA testing	Aug (Mar-Dec; Jul-Oct)	Jul (May-Nov; Jul-Aug)	Aug (Mar-Dec; Jul-Oct)	0.2	
Month of enrollment	Sep (Jan-Dec; Aug-Nov)	Sep (Jan-Nov; Aug-Oct)	Oct (Jan-Dec; Aug-Nov)	0.2	
Initial IFA titer	64 (64-128)	256 (128-512)	128 (128-128)	<0.001‖	
By titer					
64	46 (55)	4 (22)	42 (64)	0.002	Comparison of 64 vs. >64
128	20 (24)	4 (22)	16 (24)		
256	6 (7)	3 (17)	3 (5)		
512	8 (10)	5 (28)	3 (5)		Subjects D, J, L, O, and R
1024	4 (5)	2 (11)	2 (3)		Subjects E and P
≥512**	12 (14)	7 (39)	5 (8)**	0.003	Comparison of ≥512 vs. <512
Enrollment IFA titer	64 (<64-128)	256 (128-512)	64 (<64-128)	<0.001‖	
By titer					
<64 (IFA negative††)	24 (29)	2 (11)	22 (33)	0.06	Comparison of <64 vs. ≥64
64	25 (30)	1 (6)	24 (36)		
≤64	49 (58)	3 (17)	46 (70)	<0.001	Comparison of ≤64 vs. >64
128	19 (23)	6 (33)	13 (20)		
256	8 (10)	2 (11)	6 (9)		
512	5 (6)	5 (28)	0		Subjects A, J, N, P, and R
1024	3 (4)	2 (11)	1 (2)**		Subjects E and O
≥512**	8 (10)	7 (39)	1 (2)**	<0.001	Comparison of ≥512 vs. <512
Subtotal who received anti-*Babesia* treatment	10	6	4		Self-reported data; could be incomplete
Treated before enrollment	6 (7)	2 (11)	4 (6)	0.4	Comparison of pretreated vs. not pretreated
Treated after enrollment	4	4	0		
Study specimens; total‡‡	5 (1-17; 3-10); n = 540	11 (4-17; 6-14); n = 181	5 (1-13; 3-7); n = 359	<0.001	
Positive specimens; total		2 (1-4); n = 30			
Interval (days)					
Initial specimen to enrollment specimen**	51 (9-296; 28-67)	44 (25-94; 28-55)	52 (9-296; 28-71)**	0.3	
Initial specimen to last positive specimen		75 (28-770; 54-229)			Does not constitute duration of infection
Initial specimen to last study specimen	409 (26-1275; 232-757)	740 (346-1141; 372-1078)	366 (26-1275; 193-624)	0.001	
Enrollment specimen to last study specimen	348 (0-1087; 184-693)	677 (295-1087; 329-1023)	318 (0-1057; 150–566)	<0.001	

* Data are reported as median (range; IQR), number (%), number/total number (%), or median (IQR). Unless otherwise specified, number (%) refers to persons; percentages might not total 100 because of rounding. The word initial refers to the preenrollment specimen or testing in the seroprevalence study. The 18 persons who ever tested positive for evidence of parasitemia are referred to as Subjects A through R (Fig. 1).

† The 77 Connecticut donors include 75 Connecticut residents (16 tested positive) and two Rhode Island residents (one tested positive). The seven Massachusetts donors include six Massachusetts residents (one tested positive) and one Vermont resident.

‡ All 84 enrollees completed the "short" questionnaire at least once. Among the 80 who completed the "long" questionnaire, 76 (95%) described their locale as rural (49 persons) or suburban (31 persons).

§ All 83 enrollees with available data reported at least one of the following outdoor activities in potential risk areas: doing lawn or yard work, with or without clearing land or brush (79 of 81 persons); gardening (59 of 75); walking (69 of 77); or hiking (48 of 75).

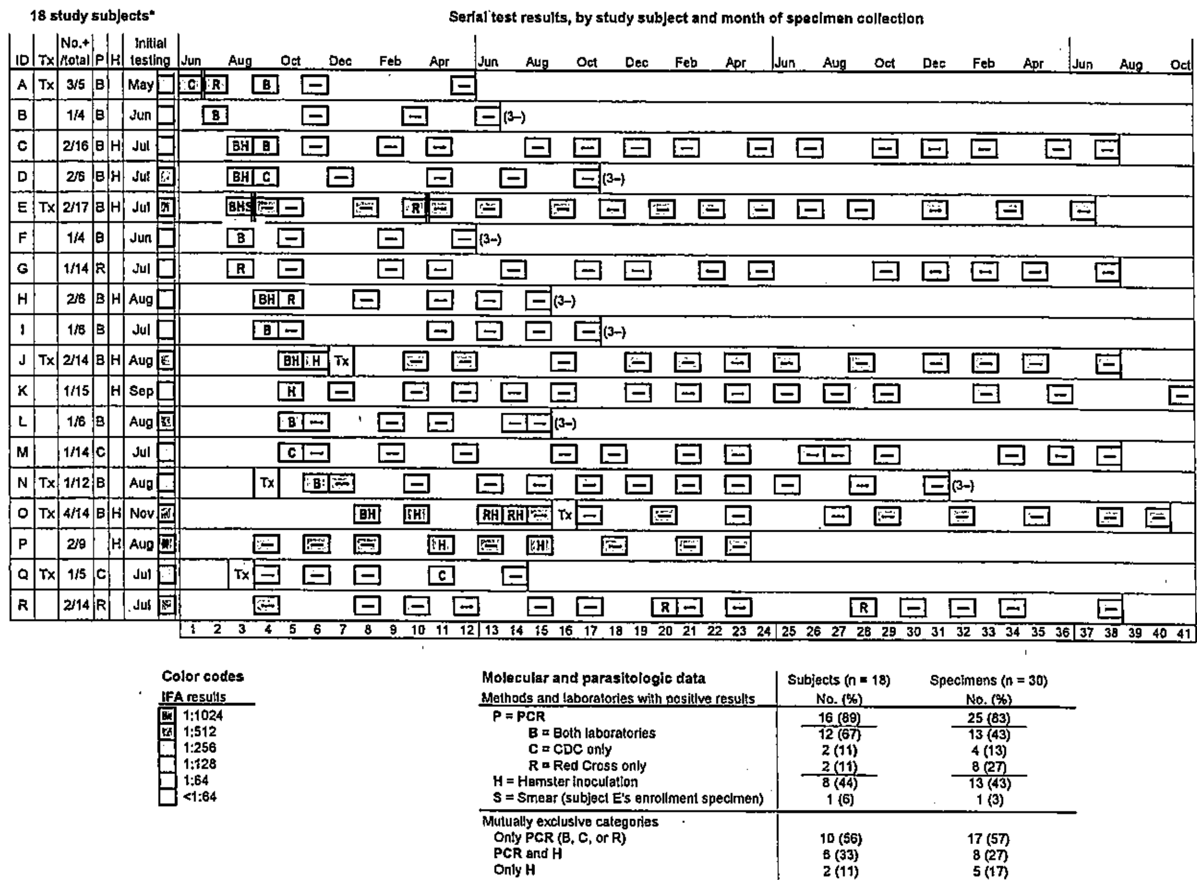
‖ The results were comparable if data for Subjects G, M, Q, and R, who had evidence of parasitemia only by PCR, at one laboratory (Fig. 1), were excluded.

¶ Overall, 10 (56%) of the 18 persons with evidence of parasitemia had their initial IFA testing in 2000; the eight (44%) whose initial testing was during 2001 to 2003 account for five (63%) of the eight total persons who tested positive by hamster inoculation.

** Of the 66 without evidence of parasitemia, five (8%) had IFA titers of at least 512 before enrollment, only one of whom had a titer of at least 512 on enrollment (see text); three of the other four persons had prolonged intervals between their initial and enrollment specimens (i.e., 71, 120, and 218 days).

†† Of the 24 who were seronegative on enrollment, 12 (14% of 84) did not have any IFA-positive study specimens, one of whom (Subject F) had positive PCR results at both laboratories on enrollment (Fig. 1).

‡‡ Of the 540 study specimens, seven (7%) were tested by PCR only at CDC (vs. also at the ARC): the final three specimens from each of two subjects (E and R) who previously had had positive results (Fig. 1), and the penultimate specimen from one of the 66 subjects who never had evidence of parasitemia by any method (PCR, hamster inoculation, or blood smear). Overall, six (9%) of the 66 persons without evidence of parasitemia provided only one study specimen.



Molecular and parasitologic data Methods and laboratories with positive results	Subjects (n = 18) No. (%)	Specimens (n = 30) No. (%)
P = PCR	16 (89)	25 (83)
B = Both laboratories	12 (67)	13 (43)
C = CDC only	2 (11)	4 (13)
R = Red Cross only	2 (11)	8 (27)
H = Hamster inoculation	8 (44)	13 (43)
S = Smear (subject E's enrollment specimen)	1 (6)	1 (3)
Mutually exclusive categories		
Only PCR (B, C, or R)	10 (56)	17 (57)
PCR and H	6 (33)	8 (27)
Only H	2 (11)	5 (17)

Fig. 1. Characteristics of and serial laboratory data for the 18 *B. microti*–seropositive study subjects with evidence of parasitemia. These 18 subjects (A through R) provided a total of 181 study specimens, 30 (17%) of which tested positive for evidence of parasitemia by at least one of three methods: PCR analysis (P), at the CDC (C), the ARC (R), or both laboratories (B); hamster inoculation (H); and blood-smear examination (S). The specimens were tested by PCR at both laboratories, with the exception of the final three specimens from Subjects E and R, which were tested only at CDC. See right bottom for a summary of the molecular and parasitologic results; percentages might not total 100 because of rounding. See left bottom for the color codes used to indicate the IFA results. The left-hand columns specify each subject's letter identification (ID), self-reported history of anti-*Babesia* treatment (Tx), and the numbers of positive and total study specimens (No.+/total). The P and H columns indicate which subjects ever had positive results by PCR and hamster inoculation, respectively. In Column P, the summary PCR results (B, C, R, or blank) are mutually exclusive. The initial-testing column specifies the month of collection and the IFA results for the preenrollment specimen tested in a separate study. In the timelines, the 6-year study period was normalized to 3.4 years (41 months); data for the eight subjects whose initial testing was after 2000 were shifted back from 1 to 3 years. The results for the 181 study specimens are provided by month of collection (see column headers); because the IFA results are provided for the preenrollment specimen as well as all 181 study specimens, the data can be reanalyzed ad hoc using different criteria for classifying a specimen as IFA positive (and, therefore, for considering a person eligible to enroll in the study and for releasing the study subject after <3 years of monitoring). For the pertinent subjects, the timing of therapy is shown; for Subjects A and E, the timing is indicated by a double-lined border between specimens in consecutive months (E was treated twice). The 1-month data columns are numbered (see footers) to facilitate ad hoc calculations of intervals. The data boxes for the 30 specimens with evidence of parasitemia include the pertinent letters for the positive molecular or parasitologic results and the background color indicative of the IFA results. The data boxes for the other 151 study specimens have a centered negative sign; a white box with a negative sign indicates that the IFA results also were negative (<64). For seven subjects, the final three specimens tested negative by all study methods, as indicated by "(3–)"; their last three specimens were collected over a median of 6 months (range, 4-7 months). Because IFA results are provided for the preenrollment specimen and all study specimens, the data can be reanalyzed using different criteria for classifying a specimen as IFA positive. *The 18 subjects are listed in order of the month of their first study specimen with evidence of parasitemia and by the number and clustering of positive specimens; the first 15 subjects tested positive on enrollment. Several subjects found small ticks attached to their skin at various intervals before their preenrollment testing: the interval was not more than 1 month for Subjects E, L, and Q and was approximately 3 months for Subject J. Subjects C, D, and G also reportedly found small attached ticks; but the timing was unclear. For Subjects G, M, and Q—each of whom had only one specimen with evidence of parasitemia, by PCR at one laboratory—CDC repeated the PCR analyses after reextracting DNA from an aliquot of the pertinent specimen. Upon retesting, CDC's results were positive for Subjects G and M and were negative for Subject Q. CDC's results for the first extraction are shown; the ARC did not retest the specimens. Of note, Subject A's first two specimens still had discordant PCR results on repeat testing at CDC.

in November, 56 days posttreatment, had positive PCR results. Subjects A and E, who were 32 and 79 years old, respectively, tested positive before and after they were treated. Subject A was treated between the first and second of her three consecutive PCR-positive specimens, which were collected in June, July, and September, 58 days posttreatment (Fig. 1). The fact that a lookback investigation of her blood donation the previous December was positive suggests that she had protracted infection: if she became infected during the preceding spring-or-summer tick season, the interval from acquisition of infection to her last PCR-positive specimen was more than 1 year. Subject E also likely had protracted infection. His enrollment specimen in August had positive results by all modalities, including blood-smear examination (<1% of the RBCs were infected). He was treated in September and was re-treated 7 months later, after PCR positivity was noted again in March.

Subject O, who did not have positive PCR results after treatment, is also noteworthy (Fig. 1): she had four consecutive specimens with evidence of parasitemia; all four tested positive by hamster inoculation, but the PCR results varied; the first two hamster-positive specimens were collected during winter months, in January and March; the other two were from June and July, approximately 1 year after the preceding tick season; and she was seropositive throughout the 3-year period from her preenrollment specimen to her final study specimen (IFA titer, 512), which was collected 2 years posttreatment.

Overall, at least three persons (A, O, and P)—two of whom (A and P) had positive lookback investigations—likely had been infected for approximately 1 year or longer when their last specimen with evidence of parasitemia was collected, which tested positive by hamster inoculation or by PCR at both laboratories (i.e., the probability of true-positive results was high). None of the 18 subjects had evidence of parasitemia when last tested; only three (A, P, and Q) withdrew before fulfilling study criteria (Fig. 1). Seven subjects were released after less than 3 years of monitoring because they had had three consecutive specimens that tested negative by all study methods, including IFA.

Among the remaining eight persons, who were seropositive when last tested but were released because they had been monitored for 3 years, five subjects—C, G, K, M, and R—had a final IFA titer of 64 or 128. In supplemental testing, serial specimens from Subjects C, G, and R were retested by IFA, in parallel, on the same day. Upon retesting, the IFA results typically were the same as those shown in Fig. 1 or differed by only one (twofold) dilution: Subject G's last four specimens had negative IFA results; Subject R's titers gradually decreased, without fluctuations (final titer, 64); and Subject C's titers still fluctuated. The other three subjects (E, J, and O) who were released after having been monitored for 3 years had a final titer of 512, despite having been treated and monitored for at least 2 years posttherapy. If Subject P (who did not report receipt of treatment) is counted, a total of four persons (22% of 18) had a titer of 512 when last tested. Of interest, one of the 66 participants who never had demonstrable parasitemia had prolonged high-level seropositivity: the IFA titer was 1024 for all 11 specimens he provided over a 2-year period (data not shown).

DISCUSSION

We assessed the course and laboratory markers of *B. microti* infection in settings relevant to transfusion medicine by conducting a multiyear longitudinal study among prospectively identified seropositive donors in *Babesia*-endemic areas. The strengths of the study included the collection of serial specimens, the use of parasitologic as well as molecular amplification techniques, the independent performance of PCR analysis at two laboratories, and the availability of epidemiologic and clinical context. Because of the internal controls inherent to the study design, we were able to identify discordant or inconsistent laboratory results. In the data analyses, we focused on the 18 enrollees who ever tested positive for evidence of parasitemia—particularly, the 14 persons who had positive PCR results at both laboratories and/or had parasitologically confirmed infection (i.e., strong evidence of active infection), four of whom also had positive lookback investigations. None of our conclusions are dependent on data from persons who tested positive only by PCR at one laboratory or who never had evidence of parasitemia, and the study was not designed to determine the proportion of seropositive persons who had demonstrable parasitemia.

The aggregate longitudinal data underscore that persons who fulfill the eligibility criteria for donating blood can have protracted low-level parasitemia that is variably and intermittently detected by parasitologic and molecular amplification techniques. Regardless of the analytic sensitivity of the method used, the results will be negative if the target parasite or DNA is not present in the aliquots tested, which typically are several orders of magnitude smaller than the volumes transfused to adults.[1] When this study was initiated, *B. microti* two-step nested PCR analysis was considered state of the art. Although using real-time PCR and/or increasing the starting volume (e.g., for extracting and targeting DNA) could improve detection of low parasite densities, the discrepancy between the volumes tested versus transfused would remain a fundamental limitation. In the blood donor setting, negative PCR results—even for optimally collected, processed, and tested specimens—would not exclude *Babesia* infection or infectivity. The PCR positivity rates, as determined by nucleic acid testing, for donors infected with viral pathogens such as hepatitis B virus that

are associated with relatively high viremias are not applicable to *B. microti*. In general, with the exception of the window period, which was not addressed by this study, seropositivity is a more sensitive marker than PCR of *B. microti* infection, although it does not reliably distinguish between active and resolved infection.

Our findings also highlight distinctions between patient and donor settings (i.e., between clinical and transfusion medicine). Patients with acute symptomatic babesiosis typically have patent parasitemia, detectable by careful blood-smear examination.[1,3] In contrast, *Babesia*-infected persons who meet the criteria for donating blood by definition "feel well" and usually have subpatent (smear-negative) parasitemia, which may or may not be detected by methods that amplify parasites or DNA, even though the transfused inoculum may suffice to cause patent parasitemia in a susceptible recipient.[1,10] Only one enrollee in our study, an elderly man (Subject E), was documented to have a smear-positive specimen, which, as expected, also tested positive by PCR, at both laboratories and by hamster inoculation. However, PCR and in vivo positivity rates for persons with positive blood smears are not generalizable to donors with subpatent parasitemia.

In a previously described study,[19] hamsters reliably developed patent parasitemia if the intraperitoneal inoculum was at least 300 *B. microti* parasites; approximately one-third of hamsters became infected if the inoculum was as low as 30 parasites. In our study, more persons and specimens tested positive by PCR than by hamster inoculation but not necessarily in both laboratories or for consecutive specimens, and some had positive results only by the in vivo method, either by chance or because a larger volume of blood was tested. The potential role of chance detection is underscored by the higher concordance rate by person than by specimen (e.g., as exemplified by Subject A; Fig. 1). Variable detection of parasites or DNA—in aliquots of specimens collected at the same time and in serial specimens—should not be surprising in the context of low-level parasite densities that approximate a Poisson probability distribution. Especially for the 14 persons who were the focus of the analyses, false-positive PCR results were unlikely, although they cannot be excluded for some specimens. Although we did not use quantitative methods, the persons or specimens with positive results by both methods or laboratories likely had higher parasite densities than those with discordant or inconsistent results.

The overall number of persons who still were infected when they enrolled in the longitudinal study is not known, nor is the true duration of infection among the subset of enrollees who tested positive for evidence of parasitemia. However, the aggregate longitudinal data affirm the potential for otherwise healthy persons to have protracted parasitemia.[1,9,10,20-23] None of the study subjects had evidence of parasitemia when last tested, but several still had comparatively high-level seroreactivity. Potential explanations include persistent infection, with very low densities of residual or sequestered parasites not detected by the amplification methods we used,[24] reexposure or reinfection, or other antigenic stimuli.

The increasing recognition of US cases of TTB[1] has strengthened the impetus to develop, evaluate, and implement strategies to reduce the risk for transmission.[1,4-6,25-27] Our findings support the concept of year-round donor testing: the study specimens that had evidence of parasitemia were collected during 9 different months of the year (even if only hamster-positive specimens are included), which is consistent with the presence of protracted parasitemia in some donors and with the year-round occurrence of cases of TTB.[1] Almost all of the identified cases of TTB cases have been linked to RBC transfusions, which have included components that had been leukoreduced, irradiated, or cryopreserved.[1] The identified cases linked to whole blood–derived platelets[1,9,12,17] presumably were caused by residual RBCs that were infected with *B. microti* or by the presence of extracellular forms of the parasite.[1,28]

The FDA's Blood Products Advisory Committee that was convened in July 2010 supported the concept of regional (vs. other selective or universal) donor testing for evidence of *Babesia* infection;[26] the details of where to test (in which areas) and how to do so (with what types of approaches and which specific assays or protocols) have not yet been resolved. Our data underscore that donor-screening algorithms should include serologic testing and should not rely solely on molecular testing; indeed, both serologic and molecular testing have been conducted in the donor screening performed to date in selected areas under FDA-approved investigational protocols. To minimize the loss of uninfected donors (while maximizing the detection of infected donors), the definition of a seropositive result for the pertinent assay(s) should be evaluated and candidate reentry algorithms could be investigated; because negative PCR results do not exclude ongoing infection, negative seroconversion, with consistently negative serologic results thereafter (for an as-of-yet unspecified period), also would be needed. Although our study was not designed to evaluate the particulars of potential donor-screening tests or management strategies, multiple participants who had been seropositive and had evidence of parasitemia ultimately had three consecutive specimens that tested negative by all of our study methods or definitions (Fig. 1).

Donor-screening tests targeted at *B. microti* have the highest near-term priority. However, infection with other species, such as *B. duncani*, which has caused three documented cases of TTB,[22,23,29] is not detected by the available serologic or molecular tests for *B. microti*.[1] For the longer-term future, the ideal screening test would be a high-throughput, highly sensitive and specific marker of active

Babesia infection, regardless of the species. Pathogen reduction constitutes an alternative or supplemental mitigation strategy; techniques for cellular components have not yet been approved for use in the United States[4,25] but are under investigation.[30,31]

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CONFLICT OF INTEREST

The authors report no conflicts of interest or funding sources.

REFERENCES

1. Herwaldt BL, Linden JV, Bosserman E, et al. Transfusion-associated babesiosis in the United States: a description of cases. Ann Intern Med 2011;155:509-19.
2. Centers for Disease Control and Prevention. Babesiosis surveillance—18 states, 2011. MMWR Morb Mortal Wkly Rep 2012;61:505-9.
3. Vannier E, Krause PJ. Human babesiosis. N Engl J Med 2012;366:2397-407.
4. Gubernot DM, Nakhasi HL, Mied PA, et al. Transfusion-transmitted babesiosis in the United States: summary of a workshop. Transfusion 2009;49:2759-71.
5. Leiby DA. Transfusion-associated babesiosis: shouldn't we be ticked off? Ann Intern Med 2011;155:556-7.
6. Leiby DA. Transfusion-transmitted *Babesia* spp.: bull's-eye on *Babesia microti*. Clin Microbiol Rev 2011;24:14-28.
7. Tonnetti L, Eder AF, Dy B, et al. Transfusion-transmitted *Babesia microti* identified through hemovigilance. Transfusion 2009;49:2557-63.
8. Asad S, Sweeney J, Mermel LA. Transfusion-transmitted babesiosis in Rhode Island. Transfusion 2009;49:2564-73.
9. Herwaldt BL, Neitzel DF, Gorlin JB, et al. Transmission of *Babesia microti* in Minnesota through four blood donations from the same donor over a 6-month period. Transfusion 2002;42:1154-8.
10. Cangelosi JJ, Sarvat B, Sarria JC, et al. Transmission of *Babesia microti* by blood transfusion in Texas. Vox Sang 2008;95:331-4.
11. Gubernot DM, Lucey CT, Lee KC, et al. *Babesia* infection through blood transfusions: reports received by the US Food and Drug Administration, 1997-2007. Clin Infect Dis 2009;48:25-30.
12. Jacoby GA, Hunt JV, Kosinski KS, et al. Treatment of transfusion-transmitted babesiosis by exchange transfusion. N Engl J Med 1980;303:1098-100.
13. Krause PJ, Spielman A, Telford SR 3rd, et al. Persistent parasitemia after acute babesiosis. N Engl J Med 1998;339: 160-5.
14. Krause PJ, Gewurz BE, Hill D, et al. Persistent and relapsing babesiosis in immunocompromised patients. Clin Infect Dis 2008;46:370-6.
15. Ruebush TK 2nd, Chisholm ES, Sulzer AJ, et al. Development and persistence of antibody in persons infected with *Babesia microti*. Am J Trop Med Hyg 1981;30: 291-2.
16. Johnson ST, Cable RG, Tonnetti L, et al. Seroprevalence of *Babesia microti* in blood donors from *Babesia*-endemic areas of the northeastern United States: 2000 through 2007. Transfusion 2009;49:2574-82.
17. Johnson ST, Cable RG, Leiby DA. Lookback investigations of *Babesia microti*-seropositive blood donors: seven-year experience in a *Babesia*-endemic area. Transfusion 2012; 52:1509-16.
18. Persing DH, Mathiesen D, Marshall WF, et al. Detection of *Babesia microti* by polymerase chain reaction. J Clin Microbiol 1992;30:2097-103.
19. Etkind P, Piesman J, Ruebush TK 2nd, et al. Methods for detecting *Babesia microti* infection in wild rodents. J Parasitol 1980;66:107-10.
20. Wittner M, Rowin KS, Tanowitz HB, et al. Successful chemotherapy of transfusion babesiosis. Ann Intern Med 1982;96:601-4.
21. Wei Q, Tsuji M, Zamoto A, et al. Human babesiosis in Japan: isolation of *Babesia microti*-like parasites from an asymptomatic transfusion donor and from a rodent from an area where babesiosis is endemic. J Clin Microbiol 2001; 39:2178-83.
22. Herwaldt BL, Kjemtrup AM, Conrad PA, et al. Transfusion-transmitted babesiosis in Washington State: first reported case caused by a WA1-type parasite. J Infect Dis 1997;175: 1259-62.
23. Bloch EM, Herwaldt BL, Leiby DA, et al. The third described case of transfusion-transmitted *Babesia duncani*. Transfusion 2012;52:1517-22.
24. Leiby DA, Chung AP, Gill JE, et al. Demonstrable parasitemia among Connecticut blood donors with antibodies to *Babesia microti*. Transfusion 2005;45:1804-10.
25. Stramer SL, Hollinger FB, Katz LM, et al. Emerging infectious disease agents and their potential threat to transfusion safety. Transfusion 2009;49(Suppl 2): 1S-29S.
26. Food and Drug Administration. Meeting of the Blood Products Advisory Committee. Risk of *Babesia* infection by blood transfusion and potential strategies for donor testing. July 26, 2010 [cited 2013 Dec 22]. Available from: http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/BloodProductsAdvisoryCommittee/ucm205013.htm
27. Young C, Chawla A, Berardi V, et al., *Babesia* Testing Investigational Containment Study Group. Preventing transfusion-transmitted babesiosis: preliminary experience of the first laboratory-based blood donor screening program. Transfusion 2012;52:1523-9.
28. Pantanowitz L, Cannon ME. Extracellular *Babesia microti* parasites. Transfusion 2001;41:440.

29. Kjemtrup AM, Lee B, Fritz CL, et al. Investigation of transfusion transmission of a WA1-type babesial parasite to a premature infant in California. Transfusion 2002;42:1482-7.
30. Tonnetti L, Proctor MC, Reddy HL, et al. Evaluation of the Mirasol platelet reduction technology system against *Babesia microti* in apheresis platelets and plasma. Transfusion 2010;50:1019-27.
31. Tonnetti L, Thorp AM, Reddy HL, et al. Riboflavin and ultraviolet light reduce the infectivity of *Babesia microti* in whole blood. Transfusion 2013;53:860-7.

医薬品 研究報告 調査報告書

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研究報告の概要

○血小板製剤における*Brucella abortus*汚染：症例報告

背景：ブルセラ症は小型のグラム陰性球桿菌であるブルセラ属が原因の人獣共通疾患である。ウシやヤギの未殺菌乳の摂取、感染動物との直接接触あるいはエアロゾルの吸入によりヒトに感染し、ヒト－ヒト感染は非常に稀である。

症例：血小板減少症で入院中の高齢男性が、全血由来のプール血小板（AcrodoseTM Systems）を輸血された。同血小板はその後、細菌汚染が確認され、*Brucella Abortus*と同定された。輸血中、輸血後の有害事象は報告されていない。

所見：血液センターが行う細菌スクリーニング（BacT/ALERT®）により、ボトル培養の2.5日後、輸血の12.5時間後に細菌汚染があると判定された。血液センターはBacT培養陽性が判明して1時間後（輸血13.5時間後）に患者の担当医に報告し、医師は患者に広域抗生物質を投与した。患者には6カ月間、*Brucella*凝集反応検査を行ったが全て陰性であり、その後も臨床感染のエビデンスは得られなかった。血液センターは、供血の8カ月前にベネズエラから米国に移住した男性エンジニアである感染供血者を同定した。質問の結果、供血者には供血後2日間微熱があったことが判明した。血清学的検査で、*Brucella*総抗体価は1:5,120、IgG抗体価は1:320（基準値<1:20）であった。

結論：培養による血小板の品質管理検査により、特に増殖が遅い*Brusella*のような菌種では、血液供給・輸血後に細菌汚染が確認される可能性がある。本症例では、輸血反応の徴候がなかったにも拘わらず、患者の主治医への迅速な通知により、予防的に抗生物質投与を行うことが出来た。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」
赤血球液-LR「日赤」
照射赤血球液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見

患者への血小板輸血後に当該製剤の*Brucella Abortus*汚染が判明したが、主治医への迅速な通知により、患者に対する適切な対策をとることが可能であったとの報告である。

今後の対応

日本赤十字社では全献血者に問診を実施しているほか、輸血による細菌感染予防対策として全輸血用血液製剤を対象に保存前白血球除去を行っている。また、ブルセラ症の既往の申告がある場合は、完全に治癒して一定期間が経過するまで献血不適としている。今後も引き続き情報の収集に努める。

⑨

cavity was stable, with calcification and scarring. Case 2: A 23-y-old male with Job's syndrome, with right lower lobe *A. fumigatus* mycetoma (7.8 cm in largest dimension) for 8 mo, despite antimicrobial therapy. He was stable during the procedure, with 85 mL of material instilled, but became hypoxic postextubation, with increased inflammation in the right lung on imaging managed with intravenous steroids and oxygen supplementation. The following day, he had no shortness of breath or fever, but had mild blood-tinged sputum and pleuritic chest pain at site of intervention. He was discharged 3 d postprocedure. One mo post-procedure, the overall volume of the intracavitary nodule was moderately decreased (2.5 × 2 × 1.5 cm). **Conclusion:** Allogeneic cryoprecipitate can be used to create an antimicrobial-containing fibrin gel for transthoracic instillation in the treatment of persistent pulmonary mycetoma. Further study is required to determine the efficacy of this approach.

Disclosure of Commercial Conflict of Interest

C. Cantilena: No Answer; R. Chang: No Answer; A. Freeman: No Answer; G. Grimes: No Answer; M. Kolf: No Answer; S. F. Leitman: No Answer; K. Olivier: No Answer; K. West: Nothing to disclose

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C. Cantilena: No Answer; R. Chang: No Answer; A. Freeman: No Answer; G. Grimes: No Answer; M. Kolf: No Answer; S. F. Leitman: No Answer; K. Olivier: No Answer; K. West: Nothing to disclose

SP364

Acquired Factor V Inhibitor after Exposure to Human Thrombin

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Background/Case Studies: Acquired factor V (FV) inhibitors occur rarely and classically develop after exposure to bovine thrombin. The clinical presentation is variable, ranging from asymptomatic to significant bleeding. With the development of human-derived thrombin agents, bovine thrombin is less frequently used. We report a case of an acquired FV inhibitor that developed in a patient after exposure to human thrombin. **Study Design/Methods:** The study consisted of a case report and a review of the literature. **Results/Findings:** A 71-y-old female with a history of recurrent hemangiopericytoma involving the nasopharynx was admitted for embolization and resection of tumor. It is not known whether she was exposed to bovine thrombin during her prior hospitalizations elsewhere. On this admission, a porcine-derived gelatin paste mixed with human thrombin powder was used as a topical hemostatic agent. Postoperatively, she developed a deep venous thrombosis. She was started on intravenous heparin and warfarin. Two days after starting anticoagulation, the activated partial thromboplastin time (aPTT) was increased at 120.6 sec, and heparin was discontinued. The International Normalized Ratio (INR) was 3.34 on this same day, and warfarin was then held. The next day, the PT was 124.8 sec, INR 10.78, and aPTT 47.6 sec. The coagulopathy was attributed to depletion of vitamin K-dependent factors secondary to warfarin and antibiotics; therefore vitamin K was given, along with 20 units/kg of prothrombin complex concentrate (PCC) and 2 units of fresh-frozen plasma (FFP). About 15 h later, the PT, INR, and aPTT normalized. In order to resume anticoagulation, enoxaparin at 1 mg/kg twice daily was started. Within hours, the PT and aPTT started to again increase. The aPTT corrected on mixing with normal plasma, but the PT did not, which suggests the presence of an inhibitor. FV activity was reduced at 1% of normal. The FV inhibitor level was 1.4 Bethesda units/mL. The thrombin time was >300 sec (normal 19.1-26.1 sec). Despite the profound coagulation tests, the patient did not bleed, but anticoagulation was discontinued. An inferior vena cava filter was inserted. Over the next 2 wk, the patient was observed without anticoagulation, and her INR decreased to 1.1. **Conclusion:** Our review of the literature revealed only one prior reported case of FV inhibitor after exposure to human thrombin. No bleeding complications occurred in our patient, but the treatment of bleeding secondary to acquired FV inhibitors would include platelets, FFP, PCC, and recombinant factor VIIa. Topical human thrombin may pose a risk of acquired FV inhibition, especially in patients with prior exposure to bovine thrombin. Additional studies will be needed to confirm this association.

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Transfusion-transmitted Infectious Diseases: Viruses and Bacteria, Parasites, and Prions

SP365

Trends in the Incidence of Transfusion of Bacterially Contaminated Platelets over Two Decades at an Academic Medical Center

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Background/Case Studies: Several processes have been implemented to decrease the incidence of bacterial contamination of platelets, including shortening outdate time to 5 d in 1986, requirement for detection methods in 2002, culture of 24-h-old apheresis products in 2004, prepooling and culture of 24-h-old pooled products from 2007, diversion of the first part of the collection and improved skin prep procedures from 2006, and FDA approval of a point-of-issue test in 2007. As active surveillance for bacterial contamination of platelet products has been carried out at our institution since 1991, the relationships between the incidence of bacterial contamination and these interventions were assessed. **Study Design/Methods:** Platelet concentrates (PCs) are prepared by two regional blood centers supplying our 947-bed academic medical center transfusion service. These are prepared either by apheresis (AP) or from whole blood-derived (WBD) platelets, using the pool-and-store method for WBD since 2007. Samples of the apheresis and WBD pools, prepared by both blood centers, have been sampled for bacterial contamination since 2003 and 2007, respectively, with testing by an FDA-cleared culture system. Since 1991, all platelets released from our transfusion service have undergone a surveillance culture at time of issue, with inoculation of 0.1 mL of product on a blood agar plate that is incubated aerobically at 35°C for up to 48 h and quantitation of any positive cultures. **Results/Findings:** During 1991-1999, contamination rates were 2,523/million (29/11,496) for WBD pools and 393/million (10/4,241) for AP ($p < 0.001$). Introduction of prepooling WBD PC in 2007 was associated with a significant decrease in the contamination rate of WBD pools, from 2,358/million (10/4,241) in 2004-2006 to 308/million (4/12,974) in 2007-2013 ($p < 0.001$). For AP units, no significant change was noted following the introduction of early culture in 2004, although the spectrum of contaminants did change, with the proportion of Gram-negative contaminants decreasing and reactions frequently delayed. There was, however, a downward trend in the contamination rate of AP during 2007-2013 (456/million; 18/39,489) compared to 2004-2006 (690/million; 8/11,589), but this trend was not significant ($p = 0.45$). **Conclusion:** These findings document the continuing occurrence of "breakthrough" cases of bacterial contamination, despite early culture in both AP and pooled WBD platelets and the beneficial effect of prepooling and early culture of WBD platelets, which reduced bacterial contamination rates to levels comparable to those of AP units. Additional efforts, such as an additional culture during the storage period, point-of-issue testing, or use of pathogen inactivation, are needed to further reduce bacterial contamination of platelets.

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M. Jacobs: Verax,; Grants or Research SupportImmunetics, Grants or Research Support; Hemonetics, Grants or Research Support; R. Yomtovian: Nothing to disclose

SP366

***Brucella abortus* Contamination in a Unit of Platelets: A Case Report**

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Background/Case Studies: Brucellosis is a zoonotic disease caused by members of the *Brucella* spp.; it consists of small gram-negative coccobacilli that can be transmitted to humans by ingestion of unpasteurized bovine or goat raw milk products, direct contact with an infected animal, or inhalation of aerosols. Human-to-human transmission is extremely rare. **Study Design/Methods:** A thrombocytopenic elderly male in a community

hospital received a pool of 4 whole blood-derived platelet units (Acrodose™ Systems) later confirmed to be contaminated with bacteria, which was subsequently identified as *Brucella abortus* by a commercial lab and confirmed by the Florida Department of Health (FDOH). No adverse reaction was reported during or immediately after the transfusion. **Results/Findings:** The bacterial screening (BacT/AlertR) used by the blood center detected the bacterial contamination 2.5 d after the bottle inoculation and 12.5 h after transfusion. The blood center notified the patient's treating physician within 1 h after the BacT culture became positive, which was approximately 13.5 h after the transfusion took place. The patient was placed on broad-spectrum antibiotics. *Brucella* microagglutination testing (BMAT) conducted on serial serum samples collected over a 6-mo period and tested at the Centers for Disease Control and Protection (CDC) were all negative, and the recipient demonstrated no subsequent evidence of clinical infection. The blood center in collaboration with the FDOH was able to identify the infected donor, a young male engineer, who immigrated to the United States from Venezuela 8 mo before donation. Despite the patient's claim to feel well and healthy on the day of platelet donation, further questioning revealed a 2-d history of low-grade fever immediately following donation. His blood cultures and attempts at molecular amplification studies to reveal circulating *Brucella* microorganisms were negative. However, serologic studies revealed *Brucella* antibody titers of 1:5120 (total, IgM, and IgG) and IgG titers of 1:320 (reference value: ≤1:20). The donor was successfully treated by his primary physician. **Conclusion:** Culture-based platelet quality-control tests may identify bacterial contamination after the unit has been released and transfused, especially in cases of slow-growing bacteria such as *Brucella* spp. In this case, prompt notification of the patient's clinician, even in the absence of signs of a transfusion reaction, allowed for the timely administration of prophylactic broad-spectrum antibiotics and possible prevention of clinical disease. In addition, to the best of our knowledge, this represents the first case of *Brucella* contamination of a platelet product reported in the English literature.

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SP367

Bacterial Sepsis from Platelets in Rhode Island, 1999-2013

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Background/Case Studies: Bacterial sepsis remains a serious adverse event of platelet transfusion, despite the practice of bacterial culturing of platelet products prior to shipping. The true prevalence of this complication remains unknown, as no active surveillance is in place, and there is ongoing controversy regarding the relative safety of whole bloodderived pools versus apheresis platelets in this regard. Our medical systems account for approximately 85% of all platelets transfused in the state, and whole blood-derived pooled platelets account for 70% of all platelet doses. Since 2004, all platelet products (apheresis and whole blood-derived) must test negative by a culture technique prior to shipping. Furthermore, with the exception of urticaria as the sole manifestation, routine gram staining and bacterial culturing of all platelet products implicated in a reported transfusion reaction have been standard practice since 1999. **Study Design/Methods:** All transfusion reactions were examined for the 15-y period from 1999 to 2013, and septic reactions were identified. Septic reactions to platelets were defined as reactions in which the Gram's stain of the bag was positive, the culture of the bag was positive, and the clinical features were consistent with sepsis. Platelet products shipped from the Community Blood center were tabulated and separated into whole-blood pools (prestorage or poststorage) or apheresis platelets. Outdating was estimated at 8% for apheresis platelets and 15% for whole blood-derived platelets. **Results/Findings:** A total of five septic reactions were observed, three to whole blood- derived platelets and two to apheresis platelets. Notably, four of the five reactions were diagnosed after gram staining of the bag, and only one was suspected by the clinician. There were two associated deaths (2/5, 40%). Details of these reactions are shown in the Table. An estimated total of 50,651 whole blood-derived platelet doses were transfused, along with 23,768 apheresis platelet doses, giving an observed prevalence of 1 septic reaction per 16,901 whole-blood pools (95% CI, 1:5,777-1:81,967) and 1 septic reaction per 11,884 apheresis platelet doses (95% CI, 1:3,290-1:98,040) (p = 0.66). **Conclusion:** Our observed prevalence of septic reactions is much higher thanthat generally reported, which may reflect the practice of routinely gram-staining platelets implicated in a transfusion reaction. The risk of sepsis and death was similar for the two platelet product types.

Disclosure of Commercial Conflict of Interest

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Disclosure of Grants Conflict of Interest

R. Clubwala: Nothing to disclose; J. Sweeney: Nothing to disclose

TABLE.

Year	Recipient Age/Sex	Recipient Diagnosis	Pretransfusion Platelet Count (x 109/L)	Product	Storage Age	Max Temperature (F)	Clinical Features	Organism	Was Product Pre-screened for Bacteria?	Outcome
1999	43M	MDS	45	Apheresis Platelets	5 days	100	Chills Backache Dyspnea Cyanosis	Staph Epidermis	No	Non-fatal
2000	74F	AML	38	Pooled Platelets	2 days	101	Chills Dyspnea Cyanosis	Serratia Marcescens	No	Fatal
2002	65F	AML	15	Pooled Platelets	5 days	101	Rigors	Staph Epidermis	No	Non-fatal
2007	62F	AML	5	Apheresis Platelets	5 days	101.3	Chills	Staph Aureus	Yes	Fatal
2011	68M	Aplastic Anemia	14	Prepooled Platelets	5 days	104	Rigors	Staph Epidermis	Yes	Non-fatal

SP368

Characterization of *Staphylococcus epidermidis* Strains Isolated from Contaminated Platelets at Canadian Blood Services from 2008 to 2013

S Maliya[1], M Taha[1], S Ramirez-Arcos[1]. *[1]Canadian Blood Services, Ottawa, ON, Canada*

Background/Case Studies: Platelet concentrates (PCs) are highly susceptible to bacterial contamination due to their storage at 20-24°C under agitation. Our laboratory has shown that the major platelet contaminant, *Staphylococcus epidermidis*, forms surface-attached aggregates (biofilms) during platelet storage. Biofilms are more likely to escape detection during platelet screening and to have increased pathogenicity. This study was aimed at determining the prevalence of biofilm-forming *S. epidermidis* isolated from contaminated buffy coat pools and apheresis PCs at Canadian Blood Services over a 5-y period. **Study Design/Methods:** Rates of bacterial contamination in PCs from 2008 to 2013 were obtained, and 24 *S. epidermidis* strains isolated during that period were used in this study. Standard biofilm formation assays were performed in trypticase soy broth supplemented with glucose (TSBg) with overnight incubation at 37°C. A subgroup of 9 strains, including 7 biofilm-negative and 2 biofilm-positive isolates in TSBg, were tested for biofilm formation in fresh (<5-d-old) PCs under platelet storage conditions. Biofilm production was quantified by using a crystal violet method, and slime production was measured by the appearance of black colonies on Congo red agar. The presence of biofilm-associated genes (*icaA* and *icaD*) was also determined by PCR. All assays

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研究報告の概要	使用上の注意記載状況・その他参考事項等
背景：英国におけるウシ海綿状脳症（BSE）、および変異型クロイツフェルト･ヤコブ病（CJD）の出現により、医原性の伝播について懸念があり、このリスクを管理する試みは他にない。 **目的**：CJD のインシデント管理、および医原性伝播を減らすための戦略としての「リスクが高い」個人の通知を記述し、見直すことにある。 **方法**：医原性 CJD 伝播、CJD 事象パネルの役割、CJD 発生報告の数と性質、および 2012 年中頃まで「リスクが高い」とみなされた個人の記述。 **調査結果**：CJD の 77 の英国の症例は、ヒト由来成長ホルモン（64 例）、硬膜移植片（8 例）、輸血（4 例）、および血漿製品（1 例）のレシピエント間で、医原性伝播から生じた可能性が高い。伝播を抑制するために、パネルは 490 の事象を見直し、血液および血漿製品のルックバック、回収および手術器具の保存管理と処理について助言した。その上、パネルの助言に関して、およそ 6,000 人の無症候性の人々は、彼らが CJD のリスクが高いことを知り、公衆衛生上の注意を払うことを求められている。 **結論**：CJD の医原性伝播を減らすための戦略は、科学的な不確実性の前後関係の中で策定された。伝播事象の希少性は、その事例に関連した無視できる伝播リスクを示す、或いは（感染がまだ起こっていない、或いはまだ検出されていない）CJD の長期の潜伏期間および無症状表現型を示すのかもしれない。感染有病率の推定、スクリーニング検査、或いは手術器具の汚染除去の改善を含む科学的な発展は、将来のリスク管理を変えるかもしれない。	2. 重要な基本的注意 (1)略 1)略 2)現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的な vCJD 等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

報告企業の意見	今後の対応
血漿分画製剤は理論的なvCJD伝播リスクを完全には排除できないため、投与の際には患者への説明が必要である旨を2003年5月から添付文書に記載している。2009年2月17日、英国健康保護庁(HPA)はvCJDに感染した供血者の血漿が含まれる原料から製造された第Ⅷ因子製剤の投与経験のある血友病患者一名から、vCJD異常プリオン蛋白が検出されたと発表したが、日本血液製剤機構の原料血漿採取国である日本及び米国では、欧州滞在歴のある献（供）血希望者を一定の基準で除外している。また、国際獣疫事務局（OIE）により、米国は我が国と同じく「無視できるBSEリスク」の国に認定されたことからも、原料血漿中に異常型プリオン蛋白が混入するリスクは1999年以前の英国に比べて極めて低いと考える。	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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Managing the risk of iatrogenic transmission of Creutzfeldt–Jakob disease in the UK

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SUMMARY

Background: With the emergence of bovine spongiform encephalopathy (BSE) and variant Creutzfeldt–Jakob disease (CJD) in the UK, there is concern about iatrogenic transmission, and the approach to managing this risk is unique.
Aim: To describe and review CJD incident management and the notification of individuals 'at increased risk' as a strategy for reducing iatrogenic transmission.
Methods: A description of iatrogenic CJD transmission, the CJD Incidents Panel's role, the number and nature of CJD incidents reported and the individuals considered 'at increased risk' by mid-2012.
Findings: Seventy-seven UK cases of CJD are likely to have resulted from iatrogenic transmission, among recipients of human-derived growth hormone (64 cases), dura mater grafts (eight cases), blood transfusions (four cases) and plasma products (one case). To limit transmission, the Panel reviewed 490 incidents and advised on look-backs, recalls of blood and plasma products, and quarantining and disposing of surgical instruments. Additionally, on Panel advice, around 6000 asymptomatic individuals have been informed they are at increased risk of CJD and have been asked to follow public health precautions.
Conclusion: The strategy to reduce iatrogenic transmission of CJD has been developed in a context of scientific uncertainty. The rarity of transmission events could indicate that incident-related exposures present negligible transmission risks, or – given the prolonged incubation and subclinical phenotypes of CJD – infections could be yet to occur or have been undetected. Scientific developments, including better estimates of infection prevalence, a screening test, or improvements in decontaminating surgical instruments, may change future risk management.

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Introduction

In the late 1990s, with fears of a large epidemic of variant Creutzfeldt–Jakob disease (vCJD) in the UK following widespread exposure to bovine spongiform encephalopathy (BSE), the need for effective measures to reduce transmission of both vCJD and CJD through healthcare procedures (iatrogenic transmission) was recognized by the UK Health Departments.

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Whereas there have been many fewer vCJD cases than initially feared, and although the annual incidence of vCJD has declined since 2000, the continued incidence of sporadic and genetic CJD (Figure 1) and possible subclinical vCJD infections give serious cause for concern about iatrogenic transmission.[1–4]

All forms of CJD (variant, sporadic, and inherited prion disease) could be transmissible through the re-use of instruments that have had contact with potentially infectious tissue during specific procedures, as prions are resistant to conventional decontamination processes. In vCJD as tissue infectivity is more widespread, more procedures pose a risk of transmission.[5] Tissue and organ donations from infected individuals are another potential transmission route. Transmission of sporadic CJD has been reported among recipients of tissue transplants (dura mater, corneas) and of human-derived pituitary hormones (growth hormone and gonadotrophin).[6–8] Blood and plasma products are an additional transmission route in vCJD only.[9–14]

The UK strategy to reduce secondary transmission has focused on measures to safeguard donated blood and plasma products from vCJD. The strategy includes the introduction of universal leucodepletion and the importation of plasma to manufacture plasma products. To reduce the risk associated with surgery, guidance on infection control, instrument traceability and neurosurgical practice has been introduced.[15–17]

This paper aims to describe and review CJD incident management and the notification of individuals 'at increased risk' as a strategy for reducing iatrogenic transmission. This key aspect of the UK strategy is overseen by the Advisory Committee on Dangerous Pathogens Transmissible Spongiform Encephalopathy Risk Management Subgroup and, until recently, its associated committee, the CJD Incidents Panel.

Methods

Role of the CJD Incidents Panel

The CJD Incidents Panel (the Panel), which sat from 2000 to 2013, was composed of scientific and medical experts, ethicists, and lay people. The Panel was responsible for advising healthcare providers on managing incidents in which individuals may have been exposed to CJD risks through healthcare.[18] In March 2013 the Panel was dissolved, and responsibility for investigating, assessing, and managing CJD incidents (and where appropriate notifying patients) now rests with local hospitals and healthcare providers, health boards, and health protection teams. Public Health England provides assistance and advice as required.

The Panel also defined categories of individuals who can become 'at increased risk' following iatrogenic exposure. Individuals are 'at increased risk' if they are considered to have ≥1% risk of CJD in addition to the background risk in the UK population, following specific iatrogenic exposures, or if they are at risk of genetic forms of CJD. The 1% threshold level is used as a cut-off for implementing public health precautions and is not intended to be a precise measure of an individual patient's risk. The ten iatrogenic exposures which have led to individuals being considered 'at increased risk' of CJD are described in Table I.

Blood incidents

Blood and plasma are only considered transmission routes for vCJD. Blood incidents occur when an individual diagnosed with clinical vCJD previously donated or received blood components. This is identified by NHS Blood & Transplant (NHSBT) and the National CJD Research and Surveillance Unit, who run the Transfusion Medicine Epidemiological Review (TMER) study. Three patient groups are considered to have an increased vCJD risk following blood incidents and should be notified: recipients of blood from donors who later develop vCJD; blood donors to vCJD cases, and recipients of blood from donors to vCJD cases.[19–22] The latter two groups are considered 'at increased risk' as the donor to a vCJD case is considered to be a potential source of the recipient case's infection, and, if so, would be incubating the infection themselves and could have infected other blood recipients.

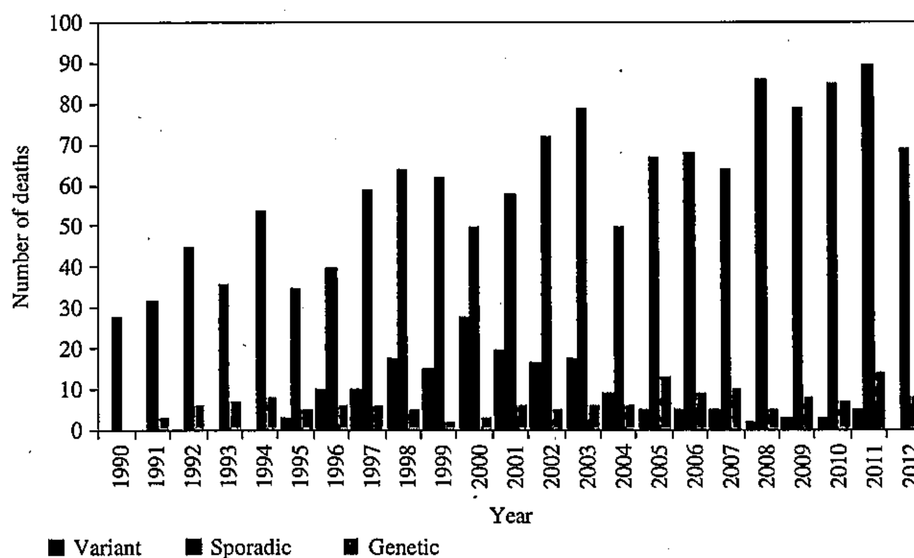

Figure 1. Deaths from Creutzfeldt–Jakob disease (CJD) in the UK 1990–2012 (confirmed and probable diagnoses). Data source: UK National CJD Research & Surveillance Unit. Data correct on 3 December 2012.

Table I
Individuals at increased risk of Creutzfeldt–Jakob disease (CJD) in the UK resulting from iatrogenic exposures (to mid-2012)

Exposure category	No. identified as 'at increased risk'	No. informed they are 'at increased risk'[a]	No. of CJD cases diagnosed	Asymptomatic infections detected at postmortem
Recipient of blood from vCJD case	67	27	3	1
Blood donor to vCJD case	112	107	0	0
Other recipient of blood donors to vCJD case	34	32	0	0
Plasma product recipient (non-bleeding disorders)	11	10	0	0
Surgical contacts of CJD case[b]	154	129	0	0
Highly transfused patient (recipient of blood from >80 donors identified at pre-surgical assessment)	10	9	0	0
Patient with bleeding disorder who received UK-sourced plasma products[c]	3872	N/K	0	1
Recipient of human-derived growth hormone[d]	1883	N/K	64	0
Recipient of human-derived gonadotrophin hormone (UK pre-1974)	Around 300	N/K	0	0
Patient who had surgery on brain or spinal cord before 1992 and may have received a human-derived dura mater graft[e]	N/K	N/K	8	0
Total	At least 6443		75	2

vCJD, variant Creutzfeldt–Jakob disease; N/K, not known.

[a] The number of individuals identified as having an increased risk of CJD is larger than the number informed of their risk, either because of deaths before notification or because a local decision was taken not to inform the individual. In most cases, particularly for the 'at increased risk' recipients of blood from vCJD cases, the discrepancy is because people died before notification.

[b] This excludes 38 patients who were previously considered 'at increased risk' and were subsequently denotified.

[c] Data source: UK Haemophilia Centres' Doctors' Organisation (UKHCDO). These are minimum numbers. Central reporting for bleeding disorder patients is incomplete, and some patients have opted out of the central UKHCDO database. Individual haemophilia centres were asked to send out standardized letters of notification to all their 'at increased risk' patients, but the exact number of patients who received these letters, and who are therefore aware of their risk, is not known.

[d] Data source: Institute of Child Health (ICH). A small number of 'at increased risk' growth hormone recipients is not included in the ICH study, so the true number 'at increased risk' will be greater. The exact number of growth hormone recipients in the ICH study currently aware of their risk is not known — due to their age at the original notification, many were informed indirectly by their parents.

[e] Data source: Heath *et al.*, who reported seven cases.[8] One further case was reported to the authors by the National CJD Research and Surveillance Unit (personal communication), bringing the total to eight.

Plasma incidents

Plasma incidents arise when an individual diagnosed with vCJD donated blood used to manufacture pooled plasma products. Depending on the potential vCJD infectivity of each product batch and the dose received, recipients may have been exposed to an infection risk.[23] Given considerations of dose and the expectation of many more implicated batches, in 2004 all recipients of UK sourced clotting factors between 1980 and 2001 were notified that they were 'at increased risk' of vCJD, irrespective of whether they were known to have received implicated batches.[24,25]

Surgical incidents

Surgical incidents occur when a patient with or 'at increased risk' of any type of CJD undergoes surgery without appropriate infection control. This may occur if the operation was performed before CJD was diagnosed, but when tissue infectivity may have been present, or before the patient was identified as 'at increased risk' of CJD. The Panel advised and set precedent on the possible risk to other patients by considering the index patient's diagnosis; the timing of the procedure in relation to symptom onset; the tissues involved; the use and decontamination of instruments after the procedure; and the procedures performed on the contacts.[15,26]

Notifying individuals 'at increased risk' of CJD

The basis for assessing individual risk varies between exposures, and a series of risk assessments underpins the risk management decisions taken.[19,21–23,26,27] The Panel/Advisory Committee on Dangerous Pathogens Transmissible Spongiform Encephalopathy Risk Management Subgroup advise that identified individuals and their clinicians should be informed of their exposure and the type of CJD to which they have been exposed, so that public health actions to limit onward transmission may be taken. When patients are assessed to be 'at increased risk', their general practitioner (GP) is contacted together with other specialist clinicians as appropriate, alerting them to their patients' risk and asking that they contact the patients and arrange an appointment to inform them of their risk. In some notifications, contact has been made directly with the individuals to inform them of their risk. The CJD Section at Public Health England [PHE; formerly the Health Protection Agency (HPA)] now provides guidance throughout this process and supplies information leaflets for patients and clinicians. The arrangements for patient notification vary and generally involve extensive discussion between the CJD Section, the appropriate consultant in health protection, the local hospital, the patient's GP and any specialist clinicians, to discuss whether there are any strong clinical or psychological objections to notification, to identify who is best placed to inform

the patient, and how this should be done. Appropriate arrangements are also made to provide additional support, such as appointments with CJD specialists, counsellors, and the CJD Support Network telephone helpline.

'At increased risk' individuals are asked to take the following precautions once they are informed of their risk: not to donate blood, organs or tissues, and to alert healthcare staff before undergoing invasive procedures so that infection control guidance can be followed. GPs are also asked to alert healthcare staff prior to their 'at increased risk' patients undergoing invasive procedures. This complements the guidance that all hospitals should assess patients for their CJD risk prior to surgery.[15]

The HPA and others established long-term public health surveillance to follow up 'at increased risk' individuals to determine whether they develop CJD, which continues through PHE. Research studies also investigate whether 'at increased risk' individuals are asymptomatically infected with CJD, by testing tissue samples, and conducting postmortems.

Ethics approval

The HPA project 'Enhanced surveillance of individuals at increased risk of CJD' has received ethical approval from the National Research Ethics Service, London Research Ethics Committee (London REC) number: 07/H0718/79, awarded in 2008.

Results

CJD incidents reported to the Panel

Between 2000 and the end of June 2012, 452 surgical incidents, 29 blood incidents and 11 plasma incidents were reported to the CJD Incidents Panel. The Panel reviewed all of these incidents and advised on whether patients had been put at increased risk or whether products or instruments should be removed from use to prevent onward transmission. The Panel also advised on several patient notification exercises, described below.

Patients exposed to 'an increased risk' of CJD following CJD incidents

At least 6443 individuals have been identified as 'at increased risk' of CJD following healthcare procedures (Table I).

Recipients of human-derived hormones and tissues

In all, 1883 individuals received human-derived growth hormone and around 300 are thought to have received human gonadotrophin hormone. The number of individuals who received human-derived dura mater grafts during spinal surgery is unknown. These individuals are considered 'at increased risk' of iatrogenic CJD, but not of vCJD, as the practices of using human-derived growth hormone, gonadotrophin, and dura mater grafts were stopped prior to the emergence of vCJD. No other organ or tissue incidents have been identified.

Individuals put at increased risk through blood transfusions

The TMER study identified that 18 vCJD patients had donated blood that was used to treat 67 patients (Figure 2). A total of 112 donors who donated blood to 10 patients with vCJD and 34 individuals who also received blood from these donors were identified.

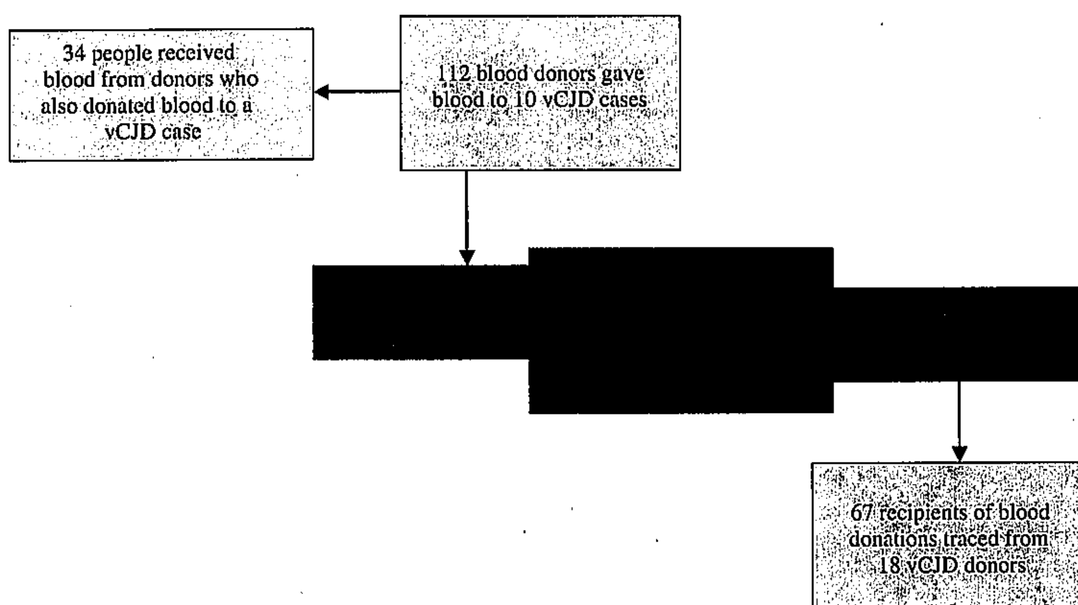

Figure 2. Patients at increased risk traced to a variant Creutzfeldt–Jakob disease (vCJD) case through blood donations. Data source: Transfusion Medicine Epidemiological Review (TMER) study.

Recipients of plasma products

At least 3872 bleeding disorder patients are considered 'at increased risk' of vCJD, according to the UK Haemophilia Centres Doctor's Organisation [data correct on 30 June 2012]. Nearly 800 received plasma products from one of 25 implicated batches to which 11 donors who later developed vCJD contributed plasma. A further 11 patients also received products from these implicated batches for other conditions. Products from these implicated batches were recalled, and, as some products had been exported, appropriate information was forwarded to relevant authorities and international agencies, to enable them to make their own risk management decisions.[25,28]

Individuals exposed through surgery

One hundred and fifty-four individuals are considered 'at increased risk' of various forms of CJD following surgery.

Secondary cases of CJD in the UK

Seventy-seven secondary transmission events have been identified in the UK (Table I). There have been 75 clinical cases of CJD, with 64 cases among recipients of human-derived growth hormone, eight cases in recipients of dura mater grafts, all of whom developed either sporadic or familial CJD, and three cases of vCJD developed in recipients of blood from donors who later developed vCJD. Of the 33 post-mortems conducted on 'at risk' individuals, most of which have been performed by CJD specialists at the NCJDRSU and the National Prion Clinic, there have been two cases of asymptomatic vCJD infection detected, one in a blood transfusion recipient and one in a recipient of implicated plasma products (data correct on 8 November 2013).

Risk of transmission associated with different exposures

Since monitoring began in 2000, transmission of CJD has been recorded in four of the 10 healthcare exposure routes considered to put individuals at increased risk of CJD, described in Table I. The proportion of 'at increased risk' individuals infected with CJD (both clinical cases and asymptomatic infection) is 6.0% among recipients of blood transfusions from vCJD cases, 3.4% among growth hormone recipients, and <0.01% among plasma product recipients. In six of the risk groups, no transmission has been detected in the UK since 2000 (Table I).

Patient notifications

The intention is that the majority of individuals 'at increased risk' of CJD should have been informed of their exposure. There is some uncertainty over the exact number of bleeding disorder patients or recipients of growth hormone notified, as central reporting of notified bleeding disorder patients is incomplete and most growth hormone recipients were notified indirectly through their parents. We are unaware of the number of notified recipients of human gonadotrophin hormone or possible dura mater drafts. In addition, given the uncertainty surrounding transmission routes, advances in scientific understanding may lead to a revision of risk assessments and could affect whether a particular exposure is considered to put someone at increased risk. Following changes in the assessment of tissue infectivity and decontamination effectiveness, 38 patients previously considered 'at increased risk' following certain types of surgery have been informed they are no longer 'at increased risk'.

Advice on managing potentially contaminated surgical instruments

Of the 452 surgical incidents reported to the Panel from 2000 to the end of June 2012, the Panel advised immediate instrument quarantine in 160 incidents (35%) pending further investigation. In 90 (56%) of these 160 incidents, after investigation the Panel advised that the instruments should be permanently removed from general use (they could be quarantined, kept for exclusive use of the index patient, refurbished, or destroyed) and that in the other 70 incidents the instruments could be returned to general use. In some incidents, whereas instruments may have originally posed an infection risk, no action was taken as the instruments were untraceable or, as in at least 43 incidents, the instruments had been through a threshold number of decontamination cycles (10 for medium infectivity tissue and 20 for high infectivity tissue) prior to the incident reporting, and were no longer considered an infection risk.

Discussion

The potential for ongoing secondary transmission of CJD through medical procedures remains a serious concern in the UK. Strategies to reduce the risk of iatrogenic CJD transmission, including the operation of the CJD Incidents Panel, have been in place for more than a decade. The CJD Incidents Panel was dissolved at the end of March 2013. Local teams are now responsible for investigating, assessing, and managing CJD incidents and any patient notifications using guidance that reflects the precedents set by the Panel.

This is the first time that the UK strategy of reducing iatrogenic CJD transmission through incident management and patient notifications has been described in the scientific literature. The data presented in this paper provide a unique insight into the risk associated with different potential routes of iatrogenic transmission of CJD.

However, interpretation of this data on outcomes, number of incidents, and incidence of CJD infection in the 'at increased risk' cohort is difficult. The Panel was an advisory body: local clinical teams were not required to report incidents or to follow Panel advice. The number of surgical incidents may therefore be underestimated. CJD is a rare disease with a long incubation period. 'At increased risk' patients often have a relatively short life expectancy given their medical conditions. Diagnosing asymptomatic infection requires testing specific tissues that are most readily available at postmortem. The numbers in the exposure categories are small, and few postmortems have been conducted when 'at increased risk' individuals die; therefore some asymptomatic infections may have been missed.

It is not yet clear whether most of the putative exposure categories, particularly those where transmission events have

not been identified, are indeed risks for CJD transmission, and therefore warrant the precautions described above.

The current risk management strategy is based on risk assessment modelling, which has generally used precautionary input values. Information on the outcomes of patient cohorts exposed to possible iatrogenic risks can inform the interpretation and development of these risk assessments. Interpretation of the outcomes of these cohorts requires consideration of the small sample sizes, short follow-up periods and low uptake of postmortems. Given these uncertainties, the effectiveness of the risk management strategy adopted in the UK cannot be fully evaluated in order to determine whether the significant costs, to both individuals 'at increased risk' and healthcare providers, are justified by preventing new CJD cases.

A number of potential scientific developments may affect national CJD health protection strategy in the future. These include: the results of ongoing studies investigating UK vCJD infection prevalence; greater understanding of iatrogenic transmission through the surveillance of individuals 'at increased risk'; the development and implementation of effective prion decontamination; and the development and implementation of an effective test for asymptomatic prion infection. As evidence in these fields accrues and uncertainties diminish, a less precautionary approach to managing iatrogenic CJD risks may be possible.

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Conflict of interest statement
None declared.

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References

1. Cousens SN, Vynnycky E, Zeidler M, Will RG, Smith PG. Predicting the CJD epidemic in humans. *Nature* 1997;385:197–198.
2. Ghani AC, Ferguson NM, Donnelly CA, Anderson RM. Predicted vCJD mortality in Great Britain. *Nature* 2000;406:583–584.
3. National CJD, Surveillance and Research Unit. *Nineteenth annual report 2012: Creutzfeldt–Jakob disease surveillance in the UK.* Edinburgh: NCJDRU; 2012.
4. Clarke P, Ghani AC. Projections of the future course of the primary vCJD epidemic in the UK: inclusion of subclinical infection and the possibility of wider genetic susceptibility. *J R Soc Interface* 2005;2:19–31.
5. Ironside JW, McCardle L, Horsburgh A, Lim Z, Head MW. Pathological diagnosis of variant Creutzfeldt–Jakob disease. *Acta Pathol Microbiol Immunol Scand* 2002;110:79–87.
6. Brown P, Preece M, Brandel JP, *et al.* Iatrogenic Creutzfeldt–Jakob disease at the millennium. *Neurology* 2000;55:1075–1081.
7. Swerdlow AJ, Higgins CD, Adlard P, Jones ME, Preece MA. Creutzfeldt–Jakob disease in United Kingdom patients treated with human pituitary growth hormone. *Neurology* 2003;61:783–791.
8. Heath CA, Barker RA, Esmonde TF, *et al.* Dura mater-associated Creutzfeldt–Jakob disease: experience from surveillance in the UK. *J Neurol Neurosurg Psychiatry* 2006;77:880–882.
9. Hunter N, Foster J, Chong A, *et al.* Transmission of prion diseases by blood transfusion. *J Gen Virol* 2002;83:2897–2905.
10. Llewelyn CA, Hewitt PE, Knight RS, *et al.* Possible transmission of variant Creutzfeldt–Jakob disease by blood transfusion. *Lancet* 2004;363:417–421.
11. Hewitt PE, Llewelyn CA, Mackenzie J, Will RG. Creutzfeldt–Jakob disease and blood transfusion: results of the UK Transfusion Medicine Epidemiological Review study. *Vox Sang* 2006;91:221–230.
12. Peden A, McCardle L, Head MW, *et al.* Variant CJD infection in the spleen of a neurologically asymptomatic UK adult patient with haemophilia. *Haemophilia* 2010;16:296–304.
13. Peden AH, Head MW, Ritchie DL, Bell JE, Ironside JW. Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. *Lancet* 2004;364:527–529.
14. Wilson K, Code C, Ricketts MN. Risk of acquiring Creutzfeldt–Jakob disease from blood transfusions: systematic review of case–control studies. *BMJ* 2000;321:17–19.
15. Advisory Committee on Dangerous Pathogens Transmissible Spongiform Encephalopathy Risk Management Subgroup (ACDP TSE RMS). *Transmissible spongiform encephalopathy agents: safe working and the prevention of infection.* London: Department of Health; 1998.
16. Department of Health. *Health Service Circular 2000/032: decontamination of medical devices.* London: DH; 2000.
17. National Institute for Health and Clinical Excellence. *Patient safety and reduction of risk of transmission of Creutzfeldt–Jakob disease (CJD) via interventional procedures.* London: NICE; 2006.
18. CJD Incidents Panel. *CJD Incidents Panel Framework Document: Management of possible exposure to CJD through medical procedures.* London: Health Protection Agency; 2005.
19. Department of Health. *Assessing the implications for blood donors if recipients are infected with vCJD.* London: DH; 2005.
20. Hewitt PE, Moore C, Soldan K. vCJD donor notification exercise: 2005. *Clin Ethics* 2006;1:172–178.
21. Department of Health. Other recipients of blood from donors to vCJD cases: implications of risk assessment and options for action. Paper for CJD Incidents Panel, 7 September 2005.
22. Bennett PG, Dobra S, Gronlund J. The implications for blood donors if a recipient develops vCJD. *OR Insight* 2006;19:3–13.
23. Det Norske Veritas Consulting. *Risk assessment of exposure to vCJD infectivity in blood and blood products.* London: DNVC; 2003.
24. CJD Incidents Panel. *Fourth annual report, 1st September 2003 to 31st August 2004.* London: Health Protection Agency; 2004.
25. Millar CM, Connor N, Dolan G, *et al.* Risk reduction strategies for variant Creutzfeldt–Jakob disease transmission by UK plasma products and their impact on patients with inherited bleeding disorders. *Haemophilia* 2010;16:305–315.
26. Department of Health. *Risk assessment for transmission of vCJD via surgical instruments: a modelling approach and numerical scenarios.* London: DH; 2001.
27. Department of Health. *Assessing the risk of vCJD transmission via surgery: an interim review.* London: DH; 2005.
28. Health Protection Agency. *Notification of potential exposure to variant Creutzfeldt–Jakob disease through plasma products: report to the CJD Incidents Panel by the HPA.* London: HPA; 2004.

医薬品 研究報告 調査報告書

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研究報告の概要

○HIV感染患者におけるE型肝炎ウイルス(HEV)重複感染の発生率と自然経過
目的: スペインのHIV感染者におけるHEV感染の有病率、発生率及び関連因子を調べ、HIV/HEV重複感染の自然経過を観察するため、前向きコホート研究を行った。
方法: 613人のHIV感染者を対象に、調査開始時と2年後の血清中のHEV IgG抗体の存在を調べ、陽性となったサンプルはHEV RNAについても調べた。セロコンバージョンした患者では、肝機能検査結果の変化、調査開始時と最終時点との間に採取したサンプルにおけるHEV IgM抗体及びHEV RNAを分析した。
結果: 調査開始時に162人(26%)がHEV IgG抗体陽性であった。HEV感染率は女性と比べ男性の方が多く(28%vs. 18%; P=0.022)、年齢とともに直線的に上昇した(40歳未満で16%、40～49歳で26%、50歳以上で44%; P=0.000002)。1人が調査開始時にHEV RNAを有していた。HEV抗体陰性の18人(4%)が追跡期間中にセロコンバージョンを起こした。セロコンバージョンを予測する因子はなかった。これらの患者のうち1人が急性肝炎を発症し、4人が軽微な高トランスアミナーゼ血症を来した。セロコンバージョンから慢性HEV感染に移行した例はなかった。調査開始時にHEV抗体陽性であった患者の19%にセロリバージョン(抗体の消失)が見られた。これらの患者は、抗体陽性のままの患者と比べてCD4細胞数が500/μL未満である割合が高かった(77%vs. 46%; P=0.004)。
結論: スペインのHIV感染者にはHEV感染が多く、年齢とともに増加するが、慢性感染への移行は稀である。急性HEV感染症例は、臨床的、生化学的に発症せず、見過ごされることが多い。

使用上の注意記載状況・その他参考事項等

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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
スペインにおいてHIV感染者におけるHEV重複感染は多く、年齢とともに増加するが、慢性感染へ移行することは稀であるとの報告である。	日本赤十字社では、厚生労働科学研究「経口感染によるウイルス性肝炎(A型及びE型)の感染防止、病態解明、遺伝的多様性及び治療に関する研究」班と共同して、献血者におけるHEV感染の疫学調査を行っている。HEV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。

Incidence and natural history of hepatitis E virus coinfection among HIV-infected patients

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Objectives: To know the prevalence, incidence and factors associated with hepatitis E virus (HEV) infection in HIV-infected individuals in Spain, as well as to provide information on the natural history of HIV/HEV coinfection.

Design: Prospective cohort study.

Methods: Serum HEV IgG antibodies were tested in 613 HIV-infected patients at baseline and 2 years thereafter. Positive samples were tested for HEV-RNA. In patients with seroconversion, changes in liver function tests, serum HEV IgM antibodies and HEV RNA in samples collected between the baseline and the final time points were analyzed.

Results: One hundred and sixty-one (26%) patients tested positive for serum HEV IgG antibodies at baseline. HEV exposure was more common in men than in women (28 vs. 18%; $P = 0.022$) and increased linearly with age: 16, 26 and 44% in younger than 40, from 40 to 49 and older than 50 years, respectively ($P = 0.000002$). One patient bore the serum HEV-RNA at baseline. Eighteen (4%) HEV-seronegative patients seroconverted during the follow-up. None of the factors predicted seroconversion. One patient with seroconversion developed acute hepatitis and four mild hypertransaminasemia without another apparent cause. No case of seroconversion evolved to chronic HEV infection. Seroreversion was detected in 19% of the HEV-seropositive patients at baseline. Patients with seroreversion showed more commonly $CD4^+$ cell counts below 500 cells/μl than those who remained seropositive (77 vs. 46%; $P = 0.004$).

Conclusions: Exposure to HEV among HIV-infected patients in Spain is very common, and this increases with age. Evolution to chronic infection is extremely unusual. Most cases of acute HEV infection seem to be clinically and biochemically unexpressive, therefore going unnoticed. © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins

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Keywords: acute hepatitis, epidemiology, hepatitis E virus, HIV, liver disease

Introduction

Hepatitis E virus (HEV) infection has been reported to be a cause of unexplained liver disease in HIV-infected patients [1–3]. In this setting, HEV infection may become chronic and rapidly evolve to cirrhosis [3,4]. Several cross-sectional studies have reported prevalence figures of HEV seropositivity in HIV-infected patients ranging from 1.5% in Paris to 45.3% in Ghana [5–10]. Studies including a HIV-seronegative control population [5,10] did not find differences in the rate of HEV exposure between HIV-infected patients and controls. However, no longitudinal studies aimed to assess the incidence of HEV infection in HIV-infected individuals have been

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reported so far. Consequently, risk factors for this coinfection have not been established.

Little is also known about the natural history of HEV infection in HIV-coinfected patients. Few patients with positive HEV serology show detectable plasma HEV-RNA [6,7,10,11], which suggests that chronic HEV infection is uncommon, but the precise rate of evolution to chronic hepatitis E remains unclear. In acute hepatitis E, serum antibodies (both IgG and IgM) become detectable at the time of clinical onset [12]. IgM anti-HEV antibodies remain detectable for 3–12 months, whereas IgG anti-HEV antibodies persist for years [12]. However, whether HIV modifies the serology pattern in HEV infection or not is unknown. It has been suggested that most cases of autochthonous HEV infection in developed countries are subclinical [12]. But, again, information on the rate of symptomatic hepatitis E in the setting of HIV coinfection is lacking.

It is critical to gain more information on the incidence, risk factors and natural history of HEV infection in HIV-infected individuals. In fact, a HEV vaccine has shown to be effective, although it is not commercially available everywhere [12,14]. The proper selection of candidates for vaccination will require knowing the precise incidence of HEV infection in specific areas, as well as the subpopulations with the highest risk for infection. Likewise, if chronic hepatitis E was relatively common, surveillance could be warranted, since it is a potentially curable disease, which, if untreated, may evolve to end-stage liver disease. Because of these reasons, we undertook the present study, whose objectives were, on one hand, to know the prevalence, incidence and factors associated with HEV infection in HIV-infected individuals in Spain, and, on the other hand, to provide further information on the natural history of this disorder.

Methods

Design and study population

All patients belonging to a cohort of HIV-infected patients, prospectively followed at the Unit of Infectious Diseases of a tertiary-care hospital from Southern Spain, were offered to be included in this study. All those who gave informed consent were considered eligible for this study. Patients from this cohort attended the hospital at least once every 6 months. In each visit, a clinical evaluation, as well as blood tests, including HIV viral load, $CD4^+$ cell counts and liver function tests, were carried out. Similarly, a serum sample from each patient was collected and frozen at −80°C in each visit. The patients selected to participate in this study were those from this cohort who attended the outpatient clinic within the first 6 months of 2009. The first visit during this period was considered as the baseline time point. The follow-up visit for this study was the first time that the included patients were seen during the first semester of 2011. As explained below in more detail, in patients in whom HEV seroconversion was detected, data and samples collected every 6 months between the baseline and the follow-up visits were also analyzed.

Hepatitis E virus serology, polymerase chain reaction and case definitions

Hepatitis E virus serology and PCR determinations were conducted in cryopreserved specimens. All baseline and follow-up samples were tested for HEV IgG antibodies by a PE2 enzyme immunoassay (EIA) (Wantai HEV-IgG ELISA kit, Beijing Wantai Biological Pharmacy Enterprise Co. Ltd, Beijing, China). All samples were tested in duplicate and only samples showing results above the cut-off in both tests were considered positive. The presence of serum HEV-RNA was determined by a real-time PCR assay, using commercially available kits (ampliCube HEV; Mikrogen GmbH, Neuried, Germany; and QiagenOne-Step RT-PCR kit; Qiagen GmbH, Hilden, Germany) in all samples that turned out to be positive for HEV IgG antibodies.

Patients who tested positive for serum antibodies were considered as exposed to HEV. The detection of a positive EIA result in the follow-up sample in a patient seronegative at baseline was considered as seroconversion. On the contrary, when a patient exposed to HEV at baseline cleared serum HEV antibodies, the case was considered as seroreversion. Active HEV infection was diagnosed when serum HEV-RNA was detected in a patient exposed to HEV. Chronic HEV infection was considered when plasma HEV-RNA was diagnosed in a patient for more than 6 months.

In order to obtain further data about acute HEV infection, in patients with seroconversion, all cryopreserved samples taken every 6 months between the baseline and the final study time points were tested for serum IgM antibodies, also by EIA (Wantai HEV-IgM ELISA kit, Beijing Wantai Biological Pharmacy Enterprise Co. Ltd), as well as for serum HEV-RNA. In these patients, changes in liver function tests at the time when these samples had been collected were also analyzed. Levels of alanine aminotransferase (ALT) or aspartate aminotransferase (AST) higher than 40 IU/ml were considered as hypertransaminasemia. ALT or AST elevations higher than 5-fold this threshold, in patients with normal baseline figures, or 3.5-fold the baseline values in those with abnormal values, were considered as indicative of acute hepatitis.

Statistical study

The categorical variables are showed as number {percentage [95% confidence interval (CI)]} and the continuous ones as median (quartile 1–quartile 3). Frequencies were compared by the chi-square test or the Fisher's test, when there was at least one cell with an expected frequency lower than 5. The Student's *t* test was

used for comparing continuous variables following a normal distribution, and the Mann–Whitney U test when the latter condition was not met. The median was used as a cut-off value when continuous variables were categorized, unless otherwise specified. Variables associated with HEV exposure, as well as those associated with HEV seroconversion, in the univariate analysis with a P value lower than 0.2 were entered in the logistic regression analyses, adjusted for age and sex, where HEV exposure and HEV seroconversion were the dependent variables. The statistical analysis was carried out using the SPSS statistical software package release 22.0 (IBM SPSS Inc, Chicago, Illinois, USA).

Ethical issues

The study was designed and conducted following the Helsinki declaration. The Ethics committee of the Hospital Universitario de Valme approved the study. All patients gave written informed consent to participate.

Results

Features of the study population

Six hundred and thirteen (99%) out of 618 patients who were offered to participate in the study accepted to do it; 595 (97%) of them were born in Spain. Most patients (98%) were Caucasian. Four hundred and eighty-seven (79%) patients were men. The median (Q1–Q3) age of the population was 44 (39–47) years. The risks factors for HIV infection were intravenous drug use in 379 (62%) patients, male homosexual intercourse in 88 (14%), heterosexual intercourse in 130 (21%) and unknown in 16 (3%) patients. Five hundred and fifty-five participants (90%) were receiving antiretroviral therapy at the start of the study. At baseline, the median (Q1–Q3) $CD4^+$ cell count was 479 (280–669)/μl. The median (Q1–Q3) plasma level of AST and ALT were 33 (23–52) and 34 (21–58) IU/ml, respectively. A hepatic transient elastometry, carried out using a FibroScan device, was available in 218 patients at the beginning of the study; liver stiffness was higher than 7 kPa in 110 (50%) of them. The remaining characteristics of the population studied are displayed in Table 1.

Hepatitis E virus exposure at baseline

One hundred and sixty-one [26%; 95% confidence interval (CI) 23–30%] patients tested positive for serum HEV IgG antibodies by EIA at baseline. The factors associated with HEV exposure at baseline are shown in Table 1. In the multivariate analysis (Table 1), only age and male sex were independently associated with exposure to HEV. Namely, the rate of HEV exposure increased linearly with age (Fig. 1). One (0.6%; 95% CI 0.01–3.4%) exposed patient had detectable HEV-RNA in the serum.

The plasma levels of AST, ALT, gamma-glutamyl transpeptidase (GGT) and liver stiffness were similar between HEV-exposed and unexposed patients in the overall population. However, when the anti-HCV-seronegative subpopulation was considered separately, the HEV-exposed patients showed more commonly abnormal values of AST, GGT and liver stiffness (Table 2).

Hepatitis E virus seroconversion and seroreversion

Ninety-eight (16%) individuals were lost to follow-up during the study period. One hundred and forty of the remaining patients had turned out to be HEV-seropositive at baseline. Therefore, HEV seroconversion at the follow-up time point could only be assessed in 375 patients. Eighteen (4.8%; 95% CI 2.8–7.4%) of these patients had seroconverted at the follow-up time point, which yielded an average yearly seroconversion incidence of 2.4%. The rate of seroconversion tended to be higher among patients with $CD4^+$ cell counts lower than 200 cells/μl and among those whose daily alcohol intake was at least 50 g. However, no factor was independently associated with seroconversion in the multivariate analysis (Table 3). Similarly, there was no association between baseline or follow-up liver function tests and seroconversion.

Twenty-six (19%; 95% CI 12–26%) out of the 140 HEV-seropositive patients at baseline, who were analyzed at the follow-up time point, seroreverted. Among the patients with seroreversion, 20 (77%) had $CD4^+$ cell counts lower than 500/μl, whereas the corresponding figure among individuals who remained seropositive was 52 (46%) ($P = 0.004$). No other factor was associated with loss of serum HEV antibodies.

Findings in patients with seroconversion

Among the 18 patients with seroconversion, none showed clinical symptoms of acute hepatitis. Similarly, hyperbilirubinemia greater than 2 mg/dl was not observed in any of these patients during the study period. One patient with seroconversion developed transaminase elevation consistent with acute hepatitis, with no other apparent cause. No IgM anti-HEV or serum HEV-RNA was detected in this patient. Persistent or transient hypertransaminasemia, not fulfilling criteria for acute hepatitis, was detected in nine (50%) patients in the determinations performed between the baseline and the follow-up time points. Five of these patients had another cause for elevated liver function tests, specifically active hepatitis C virus infection in four cases and alcohol intake greater than 50 g/day in the remaining one. Serum HEV IgM antibodies were detected in a sample from a patient with transient hypertransaminasemia and in three consecutive samples from another one who seroconverted, but who did not show abnormal liver function tests in any of the five sequential blood samples analyzed.

Table 1. Associations between hepatitis E virus exposure and other factors at baseline ($N = 613$).

Parameters	N (%)[a]	No. (%)[b] HEV-seropositive	P univariate	Adjusted OR (95% CI)	P multivariate
Age (years)					
≤44	334 (55)	68 (20)	0.0003	1.06 (1.03–1.09)[c]	0.000004
>44	279 (45)	93 (33)			
Sex					
Male	487 (79)	138 (28)	0.022	1.71 (1.02–2.86)	0.040
Female	126 (21)	23 (18)			
Risk factor for HIV					
Male homosexual sex	88 (14)	18 (20)	0.181	1.55 (0.86–2.78)	0.144
Other	525 (86)	143 (27)			
Area of residence					
Rural	43 (7)	14 (33)	0.331	–	–
Urban	570 (93)	147 (26)			
Professional exposure to swine					
Yes	64 (10)	19 (30)	0.511		
No	549 (90)	142 (26)			
Daily alcohol intake					
<50 g	515 (84)	134 (26)	0.711		
≥50 g	98 (16)	27 (28)			
Serum anti-HCV					
Positive	417 (68)	109 (26)	0.918	–	–
Negative	196 (32)	52 (26)			
Serum HBsAg					
Positive	25 (4)	4 (16)	0.234		
Negative	588 (96)	157 (27)			
Cirrhosis[d]					
Yes	39 (18)	13 (33)	0.201	–	–
No	179 (82)	42 (23)			
CDC clinical category					
C	166 (27)	50 (30)	0.186	1.08 (0.72–1.62)	0.714
A or B	447 (73)	111 (25)			
Plasma HIV-RNA load (copies/ml)					
<20	411 (67)	108 (26)			
≥20	202 (33)	53 (26)	0.992	–	–
$CD4^+$ cell count (cells/μl)					
<200	86 (14)	18 (21)	0.225	–	–
≥200	527 (86)	143 (27)			

CDC, Centers for Disease Control and Prevention; CI, confidence interval; HBsAg, hepatitis B surface antigen; HEV, hepatitis E virus; OR, odds ratio.
[a]Percentage of the overall population.
[b]Percentage in each category.
[c]For each year older.
[d]Available in 218 patients.

In no patient with seroconversion, serum HEV-RNA was found. Conversely, HEV viremia was detected in two patients who remained HEV-seropositive throughout the follow-up, one of them being also viremic at baseline. Both patients showed abnormal liver function tests during the follow-up period.

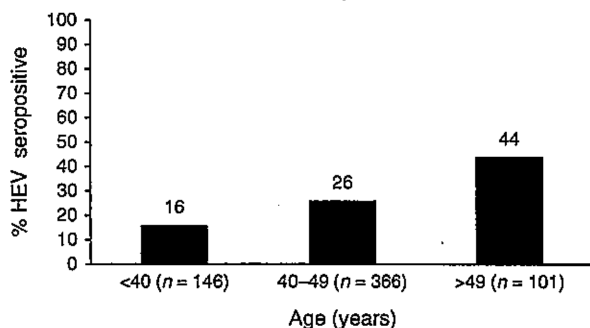

Fig. 1. Proportion of patients exposed to hepatitis E virus (HEV IgG-seropositive by EIA) at baseline according to age. P for linear trend = 0.000002.

Discussion

The results presented herein show that the rate of exposure to HEV among HIV-infected patients in southern Spain is very high, as proven by an elevated prevalence of patients bearing serum IgG antibodies against HEV, as well as by a high incidence of seroconversion for this marker. The rate of exposure to HEV infection in our area is higher than that reported in other zones in Europe [7–11]. However, the frequency of chronic HEV infection is exceedingly low. In this study, less than 1% of HEV-seropositive individuals tested positive for serum HEV-RNA at baseline and no patient evolved to chronic HEV infection after seroconversion.

Table 2. Liver function tests, liver stiffness and other parameters according to hepatitis E virus exposure at baseline in hepatitis C virus-seronegative patients ($N=196$).

Parameter	HEV-exposed ($n=52$)	HEV-unexposed ($n=144$)	P
	No. (%)	No. (%)	
ALT >40 IU/ml	11 (21)	23 (16)	0.398
AST >40 IU/ml	9 (17)	5 (3)	0.002
GGT >50 IU/ml	18 (35)	28 (19)	0.027
Liver stiffness >7 kPa[a]	4 (50)	3 (13)	0.053
Positive serum HBsAg	1 (2)	9 (6)	0.230
Daily alcohol intake ≥50 g	3 (6)	4 (3)	0.315
$CD4^+$ cell count <200 cells/μl	6 (11)	13 (9)	0.580

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; HBsAg, hepatitis B surface antigen; HEV, hepatitis E virus.
[a]Available in 31 patients.

Hepatitis E virus exposure increased with age, reaching a maximum in patients older than 50 in the population analyzed here. A similar association has been reported in some studies [10,12,13], but not in others [7,14]. Likewise, HEV exposure was more common in male patients, as it has also been reported previously [12,14]. No factor independently associated with seroconversion was identified in this study. Lack of statistical power might be the underlying cause of this finding. A trend to an association between serum HEV seroconversion and higher alcohol intake, as well as with lower $CD4^+$ cell count, was observed in the univariate analysis, but it did not remain significant in the multivariate study. With regards to alcohol intake, it might be hypothesized that alcohol-induced liver damage might increase the risk of HEV infection, since HEV seropositivity has been found to be more common in patients with chronic liver disease in other studies [11,13,15]. Whether there is an actual association between the risk of acute HEV infection and lower $CD4^+$ cell counts or not will need further studies to be clarified.

The rate of HEV antibody seroreversion was high in this study. It is well documented that HEV IgG antibodies rapidly decline in the first 3 months after the onset of acute hepatitis E, but 14 months afterwards, most patients still remain seropositive [16]. Seroreversion was associated with $CD4^+$ cell counts lower than 500 cells/μl. This

Table 3. Associations between serum hepatitis E virus IgG antibody seroconversion during the follow-up and potential predictors ($N=375$).

Parameter	N (%)[a]	No (%) with[b] HEV IgG seroconversion	P univariate	Adjusted OR (95% CI)	P multivariate
Age (years)					
≤44	220 (59)	9 (4)	0.444	1.02 (0.95–1.09)	0.560
>44	155 (41)	9 (6)			
Sex					
Male	291 (77)	15 (5)	0.773	1.04 (0.27–3.90)	0.958
Female	84 (23)	3 (4)			
Risk factor for HIV					
Male homosexual sex	63 (17)	2 (3)	0.749	–	–
Other	312 (83)	16 (5)			
Area of residence					
Rural	20 (5)	2 (10)	0.248	–	–
Urban	355 (95)	16 (4)			
Professional exposure to swine					
Yes	38 (10)	3 (8)	0.410	–	–
No	337 (90)	15 (4)			
Daily alcohol intake					
<50 g	326 (87)	13 (4)	0.071		0.118
≥50 g	49 (13)	5 (10)		2.47 (0.80–7.69)	
Serum anti-HCV					
Positive	243 (65)	11 (5)	0.737	–	–
Negative	132 (35)	7 (5)			
Cirrhosis[c]					
Yes	18 (13)	1 (6)	0.354	–	–
No	116 (87)	2 (2)			
CDC clinical category					
C	99 (26)	5 (5)	1.000	–	–
A or B	276 (74)	13 (5)			
Plasma HIV-RNA load (copies/ml)					
<20	261 (70)	12 (5)			
≥20	114 (30)	6 (5)	0.782	–	–
$CD4^+$ cell count (cells/μl)					
<200	50 (13)	5 (10)	0.076		0.123
≥200	325 (87)	13 (4)		2.38 (0.79–7.18)	

CDC, Centers for Disease Control and Prevention; CI, confidence interval; HBsAg, hepatitis B surface antigen; HEV, hepatitis E virus; OR, odds ratio.
[a]Percentage of the overall population.
[b]Percentage in each category, for each year older.
[c]Available in 134 patients.

finding is in line with that observed in the Swiss HIV cohort, where plasma HEV antibodies are less common in patients with $CD4^+$ cell counts below 100 cells/μl [9]. Also, although very infrequently [17], patients with this level of immunosuppression may show persistent plasma HEV viremia without serum antibodies [9]. Altogether, these data suggest that HIV infection, particularly when immunosuppression is advanced, impairs the humoral response against HEV, leading to a shorter duration of circulating serum antibodies. This fact might reduce the efficacy of future HEV vaccines in this subpopulation. In addition, the high rate of seroreversion leads cross-sectional studies based on HEV IgG antibody determinations to underestimate the actual frequency of prior exposure to HEV.

Patients developing seroconversion in this study were asymptomatic. After determinations conducted every 6 months, transaminase elevation consistent with acute hepatitis was observed only in one patient, whereas four out of 18 (22%) showed mild hypertransaminasemia, not meeting the criteria for acute hepatitis, and without other apparent cause. Serum HEV IgM was detected transiently in two (11%) patients and HEV-RNA in none of them. These data confirm that the vast majority of cases of infection with HEV genotype 3, the viral genotype usually detected in our area [4,7,18], are asymptomatic or go unrecognized [12,18]. The results of this study suggest that liver function test elevation, serum HEV IgM antibodies and HEV viremia might be absent, or present, just for a time too short to be detected in a semi-annual follow-up in most HIV-infected patients with acute HEV infection. An alternative explanation might be that some of the HEV IgG antibody results in seroconverters in this study were actually false-positive or false-negative. Indeed, variability in sensitivity and specificity of HEV IgG antibody test is considerable, which influences the prevalence results obtained using different procedures [19]. However, the PE2 assay used in this study has been shown to be more sensitive than other tests for the diagnosis of HEV genotype 3 infection in comparative studies [20,21], with a sensitivity of 98% [21], a specificity of 97.8% [22] and an area under the receiver-operating characteristic curve of 0.971 [20]. Because of this, it is considered appropriate for seroepidemiological studies, without requiring confirmatory testing [21]. According to these data, we believe that false-positive or negative results of serological assays are very unlikely to have accounted for the data shown in this study regarding patients with seroconversion.

There was an association between HEV seropositivity and elevated liver function tests, specifically GGT and AST, as well as with high liver stiffness. Also, both HEV exposure at baseline and seroconversion were more common among patients with cirrhosis, although statistically significant differences were not reached. These data, which are in agreement with those found in the previous studies [11,13,15], raise the question of whether liver damage is a cause or a consequence of HEV infection. In our opinion, the results of this study support that pre-existing liver disease enhances the risk of HEV infection, rather than that HEV exposure is the cause of chronic liver damage. In fact, plasma HEV was uncommonly detected in HEV-seropositive patients in this survey. Therefore, chronic HEV infection should not underlie these hepatic abnormalities. Another possible explanation for the association between HEV exposure and liver damage is that HEV-seropositive patients with markers of hepatic injury had suffered from a severe liver lesion during acute infection, of which residual chronic damage was a result, even after viral clearance. However, no patient with seroconversion showed clinical or biochemical data consistent with mild or no liver injury. This leads us to think that severe liver disease during acute HEV infection is not common among HIV-infected patients in our area.

The main limitation of this study is that the number of seroconversions detected could have been insufficient to identify some factors associated thereof. Conversely, we were able to detect an association between seroreversion and $CD4^+$ cell count lower than 500 cells/μl. This, along with the fact that no factor apart from older age and male sex were associated with exposure to HEV at baseline, lead us to think that relevant risk factors for HEV seroconversion have not been overlooked in this study. Nonetheless, further studies are required to re-assess this point. In addition, the 2-year interval from baseline to follow-up time points could have been long enough to allow that some patients seroconverted and quickly seroreverted afterwards, thus being seronegative at both determinations conducted in this study. If this was the case, we had underestimated the rate of HEV seroconversion in HIV-infected individuals. Given that most patients remained seropositive 14 months after acute HEV infection in a prior study [16], we think this is unlikely. However, since HEV seroreversion is common in HIV-infected patients, as proven herein, we cannot completely rule out this possibility. In any case, this study includes a large population of HIV-infected patients tested for HEV antibody and, to our knowledge, is the only study including a follow-up of HEV-seronegative and seropositive individuals, and this is its main strength.

Unexplained liver disease is found in around 10% of the HIV-infected patients [23,24]. This disorder is sometimes attributable to drug-induced liver injury [24] or metabolic abnormalities [23]. According to the results of this study, although HEV exposure is quite common among HIV-infected patients, chronic hepatitis E has only a minor role as a cause of liver disease of unknown origin in this setting. Because of this, clinicians should be aware of hepatitis E as a possible source of chronic liver damage, but systematic screening for this agent is not warranted. On the contrary, HEV antibody testing should be limited to patients with data consistent with liver

disease and no other apparent reason. Likewise, as chronic hepatitis E is very uncommon and most cases of acute HEV infection are subclinical, there will not be clear reasons to prioritize HEV vaccine, once completely developed, in HIV-infected patients in our area. Certainly, HEV exposure is very high, but whether it is higher or not than in the general population remains unknown. In areas where this topic has been investigated, the prevalence of HEV seropositivity was similar in HIV-infected people and in control groups [5,10]. This is another reason to believe that HIV-infected patients should not have preference for vaccination. In any case, this is a point that will require further investigation.

Acknowledgements

Role of authors in the study: A.R.: study design, serology and PCR determinations, and manuscript revision; A.R.-J.: study design, serology and PCR determinations, and manuscript revision; C.C.: data collection, result analysis, and manuscript revision; E.P.-N., sample management and manuscript revision; J.M.: study design, data collection, result analysis, and manuscript revision; J.A.P.: study design, result analysis, and manuscript writing; L.M.R.: study design, laboratory determinations, result analysis, and manuscript revision; M.P., study design, serology and PCR determinations, and manuscript revision; N.M.: study design, result analysis, and manuscript revision; P.M.: data collection, result analysis, and manuscript revision.

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Conflicts of interest

There are no conflicts of interest.

References

1. Crum-Cianflone NF, Curry J, Drobeniuc J, Weintrob A, Landrum M, Ganesan A, *et al.* **Hepatitis E virus infection in HIV-infected persons.** *Emerg Infect Dis* 2012; **18**:502–506.
2. Sellier P, Mazeron MZ, Tesse S, Badsi E, Evans J, Magnier JD, *et al.* **Virus infection in HIV infected patients with elevated serum transaminases levels.** *Virol J* 2011; **8**:171.
3. Jagjit Singh GK, Ijaz S, Rockwood N, Farnworth SP, Devitt E, Atkins M, *et al.* **Chronic hepatitis E as a cause for cryptogenic cirrhosis in HIV.** *J Infect* 2013; **66**:103–106.
4. Neukam K, Barreiro P, Macías J, Avellón A, Cifuentes C, Martín-Carbonero L, *et al.* **Chronic hepatitis E in HIV patients: rapid progression to cirrhosis and response to oral ribavirin.** *Clin Infect Dis* 2013; **57**:465–468.
5. Ramezani A, Velayati AA, Khorami-Sarvestani S, Eslamifar A, Mohraz M, Banifazl M, *et al.* **Hepatitis E virus infection in patients infected with human immunodeficiency virus in an endemic area in Iran.** *Int J STD AIDS* 2013; 24:769–774.
6. Feldt T, Sarfo FS, Zoufaly A, Phillips RO, Burchard G, van Lunzen J, *et al.* **Hepatitis E virus infections in HIV-infected patients in Ghana and Cameroon.** *J Clin Virol* 2013; **58**:18–23.
7. Mateos-Lindemann ML, Diez-Aguilar M, Galdamez AL, Galán JC, Moreno A, Pérez-Gracia MT. **Patients infected with HIV are at high-risk for hepatitis E virus infection in Spain.** *J Med Virol* 2014; **86**:71–74.
8. Maylin S, Stephan R, Molina JM, Peraldi MN, Scieux C, Nicand E, *et al.* **Prevalence of antibodies and RNA genome of hepatitis E virus in a cohort of French immunocompromised.** *J Clin Virol* 2012; **53**:346–349.
9. Kenfak-Foguena A, Schoni-Affolter F, Burgisser P, Witteck A, Darling KE, Kovari H, *et al.* **Hepatitis E virus seroprevalence and chronic infections in patients with HIV, Switzerland.** *Emerg Infect Dis* 2011; 17:1074–1078.
10. Keane F, Gompels M, Bendall R, Drayton R, Jennings L, Black J, *et al.* **Hepatitis E virus coinfection in patients with HIV infection.** *HIV Med* 2012; **13**:83–88.
11. Jardi R, Crespo M, Homs M, Van den Eyden E, Girones R, Rodríguez Manzano J, *et al.* **HIV, HEV and cirrhosis: evidence of a possible link from eastern Spain.** *HIV Med* 2012; 13:379–383.
12. Hoofnagle JH, Nelson KE, Purcell RH. **Hepatitis E.** *N Engl J Med* 2012; **367**:1237–1244.
13. Kondili LA, Chionne P, Porcaro A, Madonna E, Taffon S, Resuli B, *et al.* **Seroprevalence of hepatitis E virus (HEV) antibody and the possible association with chronic liver disease: a case-control study in Albania.** *Epidemiol Infect* 2006; **134**:95–101.
14. Kumar S, Subhadra S, Singh B, Panda BK. **Hepatitis E virus: the current scenario.** *Int J Infect Dis* 2013; **17**:e228–e233.
15. Atiq M, Shire NJ, Barrett A, Rouster SD, Sherman KE, Shata MT. **Hepatitis E virus antibodies in patients with chronic liver disease.** *Emerg Infect Dis* 2009; **15**:479–481.
16. Myint KS, Endy TP, Shrestha MP, Shrestha SK, Vaughn DW, Innis BL, *et al.* **Hepatitis E antibody kinetics in Nepalese patients.** *Trans R Soc Trop Med Hyg* 2006; **100**:938–941.
17. Rivero-Juárez A, Martinez-Dueñas L, Martinez-Peinado A, Camacho A, Cifuentes C, Gordon A, *et al.* **Chronic hepatitis E virus infection is uncommon in HIV-infected patients.** *Conference on Retroviruses and Opportunistic Infections*, March 3–6 2014. Boston, United States of America (Abstract 633).
18. Kamar N, Bendall R, Legrand-Abravanel F, Xia N, Ijaz S, Izopet J, *et al.* **Hepatitis E.** *Lancet* 2012; **379**:2477–2488.
19. Wenzel JJ, Preiss J, Schemmerer M, Huber B, Jilg W. **Test performance characteristics of Anti-HEV IgG assays strongly influence hepatitis E seroprevalence estimates.** *J Infect Dis* 2013; **207**:497–500.
20. Pas SD, Streefkerk RH, Pronk M, de Man RA, Beersma MF, Osterhaus AD, van der Eijk AA. **Diagnostic performance of selected commercial HEV IgM and IgG ELISAs for immunocompromised and immunocompetent patients.** *J Clin Virol* 2013; **58**:629–634.
21. Bendall R, Ellis V, Ijaz S, Ali R, Dalton H. **A comparison of two commercially available anti-HEV IgG kits and a re-evaluation of anti-HEV IgG seroprevalence data in developed countries.** *J Med Virol* 2010; 82:799–805.
22. Abravanel F, Chapuy-Regaud S, Lhomme S, Miedougé M, Peron JM, Alric L, *et al.* **Performance of anti-HEV assays for diagnosing acute hepatitis E in immunocompromised patients.** *J Clin Virol* 2013; **58**:624–628.
23. Rivero-Juarez A, Camacho A, Merchante N, Pérez-Camacho I, Macias J, Ortiz-García C, *et al.* **Incidence of liver damage of uncertain origin in HIV patients not co-infected with HCV/HBV.** *PLoS One* 2013; **8**:e68953.
24. Merchante N, Pérez Camacho I, Mira JA, Rivero A, Macías J, Camacho A, *et al.* **Prevalence and risk factors for abnormal liver stiffness in HIV-infected patients without viral hepatitis coinfection: role of didanosine.** *Antivir Ther* 2010; **15**:753–763.

別紙様式第2-1

No. 13

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分		総合機構処理欄
				2014. 10. 1	該当なし		
一般的名称	解凍人赤血球液		研究報告の公表状況	CDC/CDC Newsroom/Press Release; Available from: http://www.cdc.gov/media/releases/2014/s930-ebola-confirmed-case.html		公表国	
販売名（企業名）	解凍赤血球液-LR「日赤」（日本赤十字社） 照射解凍赤血球液-LR「日赤」（日本赤十字社）					米国	

研究報告の概要

○米国で初のエボラ症例を確認
米国疾病管理予防センター（CDC）は9月30日、リベリアへ渡航しテキサス州ダラスに帰国した男性に、米国で診断された初のエボラ出血熱症例を確認した。患者は、西アフリカを出発するときには症状がなかったが、9月20日に米国に到着してから4日後に発症した。9月26日に病院を受診し、その後エボラ出血熱と一致する症状が発現し、28日に入院した。患者の渡航歴及び症状に基づき、CDCからエボラ出血熱の検査を推奨されたため、病院は患者を隔離し、CDCと検査対応ネットワークの参画下にあるテキサス検査室に検体を送付した。CDC及びテキサス州保健局は病院に検査結果を報告し、病院は患者に告知した。CDCのチームが調査を支援するためにダラスに派遣されている。
患者は西アフリカからのフライト中は症状を示していなかった。エボラ出血熱は症状が現われていない時は感染性を持たないため、CDCは、患者と同便の飛行機に乗り合わせた人々を監視することを推奨していない。

使用上の注意記載状況・その他参考事項等

解凍赤血球液-LR「日赤」
照射解凍赤血球液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見

リベリアからテキサス州ダラスに帰国した男性に、米国で初となるエボラ出血熱が確認されたとの報告である。

今後の対応

日本赤十字社では、輸血感染症対策として受付時に海外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。今後も引き続き情報の収集に努める。

⑫

Press Release

For Immediate Release: Tuesday, September 30, 2014
Contact: Media Relations (http://www.cdc.gov/media)
(404) 639-3286

CDC and Texas Health Department Confirm First Ebola Case Diagnosed in the U.S.

Hospitalized patient had recently returned from West Africa; active contact tracing underway.

The Centers for Disease Control and Prevention (CDC) confirmed today, through laboratory tests, the first case of Ebola (http://www.cdc.gov/vhf/ebola/) to be diagnosed in the United States in a person who hac traveled to Dallas, Texas from Liberia. The patient did not have symptoms when leaving West Africa, but developed symptoms approximately four days after arriving in the U.S. on Sept. 20.

The person fell ill on Sept. 24 and sought medical care at Texas Health Presbyterian Hospital of Dall on Sept. 26. After developing symptoms consistent with Ebola, he was admitted to hospital on Sept. 28. Based on the person's travel history and symptoms, CDC recommended testing for Ebola. The medical facility isolated the patient and sent specimens for testing at CDC and at a Texas lab participating in the CDC's Laboratory Response Network (http://www.bt.cdc.gov/lrn/). CDC and the Texas Health Department reported the laboratory test results to the medical center to inform the patient. A CDC team is being dispatched to Dallas to assist with the investigation.

"Ebola can be scary. But there's all the difference in the world between the U.S. and parts of Africa where Ebola is spreading. The United States has a strong health care system and public health professionals wh will make sure this case does not threaten our communities," said CDC Director, Dr. Tom Frieden, M.D., M.P.H. "While it is not impossible that there could be additional cases associated with this patient in the coming weeks, I have no doubt that we will contain this."

The ill person did not exhibit symptoms of Ebola during the flights from West Africa and CDC does not recommend that people on the same commercial airline flights undergo monitoring, as Ebola is contagious only if the person is experiencing active symptoms. The person reported developing symptom several days after the return flight. Anyone concerned about possible exposure may call CDC-Info (http://www.cdc.gov/cdc-info/) at 800-CDC-INFO for more information.

CDC recognizes that even a single case of Ebola diagnosed in the United States raises concerns. Knowing the possibility exists, medical and public health professionals across the country have been preparing to respond. CDC and public health officials in Texas are taking precautions to identify people who have had close personal contact with the ill person, and health care professionals have been reminded to use meticulous infection control at all times.

We do know how to stop Ebola's further spread: thorough case finding, isolation of ill people, contacting people exposed to the ill person, and further isolation of contacts if they develop symptoms. The U.S. public health and medical systems have had prior experience with sporadic cases of diseases such as Ebola. In the past decade, the United States had 5 imported cases of viral hemorrhagic fever (VHF) diseases similar to Ebola (1 Marburg, 4 Lassa). None resulted in any transmission in the United States.

CDC has been anticipating and preparing for a case of Ebola in the United States. We have been:

- Enhancing surveillance and laboratory testing capacity in states to detect cases
- Developing guidance and tools for health departments to conduct public health investigations
- Providing recommendations for healthcare infection control and other measures to prevent disease spread

- Providing guidance for flight crews, Emergency Medical Services units at airports, and Customs and Border Protection officers about reporting ill travelers to CDC
- Disseminating up-to-date information to the general public, international travelers, and public health partners

The data health officials have seen in the past few decades since Ebola was discovered indicate that it is not spread through casual contact or through the air. Ebola is spread through direct contact with bodily fluids of a sick person or exposure to objects such as needles that have been contaminated. The illness has an average 8-10 day incubation period (although it ranges from 2 to 21 days); CDC recommends monitoring exposed people for symptoms a complete 21 days. People are not contagious after exposure unless they develop symptoms.

More information is available at www.cdc.gov/ebola (http://www.cdc.gov/ebola) .

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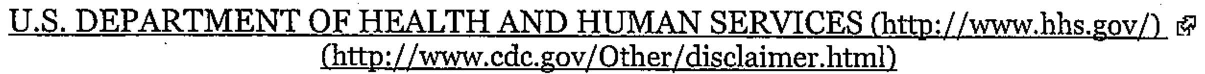

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES (http://www.hhs.gov/) (http://www.cdc.gov/Other/disclaimer.html)

Centers for Disease Control and Prevention 1600 Clifton Road Atlanta, GA 30329-4027, USA
800-CDC-INFO (800-232-4636) TTY: (888) 232-6348 - Contact CDC–INFO

別紙様式第２－１

医薬品　研究報告　調査報告書

識別番号・報告回数		報告日	第一報入手日 2014 年 9 月 9 日	新医薬品等の区分 該当なし	機構処理欄
一般的名称	ﾃｸﾈﾁｳﾑ人血清ｱﾙﾌﾞﾐﾝ（^{99m}Tc）	研究報告の公表状況	mBio. 2014 Jul 22;5(4)	公表国 サウジアラビア	
販売名（企業名）	テクネアルブミンキット （富士フイルムＲＩファーマ株式会社）				

研究報告の概要	使用上の注意記載状況・その他参考事項等
＜要約＞中東呼吸器症候群コロナウイルス（MERS-CoV）は、2012 年以降アラブ半島で広がっている新規のβコロナウイルスで、人で深刻な呼吸器感染症を引き起こしている。コウモリが人の MERS-CoV 感染症に関与することが示唆されているものの、コウモリと MERS-CoV との直接的な関連は不明確である。一方、血清学的およびウイルス学的データでは、MERS-CoV の潜在的な保有動物として、ヒトコブラクダが示唆される。最近我々はラクダと MERS-CoV に感染したラクダの所有者（患者）から MERS-CoV を分離し、感染したラクダから患者へ MERS-CoV が直接伝播した証拠を提示した。ここで、我々はこの調査の範囲を広げ、我々の以前の調査で感染したラクダを保護した同じ納屋から収集された空気サンプルで、同一の MERS-CoV RNA フラグメントが検出されたことを提示する。これらのデータは、ラクダと人においてウイルスが検出されたと同時に、この農場でウイルスが循環していたことを意味しており、それは MERS-CoV で起こりうる空気感染について更なる調査を必要とする。 ＜重要性＞この調査は、MERS-CoV の世界的な脅威をコントロールするための感染制御法と、連続監視の重要性を明確にハイライトする。現在の MERS-CoV の伝播は制限されているように見えるが、我々は、ラクダとの接触を最小限にし（特に免疫不全の人）、感染した患者に対処する際の感染防止と管理方法、安全衛生上の適切な取り扱いを助言する。また、何らかの動物暴露を受けて疫学調査および臨床検査が行われた MERS-CoV 症例の詳細な病歴は、動物種特定のために考慮されなければならない。	特になし

報告企業の意見	今後の対応
MERS-CoV の発生源および伝播様式については、これまではっきりと判っていなかったが、人の中間宿主または未知の保有宿主からの人畜共通感染が示唆されていた。著者は、以前の調査で感染したラクダとラクダの所有者（患者）から MRES-CoV を分離し、ラクダから患者への異種間の直接伝播を示唆していたが、本報告では患者の所有するラクダの納屋から得た空気サンプルから MERS-CoV に特異的な遺伝子配列の存在を確認した。更にこの遺伝子配列が、以前の調査でこの納屋で感染したラクダと患者から得たウイルス分離株の対応領域に 100%一致することが判明した。著者は、特に感染した患者と動物に直接接触する仕事をしている人々に対し、MERS-CoV の空気感染の可能性について示唆している。 本報告は、重大な感染症かつ新規感染経路に該当すると考えられ、感染症定期報告の対象と判断する。	本研究報告は、ヒト血液を原料とする血漿分画製剤とは直接関連がないことから、現時点で当該生物由来製品に関し、措置等を行う必要はないと判断する。

MedDRA/J Version(17.1)

Detection of the Middle East Respiratory Syndrome Coronavirus Genome in an Air Sample Originating from a Camel Barn Owned by an Infected Patient

Esam I. Azhar, Anwar M. Hashem, Sherif A. El-Kafrawy, et al.
2014. Detection of the Middle East Respiratory Syndrome Coronavirus Genome in an Air Sample Originating from a Camel Barn Owned by an Infected Patient. mBio 5(4): .
doi:10.1128/mBio.01450-14.

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OBSERVATION

Detection of the Middle East Respiratory Syndrome Coronavirus Genome in an Air Sample Originating from a Camel Barn Owned by an Infected Patient

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ABSTRACT **Middle East respiratory syndrome coronavirus (MERS-CoV) is a novel betacoronavirus that has been circulating in the Arabian Peninsula since 2012 and causing severe respiratory infections in humans. While bats were suggested to be involved in human MERS-CoV infections, a direct link between bats and MERS-CoV is uncertain. On the other hand, serological and virological data suggest dromedary camels as the potential animal reservoirs of MERS-CoV. Recently, we isolated MERS-CoV from a camel and its infected owner and provided evidence for the direct transmission of MERS-CoV from the infected camel to the patient. Here, we extend this work and show that identical MERS-CoV RNA fragments were detected in an air sample collected from the same barn that sheltered the infected camel in our previous study. These data indicate that the virus was circulating in this farm concurrently with its detection in the camel and in the patient, which warrants further investigations for the possible airborne transmission of MERS-CoV.**

IMPORTANCE **This work clearly highlights the importance of continuous surveillance and infection control measures to control the global public threat of MERS-CoV. While current MERS-CoV transmission appears to be limited, we advise minimal contact with camels, especially for immunocompromised individuals, and the use of appropriate health, safety, and infection prevention and control measures when dealing with infected patients. Also, detailed clinical histories of any MERS-CoV cases with epidemiological and laboratory investigations carried out for any animal exposure must be considered to identify any animal source.**

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The Middle East respiratory syndrome coronavirus (MERS-CoV) is a novel human pathogen associated with severe respiratory symptoms and renal failure (1, 2). Since its emergence in 2012, there has been up to 536 laboratory-confirmed infections and 145 deaths in at least 17 countries in Asia, Africa, Europe, and North America (3). Most of these cases originated from countries in or around the Arabian Peninsula, particularly Saudi Arabia (3). The ability of this virus to infect those in close contact with an infected individual, such as family members and health care personnel, as well as the associated high mortality rate may represent a global public health threat (4–7).

Although the source of MERS-CoV and its mode of transmission are not fully understood, zoonotic transmission from an unknown reservoir or through an intermediate host to humans was suggested (7–9). Phylogenetic analysis showed that MERS-CoV belongs to bat-associated clade 2c betacoronaviruses (2, 9, 10). Detection of MERS-CoV-related viruses in Old World insectivorous bats from the family *Vespertilionidae* (11, 12) and the isolation of small genomic fragments identical to the sequence of the Erasmus Medical Center (EMC)/2012 MERS-CoV Essen isolate (GenBank accession number KC875821) from a *Taphozous perforatus* bat in Saudi Arabia (13) suggested that insectivorous bats could be the original source of MERS-CoV. However, due to the limited direct contact between humans and bats and to the detection of neutralizing antibodies to MERS-CoV in dromedary camels from countries like Oman, the United Arab Emirates, Qatar, Egypt, and Saudi Arabia (8, 14–16), dromedary camels were proposed to be involved in the cross-species transmission of MERS-CoV. This was further supported by the detection and partial genome sequencing of MERS-CoV RNA in samples collected from camels in Qatar and Saudi Arabia (17, 18). Anti-MERS-CoV antibodies have also been detected in samples collected from dromedary camels in Saudi Arabia since 1992 (18). While these studies provide a convincing link between humans, camels, and MERS-CoV and indicate that MERS-CoV has been circulating in dromedary camels for a long time, they do not prove that camels passed the virus to humans.

Recently, several MERS-CoV isolates were obtained from nasal

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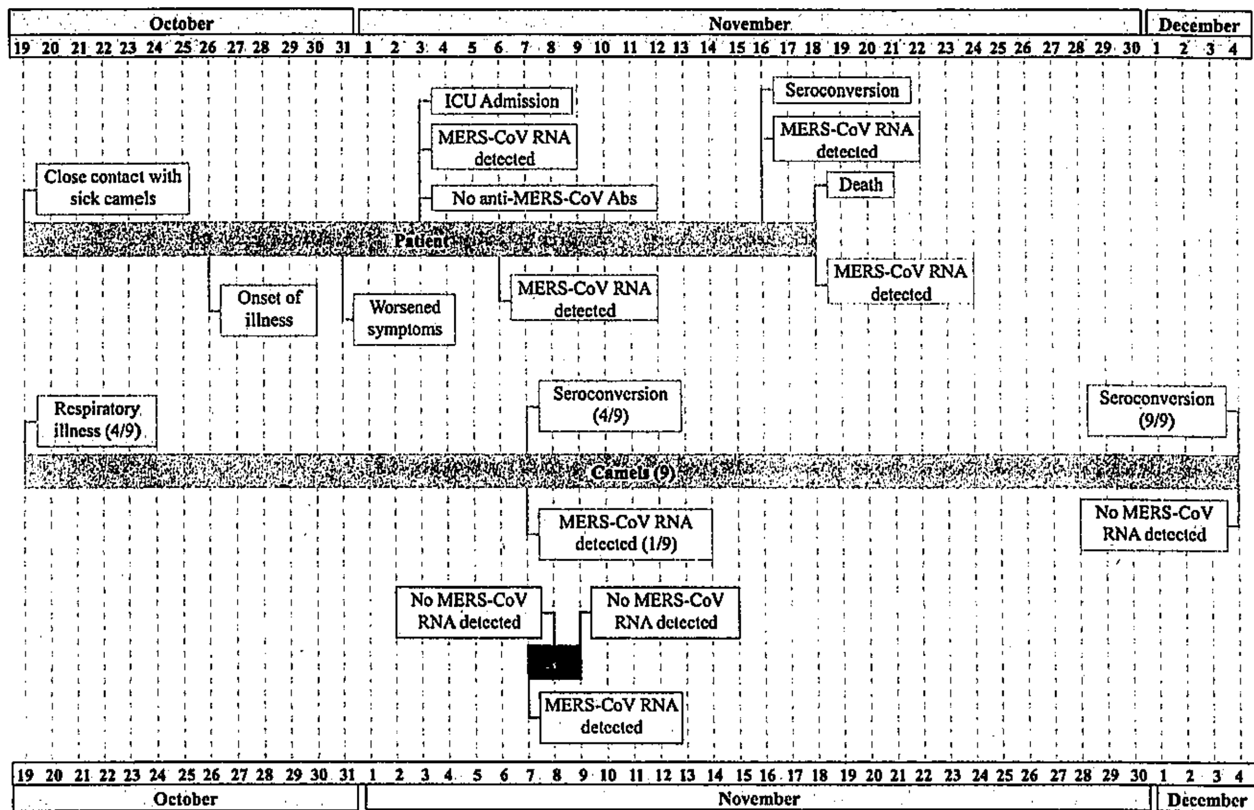

FIG 1 Timeline of the main events in the study. ICU, intensive care unit; Abs, antibodies.

samples from dromedary camels in Saudi Arabia, and their consensus genome sequences were found to be similar to published human MERS-CoV sequences, supporting the role of dromedary camels in human MERS-CoV infections (19). In another report, we also provided evidence for the direct cross-species transmission of MERS-CoV from infected camels to their owner (20). Serological data suggested that the virus was circulating in the herd before infecting the patient. Additionally, we showed based on reverse transcription-PCR (RT-PCR), viral isolation, and full-genome sequencing that both MERS-CoV-Jeddah-human-1 (accession number KF958702) and MERS-CoV-Jeddah-camel-1 (accession number KF917527) isolates were 100% identical and contain characteristic mutations compared to other reported sequences, suggesting direct cross-species transmission from the camels to the patient.

Here, we tried to extend our previous study and to examine whether air could play a role in MERS-CoV transmission. To this end, three air samples were collected from the camels' barn on three consecutive days as shown in Fig. 1. All samples were screened by real-time RT-PCR targeting the upstream region of the E gene (UpE region) of MERS-CoV. Interestingly, only the air sample collected on 7 November 2013, the same day that one of the nine camels in the same barn tested positive for MERS-CoV (Fig. 1), tested positive for the UpE region. The two other air samples were negative for UpE by RT-PCR. Two other confirmatory real-time RT-PCR assays targeting the open reading frame 1a (ORF1a) and ORF1b regions confirmed the UpE-positive result of the first air sample, as shown in Table 1.

To further confirm these results, extracted RNA was subjected to partial genome sequencing of a 665-nucleotide (nt) segment in ORF1a (accession number KJ740999), a 706-nt segment in the RNA-dependent RNA polymerase (RdRp) (accession number KJ741000), a 688-nt segment in ORF1b (accession number KJ741001), a 452-nt segment in UpE (accession number KJ741002), and a 403-nt segment in the nucleocapsid (N) region (accession number KJ741003) of the viral genome. Here, we con-

TABLE 1 Results of a real-time RT-PCR for MERS-CoV RNA in air samples

Day of air sample collection[a]	Threshold cycle of the real-time RT-PCR for detection of indicated MERS-CoV RNA[b]		
	UpE	ORF1a	ORF1b
1	34.7	34.4	33.3
2	ND	ND	ND
3	ND	ND	ND

[a] Samples were collected starting on 7 November 2013.
[b] ND, not detected.

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firmed the presence of MERS-CoV-specific sequences in the first air sample and found that these fragments are 100% identical to the corresponding regions in our previous isolates MERS-CoV-Jeddah-human-1 and MERS-CoV-Jeddah-camel-1, obtained from the patient and the infected camel in this barn, respectively (see Fig. S1 in the supplemental material). Of note, further partial genome sequencing of a 697-nt segment of the viral RNA in the ORF1a region (accession number KJ740998) from the air sample showed that the virus is identical to the original isolates obtained from the nasal samples collected from the patient and the infected camel, without the cell culture-adapted mutation (T10154C) observed in our earlier report (see Fig. S2 in the supplemental material).

These data confirm our previous report (20) and show evidence for the presence of the airborne MERS-CoV genome in the same barn that was owned by the patient and housed the infected camels. The detection of viral RNA in the air sample collected on the same day that one of the camels' samples tested positive for MERS-CoV and the fact that all genome sequences obtained from the air sample were identical to those from the camel and the patient samples suggest that the detected viral RNA originated from the camels.

MERS-CoV was reported to be more stable than influenza A H1N1 virus under different environmental conditions on surfaces or in aerosols (21). Specifically, viable MERS-CoV was recovered from surfaces after 48 h at 20°C and 40% relative humidity and after 24 h at 30°C and 30% relative humidity. Similarly, the viability of MERS-CoV decreased by 7% only in aerosols when the virus was incubated at 20°C with 40% relative humidity. However, virus isolation in cell culture was unsuccessful from the air sample collected in the current study, which may be due to a loss of viral infectivity in the collected air sample. Therefore, further studies are clearly needed to confirm the viability of MERS-CoV at different environmental conditions and to confirm its infectivity. Nonetheless, while other routes of transmission, such as droplet contact or fomite transmission, may be involved, the detection of MERS-CoV RNA in the air sample from this barn concurrently with its detection and isolation from the infected camel and the onset of symptoms in the patient warrants further investigations for the possible airborne transmission of MERS-CoV.

The shedding of MERS-CoV into the environment is supported by several reports, including report of the nosocomial infection of immunocompromised patients and the infection of those in close contact with patients, such as family members and health care workers (4–7). Furthermore, the detection of MERS-CoV-neutralizing antibodies and its genome in dromedary camels (8, 14–18) clearly suggest that these animals may play an important role in MERS-CoV transmission to humans. To our knowledge, this is the first report on the possible risk of airborne transmission of MERS-CoV, especially to personnel working directly with infected patients or animals. Our data suggest that camels may be a source of infectious MERS-CoV, which can be transmitted to humans within confined spaces. These results also suggest that air sampling might be a useful approach to investigate the role of the airborne transmission of MERS-CoV spread and shedding. Further studies are urgently needed to fully understand the role of camels in the transmission of MERS-CoV and whether airborne transmission plays a role in MERS-CoV spread in order to implement control and prevention measures to prevent the transmission of this deadly virus.

Air sampling procedure. Air samples were collected from the camels' barn on three consecutive days, with day 1 (7 November 2013) being the same day that one of the nine camels was positive for MERS-CoV by real-time RT-PCR. Samples were collected using the MD8 airscan sampling device (Sartorius) and sterile gelatin filters (80 mm in diameter and 3-μm pore size; type 17528-80-ACD; Sartorius). Air was sampled at a speed of 50 liters/min for 20 min. Filters were dissolved in 5 ml viral transport medium (VTM) and stored at −80°C until analyzed.

Real-time RT-PCR. RNA was extracted from the dissolved filter solution using a QIAamp viral RNA minikit (Qiagen, Germany) according to manufacturer's instructions. Eluted RNA was screened for the UpE region using the real-time RT-PCR assay on a Rotor-Gene Q real-time PCR machine (Qiagen, Germany) as previously described (22). Samples were also tested by real-time RT-PCR for the ORF1a and ORF1b regions for confirmation as described previously (22).

Sequencing and alignment. Further confirmation was performed by partially sequencing the UpE, ORF1a, ORF1b, RdRp, and N regions of the viral genome as per the WHO recommendations (23). In addition, one region containing unique mutations in isolates obtained in our previous report (20) were also sequenced. Sequencing was performed as described previously (20). Sequences were aligned with the genome of the MERS-CoV-Jeddah-camel-1isolate (KF917527) obtained in our pervious study using Geneious 7.0.6 software.

Nucleotide sequence accession numbers. Sequences obtained in this study were deposited in GenBank and given the following accession numbers: MERS-CoV-Jeddah-air-1-2014-ORF1a-partial cds-1, KJ740998; MERS-CoV-Jeddah-air-1-2014-ORF1a-partial cds-2, KJ740999; MERS-CoV-Jeddah-air-1-2014-RdRp-partial, KJ741000; MERS-CoV-Jeddah-air-1-2014-ORF1b-partial, KJ741001; MERS-CoV-Jeddah-air-1-2014-UpE-partial, KJ741002; and MERS-CoV-Jeddah-air-1-2014-N protein-partial, KJ741003.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.01450-14/-/DCSupplemental.

Figure S1, PDF file, 1.3 MB.

Figure S2, PDF file, 0.1 MB.

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REFERENCES

1. Bermingham A, Chand MA, Brown CS, Aarons E, Tong C, Langrish C, Hoschler K, Brown K, Galiano M, Myers R, Pebody RG, Green HK, Boddington NL, Gopal R, Price N, Newsholme W, Drosten C, Fouchier RA, Zambon M. 2012. Severe respiratory illness caused by a novel coronavirus, in a patient transferred to the United Kingdom from the Middle East, September 2012. Euro Surveill. 17:20290. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20290.
2. Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA. 2012. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N. Engl. J. Med. 367:1814–1820. http://dx.doi.org/10.1056/NEJMoa1211721.
3. World Health Organization. 2014. Middle East respiratory syndrome coronavirus (MERS-CoV) summary and literature update—as of 9 May 2014. http://www.who.int/csr/disease/coronavirus_infections/MERS_CoV_Update_09_May_2014.pdf?ua=1. Accessed 4 June 2014.
4. Assiri A, McGeer A, Perl TM, Price CS, Al Rabeeah AA, Cummings DA, Alabdullatif ZN, Assad M, Almulhim A, Makhdoom H, Madani H,

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Alhakeem R, Al-Tawfiq JA, Cotten M, Watson SJ, Kellam P, Zumla AI, Memish ZA, KSA MERS-CoV Investigation Team. 2013. Hospital outbreak of Middle East respiratory syndrome coronavirus. N. Engl. J. Med. 369:407–416. http://dx.doi.org/10.1056/NEJMoa1306742.

5. Guery B, Poissy J, el Mansouf L, Séjourné C, Ettahar N, Lemaire X, Vuotto F, Goffard A, Behillil S, Enouf V, Caro V, Mailles A, Che D, Manuguerra JC, Mathieu D, Fontanet A, van der Werf S, MERS-CoV Study Group. 2013. Clinical features and viral diagnosis of two cases of infection with Middle East Respiratory Syndrome coronavirus: a report of nosocomial transmission. Lancet 381:2265–2272. http://dx.doi.org/10.1016/S0140-6736(13)60982-4.
6. Health Protection Agency (HPA) UK Novel Coronavirus Investigation team. 2013. Evidence of person-to-person transmission within a family cluster of novel coronavirus infections, United Kingdom, February 2013. Euro Surveill. 18:pii=20427. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20427.
7. Memish ZA, Zumla AI, Al-Hakeem RF, Al-Rabeeah AA, Stephens GM. 2013. Family cluster of Middle East respiratory syndrome coronavirus infections. N. Engl. J. Med. 368:2487–2494. http://dx.doi.org/10.1056/NEJMoa1303729.
8. Perera RA, Wang P, Gomaa MR, El-Shesheny R, Kandeil A, Bagato O, Siu LY, Shehata MM, Kayed AS, Moatasim Y, Li M, Poon LL, Guan Y, Webby RJ, Ali MA, Peiris JS, Kayali G. 2013. Seroepidemiology for MERS coronavirus using microneutralisation and pseudoparticle virus neutralisation assays reveal a high prevalence of antibody in dromedary camels in Egypt, June 2013. Euro Surveill. 18:pii=20574. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20574.
9. Cotten M, Lam TT, Watson SJ, Palser AL, Petrova V, Grant P, Pybus OG, Rambaut A, Guan Y, Pillay D, Kellam P, Nastouli E. 2013. Full-genome deep sequencing and phylogenetic analysis of novel human betacoronavirus. Emerg. Infect. Dis. 19:736B–742B. http://dx.doi.org/10.3201/eid1905.130057.
10. van Boheemen S, de Graaf M, Lauber C, Bestebroer TM, Raj VS, Zaki AM, Osterhaus AD, Haagmans BL, Gorbalenya AE, Snijder EJ, Fouchier RA. 2012. Genomic characterization of a newly discovered coronavirus associated with acute respiratory distress syndrome in humans. mBio 3(6):e00473-12. http://dx.doi.org/10.1128/mBio.00473-12.
11. Ithete NL, Stoffberg S, Corman VM, Cottontail VM, Richards LR, Schoeman MC, Drosten C, Drexler JF, Preiser W. 2013. Close relative of human Middle East respiratory syndrome coronavirus in bat, South Africa. Emerg. Infect. Dis. 19:1697–1699. http://dx.doi.org/10.3201/eid1910.130946.
12. Annan A, Baldwin HJ, Corman VM, Klose SM, Owusu M, Nkrumah EE, Badu EK, Anti P, Agbenyega O, Meyer B, Oppong S, Sarkodie YA, Kalko EK, Lina PH, Godlevska EV, Reusken C, Seebens A, Gloza-Rausch F, Vallo P, Tschapka M, Drosten C, Drexler JF. 2013. Human betacoronavirus 2c EMC/2012-related viruses in bats, Ghana and Europe. Emerg. Infect. Dis. 19:456–459. http://dx.doi.org/10.3201/eid1903.121503.
13. Memish ZA, Mishra N, Olival KJ, Fagbo SF, Kapoor V, Epstein JH, Alhakeem R, Durosinloun A, Al Asmari M, Islam A, Kapoor A, Briese T, Daszak P, Al Rabeeah AA, Lipkin WI. 2013. Middle East respiratory syndrome coronavirus in bats, Saudi Arabia. Emerg. Infect. Dis. 19:1819–1823. http://dx.doi.org/10.3201/eid1911.131172.
14. Reusken CB, Haagmans BL, Müller MA, Gutierrez C, Godeke GJ, Meyer B, Muth D, Raj VS, Smits-De Vries L, Corman VM, Drexler JF, Smits SL, El Tahir YE, De Sousa R, van Beek J, Nowotny N, van Maanen K, Hidalgo-Hermoso E, Bosch BJ, Rottier P, Osterhaus A, Gortázar-Schmidt C, Drosten C, Koopmans MP. 2013. Middle East respiratory syndrome coronavirus neutralising serum antibodies in dromedary camels: a comparative serological study. Lancet Infect. Dis. 13:859–866. http://dx.doi.org/10.1016/S1473-3099(13)70164-6.
15. Alexandersen S, Kobinger GP, Soule G, Wernery U. 2014. Middle East Respiratory Syndrome coronavirus antibody reactors among camels in Dubai, United Arab Emirates, in 2005. Transbound. Emerg. Dis. 61:105–108. http://dx.doi.org/10.1111/tbed.12212.
16. Hemida MG, Perera RA, Wang P, Alhammadi MA, Siu LY, Li M, Poon LL, Saif L, Alnaeem A, Peiris M. 2013. Middle East respiratory syndrome (MERS) coronavirus seroprevalence in domestic livestock in Saudi Arabia, 2010-2013. Euro. Surveill. 18:20659. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20659.
17. Haagmans BL, Al Dhahiry SH, Reusken CB, Raj VS, Galiano M, Myers R, Godeke GJ, Jonges M, Farag E, Diab A, Ghobashy H, Alhajri F, Al-Thani M, Al-Marri SA, Al Romaihi HE, Al Khal A, Bermingham A, Osterhaus AD, Alhajri MM, Koopmans MP. 2014. Middle East respiratory syndrome coronavirus in dromedary camels: an outbreak investigation. Lancet Infect. Dis. 14:140–145. http://dx.doi.org/10.1016/S1473-3099(13)70690-X.
18. Alagaili AN, Briese T, Mishra N, Kapoor V, Sameroff SC, de Wit E, Munster VJ, Hensley LE, Zalmout IS, Kapoor A, Epstein JH, Karesh WB, Daszak P, Mohammed OB, Lipkin WI. 2014. Middle East respiratory syndrome coronavirus infection in dromedary camels in Saudi Arabia. mBio 5(2):e00884-14. http://dx.doi.org/10.1128/mBio.00884-14.
19. Briese T, Mishra N, Jain K, Zalmout IS, Jabado OJ, Karesh WB, Daszak P, Mohammed OB, Alagaili AN, Lipkin WI. 2014. Middle East respiratory syndrome coronavirus quasispecies that include homologues of human isolates revealed through whole-genome analysis and virus cultured from dromedary camels in Saudi Arabia. mBio 5(3):e01146-14. http://dx.doi.org/10.1128/mBio.01146-14.
20. Azhar EI, El-Kafrawy SA, Farraj SA, Hassan AM, Al-Saeed MS, Hashem AM, Madani TA. 2014. Evidence for camel-to-human transmission of MERS-coronavirus. N. Engl. J. Med. 370:2499–2505. http://dx.doi.org/10.1056/NEJMoa1401505.
21. van Doremalen N, Bushmaker T, Munster VJ. 2013. Stability of Middle East respiratory syndrome coronavirus (MERS-CoV) under different environmental conditions. Euro Surveill. 18:pii=20590. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20590.
22. Corman VM, Müller MA, Costabel U, Timm J, Binger T, Meyer B, Kreher P, Lattwein E, Eschbach-Bludau M, Nitsche A, Bleicker T, Landt O, Schweiger B, Drexler JF, Osterhaus AD, Haagmans BL, Dittmer U, Bonin F, Wolff T, Drosten C. 2012. Assays for laboratory confirmation of novel human coronavirus (hCoV-EMC) infections. Euro Surveill. 17:pii=20334. http://www.eurosurveillance.org/viewarticle.aspx?articleid=20334.
23. World Health Organization. 2013. Laboratory testing for Middle East respiratory syndrome coronavirus. Interim recommendations, September 2013. http://www.who.int/csr/disease/coronavirus_infections/MERS_Lab_recos_16_Sept_2013.pdf. Accessed March 2014.

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                                     11,170     11,180     11,190     11,200     11,210     11,220     11,230     11,240     11,250     11,260     11,270     11,280
                                 GTTTGACTTA TGCAAACATA GTCTACGAGC CCACTACTCC CATTTCGTCA GCGCTGATTG CAGTTGCAAA TTGGCTTGCC CCCACTAATG CTTATATGCG CACTACACAT ACTGATATTG
KF917527|MERS-CoV-Jeddah-Camel-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KF958702|MERS-CoV-Jeddah-Human-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KJ740998|MERS-CoV-Jeddah-air-1-ORF1a                     .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

                                     11,290     11,300     11,310     11,320     11,330     11,340     11,350     11,360     11,370     11,380     11,390     11,400
                                 GTGTCTACAT TAGTATGTCA CTTGTATTAG TCATTGTAGT GAAGAGATTG TACAACCCAT CACTTTCTAA CTTTGCGTTA GCATTGTGCA GTGGTGTAAT GTGGTTGTAC ACTTATAGCA
KF917527|MERS-CoV-Jeddah-Camel-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KF958702|MERS-CoV-Jeddah-Human-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KJ740998|MERS-CoV-Jeddah-air-1-ORF1a .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

                                     11,410     11,420     11,430     11,440     11,450     11,460     11,470     11,480     11,490     11,500     11,510     11,520
                                 TTGGAGAAGC CTCAAGCCCC ATTGCCTATC TGGTTTTTGT CACTACACTC ACTAGTGATT ATACGATTAC AGTCTTTGTT ACTGTTAACC TTGCAAAAGT TTGCACTTAT GCCATCTTTG
KF917527|MERS-CoV-Jeddah-Camel-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KF958702|MERS-CoV-Jeddah-Human-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KJ740998|MERS-CoV-Jeddah-air-1-ORF1a .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

                                     11,530     11,540     11,550     11,560     11,570     11,580     11,590     11,600     11,610     11,620     11,630     11,640
                                 CTTACTCGCC ACAGCTTACA CTTGTGTTTC CGGAAGTGAA GATGATACTT TTATTATACA CATGTTTAGG TTTCATGTGT ACTTGCTATT TTGGTGTCTT CTCTCTTTTG AACCTTAAGC
KF917527|MERS-CoV-Jeddah-Camel-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KF958702|MERS-CoV-Jeddah-Human-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KJ740998|MERS-CoV-Jeddah-air-1-ORF1a .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

                                     11,650     11,660     11,670     11,680     11,690     11,700     11,710     11,720     11,730     11,740     11,750     11,760
                                 TTAGAGCACC TATGGGTGTC TATGACTTTA AGGTCTCAAC ACAAGAGTTC AGATTCATGA CTGCTAACAA TCTAACTGCA CCTAGAAATT CTTGGGAGGC TATGGCTCTG AACTTTAAGT
KF917527|MERS-CoV-Jeddah-Camel-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KF958702|MERS-CoV-Jeddah-Human-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KJ740998|MERS-CoV-Jeddah-air-1-ORF1a .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

                                     11,770     11,780     11,790     11,800     11,810     11,820     11,830     11,840     11,850     11,860     11,870     11,880
                                 TAATAGGTAT TGGCGGTACA CCTTGTATAA AGGTTGCTGC TATGCAGTCT AAACTTACAG ATCTTAAATG CACATCTGTG GTTCTCCTCT CTGTGCTCCA ACAGTTACAC TTAGAGGCTA
KF917527|MERS-CoV-Jeddah-Camel-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KF958702|MERS-CoV-Jeddah-Human-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KJ740998|MERS-CoV-Jeddah-air-1-ORF1a .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

                                     11,890     11,900     11,910     11,920     11,930     11,940     11,950     11,960     11,970     11,980     11,990     12,000
                                 ATAGTAGGGC CTGGGCTTTC TGTGTTAAAT GCCATAATGA TATATTGGCA GCAACAGACC CCAGTGAGGC TTTCGAGAAA TTCGTAAGTC TCTTTGCCAC TTTAATGACT TTTTCTGGTA
KF917527|MERS-CoV-Jeddah-Camel-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KF958702|MERS-CoV-Jeddah-Human-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KJ740998|MERS-CoV-Jeddah-air-1-ORF1a ......
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(B)

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                                     14,890     14,900     14,910     14,920     14,930     14,940     14,950     14,960     14,970     14,980     14,990     15,000
                                 AGTGGTTGTT AATAATTTAG ACAAGAGTGC TGGCCATCCT TTTAATAAGT TTGGCAAAGC TCGTGTCTAT TATGAGAGCA TGTCTTACCA GGAGCAAGAT GAACTCTTTG CCATGACAAA
KF917527|MERS-CoV-Jeddah-Camel-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KF958702|MERS-CoV-Jeddah-Human-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KJ741000|MERS-CoV-Jeddah-air-1-RdRp                                                                                                                 ..........

                                     15,010     15,020     15,030     15,040     15,050     15,060     15,070     15,080     15,090     15,100     15,110     15,120
                                 GCGTAACGTC ATTCCTACCA TGACTCAAAT GAATCTAAAA TATGCTATTA GTGCTAAGAA TAGAGCTCGC ACTGTTGCAG GCGTGTCCAT ACTTAGCACA ATGACTAATC GCCAGTACCA
KF917527|MERS-CoV-Jeddah-Camel-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KF958702|MERS-CoV-Jeddah-Human-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KJ741000|MERS-CoV-Jeddah-air-1-RdRp .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

                                     15,130     15,140     15,150     15,160     15,170     15,180     15,190     15,200     15,210     15,220     15,230     15,240
                                 TCAGAAAATG CTTAAGTCCA TGGCTGCAAC TCGTGGAGCG ACTTGCGTCA TTGGTACTAC AAAGTTCTAT GGTGGCTGGG ATTTCATGCT TAAAACATTG TACAAAGATG TTGATAATCC
KF917527|MERS-CoV-Jeddah-Camel-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KF958702|MERS-CoV-Jeddah-Human-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KJ741000|MERS-CoV-Jeddah-air-1-RdRp .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

                                     15,250     15,260     15,270     15,280     15,290     15,300     15,310     15,320     15,330     15,340     15,350     15,360
                                 GCATCTTATG GGTTGGGATT ACCCTAAGTG TGATAGAGCT ATGCCTAATA TGTGTAGAAT CTTCGCTTCA CTCATATTAG CTCGTAAACA TGGCACTTGT TGTACTACAA GGGACAGATT
KF917527|MERS-CoV-Jeddah-Camel-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KF958702|MERS-CoV-Jeddah-Human-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KJ741000|MERS-CoV-Jeddah-air-1-RdRp .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

                                     15,370     15,380     15,390     15,400     15,410     15,420     15,430     15,440     15,450     15,460     15,470     15,480
                                 TTATCGCTTG GCAAATGAGT GTGCTCAGGT GCTAAGCGAA TATGTTCTAT GTGGTGGTGG TTACTACGTC AAACCTGGAG GTACCAGTAG CGGAGATGCC ACCACTGCAT ATGCCAATAG
KF917527|MERS-CoV-Jeddah-Camel-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KF958702|MERS-CoV-Jeddah-Human-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KJ741000|MERS-CoV-Jeddah-air-1-RdRp .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

                                     15,490     15,500     15,510     15,520     15,530     15,540     15,550     15,560     15,570     15,580     15,590     15,600
                                 TGTCTTTAAC ATTTTGCAGG CGACAACTGC TAATGTCAGT GCACTTATGG GTGCTAATGG CAACAAGATT GTTGACAAAG AAGTTAAAGA CATGCAGTTT GATTTGTATG TCAATGTTTA
KF917527|MERS-CoV-Jeddah-Camel-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KF958702|MERS-CoV-Jeddah-Human-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KJ741000|MERS-CoV-Jeddah-air-1-RdRp .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

                                     15,610     15,620     15,630     15,640     15,650     15,660     15,670     15,680     15,690     15,700     15,710     15,720
                                 CAGGAGCACT AGCCCAGACC CCAAATTTGT TGATAAATAC TATGCTTTTC TTAATAAGCA CTTTTCTATG ATGATACTGT CTGATGACGG TGTCGTTTGC TATAATAGTG ATTATGCAGC
KF917527|MERS-CoV-Jeddah-Camel-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KF958702|MERS-CoV-Jeddah-Human-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KJ741000|MERS-CoV-Jeddah-air-1-RdRp .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
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(C)

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                                     18,010     18,020     18,030     18,040     18,050     18,060     18,070     18,080     18,090     18,100     18,110     18,120
                                 GATTGTAACT GGCCTTTTTA AAGATTGCTC TAGAGAAACT TCTGGCCTCT CACCTGCTTA TGCACCAACA TACGTTAGTG TTGATGACAA GTATAAGACG AGTGATGAGC TTTGCGTGAA
KF917527|MERS-CoV-Jeddah-Camel-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KF958702|MERS-CoV-Jeddah-Human-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KJ741001|MERS-CoV-Jeddah-air-1-ORF1b                                                                                                       ........ ..........

                                     18,130     18,140     18,150     18,160     18,170     18,180     18,190     18,200     18,210     18,220     18,230     18,240
                                 TCTTAATTTA CCCGCAAATG TCCCATACTC TCGTGTTATT TCCAGGATGG GCTTTAAACT CGATGCAACA GTTCCTGGAT ATCCTAAGCT TTTCATTACT CGTGAAGAGG CTGTAAGGCA
KF917527|MERS-CoV-Jeddah-Camel-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KF958702|MERS-CoV-Jeddah-Human-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KJ741001|MERS-CoV-Jeddah-air-1-ORF1b .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

                                     18,250     18,260     18,270     18,280     18,290     18,300     18,310     18,320     18,330     18,340     18,350     18,360
                                 AGTTCGAAGC TGGATAGGCT TCGATGTTGA GGGTGCTCAT GCTTCCCGTA ATGCATGTGG CACCAATGTG CCTCTACAAT TAGGATTTTC AACTGGTGTG AACTTTGTTG TTCAGCCAGT
KF917527|MERS-CoV-Jeddah-Camel-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KF958702|MERS-CoV-Jeddah-Human-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KJ741001|MERS-CoV-Jeddah-air-1-ORF1b .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

                                     18,370     18,380     18,390     18,400     18,410     18,420     18,430     18,440     18,450     18,460     18,470     18,480
                                 TGGTGTTGTA GACACTGAGT GGGGTAACAT GTTAACGGGC ATTGCTGCCC GTCCTCCACC AGGTGAACAG TTTAAGCACC TCGTGCCTCT TATGCATAAG GGGGCTGCGT GGCCTATTGT
KF917527|MERS-CoV-Jeddah-Camel-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KF958702|MERS-CoV-Jeddah-Human-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KJ741001|MERS-CoV-Jeddah-air-1-ORF1b .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

                                     18,490     18,500     18,510     18,520     18,530     18,540     18,550     18,560     18,570     18,580     18,590     18,600
                                 TAGACGACGT ATAGTGCAAA TGTTGTCAGA CACTTTAGAC AAATTGTCTG ATTACTGTAC GTTTGTTTGT TGGGCTCATG GCTTTGAATT AACGTCTGCA TCATACTTTT GCAAGATAGG
KF917527|MERS-CoV-Jeddah-Camel-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KF958702|MERS-CoV-Jeddah-Human-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KJ741001|MERS-CoV-Jeddah-air-1-ORF1b .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

                                     18,610     18,620     18,630     18,640     18,650     18,660     18,670     18,680     18,690     18,700     18,710     18,720
                                 TAAGGAACAG AAGTGTTGCA TGTGCAATAG ACGCGCTGCA GCGTACTCTT CACCTCTGCA ATCTTATGCC TGCTGGACTC ATTCCTGCGG TTATGATTAT GTCTACAACC CTTTCTTTGT
KF917527|MERS-CoV-Jeddah-Camel-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KF958702|MERS-CoV-Jeddah-Human-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KJ741001|MERS-CoV-Jeddah-air-1-ORF1b .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

                                     18,730     18,740     18,750     18,760     18,770     18,780     18,790     18,800     18,810     18,820     18,830     18,840
                                 CGATGTTCAA CAGTGGGGTT ATGTAGGCAA TCTTGCTACT AATCACGATC GTTATTGCTC TGTCCATCAA GGAGCTCATG TGGCTTCTAA TGATGCAATA ATGACTCGTT GTTTAGCTAT
KF917527|MERS-CoV-Jeddah-Camel-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KF958702|MERS-CoV-Jeddah-Human-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KJ741001|MERS-CoV-Jeddah-air-1-ORF1b .......... .......... .......... .......... .......... .......... ..........
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(D)

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                                          27,370     27,380     27,390     27,400     27,410     27,420     27,430     27,440     27,450     27,460     27,470     27,480
                                    GTGCACTTAT ATTAGCCGTT TTAGTAAGAT TAGCCTAGTT TCTGTAACTG ACTTCTCCTT AAACGGCAAT GTTTCCACTG TTTTCGTGCC TGCAACGCGC GATTCAGTTC CTCTTCACAT
KF917527|MERS-CoV-Jeddah-Camel-1    .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KF958702|MERS-CoV-Jeddah-Human-1    .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KJ741002|MERS-CoV-Jeddah-air-1-UpE                                 ..... .......... .......... .......... .......... .......... .......... .......... .......... ..........
                                          27,490     27,500     27,510     27,520     27,530     27,540     27,550     27,560     27,570     27,580     27,590     27,600
                                    AATCGCCCCG AGCTCGCTTA TCGTTTAAGC AGCTCTGCGC TACTATGGGT CCCGTGTAGA GGCTAATCCA TTAGTCTCTC TTTGGACATA TGGAAAACGA ACTATGTTAC CCTTTGTCCA
KF917527|MERS-CoV-Jeddah-Camel-1    .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KF958702|MERS-CoV-Jeddah-Human-1    .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KJ741002|MERS-CoV-Jeddah-air-1-UpE  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
                                          27,610     27,620     27,630     27,640     27,650     27,660     27,670     27,680     27,690     27,700     27,710     27,720
                                    AGAACGAATA GGGTTGTTCA TAGTAAACTT TTTCATTTTT ACCGTAGTAT GTGCTATAAC ACTCTTGGTG TGTATGGCTT TCCTTACGGC TACTAGATTA TGTGTGCAAT GTATGACAGG
KF917527|MERS-CoV-Jeddah-Camel-1    .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KF958702|MERS-CoV-Jeddah-Human-1    .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KJ741002|MERS-CoV-Jeddah-air-1-UpE  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
                                          27,730     27,740     27,750     27,760     27,770     27,780     27,790     27,800     27,810     27,820     27,830     27,840
                                    CTTCAATACC CTGTTAGTTC AGCCCGCATT ATACTTGTAT AATACTGGAC GTTCAGTCTA TGTAAAATTC CAGGATAGTA AACCCCCTCT ACCACCTGAC GAGTGGGTTT AACGAACTCC
KF917527|MERS-CoV-Jeddah-Camel-1    .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KF958702|MERS-CoV-Jeddah-Human-1    .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KJ741002|MERS-CoV-Jeddah-air-1-UpE  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......
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(E)

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                                          29,410     29,420     29,430     29,440     29,450     29,460     29,470     29,480     29,490     29,500     29,510     29,520
                                    TTCAATTGAA TAAACTCGGC ACTGAGGACC CACGTTGGCC CCAAATTGCT GAGCTTGCTC CTACAGCCAG TGCTTTTATG GGTATGTCGC AATTTAAACT TACCCATCAG AACAATGATG
KF917527|MERS-CoV-Jeddah-Camel-1    .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KF958702|MERS-CoV-Jeddah-Human-1    .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KJ741003|MERS-CoV-Jeddah-air-1-N                                                             .. .......... .......... .......... .......... .......... .......... ..........
                                          29,530     29,540     29,550     29,560     29,570     29,580     29,590     29,600     29,610     29,620     29,630     29,640
                                    ATCATGGCAA CCCTGTGTAC TTCCTTCGGT ACAGTGGAGC CATTAAACTT GACCCAAAGA ATCCCAACTA CAATAAGTGG TTGGAGCTTC TTGAGCAAAA TATTGATGCC TACAAAACCT
KF917527|MERS-CoV-Jeddah-Camel-1    .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KF958702|MERS-CoV-Jeddah-Human-1    .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KJ741003|MERS-CoV-Jeddah-air-1-N    .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
                                          29,650     29,660     29,670     29,680     29,690     29,700     29,710     29,720     29,730     29,740     29,750     29,760
                                    TCCCTAAGAA GGAAAAGAAA CAAAAGGCAC CAAAAGAAGA ATCAACAGAC CAAATGTCTG AACCTCCTAA GGAGCAGCGT GTGCAAGGTA GCATCACTCA GCGCACTCGC ACCCGTCCAA
KF917527|MERS-CoV-Jeddah-Camel-1    .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KF958702|MERS-CoV-Jeddah-Human-1    .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
KJ741003|MERS-CoV-Jeddah-air-1-N    .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
                                          29,770     29,780     29,790     29,800     29,810     29,820     29,830     29,840     29,851
                                    GTGTTCAGCC TGGTCCAATG ATTGATGTTA ACACTGATTA GTGTCACTCA AAGTAACAAG ATCGCGGCAA TCGTTTGTGT TTGGTAACCC C
KF917527|MERS-CoV-Jeddah-Camel-1    .......... .......... .......... .......... .......... .......... .......... .......... .......... .
KF958702|MERS-CoV-Jeddah-Human-1    .......... .......... .......... .......... .......... .......... .......... .......... .......... .
KJ741003|MERS-CoV-Jeddah-air-1-N    .......... .......... .......... .......... .......... .......... .......... .......... .......... .
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Figure S1. Alignment of MERS-CoV genome sequences obtained from the air sample, the camel's and the patient's isolates. Partial genome sequences obtained from the air sample were aligned with corresponding regions from both isolates for **(A)** ORF1a, **(B)** RdRp, **(C)** ORF1b, **(D)** UpE and **(E)** N regions.

Identity						
	10,150	10,160	10,170	10,180	10,190	10,200
MERS-CoV-Jeddah-Camel-1 (culture isolate)	TGTGCCCAGC	TGATCAGTTG	TCTGATCCTA	ATTATGATGC	CTTGTTGATT	TCTATGACTA
MERS-CoV-Jeddah-Camel-1 (original nasal isolate)	....	...C......	....	....	....	....
MERS-CoV-Jeddah-air-1-ORF1a	....	...C......	....	....	....	....

Figure S2. MERS-CoV genome in the air sample is identical to the original MERS-CoV-Jeddah-camel-1 isolate without the cell-culture adapted mutation. Partial genome sequence obtained from the air sample was aligned with the corresponding region from the original camel nasal sample and the cell culture isolate sequences containing the cell-culture adapted mutation at position 10154.

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化粧品

識別番号・報告回数			報告日	第一報入手日 2014 年 09 月 03 日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	乾燥濃縮人アンチトロンビンⅢ		研究報告の公表状況	YOMIURI ONLINE（読売新聞） /2014/09/02	公表国 日本	
販売名 （企業名）	①ノイアート静注用 500 単位　（日本血液製剤機構） ②ノイアート静注用 1500 単位（日本血液製剤機構）					

研究報告の概要

中国農業省は 1 日、黒竜江省ハルビン市の飼育場で死んだガチョウから、鳥インフルエンザ H5N6 型ウイルスが検出されたと発表した。
同市では 8 月 28 日、飼育されていたガチョウ約 1 万 8000 羽が大量死しているのが見つかり、原因を調査していた。一帯を封鎖し、感染の恐れのある鳥約 6 万 9000 羽を殺処分した。
中国では 5 月、四川省南充市の養鶏場で同型ウイルスが検出され、感染した男性（49）が肺炎で死亡した。同型感染による世界初の死亡例とみられる。

使用上の注意記載状況・その他参考事項等

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

報告企業の意見

インフルエンザウイルス（Influenza virus）は、オルソミクソウイルス科（Orthomyxoviridae）に属する A 型インフルエンザウイルス（Influenzavirus A）、B 型インフルエンザウイルス（Influenzavirus B）、C 型インフルエンザウイルス（Influenzavirus C）の 3 属を指す。ウイルスの大きさは直径 80～120nm の球形粒子で、エンベロープを有する 1 本鎖 RNA ウイルスで、万一原料血漿にインフルエンザウイルスが混入したとしても、HIV-1、或いは BVDV 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。

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。

中国で死んだガチョウからH5N6型鳥インフル

YOMIURI ONLINE（読売新聞） 2014/09/02

中国農業省は1日、黒竜江省ハルビン市の飼育場で死んだガチョウから、鳥インフルエンザH5N6型ウイルスが検出されたと発表した。

同市では8月28日、飼育されていたガチョウ約1万8000羽が大量死しているのが見つかり、原因を調査していた。一帯を封鎖し、感染の恐れのある鳥約6万9000羽を殺処分した。

中国では5月、四川省南充市の養鶏場で同型ウイルスが検出され、感染した男性(49)が肺炎で死亡した。同型感染による世界初の死亡例とみられる。

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2014. 10. 4	該当なし	
一般的名称	人赤血球液		研究報告の公表状況	Emerging Infectious Disease Journal, Vol.20 No.10; Available from:	公表国	
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社) 赤血球液-LR「日赤」(日本赤十字社) 照射赤血球液-LR「日赤」(日本赤十字社)				ドイツ	

研究報告の概要

○旅行者によりケニアから欧州に持ち込まれたオニョンニョンウイルス(ONNV)
1956年にウガンダの発熱患者において初めて分離されたONNVは、トガウイルス科に属する蚊媒介性のRNAウイルスである。遺伝的、血清学的にチクングニヤウイルス(CHIKV)と関連があるが、CHIKVとは異なりアフリカ大陸以外には存在せず、これまで他地域への輸入症例は報告されていなかった。
2013年10月14日、7日間の東アフリカ旅行から2日前に帰国した60歳のドイツ人女性が、旅行中から繰り返す発熱を訴えて受診した。発症から5日後に患者の熱は39℃に達し、頸部リンパ節腫脹、悪心、手足の小関節痛を伴い3日間持続した。顔、手足には浮腫が見られ、臨床検査では軽度のCRPの上昇が示された。血算値と肝機能検査は基準値内であった。血液塗抹標本 によるマラリア検査は陰性であった。IFA法により入院時の血清サンプルからONNV IgMとIgG抗体及びCHIKV IgMとIgG抗体が検出された。発症26日後の血清サンプルにおけるIFAでは、ONNV IgG抗体価が1/4に減少し、ウイルス中和試験によりONNVに特異的な中和抗体の存在が確認された。CHIKVに対する交差性中和抗体も検出されたが、ONNVの抗体価(1:1280)よりもかなり低かった(1:80)。発症10日後に患者は自然治癒した。
これは、欧州に輸入されたONNV感染の検査確定症例である。本症例は東アフリカでCHIKVと共循環するONNVが、CHIKV感染に類似した症状を引き起こす事実を示す。アルボウイルス疾患が疑われる旅行者の中でも、特にアルファウイルス属の血清学的交差反応を考慮することが重要である。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
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照射赤血球液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
7日間の東アフリカ旅行から帰国したドイツ在住者にONNV感染が発生したとの報告である。	日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。今後も引き続き情報の収集に努める。

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distance, and maximum parsimony methods. Mol Biol Evol. 2011;28:2731–9. http://dx.doi.org/10.1093/molbev/msr121

9. Duraisamy R, Rota PA, Palani G, Elango V, Sambasivam M, Lowe L, et al. Molecular characterization of wild-type measles viruses in Tamil Nadu, India, during 2005–2006: relationship of genotype D8 strains from Tamil Nadu to global strains. J Med Virol. 2012;84:348–57. http://dx.doi.org/10.1002/jmv.22244
10. Vaidya SR, Wairagkar NS, Raja D, Khedekar DD, Gunasekaran P, Shankar S, et al. First detection of measles genotype D7 from India. Virus Genes. 2008;36:31–4. http://dx.doi.org/10.1007/s11262-007-0172-2

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O'nyong-nyong Virus Infection Imported to Europe from Kenya by a Traveler

To the Editor: O'nyong-nyong virus (ONNV) is a mosquitoborne RNA virus of the *Togaviridae* family. The virus was first isolated in June 1959 from serum samples from febrile patients in the northern province of Uganda (*1*). Unlike other members of the alphavirus genus, ONNV is primarily transmitted by anopheline mosquitoes (*2*). ONNV is genetically and serologically related to chikungunya virus (CHIKV) (*1*), but is restricted to the African continent. The clinical picture resembles CHIKV infection, i.e., a self-limited febrile illness characterized by headache, rash, and joint pain. In contrast to CHIKV, ONNV is reported to cause lymphadenopathy more often and affected joints do not show effusions (*3*).

ONNV caused 2 large-scale epidemics in East Africa during 1959–1962 and in 1996. The first instance had spread from Uganda south to Mozambique and westward to Senegal. Comprising >2 million cases in east Africa alone, this first epidemic ranked among the largest mosquitoborne virus outbreaks recorded (*4*). After an absence of reported cases for 35 years, a second ONNV epidemic occurred in Uganda (*3*–*4*). Patients had fever, a maculopapular rash, pruritis, myalgia, and arthralgia of large joints. Lymphadenitis, most often of the posterior cervical spine region, was also observed (*3*). Despite the virus' potential to cause large outbreaks and its endemicity in the vast geographic area of East Africa, and at least sporadic occurrence in West Africa, imported cases to other areas have not been reported.

On October 14, 2013, a 60-year-old woman residing in Germany who had returned home 2 days before from a 7-week vacation in East Africa sought medical attention at the University Medical Center, Section of Clinical Tropical Medicine, in Heidelberg for recurring fever and illness that began during her travel. She and her husband had traveled from Kenya to Uganda, Rwanda, Tanzania, and back to Kenya, along the shore of Lake Victoria. Bed nets and malaria prophylaxis were used regularly. On October 9, she had experienced the first episode of fever, general malaise, arthralgia, and nausea while staying at the lake shore near the city of Kisumu, Kenya. Fever had persisted until October 12. Thin and thick blood films, examined in a local hospital and later in Nairobi, did not show malarial parasites.

October 14 was day 5 of symptom onset. Her fever reached 39°C and lasted 3 more days. It was accompanied by cervical spine and nuchal lymphadenopathy, nausea, and arthralgia of the small joints of her hands and feet. A maculopapular rash developed, which covered her face, hands, feet, and trunk. Her face, hands, and feet were edematous. Laboratory tests on admission to the medical center revealed a slightly elevated C-reactive protein level of 13 mg/L (reference level<5). Full blood count and results of liver function tests were within reference ranges. Thin and thick blood films were examined again and were negative for *Plasmodium* spp. A serum sample from the day of admission showed anti-ONNV IgM and IgG and anti-CHIKV IgM and IgG in the indirect immunofluorescence assay, according to Tappe et al. (*5*, Table). Serology for dengue virus and generic alphavirus reverse transcription PCR (*6*) were negative. A 4-fold anti-ONNV IgG titer decrease in the indirect immunofluorescence assay was demonstrated in the second serum sample, which was collected 26 days after disease onset (Table). The presence of ONNV-specific neutralizing antibodies in the second serum sample was confirmed by a virus neutralization test. Cross-neutralizing antibodies against CHIKV were detected also, but with a notably lower titer (1:80) when compared with the ONNV titer (1:1,280) (Table). Ten days after symptom onset, the patient recovered spontaneously. Her husband had no symptoms of illness during travel or after returning.

We report the laboratory-confirmed case of an ONNV infection imported into Europe. This patient most likely was infected in the eastern part of Kenya (Kisumu region), where she had stayed during the 2 weeks before symptom onset. The case highlights the fact that ONNV infections, which occur sympatrically with CHIKV infections in East Africa, lead to symptoms resembling CHIKV infection. The clinical and laboratory findings emphasize the importance of a careful diagnostic and clinical assessment of travelers

Table. Results of serologic analysis of a German traveler from Kenya with O'nyong-nyong virus infection, October 2013*

Virus	Immunofluorescence assay†				Virus neutralization test, 26 d after symptom onset
	5 d after symptom onset		26 d after symptom onset		
	IgG	IgM	IgG	IgM	
O'nyong-nyong	1:160	1:160	1:2,560	1:1,280	1:1,280
Chikungunya	1:80	1:80	1:2,560	1:320	1:80
Sindbis	Neg	Neg	1:640	Neg	ND
Semliki Forest	Neg	Neg	Neg	Neg	ND

*Neg, negative; ND, not done.
†Immunofluorescence assay and virus neutralization test results of <1:20 were considered negative.

with suspected arboviral disease, and consideration of the well-known serologic cross-reactions in the alphavirus group. Because of the serologic and clinical similarities of ONNV and CHIKV infections, it remains unclear how many true ONNV infections in travelers have been diagnosed as CHIKV infections. Similar to other arboviruses, especially CHIKV and dengue viruses (*7,8*), ONNV might have the potential to spread to areas outside of Africa. There are no known invasive anopheline vectors for ONNV in Europe, but it was demonstrated that the culicine mosquito species *Aedes aegypti*, found in some parts of Europe (*8*), might be a competent vector for ONNV (*9*). Thus, it will be critical to study the vector competence of both the indigenous anopheline and culicine mosquitoes for ONNV in Europe.

Acknowledgments

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This letter is dedicated to the late Ursula Herrmann (1927–2014) for making this study possible.

Dennis Tappe,[1] Annette Kapaun,[1] Petra Emmerich, Renata de Mendonca Campos, Daniel Cadar, Stephan Günther, and Jonas Schmidt-Chanasit

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References

1. Williams MC, Woodall JP. O'nyong-nyong fever: an epidemic virus disease in East Africa. II. Isolation and some properties of the virus. Trans R Soc Trop Med Hyg. 1961;55:135–41. http://dx.doi.org/10.1016/0035-9203(61)90017-7
2. Corbet PS, Williams MC, Gillett JD. O'Nyong-Nyong fever: an epidemic virus disease in East Africa. IV. Vector studies at epidemic sites. Trans R Soc Trop Med Hyg. 1961;55:463–80. http://dx.doi.org/10.1016/0035-9203(61)90095-5
3. Rwaguma EB, Lutwama JJ, Sempala SD, Kiwanuka N, Kamugisha J, Okware S, et al. Emergence of epidemic O'nyong-nyong fever in southwestern Uganda, after an absence of 35 years. Emerg Infect Dis. 1997;3:77. http://dx.doi.org/10.3201/eid0301.970112
4. Lutwama JJ, Kayondo J, Savage HM, Burkot TR, Miller BR. Epidemic O'Nyong-Nyong fever in southcentral Uganda, 1996–1997: entomologic studies in Bbaale village, Rakai District. Am J Trop Med Hyg. 1999;61:158–62.
5. Tappe D, Schmidt-Chanasit J, Ries A, Ziegler U, Müller A, Stich A. Ross River virus infection in a traveller returning from northern Australia. Med Microbiol Immunol (Berl). 2009;198:271–3 http://dx.doi.org/10.1007/s00430-009-0122-9.
6. Eshoo MW, Whitehouse CA, Zoll ST, Massire C, Pennella TT, Blyn LB, et al. Direct broad-range detection of alphaviruses in mosquito extracts. Virology. 2007;368:286–95. http://dx.doi.org/10.1016/j.virol.2007.06.016
7. Schmidt-Chanasit J, Emmerich P, Tappe D, Gunther S, Schmidt S, Wolff D, et al. Autochthonous dengue virus infection in Japan imported into Germany, September 2013. Euro Surveill. 2014;19:20681.
8. Van Bortel W, Dorleans F, Rosine J, Blateau A, Rousset D, Matheus S, et al. Chikungunya outbreak in the Caribbean region, December 2013 to March 2014, and the significance for Europe. Euro Surveill. 2014;19:20759.
9. Vanlandingham DL, Hong C, Klingler K, Tsetsarkin K, McElroy KL, Powers AM, et al. Differential infectivities of o'nyong-nyong and chikungunya virus isolates in *Anopheles gambiae* and *Aedes aegypti* mosquitoes. Am J Trop Med Hyg. 2005;72:616–21.

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The Public Health Image Library (PHIL)
http://phil.cdc.gov/phil

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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 2014. 10. 4	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	人赤血球液		研究報告の公表状況	Emerging Infectious Disease Journal, Vol.20 No.10; Available from: http://wwwnc.cdc.gov/eid/article/20/10/14-0353_article	公表国 チリ	
販売名（企業名）	赤血球濃厚液-LR「日赤」（日本赤十字社） 照射赤血球濃厚液-LR「日赤」（日本赤十字社） 赤血球液-LR「日赤」（日本赤十字社） 照射赤血球液-LR「日赤」（日本赤十字社）					

研究報告の概要

○家族内及び院内におけるアンデスハンタウイルスのヒトーヒト感染、2011年チリ南部
アンデスハンタウイルス（ANDV）はチリにおけるハンタウイルス心肺症候群の原因であり、またヒトからヒトへの感染が証明された唯一のハンタウイルスである。
発端患者への接触後に2人の家族と2人の医療従事者が発症した、5人のANDV感染症例を報告する。疫学調査及びウイルス分離株の解析の結果、4人の二次症例患者に関するヒトーヒト感染が確認された。ANDV感染患者または彼らの体液に直接触れる医療従事者は、ウイルス感染を防ぐための予防措置をとらなくてはならない。さらに、ANDV感染確定患者に接触したか、ウイルスに環境曝露した可能性のある者は全て、42日間（ヒト間感染最大潜伏期間）臨床症状を監視する必要がある。

使用上の注意記載状況・その他参考事項等

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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
発端患者への接触後、4人（2人の医療従事者を含む）にANDVのヒトーヒト感染が発生したとの報告である。	日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、帰国（入国）後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。今後も引き続き情報の収集に努める。

⑯

Person-to-Person Household and Nosocomial Transmission of Andes Hantavirus, Southern Chile, 2011

Constanza Martinez-Valdebenito, Mario Calvo, Cecilia Vial, Rita Mansilla, Claudia Marco, R. Eduardo Palma, Pablo A. Vial, Francisca Valdivieso, Gregory Mertz, and Marcela Ferrés

Andes hantavirus (ANDV) causes hantavirus cardiopulmonary syndrome in Chile and is the only hantavirus for which person-to-person transmission has been proven. We describe an outbreak of 5 human cases of ANDV infection in which symptoms developed in 2 household contacts and 2 health care workers after exposure to the index case-patient. Results of an epidemiologic investigation and sequence analysis of the virus isolates support person-to-person transmission of ANDV for the 4 secondary case-patients, including nosocomial transmission for the 2 health care workers. Health care personnel who have direct contact with ANDV case-patients or their body fluids should take precautions to prevent transmission of the virus. In addition, because the incubation period of ANDV after environmental exposure is longer than that for person-to-person exposure, all persons exposed to a confirmed ANDV case-patient or with possible environmental exposure to the virus should be monitored for 42 days for clinical symptoms.

Hantavirus cardiopulmonary syndrome (HCPS) is caused by infection with New World hantaviruses. First described in 1993 in the southwestern United States, HCPS has been documented throughout the Americas (*1*,*2*). For human cases, the mean incubation period of hantavirus infection from exposure to illness onset is 18.5 (range 7–42) days (*3*). As of December 31, 2013, a total of 848 human HCPS cases had been reported in Chile; the case-fatality rate has ranged from 32% to 35% per year (*4*).

The sole confirmed etiologic agent of HCPS in Chile is Andes virus (ANDV). Human infection with this virus occurs from exposure to contaminated excreta and secretions of rodents of the family *Cricetidae*. Transmission of ANDV between rodents has been experimentally documented after exposure of seronegative rodents to inhalation of aerosolized infected rodent secretions (*5*). ANDV is endemic in Chile and Argentina and is the only hantavirus for which person-to-person transmission has been documented. Person-to-person transmission of ANDV occurs mainly in family clusters or, less commonly, after activities in which close contact with an infected case-patient has occurred, primarily during the disease prodrome (*6*–*8*). A prospective study in Chile found that sexual partners and other close household contacts of ANDV-infected persons showed a 10-fold higher risk of acquiring the virus than household contacts who did not share bed or bedroom with the index case-patient (*3*,*9*).

Nosocomial transmission of ANDV has been a matter of concern for infection control practice and for health care workers who provide care for these patients, and in particular for workers who perform invasive procedures. In Argentina, person-to-person transmission of ANDV was documented in a physician who acquired infection after exposure to an ANDV-infected patient shortly after onset of the febrile prodrome (*7*,*8*). Although person-to-person transmission in Chile has been epidemiologically documented (*10*), nosocomial transmission has not been reported. Seroprevalence studies conducted among health care workers in hospitals in Chile where patients with ANDV infection have been treated have reported that health care workers exhibited ANDV IgG antibody at a proportion similar to that of the general population (*11*,*12*). Similarly, a study in the southwestern United States found no evidence of nosocomial transmission of another hantavirus, Sin Nombre virus (*13*).

We describe an outbreak of 5 cases of ANDV infection that occurred in a small, rural community in southern Chile in 2011. We present the epidemiologic and the clinical features of the cases, along with the molecular analysis of the

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virus strains from each case. Epidemiologic and virus sequence analyses support person-to-person transmission of ANDV in 4 of these cases, including nosocomial transmission in 2 cases.

Materials and Methods

Study Population

A case cluster of 5 human case-patients, including 2 persons involved in health care, occurred in Corral, Los Rios, Chile, during February–April 2011. Clinical history and information from epidemiologic questionnaires were obtained for each patient; all 5 had an acute febrile illness and signs and symptoms compatible with hantavirus infection. Acute infection was confirmed by detection of IgM against viral nucleoprotein antigen and real-time reverse transcription PCR (RT-PCR) targeting the small RNA segment of ANDV in blood samples obtained from these patients during the acute illness (*14,15*). Samples from 7 additional patients who had had HCPS in the same geographic region in previous years were used as controls for virus sequence analysis. All participants signed an informed consent approved by an ethics committee.

Geographic and Demographic Features of Corral

Corral is a coastal town (39°52′0″ S, 73°25′60″ W) located 15 km west of Valdivia, the capital of the Los Rios region in Chile; the town is in the foothills of a coastal mountain range in the Valdivian rainforest ecoregion (*16*). The population is ≈5,433 inhabitants. Corral has 1 primary care hospital with 5 beds (hospital I); all patients with complications are transferred to a regional care center in Valdivia that has intensive care facilities (hospital II). Since 1997, a total of 13 cases of hantavirus infection have been reported in Corral, including the 5 cases described in this report (*17*). Prior to this report, the last 2 confirmed cases were in 2008 and 2010.

Outbreak Description

On March 20, 2011, two suspected cases of hantavirus infection were reported. The patients were a 31-year-old woman (case-patient B) who worked as a nursing assistant at hospital I and a 53-year-old woman (case-patient C). Both lived near Corral. In addition to these cases, in late February, a 73-year-old man (case-patient A), the spouse of case-patient C, had been transferred from hospital I to hospital II for treatment of a pulmonary disease and evaluated for hantavirus infection; initial serologic testing results at a national reference laboratory were negative. On March 22, a fourth patient (case-patient D), a 60-year-old female housekeeper at hospital I, was admitted to hospital II with respiratory failure; she died a few hours later. A fifth patient (case-patient E), a 34-year-old man who was the husband of case-patient B, was hospitalized on April 3 at hospital II. On April 3, an epidemiologic investigation was initiated by the Health and Epidemiology Service, including investigation of infection control measures used at hospital I.

Genetic Characterization of the Virus

RNA was obtained from patients' leukocytes from diagnostic samples and extracted by using the High Pure Viral RNA Kit (Roche Diagnostic GmbH, Roche Applied Science, Mannheim, Germany), according to the manufacturer's instructions. For segment amplification, heminested RT-PCR was performed as described previously (*16*). Two portions of the medium segment, Gn and Gc, were amplified (Table 1), and the amplicons underwent agarose gel purification and sequencing in both directions. The chromatogram of each sequence was analyzed and aligned to generate a consensus sequence by using BioEdit version 7.1.11 (http://www.mbio.ncsu.edu/bioedit/bioedit.html). Twelve sequences were aligned by using ClustalW (http://www.clustal.org). Sequences were phylogenetically analyzed by conducting maximum-likelihood (ML) and Bayesian methodology on the concatenated Gn and Gc sequences. For ML, PAUP* version 4.0 (*18*) was used for a heuristic search with 100 random additions and branch swapping via tree-bisection-reconnection (*19*). jModeltest 3.7 was used to choose the best-fitting model of sequence evolution (*20*). The corrected Akaike information criterion (Akaike 1974) identified the Kimura 81 unequal base frequencies + gamma model (K81uf + Γ) as optimal (–lnL = 1251.2770, Akaike information criterion = 2515.7539, G = 1.5780), with base frequencies A = 0.2868, C = 0.3132, G = 0.0670, and T = 0.3329. Reliability of nodes in the ML tree was estimated by bootstrap analysis (*21*) obtained after 1,000 pseudo-replicates. The tree was rooted on the basis of the outgroup criterion by using the ANDV sequence available in GenBank (accession no. NC_003467.2). Sequences also were analyzed in a Bayesian framework to estimate the posterior probabilities of phylogenetic trees. Ten million phylogenetic trees were generated; the first 1,000 trees of the sample were removed to avoid including trees before convergence of the Markov Chain. As 2 independent molecular markers were used, a general likelihood–based mixture model of sequence evolution was applied as described (*22*). This model accommodates cases in which different sites in the alignment evolved in qualitatively distinct ways but does not require prior knowledge of these patterns or

Table 1. Primers used for M segment amplification and sequencing of Andes hantavirus

Primer identification	Primer sequences, 5′ → 3′
GN1+	TAGTAGTAGACTCCGCAAGAAGAAG
GN534–	TCCTGCTKKTAAACACACTAGCCAT
GC94+	TGCAAATGATTGTGTTAGTAACACCA
GC674–	GTATTAGAGCCCCTAGCACAGGTT

partitioning data. These analyses were conducted by using Bayes Phylogenies software (*22*). To find the best mixture model of evolution, the number of general time reversible matrices was estimated by using a reversible-jump Markov chain Monte Carlo method (*23*).

Results

Laboratory and Epidemiologic Investigations

IgM and IgG against ANDV were detected in serum samples, and ANDV RNA was detected by RT-PCR in blood for all 5 patients in the cluster (Table 2; Figure 1). Case-patient A, the 73-year-old man, was identified as the index case-patient of the cluster.

Case-patient A lived in a small settlement near Corral. His main risk activity was the cleaning of a home cellar where he was moving tiles on February 5. The cellar was heavily contaminated with rodent feces. The patient was admitted to hospital I on February 24 after 3 days of fever, dry cough, weakness, and progressive dyspnea. During hospitalization, he experienced progressive respiratory compromise, productive cough, and intense sweating that required frequent changes of gowns, sheets, and blankets. On February 26, he was transferred to the critical care unit at hospital II for mechanical ventilation. Serum samples were sent to the National Reference Laboratory 11 days after onset of his symptoms; results were negative for ANDV IgM. When the epidemiologically related hantavirus case-patients were admitted to hospital II, ANDV IgM testing was repeated, 24 days after onset of his symptoms, and results were positive. Case-patient A died on March 26 after 28 days of mechanical ventilation and use of vasoactive drugs.

Case-patient B, a nursing assistant at hospital I, exhibited a fever on March 17. She was hospitalized on March 20 and the same day was transferred from hospital I to the intensive care unit at hospital II. Severe shock and respiratory failure developed, and high doses of vasopressors and mechanical ventilation were required. A diagnosis of HCPS caused by ANDV infection was confirmed after 8 days of symptoms, and she was discharged on April 11. This patient had direct contact with case-patient A at hospital I from February 24–26, during his febrile prodrome and progression to the cardiopulmonary phase. She changed the patient's clothes, sheets, and blankets because he perspired profusely. In addition, having met the patient previously, she greeted case-patient A with a kiss on his cheek several times during his hospitalization.

Table 2. Clinical and epidemiologic features of 5 patients involved in outbreak of ANDV infection, Chile, 2011*

Feature	Case-patient A†	Case-patient B	Case-patient C	Case-patient D	Case-patient E
Age, y/sex	73/M	31/F	53/F	60/F	34/M
Occupation	Farmer	Nursing assistant at hospital	Teacher	Cleaning personnel at hospital	Car mechanic
Relationship to other case-patients	Husband of case-patient C	Health care provider for case-patient A	Wife of case-patient A	Health care assistant for case-patient A	Husband of case-patient B
Date of symptom onset	Feb 21	Mar 17	Mar 18	Mar 18	Apr 2
Date of hospitalization	Feb 24	Mar 20	Mar 20	Mar 22	Apr 3
Signs and symptoms					
Fever	Yes	Yes	Yes	Yes	Yes
Respiratory symptoms‡	Yes	Yes	No	Yes	Yes
Gastrointestinal symptoms§	No	No	Yes	Yes	No
Other symptoms¶	No	Yes	Yes	Yes	Yes
Mechanical ventilation, d	28	8	0	1	6
Hospitalization, d	30	22	12	1	17
Outcome	Died	Survived	Survived	Died	Survived
Days from environmental exposure to onset of symptoms	16	25–26	41	7–45	41–42
Days from exposure to hantavirus case-patient to onset of symptoms	NA	19–21	22–25	18–20	13–27
Laboratory test results on admission					
Platelet count, × 10^3/µL	51	56	108	101	147
Leukocytes, × 10^3 cells/µL	4,67	5,46	1,21	3,92	11,46
Hematocrit, %	52	39	39	45	44
Lymphocytes, %	12	7	39	19	7
Immunoblasts, %	Yes	Yes	Yes	NR	Yes
IgM/IgG for ANDV	Negative#	Positive	Positive	Positive	Positive
RT-PCR ANDV in blood cells	ND	Positive	Positive	Positive	Positive

*ANDV, Andes virus; NA, not applicable; NR, not reported; RT-PCR, reverse transcription PCR; ND, not done.
†Index case-patient.
‡Dry cough, dyspnea, cyanosis, crepitus.
§Vomiting, diarrhea, nausea.
¶Severe headache, meningeal signs, myalgia, arthralgia, conjunctival infection, chills, photophobia, facial edema.
#On hospital admission. Repeat testing after 24 days yielded positive results.

She also had close contact with her husband at their home from the time she cared for the index case-patient through the first 3 days of her illness. She recalled possible environmental exposure from camping at 2 local beaches during February 1–4 and February 19–20; she collected wood and cleaned the area to set up tents.

Case-patient C was the spouse of case-patient A. She shared the same bed and cared for him during his febrile prodrome but denied that they had sexual activity after symptom onset. She entered the contaminated cellar with her husband but did not participate actively in his work in this area. On March 18, twenty-five days after her husband's illness onset and 41 days after they entered the cellar, she exhibited mild fever, severe headache, myalgia, and photophobia . She sought medical attention at hospital II while her husband was still hospitalized, and acute ANDV infection was confirmed on March 24. Her chest radiograph results were normal. Her most remarkable symptoms were headache and irritability, and she had meningeal signs. Testing of cerebrospinal fluid (CSF) showed 8 white mononuclear cells, normal glucose levels, and a slightly elevated protein level of 0.5 g/L. CSF testing by RT-PCR for ANDV and ELISA for ANDV-specific IgG yielded negative results.

Case-patient D, a housekeeper at hospital I, had fever, abdominal pain, and vomiting develop on March 18. Two days later, she was hospitalized at hospital I, and 4 days later, she was transferred to hospital II, where severe shock and respiratory failure developed. She died a few hours after admission to hospital II. Her diagnosis was confirmed by positive results of serologic testing and RT-PCR for ANDV. She had direct and indirect contact with case-patient A while he was at hospital I. She entered his room and helped the nursing assistant (case-patient B) change his clothes and remove his sheets and bedclothes for washing.

Case-patient E, the husband of case-patient B, had fever, headache, myalgia, and back pain develop on April 2, and he was admitted to hospital II on April 3. Serologic testing for ANDV IgM and IgG after 5 days of symptoms yielded negative results, but results of RT-PCR for ANDV RNA were positive. IgM and IgG seroconversion were confirmed 10 days after symptom onset. The person-to-person exposure period for this patient was March 6–20; his possible environmental exposure exceeded the known incubation period for ANDV (*11*). Shock and respiratory failure developed, and he required mechanical ventilation and vasopressors but survived.

Environmental Investigation

Rodent trapping was performed for 2 and 3 nights, respectively, at the 2 sites where case-patients reported possible environmental exposure: the cellar of the home of

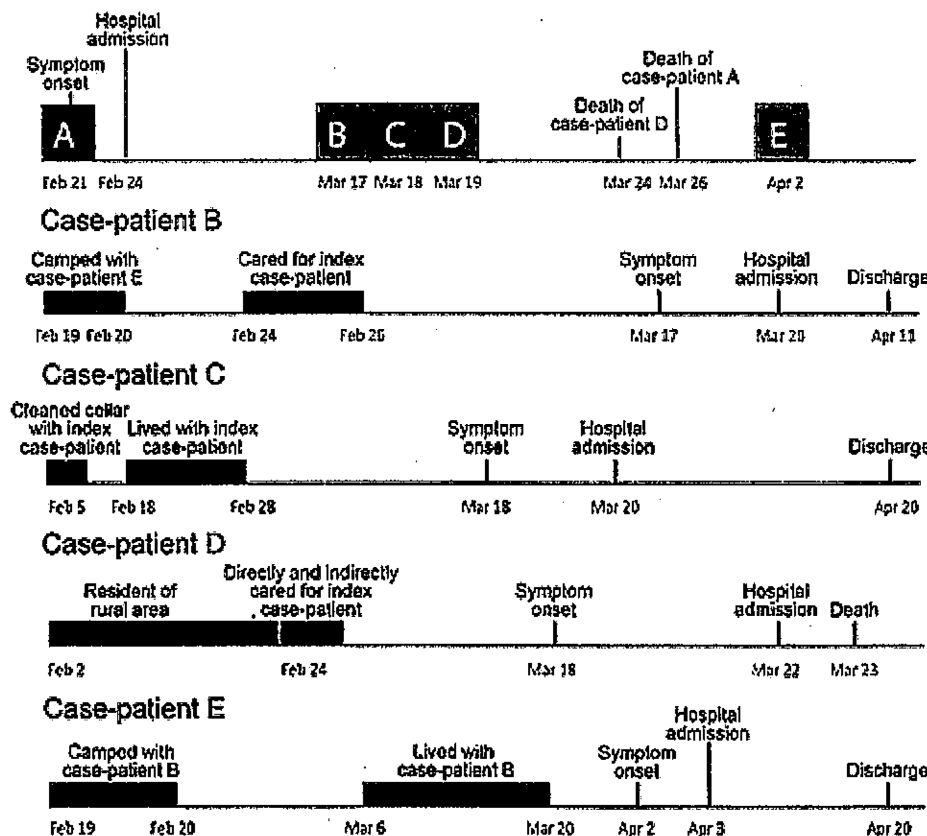

Figure 1. Timelines showing progression and key events related to each case-patient (A–E) in a cluster of 5 Andes hantavirus cases, southern Chile, 2011. Blue boxes along timeline for index case-patient (A) indicate date of illness onset for subsequent case-patients; green boxes indicate environmental exposures (exposure for case-patient A was the same as for case-patient C); red boxes indicate contact with other case-patients.

case-patients A and C and a camping area used by case-patients B and E (Table 3). Rodent serum samples were tested for ANDV antibodies by strip immunoblot assay (*24*); results were positive for 1 *Abrothrix longipilis* rodent trapped at the camping site. However, RT-PCR results for this sample were negative, and testing of rodents trapped at the home of case-patients A and C yielded negative results.

Viral Molecular Analysis

A portion of 942 bp of the ANDV small RNA segment was amplified and sequenced from samples of each of the 5 patients in the case cluster. Sequences aligned by using ClustalW showed 100% identity (data not shown), an observation consistent with the high degree of conservation of the small segment among hantaviruses (*7,25*).

Virus variability was established by comparing a portion of 914 bp of the highly variable ANDV medium RNA segment. The sequences obtained for the 2 medium segments encoding the ANDV glycoproteins Gn and Gc were compared separately (data not shown) and concatenated. Results were visualized in the identity matrix of concatenated sequences and showed that the concatenated sequences derived from the 5 cases in the cluster were similar to each other but differed from viral sequences from 7 patients who acquired ANDV in the same community in previous years (Table 4). The molecular identity of the concatenated Gn and Gc sequences between cases ranged from 99% to 100%, whereas the comparison with control sequences from the same geographic region ranged from 97% to 99%. These values show higher identity between the sequences derived from the cluster cases compared with other human cases from the same geographic region from previous years. All sequences obtained in this study have been deposited in GenBank (accession nos. KC567258–KC567281).

The phylogenetic analyses through ML and Bayesian methods revealed similar topologic results; thus, a single tree is shown (Figure 2). Results show 2 major groups with strong support provided by the bootstrap and posterior probability values. The group of samples that included the Corral cases is clearly separated from other major clustering that includes ANDV sequences from other localities in Chile.

Discussion

ANDV is the only hantavirus for which person-to-person transmission has been reported (*7*). Our study of a case cluster in Chile provides epidemiologic and molecular evidence that strongly supports the conclusion that 4 of 5 cases resulted from person-to-person transmission of ANDV, including 2 cases of nosocomial transmission.

Most of the reports of person-to-person transmission of ANDV share common traits that constitute potential risk factors for virus spread (*7–9*). These features were also observed in this cluster. First, the period of the disease during which the acute case-patient and the household contact or health care personnel have close contact is primarily the febrile prodrome phase, when symptoms are nonspecific for hantavirus. Second, the number of days from exposure to an index case-patient and the onset of symptoms among additional cases ranges from 12 to 27 days (*7,26*), consistent with the intervals observed in our report. In the 2 cases for which environmental exposure was reported, the estimated incubation period after that exposure exceeded the longest reported incubation range of 42 days for ANDV (*3,11*). In contrast, in these 2 cases the estimated incubation periods from exposure to a case-patient to onset of symptoms was 13–27 days. Finally, the viral genetic characterization established that viruses from the case cluster shared a high nucleotide sequence identity in Gn and Gc fragments, the most variable viral genomic regions (*6*).

During the prodrome, when symptoms are nonspecific, consideration of ANDV infection and early diagnosis might be triggered by a history of environmental exposure (*1,2*) or close exposure to another confirmed case-patient within the known incubation period (*3,6*). In this cluster, all the cases appeared in a geographic region that is considered an endemic risk area for hantavirus (*26,27*). However, no other cases had occurred in this town since 2010, and our epidemiologic and virus sequence analysis showed that the main risk factor for all the 4 additional cases was the patient's close contact with a symptomatic, HCPS case-patient (*6,28*).

One case of nosocomial transmission of the virus has been previously reported in Argentina (*7*), and evidence of this transmission has been sought in Chile (*12*). We document 2 cases of nosocomial transmission of ANDV, from the index case-patient to a nursing assistant and to a housekeeper, even though their contact with the patient was limited to kissing the patient on the cheek and to

Table 3. Results of environmental investigation for 4 cases of ANDV infection, Chile, 2011*

Case-patients	Days after case-patient diagnosis	No. trapping nights	No. trapped rodents	No. traps per night	Rodent species trapped	SIA results, n = 24	RT-PCR results
B and E	63	3	46	57, 40, 40	*Abrothrix longipilis, A. olivaceus,* other *Abrothrix* sp., *Oligoryzomys longicaudauts*	1 positive (*A. longipilis*)	Negative
A and C	90	2	9	68, 68	*A. olivaceus, O. longicaudauts, Rattus norvegicus, R. rattus*	Negative	ND

*SIA, strip immune assay; RT-PCR, reverse transcription PCR; ND, not done.

Table 4. Identity matrix of concatenated Gn and Gc sequences of ANDV isolates from the 5 case-patients in this study compared with sequences from ANDV samples from previous case-patients in the same geographic region of Chile*

Sequence	Pan2010	Pai2011	Mar2010	Fut2010	C2012(1)	C2012(2)	Pan2012	C	B	E	D	A
Pan2010	–	0.972	0.991	0.985	0.964	0.971	0.989	0.961	0.961	0.955	0.961	0.961
Pai2011		–	0.974	0.973	0.983	0.994	0.976	0.984	0.984	0.978	0.984	0.984
Mar2010			–	0.987	0.970	0.975	0.995	0.961	0.961	0.955	0.961	0.961
Fut2010				–	0.964	0.974	0.990	0.960	0.960	0.953	0.960	0.960
C2012(1)					–	0.985	0.970	0.970	0.970	0.963	0.970	0.970
C2012(2)						–	0.978	0.981	0.981	0.974	0.981	0.981
Pan2012							–	0.963	0.963	0.957	0.963	0.963
C								–	1.000	0.993	1.000	1.000
B									–	0.993	1.000	1.000
E										–	0.993	0.993
D											–	1.000
A												–

*Geographic location and year are indicated for control cases (numbers in parentheses indicate multiple cases in the same year; case-patient identification letters (A–E) are given for cases from this study. – indicates alignment of the same sequence.

handling bedding and gowns (no invasive procedures). Two seroprevalence studies performed soon after recognition of hantavirus in Chile did not reveal a higher proportion of antibodies against ANDV among hospital personnel when compared with the general population (*11,12*).

In our study, ANDV infection was not diagnosed in the index case-patient until he had been ill for 31 days, which resulted in a wider time frame of exposure for health care personnel. The patient had a history of diabetes mellitus but no history of any other immunodeficiency that might explain his initial negative serologic test. However, the initial testing was not repeated, so we cannot rule out the possibility of a false-negative result.

For case-patient C, the clinical manifestation of illness was unusual because she lacked respiratory symptoms and showed meningeal irritability as the main sign of the infection. Viral RNA and specific antibodies were not detected, but a slight elevation in the CSF white blood count and protein level were seen. It is possible that viral RNA was present before CSF testing or that it was below the level of detection by RT-PCR, but the timing of her symptoms is probably inconsistent with a postinfectious process.

It is not clear why person-to-person transmission has been documented for ANDV but not for other hantaviruses. Risk factors associated with close contact, including sexual contact, deep kissing, or sleeping in the same bed or room, have been identified in a prospective study of household contacts of index case-patients with HCPS (*9*). As such, respiratory secretions, saliva, or both may be involved in transmission. Puumala virus RNA has been detected by RT-PCR but not by cell culture in saliva from patients who had hemorrhagic fever with renal syndrome (*29*). The antiviral activity of different human saliva concentrations has been experimentally tested against Hantaan virus, Puumala virus, and ANDV; ANDV was least sensitive to the antiviral effect of saliva (*30*). RT-PCR testing has found ANDV RNA in previous and ongoing studies in blood and in body fluids, including gingival crevicular fluid, saliva, endotracheal fluid, and urine (*31*). ANDV was isolated from blood obtained from a child in Chile before the onset of symptoms or development of ANDV antibodies (*32*), and studies are ongoing to determine which, if any, of the body fluids positive by RT-PCR also contain infectious virus.

To characterize and compare the outbreak viral sequences, we used as reference material a selection of sequences from strains obtained 2 or 3 years earlier in the same ecogeographic region near Corral. All 5 medium fragments obtained from case-patient isolates in this cluster were highly similar to each other but were more distantly related to the reference sequences. The strong relatedness of the viruses in the Corral cluster is supported by high bootstrap and posterior probability values in the phylogenetic analyses. Furthermore, the small segment showed 100% identity between the 5 sequences in this cluster. The dates of exposure to high-risk environments or to persons with ANDV infection, known incubation periods, and 100% sequence identify all support a conclusion of person-to-person transmission (*7*). Our data showed 99%–100% identity for a fragment of 913 bp of the medium segment, supporting identity using different sequences. However, we did not include noncoding region fragments, which might provide additional confirmation of identity.

Our study documents a small but definite risk of nosocomial acquisition of ANDV infection for personnel who care for patients, including handling of bedding and gowns. After this investigation, the Ministry of Health of Chile has recommended, in addition to strict adherence to universal precautions, the use of droplet precautions when ANDV infection is suspected. Use of N95 respirator masks, designed to prevent the inhalation of airborne particles, is recommended for those procedures associated with aerosolization of viral contaminated secretions (e.g., respiratory, saliva) when procedures such as suction or intubation are performed. However, this recommendation should be extended to all personnel who have any kind of direct contact with patients or body fluids, including bedding and gowns.

Finally, all close household contacts and health care personnel exposed to a confirmed ANDV case-patient should be closely monitored for signs and symptoms of

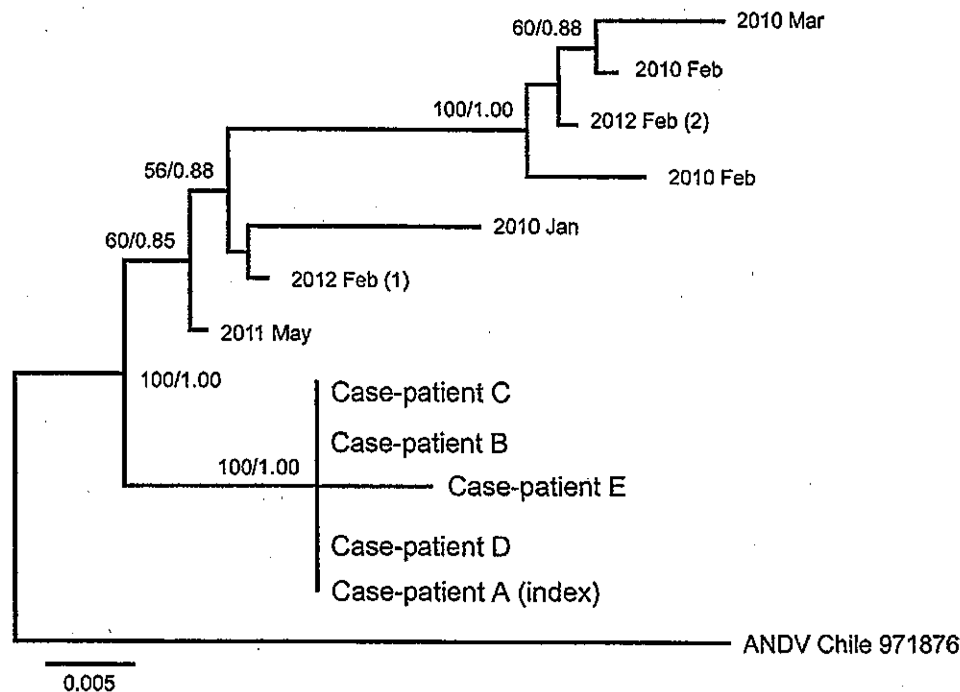

Figure 2. Phylogenetic analyses of the medium RNA segment (Gc and Gn) of concatenated sequences of Andes hantavirus (ANDV). Isolates from the case-patients (A–E) from the 2011 outbreak in Chile were compared with control samples from the same geographic region (indicated by year isolated; number in parentheses indicates multiple isolates from the same year) and an ANDV sequence from GenBank (bottom isolate on tree; accession no. NC_003467.2). Scale bar indicates substitutions per site.

infection, such as fever, myalgia, headache, and abdominal pain, during the entire documented incubation period of 42 days, even though in person-to-person transmission of ANDV, the onset of symptoms has usually occurred 12–27 days after close contact with a sick patient (*6*,*9*). ANDV RT-PCR should be performed in addition to testing for specific IgM in any exposed contact in whom fever develops within the incubation period, particularly if testing is done within a few days of the onset of fever and before onset of the cardiopulmonary phase. Results of ANDV RT-PCR on blood cells may be positive as early as 5–15 days before onset of symptoms or detection of ANDV antibody (*9*). As we have documented, RT-PCR can detect ANDV RNA in the rare, symptomatic patient in whom seroconversion is delayed.

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References

1. Pini N. Hantavirus pulmonary syndrome in Latin America. Curr Opin Infect Dis. 2004;17:427–31. http://dx.doi.org/10.1097/00001432-200410000-00007
2. Schmaljohn C, Hjelle B. Hantaviruses: a global disease problem. Emerg Infect Dis. 1997;3:95–104. http://dx.doi.org/10.3201/eid0302.970202
3. Vial PA, Valdivieso F, Mertz G, Castillo C, Belmar E, Delgado I, et al. Incubation period of hantavirus cardiopulmonary syndrome. Emerg Infect Dis. 2006;12:1271–3. http://dx.doi.org/10.3201/eid1208.051127
4. Chile Ministry of Health. Resumen ejecutivo de Hantavirus, semana epidemiológica 1 a 52, dic 2012 [cited 2014 Feb 28]. http://epi.minsal.cl/epi/html/bolets/reportes/Hantavirus/hantavirus_SE522013.pdf
5. Padula P, Figueroa R, Navarrete M, Pizarro E, Cadiz R, Bellomo C, et al. Transmission study of Andes hantavirus infection in wild sigmodontine rodents. J Virol. 2004;78:11972–9. http://dx.doi.org/10.1128/JVI.78.21.11972-11979.2004
6. Martinez VP, Bellomo C, San Juan J, Pinna D, Forlenza R, Elder M, et al. Person-to-person transmission of Andes virus. Emerg Infect Dis. 2005;11:1848–53. http://dx.doi.org/10.3201/eid1112.050501
7. Padula PJ, Edelstein A, Miguel SD, Lopez NM, Rossi CM, Rabinovich RD. Hantavirus pulmonary syndrome outbreak in Argentina: molecular evidence for person-to-person transmission of Andes virus. Virology. 1998;241:323–30. http://dx.doi.org/10.1006/viro.1997.8976
8. Wells RM, Sosa Estani S, Yadon ZE, Enria D, Padula P, Pini N, et al. An unusual hantavirus outbreak in southern Argentina: person-to-person transmission? Hantavirus Pulmonary Syndrome Study Group for Patagonia. Emerg Infect Dis. 1997;3:171–4. http://dx.doi.org/10.3201/eid0302.970210
9. Ferrés M, Vial P, Marco C, Yanez L, Godoy P, Castillo C, et al. Prospective evaluation of household contacts of persons with hantavirus cardiopulmonary syndrome in Chile. J Infect Dis. 2007;195:1563–71. http://dx.doi.org/10.1086/516786

10. Toro J, Vega JD, Khan AS, Mills JN, Padula P, Terry W, et al. An outbreak of hantavirus pulmonary syndrome, Chile, 1997. Emerg Infect Dis. 1998;4:687–94. http://dx.doi.org/10.3201/eid0404.980425
11. Castillo C, Villagra E, Sanhueza L, Ferres M, Mardones J, Mertz GJ. Prevalence of antibodies to hantavirus among family and health care worker contacts of persons with hantavirus cardiopulmonary syndrome: lack of evidence for nosocomial transmission of Andes virus to health care workers in Chile. Am J Trop Med Hyg. 2004;70:302–4.
12. Chaparro J, Vega J, Terry W, Vera JL, Barra B, Meyer R, et al. Assessment of person-to-person transmission of hantavirus pulmonary syndrome in a Chilean hospital setting. J Hosp Infect. 1998;40:281–5. http://dx.doi.org/10.1016/S0195-6701(98)90304-8
13. Vitek CR, Breiman RF, Ksiazek TG, Rollin PE, McLaughlin JC, Umland ET, et al. Evidence against person-to-person transmission of hantavirus to health care workers. Clin Infect Dis. 1996;22:824–6. http://dx.doi.org/10.1093/clinids/22.5.824
14. Kramski M, Meisel H, Klempa B, Kruger DH, Pauli G, Nitsche A. Detection and typing of human pathogenic hantaviruses by real-time reverse transcription-PCR and pyrosequencing. Clin Chem. 2007;53:1899–905. http://dx.doi.org/10.1373/clinchem.2007.093245
15. Padula PJ, Rossi CM, Della Valle MO, Martinez PV, Colavecchia SB, Edelstein A, et al. Development and evaluation of a solid-phase enzyme immunoassay based on Andes hantavirus recombinant nucleoprotein. J Med Microbiol. 2000;49:149–55.
16. Medina RA, Torres-Perez F, Galeno H, Navarrete M, Vial PA, Palma RE, et al. Ecology, genetic diversity, and phylogeographic structure of Andes virus in humans and rodents in Chile. J Virol. 2009;83:2446–59. http://dx.doi.org/10.1128/JVI.01057-08
17. Sotomayor Proschle V. Enfrentamiento de una enfermedad emergente: hantavirus en Corral equipo respuesta rápida. Santiago (Chile): Ministerio de Salud; 2011.
18. Swofford DL, Waddell PJ, Huelsenbeck JP, Foster PG, Lewis PO, Rogers JS. Bias in phylogenetic estimation and its relevance to the choice between parsimony and likelihood methods. Syst Biol. 2001;50:525–39. http://dx.doi.org/10.1080/106351501750435086
19. Takahashi K, Nei M. Efficiencies of fast algorithms of phylogenetic inference under the criteria of maximum parsimony, minimum evolution, and maximum likelihood when a large number of sequences are used. Mol Biol Evol. 2000;17:1251–8. http://dx.doi.org/10.1093/oxfordjournals.molbev.a026408
20. Posada DC, Crandall KA. MODELTEST: testing the model of DNA substitution. Bioinformatics. 1998;14:817–8. http://dx.doi.org/10.1093/bioinformatics/14.9.817
21. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution. 1985;39:783–91. http://dx.doi.org/10.2307/2408678
22. Meade A. A phylogenetic mixture model for detecting pattern-heterogeneity in gene sequence or character-state data. Syst Biol. 2004;53:571–81. http://dx.doi.org/10.1080/10635150490468675
23. Pagel M, Venditti C, Meade A. Large punctuational contribution of speciation to evolutionary divergence at the molecular level. Science. 2006;314:119–21. http://dx.doi.org/10.1126/science.1129647
24. Hjelle B, Jenison S, Torrez-Martinez N, Herring B, Quan S, Polito A, et al. Rapid and specific detection of Sin Nombre virus antibodies in patients with hantavirus pulmonary syndrome by a strip immunoblot assay suitable for field diagnosis. J Clin Microbiol. 1997;35:600–8.
25. Cao ZW, Zuo SQ, Gong ZD, Zhan L, Bian CL, Zhang PH, et al. Genetic analysis of a hantavirus strain carried by Niviventer confucianus in Yunnan province, China. Virus Res. 2010;153:157–60. http://dx.doi.org/10.1016/j.virusres.2010.06.011
26. Murúa R, Navarrete M, Cadiz R, Figueroa R, Padula P, Zaror L, et al. Hantavirus pulmonary syndrome: current situation among rodent reservoirs and human population in the 10th region, Chile [in Spanish]. Rev Med Chil. 2003;131:169–76. http://dx.doi.org/10.4067/S0034-98872003000200006
27. Pedrosa PB, Cardoso TA. Viral infections in workers in hospital and research laboratory settings: a comparative review of infection modes and respective biosafety aspects. Int J Infect Dis. 2011;15:e366–76. http://dx.doi.org/10.1016/j.ijid.2011.03.005
28. Padula PJ, Colavecchia SB, Martinez VP, Gonzalez Della Valle MO, Edelstein A, Miguel SD, et al. Genetic diversity, distribution, and serological features of hantavirus infection in five countries in South America. J Clin Microbiol. 2000;38:3029–35.
29. Pettersson L, Klingstrom J, Hardestam J, Lundkvist A, Ahlm C, Evander M. Hantavirus RNA in saliva from patients with hemorrhagic fever with renal syndrome. Emerg Infect Dis. 2008;14:406–11. http://dx.doi.org/10.3201/eid1403.071242
30. Hardestam J, Lundkvist A, Klingstrom J. Sensitivity of Andes hantavirus to antiviral effect of human saliva. Emerg Infect Dis. 2009;15:1140–2. http://dx.doi.org/10.3201/eid1507.090097
31. Ferres M, Martinez C, Godoy P, Cuiza A, Valdivieso F, Hjelle B, et al. Presence of Andes virus genome in gingival crevicular fluid during acute hantavirus infection. Abstract presented at: VIII International Conference on HFRS, HPS & Hantavirures; May 22, 2010; Athens, Greece.
32. Galeno H, Mora J, Villagra E, Fernandez J, Hernandez J, Mertz GJ, et al. First human isolate of hantavirus (Andes virus) in the Americas. Emerg Infect Dis. 2002;8:657–61. http://dx.doi.org/10.3201/eid0807.010277

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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）	赤血球濃厚液-LR「日赤」（日本赤十字社） 照射赤血球濃厚液-LR「日赤」（日本赤十字社） 赤血球液-LR「日赤」（日本赤十字社） 照射赤血球液-LR「日赤」（日本赤十字社）					

研究報告の概要

○ヒト住血吸虫症：欧州の新たな脅威
住血吸虫症が欧州の疾患になりつつあるという認識は重要である。
地中海地域は住血吸虫の中間宿主である淡水巻貝の一種*Bulinus*の生息地であり、また、気候温暖化により住血吸虫にとって良好な環境が生まれ、南欧での地域内感染が発生し易い条件となっている。この10年間の欧州各地での土着症例に加え、フランスのコルシカ島における尿路住血吸虫症の発生は、住血吸虫症が今や欧州全体の懸念となったことを意味するかも知れない。
また、多くの旅行者が、流行地域で水への曝露を通じて汚染された後に帰国する。欧州各国の国軍では、アフリカ（特にコートジボワール、マリ、中央アフリカ共和国）での任務のため、多くの住血吸虫症の症例が見られる。臨床検査の感度、特異度は低く、患者の3人に1人は無症状である。
欧州の医師はこの新たな状況に対処しなくてはならない。原因不明の慢性泌尿器症状や消化器症状があれば、住血吸虫症を疑い、このような患者や流行地域で汚染された可能性のある水に曝露した旅行者には、症状の有無に拘わらず体系的なスクリーニングを実施し、地域感染拡大を監視するための疫学的サーベイランスを行うべきである。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」
赤血球液-LR「日赤」
照射赤血球液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
土着症例及び輸入感染症例が増加し、欧州では住血吸虫症が新たな脅威となっているとの報告である。	日本赤十字社では、輸血感染症対策として受付時に海外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、住血吸虫症の既往の申告がある場合は、献血不適としている。今後も新興・再興感染症に関する情報収集に努める。

⑰

JRC2014T-038

1 Silver KL, Singer PA. A focus on child development. Science 2014; 345: 121.
2 Yousafzai AK, Rasheed MA, Rizvi A, Armstrong R, Bhutta ZA. Effect of integrated responsive stimulation and nutrition interventions in the Lady Health Worker programme in Pakistan on child development, growth, and health outcomes: a cluster-randomised factorial effectiveness trial. Lancet 2014; published online June 17. http://dx.doi.org/10.1016/S0140-6736(14)60455-4.
3 Walker SP, Chang SM, Powell CA, Grantham-McGregor SM. Effects of early childhood psychosocial stimulation and nutritional supplementation on cognition and education in growth-stunted Jamaican children: prospective cohort study. Lancet 2005; 366: 1804–07.
4 Gertler P, Heckman J, Pinto R, et al. Labor market returns to an early childhood stimulation intervention in Jamaica. Science 2014; 344: 998–1001.

Global health and the media

We commend Pamela Das and Gabriela Sotomayor for shining a light on WHO and its recent handling of communications at the 67th World Health Assembly (June 21, p 2102).[1] What this article also touches on is the role of the media and global health more broadly.

Health advocates already struggle to make core public health issues headline news and at the forefront of people's minds. With a record-breaking number of agenda items, World Health Assembly briefings with the media should have been proportionate to new developments and changes being made to communicate these effectively and appropriately.

The media have a responsibility to communicate with the public responsibly. And health advocates have a responsibility to communicate stories with the media. History has shown that when they do, positive change might be stimulated—as seen with HIV and enhanced access to antiretrovirals. The media is a powerful force for good and WHO needs to prioritise these relationships. Neglect the media and the issues are overlooked.

A report[2] by independent think tank Chatham House on governance and WHO suggests the complexity of WHO as an organisation. With reform on the agenda, media and communication necessitate a central role.

Article 2 of the WHO constitution, about the functions of the organisation, emphasises the need "to provide information, counsel and assistance in the field of health",[3] which is clearly essential "to assist in developing an informed public opinion among all peoples on matters of health"[3] and needs partnership with the media to achieve that goal.

Systems need to be in place to respond to questions and points of clarification by those tasked with spreading new ideas and developments—otherwise an absence of clarity will lead to no dissemination. Twitter and Facebook posts cannot substitute briefings with the press. We urge WHO to respond in time for the 68th World Health Assembly.

We declare no competing interests.

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1 Das P, Sotomayor G. WHO and the media: a major impediment to global health? Lancet 2014; 383: 2102–04.
2 Clift C. What's the World Health Organization for? Final Report from the Centre on Global Health Security Working Group on Health Governance. 2014. http://www.chathamhouse.org/sites/files/chathamhouse/field/field_document/20140521WHOHealthGovernanceClift.pdf (accessed June 30, 2014).
3 WHO. Constitution of the World Health Organization. 2005. http://apps.who.int/gb/bd/PDF/bd47/EN/constitution-en.pdf (accessed June 30, 2014).

Human schistosomiasis: an emerging threat for Europe

In their Seminar, Daniel Colley and colleagues (June 28, p 2253)[1] described the epidemiology of human schistosomiasis, but it is important to acknowledge that schistosomiasis is now becoming a European disease. The Mediterranean area is a former settlement of *Bulinus* and climate warming creates favourable conditions for local transmission in southern Europe. The emergence of urinary schistosomiasis in Corsica (France), with a decade of native cases around Europe, might mean that schistosomiasis is now a cause for concern in Europe.[2,3] Also, many travellers (migrants or tourists) come back from endemic areas after being contaminated through contact with water. Among travellers, the European armed forces have many cases of schistosomiasis because of their deployments in Africa (especially Côte d'Ivoire, Mali, and Central African Republic).[4] Clinical examination has low sensibility and specificity (one of three people are asymptomatic).[1,4,5]

European physicians have to manage this new situation. Medical education enhancement would improve their clinical sensibility. Nowadays, unexplained chronic urinary or digestive symptoms should evoke suspicion of schistosomiasis. Biological screening should be systematically done in these patients and in travellers with water contact in endemic countries, whatever their symptomatology. Finally, epidemiological surveillance should permit the detection of clusters around cases and monitor the spread of the local transmission.

We declare no competing interests.

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1 Colley DG, Bustinduy AL, Secor WE, King CH. Human schistosomiasis. Lancet 2014; 383: 2253–64.
2 ProMED-mail. Schistosomiasis—France: (Corsica). Archive Number 20140518.2480187. 2014. http://www.promedmail.org (accessed Sept 3, 2014).

3 Holtfreter M, Mone H, Muller-Stover I, Mouahid G, Richter J. *Schistosoma haematobium* infections acquired in Corsica, France, Aug 2013. *Euro Surveill* 2014; 19: 208–21.
4 Soentjens P, Clerinx J, Aerssens A, Cnops L, Van Esbroeck M, Bottieau E. Diagnosing acute schistosomiasis. *Clin Infect Dis* 2014; 58: 304–05.
5 Meltzer E, Schwartz E. Schistosomiasis: current epidemiology and management in travelers. *Curr Infect Dis Rep* 2013; 15: 211–15.

Calling tuberculosis a social disease—an excuse for complacency?

In the June 28 issue of *The Lancet*, Ali Mohsin re-discussed the paradigm of tuberculosis as a social disease.[1] The social element of tuberculosis is certainly important because there is strong evidence that tuberculosis does flourish in poverty, but this has several pitfalls. First, reservation of an exceptional social disease status for tuberculosis might, paradoxically, be detrimental, especially if the designation social suggests that poverty eradication is necessary to eliminate tuberculosis. Second, a paucity of studies show that social interventions have an effect on tuberculosis transmission and incidence.[2] Third, associating tuberculosis with poverty is a driver of stigma in communities.[3]

Is HIV any less social than tuberculosis? The HIV pandemic has taught us that with political will, adequate funding, community mobilisation, and scientific resources, the huge barriers of poverty and social deprivation can be overcome. On the contrary, given the dismal success of eliminating tuberculosis, perhaps the biomedical community has used the social paradigm as an excuse to underperform.

The emergence of drug-resistant tuberculosis is iatrogenic and suggests that the current biomedical and public health approaches for tuberculosis are failing. The time has come to use the successful HIV recipe (political will, money, activism, and brains) for tuberculosis. We have had enough of using the paradigms, diagnostics, and drugs of the previous century. We need a shift for tuberculosis: the one most important ingredient of a new paradigm is not biomedical or social, it is urgency.

We declare no competing interests.

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1 Ali M. Treating tuberculosis as a social disease. *Lancet* 2014; 383: 2195.
2 Bhargava A, Pai M, Bhargava M, Marais BJ, Menzies D. Can social interventions prevent tuberculosis?: the Papworth experiment (1918–1943) revisited. *Am J Respir Crit Care Med* 2012; 186: 442–49.
3 Kielstra P. Ancient enemy, modern imperative. A time for greater action against tuberculosis. 2014. http://www.economistinsights.com/sites/default/files/Ancient%20enemy%20modern%20imperative.pdf (accessed July 8, 2014).

Cardiovascular outcome trials of glucose-lowering strategies in type 2 diabetes

We agree with Rury Holman and colleagues (June 07, p 2008)[1] that prospective cardiovascular outcome studies of glucose-lowering drugs cannot solve the problem of how to help patients and clinicians make decisions. We support the authors' proposal for exploitation of electronic health records to do large, low-cost, pragmatic randomised trials measuring real-world outcomes.[2] These trials should be the standard for all newly licensed drugs.

These trials, however, will permit assessment of only previously licensed drugs. We have published an analysis,[3] which suggests the need to rethink the criteria for approval, registration, and clinical use of new drugs. Even with optimistic assumptions, including cardiovascular benefit, we have estimated that more than 90% of people started on such treatment will not benefit. A 1% reduction in HbA_{1c} would add only about 10 months of quality-adjusted life for a 45 year old and 6 weeks for a 75 year old. But such gains would be completely eliminated by any treatment deemed, by the patient, to reduce the quality of life by more than 3%, a figure below that generally cited for injectable drugs. On this basis, even a drug for diabetes that improves cardiovascular outcomes might be a poor choice for many patients.

A Dowsett, Public Health England/Science Photo Library

These measures of likely health gains matter because such treatments, although potentially providing benefit in aggregate outcomes, are being used for individual benefit. The patient should be the one who makes choices about treatment once they are fully informed of potential benefits, burdens, and harms. When these factors are closely balanced, and when patients vary in the weight they give to different factors, good quality information that is clearly communicated becomes particularly important. Data about glucose lowering falls far short of what licensing and regulatory bodies, clinicians, and patients need from new drugs for diabetes.

We declare no competing interests.

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1 Holman RR, Sourij H, Califf RM. Cardiovascular outcome trials of glucose-lowering drugs or strategies in type 2 diabetes. *Lancet* 2014; 383: 2008–17.
2 Staa TP, Goldacre B, Gulliford M, et al. Pragmatic randomised trials using routine electronic health records: putting them to the test. *BMJ* 2012; 344: e55.
3 Vijan S, Sussman JB, Yudkin JS, Hayward RA. The effect of patients' risks and preferences on health gains with glucose lowering in type 2 diabetes. *JAMA Internal Med* 2014; 174: 1227–34.

Ｂ　個別症例報告概要

○ 総括一覧表
○ 報告リスト

平成２７年２月２５日
（平成２６年１１月～平成２７年１月受理分）

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）。

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23回	23 -1	感染症および寄生虫症	C型肝炎	ドイツ	男性	14ヶ月	2014/5/5	不明	自発報告	外国製品	14000010	2014/6/4	17.0	
第23回	23 -2	臨床検査	B型肝炎表面抗体陽性	アメリカ	女性	不明	不明	不明	自発報告	外国製品	14000020	2014/9/10	17.0	

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
18	26-Dec-14	140773	バクスター(株)	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	無	有	無

感染症定期報告に関する今後の対応について

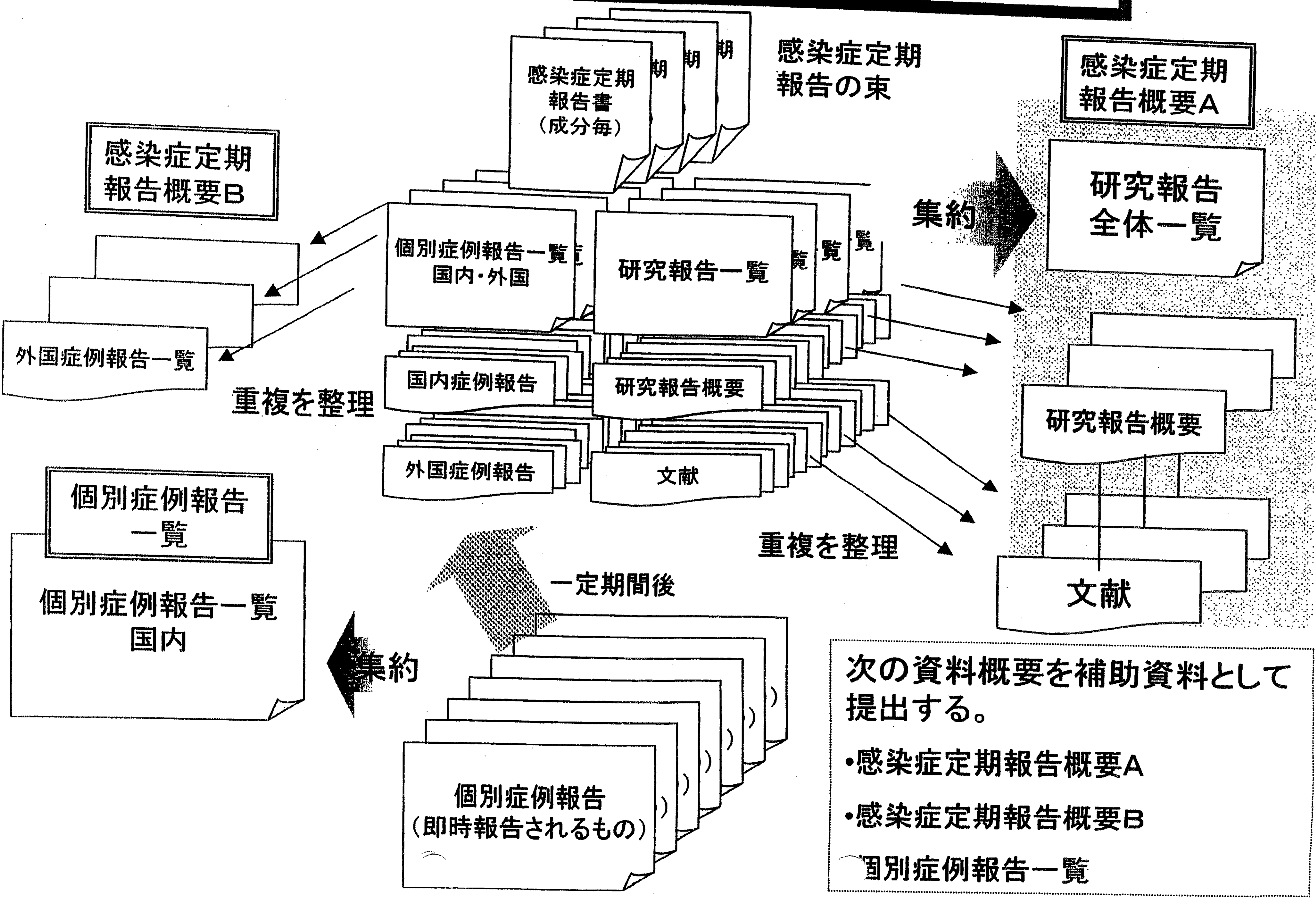
平成１６年度第５回
運営委員会確認事項
（平成１６年９月１７日）

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
感染症定期報告書
（成分毎）
感染症定期報告の束
感染症定期報告概要B
個別症例報告一覧
国内・外国
研究報告一覧
国内症例報告
研究報告概要
外国症例報告
文献
外国症例報告一覧
重複を整理
集約
感染症定期報告概要A
研究報告
全体一覧
研究報告概要
文献
重複を整理
個別症例報告
一覧
個別症例報告一覧
国内
集約
一定期間後
個別症例報告
（即時報告されるもの）
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