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研究報告の概要

○ドイツの輸血後E型肝炎、2013年
免疫抑制状態にある40歳代男性患者が2013年7月4日に血小板輸血を受け、その後HEV IgM及びIgG抗体陽性となり、HEV RNAが検出され、HEV感染及びその慢性化が確認された。輸血による感染を疑い遡及調査を開始したところ、無症候性供血者による7月1日に採血されたHEV IgM及びIgG抗体陰性のウイルス血症供血(成分採血血小板)が確認された。当該供血は血漿中に120 IU HEV RNA/mLを含み、患者には7,056 IU HEV RNAを含む製剤が輸血されていた。供血と患者からのHEV分離株のヌクレオチド配列は100%一致し、輸血による感染が証明された。また、同じ当該供血由来製剤を輸血された先天性心疾患を有する男児が輸血8カ月後の検査でHEV IgG抗体陽性、IgM抗体判定保留、HEV RNA陰性であり、輸血により感染していた可能性がある。
本症例では、リアルタイムRT-PCRの検出限界に近い低ウイルス濃度でも輸血によるHEV感染が成立することが示された。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」120
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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
ドイツで血小板製剤を輸血された免疫不全患者にHEV感染が発生し、リアルタイムRT-PCRの検出限界に近い低ウイルス濃度でも輸血によるHEV感染が成立することが示されたとの報告である。	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。今後もHEV感染の実態に関する情報の収集及び安全対策に努める。

①

Transfusion-transmitted hepatitis E in Germany, 2013

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The reported IgG seroprevalence against hepatitis E virus (HEV) in German blood donations is 6.8%, and HEV RNA detected in 0.08%, but documented evidence for HEV transmission is lacking. We identified two donations from a single donor containing 120 IU HEV RNA/mL plasma and 490 IU/mL. An infectious dose of 7,056 IU HEV RNA was transmitted via apheresis platelets to an immunosuppressed patient who developed chronic HEV. Further, transmission was probable in an immunocompetent child.

Hepatitis E virus (HEV) infection was diagnosed in December 2013 in Germany. Retrospective analysis identified the event as the first transfusion-associated hepatitis E virus (HEV) infection in the country. Here, we report baseline virological data on the case.

Case description

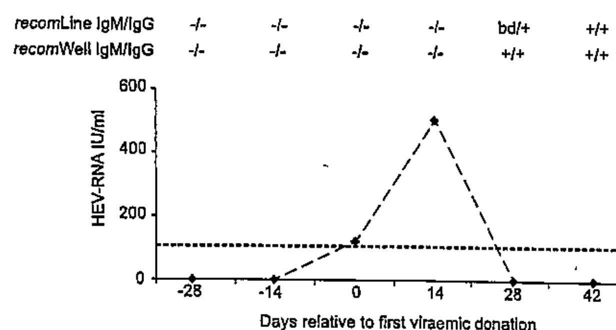
The patient (recipient 1), an immunocompromised man in his 40s, was positive for anti-HEV IgM and IgG using a recomLine HEV assay (Mikrogen, Munich, Germany), and HEV RNA was detected by real-time RT-PCR (Altona Diagnostics, Hamburg, Germany). Retrospective analysis showed that he had been chronically infected with HEV since 24 July 2013, when HEV RNA was detected for the first time. When reviewing the medical charts it was noticed that the patient had received apheresis platelets from a single donor on 4 July 2013.

A lookback procedure was initiated and two viraemic donations of this donor were identified. The donor was a man in his 40s and asymptomatic around the time of the blood donations. He donated blood regularly every 14 days. The first viraemic donation (donation 1, day 0) was from 1 July 2013 and contained 120 IU HEV RNA/mL plasma, and the second donation (donation 2, day 14) was from 15 July 2013 and contained 495 IU HEV RNA/mL plasma (Figure 1). This corresponds to an infectious dose of 7,056–8,892 IU HEV RNA in a total volume of 196–247 mL apheresis platelets transfused for donation 1 (assuming a residual plasma volume of 0.33 mL per 1 mL apheresis platelets). For donation 2, an infectious dose of 30,888–37,273 IU HEV RNA was calculated. Real-time RT-PCR results were confirmed using a nested RT-PCR protocol [1]. All other donations (n=4) of this donor before and after donations 1 and 2 tested negative by real-time RT-PCR and by nested RT-PCR (Figure 1).

The HEV nucleotide sequence of a 242 bp fragment of the ORF1 region was amplified and sequenced from donations 1 and 2 and from recipient 1 [1]. Phylogenetic analysis showed that the samples clustered together and were closely related to HEV genotype 3f, which is prevalent in Germany (Figure 2). The nearly complete nucleotide sequence (6,688 nt, GenBank accession number KJ873911) of the HEV isolate from recipient 1 was determined and compared to sequences from

Figure 1

Hepatitis E virus RNA concentration and serology results in an asymptomatic blood donor, Germany, 2013

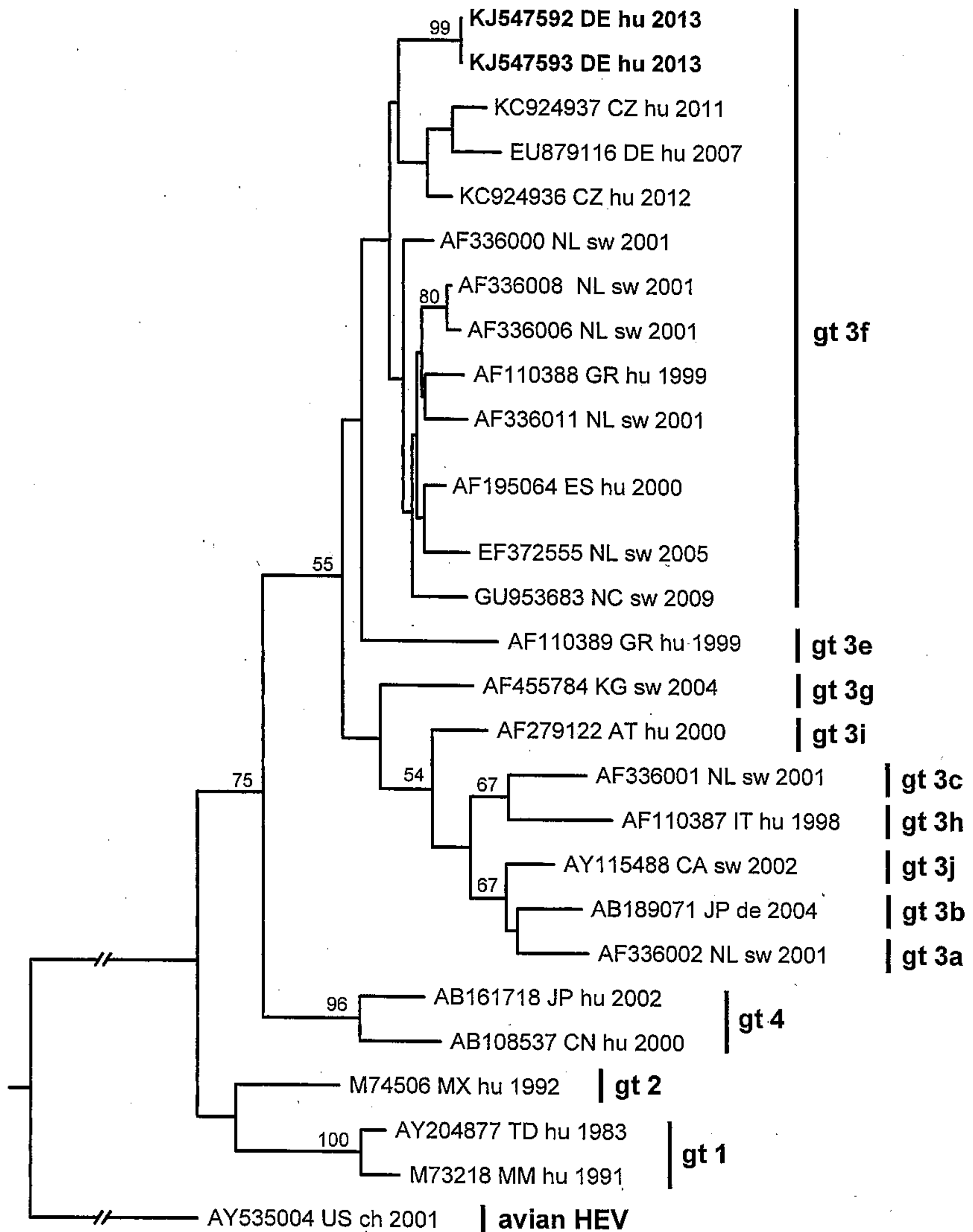


HEV: hepatitis E virus; IU: international units.

Viral RNA concentration is given on the y-axis in IU/mL as indicated by diamonds. The thin broken line indicates the limit of detection of real-time RT-PCR (Altona Diagnostics). Symbol – denotes a negative result as measured by recomLine or recomWell assay, symbol + denotes a positive result, bd indicates borderline result.

Figure 2

Rooted maximum likelihood phylogenetic consensus tree for ORF1 nucleotide sequences of selected hepatitis E virus isolates



AT: Austria; CA: Canada; ch: chicken isolate; CN: China; CZ: Czech Republic; DE: Germany; ES: Spain; GR: Greece; gt: genotype; hu: human; IT: Italy; JP: Japan; KG: Kyrgyzstan; MM: Myanmar; MX: Mexico; NC: New Caledonia; NL: Netherlands; sw: swine; TD: Chad; US: United States.

The sequences of the presented cases (KJ547592 and KJ547593, bold) cluster in subgenotype 3f. The selected sequences represent the nearest homologues in GenBank and typical members of genotype 1, 2 and 4 [15]. An avian hepatitis E virus sequence was used as an outgroup. Numbers at the nodes indicate bootstrap values of greater than 50%. Sequences are denoted by GenBank identification number, country, International Organization for Standardization country code, source, and year of isolation (or publication).

Table

Characteristics and outcome of recipients of hepatitis E virus-positive donations, Germany, 2013 (n=5)

	Transfusion recipients				HEV status		
	Transfused infectious dose HEV RNA	Recipient, sex and age	Immuno-compromised	Outcome	HEV status determined, time after transfusion	HEV PCR	Anti HEV IgG status
Blood products from donation 1							
Apheresis platelets (196 mL)	7,056 IU	#1, male 47 years	Yes	Chronic HEV infection	6 months	Positive	Positive
Apheresis platelets (247 mL)	8,892 IU	#2, male 6 years	No	Probable HEV infection	8 months	Negative	Positive
Apheresis platelets (243 mL)	8,748 IU	#3, female 70 years	Yes	Died, sepsis	NA	NA	NA
Blood products from donation 2							
Apheresis platelets (208 mL)	30,888 IU	#4, male 71 years	Yes	No HEV infection	5 months	Negative	Negative
Apheresis platelets (251 mL)	37,273 IU	#5, male 71 years	No	Died, arrhythmia	NA	NA	NA
Apheresis platelets (249 mL)	36,976 IU	#5, male 71 years	No	Died, arrhythmia	NA	NA	NA

HEV: hepatitis E virus; NA= Not applicable.

donation 2 (4,251 nt, KJ873912). The nucleotide sequences were 100% identical proving transfusion-associated transmission.

In donation 1 and 2, anti-HEV IgG and IgM were not detected using two different serological HEV assays (recomLine HEV and recomWell HEV, Mikrogen, Munich, Germany). Seroconversion of the donor was observed 14 days after donation 2 (Figure 1). Levels of alanine aminotransferase, aspartate aminotransferase, bilirubin and gamma-glutamyl transferase were within normal range from days −28 to 42 relative to the first HEV RNA-positive donation. Detailed anamnestic exploration of possible risk factors for HEV infection (e.g. occupational exposure to pigs) remained inconclusive and the travel history was negative.

Another four recipients were identified, who had received apheresis platelets from donations 1 or 2 (Table). An immunocompetent child with a history of congenital heart disease tested positive for anti-HEV IgG and borderline for anti-HEV IgM (recomLine HEV and recomWell HEV) in a single sample eight months after receiving apheresis platelets from donation 1. Real-time RT-PCR from this sample was negative (Table). Clinical symptoms suggestive of acute HEV infection were not reported. The available samples from the remaining recipients were all negative for HEV markers (Table). Two patients died for reasons other than HEV infection.

Discussion

HEV recently emerged as a transfusion-transmissible pathogen, with reports from France, the United Kingdom, and Japan [2-4]. In Europe, the vast majority of autochthonous HEV infections are caused by HEV genotype 3 (gt-3) and are linked to the consumption of contaminated food. In general, HEV gt-3 infection remains asymptomatic or presents as mild self-limited acute hepatitis [5]. HEV IgG seroprevalence in Europe ranges from 17% in Germany to 26% in France among the general population, indicating widespread contact with HEV [6,7]. A HEV IgG seroprevalence of 6.8% was determined among German blood donors in 2011, and HEV RNA was detected in 0.08% of donations [8,9]. Juhl et al. reported an HEV IgG incidence in donors of 0.35% per year [9]. A total of 7.4 million blood products were administered in Germany in 2013, and between 1,600 and 5,900 HEV RNA-positive blood donations could be occurring in Germany per year [8,10]. In the Netherlands, one HEV-positive donation per day was reported, which implies that transmission by transfusion could be a likely event in both countries [11].

An estimated 30–40% of blood products in Germany were transfused to immunocompromised patients and these patients are at risk of developing chronic HEV gt-3 infection with increased mortality [5]. Sequence analysis of HEV strains from the Czech Republic, Germany and the Netherlands showed close homology indicating a geographically confined circulation [8]. This is supported by the high degree of sequence identity of our and recent Czech and Dutch sequences. Zoonotic transmission from pigs to humans seems to be the major mode of infection, but occupational exposure to pigs was not reported in our case [6].

Two important observations were made in this study. Firstly, we could show that the infectious dose required for HEV infection seems to be low, i.e. HEV RNA

concentrations close to the limit of detection of the real-time RT-PCR. Low levels of HEV RNA in asymptomatic donors have already been reported but without evidence for transmission [8,9]. Interestingly, Juhl et al. speculated that viraemia of around 125 IU/mL in the presence of anti-HEV IgM was not sufficient for transfusion-associated infection [9]. However, it is not clear if HEV antibodies can prevent infection. A recent study showed that infectious HEV could be propagated in cell culture in the presence of HEV-specific antibodies, suggesting that they do not efficiently reduce virus infectivity [12]. In addition, a clinical study demonstrated that anti-HEV IgG did not uniformly protect against re-infection [13].

Secondly, the duration of viraemia in our asymptomatic donor did not exceed 45 days, based on the time interval between the last and the first HEV RNA-negative donation. The interval of 14 days between first and last HEV RNA-positive donation was even shorter than the 27 to 58 days reported by Slot et al., but could be due to the shorter sampling interval in our study [11]. From our and previously published data it is obvious that highly sensitive methods would be required if screening for HEV RNA were to be considered for blood products.

The second HEV transfusion-associated transmission possibly occurred in a child. However, we were not able to definitely prove transmission since only one sample was available. In light of the very low HEV seroprevalence among children in Germany it seems probable that this child was infected by donation 1 [14]. It remains unclear why transfusion of donation 2 with a fourfold higher HEV RNA concentration did not result in infection, but this could be related to host factors.

To conclude, we could demonstrate that transmission of HEV by asymptomatic donors with low-level viraemia is possible. Current German guidelines in transfusion medicine do not recommend testing for HEV. Importantly, with regard to the possible severe consequences of transfusion-associated transmission of HEV, especially in immunocompromised patients, the necessity of screening for HEV RNA needs to be discussed in countries with a high HEV prevalence. However, more data regarding the HEV disease burden due to blood transfusions are needed before recommendations can be made.

Conflict of interest

None declared

Authors' contributions

DH, HH, MP wrote the manuscript. DB, PH, ES, RT took part in the clinical management of the patient. MU, TC, FE, RH, CSH took part in the look-back procedure. JJW, MP collaborated in molecular biology techniques. OK, SM, RU collaborated on the public health investigation. All authors participated in the investigation. All authors read and approved the final manuscript.

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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. 7. 28	新医薬品等の区分 該当なし	総合機構処理欄
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研究報告の概要

○血液製剤中のE型肝炎ウイルス(HEV):イングランド南東部における有病率と伝播の研究
背景:イングランドの住民におけるジェノタイプ3の感染率は不明であるがおそらく広範囲にわたると考えられ、プール血漿製剤からも検出されている。輸血によるHEV感染の頻度と転帰は分かっていない。
方法:2012年10月8日～2013年9月30日の間、イングランド南東部で採取した225,000供血血液について、後方視的にHEV RNAをスクリーニングした。HEV陽性血液は、血清学的検査及びゲノム系統発生解析を行った。これらの供血血液を輸血された受血者を特定し、転帰を調べた。
結果:79人の供血者がHEVジェノタイプ3のウイルス血症であった(1/2,848、0.04%)。ウイルス血症供血者の多くは供血時に抗体陰性であった。79供血血液から129製剤が製造され、うち62製剤は感染が判明する前に輸血されていた。これらの製剤の受血者43人の追跡調査の結果、18人(42%)に感染が認められた。1人のみが軽度の輸血後肝炎を発症した。10人が免疫抑制状態にあり、セロコンバージョンが抑制されたことによりウイルス血症期間が延長した。そのうち3人の受血者はリバビリン治療または免疫抑制療法の変更により、長期間のHEV感染が消退した。
考察:これらの結果は、イングランドの住民及び供血者においてHEVジェノタイプ3感染が広がっていることを示している。輸血感染が急性疾患を引き起こすことは稀であるが、免疫抑制患者では感染が持続することがある。現在、供血血液に対してHEVスクリーニングは実施していないため、HEVに慢性感染している受血者を同定し、適切な抗ウイルス療法を行うための対策が必要である。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
イングランド南東部の供血血液における後方視的なHEV RNA検査を行ったところ、79人(0.04%)の供血者がHEVジェノタイプ3のウイルス血症であり、これらの供血血液からの血液製剤を輸血された受血者18人に感染が確認された。この研究から同地域にHEVジェノタイプ3感染が広がっていることが示唆されたとの報告である。	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。今後もHEV感染の実態に関する情報の収集及び安全対策に努める。

②

Hepatitis E virus in blood components: a prevalence and transmission study in southeast England

Patricia E Hewitt, Samreen Ijaz, Su R Brailsford, Rachel Brett, Steven Dicks, Becky Haywood, Iain T R Kennedy, Alan Kitchen, Poorvi Patel, John Poh, Katherine Russell, Kate I Tettmar, Joanne Tossell, Ines Ushiro-Lumb, Richard S Tedder

Summary

Background The prevalence of hepatitis E virus (HEV) genotype 3 infections in the English population (including blood donors) is unknown, but is probably widespread, and the virus has been detected in pooled plasma products. HEV-infected donors have been retrospectively identified through investigation of reported cases of possible transfusion-transmitted hepatitis E. The frequency of HEV transmission by transfusion and its outcome remains unknown. We report the prevalence of HEV RNA in blood donations, the transmission of the virus through a range of blood components, and describe the resulting morbidity in the recipients.

Methods From Oct 8, 2012, to Sept 30, 2013, 225 000 blood donations that were collected in southeast England were screened retrospectively for HEV RNA. Donations containing HEV were characterised by use of serology and genomic phylogeny. Recipients, who received any blood components from these donations, were identified and the outcome of exposure was ascertained.

Findings 79 donors were viraemic with genotype 3 HEV, giving an RNA prevalence of one in 2848. Most viraemic donors were seronegative at the time of donation. The 79 donations had been used to prepare 129 blood components, 62 of which had been transfused before identification of the infected donation. Follow-up of 43 recipients showed 18 (42%) had evidence of infection. Absence of detectable antibody and high viral load in the donation rendered infection more likely. Recipient immunosuppression delayed or prevented seroconversion and extended the duration of viraemia. Three recipients cleared longstanding infection after intervention with ribavirin or alteration in immunosuppressive therapy. Ten recipients developed prolonged or persistent infection. Transaminitis was common, but short-term morbidity was rare; only one recipient developed apparent but clinically mild post-transfusion hepatitis.

Interpretation Our findings suggest that HEV genotype 3 infections are widespread in the English population and in blood donors. Transfusion-transmitted infections rarely caused acute morbidity, but in some immunosuppressed patients became persistent. Although at present blood donations are not screened, an agreed policy is needed for the identification of patients with persistent HEV infection, irrespective of origin, so that they can be offered antiviral therapy.

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Introduction

Hepatitis E was first recognised as a clinical disease in 1978 as an outbreak of epidemic non-A, non-B hepatitis in Kashmir.[1] In 1990, faecal extracts from cases in a Russian military camp were shown to be infectious orally in people and domestic pigs,[2,3] with the infective agent hepatitis E virus (HEV) being partly sequenced the same year.[4] There are four HEV genotypes—1 and 2 (human viruses), and 3 and 4 (animal viruses) that infect human beings zoonotically. The results of a recent population-based seroprevalence study in England and Wales suggested that the prevalence of infection is more common than would be expected from an imported infection and that 25% of adults in the sixth and seventh decades of life are seropositive.[5]

In the UK, the numbers of cases of hepatitis E have increased every year since 2010[6] and this increase is associated with the emergence of a viral phylotype not previously seen. HEV is now the most common infective cause of acute enterically transmitted viral hepatitis and is detected in people who have not travelled outside the UK.[7] The association between hepatitis E and consumption of processed pork products makes hepatitis E a likely zoonosis in the UK[8] and other countries where viral sequencing has implicated food containing animal products from pigs,[9] boar,[10] and deer.[11]

The first transmission in the UK of HEV from a blood component reported in 2006[12] was identified retrospectively in the recipients of blood components from a donor diagnosed with hepatitis E 24 days after donation. Post-transfusion hepatitis E seems unusual and since the first report eight post-transfusion HEV enquiries (two in 2013, five in 2012, and one in 2011) have been notified to the National Health Service Blood and Transplant (NHSBT). Only the two most recent cases

were confirmed to be linked to components from an infected donor. HEV RNA in UK plasma pools[13,14] and serological evidence of recent HEV infection in donors[15] have also been documented, indicating a turnover of HEV in donors, but neither finding provides a measure of the prevalence of viraemia. Intercurrent immuno-suppression, common in component recipients, can delay viral clearance and lead to viral persistence in patients with solid organ transplant[16] and HIV infection.[17,18] These findings have raised the question of whether the hazard from HEV infection in donors ought to be defined. We report here the prevalence of HEV RNA in blood donors, the transmission of HEV by a range of components, and we describe the resulting morbidity in recipients.

Methods

This study and related protocols were presented to the London Bridge Research Ethics Committee (reference 12/LO/0987) and approval was received in September, 2012. An overarching data monitoring committee maintained an independent continuous review of the progress of the study. A study steering group, reporting to the data monitoring committee, reviewed all aspects of the study on a weekly basis. Identification and clinical follow-up of exposed recipients were coordinated by the NHSBT in accordance with existing protocols for the discharge of the duty of care to recipients of components carrying previously unidentified risks.

From Oct 8, 2012, to Sept 30, 2013, plasma samples from individual donations collected in the South East

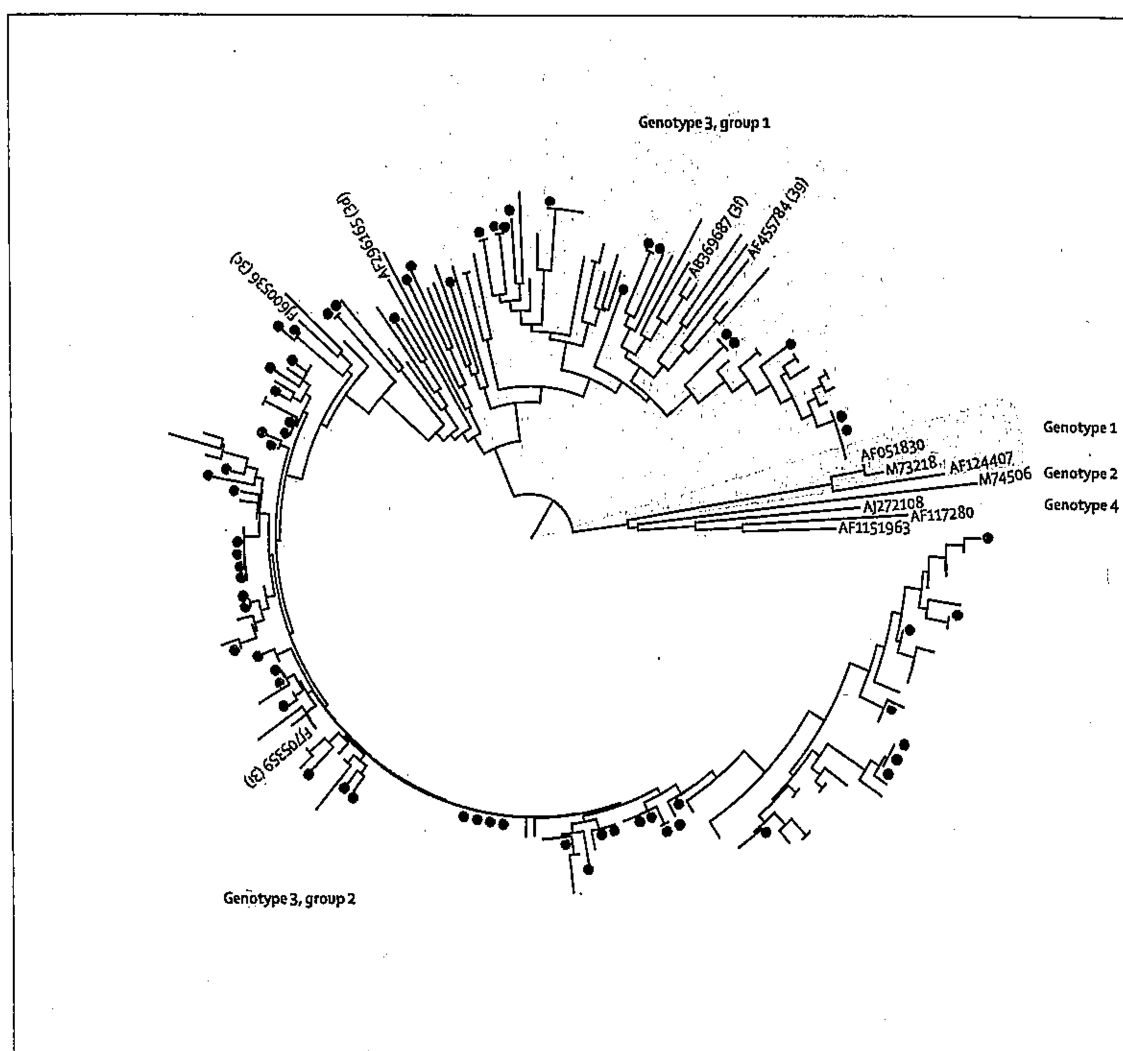

Figure 1: **Phylogenetic tree based on partial of open reading frame 2 nucleotide sequences from cases of HEV infection**
The genotype 3 sequences are from acute hepatitis E cases diagnosed in England and Wales, UK, during the study period and are shown as unlabelled branches. Sequences from 54 HEV-infected blood donors are shown as blue dots and 12 HEV-infected recipients are shown as red dots. Accession numbers for reference sequences are given. HEV=hepatitis E virus.

England from consenting donors were dispatched from NHSBT Filton, Bristol, to NHSBT Colindale, London, UK, where minipools of 24 donations, or fewer if a full 24 set was not available at the time of pooling, were assembled and extracted on the QiaSymphony (Qiagen, Crawley, UK; virus-specific cell-free protocol). At any point in time, staff and equipment limitations determined whether all sequential donations were taken for pooling or discarded. 9382 minipools were screened for HEV RNA during the study. HEV RNA was detected with an internally controlled RT-PCR[19] (detection limit 22 IU/mL). Briefly, extracted nucleic acid in 10 µL was reverse transcribed in 25 µL with Quantitect Probe rt-pcr (Qiagen, Crawley, UK) and then amplified through 45 cycles. Sample reactivity was ascribed an RNA value in IU/mL by comparison with a standard curve of serial $\log_{10}$ dilutions in normal human plasma of a high titre sample of known potency in WHO international units. Reactive pools were resolved to individual donations that were then subjected to HEV RNA detection, quantification, phylogeny, and serology. Plasma RNA was amplified, sequenced, and subjected to phylogenetic analysis across part of the open reading frame 2 as previously described.[5]

HEV antibody was detected with the Wantai IgM and IgG detection assays (Fortress Diagnostics, Antrim, Northern Ireland, UK) in accordance with the manufacturer's instructions.

Unused blood components remaining in the NHSBT inventory were discarded and those already issued were recalled. A standard look back (ie, check) was initiated for all transfused components. The involved hospital transfusion team was asked to identify the recipient and the clinical team providing patient care. The clinical team (or family doctor if the recipient had been discharged) was advised of the possible exposure to HEV and sent information about HEV and a suggested recipient follow-up plan.

Through the attending clinician, clinical information on all of the recipients was sought. Where possible, blood samples were collected during the follow-up. Negative serology at 16 weeks post-transfusion and an absence of HEV RNA at any stage indicated the lack of transmission. The detection of plasma RNA at any stage or seroconversion or serological markers of recent infection indicated transmission. Any recipient with viraemia was monitored until HEV RNA clearance and the development of both IgG and IgM.

Role of the funding source

This study was jointly funded by Public Health England and the NHSBT. Pooling was done on NHSBT premises. Serology, molecular testing, and phylogenetic analysis were done on Public Health England premises. Donor records, including consent for testing, enrolment, and clinical data were maintained by the NHSBT. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

9382 minipools, comprising 225 000 individual donations, were screened and 79 donations containing HEV RNA were identified, giving a prevalence of about one in 2848 donations (0·04%). 56 (71%) donors were sero-negative (negative for anti-HEV IgM and anti-HEV IgG). The median viral load was 3900 IU/mL (range 50 to $2 \cdot 37 \times 10^6$) and was $0 \cdot 5 \log_{10}$ higher in index donations that were antibody negative. 54 (68%) of 79 donor samples could be genotyped and all had a genotype 3 virus (figure 1).

129 components were manufactured from 79 donations (table 1). Red cells comprised the largest number (71 [55%]) followed by platelets (39 [30%]), but, because of discard or recall, only 62 (48%) components were given as transfusions to 60 recipients: one patient received two aliquots of an apheresis platelet donation, and another received two separate HEV-containing components (table 1). Platelets were the most commonly transfused virus-containing blood component (table 1).

Of the 60 patients given blood components from HEV-infected donors, one declined investigation. 16 patients were not available for follow-up: nine died soon after transfusion and before follow-up, five were terminally ill or incapacitated and therefore the initiation of HEV monitoring was thought to be inappropriate, and two had returned to their country of origin. In no case did the

	Blood components associated with HEV-viraemic donation	Blood components recalled or discarded	Blood components transfused
Red blood cells	71	48 (68%)	23 (32%)
Pooled platelets	15	3 (20%)	12 (80%)
Apheresis platelets	24	1 (4%)	23 (96%)
Fresh frozen plasma	12	9 (75%)	3 (25%)
Cryoprecipitate	6	6 (100%)	0
Pooled granulocytes	1	0	1 (100%)
Total	129	67 (52%)	62 (48%)

Data are number or number (%).

Table 1: Blood components associated with viraemic donations

	Recipients of blood components	Infected recipients	Uninfected recipients
Red blood cells	16	4 (25%)	12 (75%)
Pooled platelets	10	4 (40%)	6 (60%)
Apheresis platelets	14	7 (50%)	7 (50%)
Fresh frozen plasma	2	2 (100%)	0
Pooled granulocytes	1	1 (100%)	0
Total	43	18 (42%)	25 (58%)

Data are number or number (%).

Table 2: Association between transfused blood components and transmission of hepatitis E virus in 43 of 60 exposed patients in whom follow-up was possible

	Primary diagnosis	Inferred immune suppression	Weeks to RNA positivity	Weeks to first detection of antibody	Duration of infection (weeks)*	Viral clearance	Alanine aminotransferase (IU/ml)	Comment
Patients 1–8								
Patient 1	Cardiac surgery	None	Marker not detected	8	NA	Yes	Not raised	No illness
Patient 2	Cardiac surgery	None	Marker not detected	14	NA	Yes	No information	No illness
Patient 3	Gastrointestinal bleeding	None	Marker not detected	6	NA	Yes	Not raised	No illness
Patient 4	Cardiac surgery	None	5	5	7	Yes	375, week 7	Mild jaundice
Patient 5	Sepsis	None	2	10	10	Yes	42, week 2	No information
Patient 6	Myelodysplastic syndrome	Mild	Marker not detected	6	NA	Yes	Not elevated	No illness
Patient 7	Myelodysplastic syndrome	Mild	Marker not detected	3	NA	Yes	No information	No information
Patient 8	Myelodysplastic syndrome	Mild	14	28	28	Yes	101, week 21	No information
Median for patients 1–8	..	..	5	7	10	..	..	..
Patients 9–14								
Patient 9	Aplastic anaemia	Moderate	8	Marker not detected	>12	Not	43, week 4	Sepsis death†
Patient 10	Metastatic cancer	Moderate	Marker not detected	6	NA	Yes	No information	No information
Patient 11	Aplastic anaemia	Moderate	4	10	>10	Not	200, week 7	Cardiac death†
Patient 12	Acute renal failure	Moderate	3	11	11	Yes	148, week 9	Steroid reduction
Patient 13	Non-Hodgkin lymphoma	Moderate	13	13	>43	No	No information	No information
Patient 14	Acute myeloid leukaemia	Moderate	12	21	25	Yes	1380, week 20	No information
Median for patients 9–14	..	..	8	11	18	..	..	..
Patients 15–18								
Patient 15	Acute myeloid leukaemia	High	17	38	>40	No	Not elevated	Deceased
Patient 16	Acute myeloid leukaemia	High	7	Marker not detected	16	Yes	Not elevated	11 weeks of Ribavirin
Patient 17	Failed transplant	High	7	Marker not detected	>10	Not	295‡, week 7	Sepsis death†
Patient 18	Multi organ transplant	High	11	37	44	Yes	40, week 22	Reduction of drug dose
Median for patients 15–18	..	..	9	37·5	30	..	..	..

Data are number, unless otherwise indicated. Median values are calculated from the numerate values in the table. NA=not applicable. *Period from transfusion to last detection of hepatitis E virus RNA; marked > when still viraemic after the end of follow-up. †Recipient died during follow-up, so relevant data excluded from numerical analysis. ‡Transaminitis thought to be secondary to abdominal sepsis and haematoma.

Table 3: Outcome in 18 recipients infected by transfusion of a blood component from a viraemic donor, ranked by immunosuppression

clinical team judge that HEV had contributed to any illness or to death. Therefore, 43 patients were followed up (table 2).

Six patients (1–3, 6, 7, and 10) had serological markers of the recent development of antibody (seroconversion) when first tested at a median of 6 weeks (range 3–14 weeks) after transfusion (table 3). High concentrations of anti-HEV IgG (sample/cutoff [S/CO] >20) were detected in all samples, IgM was detected in one sample (S/CO 1·2), and borderline IgM (S/CO 0·7–0·9) was detected in three samples. A further 12 recipients were viraemic at one or more timepoints in the post-transfusion period (table 3). Taking both groups together, the overall transmission rate was 42% (18 of 43 exposed patients), supported by the finding of sequences in each of the 12 viraemic recipients that were identical to sequences from the involved donors (figure 1). 25 recipients were judged to not have been infected, 16 of whom had no serological evidence of HEV infection at 16 weeks after transfusion and nine who were both seronegative and non-viraemic at 8 weeks or longer after transfusion.

The components associated with transmission of HEV to recipients are shown in table 2; red blood cells seemed to be the component least likely to transmit infection. HEV antibody was detected in four (22%) of 18 donations associated with virus transmission and in 13 (52%) of 25 donations not associated with

transmission. The antibody levels were much lower in the four donations that resulted in transmission than in the 13 that did not (figure 2A). The HEV viral load was about 1·5 $\log_{10}$ higher in the donations that transmitted than in those that did not (figure 2B).

Follow-up of the infected recipients showed a varied response to infection, reflecting their overall clinical state and inferred degree of immunosuppression. The median times for seroconversion and duration of infection increased in patients as the degree of immunosuppression increased (table 3). Eight patients (1–8) were deemed to be immunocompetent or only mildly immunosuppressed (table 3). Five patients cleared their infection without having detectable viraemia, the other three recipients cleared their RNA in a median of 10 weeks (table 3). Six patients (9–14) with varying degrees of moderate immunosuppression had a longer median time of 11 weeks to seroconversion and a median duration of viraemia of 18 weeks (table 3). Four patients (15–18) were judged to be heavily immunosuppressed. In these patients, seroconversion was either very delayed (week 38 for patient 15 and week 37 for patient 18) or was not detected.

In three viraemic recipients, one moderately (patient 12) and two severely immunosuppressed (patients 16 and 18), an elective decision was made to induce viral clearance. In patient 12, steroid dose reduction and withdrawal of additional immunosuppressive drugs 9 weeks after transfusion led to seroconversion and viral clearance over 3 weeks. In patient 18, changes in immunosuppressive therapy coincided with the onset of seroconversion at 37 weeks and subsequent viral clearance from both stool and plasma. In patient 16, 2 weeks of ribavirin was given between cycles of chemotherapy at 12 weeks after transfusion and led to a 1000 times reduction in HEV RNA concentrations but not to clearance. Further ribavirin treatment starting at 19 weeks after transfusion led to viral clearance in the absence of a detectable antibody response.

Clinical hepatitis was reported in only one recipient (patient 4), whose indication for transfusion was a cardiac surgical procedure (table 3). 5 weeks after transfusion, the patient consulted with the family doctor and was confirmed to have hepatitis, associated with HEV seroconversion. Four other recipients (patients 8, 11, 12, and 14) had asymptomatic transaminitis coincident with seroconversion, which was triggered in patient 12 by a change in therapy. Transaminitis was marked in patient 14 in whom plasma alkaline phosphatase was also elevated for 1 week before the first development of anti-HEV antibodies. No infected patient was reported to have neurological disease.

Discussion

The prevalence of blood donations containing HEV RNA was higher than anticipated in the planning of the project. When projected across the country, and allowances are made for the duration of a detectable viraemia for 8 weeks, a prevalence of one in 2848 indicates that about 80 000–100 000 acute HEV infections are likely to have occurred in England during the year of the study. This is close to the modelled estimate[20] and shows a truly sizeable zoonosis, including both group 1 and group 2 viruses of genotype 3 HEV,[7] which was also transmitted to the donors identified in this study (figure 1). Similar prevalences of viraemia have been reported in Sweden and Germany,[21–23] suggesting that this zoonosis is also widespread across the European continent, further supported by a recently reported case of post-transfusion HEV in France (panel).[24]

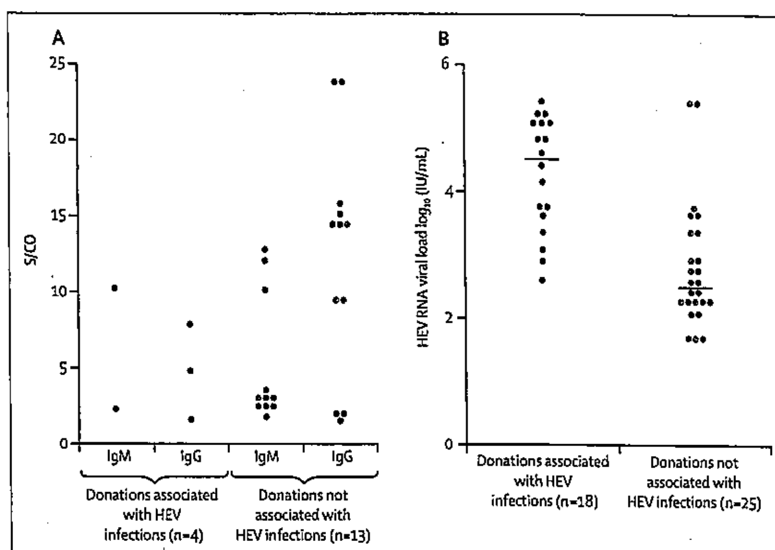

Figure 2: **Data spread plot of HEV IgM and IgG antibody levels in donors whose components transmitted HEV compared with those that did not (A) and HEV RNA levels in donors whose components transmitted HEV compared with those that did not (B)**

In (B), the bars indicate median viral load values for donations that were or were not associated with HEV infections (4·53 IU/mL [range 2·61–5·41] vs 2·57 IU/mL [1·70–5·49], p<0·0001). S/CO=sample/cutoff.

The inevitable delay between donation and the identification of a viraemic donor meant that when recall of components was started, a high proportion of the short shelf-life components had already been transfused and most of the recalled units were inevitably of the longer shelf-life red cell and frozen components. This might have altered the profile of recipients towards those who were immunosuppressed and requiring platelet support.

Two linked variables in the donor plasma that were associated with transmission were the anti-HEV status of the donation and the level of virus in the plasma (figure 2A, 2B). Overall, donations containing antibody were less likely to transmit and, when they did, there was a trend for lower levels of anti-HEV to be associated with transmission. Donations associated with transmission had significantly higher levels of plasma RNA (p<0·0001) than did those not associated with transmission, but overall viral RNA levels were ten times lower in viraemic donors than in the plasma of patients presenting with acute clinical hepatitis E (median $6·2 \times 10^4$ IU/ml, range 20 to

Panel: Research in context

Systematic review
We searched MedLine for articles published in the 7 years up to December, 2013, on the topic of HEV and blood safety. We used a range of keywords including "hepatitis E", "HEV", "blood safety", "transmission", "blood donors", and "recipients". In the past 10 years hepatitis E virus (HEV) has been increasingly recognised as a zoonotic infection in high-income countries where it was previously thought to be an imported infection. Current infection in blood donors and a small number of post-transfusion cases from some countries indicate a potential for transmission by transfusion. No systematic analysis of transmission rates and clinical effect of transfusion-transmitted HEV exists in published literature.

Interpretation
We have defined the prevalence of viraemic donors and transmission to recipients. Spontaneous clearance without clinical disease was common, despite delayed seroconversion, and resulting acute illness was rare. Our data are from the first reported systematic study of HEV transmission from donors infected by an extensive but largely non-apparent zoonosis in England. On a clinical basis alone, the resulting minimal burden of disease does not signal a pressing need for donation screening at this time.

$4 \cdot 2 \times 10^7$; unpublished data). In this study it was not possible to ascertain the serological status of the recipient before transfusion because of the unavailability of samples.

The numbers of components in each category were insufficient for a robust attribution of transmissibility, though there is clearly a trend for those components that contain larger plasma volumes, principally fresh frozen plasma and platelet components, to transmit more readily. Despite this, in some instances apparently susceptible individuals who were challenged with components prepared from donors with high-level HEV viraemia did not become infected, raising the question of whether some people are innately resistant to infection or whether coincidental administration of antibody-containing components from other donors might also have mitigated the risk of infection.

Table 3 shows that the immunological integrity of the host materially alters the time course of the post-transfusion infection. Increasing immunosuppression prolongs viraemia and delays seroconversion. Although eight of 12 viraemic recipients underwent seroconversion, coinciding in some with a biochemical transaminitis, seroconversion does not necessarily bring about clearance and can still be followed by long-term viraemia (patients 13 and 15). At the other end of this range, four heavily immunosuppressed patients either did not produce anti-HEV or had very delayed seroconversion and exhibited prolonged viraemia as described previously in recipients of solid organ transplants.[16]

What is of concern in this small series is that ten patients infected through transfused components seemed likely to be at the beginning of long-term persistence. Two patients (8 and 14) cleared viraemia spontaneously late after infection, four (9, 11, 15, and 17) remained viraemic at time of their deaths, and four (12, 13, 16, and 18) were at risk of chronic liver disease and requiring continued monitoring and possible intervention. In three cases, the decision to attempt viral clearance was made. Indirect antiviral intervention with electively reduced immunosuppression led to seroconversion and viral clearance in two recipients (10 and 18). Direct antiviral intervention with ribavirin led to resolution of the infection without seroconversion in patient 16. The fourth patient (13) remains the only persistently infected recipient a year after transfusion.

Our findings confirm the potential danger of transfusion-transmitted HEV in the transplant and haemoncology settings but also the susceptibility of this persistent infection to immune clearance. Persistent infection might be more of a hazard for recipients of solid organ transplant in whom the immunosuppression is unremitting than for recipients of stem cell transplantation in which immune recovery might be expected. Based on the finding of little acute morbidity, there is no indication to alter previously optimised treatment pathways for patients who have been exposed or infected with HEV. Two-thirds of patients are likely to clear infection spontaneously, and when long-term persistence develops intervention can be undertaken electively.[15] Immune recovery is the desired outcome in many haematological situations and this alone might well bring about viral clearance that might also be associated, like seroconversion, with an illness during viral clearance.[26]

Since HEV infection transmits through transfusion and the incidence of acute infection in donors from the southeast of England is high, about 1200 HEV-containing components are likely to be released for transfusion purposes each year in England. Most infections will not be identifiable through any acute illness in the immediate post-transfusion period but might present much later at the time of immune reconstitution or as a manifestation of long-term chronic liver disease, especially in solid organ transplants when an association with transfusion might not be made. One way of mitigating unfavourable outcomes would be to introduce routine yearly screening for persistent HEV infection in all transplant patients with an option to treat those who are chronically infected independent of the route of infection.

Setting aside this option, what would be a proportionate response to this zoonosis? Is it possible to deal with the source of infection that is likely to be foods containing pork,[8] perhaps advising patients at risk to modify their diet as is done for listeria? A societal change reverting to the old principle of extended cooking of pork would not fit with the tendency to consume it lightly cooked now that trichinosis is no longer a perceived hazard.[27] Addressing animal husbandry and determining how to

control HEV in pig herds remains a possibility, although an easily transmissible enteric agent like HEV[28] will be more difficult to control than trichinella. Alternatively, screening of blood donations, at substantial cost, on the basis of reducing the risk of long-term infection, would remove the bulk of the transfusion hazard but still allow the dietary risk to transplant patients; this issue was addressed in Toulouse, France, by the removal of the figatelu liver sausage from the hospital diet.[9] Nevertheless, every donor exposure in England and Wales will increase the likelihood of recipient infection by one in 3000, and if a recipient in 1 year of treatment were to be exposed to components from 20 donors the accumulated yearly risk from transfusion would be one in 150 compared with a dietary risk of one in 500–1000, modelled on the yearly seroconversion rate of 0·1–0·2%. Our experience in this study, however, indicates that the burden of harm engendered by HEV acquisition through transfusion is very slight and from a clinical perspective alone there seems no pressing need to move rapidly with the introduction of donation screening. The broader issues of HEV and blood safety, including the need for donation screening,[29] will be addressed in the UK later this summer after the recent commissioning of a short-life expert committee of the UK Departments of Health Advisory Committee on the Safety of Blood, Tissues and Organs to consider these matters within the context of a financially constrained health service.

The magnitude of the current zoonosis in Europe is shown by both more cases of hepatitis E being reported for England in 2012 than in 2011,[7] and an increase in prevalence of HEV antibody in young Dutch blood donors (Zaaijer H, Sanquin Blood Supply Foundation, personal communication). It should be borne in mind that HEV disease in England and Wales shows considerable temporal variation, and though the magnitude of the risk now possibly justifies intervention[30] it is unlikely that the high frequency of acute infection will be maintained indefinitely; this provides another complication in the decision of an appropriate response to this interesting and rather unexpected transfusion-associated infection in the UK.

Contributors

RST, SI, KIT, and PEH designed the study. AK managed and coordinated the collection and subsequent pooling of blood samples used in this study. PEH and IUL oversaw the follow-up of the recipients. SRB, KR, ITRK, JT, IUL, and PEH contacted the clinical teams or family doctors of the recipients and coordinated the follow-up of these patients. RST, PEH, and IUL worked with the clinical teams in the management of the recipients. RB, SD, BH, PP, and JP undertook all the laboratory work for this project and were all involved in the analysis of the generated data. SI and RST oversaw the delivery and interpretation of the laboratory aspects for this study. KT was responsible for the overall management of the study and also for data handling and storage. PEH, SI, and RST wrote the manuscript. PEH and SI contributed equally to the preparation of the manuscript. All authors reviewed and commented on the text.

Declaration of interests

We declare no competing interests.

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医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分		総合機構処理欄
				2014. 8. 7	該当なし		
一般的名称	新鮮凍結人血漿		研究報告の公表状況	Al-Hajjar SH, Frayha HH, Al-Hazmi M, Batawi R, McIntosh K, Sax PE, Al-Thawadi S, Al-Jumaah S, Busch MP, Hanhauser E, Kuritzkes DR, Li JZ, Henrich TJ. AIDS. 2014 Jun 19;28(10):1539-41. doi: 10.1097/QAD.0000000000000268.		公表国 サウジアラビア	
販売名（企業名）	新鮮凍結血漿-LR「日赤」120（日本赤十字社） 新鮮凍結血漿-LR「日赤」240（日本赤十字社） 新鮮凍結血漿-LR「日赤」480（日本赤十字社）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
○不注意によるHIV-1感染血液輸血後に曝露後予防を用いた感染の防止 曝露後予防（PEP）は一般的に職業的・非職業的なHIV-1曝露の際に処方されるが、感染血液の輸血時における文書化されたエビデンスは限られている。本報告は、HIV感染赤血球製剤を輸血された鎌状赤血球症の12歳女児の症例である。患者は血管閉塞性クリーゼ治療のため入院し、32時間前に供血された赤血球製剤を1本輸血された。数時間後、人為的ミスによりHIV-1に汚染されている血液を輸血されたことが判明した。供血血液はHIV-1抗体陽性であり、ウイルス量が9,740コピー/mL（サブタイプC）であることが確認された。供血者は抗レトロウイルス治療（ART）を受けていなかった。輸血の約24時間後に、患者にテノフォビル、エムトリシタビン、リトナビルでブーストしたダルナビル（後にロピナビルに変更）及びラルテグラビルによる治療が開始された。輸血約24時間後の患者の血液検査は、HIV抗体陽性で血漿中のHIV-1 RNAは陰性であった。ウェスタンブロットのバンドパターンは、供血者と患者サンプルで一致した。患者は13週間のARTを受け、その8カ月後には全てのHIV検査で陰性であった。HIVはARTを中止した後に患者の血液から検出されず、時間経過と共に抗体が消滅したこれらの所見は、PEPによるHIV感染予防が成功したことを示唆する。	新鮮凍結血漿-LR「日赤」120 新鮮凍結血漿-LR「日赤」240 新鮮凍結血漿-LR「日赤」480 血液を介するウイルス、細菌、原虫等の感染 vCJD等の伝播のリスク

報告企業の意見	今後の対応
HIV-1感染血液を輸血された患者に抗レトロウイルス薬による曝露後予防を行い、HIV-1感染の防止に成功したことが示唆されたとの報告である。	日本赤十字社では、化学発光酵素免疫測定法（CLEIA）による抗体検査を行い、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。

③

by 300%; the authors suggested that TDF elimination could have been reduced by the inhibition of renal drug transporters by TVR [7]. In our case, a major role of TDF in renal impairment does not seem probable since TDF withdrawal did not restore kidney function. Nevertheless, TDF plasma level was not measured and therefore we can not rule out an accumulation of TDF thorough renal drug transporters inhibition by TVR.

Telaprevir is mainly metabolized by the CYP3A4, acting as both a substrate and an inhibitor. This is of particular importance for the treatment of HIV patients, as the use of ritonavir-boosted HIV protease inhibitors (HIV-IPr) is sometimes indispensable. Currently, the only HIV-PIr recommended for coadministration with TVR is ATVr. An increase in the ATV minimum concentration by 85% was seen when ATV and TVR were combined in healthy volunteers, which led to an increased risk of symptomatic hyperbilirubinemia [8]. In our case, TVR initiation probably led to a rise in ATV concentrations since an unexpected, abnormally high plasma ATV level was seen 60 h after the last dose, at a value similar to the maximum concentration described for the drug [5]. This fact had its clinical and laboratory correlation in the form of nausea, vomits, a rise in hepatic enzymes, and hyperbilirubinemia, which ultimately contributed to renal failure through intravascular volume depletion and dehydration. To the best of our knowledge, an unexpected plasma ATV elevation leading to such a major side effect of the drug has not been described previously, and its mechanism is uncertain, since it might not be attributed only to CYP3A4 mediation. A hypothetical reduction in ATV renal excretion by inhibition of renal drug transporters in the presence of TVR might have been involved in the overexposure to this drug seen in our patient.

In conclusion, even though major drug–drug interactions between ATV and TVR have not been seen in clinical trials, we report a serious adverse event that was likely related to a major rise in plasma ATV concentration in the presence of TVR. Various mechanisms that are currently incompletely defined may have been implicated in this unexpected drug–drug interaction. It seems advisable to closely monitor patients on TVR therapy, particularly if additional risk factors or co-medications are present. The effect of TVR on renal and hepatic drug transporters and its related clinical implications deserve further investigation.

Conflicts of interest

Daniel Podzamczer has received research grants and/or honoraria for advisories and/or conferences from Boehringer Ingelheim, GSK, Viiv, Pfizer, BMS, Abbott, Gilead, Janssen and Merck; Elena Ferrer and Eva Van den Eynde have received honoraria for lectures from Boehringer Ingelheim, GSK, Viiv, Pfizer, BMS, Abbott, Gilead, Janssen and Merck.

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Prevention of HIV-1 transmission with postexposure prophylaxis after inadvertent infected blood transfusion

Although postexposure prophylaxis (PEP) is commonly prescribed in the setting of occupational and non-occupational HIV-1 exposures [1,2], there is limited evidence documenting efficacy in the setting of transfusion with infected blood. Furthermore, there is a paucity of data regarding blood-borne exposures that lead to passive transfer of antibodies against HIV [3,4]. We describe the efficacy of PEP in such a situation using highly sensitive assays for HIV-1 DNA and low-level residual viremia.

We report the case of a 12-year-old girl with sickle-cell disease admitted for management of a vaso-occlusive crisis who inadvertently received HIV-infected packed red blood cells (PRBCs). She required intermittent PRBC transfusions since the age of 2 years, with the last transfusion 5 years ago. Her white blood cell count was 10 100 per μl, and haemoglobin 9.6 g/dl. Haemoglobin electrophoresis revealed 57% haemoglobin S. During her admission, she was transfused with one PRBC unit that was collected 32 h prior to administration.

Despite the standard practice of prescreening blood products, the laboratory at the public hospital in the Kingdom of Saudi Arabia became aware that the PRBCs were contaminated with HIV-1 within hours of her transfusion as a result of human error involving mixing an un-screened bag with screened bags. The donor blood was discovered to be HIV-1 antibody positive and subsequently determined to have a viral load of 9740 copies/ml (subtype C); the donor was not receiving antiretroviral therapy (ART). The Ministry of Health conducted an in-depth investigation and halted blood transfusions at the responsible blood blank. Approximately 24 h after transfusion, the patient was started on tenofovir, emtricitabine, ritonavir-boosted darunavir (subsequently changed to lopinavir) and raltegravir. Blood tests were positive 24 h after transfusion for HIV antibodies by ELISA and confirmatory western blot, but negative for HIV-1 DNA and plasma HIV-1 RNA by PCR. The pattern of reactive bands on western blot was identical for samples obtained from the donor and patient (gp120, gp41, gp31, p24 and p17). Genotyping revealed that she was CCR5 wild-type.

The patient demonstrated no signs or symptoms of acute infection during 13 weeks of ART in a tertiary care centre. Testing of donor blood revealed no HIV-1 resistance to the antiretrovirals chosen. Longitudinal testing of the patient's plasma and peripheral blood mononuclear cells (PBMCs) was performed by both clinical laboratories and by sensitive research assays with thresholds of detection down to 0.1 HIV-1 DNA copies/10^6 PBMCs and 0.4 RNA copies/ml of plasma during and after ART. All tests were negative prior to and 8 months after ART interruption. She continued to have declining but detectable HIV-1 antibodies with positive confirmatory line immunoassay up to 5 months after transfusion, but confirmatory testing was negative by

Table 1. HIV-1 DNA, plasma RNA and antibody tests results before and after infected blood transfusion.

		Clinical laboratory testing					Research laboratory testing		
Day posttransfusion	Clinical course	HIV ab ELISA/ WB[a]	Architect HIV-1/2 Ag/Ab Combo[b]	Line immunoassay confirmation[c]	HIV-1 DNA (Blood)[d]	Quantitative HIV-1 RNA PCR[e]	HIV-1 DNA (copies/10^6 PBMC)	HIV-1 plasma RNA by SCA (copies/ml)	HIV Ab by VITROS assay
Donor									
-1		Positive				9740 copies/m			
Patient									
-1		Negative							
0	Transfusion								
1	Start ART	Positive			ND	ND			
4			463.44	Pos	ND	ND			
34					ND	ND			
50			132.74	Pos	ND	ND			
78					ND	ND			
82			33.89	Pos	ND	ND		<0.4[g]	1.7[h]
91	Stop ART								
98					ND	ND			
105					ND	ND			
112			8.43	Pos	ND	ND	<0.12[f]	<0.4	0.35
119					ND	ND			
126			4.5	Pos	ND	ND			
133					ND	ND			
140			2.73	Pos	ND	ND	<0.1	<0.4	0.1
154			1.62	Pos	ND	ND			
175			0.69	Neg	ND	ND			
240			0.19	Neg	ND	ND			

Ab, antibody; Ag, antigen; ART, antiretroviral therapy; ND, not detected; SCA, single copy HIV-1 RNA assay; WB, western blot.
[a]Screening HIV-1 enzyme-linked immunoassay with western blot confirmation at transfusing healthcare facility.
[b]Abbot Architect HIV antibody/antigen combination chemiluminescent microparticle immunoassay (the presence of antigen and antibody are not differentiated); value = relative light units (RLU; positive assay cutoff value = mean calibrator RLU value × 0.40).
[c]INNO-Lia HIV I/II Line Immuno Assay (LIA) used to confirm the presence of antibodies against the HIV-1/HIV-2.
[d]HIV-1 proviral DNA qualitative detection by PCR performed at Mayo Laboratories (lower limit of detection = 66 copies/ml whole blood).
[e]Measured by quantitative real-time PCR (detection threshold = 40 RNA copies/ml of plasma).
[f]Threshold of detection in DNA copies/10^6 PBMCs (no DNA detected at all time-points tested).
[g]Threshold of detection in RNA copies/ml of plasma (no RNA detected at all time-points tested).
[h]Signal/cutoff value from VITROS anti-HIV-1 + 2 assay.

month 6. Viral load testing 8 months following exposure remained negative (Table 1).

We report the successful use of combination ART PEP following large-volume transfusion of HIV-infected blood from a viremic donor with passive transfer of antibodies to HIV-1. The observation that no HIV was detected in her blood after stopping ART and that antibody levels disappeared over time strongly suggests that PEP successfully prevented HIV acquisition. The overall transmission rate from HIV-1 antibody positive blood transfusions was 89% in one study with non-transmission attributed to lower viral load and prolonged blood-product storage [5]. HIV-1 transmission from transfusion of PRBCs stored for less than 48 h is essentially 100% regardless of viral load [5]. Furthermore, experiments of simian immunodeficiency virus primary infection in primate models suggest that infectivity of plasma virus from acute infection is higher than set-point virus, but the correlation between cell-associated HIV-1 DNA levels and transmissibility is poorly understood [6,7]. Our patient received effective prophylaxis despite transfusion with PRBCs stored for less than 36 h, but was from a donor with relatively low viral load.

Passive transfer of HIV-1 antibodies after occupational exposure has been documented but is rare [3,4]. For example, one individual became infected with resistant virus despite the use of zidovudine 2 h after a deep laceration with contaminated blood, whereas another individual was not infected after transfusion with a contaminated unit of PRBCs from a donor with a low viral load (2000 copies/ml); she was started on zidovudine, lamivudine and indinavir 19 days after exposure. These cases illustrate the sensitivity of current antibody testing platforms and cautions against presuming that individuals have preexisting HIV-1 infection, and not initiating or stopping PEP due to the presence of antibody reactivity immediately following contaminated blood exposure. Highly sensitive laboratory assays may also help guide the duration of PEP to guarantee the prevention of infection.

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Conflicts of interest

There are no conflicts of interest.

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医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2014. 5. 8	該当なし	
一般的名称	新鮮凍結人血漿		研究報告の公表状況	Wang X, Tan J, Zhao J, Ye Z, Hewlett I. BMC Infect Dis. 2014 Apr 8;14:192. doi: 10.1186/1471-2334-14-192.	公表国	
販売名（企業名）	新鮮凍結血漿-LR「日赤」120（日本赤十字社） 新鮮凍結血漿-LR「日赤」240（日本赤十字社） 新鮮凍結血漿-LR「日赤」480（日本赤十字社）				米国	

研究報告の概要

○高病原性トリインフルエンザA（H5N1）ウイルスは輸血でフェレットに感染する

背景：高病原性トリインフルエンザA（H5N1）ウイルスは、重症症例においてウイルス血症が見られ、肺以外の臓器にも感染することが示されている。血中ウイルスの感染性と輸血によるウイルス伝播の可能性については調査されていない。

方法：H5N1ウイルスに感受性のあるフェレットを用いて、輸血によるウイルス伝播について評価した。CDCから入手したH5N1ウイルス株（A/Vietnam/1203/04）は孵化鶏卵を用いて培養後、50%卵感染量（EID_{50}）を定めた。低用量（$1.0\times10^{2.6}$EID50/mL）または高用量（$1.0\times10^{3.6}EID_{50}$ /mL）のウイルスを、血液ドナーとなるフェレットにそれぞれ経鼻感染させ、感染後2、4、6及び10日目に採血し、レシピエント動物に輸血した。

結果：低用量のウイルスを接種されたドナーフェレットからの血液を輸血されたレシピエントの2/12匹（16.67%）、高用量ドナーフェレットの血液を輸血されたレシピエントの7/12匹（58.33%）においてウイルス血症が確認された。低用量レシピエントの1/12匹（8.9%）及び高用量レシピエントの6/12匹（50%）は輸血後11日以内に死亡した。高濃度のウイルスRNAはウイルス血症早期の血液を輸血されたレシピエントにおいて検出され、致死率と相関した。

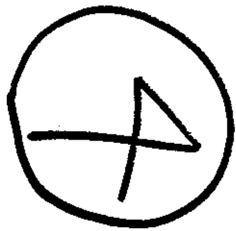
結論：これらのデータは、高病原性トリインフルエンザA（H5N1）ウイルスがフェレットにおいて輸血により伝播しうることを示唆する。H5N1ウイルスに感染したフェレットにおいて低濃度のウイルス血症は発症期付近または発症後に検出された。これらの知見はH5N1ウイルスの発症機序や伝播率に影響を与えるものである。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
高病原性トリインフルエンザA（H5N1）ウイルスは、フェレットを用いた実験で輸血により感染したとの報告である。	日本赤十字社では、問診で発熱などの体調不良者を献血不適としている。また、高病原性トリインフルエンザA(H5N1)が発生した場合には、感染の疑いがある家禽と密接な接触をした当該飼養農場の関係者及び防疫作業の従事者からの献血を不適としている。今後も引き続き情報の収集に努める。



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BMC Infectious Diseases

RESEARCH ARTICLE Open Access

Highly pathogenic avian influenza A virus (H5N1) can be transmitted in ferrets by transfusion

Xue Wang[1*], Jiying Tan[1], Jiangqin Zhao[1], Zhiping Ye[2] and Indira Hewlett[1*]

Abstracts

Background: Highly pathogenic avian influenza A virus has been shown to infect organs other than the lung, and this is likely to be mediated by systemic spread resulting from viremia which has been detected in blood in severe cases of infection with avian H5N1 viruses. The infectivity of virus in blood and the potential for virus transmission by transfusion has not been investigated.

Methods: Using a susceptible ferret animal model, we evaluated viremia and transmission by blood transfusion. Blood was collected on day 2, 4, 6, and 10 post-infection (or before death) from donor ferrets infected with either low dose ($1.0 \times 10^{2.6}$ EID_{50}/ml) or high dose ($1.0 \times 10^{3.6}$ EID_{50}/ml) of H5N1 virus, A/VN/1203/04 and transfused to recipient animals.

Results: Viremia was observed in 2/12 (16.67%) recipients that received blood from donor ferrets infected with low dose and 7/12 (58.33%) recipients who received blood from high dose infected donors. 1/12 (8.3%) low dose recipients and 6/12 (50%) high dose recipients died within 11 days after transfusion. Increased changes in body weight and temperatures were observed in high dose recipients, and high levels of viral RNA were detected in recipient ferrets after transfusion of blood from the early viremic phase, which also correlated with adverse impact on their survival.

Conclusion: These data suggest that highly pathogenic avian influenza A virus, H5N1, is transmissible by blood transfusion in ferrets. Low levels of viremia were detected around the time of onset of symptoms and later in ferrets infected with highly pathogenic H5N1 virus. These findings may have implication for pathogenesis and transmissibility of H5N1.

Keywords: H5N1, Transfusion, Viremia, Ferrets

Background

Influenza A viruses belong to the *Orthomyxoviridae* family of RNA viruses that contain eight segments of negative sense RNA [1]. There are several subtypes, numbered according to antigen HA and NA, containing sixteen different HA antigens (H1 to H16) and nine different NA antigens (N1 to N9) have been identified to date. Influenza A viruses evolve with high mutation rates and can occasionally cross the species barrier. Highly pathogenic avian influenza virus H5N1 originated in poultry and has been occasionally transmitted to humans resulting in high mortality [2]. There have been no reports thus far to indicate that H5N1 is readily transmissible from human to human.

H5N1 virus continues to circulate among poultry in many countries in Asia, Africa, and Europe, occasionally spreading to humans. In 1997, avian influenza A virus subtype H5N1 emerged capable of infecting humans with a highly fatal disease outcome (fatality rate of ~60%) [3,4]. The primary pathologic process that causes death is fulminant viral pneumonia [3,5]. High replication efficiency, broad tissue tropism and systemic replication seem to determine the pathogenicity of H5N1 viruses in animals [2,6]. A human isolate A/Vietnam/1203/04 (H5N1) was reported to be highly pathogenic and the severity of disease was associated with broad tissue tropism and high virus titers in multiple organs, including the brain in ferrets [6].

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Some studies have shown that H5N1 virus is found exclusively in the respiratory tract (mainly in the lung) [7,8]. Other studies report the presence of H5N1 viruses in many extrapulmonary organs, such as intestine, liver, and brain [9-14]. Viral RNA has been detected in nasopharyngeal aspirates ranging from 1 day up to 15 days after disease onset [15,16]. Viral replication appears to be prolonged in H5N1 influenza because viral loads when plotted against time did not show a clear decline in a large group of H5N1 patients [17]. These findings demonstrate that H5N1 can be detected in brain, intestine, liver, lymph nodes, placenta, and fetal lung and cause coma, diarrhea, and encephalopathy in children [18], in addition to pneumonia.

Early in the 1960s, viremia was found in patients infected with influenza A virus in Asia [11,12]. Since then, other groups have reported that influenza viral RNA could be detected in blood from infected patients [17,19-22]. It has also been reported that detectable viral RNA in the blood of humans was associated with fatal outcomes while no viral RNA was detected in the blood of surviving H5N1-infected individuals [17].

Ferrets are an excellent mammalian animal model for studies of influenza virus pathogenicity and host immunity, and disease manifestations of influenza virus infection in ferrets closely resemble those in humans [6]. Recently, we found that infection with a human isolate, A/Vietnam/1203/04 (H5N1), resulted in viremia in the ferret model, which positively correlated with animal death [6]. Viral RNA could also be detected in brain, lung, ileum, nasal turbinate, and nasal wash. Although influenza viruses in blood and plasma are very stable [23], it is unclear whether the influenza virus can be transmitted by blood transfusion. Here, we performed studies in ferrets infected with the H5N1 strain to determine whether influenza A virus can be transmitted through blood transfusion.

Methods

Virus and its titer determination

A H5N1 strain, A/Vietnam/1203/04, was obtained from the Centers for Disease Control and Prevention (Atlanta, GA) (CDC#2004706280, E1/E3 (1/19/07) and amplified in 10–day old embryonated hen's eggs (CBT Farms, Chestertown, MD). The virus was maintained at −80°C until use. For infectious titer determination 10-fold dilutions of virus stock was inoculated into 10-day old embryonated hen's eggs (4–8 eggs per dilution) and the eggs were incubated for 48 hours. 50 μl of allantoic fluid was then collected from each egg and added to a microtiter plate. 50 μl of 0.5% turkey red blood cells (tRBCs) were added to all wells and plates were incubated for 30 minutes at room temperature. Plates were read for agglutination or non-agglutination. The 50% endpoint was determined by the method of Reed and Muench [24] from virus dilutions testing positive for hemagglutinin activity in tRBCs. Data were expressed as 50% egg infectious dose (EID_{50}) per milliliter.

Ferret inoculation and transfusion

Forty two adult male ferrets (Triple F Farms, Sayre, PA) that were 6 ~ 7 months of age and seronegative for representative currently circulating human influenza A strains and the challenge virus prior to initiation of the study were identified for the study. Ferrets were housed and cared for at BIOQUAL, Inc. (Rockville, MD). The BIOQUAL, Inc. IACUC had approved the animal care and use proposal, ACUP # 11-3056-71, prior to start of the study. For all procedures, the ferrets were lightly anesthetized with a solution of ketamine/xylazine formulated to provide doses of 25 mg/kg ketamine and 2.0 mg/kg xylazine to each animal. The animals (donors) were inoculated intranasally with 500 μl of virus, approximately 250 μl to each nare. Six donor ferrets were infected with low dose, about $1.0 \times 10^{2.6}$ EID_{50}/ml of virus and the other 6 donors were infected with high dose of about $1.0 \times 10^{3.6}$ EID_{50}/ml of virus. For each challenge dose, the donor animals were divided into two groups; blood was collected on days 2 and day 6 post-infection from one of these groups and on days 4 and day 10 post-infection from the other group. At these time points about 2 ml of freshly collected blood was transfused into its appropriate recipient (see Table 1 for details). To reduce the risk of a minor allergic reaction the recipient ferrets were administered a dose of anti-histamine (Benadryl, 2 mg/kg, IM) 5 min. prior to the transfusion. A 24 gauge IV catheter was then inserted into the cephalic vein and a blood line with filter was connected. The anti-coagulated (acid citrate dextrose (ACD). 0.48% (w/v) citric acid. 1.32% (w/v) sodium citrate. 1.47% (w/v) glucose) blood from one donor was slowly pushed through the line over a 10–15 minute period. In total, 24 recipients received blood from H5N1-infected animals and 3 animals received blood from animals treated with equal volume of PBS in each nare as controls.

Clinical signs of infection, weight, and temperatures were recorded twice daily. Activity scores were assigned as follows: 0, alert and playful; 1, alert but playful only when stimulated; 2, alert, but not playful when stimulated; and 3, neither alert nor playful when stimulated. Ferrets that showed signs of severe disease (prolonged fever; diarrhea; nasal discharge interfering with eating, drinking, or breathing; severe lethargy; or neurological signs) or had >20% weight loss were euthanized immediately. Euthanasia was performed on ferrets that had been sedated using IM inoculation with a ketamine HCl (25 mg/kg) and xylazine (2 mg/kg) solution. Blood was obtained from recipients on days 2, 4, and 8 post-transfusion (or before animal death) and analyzed for viral loads using RT-qPCR.

Table 1 Study design of donors and their appropriate recipients

Donor group	Animal code	Day blood collected	Recipient code
Low dose	51	2	63
		6	67
	52	2	64
		6	68
	53	2	66
		6	69
	54	4	70
		10	73
	55	4	71
		10	74
	56	4	72
		10	75
High dose	57	2	76
		6	79
	58	2	77
		6	80
	59	2	78
		6	81
	60	4	82
		10	85
	61	4	83
		10	86
	62	4	84
		10	87
PBS	49	4	88
	50	6	89
	62	10	90

These studies were performed in a Biosafety level 3 enhanced laboratory.

Nasal wash

Nasal wash samples were collected from all ferrets on days 0, 2, 4, 6, and 8 for viral load determination. Briefly, the ferret was sedated with ketamine/xylazine and placed in the laminar flow hood, draped over a small box. With the nose pointed upwards, 3 ml PBS/gentamicin/0.5% BSA solution is slowly instilled into the nostrils using a 24 gauge ¾" plastic catheter connected to a 3 ml syringe. The nasal wash was collected into a 15 ml sterile conical tube, spun down to remove cell debris. 0.5 ml sample was used for immediate isolation of viral RNA, and an additional sample used for the $TCID_{50}$ assay. These procedures were performed in a Biosafety level 3 enhanced laboratory.

RT-qPCR

Quantitative real-time RT-PCR was used for detection of viral RNA in blood and nasal wash. 500 μl of blood or nasal wash were used to isolate nucleic acids by using the QIAamp Viral RNA Mini Kit (Valencia, CA 91355) according to the manufacturer's protocol. We designed a set of primers and probes for the matrix gene, M, of the avian H5N1 influenza A virus, according to GenBank database. The forward primer was 5'-CGTCAGGCCC CCTCAAA-3', and the reverse primer was 5'-GGTGTT CTTTCCTGCAAAGA-3'. The TaqMan probe was oligonucleotide 5'-TCAAGTTTCTGTGCGATCT-3', coupled with a reporter dye [6-carboxy fluorescein] (FAM) at the 5' end, a non-fluorescent quencher and a minor groove binder (MGB), that served as a Tm enhancer, at the 3' end. The nucleic acids were amplified and detected in an automated TaqMan 7500 Analyzer by using QuantiTect™ Probe RT-PCR kit (Qiagen Inc., Valencia, CA). The 25-μl PCR mixture consisted of 100 nM primers and 100 nM probe. Following three thermal steps at 55°C for 5 min, at 50°C for 30 min and at 95°C for 10 min, 45 cycles of two-step PCR at 95°C for 15 s and at 60°C for 1 min were performed. The limit of detection was 1 fg of virus RNA per reaction with the TaqMan assay initial sample dilution at 1:10.

$TCID_{50}$ assay

The nasal wash and plasma samples were added in 10-fold graded dilutions to 96-well round-bottomed tissue culture plates. As a positive control, 10-fold dilutions of the challenge virus were included in each experiment. MDCK cells were then added to all wells and the plates incubated for 48 hr at 37°C, 5% CO_2. Following this period 50 μL from each well was transferred to a 96-well V-bottomed microtiter plate, 0.5% turkey RBC added to all wells and the presence of virus detected by hemagglutination as a readout. The 50% endpoint was determined by the method of Reed and Muench [17] from virus dilutions testing positive for hemagglutinin activity in Turkey Red Blood Cells (tRBC). Data were expressed as 50% tissue culture infective dose ($TCID_{50}$) per milliliter.

Statistical analysis

The log rank test was used for comparing survival curves and the unpaired Student's *t* test was used for other data analyses as indicated, and a value of $p < 0.05$ was considered significant.

Results

Transfusion of viremic blood was associated with fatal outcome in recipients

We infected 6 ferrets (donors) with $1.0 \times 10^{2.6}$ EID_{50}/ml (low dose) and another 6 animals with $1.0 \times 10^{3.6}$ EID_{50}/ml (high dose) of H5N1 virus, strain A/VN/1203/04. As shown

in Figure 1A, mortality in donors occurred at a higher rate with high dose compared with low dose. Blood was collected from infected animals (donors) as scheduled (Table 1) or before the death of the animal and about 2 ml of the fresh blood was transfused to their appropriate recipients (Table 1). Fatal outcomes were observed in some recipient ferrets (Figure 1B). About 50% of ferrets died within 12 days post-transfusion of blood from ferrets that were infected with a high dose of virus compared with 10% of recipients from the low dose virus infected group (Figure 1B). All control animals remained healthy and symptom-free throughout the duration of the study. These data suggested that the fatal outcomes observed were likely to be associated with virus infection rather than the result of the transfusion procedure.

Changes in body weight (kg) and temperature (°C) were also recorded and calculated in Figure 2 and Table 2. In general, animals that received blood from ferrets infected with high dose of virus showed decreased body weight and increased body temperature relative to the recipients that received blood from ferrets infected with low dose of virus.

Viral RNA could be detected in the blood of both donors and recipients

Transfusion of blood from ferrets infected with high dose of virus resulted in the death of recipient ferrets which positively correlated with levels of viral RNA [6]. We measured viral RNA in ferret blood collected from donors on the day of transfusion, or from recipients on day 2, 4, 8 post-transfusion as scheduled or before the recipient died using the RT-qPCR assay. Viral RNA detection in blood from donors and/or their appropriate recipients are shown in Table 3. In the low dose group, viral RNA in blood could be detected only in one donor (1/6 ferrets) on day 10 post-infection; while 5/6 of donors displayed viral RNA in their blood in the high dose group. Partial identity of the viral matrix gene sequences with wild type sequence was found in the blood of some recipients, which were analyzed using cDNA sequencing (Additional file 1: Figure S1) which provided direct evidence of viremia.

Table 3 also showed that viral RNA could be detected in the blood of 3/12 (25%) ferrets that received blood from donors infected with low-dose of virus and 7/12 (58.33%) recipients that received blood from high-dose infected donors. These data indicate that highly pathogenic avian influenza A virus (H5N1) can be transmitted through blood transfusion. Most recipients had detectable viral RNA in blood collected on day 2 post-transfusion while donor animals showed viremia after day 2 post-infection (Figure 3A). We also found that most viremic recipients died while recipients without viremia had 100% survival (Figure 3B), indicating that viremia is associated with fatal outcomes in ferrets infected with highly pathogenic H5N1 influenza virus.

To understand the relationship of virus load in blood with animal death, we recorded animal death and detectable virus load in blood. As shown in Table 4, viral RNA levels greater than 8×10^4 fg/ml in blood were associated with higher number of fatalities, while some recipients with viral RNA of more than 3×10^4 fg/ml in blood also did not survive. These data indicate that transfusion of lower amount of virus load in blood could also result in recipient animal death, and that high titers of viremia are a strong predictor of fatality in the H5N1-infected host.

Higher levels of viral RNA were found in the blood of some recipients after transfusion of blood collected in the early viremic phase

Although viral RNA could not be detected in the blood of animal #54, #56 from the low dose group and animal #60

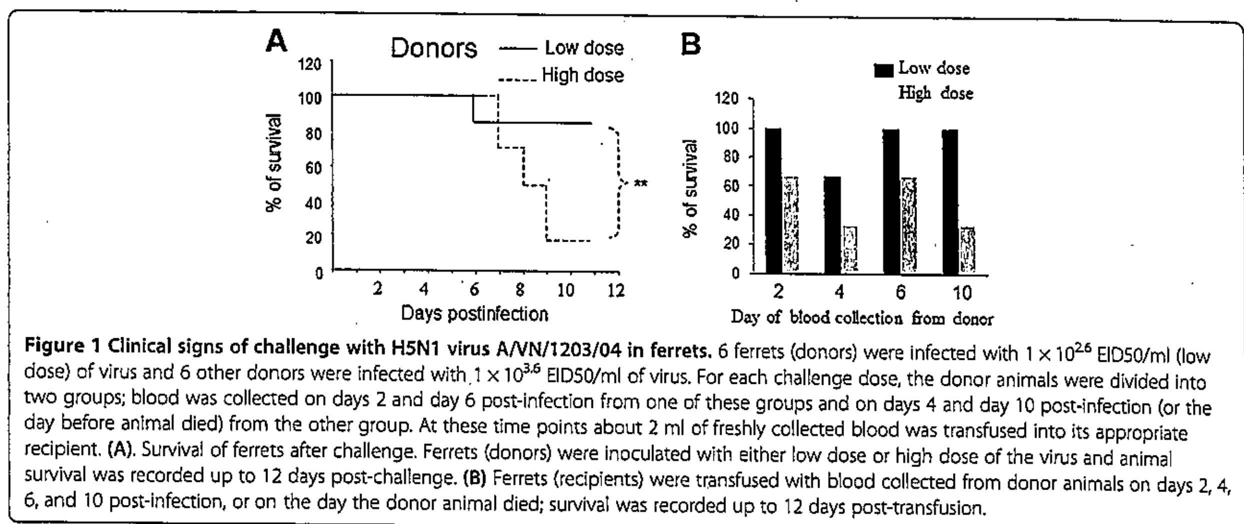

Figure 1 Clinical signs of challenge with H5N1 virus A/VN/1203/04 in ferrets. 6 ferrets (donors) were infected with $1 \times 10^{2.6}$ EID50/ml (low dose) of virus and 6 other donors were infected with $1 \times 10^{3.6}$ EID50/ml of virus. For each challenge dose, the donor animals were divided into two groups; blood was collected on days 2 and day 6 post-infection from one of these groups and on days 4 and day 10 post-infection (or the day before animal died) from the other group. At these time points about 2 ml of freshly collected blood was transfused into its appropriate recipient. **(A)**. Survival of ferrets after challenge. Ferrets (donors) were inoculated with either low dose or high dose of the virus and animal survival was recorded up to 12 days post-challenge. **(B)** Ferrets (recipients) were transfused with blood collected from donor animals on days 2, 4, 6, and 10 post-infection, or on the day the donor animal died; survival was recorded up to 12 days post-transfusion.

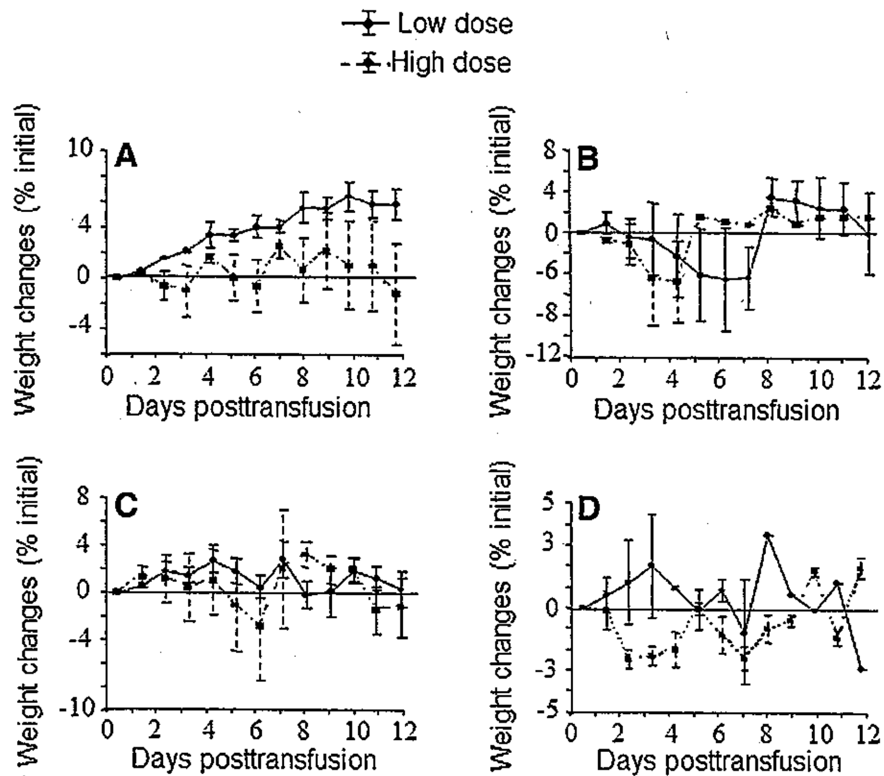

Figure 2 Changes in weights of ferrets (recipients) transfused with blood from infected donor animals. Ferrets (recipients) were transfused with blood from infected donor animals on the day post-infection when blood was collected, day 2 **(A)**, 4 **(B)**, 6 **(C)**, 10 **(D)**. The weights of ferrets were measured daily. The loss or gain of weight was calculated for each ferret as the percent change in the initial mean starting weight on day 0. Values are the averages ± SD for the ferret(s) alive for each group.

Table 2 Changes in body temperatures of recipients animals

Day	Day 2	Collected	Day 4	Collected	Day 6	Collected	Day 10	Collected
Post- transfusion	Low dose	High dose	Low dose	High dose	Low dose	High dose	Low dose	High dose
0	0.00 ± 0.76	0.00 ± 1.93	0.00 ± 1.73	0.00 ± 1.02	0.00 ± 0.96	0.00 ± 1.40	0.00 ± 0.91	0.00 ± 1.54
1	2.00 ± 1.04	2.54 ± 0.30	0.63 ± 1.13	3.50 ± 1.13	1.23 ± 0.47	1.50 ± 0.95	0.03 ± 1.51	−1.30 ± 0.29
2	0.83 ± 0.72	2.44 ± 2.00	1.43 ± 1.97	4.53 ± 1.45	1.13 ± 0.50	2.27 ± 2.44	1.00 ± 1.08	−0.13 ± 1.28
3	3.43 ± 0.44	4.44 ± 0.71	0.50 ± 2.19	2.93 ± 1.54	1.83 ± 0.25	2.60 ± 1.31	1.73 ± 0.65	−1.13 ± 2.18
4	2.06 ± 1.82	2.77 ± 2.83	−0.80 ± 1.80	−0.62 ± 1.20	1.97 ± 1.88	3.20 ± 1.81	−1.94 ± 0.95	−1.63 ± 3.25
5	1.10 ± 1.48	2.07 ± 0.00	−1.03 ± 0.98	4.73 ± 0.00	2.37 ± 0.76	2.90 ± 1.57	2.40 ± 0.55	0.82 ± 0.35
6	1.06 ± 0.78	0.02 ± 1.48	−0.73 ± 2.24	3.93 ± 0.00	2.97 ± 0.72	2.75 ± 2.76	0.36 ± 1.80	−1.93 ± 3.54
7	0.46 ± 0.68	3.02 ± 1.91	−1.05 ± 0.07	3.23 ± 0.00	0.77 ± 1.80	3.20 ± 2.40	1.83 ± 2.19	−0.88 ± 0.35
8	0.46 ± 1.16	1.37 ± 1.27	0.65 ± 1.34	3.43 ± 0.00	1.07 ± 0.35	2.50 ± 2.69	0.26 ± 0.91	0.07 ± 0.00
9	0.20 ± 1.22	1.92 ± 2.47	−0.60 ± 0.71	1.73 ± 0.00	1.27 ± 1.72	3.50 ± 2.55	1.53 ± 0.26	0.07 ± 0.00
10	0.30 ± 0.45	2.12 ± 1.91	−2.95 ± 1.63	3.83 ± 0.00	1.13 ± 0.71	2.40 ± 2.69	−1.64 ± 2.19	0.07 ± 0.00
11	1.20 ± 0.57	0.32 ± 2.90	1.30 ± 1.56	2.73 ± 0.00	2.90 ± 0.75	3.85 ± 2.47	−0.17 ± 1.77	0.07 ± 0.00
12	1.33 ± 2.19	−1.78 ± 0.64	−1.70 ± 0.57	−0.87 ± 0.00	−0.23 ± 1.22	2.10 ± 0.14	2.13 ± 0.69	0.07 ± 0.00
Average	1.11	1.77	−0.33	2.54	1.42	2.52	0.58	−0.71

Note: Ferrets (recipients) were transfused with blood from infected donor animals on the day when blood was collected (Day collected), and body temperatures were monitored daily by the use of subcutaneous implantable temperature transponders for 12 days post-transfusion. Each data point represents the mean value ± SD for the surviving ferrets.

Table 3 Viral load (fg/ml) in blood of ferrets (donors) infected with H5N1 virus, A/VN/1203/04 and the ferrets (recipients) transfused with blood from their appropriate donors using the RT-qPCR

	Donors				Recipients			
Group	Animal code	Day post-infection	Virus load (fg/ml) (mean ± SD)	Survival (days posttransfusion)	Animal code	Day post-challenge	Virus load (fg/ml) (mean ± SD)	Survival (days posttransfusion)
Low dose	51	2	0[a]	>12	63	2,4,8	0	>12
		6	0	>12	67	2,4,8	0	>12
	52	2	0	>12	64	2,4,8	0	>12
		6	0	>12	68	2,4,8	0	>12
	53	2	0	>12	66	2,4,8	0	>12
		6	0	>12	69	2,4,8	0	>12
	54	4	0	>12	70	2	$8.12 \times 10^4 \pm 5.17 \times 10^2$	6
						4	$5.77 \times 10^5 \pm 6.90 \times 10^3$	
						6	$4.02 \times 10^6 \pm 3.94 \times 10^5$	
		10	0	>12	73	2,4,8	0	>12
	55	4	0	>12	71	2,4,8	0	>12
		10	$7.62 \times 10^4 \pm 1.31 \times 10^3$	>12	74	2,4,8	0	>12
	56	4	0	>12	72	2,4,8	0	>12
		10	0	>12	75	4	$1.33 \times 10^4 \pm 9.59 \times 10^3$	>12
High dose	57	2	0	7	76	2,4,8	0	>12
		6	$4.94 \times 10^5 \pm 5.27 \times 10^4$		79	8	$2.11 \times 10^4 \pm 4.23 \times 10^3$	>12
	58	2	0	>12	77	2,4,8	0	>12
		6	0		80	2,4,8	0	>12
	59	2	$7.62 \times 10^4 \pm 3.57 \times 10^3$	6	78	2	$2.30 \times 10^6 \pm 2.58 \times 10^4$	4
		6	$1.93 \times 10^6 \pm 6.95 \times 10^5$		81	2	$2.01 \times 10^4 \pm 1.60 \times 10^3$	5
						4	$8.59 \times 10^4 \pm 5.63 \times 10^3$	
	60	4	0	7	82	2	$4.51 \times 10^6 \pm 9.65 \times 10^4$	4
		7	$2.12 \times 10^5 \pm 2.35 \times 10^4$		85	2	$5.81 \times 10^5 \pm 3.65 \times 10^4$	7
						4	$1.18 \times 10^7 \pm 5.99 \times 10^5$	
	61	4	0	8	83	2,4,8	0	>12
		8	$2.28 \times 10^6 \pm 5.94 \times 10^5$		86	4	$4.79 \times 10^4 \pm 2.54 \times 10^3$	11
	62	4	$5.10 \times 10^4 \pm 2.37 \times 10^3$	8	84	2	$9.76 \times 10^4 \pm 3.14 \times 10^3$	5
						4	$1.67 \times 10^7 \pm 5.96 \times 10^5$	
		8	$1.33 \times 10^4 \pm 6.59 \times 10^2$		87	2,4,8	0	>12
PBS	49	4	0		88	8	0	>12
	50	6	0		89	8	0	>12
	62	10	0		90	8	0	>12

Note: a) 0 indicates that viral load could not be detectable in our experiment condition.

from the high dose group, viremia was detected in the blood of their recipients. Also some recipients given blood from the high dose group had higher levels of viral RNA than their donors on the day that blood was collected (Table 3). Figure 3C showed that 1/1 (100%), or 4/4 (100%) recipients of blood collected from donors on day 2 or day 4 post-infection displayed increased viral RNA in their blood (also see Table 3) after transfusion in both virus dose groups relative to their donors and mortality was 100%. Some recipients that survived did not show high amounts of viral RNA in their blood after transfusion of blood from donors at day 6 post-infection or later. These data indicated that higher titers of viral RNA could be detected in recipients of blood collected in the early viremic phase compared with the late viremic phase.

Viral loads were detectable in nasal wash from recipients

Previously we reported that virus could be detected more frequently in the nasal washes of animals in the high dose group compared with those in the low dose

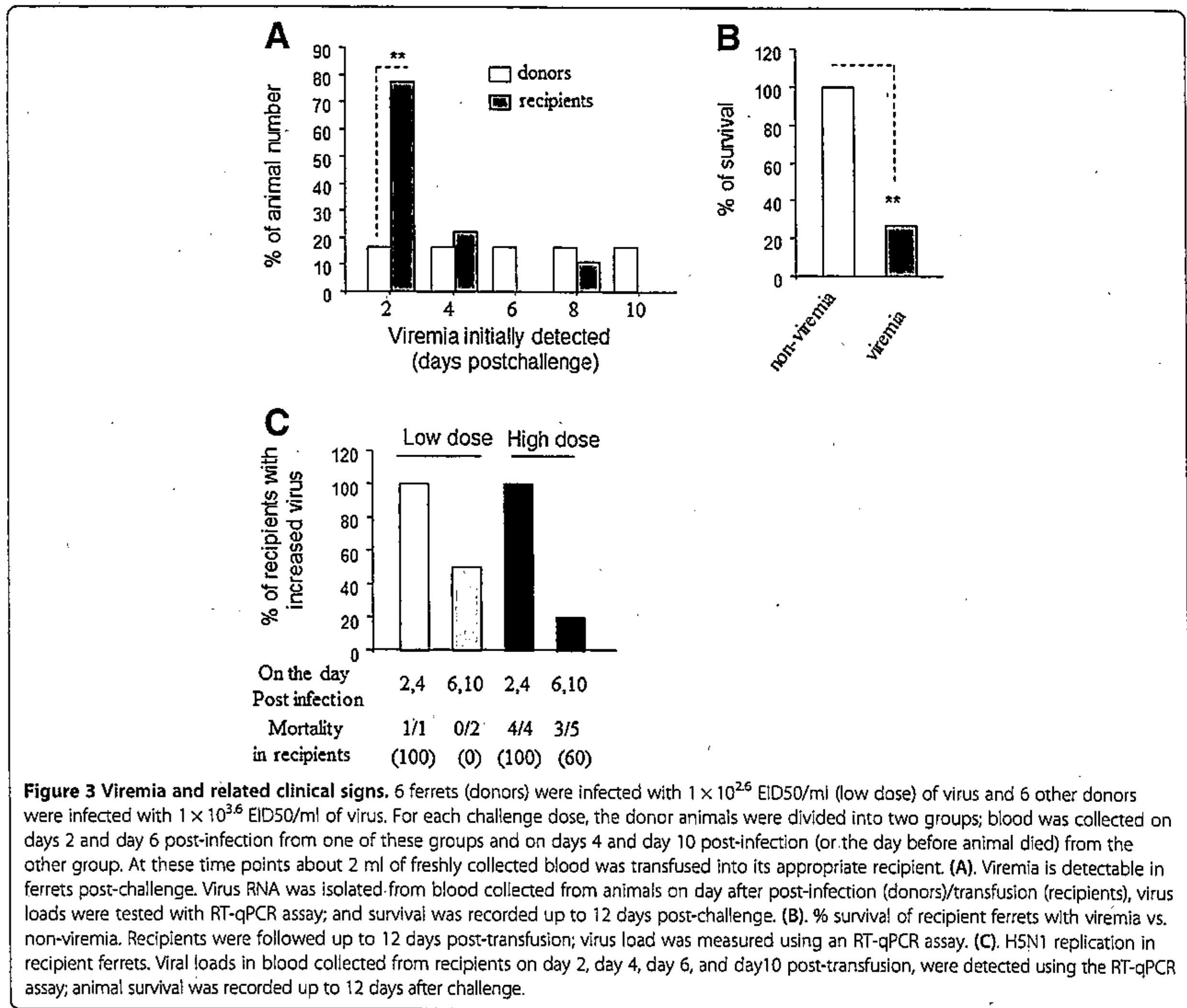

Figure 3 Viremia and related clinical signs. 6 ferrets (donors) were infected with $1 \times 10^{2.6}$ EID50/ml (low dose) of virus and 6 other donors were infected with $1 \times 10^{3.6}$ EID50/ml of virus. For each challenge dose, the donor animals were divided into two groups; blood was collected on days 2 and day 6 post-infection from one of these groups and on days 4 and day 10 post-infection (or the day before animal died) from the other group. At these time points about 2 ml of freshly collected blood was transfused into its appropriate recipient. **(A)**. Viremia is detectable in ferrets post-challenge. Virus RNA was isolated from blood collected from animals on day after post-infection (donors)/transfusion (recipients), virus loads were tested with RT-qPCR assay; and survival was recorded up to 12 days post-challenge. **(B)**. % survival of recipient ferrets with viremia vs. non-viremia. Recipients were followed up to 12 days post-transfusion; virus load was measured using an RT-qPCR assay. **(C)**. H5N1 replication in recipient ferrets. Viral loads in blood collected from recipients on day 2, day 4, day 6, and day10 post-transfusion, were detected using the RT-qPCR assay; animal survival was recorded up to 12 days after challenge.

group. Viral load was detected in nasal wash samples on day 2 in both groups [6]. In this study, we tested viral loads in the nasal wash from recipients and viral loads were detected in these samples (Table 5). 8/10 of recipients with viremia had detectable viral loads in the nasal wash, and two recipient animals (#81 and #79) with viremia did not have detectable virus in the nasal wash (compared Table 3 to Table 5). As shown in Table 5, there was a positive association of viral load measured using both RT-qPCR and $TCID_{50}$ assays with lack of survival; generally detection of a higher viral load in nasal wash was associated with a decreased chance of recipient survival.

Viral load testing using the RT-qPCR assay was more sensitive than the $TCID_{50}$ assay. Twelve recipients displayed detectable viral loads in nasal wash using RT-qPCR assay, while only 8/12 of animals showed positive viral loads in the nasal wash ($lg(TCID_{50}/ml) > 0.5$) using $TCID_{50}$ assay. These data indicate that H5N1 can be transmitted by transfusion of viremic blood and that transfusion resulted in increased virus levels in the recipient as evidenced by detectable viral load in the nasal wash of recipients using both RT-qPCR assay and $TCID_{50}$ assays. High viral loads in blood and nasal wash of recipients correlated with fatal outcomes in these animals.

In summary, 4/12 recipients (33.33%) who received blood from donor ferrets infected with low dose displayed viral RNA in blood and/or nasal washes. 10 of 12 recipients (83.33%) who received blood from donor ferrets infected with high dose had detectable viral RNA in blood and/or nasal wash.

Discussion

Although H5N1 infection of humans has been associated primarily with infection of the respiratory tract, dissemination of virus to other organs, such as the brain, has also been reported in several cases. Virus dissemination from

Table 4 The relationship between viral load (fg/ml) in blood and ferret death (days post-challenge)

Group	Donors			Recipients		
	Animal code	Survival days		Animal code	Survival days	
		$<8\times10^4$	$>8\times10^4$		$<3\times10^4$	$>3\times10^4$
Low dose	54	>12		70		6
	55	>12		74	>12	
	56	>12		75	>12	
High dose	57		7	79	>12	
	59		6	78		4
				81		5
	60		7	82		4
				85		7
	61		8	86		11
	62	8		84		5
				87	>12	

Note: data shown here only represented either animal of both donor and its appropriate recipient that viral RNA can be detectable in their blood under our experiment condition. And the animals with non-detectable (nd) viral RNA in blood did not show here.

lungs to extrapulmonary tissues most likely occurs by viremia. The isolation of highly pathogenic influenza H5N1 virus from the blood of 2 patients and the detection of viral RNA by RT-qPCR in the blood of 9 of 16 patients suggest that viremia can occur at high levels and for prolonged periods in people with symptoms of highly pathogenic influenza H5N1 virus infection [25]. Thus far, it has been shown that infection with both avian H5N1 virus [14,17] and pandemic H1N1 (swine) virus [22] can result in viremia, which is associated with severe disease manifestations and fatal outcomes.

Ferrets develop a productive infection after inoculation with human and avian influenza viruses without prior adaptation of the virus. Experimental influenza virus infections of ferrets have been used to model different aspects of influenza in humans [26]. The human isolate A/Vietnam/1203/04 (H5N1) is one of the most pathogenic virus isolates; and severity of disease was associated with broad tissue tropism and high virus titers in multiple organs, including the brain. High fever, weight loss, anorexia, extreme lethargy, and diarrhea were observed as major clinical signs and symptoms [6]. In addition, viral RNA is frequently detected in blood one or two days before death and viremia is associated with lethal outcomes [6].

Detection of virus in nasal washes is a key biomarker for influenza virus infection. In our study we showed that transfusion of viremic blood resulted in virus transmission in the recipient and that virus could be detected in nasal wash using RT-qPCR and $TCID_{50}$ assays. Our study also showed that viremia correlated with detectable viral load in the nasal wash, and high amounts of virus in nasal wash as detected by the $TCID_{50}$ assay was associated with recipient death (Tables 3 and 4).

Transfusion of blood collected on day 2 or day 4 post-infection resulted in higher levels of viral RNA in the blood of recipient ferrets and 100% fatality compared with levels observed in ferrets who received blood at from day 6 or later post-infection. One explanation may be that at the later periods of infection antibody production could

Table 5 Viral loads assay in nasal wash from recipient ferrets that viral RNA could be detectable using either RT-qPCR or $TCID_{50}$ assay

Group	Animal code	Day post- transfusion	Viral RNA (fg/ml) (mean ± SD)	lg ($TCID_{50}$/ml)	Survival (days post-transfusion)
Low dose	70	2	$1.20\times10^4\pm5.98\times10^3$	<0.5	6
		6	$1.36\times10^7\pm4.60\times10^5$	3.6	
	74	2	$6.58\times10^6\pm2.54\times10^4$	0.5	>12
	75	13	$1.63\times10^4\pm3.92\times10^3$	<0.5	>12
	69	14	$2.01\times10^4\pm2.24\times10^3$	<0.5	>12
High dose	78	2	$9.09\times10^4\pm6.82\times10^3$	1	4
	82	2	$2.11\times10^6\pm4.13\times10^5$	1.5	4
	84	2	$7.73\times10^4\pm1.29\times10^3$	<0.5	5
		4	$6.53\times10^6\pm5.43\times10^5$	3.84	
	85	2	$2.75\times10^8\pm1.14\times10^7$	4.84	7
		3	$2.51\times10^7\pm8.52\times10^6$	0.5	
	86	2	$2.72\times10^4\pm2.45\times10^3$	1.5	11
	87	2	$1.32\times10^4\pm5.97\times10^3$	1.5	>12
	83	4	$4.52\times10^4\pm2.57\times10^3$	<0.5	>12
	80	14	$1.71\times10^4\pm9.98\times10^3$	<0.5	>12

have been initiated which would reduce potential for transmission by immune complex formation (Additional file 1: Table S1). However, these aspects of influenza transmission by viremic blood would need to be explored further.

The highly pathogenic influenza virus H5N1 has been shown to infect multiple human organs other than the lungs, suggesting that H5N1 can replicate in these organs. Quantitative RT-PCR showed that high viral load is associated with increased host responses [14]. High viral loads have been found in lung, brain and blood from ferrets infected with H5N1, A/VN/1203/04 and virus could replicate in these tissues and damage cells in these organs [6]. It has been reported that Influenza A virus can infect and replicate in T lymphocytes and peripheral blood mononuclear cells (PBMCs) [27], primary T cells, and Jurkat cells [28]. Currently, it is not known whether H5N1 viruses replicate in PBMCs and the type of cells in PBMCs that could support replication needs further investigation.

Conclusions

In conclusion, our study has shown that highly pathogenic influenza A virus H5N1 can be transmitted through blood transfusion in a susceptible ferret model under certain conditions. These findings suggest that highly pathogenic influenza strains may have broader tropism and therefore may be transmitted by mechanisms other than the nasopharyngeal route. Although our current study does not provide direct evidence of virus replication in blood, H5N1 replication in blood cells may warrant further exploration.

Additional file

Additional file 1: Table S1. Hemagglutination inhibition assay with blood from ferrets challenged with H5N1 virus, A/VN/1203/04. **Figure S1.** Alignment and compare of partial sequence of wild type (infection) M (matrix) gene of H5N1 virus with these sequenced from bloods of some recipients.

Competing interests

The authors declare that there are no competing interests.

Authors' contributions

IH conceived of the study. XW, IH designed the experiments. XW, JT, JZ, ZY performed study and data analysis. XW and IH wrote the paper. The authors read and approved the final manuscript.

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研究報告の概要

○供血者からのマラリアDNAの検出：マラリア持続感染の証拠
背景：英国輸血サービスは10年以上にわたり、リスク供血者の供血スクリーニングとしてマラリア抗体検査を行ってきた。供血者には多くのマラリア流行国からの移民が含まれており、これまでの輸血感染マラリアの経験上、このような供血者の一部には原虫血症が持続していることから、臨床的評価が必要である。
方法：マラリア抗体スクリーニングに反応した供血者は、マラリアDNAについてさらに検査された。
結果：2010年4月～2013年8月まで、マラリアリスクが特定された合計138,782供血血液において抗体スクリーニングを実施した。このうち4,302検体(3.1%)は一次抗体検査に反応し、1,170検体(27.2%)の抗体陽性が確認され(2,059検体は陰性、1,073検体は判定保留)、マラリアDNA検査で14検体が陽性となった(熱帯熱マラリア3例、三日熱マラリア5例、卵形マラリア3例、四日熱マラリア2例及び熱帯熱/四日熱マラリアの重複感染1例)。14人中3人は、後にマラリア罹患歴を申告した。14人のマラリアリスクは全て、旅行歴ではなく居住歴(アフリカ、インド及びパキスタン)であり、流行地から英国に滞在してから平均で2.6年(範囲1～7年)が経過していた。
結論：マラリア原虫血症は健康な供血者にも見られることがあり、供血者におけるマラリアリスク対策にはそのような供血者の存在を考慮に入れる必要がある。マラリア抗体スクリーニングを行っていない国は、流行国への居住経験がある供血者の血液を採取する際に細心の注意を払うべきであり、一時的な供血延期措置では不十分である。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見

英国において2010年4月～2013年8月まで合計138,782供血血液にマラリアリスクが特定された。このうち、マラリアリスク地域に居住歴のある14人にマラリアDNAが確認され、最長7年間原虫血症が持続していたとの報告である。

今後の対応

日本赤十字社では、輸血感染症対策として問診時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、問診時にマラリアの既往歴を確認し、該当する場合は献血不適としている。さらに、マラリア流行地への旅行者または居住経験者の献血を一定期間延期している(1～3年の延期を行うとともに、帰国(入国)後マラリアを思わせる症状があった場合は、感染が否定されるまで、献血を見合わせる)。平成26年4月よりマラリアの献血制限対象地域を拡大し、問診を強化している。今後も引き続き、マラリア感染に関する新たな知見及び情報の収集、対応に努める。

⑤

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Detection of malarial DNA in blood donors – evidence of persistent infection

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Vox Sanguinis

Background and Objectives The English transfusion service has screened donations from malaria-risk donors for malarial antibodies for over 10 years. The donor population includes migrants from many malaria-endemic countries and, from our experiences with post-transfusion malaria, some of these may remain parasitaemic and need clinical review.

Materials and Methods Malarial antibody screen-reactive donations with serological evidence of malaria identified by the reference laboratory were further investigated for the presence of malarial DNA.

Results Malarial DNA was found in 14 of 1955 samples investigated; three *P. falciparum*, five *P. vivax*, three *P. ovale*, two *P. malariae* and one dual parasitaemia *P. falciparum/P. malariae*. All of these were donors whose malaria risk was residency rather than travel.

Conclusion Malarial parasitaemia in healthy donors occurs, and donor malaria-risk strategies must take into account the possibility of such donors presenting. Countries not utilizing malarial antibody screening should consider carefully the collection of donations from donors previously resident in endemic countries; temporary deferral is insufficient.

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Key words: malarial DNA, malarial serology, malaria-risk, parasitaemia.

Introduction

Malaria may be transmitted through transfusion of red cell products or components containing sufficient red cells. Transmission of malaria parasites into a malaria naïve individual in this way is likely to give rise to serious clinical consequences, with a high risk of mortality from at least one plasmodium species, if not identified and treated quickly.

Although UK is not endemic for malaria, its blood supply carries a specific malaria risk either from donors born and raised in UK travelling to endemic areas or from donors who were born in or have lived in endemic countries. To address these risks, the English transfusion service (NHS Blood & Transplant – NHSBT) implemented a strategy of routine malarial antibody screening of donors identified to have a malaria risk [1, 2]. The screening outcomes are either a negative result with the assumption of absence of infection – the donation is released to inventory, or a reactive result – the donation is discarded, but requiring the status of the donor to be determined.

Although numerically the largest group of malaria-risk donors comprises those born and raised in UK and who travel to endemic areas (travellers), the highest risk of malaria infection rests with those donors whose risk is being born in, or having previous residency in, an endemic area. Whilst the majority of such individuals whose screen reactivity is subsequently confirmed to be specific are demonstrating serological response to past, resolved, infection, a small number may still be infected

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with a persistent low-level parasitaemia. These donors are likely to be those categorized as 'semi-immune', infected with high-titre antibody and persistent low-level parasitaemia. Such individuals originate from endemic areas with high levels of infection with frequent exposure to malaria parasites, leading to a dynamic balance between the infection and the immune response to it. The existence of such donors in UK has been demonstrated through previous transfusion transmissions of malaria [1]. Within NHSBT, such donors are most likely to have been born in or lived on the African continent, usually sub-Saharan Africa, or the Indian subcontinent.

Although such donors are asymptomatic, apparently well and have been assessed according to guidelines [2], there remains the risk that their 'semi-immune' status may change with the consequent development of symptoms and clinical disease. Transfusion services have a responsibility to ensure that donors are informed of any health risks identified during both donor and donation screening processes. NHSBT therefore initially determined that any malarial antibody screen-reactive donor whose reactivity was confirmed to be specific with a scorable immunofluorescence (IFAT) titre should be further investigated for the presence of plasmodial DNA.

In April 2010, NHSBT's National Transfusion Microbiology Reference Laboratory (NTMRL) introduced malarial DNA testing into its malaria confirmatory algorithm, testing screen-reactive donations that had the presence of specific malarial antibodies confirmed, but with an IFAT titre of 1:80 or higher. DNA testing was performed using two RT PCRs, one specific for *P. falciparum* the other for *P. vivax/malariae/ovale*, modified from the method of Bass [3]. Subsequently, the criteria for selection for DNA testing were extended to include donors with any confirmatory antibody reactivity.

This article presents the DNA data to date, confirming the presence of parasitaemia in asymptomatic blood donors, many of whom had not visited an endemic area for a number of years. These data are reviewed both in terms of the findings themselves and importantly in relation to donor selection guidelines and the identification and management of malaria-risk donors.

Methods

Donation samples

Samples from malaria-risk donors were additionally screened for malarial antibodies in the NHSBT Testing Laboratories using the Trinity Biotech (previously Lab21) Malaria Ab EIA, product code 60127/129 (Trinity Biotech, Newmarket, UK). Screen-reactive samples were referred to NTMRL for confirmatory testing.

Malaria confirmatory serology

Serological confirmation is performed using two additional, evaluated, commercial malarial antibody assays and an in-house *P. falciparum* antibody immunofluorescence test (IFAT). Initially, the confirmatory assays were the DiaPro Malaria Ab, product code MALAB.CE (DiaPro, Milan, Italy) and the Bio-Rad Malaria Ab EIA, product code 750001 (Diamed AG, Cressier s/Morat, Switzerland); subsequently, the Bio-Rad/Diamed assay was withdrawn from the market for commercial reasons. This was replaced by the Cellabs Malaria Ab EIA, product code Z1KMC3 (Cellabs, Brookvale, Australia).

The IFAT is an in-house assay using *P. falciparum*-infected cells, used in NTMRL since 2002 [4]. Cultured K1 strain *P. falciparum*-infected human red cells synchronized at the schizont stage are spotted onto multispot glass slides. Samples are diluted 1:30 and antibody detected using fluorescent-labelled anti-human IgG (Alexa Fluor 488; Stratech, Newmarket, UK). Screen positives are titrated from 1:40 to 1:640. Samples titrating to 1:80 or higher are recorded as positive.

Samples giving an overall consistent pattern of reactivity with at least two of the confirmatory assays are reported as 'Positive'. Samples with inconsistent reactivity in the confirmatory assays and low-level reactivity in the screening assay or with patterns of reactivity that could not be considered as 'Positive' are reported as 'Inconclusive' and a fresh sample requested together with any specific and relevant history.

Initially, only samples confirmed to contain malarial antibodies and that were IFAT positive were additionally tested for malarial DNA. Subsequently, all samples with reactivity in any of the malarial serology confirmatory assays were investigated for the presence of malarial DNA.

Malaria antigen detection

Samples found to be malaria DNA positive were investigated to determine whether malarial antigen detection could be an alternative to DNA testing or could prove useful as an additional confirmatory test.

Malaria antigen detection was performed using the BinaxNOW Malaria Antigen rapid test (Alere, Stockport, UK), which detects histidine-rich protein 2 (HRP2) as a marker of *P. falciparum* parasitaemia and pan-plasmodial aldolase as a marker of infection with any human malaria; and the Cellabs Malaria Ag EIA for HRP2 (Cellabs, Brookvale, Australia), both following the manufacturer's instructions, except that the BinaxNOW assay was pre-evaluated using fresh and frozen/thawed whole-blood preparations; the samples of malarial DNA-positive donations are stored at −40°C. The relative sensitivity of both assays was

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assessed using the WHO 1st International Standard for *P. falciparum* (1×10^7 iu/ml) (NIBSC, South Mimms, England), diluted from 1×10^7 to 0 iu/ml in tenfold dilutions in whole blood. The samples were tested as fresh dilutions (BinaxNOW) and frozen at –40°C for at least 24hr, thawed at 37°C and tested (Cellabs Malaria Ag EIA and BinaxNOW). The stored samples from the malarial DNA-positive donations were thawed at 37°C and tested undiluted.

Malarial DNA detection

From April 2010, samples from confirmed malaria serology-positive donations with an IFAT titre of 1:80 or greater were tested for malarial DNA. From February 2013, samples from donations with reactivity in any of the confirmatory serology assays were tested for malarial DNA.

Prior to DNA extraction, the buffy coat is removed from each sample. The samples are then re-mixed and the DNA extracted using the Qiagen whole-blood extraction kit, product code 951054, run on the Qiagen EZ1 Advanced XL (Qiagen Ltd, Manchester, UK). Extracts are stored at –40°C prior to performing PCR.

Detection of plasmodial DNA is performed using a modification, addition of an internal control, to the real-time Taqman PCR method of Bass [3]. The published methodology is a single tube duplex utilizing a single set of primers with two probes, one specific for *P. falciparum* (PF), the other specific for a sequence common to *P. ovale*, *P. vivax* and *P. malariae* (OVM), but does not include an internal control (IC). To include an IC, and retain sensitivity and specificity, the assay was split into two specific assays and run as a pair, both having an IC. The plasmodium-specific primer and probe sequences are as published, but the fluorophore used for the OVM probe was changed from VIC to FAM. The IC is a previously used sequence of the human β-globin gene, the specific primers and probe, which are included in both PCRs. Table 1 details probe and primer sequences and reaction concentrations.

Five microlitre of DNA extract is added to a 25 μl total volume reaction mix containing 12·5 μl of Agilent Brilliant Multiplex QPCR master mix (Agilent Technologies, Wokingham, UK), 1 μl each of malaria-specific primers, 0·5 μl of IC primers, 0·5 μl of malaria-specific probe, and 0·5 μl of IC probe (TIB Molbiol, Berlin, Germany) and 3·5 μl dH_2O. The PCR is performed on the Strategene MX3005p real-time PCR system (Stratech, Newmarket, UK), parameters: 95°C for 15 min, followed by 55 cycles of 95°C for 60 s and 60°C for 60 s.

DNA quantitation

Plasmodial DNA levels in the *P. falciparum* DNA-positive donors were estimated using the *P. falciparum* PCR with a standard curve prepared from the 1st WHO *Plasmodium falciparum* DNA standard (NIBSC, South Mimms, England) set at 1 IU/ml (approximately 0·5 gEq/ml). The lack of suitable standardized material currently prevents the quantitation of the three other human *plasmodium* species in this laboratory; suitable approaches are being investigated.

Referral of DNA-positive donors

Attempts are made to contact the DNA-positive donors to offer clinical advice. The DNA-positive donors were re-contacted at least 3 months after the initial contact to determine whether they had taken up the offer of specialist advice.

Results

Malarial antibody screening and confirmation

From April 2010 until the end of August 2013, a total of 138,782 donations were identified as malaria risk and screened for malarial antibodies. Of these, 4302 (3·1%)

Table 1 Sequences of malaria-specific and internal control primers and probes

Primer/probe	Sequence	Reaction concentration
P. falciparum probe	5′ 6FAM-TCTGAATACGAATGTC-MGBNFQ 3′	200 nM
P. falciparum forward primer	5′ GCTTAGTTACGATTAATAGGAGTAGCTTG 3′	300 nM
P. falciparum reverse primer	5′ GAAAATCTAAGAATTTCACCTCTGACA 3′	300 nM
P. o/v/m probe	5′ 6FAM-CTGAATACAAATGCC-MGBNFQ -3′	100 nM
P. o/v/m forward primer	5′ GCTTAGTTACGATTAATAGGAGTAGCTTG 3′	450 nM
P. o/v/m reverse primer	5′ GAAAATCTAAGAATTTCACCTCTGACA 3′	450 nM
β-globulin probe	5′ HEX-AAGGTGAACGTGGATGAAGTTGGTGG-BHQ 3′	20 μM
β-globulin forward primer	5′ GTGCACCTGACTCCTGAGGAGA 3′	100 nM
β-globulin reverse primer	5′ CCTTGATACCAACCTGCCCAG 3′	100 nM

Bold identifies the fluorophores used for the probes.

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were reactive on the primary malarial antibody screen and the samples referred to NTMRL for confirmation.

Of those referred for confirmation, 1170 (27·2%), overall 0·84% of those screened, were reported as malarial antibody positive, 2059 (47·9%) as malarial antibody negative and 1073 (24·9%) as inconclusive. The report of 'Inconclusive' is given where, from the results of investigations on that sample, no clear conclusion could be drawn about the malarial antibody status of the donor.

Malaria antigen detection

Both the Cellabs Malaria Ag EIA and the BinaxNOW assays picked up the WHO 1st International *P. falciparum* Standard diluted to 1 × 10^5 iu/ml. No difference was seen with this dilution series when testing the BinaxNOW assay with fresh or frozen/thawed whole blood.

None of the malarial DNA-positive donations identified were detected by either malaria antigen assay.

Malarial DNA detection

From April 2010 until the end of August 2013, a total of 1955 samples were tested for malarial DNA. Of these, 1594 (81·5%), April 2010 until January 2013, were selected on the original basis of having a positive IFAT titre, and subsequently, 361 (18·5%), from February 2013 onwards, on the basis of reactivity in any of the confirmatory serology assays.

Of those tested, 14 (0·7%) were found to be DNA positive. Four (28·6%) were positive on the *P. falciparum* assay and 10 (71·4%) on the *P. ovale/vivax/malariae* assay. All 14 donors were subsequently confirmed to be malarial DNA positive, and the OVM positives speciated, by the Department of Clinical Parasitology, Hospital for Tropical Diseases, London. Thirteen of the donors were confirmed as having clear serological evidence of malaria infection, but one had been recorded as having inconclusive serology. This donation was erroneously tested for malarial DNA, but found to be DNA positive (Sample ID 209306).

The serology and DNA results, speciation and basic donor risk data for the 14 DNA-positive donors are presented in Table 2. Three (21%) of the donors were infected with *P. falciparum*, 5 (36%) with *P. vivax*, 3 (21%) with *P. ovale*, 2 (15%) with *P. malariae*, and 1 (7%) had a dual parasitaemia with both *P. falciparum* and *P. malariae* confirmed. All four *P. falciparum*-positive donors were IFAT positive, as were both of the *P. malariae* donors and two of the three *P. ovale* donors.

The donor demographics and detailed risk information for the 14 malarial DNA-positive donors are presented in Table 3. All were first-time donors. Twelve (86%) of the donors were male; mean donor age at time of donation was 27 years (range 19–44 years). All of the DNA-positive donors had a residency risk of Africa, India and Pakistan; three of the donors subsequently declared a history of malaria, one of them only 8 months prior to donation. The mean time from last return from an endemic area was 2·6 years (range 1–7 years).

The DNA quantitation data available are presented in Table 4.

Table 2 Malarial DNA-positive donors identified; serology and DNA results and declared risk

Sample ID	Serology screen result[a]	Confirmatory serology assay results[a]			IFAT titre	Plasm. Spp	Overall risk category
		DiaPro	Cellabs	Diamed			
009839	7·46	6·76/6·90	16·54/17·05	5·21/4·20	1/640	Pf	Previous residency; no history of malaria
100255	7·51	0·69/0·62	19·09/18·79	11·39/13·26	1/640	Pf	Previous residency; no history of malaria
208922	26·61	1·49/1·83	13·94/14·4	NT	1/640	Pf	Previous residency; no history of malaria
204137	86·12	0·44/0·48	25·97/25·83	7·79/8·27	1/640	Pf/Pm	Previous residency; no history of malaria
211908	7·14	0·746/1·0	13·71/13·80	NT	1/640	Pm	Previous residency; no history of malaria
216512	2·75	0·36/0·34	9·89/8·41	NT	1/320	Pm	Previous residency; no history of malaria
103461	1·82	2·39/2·48	0·83/0·92	4·07/3·55	1/80	Po	Previous residency; no history of malaria
114294	19·36	0·37/0·38	9·12/9·64	2·77/3·07	1/160	Po	Previous residency; malaria in 2006
209306	4·65	0·39/0·43	1·88/2·04	NT	Neg	Po	Previous residency; malaria in 2006
102726	94·15	4·73/4·61	23·57/23·57	7·52/6·28	Neg	Pv	Previous residency; no history of malaria
105435	96·89	2·30/2·65	12·37/12·84	4·00/4·46	Neg	Pv	Previous residency; no history of malaria
205176	76·44	5·64/5·77	6·45/8·03	4·70/4·69	Neg	Pv	Previous residency; no history of malaria
302327	99·79	5·05/5·95	9·11/9·32	NT	Neg	Pv	Previous residency; malaria in 2012
312209	76·797	10·5/10·5	5·418/5·976	NT	Neg	Pv	Previous residency; no history of malaria

[a]All immunoassay results expressed as s/co ratios.
NT, not tested.

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Table 3 Donor demographics and time since last possible exposure for malarial DNA-positive donors

Sample ID	Date bled	Plasmodium sp.	Sex	Donor age[a]	Years sincelast exposure.	Specific risk information	Take-up of advice
009839	14/06/2010	Pf	M	19	1	From Nigeria, came to UK in Sept 2009. No h/o malaria; no revisit	Donor in contact with GP
100255	31/12/2010	Pf	M	34	5	From Ivory Coast, came to UK in 2005, No h/o malaria; no revisit	No contact with donor
208922	24/06/2012	Pf	F	31	3	From Nigeria, came to UK in 2009. No h/o malaria; no revisit	Sought treatment
204137	19/03/2012	Pf/Pm	M	30	2	From Ghana, came to UK in 2009. No h/o malaria; revisit in 2010	Provided additional samples and sought treatment
211908	28/08/2012	Pm	M	31	1	From Nigeria, came to UK in 2011. No h/o malaria; no revisit	No contact with donor
216512	06/12/2012	Pm	M	35	5	From Nigeria, came to UK in 2004, last visit endemic area 2007	Donor contacted but no information available
103461	14/03/2011	Po	M	44	7	From Nigeria, came to UK in 2004. No h/o malaria; no revisit	Donor provided additional samples and sought treatment
114294	10/11/2011	Po	F	21	3	From Nigeria, came to UK 2008, malaria aged 16. No revisit	Made appointment at HTD but did not attend
209306	29/06/2012	Po	M	26	3	From South Africa, came to UK in 2009. Malaria 2006. No revisit	No contact with donor
102726	01/08/2010	Pv	M	21	1	From India, came to UK in 2009. No h/o malaria; no revisit	Donor in contact with GP
105435	28/04/2011	Pv	M	23	1	Student from India, came to UK in Oct 2010. No h/o malaria; no revisit	Initial treatment but did not attend f/u
205176	11/04/2012	Pv	M	21	1	From Pakistan, came UK in Feb 2011. No h/o malaria; no revisit	Initial advice to donor but no further information
302327	15/02/2013	Pv	M	26	1	Born India, lived in Africa and Dubai, came to UK in 2011. Subsequently declared had malaria in June 2012	Donor contacted but no information available
312209	29/08/2013	Pv	M	22	2	Born Pakistan, came to UK in 2011. No h/o malaria, no revisit	Donor contacted but no information available

[a]Donor age at time of donation.

Uptake of specialist advice

At the time of submission, 11 of 14 donors (71%) had responded to contact. Despite numerous attempts, three donors have not responded to any means of contact. Of those responding to the initial notification of their parasitaemia, seven of 11 (64%) responded to the follow-up contact asking if specialist advice had been sought. The outcomes are presented in Table 3.

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Table 4 Malarial DNA quantitation

Sample ID	*Plasmodium* spp.	Calculated *P. falciparum* DNA level
009839	Pf	No sample available
100255	Pf	5615 iu/ml
204137	Pf/Pm	70 iu/ml
208922	Pf	70987 iu/ml

Discussion

Since the implementation of malarial DNA testing, malarial DNA has been found in 14 donations out of a total of 138,782 malaria-risk donations screened. The 14 donations were obtained from 14 different donors; thirteen of these donors had confirmed serological evidence of malaria, whilst 1 had been reported as having inconclusive malarial serology. Importantly, all 14 donors are from the 'residency' risk group, none were 'travellers' (Table 2). Three of the donors subsequently admitted having malaria in the past; in one, this was only 8 months prior to donation and, if declared at donation, would have resulted in deferral. Audit of the uptake of specialist advice showed that only 7 (50%) of the DNA-positive donors had made contact and taken up the offer of specialist referral.

These findings are significant for a number of reasons; the data serve to underline the significant risk of malaria entering the English blood supply through donations from 'at-risk' (primarily residency-risk) donors; they validate the underlying rationale for NHSBT's approach to mitigating malaria risk; the serology associated with these donations demonstrates the variability in malaria antibody assays – the need for care in assay selection and in the interpretation of their results; they highlight flaws in donor selection policies that allow the blanket reinstatement of 'residency-risk' donors without the need for malarial antibody testing.

Malaria is a problem for transfusion services in non-endemic countries whose donors either travel to or include migrants from endemic areas. UK is ethnically very diverse; migrants from most parts of the world have settled here, and many attend blood collection venues to donate. Approximately 35–45,000 blood donations per year are collected by NHSBT from malaria-risk donors and require malarial antibody screening; approximately, 20% of these donors have residency as their risk (data collected but not presented, October 2013). Additionally, migrants also donate tissues, stem cells and organs.

Strategies for dealing with malaria vary from the blanket deferral of malaria-risk donors currently applied, for example, in the United States [5], to the strategy adopted by NHSBT and other countries [1, 6–9], that of up to 6 months deferral followed by malarial antibody screening. Whatever approach is taken, however, the effective identification of malaria risk in the donor is critical. Malaria risk is a cumulative issue, and potentially significant numbers of malaria-risk donors may be unnecessarily deferred if the problem is not actively managed [5]. Our experience of fatal cases of transfusion-transmitted malaria [1] together with the significant numbers of malaria-risk donors in UK required specific action; since the implementation of the current malaria guidelines, no cases of transfusion-transmitted malaria have been identified [10].

Malarial antibody screen-reactive donations are referred to NTMRL for confirmation. Serological confirmation is undertaken using additional (confirmatory) malarial antibody immunoassays together with an in-house *P. falciparum* IFAT. The status of the donor is determined from the pattern of reactivity obtained. However, in some cases, interpretation of malaria confirmatory results can be subjective, and the donor's specific malaria risk/history may be useful in reaching a conclusion about their malaria status. Within the 27% of screen-reactive donors subsequently confirmed as having serological evidence of malaria, there will be semi-immune individuals. The inclusion of malarial DNA testing as an adjunct to our malaria confirmatory algorithm was primarily to identify semi-immune donors who may benefit from clinical review.

Although our original approach included only those donors who were IFAT reactive (titre 1:80 or higher), an error resulted in the DNA testing of an IFAT-negative donation with inconclusive malaria serology. That this donation was found to be malarial DNA positive, confirmed as *P. ovale*, was of interest and concern, and triggered review of our confirmatory malarial serology in conjunction with review of the serological findings for the DNA-positive donors. This resulted in changing the malarial DNA testing selection criteria to include all donors with any serological reactivity in the confirmatory assays.

The serology data for the 14 DNA positives (Table 2) are variable. Whilst some general patterns can be seen, none of the assays used are consistent in their ability to detect malarial antibodies, both in the detection of the four human species and the detection of all examples of the same species. Specific sensitivity evaluation of the serological assays used has been performed, and in the case of the Trinity Biotech assay published some years ago [4], it is our intention to publish our evaluation of the other assays as soon as possible.

It is unfortunate that the Bio-Rad/Diamed malarial antibody assay was withdrawn from the market, making

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the serology data set incomplete, as the Bio-Rad/Diamed results that are available indicate that the assay had the best overall sensitivity of the 'confirmatory assays', matching closely that of the Trinity Biotech screening assay. As the Trinity Biotech assay is currently the only malarial antibody assay considered suitable for the use for donation screening within the UK, and other countries, the results obtained have to be taken as the 'baseline level of performance' for malaria serology, and all other assays compared with it. The assay uses the recombinant proteins *P. falciparum* MSP 1 and *P. vivax* MSP 1 & 2, and it can be seen that it signals consistently higher with *P. vivax*, and, although in most cases giving clear-cut reactivity, variably with the other three species. The Bio-Rad/Diamed assay detected all eight of the samples tested, but the Cellabs and Diapro assays demonstrated more variable and inconsistent reactivity. It has to be concluded that the performance of the available malaria antibody assays is relatively poor, thus complicating the confirmation of malarial antibody screening reactivity. Even with a combination of serology assays, the status of a significant percentage of the screen-reactive malaria-risk donors cannot be reliably determined on the index sample. The addition of malarial DNA testing provides interesting and useful data, but the majority of these donors are not parasitaemic, and therefore, DNA testing does not help resolve inconclusive malaria serology.

As would be expected, the IFAT was strongly reactive in all 4 *P. falciparum* cases, but both of the *P. malariae* cases and two of the three *P. ovale* cases were also IFAT reactive. Looking at the risk of these donors, although there is specified cross-reactivity in the IFAT, it is also possible that the IFAT reactivity results from previous *P. falciparum* infection (either dual or sequential infection) with high-titre *P. falciparum* antibody remaining, and, except for the 1 dual infection, without any detectable parasitaemia. All three *P. malariae* cases and the 2 IFAT-positive *P. ovale* cases were from West Africa where *P. falciparum* is endemic and at a high level. The IFAT-negative *P. ovale* case is from South Africa where *P. falciparum* is not present at such a high level. All of the *P. vivax* cases were from the Indian subcontinent.

The use of malaria antigen assays to help elucidate malarial antibody screening reactivity has been reported [6, 8] and in some cases used as part of their primary donation screening strategy [11, 12], although in non-endemic countries no specific benefit has been reported. Using two different types of antigen assay, but both demonstrating a similar sensitivity using the available *P. falciparum* international standard, we failed to detect antigen in any of the 14 donations. However, when the estimated sensitivity of the malaria antigen assays is compared with the estimated malarial DNA levels in these donors, it is unsurprising that the antigen assays failed to detect; their sensitivity is at least one order of magnitude lower (10^5 iu/ml) than the highest malarial DNA level in those donations whose malarial DNA could be quantitated (7×10^4 iu/ml). This is likely to be the reason why in non-endemic countries malaria antigen assays do not generate any yield; their sensitivity is too low to detect the low-level parasitaemias in semi-immune donors, but who are no longer being constantly challenged by bites from *Plasmodium* carrying mosquitoes.

Probably, the most important outcome of this work, however, is to provide a significant body of evidence to demonstrate that malaria risk, in terms of parasitaemia, can persist for a number of years since last possible exposure, without any symptoms in the individual.

Presentation of *P. malariae* long after cessation of exposure to infection is well documented, in 1 case for at least 40 years [13], but late presentation of *P. falciparum* is less well recognized, with some authorities considering that the duration of infection does not exceed 12 months [14]. Furthermore, cases of spontaneous recovery from *P. falciparum* infection in under a year by patients undergoing malaria therapy for neurosyphilis have been detailed [14]. In contrast, D'Ortenzio [15] conducted a case–control study of prolonged *P. falciparum* infection in immigrants in Paris, similar pattern of imported malaria to that seen in London. Over a 10-year period, of 2680 diagnosed cases of *falciparum* malaria, 10 cases (five pregnant women; two HIV-positive patients; three first-arrival immigrants) presented more than 3 years after arrival. Whilst pregnancy and HIV positivity would result in donor deferral, cases in first-arrival immigrants are relevant to transfusion practice.

Theunissen *et al.* [16] reported *P. falciparum* malaria in a patient who had migrated from Guinea-Conakry to Belgium more than 9 years previously with no reported travel outside Belgium during that time. The case was reported as a possible example of suitcase malaria. A friend had visited from Guinea-Conakry 2 weeks prior to his illness and stayed in his home for 7 days, though long-term persistence of parasitaemia cannot be entirely excluded.

Howden [17] also reported a case of *P. falciparum* malaria in a patient 9 years after they migrated from a malaria-endemic (Sudan) to a non-endemic area (Victoria, Australia). The patient had not been near an airport in the preceding 6 months, making airport malaria unlikely. The patient had suffered multiple episodes of unexplained fever and led ultimately to massive splenomegaly, suggesting the case represents chronic malaria, persisting over a 9-year period.

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The last confirmed case of transfusion-transmitted malaria in UK [1] was from a previous resident still parasitaemic 8 years after last return from an endemic area and led to the development and implementation of our current strategy of requiring all donors with a residency or history of malaria risk to require malarial antibody screening at least 6 months after their last return from an endemic area, no matter how long ago that was. The data generated from this work clearly demonstrate that any donor with residency or previous diagnosis of malaria as their risk must only be allowed to donate if malaria antibody negative at least 6 months after their last return from an endemic area. Overall, the NHSBT's malaria strategy has proven to be effective both in the release to inventory of the majority of donations from malaria-risk donors, and in the identification of a population of 'malaria high-risk' semi-immune donors who demonstrate the need for such strategies. The DNA screening of malarial antibody screen-reactive donors with reactivity in the malarial antibody confirmatory assays used will continue.

The Public Health England Cryptic Malaria Guidance [18] recommends that 'where the interval between leaving a malarious area and the detection of malaria parasites exceeds 6 months for Caucasian people with *P. falciparum*, 12 months for people of other ethnic groups with *P. falciparum* (as they may be semi-immune), and 18 months for all people with other types of malaria, the case should be considered as a possible cryptic case'. From the data presented in this article, the time interval that triggers consideration as a possible cryptic case should be extended in the case of migrants from endemic areas.

The data presented in this article provide a significant body of evidence for the persistence of malaria parasitaemia in malaria-risk, but otherwise healthy, individuals. Blood transfusion services in non-endemic countries that are facing challenges in the management of malaria risk in their donor population should consider the potential for long-term low-level parasitaemia in a proportion of these donors and develop strategies that eliminate the risk of transmission from such donors, but without compromising the sufficiency of the blood supply.

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輸血感染マラリアのリスクを低減化するための供血者の問診、供血の停止・再開および製品管理に関する勧告

Ⅰ．序　　文
本手引書文書は、血液および血液成分の採血を行う血液事業者に対して、輸血感染マラリアのリスクを低減化するために、血液および血液成分の供血者への問診、供血の停止・再開、および製品管理に関するFDAの勧告を提示する。本手引書に示す勧告は、原料血漿を除く全血および血液成分の採血に適用される。また、原料血漿の供血者については、連邦規則集21、640.63(c)(9)（21CFR 640.63(c)(9)）のもとに、マラリアリスクによる供血停止の対象から除く。
本手引書は、2012年6月付け「輸血感染マラリアのリスクを低減化するための供血者の問診、供血の停止・再開および製品管理に関する勧告」と題する手引書・草案を最終とした、2013年8月付けの同一タイトルの手引書（2013年8月19日、78 FR 50421）に取って代わるものであり、1994年7月26日付の「マラリアリスクに対する供血者の供血停止に関する勧告」（1994年7月26日付メモ）（参考文献1）と題する全ての登録血液事業者へのFDAメモに取って代わるものである。
本手引書では、我々はマラリアのリスクに関する血液および血液成分の供血者スクリーニングに使用可能なものとして、AABBの供血者病歴特別委員会により準備された完全版供血者病歴問診票（2008年5月付けv.1.3）および簡易版供血者病歴問診票（2012年12月付けv.1.3）用の付属資料中に示す特定のフローチャートの改訂を認める。改訂フローチャートは2014年4月付けである。
（略）

Ⅱ．背　　景
（略）

Ⅲ．定　　義
マラリア－プラスモジウム属寄生原虫により引き起こされるる感染症。供血希望者のマラリア診断は、プラスモジウム感染を示す陽性の臨床検査に基づくか、或いは血液事業者の医長によるマラリア感染歴判定に基づいて行う。マラリアおよび関連症候に関する更なる情報については、疾病管理予防センター（CDC）のウェブサイトhttto://www.cdc.gov/malaria/を参照のこと。
マラリア流行地域－供血者スクリーニング時点において、CDCの海外旅行向け健康情報（一般にイエローブックとして知られている）の最新版に示された、CDCが旅行者に抗マラリア化学的予防を推奨するあらゆるマラリア発生地域。抗マラリア化学的予防に関する最新の推奨</td><td>使用上の注意記載状況・
その他参考事項等

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

医薬品
医薬部外品　研究報告　調査報告書
化粧品

事項については、イエローブックのマラリアの章における「国別マラリア情報」表を参照されたい。イエローブックはCDCのウェブサイト http://wwwnc.cdc.gov/travel/uage/yellowbook-2012・home.htm にて入手可能である。
マラリア流行国－供血者スクリーニング時点において、イエローブックに示されたCDCが旅行者に抗マラリア化学的予防を推奨する単一、或いは複数のマラリア発生地域を有する全ての国。いかなるマラリア流行地域であれ存在する国については、当該国全体をマラリア流行の恐れがあるものとみなす。
マラリア流行国での居住－本手引書の目的上、居住とはいかなるマラリア流行地域（上の定義を参照）であれ存在する単一、或いは複数の国に5年以上連続して滞在することと定義する。居住の判定に際し、マラリア流行国を考慮するのであって、マラリア流行地域を考慮するのではない。何故なら、居住期間中に、地理上のマラリア流行地域分布が変化する可能性や、居住者が非流行地域から流行地域に旅行していた可能性があるからである。
マラリア流行地域への旅行－CDCが規定する単一、或いは複数のマラリア流行地域（上の定義を参照）へのあらゆる旅行、または当該地域を経由するあらゆる旅行をいう。マラリア流行地域への旅行を定義する際、所要時間は、24時間を超え5年未満とする。マラリア非流行地域に向かう途中で、マラリア流行地域での滞在時間が24時間を超えた場合、曝露に十分な時間であるとみなされ、供血停止措置の原因となることに留意されたい。このような一般的な例として、マラリア流行地域を経由して観光リゾートに旅行する場合やマラリア流行地域を経由して大型客船に乗る場合、船で旅行する際にマラリア流行地域に上陸する場合などが挙げられる。マラリア流行国内の非マラリア地域への旅行または当該地域を経由する旅行については、マラリア流行地域への旅行に該当しない。

Ⅳ. 勧　　告

FDA勧告の科学的根拠および更なる説明は追補に示す。

A. 供血者病歴問診票

1. FDAは、事業者に対し、本手引書で提示した勧告内容を盛り込み、完全版問診票や簡易版問診票を含む供血者病歴問診票を改訂することを勧告する。
2. FDAは、マラリアリスクに関する供血希望者を評価するために、以下の要素を含む供血者病歴問診票を改訂することを勧告する（本手引書の第Ⅲ章の定義に留意すること）。
   a. この3年間のマラリア罹患歴；
   b. マラリア流行国での居住歴；
   c. この1年間のマラリア流行地域への渡航歴；
   d. この3年間のマラリア流行地域への渡航歴（マラリア流行国居住歴を有する場合）

B. 供血の停止および再開

1. **マラリア罹患歴**
   a. マラリア罹患歴を有する供血者については、3年間供血を停止することを勧告する。
   b. 当該供血者が非流行国に居住している間、3年間マラリアの症状が認められていない場合は、その他の供血者適格基準に全て合致していれば、医長は当該供血者を受け入れるよう判定することができる。
2. **マラリア流行国での居住**
   マラリア流行国（本手引書第Ⅲ章に定義参照）での居住歴を有する供血者については、3年間供血を停止することを勧告する。3年間の停止期間中にマラリアの症状を呈することなく、他の供血適格性基準に全て合致している場合、停止期間後は供血適格とすることができる。
3. **マラリア流行地域への旅行**
   a. マラリア非流行国に居住し、マラリア流行地域（本手引書の第Ⅲ章の定義参照）に旅行、または、当該地域を経由し旅行した供血者については、当人がマラリア化学的予防措置を受けていたか否かに拘わらず、当該地域からの最終出発時から1年間、供血を停止することを勧告する。1年間の停止期間中マラリアの症状を呈することなく、他の供血適格性基準に全て合致している場合、停止期間後は供血適格とすることができる。

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

b. マラリア流行国（本手引書の第Ⅲ章の定義参照）に居住歴があり、マラリア非流行国での連続居住期間が3年未満の供血者については、マラリア流行地域に旅行後3年間は供血を停止することを勧告する。3年間の停止期間中マラリアの症状を呈することなく、他の供血適格性基準に全て合致している場合、停止期間後は供血適格とすることができる。

c. マラリア流行国に居住歴があり、マラリア非流行国で3年間連続して居住した後にマラリア流行地域に戻った場合、当該供血者については、非流行国に帰国後1年間は供血を停止することを勧告する。1年間の停止期間中マラリアの症状を呈することなく、他の供血適格性基準に全て合致している場合、停止期間後は供血適格とすることができる。

C. 製品の回収、隔離保管および血液、血液成分の出荷先に対する通知

本手引書の第Ⅳ章Bの勧告に従い血液、或いは血液成分が供血停止すべきであった供血者から採血したと判断した場合、下記の措置を講じることを勧告する。

1. 本手引書の第Ⅳ章Bの勧告に従い供血停止すべきであった供血者から輸血用、或いは製造用として細胞血液成分を採血した場合、当該供血者から採血した出荷前かつ有効期限内のいかなる細胞血液成分も隔離保管することを勧告する。
2. 本手引書の第Ⅳ章B.1の勧告に従い供血停止すべきであったマラリア臨床歴を有する供血者から輸血用、或いは製造用として採血した細胞血液成分を出荷した場合、出荷先に対して当該供血者からの有効期限内細胞血液成分を回収し、隔離保管するよう通知することを勧告する。更に、当該状況下で細胞血液成分が既に輸血されてしまった場合、出荷先に対して、受血者の主治医に輸血後3か月間マラリア感染のモニタリングが必要である旨の経緯を知らせるよう依頼すること。
3. 本手引書第Ⅳ章B.2またはB.3の勧告に従いマラリアリスクのある渡航歴、或いは居住歴を有するために供血停止すべきであった供血者から輸血用として採血した細胞血液成分を出荷した場合、出荷先に対して当該供血者からの有効期限内細胞血液成分を回収し、隔離保管するよう通知することを勧告する。
4. 本手引書の第Ⅳ章Bの勧告に従い供血停止すべきであった供血者から輸血用、或いは製造用として無細胞性血液成分（即ち、凍結血漿製剤）を採血した場合、当該供血者から採血した出荷前の有効期限内当該無細胞性血液成分を隔離保管することを勧告する。（マラリア感染リスクがきわめて低いことを踏まえて、当該無細胞性製品を出荷した場合、出荷先に対する通知は勧告していないことに留意されたい）。

D. 製品の廃棄およびラベル表示

1. 本手引書の第Ⅳ章Bの勧告に従い供血停止すべきであった供血者から採血した細胞血液成分については廃棄、或いはラベル再表示を勧告する。細胞血液成分を再表示する場合、当該血液細胞成分は、本手引書の第Ⅳ章D.3に示すように、研究用、または非注射製剤製造用、または生体外診断用試薬用原料として使用することができる。
2. 本手引書の第Ⅳ章Bの勧告に従い供血停止すべきであった供血者から意図せず採血した無細胞性血液成分は、輸血用としては不適であるが、以下のように適切な表示をすれば、研究用、または注射製剤（即ち、血漿分画製剤）や非注射製剤の製造用、または生体外診断用試薬原料として使用することができる。
3. 血液成分のラベル再表示を迅速に行うため以下の文言を用いること。
   a. 「輸血不可：マラリア原虫感染リスクがあると判定された供血者から採血した」　および
   b. 「注意：研究用としてのみ使用」　または
      「注意：代替原料がない生体外診断用試薬の原料用」　または
      「注意：非注射製剤製造用のみ」　または
      「注意：『製造用としてのみ使用』（注射用無細胞性製剤原料として製造に用いる）」

特にFDAが認めない限り、当該製品には米国承認番号を表示しないこと。また、短期供給契約を条件に、適宜、当該未承認製剤を単体であれば製造業者に送付することができる（連邦規則21 601.22）。

E. 生物学的製剤逸脱（BPD）の報告

本手引書第IV章Bに示したマラリア感染リスクのある供血者から採血した輸血用、或いは製造用の血液細胞成分を出荷した場合、可及的速やかに生物学的製剤逸脱（BPD）報告を提出するものとし、報告すべき事象が発生したことを合理的に示唆する情報を得た日から45暦日以内に提出しなければならない（連邦規則21 606.171）。
マラリア感染リスクのある供血者からの輸血用、或いは製造原料用の無細胞性血液成分を出荷した場合は、BPD報告を提出することは求められない。

V. 更なる考察
輸血用の全血および血液成分は、製造原料に転用すること、或いはラベル再表示することを目的として、マラリア感染リスクのある供血者から採血すべきではない（例：新鮮凍結血漿を転用血漿として再表示）。

VI. 改訂供血者病歴問診票（DHQ）文書の承認
A. 改訂DHQ文書の承認
AABBの供血者病歴専門作業委員会は以下の質問のためのフローチャートを改訂した。
- 完全版供血者病歴問診票（2008年5月付けv. 1. 3）
  - o この3年間、米国、或いはカナダ外に出かけたことがありますか？
  - o あなたはこれまでにマラリアに罹ったことがありますか？
- 簡易版供血者病歴問診票（2012年12月付けv. 1. 3.）
  - o 最後の供血以降、米国、或いはカナダ外に出かけたことがありますか？

FDAは改訂されたAABB DHQフローチャートが、本手引書に示す勧告に則った献血者スクリーニング用に使用可能であることを認めている。
FDAはAABB作業委員会作成のDHQ文書が容認可能であることを認識しているので、血液事業者はそれらを実施する必要はない。FDAによって製造販売承認を受けている血液事業者に対して、本手引書に含まれる勧告を反映し、確立している過去のドナー病歴問診票や付随した項目の情報も使用できる。血液事業者の付属資料は、AABB DHQ文書中のそれらと異なる手順と言い回しを含むかもしれない。

B. 容認できるDHQ文書の実施
本手引書のVI章Aに記載した改訂DHQフローチャートの正しい実行を確実にするために、FDAは以下に記す手順の使用を勧告する：
- 完全版DHQ（2014年4月付けv. 1. 3）用新フローチャートの実施
  - o フローチャート（2008年5月付け v. 1. 3）を改訂フローチャート（2014年4月付けv. 1. 3）に代える
- 簡易版DHQ（2014年4月付けv. 1. 3）用新フローチャートの実施
  - o 余分な質問のために確保した余白に完全版DHQから簡易版DHQまで次の質問を追加する：
    - この3年間、米国、或いはカナダ外に出かけたことがありますか？
  - o 血液事業者の手順において完全版DHQからこの質問のための2014年4月付けv. 1. 3を含む
  - o これらの手順を実施する時点から1年間、簡易版DHQに関するこの質問と対応するフローチャートを維持する。1年後、簡易版DHQからこの質問と対応するフローチャートを除去することができる。
  - o 同時に、簡易版DHQに関するこの現在の質問を維持する：
    - 最後の供血以降、米国、或いはカナダ外に出かけたことがありますか？
  - o この質問に関する2012年12月付け v. 1. 3のフローチャートを同じ質問に関する簡易版DHQからの2014年4月付け v. 1. 3のフローチャートに代える。
  - o 完全版DHQからの質問およびフローチャートを含む1年の間、簡易版DHQの改訂フローチャートを実施するための次の方法の一つを使うことができる。

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

- 全体的に簡易版DHQ用の2014年4月付けv.1.3フローチャートを使用、または
- マラリア流行国や流行地域への旅行に関する供血者を評価する簡易版DHQ用フローチャートの一部をスキップし、HIV-1群Oおよびv CJD流行国への旅行を評価するフローチャートの項へ進む

o 1年経過後、血液事業者がまだ準備出来ていない場合は、次のフローチャート（簡易版DHQの2014年4月付けの改訂v.1.3）を使用する。

Ⅶ. 勧告の実施

供血者供血停止に関する新勧告を反映させ、完全版問診票や簡易版問診票を含む事業者の供血者病歴問診票（DHQ）、さらに必要に応じて付属資料を改訂したうえで、本手引書に示す勧告を実施すること。

認可血液事業者は以下の要領で変更箇所をFDAに報告しなければならない。

1. 事業者独自のDHQ、および添付資料の改訂：新勧告を履行するために事業者独自のDHQ、および添付資料を改訂する場合、重大な変更として報告すること。このような変更については、FDAに連邦規則21 601.12(b) に基づき、事前承認追加申請（PAS）として提出すること。
2. FDA了解済のDHQ、および添付資料の改訂：新勧告を履行するためにFDA了解済のDHQ、および添付資料を改訂する場合、重大な変更として報告すること。このような変更については、FDAに連邦規則21 601.12(b) に基づき、事前承認追加申請（PAS）として提出すること。
3. 2014年4月付け改訂フローチャートv.1.3と共に完全版DHQ（2008年3月付けv.1.3）および簡易版DHQ（2012年12月付けv.1.3）の使用
   a. 改訂フローチャートが本手引書のⅥ章Bの手順を用いて変更なしで、全体的に履行される場合、変更は軽微であると考える。このような軽微な変更については、連邦規則21601.12(d) に基づき、年次報告によりFDAに報告すること。報告の際、手順が履行された日時を記載すること。
   b. 改訂フローチャートの形式に変更を加えることなく、内容の整合性が保たれたまま、或いはより厳格に供血停止基準を採用している場合、変更は軽微と考える。このような軽微な変更については、連邦規則21601.12(d) に基づき、年次報告によりFDAに報告すること。報告の際、手順が履行された日時を記載し、好ましいフローチャートをどの様に変更したか説明すること。
   c. 改訂フローチャートが書式設定以外の変更をして履行される場合、変更は重大であると考える。このような重大な変更については、連邦規則21601.12(d) に基づき、事前承認追加申請（PAS）としてFDAに報告すること。
   d. 改訂フローチャートがⅥ章Bとは異なる手順を使用して履行された場合、変更が重大であると考える。このような重大な変更については、連邦規則21601.12(d) に基づき、事前承認追加申請（PAS）としてFDAに報告すること。

Ⅷ. 参考文献

（省略）

報告企業の意見	今後の対応
マラリア原虫（plasmodium）は、アピコンプレックス門・胞子虫綱・コクシジウム亜綱・真コクシジウム目・住血胞子虫亜目に属する一群の単細胞動物（原生動物）で、大きさは2-3μmの卵型である。万一、原料血漿にマラリア原虫が混入したとしても、除菌ろ過等の製造工程にて除去されるものと考えている。	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。

JBPO 2014-014

Guidance for Industry

Recommendations for Donor Questioning, Deferral, Reentry and Product Management to Reduce the Risk of Transfusion-Transmitted Malaria

This guidance is for immediate implementation.

FDA is issuing this guidance for immediate implementation in accordance with 21 CFR 10.115(g)(4)(i). Submit one set of either electronic or written comments on this guidance at anytime. Submit electronic comments to http://www.regulations.gov. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. You should identify all comments with docket number [Docket No. FDA-2000-D-0187 (formerly Docket No. 2000-D-1267)].

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD), 10903 New Hampshire Ave., Bldg. 71, Rm. 3128, Silver Spring, MD 20993-0002, or by calling 1-800-835-4709 or 240-402-7800, or email ocod@fda.hhs.gov, or from the Internet at http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

For questions on the content of this guidance, contact OCOD at the phone numbers or email address listed above.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
August 2013
Updated August 2014

Guidance for Industry

Recommendations for Donor Questioning, Deferral, Reentry and Product Management to Reduce the Risk of Transfusion-Transmitted Malaria

Note: Changes have been made to update the "Guidance for Industry: Recommendations for Donor Questioning, Deferral, Reentry and Product Management to Reduce the Risk of Transfusion-Transmitted Malaria" dated August 2013, including:

- Updates to recognize revisions to certain flow charts contained in the accompanying materials for the Full-Length Donor History Questionnaire (v.1.3 dated May 2008) and Abbreviated Donor History Questionnaire (v.1.3 dated December 2012) prepared by the AABB Donor History Task Force, as acceptable for use in screening donors of blood and blood components for risk of malaria (see new section VI).
- Revised recommendations in section VII on how licensed establishments must report the implementation of the recommendations contained herein to FDA.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
August 2013
Updated August 2014

Table of Contents

I. INTRODUCTION 1
II. BACKGROUND 2
III. DEFINITIONS 4
IV. RECOMMENDATIONS 5
A. Donor History Questionnaire 5
B. Donor Deferral and Reentry 5
C. Product Retrieval and Quarantine, and Notification of Consignees of Blood and Blood Components 6
D. Product Disposition and Labeling 7
E. Reporting a Biological Product Deviation (BPD) 8
V. ADDITIONAL CONSIDERATIONS 8
VI. RECOGNITION OF THE REVISED DONOR HISTORY QUESTIONNAIRE (DHQ) DOCUMENTS 8
A. Recognition of the Revised DHQ Documents 8
B. Implementation of the Acceptable DHQ Documents 9
VII. IMPLEMENTATION OF RECOMMENDATIONS 10
VIII. REFERENCES 12

Contains Nonbinding Recommendations

Guidance for Industry

Recommendations for Donor Questioning, Deferral, Reentry and Product Management to Reduce the Risk of Transfusion-Transmitted Malaria

This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the appropriate FDA staff. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

This guidance document provides you, blood establishments that collect blood and blood components, with our, FDA's, recommendations for questioning and deferring donors of blood and blood components, allowing their reentry, and product management to reduce the risk of transfusion-transmitted malaria. The recommendations contained in this guidance apply to the collection of Whole Blood and all blood components with the exception of Source Plasma. Donors of Source Plasma are excluded from deferral due to malaria risk under Title 21 of the Code of Federal Regulations 640.63(c)(9) (21 CFR 640.63(c)(9)).

This guidance supersedes the guidance of the same title dated August 2013 (78 FR 50421, August 19, 2013), which in turn finalized the draft guidance entitled "Recommendations for Donor Questioning, Deferral, Reentry and Product Management to Reduce the Risk of Transfusion-Transmitted Malaria" dated June 2012, and superseded the FDA memorandum to all registered blood establishments entitled "Recommendations for Deferral of Donors for Malaria Risk" dated July 26, 1994 (July 26, 1994 memorandum) (Ref. 1).

In this guidance, we recognize revisions to certain flow charts contained in the accompanying materials for the Full-Length Donor History Questionnaire (v.1.3 dated May 2008) and Abbreviated Donor History Questionnaire (v.1.3 dated December 2012) prepared by the AABB Donor History Task Force, as acceptable for use in screening donors of blood and blood components for risk of malaria. The revised flow charts are dated April 2014.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidance means that something is suggested or recommended, but not required.

II. BACKGROUND

Transfusion-transmitted malaria occurs rarely, but is a serious concern in transfusion medicine (Refs. 2, 3). It has been shown to be caused by any of the following four *Plasmodium* species: *P. falciparum; P. malariae; P. ovale;* or *P. vivax*. In the absence of a licensed test for donor screening, the measure used to reduce transfusion-transmitted malaria in the United States (U.S.) has been the deferral of donors who have had a malaria infection or had a possible exposure risk to malaria. Accurate identification of donors with the potential to transmit malaria depends on the donor exposure history obtained during the donor interview, which may be facilitated through use of a donor questionnaire (Refs. 4-6).

The July 26, 1994 memorandum had the following recommendations:

- Permanent residents of non-endemic countries who travel to an area considered endemic for malaria should not be accepted as donors of Whole Blood and blood components prior to one year after departure from the endemic area. After one year after departure, such otherwise suitable prospective donors may be accepted provided that they have been free of unexplained symptoms suggestive of malaria.
- Prospective donors who have had malaria should be deferred for three years after becoming asymptomatic.
- Citizens, residents, immigrants or refugees of endemic countries should not be accepted as donors of Whole Blood and blood components prior to three years after departure from the area. After the 3-year period, otherwise suitable prospective donors may be accepted if they have remained free of unexplained symptoms suggestive of malaria.

Public comments on the July 26, 1994 memorandum and the June 2000 draft guidance on screening of donors for malaria risk raised several concerns about the need to standardize definitions used in the recommendations, and the scientific basis for the recommended deferral periods. These concerns prompted public discussions, including a meeting of the FDA Blood Products Advisory Committee (BPAC or Committee) on September 16, 1999. At that meeting, BPAC reviewed the current status of transfusion-transmitted malaria and its impact on blood safety in the United States BPAC also reviewed the usefulness of the available laboratory test methods to detect current malaria infection or to provide evidence of past exposure to malaria parasites.

On July 12, 2006, FDA convened a scientific workshop entitled "Testing for Malarial Infections in Blood Donors" to seek public discussion of scientific developments that might support donor testing for malaria infections as part of pre-donation testing, or as follow-up testing to permit a reduced deferral period for donors deferred for malaria risk (Ref. 7). There are no FDA-licensed tests to screen blood donors for malaria. Nucleic acid-based tests were deemed unsuitable for donor screening due to the limitation of the small sample size used in nucleic acid extraction; however, several speakers and panel members emphasized the value of antibody testing to reenter deferred malaria-risk donors who tested negative for malarial antibodies (Refs. 7, 8). The outcome of the workshop was summarized at the BPAC meeting held on July 13, 2006 (Ref. 9).

At the BPAC meeting on September 11, 2008, the Committee discussed donor testing for malarial antibodies as an indicator of possible exposure to malaria parasites (Ref. 10). At the meeting, FDA presented risk assessment data for three possible scenarios in which antibody testing could be of value: (1) testing all donors (universal testing); (2) reentry testing of all at-risk donors with a history of potential exposure to malaria anywhere in the world; and (3) reentry testing of only those donors who had traveled to malaria-endemic areas in Mexico. The risk assessment model assumed that donors would be deferred for four months after returning from endemic areas of Mexico or other parts of the world before antibody testing would be performed on the donor. At the meeting, two blood organizations (the American Red Cross and America's Blood Centers) also presented data from surveys showing that approximately 41% of all blood donors deferred for risk of malaria exposure had been deferred because they had traveled to malaria-endemic areas in Mexico (Refs. 10, 11). The Committee considered all three risk assessment scenarios and the possible role that antibody testing could play in identifying or reentering malaria-risk donors, especially those donors who had traveled to endemic areas in Mexico. In the end, the Committee felt that additional risk analysis would be needed, and that the analysis should account for malaria risk globally and in Mexico, with and without antibody testing.

On November 16, 2009, FDA again sought advice from BPAC on an alternative strategy to minimize donor loss associated with deferrals for malaria risk. Specifically, FDA asked the Committee to consider a new risk assessment model which was focused on travel to malaria-endemic states in Mexico, and asked whether it was acceptable to allow blood collections without any deferral from individuals who have traveled to certain Mexican states that have a low malaria transmission rate. At that meeting, FDA presented data which showed that while travel to Mexico was a major contributor to donor deferrals due to malaria risk (about 41%), from 2006-2009, malaria transmission in Mexico was shown to be very low (average 2400 malaria cases annually) and limited only to certain Mexican states (Ref. 12). The malaria transmission rate was shown to be particularly low in Quintana Roo, a Mexican state that includes Cancun and Cozumel and is known to receive a high volume of U.S. travelers. Estimates also suggested that there was a great disparity in the contribution of different Mexican states to the number of donor deferrals among U.S. travelers. Data collected by the American Red Cross and Blood Systems Research Institute suggested that in 2006, among the 10 malaria-endemic states, Quintana Roo alone contributed approximately 70% of all malaria-risk-associated donor deferrals for travel to Mexico (Refs. 12, 13). While donors deferred because of travel to Quintana Roo were a significant percentage of deferrals, FDA's risk assessment found that the calculated overall risk to the blood supply would be expected to increase by 1.1% (an absolute increase of 0.0166 infected blood unit per year, or one in 60 years) if prospective blood donors who visited Quintana Roo and another state, Jalisco, which includes the cities of Puerto Vallarta and Guadalajara, were allowed to donate blood without any deferral for malaria risk. However, the donor pool would increase by approximately 45,000 donors (79,000 blood units) each year (Ref. 13). FDA also found that the actual donor gain might be significantly higher if the Agency took into account the total donor loss due to self-deferrals and the non-return of donors deferred under the current policy (Ref. 7). After these presentations and discussion, the Committee voted 17-1 in favor of allowing blood collection, without any deferral for malaria

risk, from U.S. residents who have visited Quintana Roo. The Committee also discussed extending the proposed policy to other malaria-endemic states of Mexico that have a low malaria transmission rate.

III. DEFINITIONS

Malaria - An infectious disease caused by a parasitic protozoan of the genus *Plasmodium*. Malaria diagnosis in a prospective donor is based on a positive laboratory test indicating *Plasmodium* infection, or a determination of a history of malaria made by the blood establishment's Medical Director. For additional information regarding malaria and its associated symptoms, visit the Centers for Disease Control and Prevention (CDC) website at http://www.cdc.gov/malaria/.

Malaria-endemic area - Any areas with malaria where CDC recommends anti-malarial chemoprophylaxis in travelers in the most current version of the *CDC Health Information for International Travel* (commonly known as *The Yellow Book*) at the time the donor is screened. We recommend you access the "Malaria Information, by Country" table in the Malaria chapter of *The Yellow Book* for the most current recommendations on anti-malarial chemoprophylaxis. *The Yellow Book* is available on the CDC website at http://wwwnc.cdc.gov/travel/page/yellowbook-2012-home.htm.

Malaria-endemic country - Any country having an area or areas with malaria where CDC recommends anti-malarial chemoprophylaxis in travelers in *The Yellow Book* at the time the donor is screened. A country that has any malaria-endemic areas should be considered to be malaria-endemic in its entirety.

Residence in a malaria-endemic country - For purposes of this guidance, residence is defined as a continuous stay of longer than 5 years in a country or countries having any malaria-endemic area (see definition above). In determining residence, consideration is by malaria-endemic country and not by malaria-endemic area since the geographic distribution of malaria-endemic areas may change during the period of residence, or the resident may have traveled from a non-endemic area to an endemic area in the country during his or her stay.

Travel to a malaria-endemic area - Any travel to or through a malaria-endemic area or areas, as identified by CDC (see definition above). The duration of travel to a malaria-endemic area is defined as more than 24 hours to less than 5 years. Note that a passage greater than 24 hours through a malaria-endemic area while on route to a malaria-free area is considered a sufficient possible exposure to trigger donor deferral. Common examples of such possible exposure include passage through a malaria-endemic area to visit a tourist resort in a malaria-free area, or passage through a malaria-endemic area to board a cruise ship, or on-shore excursions into a malaria-endemic area when traveling on a ship. Travel to or through a malaria-free area within a malaria-endemic country does not constitute travel to a malaria-endemic area.

IV. RECOMMENDATIONS

FDA's scientific rationale and further explanation for our recommendations are provided in the Appendix.

A. Donor History Questionnaire

1. We recommend that you update your donor history questionnaire, including full length and abbreviated donor history questionnaires, to incorporate the recommendations provided in this guidance.

2. We recommend that the updated donor history questionnaire include the following elements to assess prospective donors for malaria risk (note definitions in section III of this guidance):

    a. A history of malaria in the past three years;

    b. A history of prior residence in a malaria-endemic country;

    c. A history of travel to a malaria-endemic area in the past one year; and

    d. A history of travel to a malaria-endemic area in the past three years, if previously a resident of a malaria-endemic country.

B. Donor Deferral and Reentry[1]

1. History of Malaria

    a. We recommend that you defer for 3 years a donor who has a history of malaria.

    b. If that donor has remained free of malaria symptoms for a 3-year period while residing in a non-endemic country, the Medical Director may decide to accept the donor, provided the donor meets all other donor eligibility criteria.

2. Residence in a Malaria-endemic Country

We recommend that you defer a donor for 3 years who had been a prior resident (as defined in section III of this guidance) in a malaria-endemic country. After the 3-year deferral period, the donor may be eligible to donate provided the donor has been free from malaria during this period and meets all other donor eligibility criteria.

[1] See Appendix for detailed scientific rationale for the recommendations contained in this guidance.

3. Travel to a Malaria-endemic Area

   a. We recommend that you defer for 1 year after the last departure from a malaria-endemic area (as defined in section III of this guidance) a donor who is a resident of a non-endemic country and who has traveled to or through any malaria-endemic area, whether or not the donor has received malaria chemoprophylaxis. After the 1-year deferral period, the donor may be eligible to donate, provided the donor has been free from malaria during this period and meets all other donor eligibility criteria.

   b. We recommend that you defer for 3 years after a visit to a malaria-endemic area a donor who is a prior resident of a malaria-endemic country (as defined in section III of this guidance) and who has been a resident of non-endemic countries for less than 3 consecutive years. After the 3-year deferral period, the donor may be eligible to donate, provided the donor has been free from malaria during this period and meets all other donor eligibility criteria.

   c. We recommend that if a prior resident of a malaria-endemic country returns to a malaria-endemic area after residence for 3 years consecutively in non-endemic countries, that you defer that donor for 1 year from the time that they return to the non-endemic country. After the 1-year deferral period, the donor may be eligible to donate, provided the donor has been free from malaria during this period and meets all other donor eligibility criteria.

C. Product Retrieval and Quarantine, and Notification of Consignees of Blood and Blood Components

We recommend that you take the following actions if you determine that blood or blood components have been collected from a donor who should have been deferred according to the recommendations in section IV.B of this guidance.

1. If you collected cellular blood components intended for transfusion or for further manufacturing from a donor who should have been deferred according to the recommendations in section IV.B. of this guidance, we recommend that you quarantine any undistributed in-date cellular blood components collected from that donor.

2. If you distributed cellular blood components intended for transfusion or for further manufacturing collected from a donor with a clinical history of malaria who should have been deferred according to the recommendation in section IV.B.1. of this guidance, we recommend that you notify consignees to retrieve and quarantine the in-date cellular blood components collected from that donor.

Additionally, in this situation, if cellular blood components have been transfused, you should encourage consignees to notify the transfusion recipient's physician of record regarding the need for monitoring of the recipient for a possible malaria infection for a period of 3 months post-transfusion.

3. If you distributed cellular blood components intended for transfusion collected from a donor who should have been deferred for malaria-risk associated with travel or prior residence according to recommendations in sections IV.B.2 or 3 of this guidance, we recommend that you notify consignees to retrieve and quarantine the in-date cellular blood components collected from that donor.

4. If you collected acellular blood components (i.e., frozen plasma products) intended for transfusion or for further manufacturing from a donor who should have been deferred according to the recommendations in section IV.B. of this guidance, we recommend that you quarantine any undistributed in-date acellular blood components collected from that donor. (Note that based on the very low risk for transmission of malaria, we are not recommending notification of consignees if you distributed such acellular products.)

D. Product Disposition and Labeling

1. We recommend that you destroy or relabel cellular blood components that were collected from a donor who should have been deferred according to the recommendations in section IV.B of this guidance. If you relabel the cellular blood components, they may be released for research, or for manufacture into noninjectable products or in vitro diagnostic reagents as described in section IV.D.3. of this guidance.

2. Although not suitable for transfusion, acellular blood components inadvertently collected from a donor who should have been deferred according to the recommendations in section IV.B. of this guidance may be released for research, or for further manufacture into injectable (i.e., plasma derivative) or non-injectable products, or in vitro diagnostic reagents, if labeled appropriately as described below.

3. You should use the following statements to prominently relabel the blood components:

   a. "NOT FOR TRANSFUSION: Collected From A Donor Determined To Be At Risk For Infection With Malaria Parasites"

   and

   b. "Caution: For Laboratory Research Only"

   or

"Caution: For Further Manufacturing into *In Vitro* Diagnostic Reagents For Which There Are No Alternative Sources"

or

"Caution: For Use in Manufacturing Noninjectable Products Only"

or

"Caution: "For Manufacturing Use Only" (used for acellular products intended for further manufacture into injectable products).

You should not label these products with a U.S. license number unless FDA specifically approves you to do so. If appropriate, unlicensed products may be shipped solely to a manufacturer of a product subject to licensure, under a short supply agreement (21 CFR 601.22).

E. Reporting a Biological Product Deviation (BPD)

If you have distributed any cellular blood components for transfusion or for further manufacturing, collected from a donor at risk for malaria according to section IV.B. of this guidance, you should report a BPD as soon as possible, but you must report within 45 calendar days from the date you acquire the information reasonably suggesting that a reportable event has occurred (21 CFR 606.171).

You are not required to report a BPD if you have distributed an acellular blood component intended for transfusion or further manufacturing from a donor at risk for malaria.

V. ADDITIONAL CONSIDERATIONS

Whole Blood and blood components intended for transfusion should not be collected from a possible malaria risk donor with the intent of converting or relabeling those products for further manufacturing use (e.g., relabeling of Fresh Frozen Plasma as recovered plasma).

VI. RECOGNITION OF THE REVISED DONOR HISTORY QUESTIONNAIRE (DHQ) DOCUMENTS

A. Recognition of the Revised DHQ Documents

The AABB Donor History Task Force has revised the flow charts for the following questions:

- The Full-Length Donor History Questionnaire, v.1.3 dated May 2008
  - In the past 3 years have been outside the United States or Canada?
  - Have you ever had malaria?

- The Abbreviated Donor History Questionnaire, v.1.3 dated December 2012
  - Since your last donation have you been outside the United States or Canada?

FDA finds the revised AABB DHQ flow charts for the questions above (v.1.3 dated April 2014) to be acceptable for use in screening blood donors consistent with the recommendations contained in this guidance.[2]

While we recognize the DHQ documents prepared by the AABB Task Force as acceptable, you are not required to implement them. You may continue to use any donor history questionnaire and accompanying materials developed by your establishment that have been revised to reflect the recommendations contained in this guidance and, for licensed blood establishments, have been approved by FDA. Your materials may include procedures and wording that are different from those in the AABB DHQ documents.

B. Implementation of the Acceptable DHQ Documents

To ensure the correct implementation of the revised DHQ flow charts described in section VI. A of this guidance), we recommend you use the process described below:

- Implementing the new flow charts for the full-length DHQ, v.1.3 dated April 2014:
  - Replace the flow charts v.1.3 dated May 2008 with the revised flow charts v.1.3 dated April 2014

- Implementing the new flow chart for the abbreviated DHQ, v.1.3 dated April 2014:
  - Add the following question from the full-length DHQ to the abbreviated DHQ in the space reserved for extra questions:
    - In the past 3 years have you been outside the United States or Canada?
  - Include the v.1.3 dated April 2014 flow chart for this question from the full-length DHQ in your procedures.

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[2] You may view v.1.3 of the DHQ documents prepared by AABB, including the revised flow charts, on the FDA website at http://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/LicensedProductsBLAs/BloodDonorScreening/ucm164185.htm. On this website, you may also view the FDA guidance documents that recognized v.1.3 of the full-length and abbreviated DHQs and accompanying materials.

- Retain this question and corresponding flow chart on the abbreviated DHQ for 1 year from the time that you implement these procedures. After 1 year, you may remove this question and the corresponding flow chart from the abbreviated DHQ.

- Concurrently, retain this current question on the abbreviated DHQ:

    - Since your last donation have you been outside the United States or Canada?

- Replace the flow chart dated v.1.3 December 2012 for this question with the v.1.3 dated April 2014 flow chart from the abbreviated DHQ for same question.

- For the one year that you are including the question and flow chart from the full-length DHQ, you may use one of the following methods to implement the revised flowchart for the abbreviated DHQ:

    - Use the v.1.3 dated April 2014 flow chart for the abbreviated DHQ in its entirety, or

    - Skip the part of the flow chart for the abbreviated DHQ that evaluates the donor for travel to malaria-endemic countries and areas and proceed to the sections on the flow chart for evaluating travel to HIV-1 Group O and vCJD endemic countries.

- At the end of the 1 year period, if you are not already doing so, use the revised v.1.3 dated April 2014 flow chart for the abbreviated DHQ in its entirety.

VII. IMPLEMENTATION OF RECOMMENDATIONS

You may implement the recommendations contained in this guidance once you have revised your donor history questionnaire (DHQ), including full-length and abbreviated DHQs, and accompanying materials as necessary to reflect the new donor deferral recommendations.

Licensed blood establishments must report the changes to FDA in the following manner:

1. Revision of your own DHQ and accompanying materials: report as a major change if revising your own DHQ and accompanying materials to implement the new recommendations. Report such a change to FDA as a prior approval supplement (PAS) under 21 CFR 601.12(b).

2. Revision of a previously FDA accepted DHQ and accompanying materials: report as a major change if you are revising the FDA accepted DHQ and accompanying materials to implement these new recommendations. Report such a change to FDA as a prior approval supplement (PAS) under 21 CFR 601.12(b).

3. Use of full-length DHQ (v.1.3 dated May 2008) and abbreviated DHQ (v.1.3 dated December 2012) with revised flow charts v.1.3 dated April 2014:

   a. If the revised flow charts are implemented without modifications and in their entirety using the process in section VI. B of this guidance, the change is considered to be minor. You must report such changes to FDA in your annual report consistent with 21 CFR 601.12(d), noting the date the process was implemented.

   b. If you make changes to the format of the revised flow charts but the content remains consistent, or you adopt stricter donor deferral criteria, the changes are considered minor. You must report such changes to FDA in your annual report under 21 CFR 601.12(d), noting the date the process was implemented and describing how you modified the acceptable flow charts.

   c. If the revised flow charts are implemented with modifications other than formatting, the change is considered to be major. You must report such changes as a prior approval supplement (PAS) consistent with 21 CFR 601.12(b).

   d. If the revised flow charts are implemented using a process that differs from that in section VI.B, the change is considered to be major. You must report such changes as a prior approval supplement (PAS) consistent with 21 CFR 601.12(b).

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APPENDIX

SCIENTIFIC RATIONALE AND FURTHER EXPLANATION FOR THE RECOMMENDATIONS

The scientific basis and further explanation for the recommendations in section IV of this guidance are as follows:

- The recommendation for a 3-year deferral of a donor following residence in a malaria-endemic country (recommendations B.2. and B.3.b.) is based on the possible presence of low-grade parasitemia in individuals with clinical immunity to malaria, or with a chronic malaria infection who have not received definitive treatment after departure from the malaria-endemic area. Although it is not known how long parasitemia can last in such persons, it is believed that most (though not all) will either develop clinical malaria or else resolve their infection over time. This is because anti-malarial immunity is thought to wane in the absence of repeated infections. Data reported by CDC showed that out of 4,229 reported cases of malaria in foreign-born residents, only 7 cases (0.2%) had an episode of clinical malaria more than three years after the patient had left a malaria-endemic country (Ref. 3). These data suggest that a deferral period of 3 years would be adequate for resolution of parasitemia in most cases. This recommendation will be reconsidered periodically based on new scientific data.

- Recommendation B.3.a of a 1-year deferral period for a donor who is a resident of a non-endemic country and who has traveled to or through a malaria-endemic area (whether or not the donor received malaria prophylaxis), is based on the malaria surveillance reports by CDC showing that out of 2,167 imported malaria cases reported between 2008-2010 for which the date of arrival and the onset of illness was known, only 2 (0.09%) experienced clinical malaria more than 1 year after their return to the U.S. (Refs. 14-16). The 1-year deferral for residents of non-endemic countries applies to the last departure from the endemic area.

- Blood centers should use the new definition of malaria-endemic area (see section III of this guidance) in deciding whether a donor had traveled to a malaria-endemic area.

Based on the current epidemiological data and the definition of malaria-endemic area in this guidance, FDA does not currently recommend deferral of donors who have traveled to the Mexican states of Quintana Roo and Jalisco; thus, these donors, if otherwise eligible, may donate. Please note that the designation of malaria-endemic areas in Mexico or in any malaria-endemic country and accordingly, a recommendation for donor deferral, are subject to change based on the most updated malaria transmission information with respect to that area, as listed in *The Yellow Book.* For example, if malaria transmission in these states changes and anti-malarial chemoprophylaxis is recommended by CDC, then the donor deferral recommendations would encompass donors who travel to these areas.

- The recommendation for a one year deferral from the time of return to a non-endemic country of a donor who was a prior resident of a malaria-endemic country and who

had not traveled to a malaria-endemic area for 3 consecutive years preceding the most recent travel to a malaria-endemic area (recommendation B.3.c.) is based on information indicating that continued exposure to malaria parasites is necessary to maintain clinical immunity (Refs. 17, 18). Consequently, we believe it is a reasonable safeguard to assume that after 3 or more continuous years of residence in a non-endemic country, the majority of prior residents of malaria-endemic areas will not maintain their clinical immunity. Thus, after 3 years of continued residence in a non-endemic country, a prior resident of a malaria-endemic country may be treated as a resident of a non-endemic country. Such individuals should be deferred for only 1 year after each return from travel to a malaria-endemic area consistent with the deferral for travelers from non-endemic countries.

- In many parts of the world, transmission of malaria and dengue can occur in the same area. FDA is aware that under the new definition of a malaria-endemic area, potentially eligible donors may have traveled to areas where dengue virus is transmitted. FDA is currently evaluating the risk of dengue virus infections in blood donors that are acquired either locally or elsewhere in the world, and may address this issue in future guidance.

- The recommendation that consignee notification include instructions for notification of the transfusion recipient or the transfusion recipient's physician of record regarding the need for monitoring of the recipient for a possible malaria infection for a period of 3 months post-transfusion (recommendation C.2.) is based on the analysis of incubation periods in 57 cases of transfusion-transmitted malaria in the U.S., in which the maximum period observed between transfusion and onset of clinical symptoms was 90 days (range 8 to 90 days) (Ref. 3). This recommendation is limited to the highest risk circumstance of unintentional release of a unit from a donor at risk of malaria, namely a unit from a donor who had a clinical history of malaria who may not have been treated or who failed to be deferred for at least 3 years.

- The recommendation to allow the use of acellular blood components inadvertently collected from a donor who was later determined to be at risk for malaria to make injectable products is based on the knowledge that licensed plasma derivatives do not transmit malaria. In addition, notification of consignees is not recommended and reporting of biological product deviation is not required for acellular components inadvertently collected and distributed from a donor at risk for malaria because of the lack of a documented case of transfusion-transmitted malaria from acellular blood components. According to a CDC surveillance study (Ref. 3), 93 cases of transfusion transmitted malaria were reported in the U.S. from 1963-1999. Among the 70 cases for which information was available, the following blood components were implicated: whole blood (63%); red cells (31%); and platelets (6%). Plasma components were not shown as a source of transfusion-transmitted malaria.

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	第88回日本感染症学会学術講演会，第62回日本化学療法学会総会合同学会(2014.06.18,19,20)/福岡県福岡市 62(SA)375/(2014.5)	公表国 日本	
販売名(企業名)	－					

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
シャーガス病の流行地域(中南米21ヵ国)から来日し25年定住している人。2013年6月の献血の際に同意を得て実施した疫学調査において、シャーガス病の原因であるTrypanosoma cruzi抗体陽性を指摘され、病院を受診した例である。自覚症状はないものの、初診時心電図にて2度房室ブロック及び心臓超音波検査で左室壁運動の低下を認めた。 全血においてTrypanosoma cruziのDNA陽性も確認され、慢性期再燃時の寄生虫血症が示唆されたとことで、献血者疫学調査にて発見された本邦初めてのシャーガス病患者であるとのこと。	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
本邦初の症例との報告であることから、報告対象としたが、シャーガス病はクルーズトリパノソーマという原虫が寄生することによって起こる疾患であり、血漿分画製剤からの原虫感染はない。	今後とも本邦初の感染症に関連する情報に留意していく。

⑦

P2-053 診断に複数回のギムザ染色を要した四日熱マラリアの2例
感

国立国際医療研究センター国際感染症センター[1]，国立国際医療研究センター研究所熱帯医学・マラリア研究部[2]
○上村　悠[1]，忽那賢志[1]，塩沢綾子[1]，的野多加志[1]，
藤谷好弘[1]，馬渡桃子[1]，竹下　望[1]，早川佳代子[1]，
金川修造[1]，加藤康幸[1]，狩野繁之[2]，大曲貴夫[1]

四日熱マラリアを2例経験したので報告する．
【症例1】生来健康な32歳日本人男性．南スーダン，ケニアから帰国12日後より発熱が出現した．発症から2日後当院を受診し発熱以外に特記すべき身体所見を認めなかった．マラリア迅速検査は陰性で，血液ギムザ染色でマラリア原虫を認めなかった．発症から6日後，再度発熱で受診した際に行ったギムザ染色でマラリア原虫を認めマラリアと診断した．同日入院加療を開始した後，遺伝子検査で四日熱マラリアと確定診断した．3日間の治療，ギムザ染色で原虫の消失を確認し，入院4日目に退院となった．
【症例2】生来健康な36歳日本人男性．アンゴラから帰国後翌日より，発熱，頭痛が出現した．発症翌日当院を受診し発熱以外に特記すべき身体所見を認めなかった．マラリア迅速検査は陰性で，ギムザ染色でマラリア原虫を認めなかった．発症から7日後，再々度行った血液検査でマラリア原虫を認めマラリアと診断した．入院時，不穏症状を認め重症マラリアとして入院加療を開始した．その後，遺伝子検査で四日熱マラリアと確定診断した．3日間の治療，ギムザ染色で原虫の消失を確認し，入院4日目退院となった．
【考察】いずれの症例も診断にあたり複数回の検査を必要としたが，低い寄生率が偽陰性の原因であったと推測した．特に寄生率の上がりにくい非熱帯熱マラリアでは繰り返し検査を行うことが重要と考えられる．

G1414101

P2-054 アーテメター・ルメファントリン合剤による治療後に発熱が遷延し溶血性貧血を認めた熱帯熱マラリアの1例
感

京都市立病院感染症内科
○土戸康弘，篠原　浩，栃谷健太郎，清水恒広

【背景】アーテスネートは重症マラリアの第一選択薬としてWHOガイドラインで推奨されている．2013/1に米国CDCよりアーテスネートによる熱帯熱マラリア治療後の遅延型溶血性貧血について注意喚起が出された．【症例】20代日本人女性．現地調査のため201X/8/9から9/14までケニア・ウガンダに滞在．9/5から発熱を認め解熱薬を内服し解熱．9/10から再度発熱が続き，9/15帰国し9/16当院受診．体温40.4℃，脈拍120/分，血圧79/53mmHg，呼吸数18/分，SpO2 96%（室内気），肝脾腫あり．WBC 7000/μl，Hb 14.1g/dl，Plt 13.7×104/μl，AST 97 IU/l，ALT 83IU/l，LDH 374IU/l，T-Bil 0.8mg/dl．末梢血ギムザ染色：赤血球内に複数の輪状体（寄生率4.5%）．熱帯熱マラリアと診断し第1病日のみ塩酸キニーネとドキシサイクリン内服，寄生率が7.9%に増加した第2病日より熱帯病治療研究班から供与されたアーテメター・ルメファントリン内服に変更，3日間投与を行った．第4病日に解熱，第5病日に原虫消失を確認し退院．しかし38℃台の発熱が再出現し第12病日に溶血性貧血（Hb 8.8g/dl，LDH・Bil上昇，ハプトグロビン低値）を認めた．直接クームス試験陰性，ギムザ染色ではマラリア原虫は認めず，アーテメター・ルメファントリンによる副作用が疑われた．【考察】因果関係は明らかではないが，注意喚起を参考に，アーテメター・ルメファントリンによる治療後も遅延型溶血性貧血の出現に注意が必要である．

X1480019

P2-055 本邦で初めて献血者疫学調査にて発見されたChagas'病患者の1例
感

那須赤十字病院リウマチ科[1]，那須赤十字病院呼吸器内科[2]，日本赤十字社中央血液研究所[3]，那須赤十字病院循環器内科[4]，防衛医科大学校感染症・呼吸器内科[5]，獨協医科大学国際協力支援センター熱帯病寄生虫病室[6]，獨協医科大学感染制御センター[7]，獨協医科大学越谷病院臨床検査科[8]
○池野義彦[1]，阿久津郁夫[2]，三浦左千夫[3]，矢野秀樹[4]，
大口真寿[5]，福島史哉[2]，近江史人[2]，村山慶樹[2]，
前田卓哉[5]，千種雄一[6]，桐木雅史[6]，奥住捷子[7]，
吉田　敦[7]，春木宏介[8]

Chagas'disease は *Trypanosoma cruzi*（*T.cruzi*）感染によって生じる疾患であり，世界で700～800万人の感染者がいると推計される．中南米21カ国が流行地域であり，交通手段の発達，地域間の人的交流の増加などにより，非流行地域においては，流行地域からの感染者の移入，また輸血や臓器移植での感染伝播が問題となる．流行地域から来日され約25年定住されている方．2013年6月に実施した献血の際，同意を得て実施した疫学調査で *T.cruzi* 抗体陽性を指摘され当院受診した．自覚症状はないものの，初診時心電図にて2度房室ブロック及び心臓超音波検査で左室壁運動の全周性にわたる低下を確認した．全血 *T.cruzi*-DNA も陽性を認められ，慢性期再燃性のParasitemiaの存在が示唆された．本邦には流行地域から約30万人の方が在留されており，3000人程度の感染者がいると推計される．カナダ，米国及びスペインで輸血製剤を介したChagas'病感染が報告され，非流行地域においても，輸血感染予防対策の必要性が指摘され，対策が進められている．本邦でも2012年10月より本人及び母が流行地域出身の人，もしくは4週間以上流行地域に滞在した人から供出された血液は血漿分画製剤原料としての使用に制限し，2013年4月からは疫学調査を実施している．しかしながら，献血疫学調査対象者は当該在留者の約3%程度と少なく，日本においても献血者に限らず，当該在留者全員の抗体検査を強力に進める必要がある．

G1414102

P2-056 Sequential change in IgG to the filarial crude antigens of the patient with Loiasis
感

国立国際医療研究センター国際感染症センター
○馬渡桃子，小林鉄郎，杉原　淳，早川佳代子，藤谷好弘，
忽那賢志，竹下　望，金川修造，加藤康幸，大曲貴夫

A 52-year-old Japanese female presented to our hospital complaining of discomfort in her conjunctiva and recurrent skin nodular lesion. Since 2 months ago, she had been traveling in Africa (Cameroon and other 3 countries) for 7 weeks. She was bitten by a mango fly in the neck during the stay in Cameroon. On the day of her visit to our hospital, her left eyelid was edematous, but no larval body was found in her conjunctiva. Count of blood cell was normal, and she did not have eosinophilia. Her blood smear sample revealed no microfilaria. However, she had a higher level of antibody to Brugia pahangi, which is known to have crossed reactivity to Loa Loa. Considering her high risk factors and serologic test result, we treated her with empiric Albendazole targeting possible Loiasis. She developed left arm swelling on day 5 of treatment, which gradually improved in one week. The skin nodular lesion has not appeared after treatment. Serum IgG to the filarial crude antigens increased just after treatment, and 4 months after that, the IgG decreased. The sequential change in IgG to the filarial crude antigens may help us to estimate the clinical response to treatment.

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
				2014. 6. 6	該当なし	
一般的名称	新鮮凍結人血漿		研究報告の公表状況	Martin DL, Goodhew B, Czaicki N, Foster K, Rajbhandary S, Hunter S, Brubaker SA. PLoS One. 2014 Apr 23;9(4):e95398. doi: 10.1371/journal.pone.0095398. eCollection 2014.	公表国 米国	
販売名（企業名）	新鮮凍結血漿-LR「日赤」120（日本赤十字社） 新鮮凍結血漿-LR「日赤」240（日本赤十字社） 新鮮凍結血漿-LR「日赤」480（日本赤十字社）					

研究報告の概要

○低温保存後の*Trypanosoma cruzi*（*T.cruzi*）の生存性：組織保存に関する潜在的な影響
シャーガス病の原因であるT.cruziは、典型的な昆虫媒介の他、臓器移植や血液製剤の輸血によって感染する場合がある。感染したヒト細胞、組織及びそれらに由来する製品の*T.cruzi*伝播性は、製品の製造工程及び保存状況下で生き残る原虫に依存する。本研究において、室温、低温、冷凍下で様々な期間保存した場合の*T.cruzi*の生存性を調査した。
室温下：ヒト血液にスパイクされた*T.cruzi*は、24時間後にも感染性を保っていた。
低温下（1〜10℃）：感染細胞を24時間及び48時間保存後、原虫の生存率は変化しなかった。14日後には運動性を有する原虫は見られなくなり、28日保存後には2/13例に生存原虫が見られたものの、1カ月後には消失した。
冷凍下（−80℃）：凍結防止剤がない状態でも、感染細胞を1年間保存後に生存原虫が見られた。保存から30、60、120、365日後の生存原虫の数は、凍結防止剤を用いた場合の方が用いない場合よりも有意に多かった。
*T.cruzi*は冷蔵と冷凍保存の状態で長期間生存できることから、このような状況で保存される*T.cruzi*感染細胞・組織由来製品が感染性を持つことを示唆している。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見

低温、冷凍下で様々な期間保存した場合の*T.cruzi*の生存性を調査したところ、長期間生存することが示され、このような状況で保存される*T.cruzi*感染細胞・組織由来製品が感染性を持つことを示唆しているとの報告である。

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。

8

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Trypanosoma cruzi Survival following Cold Storage: Possible Implications for Tissue Banking

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Abstract

While *Trypanosoma cruzi*, the etiologic agent of Chagas disease, is typically vector-borne, infection can also occur through solid organ transplantation or transfusion of contaminated blood products. The ability of infected human cells, tissues, and cellular and tissue-based products (HCT/Ps) to transmit *T. cruzi* is dependent upon *T. cruzi* surviving the processing and storage conditions to which HCT/Ps are subjected. In the studies reported here, *T. cruzi* trypomastigotes remained infective 24 hours after being spiked into blood and stored at room temperature (N = 20); in 2 of 13 parasite-infected cultures stored 28 days at 4°C; and in samples stored 365 days at −80°C without cryoprotectant (N = 28), despite decreased viability compared to cryopreserved parasites. Detection of viable parasites after multiple freeze/thaws depended upon the duration of frozen storage. The ability of *T. cruzi* to survive long periods of storage at +4 and −80°C suggests that *T. cruzi*-infected tissues stored under these conditions are potentially infectious.

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Introduction

Trypanosoma cruzi is a parasite that causes human Chagas disease (American trypanosomiasis). Transmission typically occurs in areas of Latin America where substandard housing provides a habitat for the triatomine bug vectors that deposit the parasite in fecal matter during a nighttime blood meal. The parasite can enter the bloodstream through the skin via any break in the skin or through contact with a mucous membrane. In the vertebrate host, the parasite exists in both intracellular and extracellular forms. Extracellular trypomastigotes can invade virtually any nucleated cell type. Once inside the cell, trypomastigotes transform to amastigotes and replicate [1]. Amastigotes transform back to trypomastigotes after approximately nine rounds of replication over 4–7 days and escape the cell. The released trypomastigotes can be taken up by a triatomine vector during a blood meal or can propagate the infection *in vivo* by infecting other host cells. The acute phase of infection lasts for 1–2 months, during which the parasite has a broad tissue distribution and parasitemia is patent. After the acute phase, parasites persist primarily, but not exclusively, in muscle tissue, and the predominant clinical pathology is cardiomyopathy. Although infection of the host is life-long, parasites are rarely seen in the blood during chronic infection, and even sensitive polymerase chain reaction (PCR) assays only detect parasites in the blood of up to 66% of chronically-infected individuals [2].

To date, 23 cases of vector-borne infection have been identified in the United States (U.S.) [3]. Other potential routes of human transmission in the United States include congenital infection and infection via transplantation and transfusion [4,5]. An estimated 300,000 *T. cruzi*-infected individuals reside in the U.S. [6]. In the U.S., nine cases of *T. cruzi* transmission from solid organ donation have been documented [5,7,8]. Eight reported cases of *T. cruzi* transmission via blood transfusion have been reported; blood donor screening was implemented in the United States in 2007 [9].

The risk of transmission through transplantation of tissues from infected donors is unexplored. Tissue banks oversee the donation of a number of non-solid-organ tissues such as skin, long bone, tendons, ligaments, cornea, heart valves, musculoskeletal tissue, and nerve tissue. Unlike solid organ donation, recovery of tissue may take place up to 15–24 hours after asystole, and many tissues are processed and stored prior to transplantation [10]. While some tissues undergo minimal processing and are stored in cryoprotectant to preserve function (e.g. reproductive tissue and heart valves), other tissues undergo more extensive processing and cold storage in the absence of cryoprotectant prior to transplantation. Some tissue, such as musculoskeletal tissue, can be stored at <−40°C in the absence of cryoprotectant for up to 5 years per American Association of Tissue Bank (AATB) Standards [10]. In early 2009, the U.S. Food and Drug Administration (FDA) issued a draft Guidance for Industry (DHHS/FDA/CBER 2009) that suggested all donors of human cells, tissues, and cellular and tissue-based products (HCT/Ps) be screened and tested for antibodies to *T. cruzi*. This document identified *T. cruzi* as a "relevant communicable disease agent" and described "current data are insufficient to

identify specific effective processing methods that consistently render HCT/Ps free of *T. cruzi*." This announcement coincided with the availability of one FDA-licensed donor screening test for *T. cruzi* antibody that additionally has a claim for use in testing blood specimens from "cadaveric donors of HCT/Ps" [11]. To evaluate this disease agent's relevance to tissue types recovered from deceased donors, studies were undertaken to evaluate the survivability of *T. cruzi* using storage temperatures commonly used for transplantable tissue (i.e. ambient, refrigerated, frozen, and cryopreserved). The aim of this study was to determine the viability of *T. cruzi* parasites following room temperature and cold storage of cell lines infected with *T. cruzi* or of trypomastigotes.

Materials and Methods

Human subjects

Blood was collected from a healthy adult volunteer via venous puncture. Written informed consent was obtained under protocol 6062 approved by the New England Institutional Review Board and the Centers for Disease Control and Prevention Human Subjects Office.

Cell lines and parasite strains

Three human cell lines and one monkey-derived cell line were used in this study (HMEC-1, a human microvascular endothelial cell line; FS9, a human foreskin fibroblast cell line; Chang CONJ, a conjunctival epithelial cell line, and Vero, a green monkey kidney epithelial cell line). All cell lines were acquired from the Division of Scientific Resources at CDC. Three strains of *T. cruzi* were used (Brazil, Y, and Tc23). Brazil and Y are both long-standing laboratory strains; Tc23 was isolated from a guinea pig in the Arequipa district of Peru in 2009, passaged in cell culture, and maintained as frozen stocks (Martin, et. al, *AJHTM*, in press).

Cell infection and culture

All cells were cultured in tissue-culture treated flasks in a humidified, CO_2-rich, 37°C environment using RPMI-10 (Life Technologies, Grand Island, NY) media supplemented with 10% fetal bovine serum (FBS, Fisher Scientific, Pittsburgh PA), L-glutamine (Life Technologies), sodium pyruvate (Life Technologies), and penicillin/streptomycin (Life Technologies) (hereafter referred to as RPMI-10). All cell lines were adherent and removed from the flask for storage by incubation with 0.25% trypsin in EDTA (LifeTechnologies) for 1–3 minutes, followed by washing in RPMI-10. In this study, trypomastigotes from all parasite strains were able to infect all cell lines tested. During culture for viability testing, if cells became confluent, they were trypsinized and split into additional flasks for further culture.

Cell recovery and storage

Parasites and parasite-infected cells were stored at three temperature ranges (room temperature, refrigerated, and frozen) and various lengths of time for determination of parasite viability following storage. For room temperature storage (22–25°C), 1 ml of venous blood from a healthy, uninfected adult volunteer was spiked with 2×10^6 trypomastigotes. In some trials blood was collected in heparin, in other trials the blood was allowed to clot. Spiked blood was stored at room temperature for 24 hours, after which blood was examined on a slide for motile trypomastigotes and then plated on uninfected cells for assessment of parasite infectivity. To represent refrigerated temperature storage (from above 0°C to 10°C), cells from an infected flask were released by trypsinization and aliquoted into 15 ml conical tubes, or infected flasks themselves were placed in a refrigerator for periods of 24 h, 48 h, 72 h, 5 d, 7 d, 10 d, 14 d and 28 d. For frozen (−80°C) storage, cells from an infected flask were trypsinized and resuspended in a cryoprotectant solution (90% fetal bovine sera (FBS)/10% dimethylsulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO) as a positive control, 100% FBS, or RPMI -10. In other experiments, trypomastigotes were removed from the culture supernatant, washed, and stored in cryoprotectant solution, FBS, or RPMI-10 at 1×10^6 or 5×10^6 parasites per sample. Trypomastigotes or infected cells were aliquoted to cryogenic tubes and placed in a Mr. Frosty freezing container (Nalge Nunc, Penfield, NY, USA), to achieve a freezing rate of −1°C/minute in a −80°C freezer, and then were stored in a −80°C freezer for 24 h, 48 h, 72 h, 5 d, 14 d, 30 d, 60 d, 90 d, 120 d, and >365 days. The work flow is outlined in Table 1.

Freeze-thaw analysis

Trypomastigotes were frozen as described in RPMI-10 with or without cryoprotectant. Samples were thawed, washed once in RPMI-10, and then refrozen in the same condition (i.e. RPMI-10 alone or with cryoprotectant) as the first freeze. This was repeated for a total of 4 freeze-thaw cycles with an interval of either 1 week or >30 days between each freeze/thaw.

Measures of cell viability

In all studies, samples were cultured over uninfected mammalian cell lines at 37°C in a humidified 5% CO_2 incubator for 7 days to 3 months. Cultures were examined under 200× and 400× for the presence of motile trypomastigotes in the culture supernatant and the presence of amastigote nests in cells (see Figure 1). Either of these were indicators of viable parasites. Parasites spiked into blood for 24 h room temperature storage were examined on a slide (10 µl) for live parasites prior to reculture. To compare the number of viable parasites stored at −80°C in cryoprotectant solution, FBS alone, or RPMI-10 alone, parasites were thawed, washed once in RPMI-10, and resuspended in 1 ml of RPMI-10 before being enumerated on a hemocytometer. All samples were then added to fresh mammalian cells to determine parasite infectivity as described above.

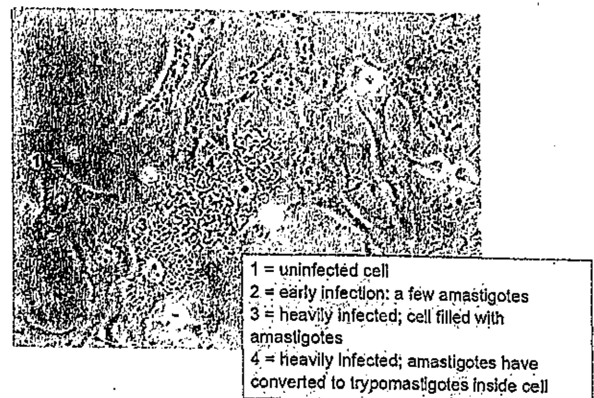

Figure 1. Vero cell culture infected with Brazil strain *T. cruzi* showing asynchronous amastigote nests inside cell. (1) uninfected cell; (2) very early infection, (3) more advanced and (4) a cell in which amastigotes have completely transformed to trypomastigotes. Note that other infected cells are present in this view as well. Photo was taken at 400× magnification.
doi:10.1371/journal.pone.0095398.g001

Photos and videos

Digital photos and videos were taken at 400× magnification or 200× magnification as listed in figure legends using an Olympus DP21 camera (Tokyo, Japan) connected to an Olympus CKX41 microscope. Videos were filmed at 15 frames per second and saved as .AVI files.

Results

Effect of room temperature storage on *T. cruzi* viability in the presence of decaying blood

Motile parasites were observed in *T. cruzi*-spiked blood under a light microscope following 24 hours of room temperature storage (Video S1). Infected cells were observed following plating with the spiked, stored blood (Figure 2). Motile, infective parasites were present in both heparinized (n = 8) and coagulated (n = 5) spiked blood samples.

Effect of refrigerated temperature storage on *T. cruzi* viability

T. cruzi-infected cells stored at refrigerated temperature for 24 h and 48 h exhibited no change in cell or parasite viability upon re-culture (Figure 3A) but showed a qualitative decrease in viable cells but not parasites when stored 72–120 hours at refrigerated temperatures (data not shown and Figure 3A). When stored between 5 and 10 days at 4°C, re-culture of supernatants over uninfected mammalian cells showed infective trypomastigotes (Figure 3B and data not shown). Following 14 days at refrigerated storage temperatures, no swimming parasites were seen in the stored flasks and no culture-positive flasks were observed after re-culture (n = 9). The majority (11/13) of *T. cruzi*-infected flasks stored 28 days at refrigerated temperatures had no viable parasites upon re-culture, but two samples showed infective parasites upon culture with uninfected cells, although these were not visible until one month later (Video S2).

Effect of frozen storage on *T. cruzi* viability

Frozen storage of *T. cruzi*-infected cells in the absence of cryoprotectant resulted in low cell and parasite viability within 24 h, but a small number of viable parasites were still observed. Viable parasites capable of infecting cells in culture were present at one year at −80°C frozen storage even in the absence of cryoprotectant (Figure 4A).

T. cruzi trypomastigotes were enumerated on a hemocytometer after storage at −80°C in the presence or absence of cryoprotectant for varying lengths of time. The number of viable parasites recovered after 30, 60, 120, and 365 days of storage from those stored with cryoprotectant was significantly higher than in

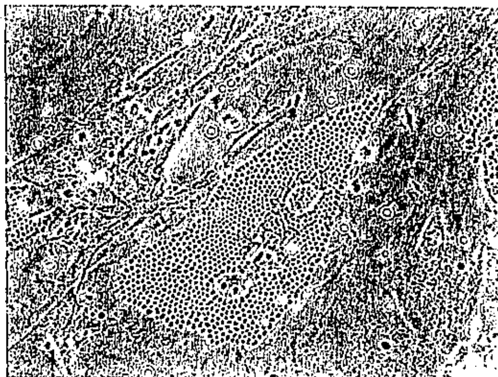

Figure 2. Infectivity of parasites stored 24 hours at RT in decaying blood product. One ml of heparinized (n = 5) or non-heparinized (n = 7) blood was spiked with 2×10^6 Y or Tc23 *T. cruzi* trypomastigotes. Blood was cultured over FS9 or HMEC-1 cells and examined for amastigote nests after 4–7 days. Pictured is a representative amastigote nest of Y strain parasites in FS9 cells. Photos were taken at 400× magnification.
doi:10.1371/journal.pone.0095398.g002

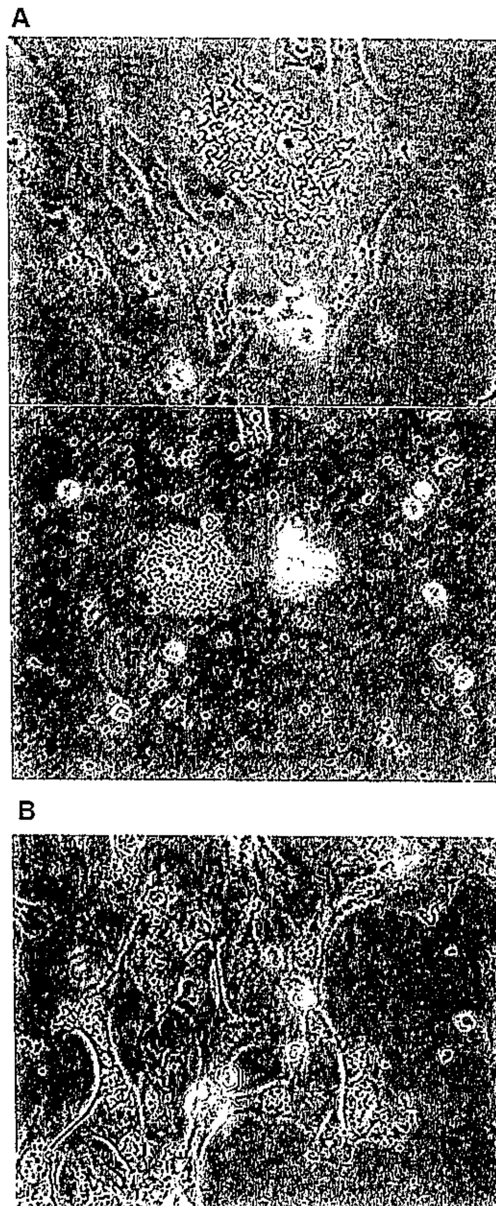

Figure 3. *T. cruzi*-infected culture cells were stored for various lengths of time at 4°C. A. Vero cells infected with Brazil *T. cruzi* after 24 h at 4°C, then re-cultured at 37°C. Figure is representative of N = 7. B. HMEC-1 cells infected with Tc23 *T. cruzi* were stored 5 d at 4°C, then the supernatants were re-cultured over fresh HMEC-1 cells. Figure is representative of N = 10 stored cultures and positive re-cultures.
doi:10.1371/journal.pone.0095398.g003

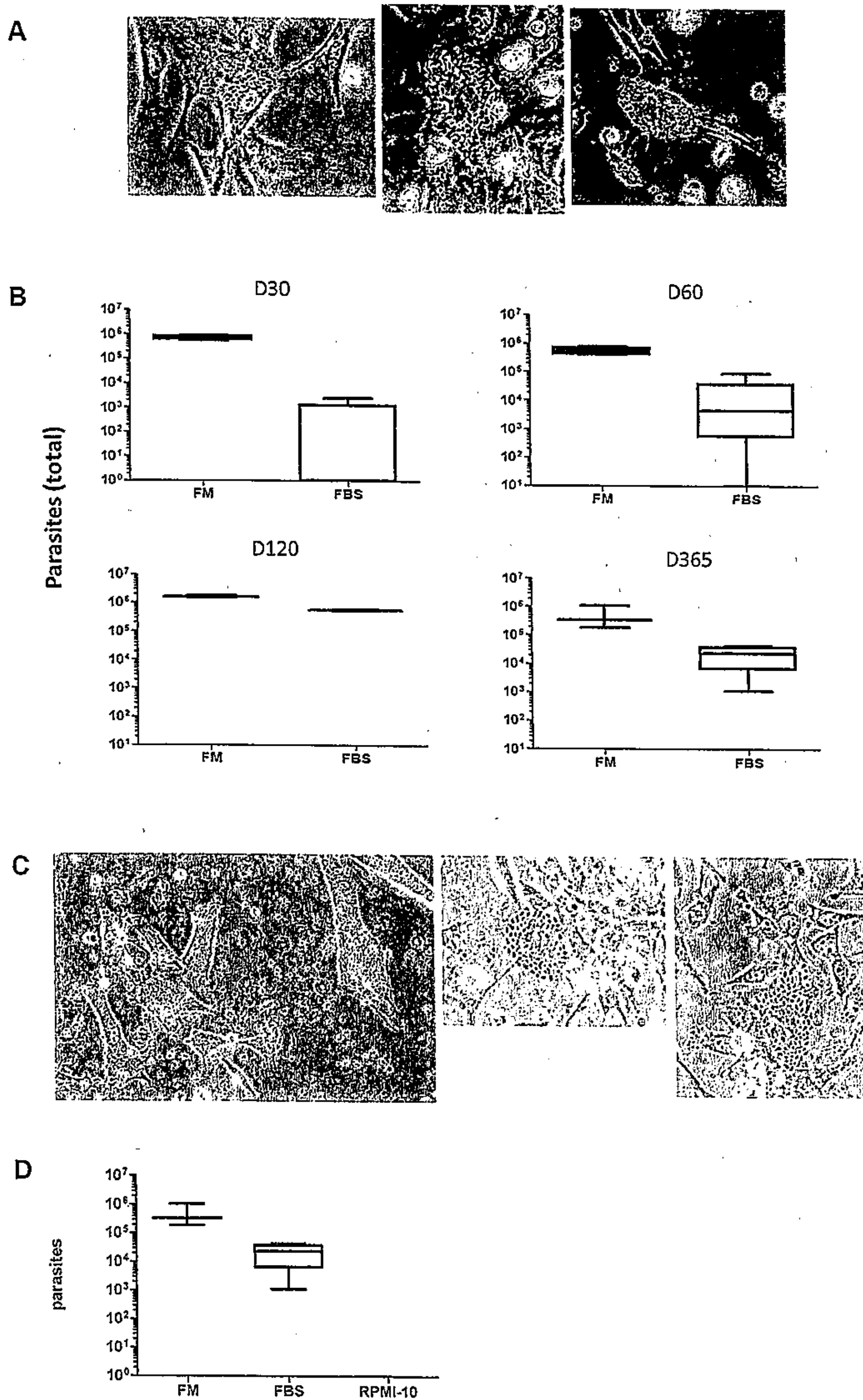

Figure 4. Viable parasites recovered after one year storage at −80°C without cryoprotection. A. *T. cruzi* (Tc23 stain)-infected HMEC-1

cells were stored in RPMI-10 at −80°C for greater than one year, then re-cultured in HMEC-1 cells. Amastigote nests were photographed at 400× magnification. Data are representative of N = 19 cultures. B. *T. cruzi* trypomastigotes (Tc23 strain) were stored with cryoprotectant (freezing media, FM) or FBS at −80°C for the indicated time period. Samples were thawed, washed in RPMI-10, and counted on a hemocytometer. Data represent at least N = 4 per group. C. *T. cruzi* trypomastigotes (Tc23 strain) were stored in RPMI-10 at -80°C for greater than one year, and then re-cultured in HMEC-1 cells. Infected cells were photographed at 400×. Data are representative of N = 5 cultures. D. *T. cruzi* trypomastigotes (Tc23 strain) were stored in FM, FBS, or RPMI-10 at −80°C for greater than one year. Samples were thawed, washed in RPMI-10, and counted on a hemocytometer. Data represent N = 4 per group.
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parasites stored without cryoprotectant, although viable parasites stored in in the absence of cryoprotectant were still observed on a hemocytometer following as long as 6 months of frozen storage (Fig 4B). After one year of frozen storage in RPMI-10, parasites could no longer be enumerated on a hemocytometer directly after thawing, but were still able to infect cell cultures (Fig 4C). Storage in 100% FBS yielded higher recovery of viable parasites, as parasites were observed on a hemocytometer after one year of frozen storage in FBS (Fig 4D). In all conditions, plating the samples from frozen storage on HMEC-1 or FS9 cells resulted in infected cultures 3 to 5 weeks later (Fig 4C).

Parasites subjected to four freeze/thaw cycles with storage in either culture media or cryoprotectant at one-week intervals were viable (Video S3). However, when the storage time between freeze/thaws was increased to greater than one month, no viable parasites or positive re-cultures were observed (data not shown).

Discussion

Because *T. cruzi* exists as both intracellular and extracellular forms, the potential for transmission from cellular and acellular tissue grafts and blood products exists. Solid organ donation of the heart [5], liver [8], kidney and kidney/pancreas [8,12–16] combination, lungs [7], bone marrow [17,18], and cord blood [19] from infected donors has resulted in transmission to recipients in both endemic and non-endemic countries. Tissue donation presents a unique scenario due to the possibility of parasites being exposed to decaying blood and tissue in the deceased donor prior to tissue recovery, undergoing chemical processing, irradiation, or lyophilization during tissue processing, and being placed in short-term storage at refrigerated temperatures and long-term storage at frozen temperatures. In this study, we addressed the effects of exposure to products from decaying blood, short-term refrigerated storage, and long-term frozen storage on *T. cruzi* parasites. The data presented herein show that *T. cruzi* is able to withstand all of these conditions, although cold storage does significantly decrease the number of viable parasites present.

Many types of tissue for transplant may be recovered from a deceased donor up to 15 hours after asystole, during which time the donor's body may not yet be stored at refrigerated temperatures [10]. Parasites present within the tissue would therefore be exposed to products from decaying blood which might impact the parasite viability. We sought to mimic this by spiking trypomastigotes into freshly-obtained venous blood and letting the parasites remain within the blood as it decayed. Viable trypomastigotes were observed by microscopic examination in either heparinized or non-heparinized blood after the 24-hour storage period, and these readily infected cell cultures.

While the vast majority of parasites die during long-term cold storage, the presence of small numbers of viable, infective parasites suggest a low potential for transmission of *T. cruzi* from stored tissue. In refrigerated and frozen conditions, the parasites showed greater resistance to death than the mammalian culture cells used. Parasites appeared to be more susceptible to refrigerated storage than frozen storage, although this was a qualitative and not quantitative observation. It is not clear from the current studies whether parasite death in refrigerated conditions is an active process induced by toxic products released from the dead culture cells or simply due to starvation. Other reports show *T. cruzi* survival following 18 days in refrigeration [20,21], so our study may underestimate the number of infective parasites following 14 days of refrigerated storage. In the absence of cryoprotectant, *T. cruzi* trypomastigotes fared better when frozen in 100% serum than in media containing 10% serum. It may therefore be important to consider the water content of tissue when thinking about *T. cruzi* survival during storage.

Tissue stored for transplantation can be thawed for testing and re-frozen. We therefore examined the viability of *T. cruzi* following up to 4 freeze-thaws. When the length of storage between freeze-thaws was short (one week), infective parasites were obtained in all cultures, but when the length of storage was extended to one month between freeze-thaws, no viable parasites were observed. While parasites were highly susceptible to the combined stress of long-term frozen storage and multiple freeze-thaws, not all tissues are routinely subjected to these conditions to ensure the destruction of *T. cruzi* parasites.

T. cruzi may not persist in all tissue in chronically-infected individuals, so knowing which tissues are affected in chronically infected individuals would be useful in future decision-making about transplantation of tissue from infected donors. The tissue distribution of *T. cruzi* in acute experimental infection of mice is extensive and involves virtually all tissues [22–26], but the extent to which *T. cruzi* persists in different tissue during chronic infection is poorly understood. A prospective study of 9 liver grafts transplanted from *T. cruzi*-infected donors to seronegative patients in Argentina showed only 2 parasitemia-positive outcomes in a one-year follow-up [27].

Some of the outcomes were difficult to quantitate due to the low sensitivity of the hemocytometer. At least 100 events need to be counted for accurate measurements; however, in many instances only one or two parasites were counted on the hemocytometer following frozen storage in RPMI-10. These data therefore give an inaccurate count, but still demonstrate the presence of motile parasites immediately after thawing. For many specimens, samples in which no parasites were seen on the hemocytometer resulted in infected cell cultures. Because we could not accurately quantify the number of viable parasites in many instances, we instead focused on the qualitative data showing live parasites following culture. Similarly, quantitation was only achievable in conditions in which a positive control group was available, such as frozen storage conditions in which cryopreservation is a well-established technique [28]. There is no analogous preservation method for *T. cruzi* parasites at refrigerated temperatures.

Cell cultures were used in this study in lieu of animal models primarily due to the failure of positive controls in pilot studies using mice; when muscle tissue from acutely-infected mice was cultured immediately after dissection, only 30% of samples (6/20) yielded positive parasite cultures. In order to show a decrease from a no storage positivity rate of 0.30 to 0.10 for cold storage conditions using paired samples with a McNemar test and Connor approximation [29], an N of 86 per condition would be required (Ryan Wiegand, personal communication), and for unpaired

samples using Fisher's exact test [30], an sample size of 180 mice per condition would be required (power analyses were performed in SAS version 9.3, SAS Institute, Inc., Cary, NC). With 72 conditions to be considered for pre- and post-recovery storage, this sample size (6192 mice) made the use of animals not feasible. The use of cell cultures leaves some questions unanswered, such as the role of the immune response in protecting against parasite infection in recipient and the potential protective effect of a large tissue mass on parasites in that tissue.

Other common preservation methods applied to tissues for transplantation, such as lyophilization, air-drying, and various chemical devitalization treatments, and mechanical agitation, are not addressed herein and are the subjects of ongoing studies. Many allograft types may be provided as acellular or decellularized after these common treatments are applied. The reported rate of confirmed *T. cruzi* infection in volunteer U.S. blood donors is approximately 1:27,500 [9], so with about 30,000 tissue donors annually in the United States, tissue banks should expect to encounter one or two *T. cruzi* infected tissue donors each year. To date, no tissue donation-associated *T. cruzi* infections have been identified. However, two of the transfusion-associated cases in the U.S. received platelet products that had been documented to be leukoreduced and irradiated [9], further showing the high resistance of *T. cruzi* to techniques commonly applied to blood products and tissue allografts. Understanding how other tissue processing techniques affect the viability of the parasite will be critical in light of the data presented here showing that viable, infective parasites remain following long-term cold storage of *T. cruzi*.

Supporting Information

Video S1 Infectivity of parasites stored 24 hours at RT in decaying blood product. One ml of heparinized (n = 5) or non-heparinized (n = 7) blood was spiked with 2×10^6 Y or Tc23 *T. cruzi* trypomastigotes. After 24 h at room temperature, blood was examined for viable trypomastigotes. Video shows swimming trypomastigote amongst red blood cells.
(AVI)

Video S2 Trypomastigotes in culture after 28 d storage at 4°C. Video is representative of 1 positive culture out of 13 total tested (1 additional positive sample was not re-cultured due to extensive yeast contamination). Video was taken at 400× magnification.
(AVI)

Video S3 Effect of multiple freeze/thaws on parasite viability in the absence of cryoprotection. Trypomastigotes were stored in RPMI-10 at −80°C for one week, between four total freeze/thaw cycles then re-cultured in HMEC-1 cells. Video was taken at a 200× magnification. Data are representative of N = 5 cultures.
(AVI)

Acknowledgments

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Author Contributions

Conceived and designed the experiments: DM SH SB. Performed the experiments: DM BG NC KF SR. Analyzed the data: DM BG NC KF SR. Contributed reagents/materials/analysis tools: DM. Wrote the paper: DM.

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No. 16

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2014. 5. 8	該当なし	
一般的名称	新鮮凍結人血漿		研究報告の公表状況	Sarkari B, Shafiei R, Zare M, Sohrabpour S, Kasraian L. J Infect Dev Ctries. 2014 Apr 15;8(4):543-7. doi: 10.3855/jidc.3831.	公表国 イラン	
販売名(企業名)	新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)					

研究報告の概要

○イラン南部の供血者における*Toxoplasma gondii*(*T.gondii*)抗体陽性率と分子診断

背景：*T.gondii*は輸血を含む様々な経路を介してヒトに感染する原虫である。この横断的研究は、健康な供血者間におけるトキソプラズマの抗体陽性率及び関連する疫学的特徴を評価することを目的とした。

方法：イラン、ファールス州の5カ所の血液センターからの合計1,480人の健常供血者がトキソプラズマ抗体(IgG、IgM)検査を受け、IgM抗体が陽性のサンプルは、PCRを行った。参加者の人口統計学的データも調査した。

結果：*T.gondii*抗体は1,480人の供血者中286人の血清から検出され、この人口の全体的な抗体陽性率である19.3%と一致した。182人(12.3%)はIgG抗体のみ陽性、81人(5.47%)はIgM抗体のみ陽性及び23人(1.6%)はIgG抗体、IgM抗体の両方が陽性であった。IgM抗体陽性被験者のうち2人(1.9%)は活動性の原虫血症であった。年齢、居住地及び教育レベルは、トキソプラズマ抗体陽性と統計的に有意に関連した(P<0.05)。

結論：この結果は、無症候性、特に原虫血症の供血者が受血者にトキソプラズマ症を伝播する可能性があることを明らかにした。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見

イラン南部において健常供血者に*Toxoplasma gondii*の抗体検査を行ったところ、陽性率が比較的高いことが示された。またIgM抗体陽性供血者の血液からDNAが検出される場合もあり、輸血によるトキソプラズマ感染のリスクが明らかにされとの報告である。

今後の対応

日本赤十字社では輸血感染症対策として、トキソプラズマ症の既往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。今後も情報の収集に努める。

⑨

Original Article

Seroprevalence and molecular diagnosis of *Toxoplasma gondii* infection among blood donors in southern Iran

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Abstract
Introduction: *Toxoplasma gondii* is a protozoan parasite which can be transmitted to human through a variety of routes including blood transfusion. This cross sectional study aimed to evaluate the seroprevalence of *Toxoplasma* infection and related epidemiological features among healthy blood donors.
Methodology: A total of 1,480 healthy blood donors from five blood service centers in Fars province were analyzed for anti-*Toxoplasma* antibodies. Blood samples were tested for anti-*T. gondii* IgG and IgM antibodies by enzyme immunoassay. IgM-positive samples were also tested for the presence of *Toxoplasma* DNA by Polymerase Chain Reaction (PCR). Demographic characteristics of participants were also recorded during samples collection.
Results: Anti *T. gondii* antibodies were detected in sera of 286 out of 1,480 blood donors corresponding to an overall seroprevalence of 19.3% in this population. From these, 182 (12.3%) were seropositive only for IgG, 81 (5.47%) were seropositive only for IgM and 23 (1.6%) were positive for both IgG and IgM. PCR detected active parasitemia in two (1.9%) of the IgM-positive subjects. Age, place of residence and level of education were statistically significant ($p < 0.05$) with seropositivity to *Toxoplasma*.
Conclusions: Our results highlighted that asymptomatic blood donors, especially those with active parasitemia, may constitute a significant risk of transmitting toxoplasmosis to susceptible recipients.

Key words: *Toxoplasma gondii*; blood donors; seroprevalence

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Introduction

Toxoplasma gondii is a protozoan which infects almost one third of world's population [1]. The seroprevalence of *Toxoplasma* varies in different countries or even in different areas of a given country. Although toxoplasmosis is a mild diseases in people with competent immune system, the disease is severe and life threatening in immunocompromised individuals [2]. Moreover, the consequences of congenital transmission of the parasite to a fetus are devastating.

The infection is usually transmitted through ingestion of oocyst-contaminated food or water, consumption of undercooked meat and vertical transmission during pregnancy [3]. Furthermore, *Toxoplasma* infection can be transmitted through organ transplantation and whole blood or white blood cell transfusion from a seropositive donor to a seronegative recipient [4].

The infection in immunocompromised individuals such as transplant recipients and HIV-positive patients can result in severe consequences including encephalitis, chorioretinitis and myocarditis [5].

Toxoplasmosis is a common infection in human and animals in all areas of Iran, including Fars province [6-7].In a recent study in this region, prevalence of *Toxoplasma* infection among animals (sheep and goats) was found to be 33.3% [7].

It has been demonstrated that *Toxoplasma* can transmit through blood transfusion [8]. Healthy seropositive blood donors, especially those who are in the acute phase of the infection, may play a major role in this case [9].

The rate of *Toxoplasma* infection in healthy blood donors varies in different areas of the world and this mainly depends on the rate of infection in the community [10-15]. In some areas, such as northeast Brazil, north India and Egypt, more than 50% of blood donors have been seropositive for *Toxoplasma*

infection [11, 14, 16]. Lower rate of infection in blood donors have been reported from Taiwan (9.3%), Thailand (9.6%), Mexico (7.4%) or Turkey (19.5%) [10, 15, 17-18].

Lack of information about the status of *Toxoplasma* infection in healthy blood donors in Iran justified this study which aimed to evaluate the seroprevalence rate of *Toxoplasma* and its relative epidemiological factors in asymptomatic healthy blood donors in southern Iran. Moreover, the study aimed to assess the possible presence of parasite DNA in healthy donors.

Methodology

Study population

The current study was conducted between July 2012 and March 2013 in Fars province. Fars is one of the 31 provinces in Iran and known as the Cultural Capital of Iran. It is situated in the south of the country and its capital is Shiraz. It has an area of 122,400 km². In 2006, this province had a population of 4.57 million people. After getting approval from the ethics committee of Shiraz University of Medical Sciences, blood samples were taken from 1,480 healthy volunteer blood donors from five branches of blood service centers in the province. These centers were in Kazeroun, Jahrom, Darab, Firouzabad and Nourabad counties. The sample size was estimated based on the population of the area. Demographicfeatures of participants were recorded during sample collection.

Serological test

Sera were obtained from the fresh whole blood of the blood donors. Moreover, buffy coat was obtained from each samples for subsequent DNA extraction. Samples were transferred from each blood service centers in each county to the serology laboratory at department of parasitology and mycology in Shiraz (Shiraz University of Medical Sciences, Shiraz, Iran). Samples were kept at -20 °C until use. Sera samples were tested for anti-*Toxoplasma* IgG and IgM, using a commercial enzyme immunoassay kit (PishtazTeb Diagnostics, Tehran, Iran).

DNA Extraction and nested PCR

DNA was extracted from the buffy coat of all of the IgM positive samples. DNA of each sample was extracted, using proteinase K and lysis buffer followed by phenol/chloroform/isoamyl extraction. Absolute ethanol was used to precipitate the DNA. Precipitated DNA was resuspended in 100 µL of double distilled water and stored at 4°C until use. Nested PCR was performed as described by Asgari *et al.* [7]. Nested primer sets were used for amplifying fragments of the B1 gene of *Toxoplasma gondii*. The outer primers which produced an amplified product of 432 bp were from bases 171 to 190 (5′-CCG TTGGTT CCG CCT CCT TC-3′) and from bases 602 to 583 (5′-GCA AAA CAG CGG CAGCGT CT-3′). Inner primers were from bases 180 to 196 (5′-CCG CCT CCT TCG TCCGTC GT-3′) and from bases 392 to 372 (5′-GTG GGG GCG GAC CTC TCT TG-3′) producing an amplified product of 213 bp.

Analysis of data

Results were analyzed by SPSS software (version 17), with a p-value<0.05 taken as statistically significant. Chi-squared and Fisher exact tests were used to compare the seroprevalence values related to the characteristics of the subjects.

Results

The mean age of participant was 39.1 (range: 20-68 years of age). Most of the subjects were aged 31-40 years. Male constituted 94.3% of participants and female were 5.7% of the subjects. Anti *T. gondii* antibodies was detected in sera of 286 out of 1,480 blood donors corresponding to an overall seroprevalence of 19.3% in this population. Of these, 182 (12.3%) were seropositive for only IgG, 81 (5.47%) were seropositive for only IgM and 23 (1.6%) were positive for both IgG and IgM. Demographic features of the blood donors are shown in Table 1.

Considering the residence of the blood donors, Kazeroun had the highest seroprevalence rate of *Toxoplasma* (25.5%) followed by Nourabad (22.7%), Darab (18.3), Firouzabad (16.3%) and Jahroum (14.7%) counties. The differences in *Toxoplasma* seropositivity and residence of participant was statistically significant ($p<0.05$). The highest seroprevalence rate of *Toxoplasma* (31.2%) was found in the age group of 41-50 years while the lowest seropositivity (16.2%) was seen in the age group of 20-30 years. The differences between age and presence of anti-*Toxoplasma* antibodies was statistically significant ($p < 0.05$).

Seroprevalenc erate of *Toxoplasma* was lower in educated people and this difference was statistically significant ($p < 0.05$). The seroprevalence rate was higher in married subjects than in unmarried ones and the differences were significant ($p < 0.05$).

Table 1. Demographic characteristics of blood donors and relative seropositivity to *T. gondii* in Fars province, southern Iran

Characteristics	Frequency (No.)	Percent (%)	Positive for anti-*Toxoplasma* antibodies (either IgG or IgM)		P value
			No.	%	
Gender					
Male	1396	94.3	264	19	> 0.05
Female	84	5.7	22	26.2	
Age group					
20-30	364	24.6	59	16.2	
31-40	455	30.7	74	16.3	< 0.05
41-50	383	25.9	87	22.7	
51 through higher	262	17.7	59	22.7	
Residence					
Kazeroun	297	20.1	73	24.6	
Jahrom	285	19.3	42	14.7	
Darab	306	20.7	56	18.3	< 0.05
Firouzabad	296	20	48	16.3	
Nourabad	296	20	67	22.7	
Marital status					
Married	1175	79.4	242	20.6	< 0.05
Unmarried	305	20.6	44	14.4	
Educational level					
Uneducated	38	2.6	8	21.1	
Primary and secondary level	603	40.9	139	23.1	< 0.05
Post-secondary level	498	33.6	81	16.3	
University level	342	23.1	56	16.4	
Occupation					
Employee	398	26.9	76	19.2	
Business	719	48.6	142	19.8	
Housewives	77	5.2	21	27.3	
Student	113	7.6	13	11.5	> 0.05
Laborer	65	4.4	13	20	
Farmer and stockbreeder	73	4.9	13	17.8	
Unemployed	35	2.4	8	22.9	
Blood group					
A	399	27	89	22.5	
B	360	24.3	66	18.3	>0.05
AB	92	6.2	19	20.7	
O	618	41.8	110	17.8	
Rh					
Positive	1354	91.5	263	19.5	>0.05
Negative	115	7.8	21	18.3	

While most of donors (44.7%) gave blood on a regular basis, 28.1% of them had no experience of blood donation before. Blood group O was the most frequent group (41.8%) and AB was the least frequent (6.2%) blood group. No correlation was found between *Toxoplasma* seropositivity and ABO or Rh blood group ($p > 0.05$). Moreover, no association was found between the occupations of participants and *Toxoplasma* seropositivity ($p > 0.05$).

All of IgM-positive samples were tested for the presence of *Toxoplasma* DNA. *Toxoplasma* DNA was detected in two of IgM-positive samples (1.9%). These two samples were positive for only IgM but not IgG. Correlation between IgM positivity and PCR results was statistically significant ($p = 0.005$).

Discussion

Presence of organism in blood during the course of infection ensures its transmission through transfusion [9]. Moreover, the ability of organism to survive in the stored blood is another factor which increases the chance of transmission through transfusion. It has been found that tachyzoites of *Toxoplasma* can survive in stored blood for several weeks [19]. During the course of active infection, *Toxoplasma* might be present in blood and this would be a real threat for blood recipients especially patients undergoing multiple transfusion or those who require blood transfusion during the course of transplantation.

The current study is the first seroprevalence study of *Toxoplasma* infection among healthy blood donors in south of Iran. Samples were taken from healthy volunteers donating blood from five blood transfusion centers in different geographical areas of Fars province, south of Iran. The Iranian blood transfusion organization (IBTO) is a nationally qualified organization which performs blood transfusion procedures. IBTO has a main center in each province and different branches in counties of each province. Recent data show that there are twenty-three blood donors per 1,000 population in Iran. More than 90% of blood donations in Iran are collected from voluntary non-remunerated blood donors and the rest is donated as family replacement donation [20].

We found an overall seroprevalence of 19.3% in blood donors. This rate of seroprevalence in healthy blood donors is more or less similar to the rates reported from Malaysia, South India, United Arab Emirates and Turkey [15-16, 21-22], but lower than those reported from Brazil, Egypt and Saudi Arabia [11,13-14].

In a study by Ormazdi *et al.*, the rate of *Toxoplasma* infection in blood donors referred to Tehran blood transfusion organization has been evaluated. Among 250 healthy volunteer blood donors, 132 (52.8%) cases have been positive for IgG and nine cases (3.6%) for IgM anti-*Toxoplasma* antibodies [23].

In the current study, differences in the seroprevalence rate of *Toxoplasma* in different donation centers of the province were statistically significant as people living in Kazeroun and Nourabadhad had a higher seroprevalence rate of *Toxoplasma* than others. This might be due to the differences in climate condition of these two areas whose temperature is milder than Firozabad, Jahrom or Darab. This might increase the chance of survival of oocysts in the environment and a higher transmission rate of *Toxoplasma* through contaminated food or soil.

High seroprevalence of *T. gondii* with age detected in this study is consistent with other studies conducted on this subject [11,17-18]. The increase in risk of acquiring *Toxoplasma* for elder people may be due to a longer lifetime exposure of these people to *Toxoplasma*-contaminated environmental sources.

Seroprevalence of *Toxoplasma* was higher in married compared to unmarried subjects in this study. Considering the association of age and *Toxoplasma* seropositivity, this might be more related to the age of the married subject, rather than to the marital status, which is higher for married participants.

The inverse rate of seroprevalence with the level of education detected in our study has been also documented in previous studies [11,14,18].

Gender was not associated with *Toxoplasma* seropositivity and it is difficult to draw any relation between sex and seroprevalance since more than 90% of people donating blood are males. The presence of IgM anti-*Toxoplasma* antibodies reflects the risk of transmission through transfusion. The seroprevalence rate of IgM anti-*Toxoplasma* antibodies in blood donors varies from 2.4 to 5% [12]. In our study, 5.4% of the blood donors were seropositive for IgM and 1.6% was tested positive for both IgG and IgM. More importantly, *Toxoplasma* DNA was detected in blood samples of two of IgM positive cases. The presence of parasitemia revealed by PCR in IgM-positive healthy blood donors ensures the likelihood of transmission of *Toxoplasma* through blood transfusion.

Conclusion

In conclusion, considering the relatively high seroprevalence rate of *Toxoplasma* infection in blood donors reported in this study, and in view of the fact

that IgM-positive individuals might have tachyzoites in their blood, toxoplasmosis should be considered as a significant transfusion risk in this region and also in any region with similar conditions. Appropriate strategies should be adapted to reduce the risk of acquiring toxoplasmosis through blood transfusion. It can be suggested that immunosuppressed recipients and pregnant women receive *T. gondii* antibody-negative blood components for transfusion.

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医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分		総合機構処理欄
一般的名称	－		研究報告の公表状況	Transfusion 54.4 (Apr 2014): 990-5.		公表国	
販売名(企業名)	－					日本	

研究報告の概要

英国において赤血球製剤による４例のクロイツフェルト・ヤコブ病(vCJD)の輸血感染例があるため、赤血球製剤の安全性は懸案事項である。検証されたスクリーニングテストが存在しないため、感染リスクを最小化するために、血液からプリオンを除去するための工程を利用することが必要である。これらの工程は可能なかぎり血中内と近い形態の感染性プリオンを使用して検証しなければならない。
人全血及び赤血球に対して、263K スクレイピー感染ハムスター脳ホモジネートの高速(遠心分離後の)上清を添加した。添加後のサンプルは白血球除去され、プリオン除去フィルター(Pall Corporation)を通過させた。他の実験として、263K スクレイピー感染ハムスターから得た赤血球に上記の処理をし、バイオアッセイにより残存する感染性を測定した。
プリオンを添加した全血及び赤血球におけるフィルターによる感染性の全体の除去量はおおよそ２桁であった。また、内因的にスクレイピーを感染させフィルターされたハムスター赤血球において感染性は検出されなかった。
プリオン除去フィルターの使用は、輸血伝播 vCJD のリスクを減らすのに役立つかもしれない。バリデーション試験におけるプリオン除去効率の過大評価を避けるため、現在の標準脳ホモジネートよりスクレイピー感染ハムスター脳ホモジネートを超遠心分離した上清を使用することがより適切であるかもしれない。

使用上の注意記載状況・その他参考事項等

重要な基本的注意
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
プリオンの除去フィルターに関する報告であるが、研究レベルでの報告であり、実用化については更なる検討結果を待つ必要があると考える。今後の更なる情報に注意していきたい。	今後ともプリオン除去に関する情報等に留意していく。

10

NIHONSEIYAKU
2008-041

Assessment of prion reduction filters in decreasing infectivity of ultracentrifuged 263K scrapie-infected brain homogenates in "spiked" human blood and red blood cells

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BACKGROUND: The safety of red blood cells (RBCs) is of concern because of the occurrence of four transfusion-transmitted variant Creutzfeldt-Jakob disease (vCJD) cases in the United Kingdom. The absence of validated screening tests requires the use of procedures to remove prions from blood to minimize the risk of transmission. These procedures must be validated using infectious prions in a form that is as close as possible to one in blood.

STUDY DESIGN AND METHODS: Units of human whole blood (WB) and RBCs were spiked with high-speed supernatants of 263K scrapie-infected hamster brain homogenates. Spiked samples were leukoreduced and then passed through prion-removing filters (Pall Corporation). In another experiment, RBCs from 263K scrapie-infected hamsters were treated as above, and residual infectivity was measured by bioassay.

RESULTS: The overall removal of infectivity by the filters from prion-spiked WB and RBCs was approximately two orders of magnitude. No infectivity was detected in filtered hamster RBCs endogenously infected with scrapie.

CONCLUSION: The use of prion-removing filters may help to reduce the risk of transfusion-transmitted vCJD. To avoid overestimation of prion removal efficiency in validation studies, it may be more appropriate to use supernates from ultracentrifugation of scrapie-infected hamster brain homogenate rather than the current standard brain homogenates.

All patients infected with the variant Creutzfeldt-Jakob disease (vCJD) agent by blood transfusion received nonleukoreduced cells sourced from asymptomatic vCJD donors.[1] No case of infection has so far been reported in recipients of leukoreduced red blood cells (RBCs), suggesting that leukoreduction is an important safety procedure. However, data from experimentally prion-infected animals show discordant results on the efficiency of leukoreduction in removing infectivity[2-5] leaving uncertainties on the real efficacy of this procedure for the safety of human blood. Moreover, partition of prion infectivity in blood constituents (i.e., RBCs, white blood cells [WBCs], platelets, and plasma) in different prion-infected sheep and rodents varies considerably[2,4-11] warning about extrapolating level of infectivity in human blood based on results produced by experimental models. Thus, it is possible that leukoreduction alone

ABBREVIATIONS: S^{HS} = high-speed supernatant; vCJD = variant Creutzfeldt-Jakob disease; WB = whole blood.

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does not guarantee the safety of blood or plasma; hence recipients of leukoreduced blood sourced from vCJD-infected donors might eventually develop disease with very long incubation periods. Despite the disappearance of clinical cases, the high prevalence (1 in 4000 to 1 in 10,000 people) of variant CJD infection estimated for the UK population[12] calls for continued attention to the risk of secondary person-to-person transmission through blood transfusions. Finally, the possible reported case of vCJD infection in an elderly hemophilia patient who received batches of Factor VIII concentrate prepared from inadvertently infected plasma pools indicates that this mode of transmission is likely and that prion infectivity is present in human plasma from vCJD-infected donors.[1]

In the past years, several "ad hoc" devices, such as resins or filters with high prion-binding capacities, have been developed to remove prion infectivity with the aim to improve the safety of blood by overcoming the possible low efficacy of leukoreduction. These devices were validated by using either human blood spiked with scrapie-infected hamster or mouse brain homogenates or blood taken from hamsters infected with the 263K strain of scrapie.[3,13-16] Spiking blood with brain homogenates, however, might overestimate the amount of prion removal because infectivity in brain tissues tends to form large aggregate that might enhance the real clearance effect of devices.[17] On the other hand, validation studies performed with blood taken from infected animals has the disadvantage to contain low levels of infectivity and therefore underestimate the potential efficiency of prion removal.

To overcome these criticisms, we spiked human whole blood (WB) and RBCs with high-speed (ultra)centrifuged homogenates prepared from brains of 263K scrapie-infected hamsters, which is likely the best available spike for representing the physical infectious particles circulating in blood.[17,18] Such preparations were used for measuring the removal of prion infectivity in blood by affinity prion reduction filters (LAPRF1 and LAPRF2, Leukotrap, Pall Corporation, East Hills, NY).[3,15,16]

MATERIALS AND METHODS

Supply of blood and RBCs

Prof. G. Girelli (Centro Trasfusionale, Policlinico Universitario "Umberto I," Rome, Italy) and Dr E. Mannella (Centro Aziendale Produzione Emocomponenti, S. Camillo-Forlanini Hospital, Rome, Italy) provided units of normal human WB in CPD anticoagulant and RBC in CPD plus saline-adenine-glucose-mannitol.

Scrapie-infected (263K strain) blood was collected by intracardiac puncture of diseased hamsters, mixed with citrate-phosphate-double dextrose and fractionated at 3500 × g for 20 minutes at 25°C. RBCs (155 mL) were collected and mixed with 62 mL of saline-adenine-glucose-mannitol. All samples were stored at 4°C.

Preparation of prion spike and filtration procedures

The hamster-adapted 263K scrapie strain was used for spiking into units of human WB and RBCs from healthy blood donors. Brains of scrapie-diseased hamsters were processed to obtain infectivity devoid of large aggregates of PrP^{TSE} according to a published procedure from our group.[17,18] Briefly, a 10% brain homogenate in phosphate-buffered saline (PBS) was sonicated (10 sonication pulses, Vibra Cell, Sonics & Materials, Inc., Newtown, CT) and clarified at low speed. The supernatant was further sonicated as above and ultracentrifuged at 200,000 × g for 30 minutes. The resulting high-speed supernatant (S^{HS}) was used for spiking 2 pooled units of WB (540 mL) and 2 pooled units of RBCs (540 mL). The ratio between S^{HS} and WB or S^{HS} and RBC was always 1:9 to keep the final concentration of 263K scrapie-infected brain at 10^{-2} g equivalent/mL (Fig. 1, left panel). Spiked WB and RBCs were leukoreduced by commercial filters (WBF3 for blood and BPF4 for RBCs, Pall Corporation), divided in two identical aliquots, and finally filtered through either LAPRF1 or LAPRF2 filters (Leukotrap, Pall Corporation).[3] RBCs from 263K scrapie-infected hamsters were leukoreduced with a BPF4 filter (Pall Medical, Pall Corporation) and then passaged through a LAPRF2 filter (Fig. 1, right panel).

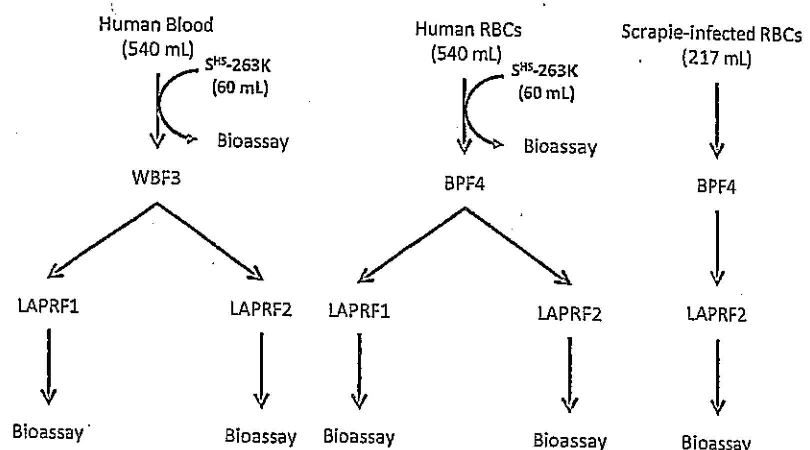

Fig. 1. Flow chart of experiments with prion-removing filters. WBF3 and BPF4 = WBC blood filter; LAPRF1 and LAPRF2 = WBC affinity prion reduction filter.

TABLE 1. Bioassay of infectivity in filtrates of S^{HS}-spiked human blood and RBCs, in prefiltered and filtered RBCs from scrapie-infected hamsters, and relative removal factors

Samples	Dilution of spiked sample (vol/vol)	Infected/ Inoculated	Infectivity (log LD_{50}/mL)	RF* (log)
WB				
Prefiltered	Undiluted†	9/9	3.4	
	10^{-1}	4/5		
	10^{-2}	4/6		
	10^{-3}	0/6		
WBF3/LAPRF1	Undiluted†	3/6	1.3	2.1
WBF3/LAPRF2	Undiluted†	1/6	<1.3	>2.1
Concentrated RBCs				
Prefiltered	Undiluted†	15/15	3.7	
	10^{-1}	9/9		
	10^{-2}	8/10		
	10^{-3}	1/10		
	10^{-4}	0/10		
BPF4/LAPRF1	Undiluted†	5/6	>1.3	<2.4
BPF4/LAPRF2	Undiluted†	11/16	1.7	2.0
	10^{-1}	3/10		
	10^{-2}	0/10		
Concentrated RBCs from scrapie-infected hamsters				
Prefiltered	Undiluted	6/84‡,§	1.5 (0.6)‖	—
BPF4/LAPRF2		0/84§	<0.2 (0.2)‖	≥0.9

* RF = removal factor, calculated as log titer of prefiltered samples minus log titer after filtration.
† Undiluted samples contain 10^{-2} gram equivalents of brain/mL.
‡ One animal was asymptomatic when killed at 514 days after inoculation but resulted positive for PrP^{Sc} in the brain at the Western blot analysis.
§ p = 0.0176 (Fisher's exact test).
‖ Title estimated as reported by Gregori and colleagues[14] and expressed in ID/mL (SD).

Bioassay

Infectivity was measured in recipient golden Syrian hamsters by intracerebral injection (50 µL/animal) of human spiked samples and scrapie-infected hamster RBCs before leukoreduction and after passage through Pall Leukotrap filters (Fig. 1). Pre- and postfiltered RBCs from scrapie-infected hamsters were subjected to four cycles of freezing and thawing before inoculation in recipient hamsters. When appropriate, samples were serially 10-fold diluted in PBS before inoculation (Table 1) and infectivity calculated by the Reed and Muench method in samples inoculated at serial dilutions or, when a single dilution was injected, estimated by the percentage (equal, lower, or higher than 1 LD_{50}) of animals that developed scrapie. In our bioassay conditions 1 LD_{50} corresponded to 1.3 log LD_{50}/mL. In RBCs from scrapie-infected hamster, infectivity was calculated by the limiting dilution method according to the Poisson distribution[14] (Table 1). Fisher's exact test (one tailed) was used to assess the significance of the different distribution of scrapie-affected animals in filtered and unfiltered RBCs from 263K scrapie-affected hamsters.

Animals were observed daily for the appearance of clinical signs and sacrificed at the terminal stage of disease or, when asymptomatic, between 17 and 18 months after inoculation. Brains were removed and divided sagittally and then half the brain was fixed in 10% buffered formalin for histologic examinations and the other half brain was frozen at −70°C for PrP^{TSE} detection by Western blot.[18] All the animals used in these studies were housed at the animal facility of the Istituto Superiore di Sanità under the supervision of the Service for Biotechnology and Animal Welfare of the ISS that warrants adherence to national and international regulations on animal welfare.

RESULTS

263K-S^{HS}–spiked human samples

Human WB and RBCs spiked with S^{HS} showed similar infectivity titers (3.4 and 3.7 log LD_{50}/mL, respectively; Table 1) suggesting that different substrates do not influence the measurement of infectivity. End-point assays of filtered samples were only performed once (RBCs through BPF4 and LAPRF2 filters) because we expected more than 2 logs of infectivity removal after passage through prion-specific filters. In other words, less than 50% of animals developed disease after inoculation with undiluted samples. However, this low percentage of diseased animals (1 in 6) occurred only in those inoculated with the WBF3- and LAPRF2-filtered sample of spiked WB (Table 1). In the other samples, 50% of hamsters developed disease when inoculated with the WBF3- and LAPRF1-filtered WB. In contrast to filtered WB samples, undiluted scrapie-contaminated RBC samples processed through BPF4 and LAPRF1 or BPF4 and LAPRF2 caused

scrapie infection in 83 and 69% of inoculated animals, respectively. The BPF4- and LAPRF2-filtered sample, however, was also end-point assayed resulting in two orders of magnitude infectivity removal. We could have used the dose–response method for estimating infectivity titer in the BPF4 and LAPRF1 RBC sample but the unexpectedly great variability of incubation periods in this and other samples, possibly due to interference with some unknown factors in blood and RBCs, made this option unfeasible.

Removal of endogenous infectivity from scrapie-infected hamster RBCs

The RBC preparation from 263K scrapie-infected hamsters showed a titer of 1.48 ID/mL (Table 1). Five of the 84 recipient hamsters developed scrapie disease with incubation periods ranging from 157 to 287 days. The sixth infected animal did not develop clinical signs but was PrP^{Sc} positive at the Western blot analysis. When the RBCs were filtered with BPF4 and LRF2 filter combination, there was no detectable infectivity in recipient intracerebrally inoculated hamsters (Table 1). No animals developed clinical signs of disease up to the end of the observation period (511-528 days) and none were PrP^{Sc} positive in the brain at the Western blot analysis. Thus, the combination of BPF4 and LAPRF2 filtration removed all endogenous infectivity, which, however, resulted only in 0.9 log reduction because of low endogenous infectivity in the original RBC sample.

DISCUSSION

Filters with high prion-binding capacity were developed for increasing the safety of blood in addition to leukoreduction and other precautionary measures to minimize the risk in relation to transfusion-transmitted vCJD.[19] Clinical trials in healthy volunteers showed that prion-removing filters are safe[20] and do not substantially alter the content of hemoglobin or RBC quality,[21] but there is still uncertainty on the cost-effectiveness of these filters for their routine use in transfusion medicine[19] and whether available validation studies are relevant in proving their real efficiency in removing prion infectivity from blood of donors with vCJD infection.[5,19]

The aim of this study was to provide further information on the efficiency of two prion-removing filters (LAPRF1 and LAPRF2) by spiking human blood and RBCs with brains of 263K scrapie-infected hamsters containing high prion infectivity in a poorly aggregated format (S^{HS}).[17,18] This ultracentrifuged spike represents a sound alternative for validation studies aimed to prove the efficiency of devices for removing prion infectivity from body fluids where prions are likely to be in the poorly aggregated forms. This poorly aggregated form of infectious prions differs from those present in brain extracts or microsomal fractions. Recently, a validation study performed with the S^{HS} spike preparation showed that several brands of nanofilters with pore sizes between 20 and 15 nm removed approximately two orders of magnitude less infectivity than previously found with "standard" spikes.[17] This report from Cardone and coworkers[17] suggests that the S^{HS} fraction contains infectious particles with a mean size smaller than 15 or 20 nm in line with the size estimated by Silveira and colleagues.[22] Thus, one must be cautious in the interpretation of process removal results based on spikes containing highly aggregated prions. Results from our current study show that previous validation experiments performed with "standard" spikes had overestimated the magnitude of the efficiency of prion removal filters by as much as two orders of magnitude (Table 2). Our data also suggest that this highly soluble, nonaggregated form of infectious prion is not removed by leukoreduction, especially considering that in our experimental setting the S^{HS}-spiked samples were initially leukoreduced before filtration with prion removal filters. This may also explain the data from Gregori and coworkers[2] that showed only 50% reduction in infectivity with leukoreduction presumably due to the removal of cell-associated infectivity and aggregated forms of infectious prions.

Our data show that there was no significant difference in infectivity reduction between the 22-layer (LAPRF2) and the 10-layer (LAPRF1) prion-specific filtering material, suggesting that the latter might be adequate for improving the safety of WB or RBCs contaminated with infectious prions. Although these devices work differently from nanofilters,[3,15] it is likely that their efficiency also depends on the level of prion aggregation, which may explain the observed difference in performances between the 10- and 22-layer filters when "standard" spike was used in an infectivity assay.[16] The level of this aggregated form of infectious prion is low in endogenously infected WB, plasma, or RBCs, and it is likely similar to that present in the spike preparation used for our study. The discrepancy between the incomplete removal of infectivity by BPF4/LAPRF2 filters challenged with RBCs spiked with S^{HS} and the complete removal of infectivity by the same BPF4 and LAPRF2 filters challenged with endogenous RBC infectivity is likely because of low endogenous infectivity in the original RBC sample. Alternatively, S^{HS} might behave differently from endogenous blood infectivity.

In conclusion, we are aware that infectivity titers of some samples were estimated with very small numbers of animals and that the final efficacy of prion-removing filters should be directly assessed in human blood with endogenous vCJD infectivity. However, this achievement would be difficult to assess in the near future because of the lack of systems able to demonstrate prion infectivity in blood of vCJD patients with the required level of

TABLE 2. Removal of blood infectivity by affinity prion filters or resins

TSE strain	Infectivity preparation	Substrate	Affinity prion filter	Residual infectivity	Reduction factor (log)	Titration assay	Reference
Scrapie 263K	1% clarified (3000 × g × 3 min) hamster BH in PBS	Human RBCs	LAPRF2*	Yes	3.7	End-point bioassay	Sowemimo-Coker et al.[3]
	0.1% clarified (12,000 × g × 15 min) and sonicated hamster BH in 0.5% sarkosyl/PBS	Human RBCs	Resin L03	Yes	4.0	Bioassay	Gregori et al.[13]
			Resin L13	Yes	4.2		
			Resin L21	Yes	0.4		
			Resin L46	Yes	3.3		
			Resin L51	Yes	4.0		
			Resin L70	Yes	2.7		
	Endogenous RBCs in 0.5% sarkosyl/PBS	None	LAPRF2*	No	>0.7	Bioassay	Sowemimo-Coker et al.[3]
			LAPRF1*	No	>1.1	Bioassay	Sowemimo-Coker et al.[15]§
			LAPRF1*	Yes	>0.7	Bioassay	Sowemimo-Coker et al.[16]§
			LAPRF2*	Yes	0.7	Bioassay	
	WB in CPD	None	Resin L13	Yes/No	>1.2	Bioassay	Gregori et al.[14]
			Resin L13A†	Yes/No	>1.2	Bioassay	
Scrapie RML	0.07% mouse brain homogenate (no details were given)	Human RBCs	LAPRF1*	Yes	1.4	Standard scrapie cell assay	Sowemimo-Coker et al.[16]
			B1451AQ	No	>2		
			B1570AI	No	>2		
			B1570AK	No	>2		
Scrapie PG127	Endogenous RBCs in CPD	None	Combination‡	No	NA‖	Bioassay	Lacroux et al.[5]
			LA plus*	Yes	NA¶		

* Pall Corporation.
† P-CAPT.
‡ ASAHI-KASEI.
§ Sowemimo-Coker et al.[16] contains updated results from a study first described in Sowemimo-Coker et al.[15]
‖, ¶ NA = not available; all (n = 5) sheep inoculated with unfiltered RBCs developed disease, while none (0/5)‖ or 1 (1/5)¶ with filtered RBCs.

sensitivity and dynamic range necessary for validation studies.[19] Thus, the findings that Pall leukoreduction in combination with the LAPRF prion-removing filters efficiently reduce low aggregated prions of approximately 100-fold should encourage the use of these filters for minimizing the risk of transfusion-transmitted vCJD in countries, such as the United Kingdom, with a high prevalence of vCJD infections.[12] Whether these devices should also be used for preventing the still uncertain possibility of sporadic CJD transmission via blood transfusion[23,24] remains to be determined.

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CONFLICT OF INTEREST

MP and FC received support from Pall Corporation to conduct this work.

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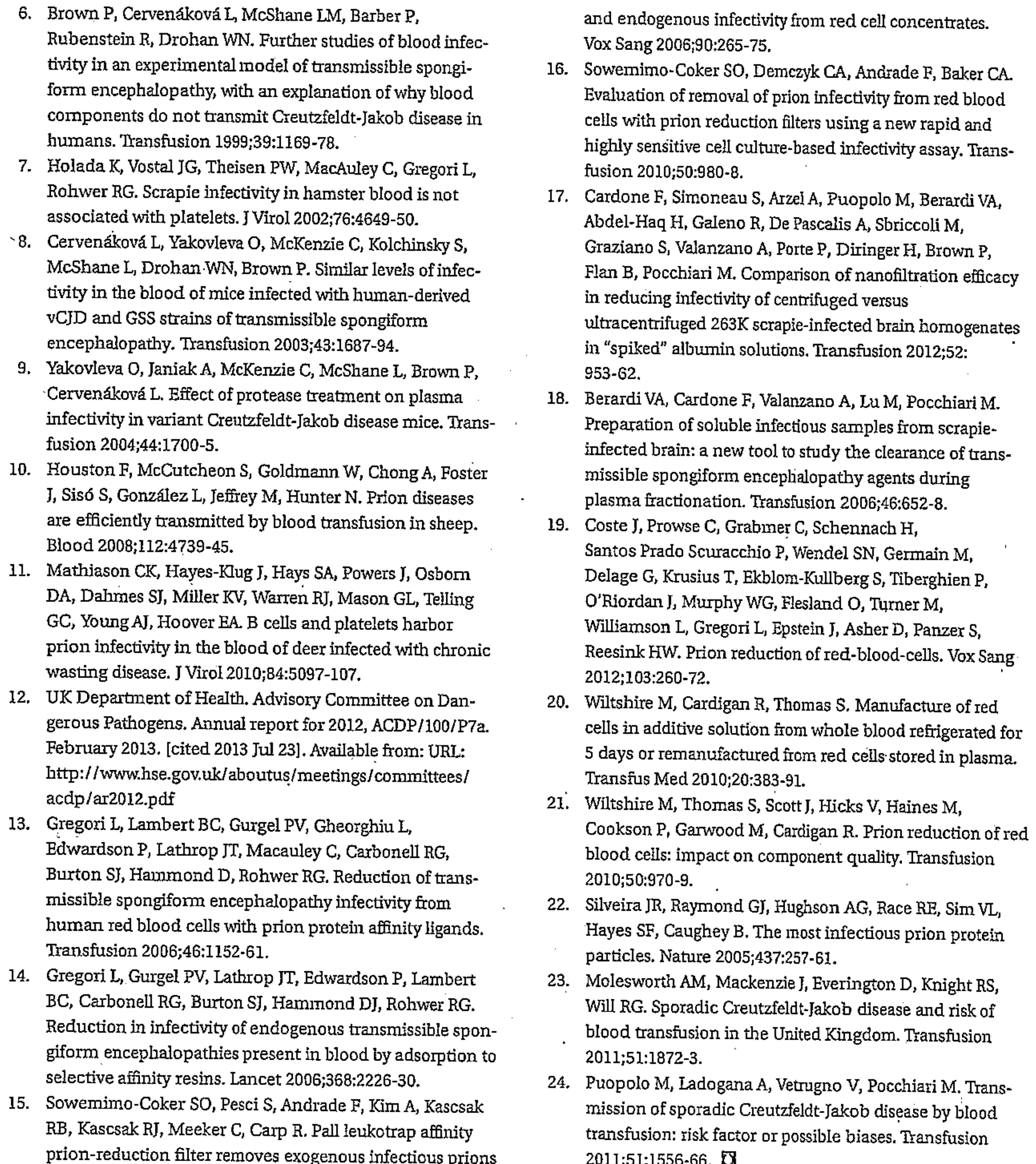

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研究報告の概要

○血液中の変異型クロイツフェルト・ヤコブ病(vCJD)と牛海綿状脳症(BSE)プリオンの発症前検出
vCJDは食品からBSEプリオンを摂取することにより発生すると考えられ、また、後に疾患を発症した無症候性供血者からの血液製剤を輸血された患者における二次的なvCJD症例が確認されている。vCJD/BSE感染者を検出するための有効なアッセイがないため、有病率は未だ不明である。そのため、vCJDの血液媒介伝播リスクは重大な懸念事項である。
本研究において、Protein Misfolding Cyclic Amplification(PMCA)法を用いたvCJD/BSEプリオンのin vitroにおける増幅の、高い効率性と特異性を得るための適切な条件と基質を初めて特定した。vCJD/BSEプリオンの由来(種)が何かにかかわらず、ヒツジQ171PrP基質が最高の増幅成績をもたらした。これらの結果は、種と基質の間のPrPアミノ酸配列の相同性が、in vitroでのvCJDプリオン増幅の重要な決定要素ではないことを示している。
次に、本方法における血液中の内在性vCJD/BSEプリオンを検出する能力を明確にした。アッセイは、ヒツジ及び霊長類の疾患モデルにおいて、発症前段階早期の感染動物の特定を可能にした(霊長類モデルでは発症の32カ月以上前)。
最後に、vCJD感染患者と健常対照からのバフィーコートを含むサンプルパネルに対し盲検試験が行われた。アッセイはvCJD感染患者4人中3人を検出し、141人の健常対照における偽陽性は見られなかった。検査されたvCJD症例の1人における陰性結果は、異なるvCJD病原体検出アッセイの結果と一致し、特定の患者におけるプリオン血症の潜在的な欠如という問題を提起する。

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
血液中のvCJD/BSEプリオンを発症前段階に検出することが出来る新たなアッセイに関する報告である。	日本赤十字社は、vCJDの血液を介する感染防止の目的から、受付時に過去の海外滞在歴を確認し、欧州等38カ国に一定期間滞在したドナーを無期限に献血延期としている。また、英国滞在歴を有するvCJD患者が国内で発生したことから、1980〜96年に1カ月以上の英国滞在歴のある人の献血を制限している。今後もプリオン検出法等の技術を含め、CJD等プリオン病に関する新たな知見及び情報の収集に努める。

11

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Preclinical Detection of Variant CJD and BSE Prions in Blood

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Abstract

The emergence of variant Creutzfeldt Jakob Disease (vCJD) is considered a likely consequence of human dietary exposure to Bovine Spongiform Encephalopathy (BSE) agent. More recently, secondary vCJD cases were identified in patients transfused with blood products prepared from apparently healthy donors who later went on to develop the disease. As there is no validated assay for detection of vCJD/BSE infected individuals the prevalence of the disease in the population remains uncertain. In that context, the risk of vCJD blood borne transmission is considered as a serious concern by health authorities. In this study, appropriate conditions and substrates for highly efficient and specific *in vitro* amplification of vCJD/BSE agent using Protein Misfolding Cyclic Amplification (PMCA) were first identified. This showed that whatever the origin (species) of the vCJD/BSE agent, the ovine Q_{171} PrP substrates provided the best amplification performances. These results indicate that the homology of PrP amino-acid sequence between the seed and the substrate is not the crucial determinant of the vCJD agent propagation *in vitro*. The ability of this method to detect endogenous vCJD/BSE agent in the blood was then defined. In both sheep and primate models of the disease, the assay enabled the identification of infected individuals in the early preclinical stage of the incubation period. Finally, sample panels that included buffy coat from vCJD affected patients and healthy controls were tested blind. The assay identified three out of the four tested vCJD affected patients and no false positive was observed in 141 healthy controls. The negative results observed in one of the tested vCJD cases concurs with results reported by others using a different vCJD agent blood detection assay and raises the question of the potential absence of prionemia in certain patients.

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Introduction

The emergence of variant Creutzfeldt Jakob Disease (vCJD) is considered a likely consequence of human dietary exposure to the Bovine Spongiform Encephalopathy (BSE) agent [1]. Both primate and sheep experimental models rapidly indicated that vCJD/BSE could be transmitted by blood transfusion [2,3]. To date, three vCJD cases and one vCJD infected but asymptomatic individual have been identified in the United Kingdom (UK), in patients that received Red Blood Cell units from donors who developed symptoms of vCJD 17 months to 3,5 years after donation [4,5]. More recently, one preclinical vCJD case was reported in the UK in a haemophiliac patient. This patient had been treated with one batch of FVIII that was manufactured using plasma from a donor who developed vCJD six months after donating blood [6].

The total number of vCJD clinical cases identified so far remains limited (225 patients worldwide at the time of writing). However the prevalence of vCJD infected and asymptomatic individuals in the BSE exposed population remains extremely uncertain [7]. A first retrospective analysis of stored lymphoid tissues indicated that vCJD prevalence in the UK could approach 1 out of 4000 individuals, though with wide confidence intervals [8]. More recently 32,441 appendix samples, collected during surgery on patients born between 1941 and 1985 were tested for abnormal prion protein accumulation. This study indicated a

Author Summary

Variant Creutzfeldt Jakob Disease (vCJD) cases were identified in patients who received blood products that had been prepared from donors who later developed the disease. The blood borne transmission of vCJD is a major concern for blood transfusion banks, plasma derived products manufacturers and public health authorities. A vCJD blood screening test would represent an ideal solution for identifying donors/blood donations that might be at risk. In this study, we describe a blood assay which is based on the *in vitro* amplification of vCJD agent by Protein Misfolding Cyclic Amplification (PMCA). In vCJD animal models (sheep and primate), the assay enabled the identification of infected individuals in a very early stage of the asymptomatic incubation phase. We also provide evidence of the high specificity and the high analytical sensitivity of this assay using blood samples from vCJD affected and healthy patients.

likely vCJD prevalence estimate of 1 in 2,000 in these age cohorts (95% Confidence Interval ranging from 1 in 3,500 to 1 in 1,250) [9].

In addition, human PrP transgenic mouse models indicated that the BSE agent can colonize lymphoid tissues without propagating to detectable levels in the brain and causing clinical disease. This suggests the possibility of silent carrying by vCJD infected individuals [10].

This data raised major concerns about the possible occurrence of inter-individual iatrogenic vCJD transmission in particular by blood and blood products. Despite a decade of efforts, there is currently no validated test that would allow the identification of vCJD infected but asymptomatic individuals or the screening of blood donations for the presence of the vCJD agent [11].

There is currently limited information related to the infectivity level and distribution in the blood components of vCJD affected patients. Bioassay testing of blood fractions from a single vCJD affected patient indicated an infectious titer of 4.45 ID per mL of blood which was approximately equivalent to the infectivity found in 1 µg of a reference vCJD brain sample [12]. Such low infectious titer makes the direct detection of prion in blood difficult to achieve. Like in various TSE animal models (mice, hamsters, sheep and cervids), a substantial part of the infectivity in this patient was associated with white blood cells (WBC) [13–16]. This suggests that WBC could be an appropriate target to detect endogenous vCJD agent presence in human blood.

Prions are primarily composed of multimers of a misfolded form (PrP^{Sc}) of the host-encoded prion protein (PrP^{C}). They propagate by recruiting and converting PrP^{C} into PrP^{Sc} and fragmentation of PrP^{Sc} multimers is thought to provide new PrP^{Sc} seeds for the conversion reaction. The Protein Misfolding Cyclic Amplification (PMCA) technology is aimed at replicating this phenomenon *in vitro*, allowing amplification of minute amounts of prions [17]. It facilitates the combining of a PrP^{C}-containing substrate with previously undetectable amounts of PrP^{Sc} by repetitive cycles of incubation and sonication leading to amplification of abnormal PrP^{Sc}. With this potential high sensitivity, PMCA has been proposed for prion detection in blood, and studies have been carried out in scrapie-infected hamsters and sheep that validated the concept that blood associated PrP^{Sc} can be amplified by PMCA [14,18,19]. However, despite the ability to amplify brain-derived vCJD agent by PMCA, the reported amplification performance was considered too limited for reliable detection in blood [20,21].

In this study, we first identified PMCA substrate and conditions that allow highly efficient and specific amplification of the vCJD/BSE prions. We then show, using white blood cells as a template, that this method enables the identification of vCJD/BSE in asymptomatic experimental animals in the early phase of the incubation period.

Methods

Ethics statement

All animal experiments have been performed in compliance with institutional and French national guidelines, in accordance with the European Community Council Directive 86/609/EEC. Primates were housed and handled in accordance with the European Directive 2010/63 related to animal protection and welfare in research, under constant internal surveillance of veterinarians, in level-3 confined facilities entirely dedicated to prion research (agreement numbers A 92-032-02 for animal care facilities, 92-189 for animal experimentation), where cynomolgus macaque is the only housed animal species. Primates were placed in individual cages (a maximum of 20 cages per room) in six separate rooms, taking into account different parameters including the experiment they belong, their ages, their sex, their affinities to each other and their hierarchical status. Social enrichment was a constant priority, through individual activities and feeding according to the infectious risk. Animals were handled under anaesthesia (including blood sampling) to limit stress and avoid injury of handlers, and euthanasia (barbiturate overdose) was performed for ethical reasons when animals lost autonomy. The blood donor animals used in this study were included in experiments that were approved by the CETEA ethical committee (French Ethical Committee N°44, approvals 12-020).

Sheep were housed in level -3 containment animal care facilities (agreement numbers C-31-555-227 for animal care facilities, 31-09-555-47 for animal experimentation). The experimental protocol (oral challenge and blood collection) was approved by the Comité d'éthique Midi Pyrénées (ref MP/05/05/01/12).

Each healthy blood donor was individually informed and gave his/her written consent for using the collected samples in a scientific study. One vCJD blood sample included in the first panel of human blood samples was collected from a French patient at the clinical stage of the disease. According to French regulation, written informed consent for the use of this sample was obtained from the next of kin. Collection, storage and use of blood samples from vCJD patient included in this study was approved by national ethical authority (PHRC ref 2004-D50-353).

The use of the second human blood sample panel that was provided by the MRC Prion Unit, London, (United Kingdom) was approved by a UK national ethical committee (authorization number 03N/022). These samples were analyzed anonymously.

Finally, the experimental protocol on animals and the use of human samples was examined and approved by the INRA Toulouse/ENVT ethics committee.

Sheep oral inoculation with BSE

TSE free sheep were produced in the Defra 'New-Zealand flock' which was a unique source able to provide animals that can be considered free from classical scrapie [22]. The animals included in our experiments were imported into France and housed in dedicated scrapie free facilities before their use in experiments. In all cases, PrP genotype was obtained by sequencing the Exon 3 of the *PRNP* gene as previously described [23,24].

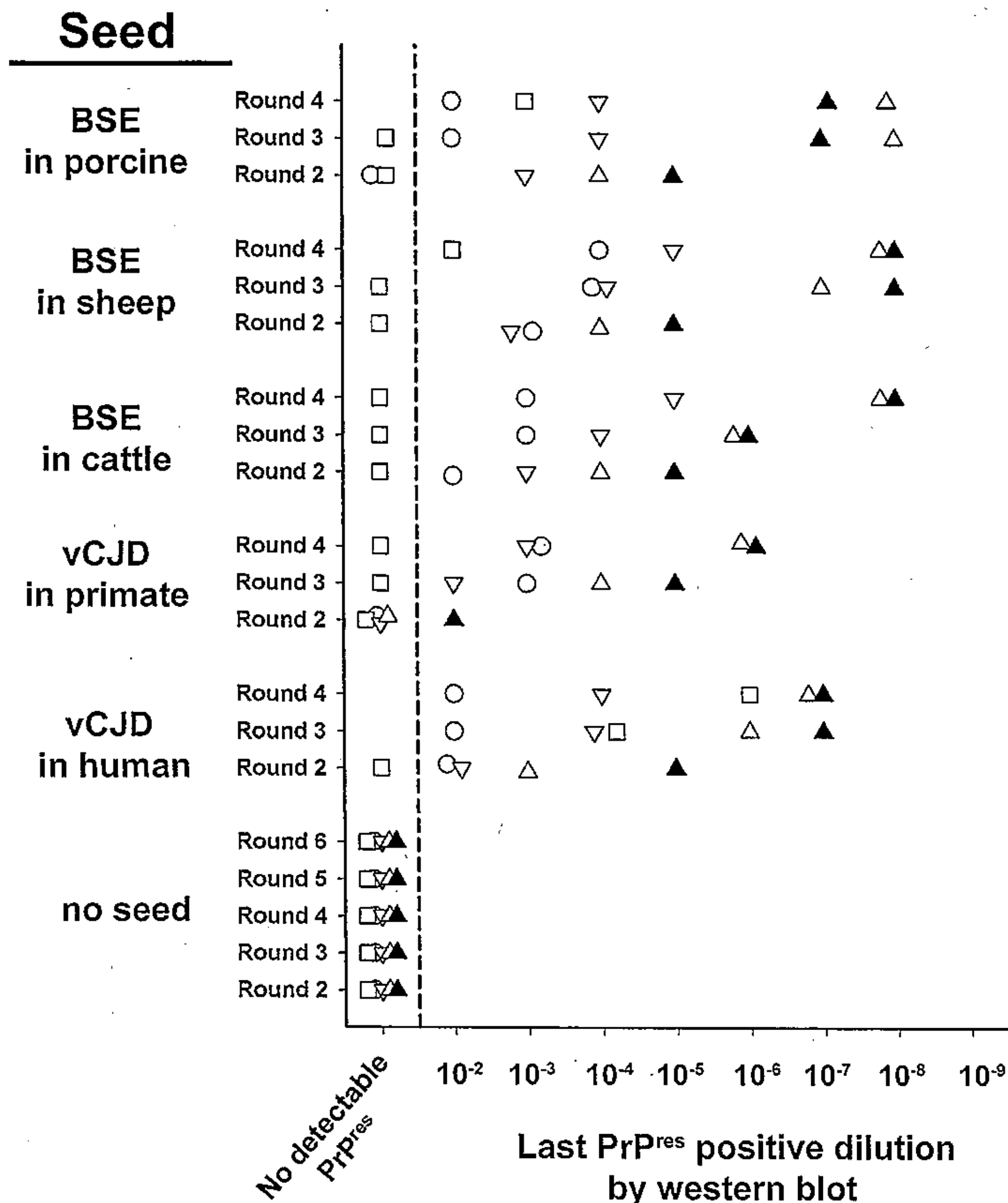

Figure 1. Relative performances of vCJD/BSE agent amplification by Protein Misfolding Cyclic Amplification using brain from transgenic mice expressing different species PrP sequences as substrate. PMCA reactions were seeded with ten-fold dilution series of vCJD/BSE brain material (10^{-2} to 10^{-9}) from different species (human, *cynomologus* macaque, bovine, ARQ sheep and porcine). PMCA substrates were prepared using brain from transgenic mice over-expressing either human (methionine 129 variant, □), bovine (▽), murine (○) or sheep (VRQ variant:△, ARQ variant:▲) Prion protein. Unseeded reactions were included as specificity control. PMCA reactions were then submitted to 2 to 6 amplification rounds each constituted with 96 cycles (30 s sonication-30 minutes incubation at 39.5°C) in a Misonix 4000 sonicator. After each round, (i) reaction products (1 volume) were mixed with fresh substrate (9 volumes) to seed the following round while (ii) a part of the same product was analysed by Western Blot (WB) for the presence of abnormal PK resistant PrP (PrP^{res} -antibody Sha31 epitope YEDRYYRE). For each round, the last dilution displaying a positive signal in WB is indicated on the graph.
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Four ARQ/ARQ sheep (6–10 months old) were orally challenged with 5 g equivalent of brain material (1% brain homogenate in glucose). The inoculum was prepared using brain from an ARQ/ARQ sheep experimentally challenged with cattle BSE. Animals were then observed until the occurrence of clinical signs and euthanized when exhibiting locomotor signs of the disease that impaired their ability to eat. White blood cells (WBC) from age and breed matched uninoculated TSE free ARQ/ARQ control animals (n = 60) were obtained by osmotic lysis of the buffy coat (one volume) with ACK solution (one volume) (NH_4Cl 0,15

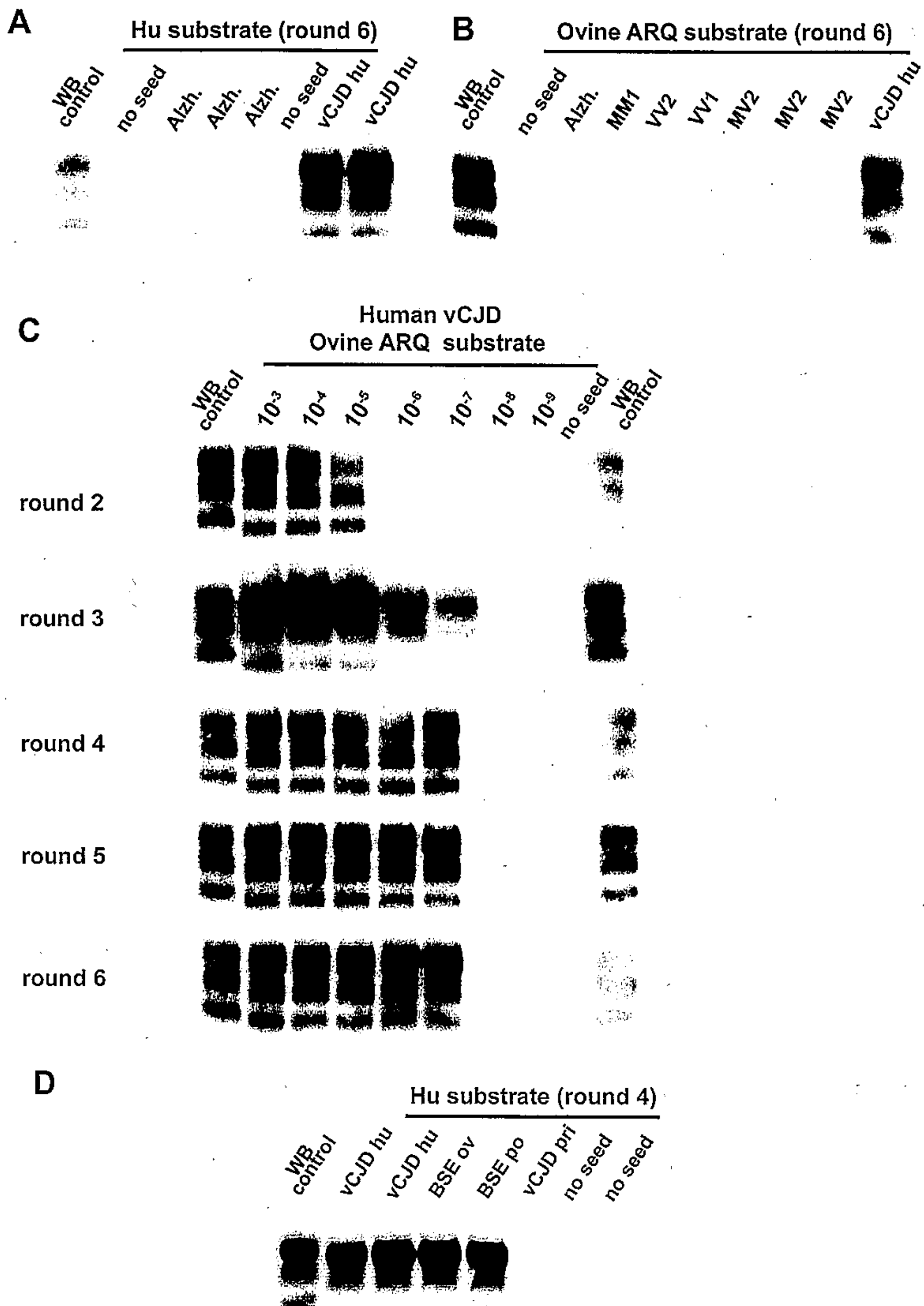
A
Hu substrate (round 6)
WB control
no seed
Alzh.
Alzh.
Alzh.
no seed
vCJD hu
vCJD hu
B
Ovine ARQ substrate (round 6)
WB control
no seed
Alzh.
MM1
VV2
VV1
MV2
MV2
MV2
vCJD hu
C
Human vCJD
Ovine ARQ substrate
WB control
10-3
10-4
10-5
10-6
10-7
10-8
10-9
no seed
WB control
round 2
round 3
round 4
round 5
round 6
D
Hu substrate (round 4)
WB control
vCJD hu
vCJD hu
BSE ov
BSE po
vCJD pri
no seed
no seed

Figure 2. vCJD/BSE, sCJD and Alzheimer seeded Protein Misfolding Cyclic Amplification reactions using brain from transgenic mice as substrate. (A–D) WB PrP^{res} detection in PMCA reactions seeded with diluted 10% brain homogenates from human vCJD patient (vCJD Hu) or sporadic CJD (sCJD MM1, MV1, MV2, VV2 and VV1), ovine BSE (BSE Ov), porcine BSE (BSE po), or vCJD in primate (vCJD pri). A Scrapie isolate (WB control) and vCJD in human (vCJD Hu) isolate were used as positive control in an immunoblot (Sha31 anti PrP monoclonal antibody: epitope: YEDRYYRE, amino acid 145–152). Unseeded reactions (no seed) and reactions seeded with brain homogenate (10^{-2} diluted, 10% - frontal cortex) from 3 Alzheimer affected patients (Alzh.) were included as specificity controls. The nature of the PMCA substrate and the number of amplification rounds are indicated.

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M, $KHCO_3$ 1 mM, Na_2EDTA 0,1 mM, pH 7.4) for 5 min RT. WBC were washed 3 times with 50 mL of PBS before being pelleted and stored at −80°C.

Control and vCJD infected primates

Captive-bred 2.5 year-old male cynomolgus macaques (*Macaca fascicularis*) were provided by Noveprim (Mauritius). Primates were checked for the absence of common primate pathogens before importation, and handled in accordance with national guidelines. One animal (Macaque 6) was transfused with 40 mL of blood from a vCJD-infected primate sampled at the terminal stage of the disease. The other primates were intravenously inoculated with clarified supernatants (obtained by centrifugation at 1,500 g for 10 minutes after extensive sonication) derived from 10 or 100 mg of brains from BSE- or vCJD-infected primates. Such intravenous inoculation route is likely to mimic the contamination as it occurs in post-transfusion vCJD secondary cases.

Primate blood samples were drawn into sodium citrate and fractionated by centrifugation at 2,000 g for 13 minutes according to the techniques classically applied in human transfusion. WBCs were obtained by osmotic lysis of buffy coat (one volume) with Easy-lyse (Dako, 9 volumes) for 10 minutes RT. WBCs were washed three times with 50 mL of PBS. Animals were handled under anesthesia to limit stress, and euthanasia was performed for ethical reasons when animals lost autonomy.

The majority of the blood samples used for vCJD agent detection were obtained from archive collections. No influence was possible on the design of blood sampling plans. The possibility of collecting multiple samples from each animal was limited by ethical constraints (reduction of stress to the primates). All samples were encoded before dispatch and tested blind. None of the primates that were involved in this experiment suffered from the myelopathic syndrome recently described in primates challenged with human and primates blood products [25].

Control and vCJD affected patients

In a first experiment related to human blood, WBC from 135 healthy volunteer human donors were prepared using the same protocol as in primates. In addition a vCJD blood sample collected in a French patient at clinical stage of the disease was tested. This blood sample was the same as the one used to measure vCJD infectivity in blood components by bioassay in a recently published study [12]. In this patient, vCJD was confirmed by both neuropathological examination and Western blot. All these samples were encoded before dispatch and testing.

In a second experiment, a panel of nine buffy coat samples was provided by the MRC Prion Unit (London, UK). This panel comprised material collected and prepared more than 10 years ago. It included three vCJD affected patients, and nine healthy patients. The blood volume that was used to prepare the buffy coat of each healthy patient varied between 3.5 to 8 mL. For one of the vCJD cases buffy coat samples were prepared using 3.5 mL of blood. In the two other vCJD cases the initial blood volume was undocumented. The nature of the anticoagulant used to collect the blood samples, the purity and the final number of WBC in the samples was not available. None of the vCJD samples that were included in this panel had been tested using the MRC vCJD blood assay described by Edgeworth et al. [26].

WBC were received as a frozen cell suspension (in 50 µL of PBS). They were re-suspended in 200 µL of PMCA amplification buffer before homogenization. The homogenates were then split in two and tested in parallel in INRA UMR 1225 (Toulouse, France) and INRA UR 892 (Jouy en Josas, France).

Brain material from vCJD (n = 4), sCJD (one MM1, one MM2, one VV1, one VV2, and one MV2) and Alzheimer's disease (n = 3) affected patients were obtained from the National Creutzfeldt-Jakob Disease Surveillance Unit (UK-Edinburgh) or from the French CJD national reference laboratories network [27].

For testing the inhibitory impact of red blood cells on vCJD amplification, red blood cells from a healthy human donor were separated from plasma and buffy coat by centrifugation (2000 g-13 min) and washed twice in PBS. Red cells were then submitted to two freezing/thawing cycles. The obtained red blood cell lysate was then used in the experiment.

PMCA substrate

Transgenic mice lines that express PrP^C of different species were used to prepare substrates: tgBov (Bovine PrP, line tg110), tga20 (murine PrP), tg338 (ovine $V_{136}R_{154}Q_{171}$ PrP), tgShXI (ovine $A_{136}R_{154}Q_{171}$ PrP variant) and tg650 (Human Met129 variant of the human PrP). All but the bovine PrP expressing mice (tgBov) were established on the same mouse PrP^{Ko} background (Zurich I) [28–30]. In each of these mouse lines relative PrP expression level in the brain, by comparison to the natural host species, was described (tga20: 10-fold–tg338: 6–8 fold, tgBov/tg110: 8-fold, tgShpXI: 3–4-fold, tg650: 6-fold) [28,31–34]

Mice were euthanized by CO_2 inhalation and perfused (intracardiac) with PBS pH 7.4/EDTA 5 mMol (40–60 mL per mouse). The brains were then harvested and snap frozen in liquid nitrogen. 10% brain homogenate was prepared (disposable UltraTurax – 3 min) in 4°C PBS pH 7–7.65+0.1% Triton X100+ 150 mM NaCl (10% Weight/vol). The substrate was then aliquoted and stored at −80°C.

In order to check the PrP^C protein level in the PMCA substrates, total protein from an aliquot of each type of substrate was quantified by bicinchoninic acid (BCA, Pierce). Five µg of proteins were mixed with an equal volume of 2X Laemmli's buffer before Western Blotting and PrP^C immunodetection (see Western blot section below, supplementary figure 1).

PMCA reaction and controls

The desired amount of WBC or Buffy coat were resuspended in 200 µL of 4°C PBS pH 7.4+150 mMoL NaCl+ 0.1 TRITON X100 and homogenized at high speed (Precess 48, Bertin, France). Samples were then spun down at 15000 g for 20 seconds and then stored at −80°C or used fresh. 7 µL of the seed were mixed with 63 µL of substrate in 0.2 mL ultrathin wall PCR tubes or 96 well microplates that contained five to eight 1 mm diameter silica/zirconium beads (Biospec Cat. No. 11079110z). Amplification was performed in a modified Misonix 4000 cup horn (see below), using

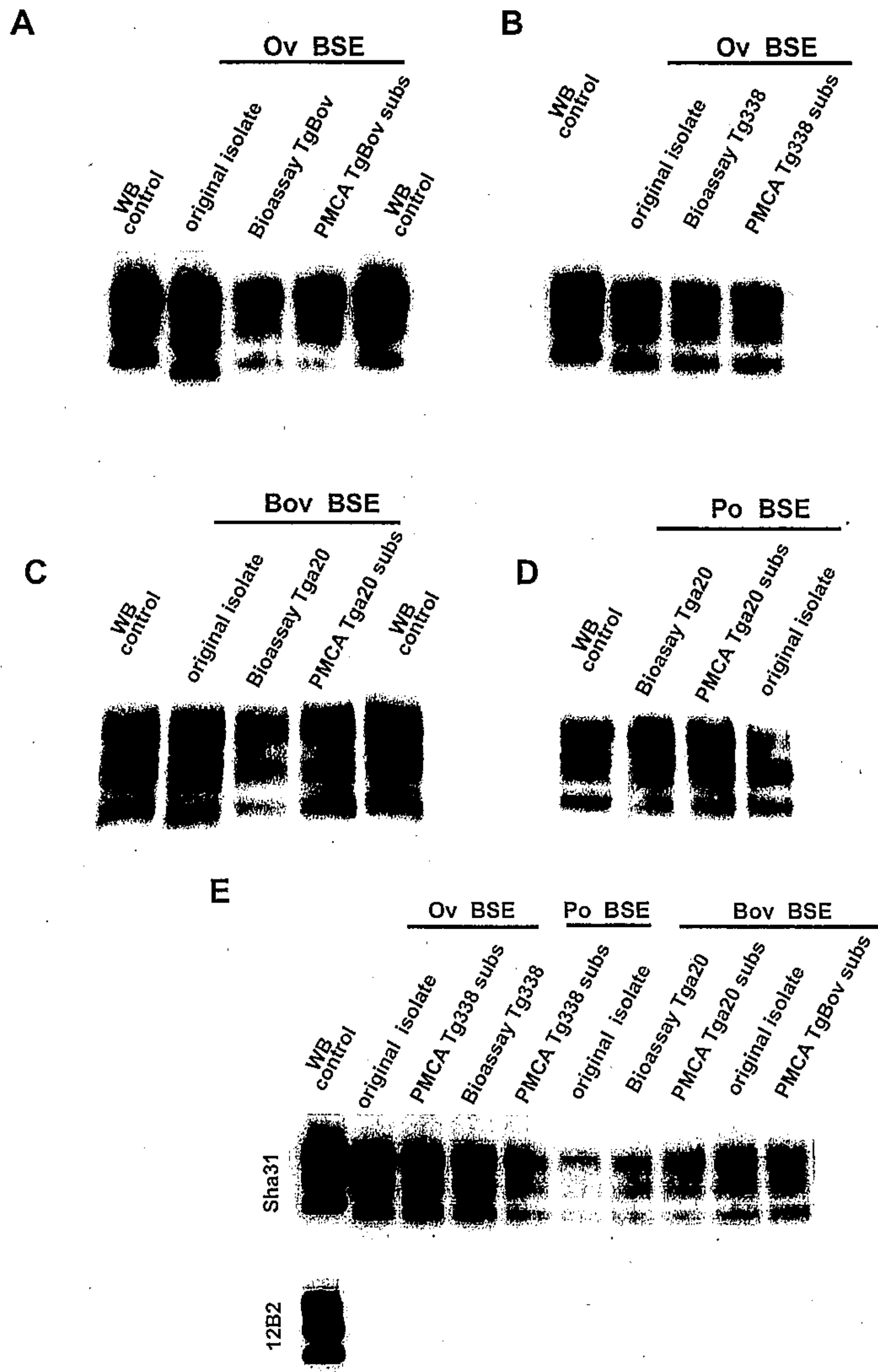

Figure 3. PrP[res] western blot (WB) profile of BSE originating from various species before and after PMCA amplification. PMCA reactions were seeded with BSE brain material (10^{-2} dilution) from different species (bovine, sheep and porcine). PMCA substrates (sub) were prepared using brain from transgenic mice over-expressing the bovine (tgBov/tg110), the murine (tga20) or the sheep VRQ variant (tg338) Prion

protein. PK digested abnormal PrP (PrP^{res}) WB profile of (i) the original isolate used for seeding the PMCA reaction, (ii) the product of the PMCA reaction and (iii) the brain of a mouse intracerebrally inoculated with the seeding isolate and belonging to the line used to prepare the PMCA substrate, were compared. **(A–D)** WB PrP^{res} signature using Sha31 anti PrP monoclonal antibody (epitope: YEDRYYRE, amino acid 145–152). **(E)** Relative immunoreactivity of Sha31 (epitope: YEDRYYRE, amino acid 145–152) and 12B2 (epitope: WGQGG, amino acid sequences 89–93) anti PrP monoclonal antibodies. In vCJD/BSE, the 12B2's epitope is cleaved by abnormal PrP PK digestion process. For this experiment, duplicate of each samples were submitted to abnormal PrP extraction before migration on two different gels and Western blotted. One of the WB membranes was probed with Sha31 while 12B2 was used for the second. On each gel a scrapie in sheep isolate was used as control (WB control).
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a water recirculation system (39.5°C). The reaction tubes/ microplates were then submitted to 96 cycles of 30 seconds sonication (power 70%) followed by a 29 minutes and 30 seconds incubation period.

After the PMCA round, 7 µL of the reaction product were added to a new tube containing fresh substrate and a new round (96 cycles) was performed. In order to limit the cross contamination risks that are linked to serial PMCA, procedures were employed that are similar to those in place for nested PCR. In particular, PMCA substrates, amplification and handling of amplified products were performed in different rooms using dedicated material.

On each PMCA run, a standard 1/10 dilution series (ovine BSE, 10% brain homogenate, 10^{-5} to 10^{-9} diluted) was included to check the amplification performance. A large batch of these controls was prepared and stored at −80°C as single use aliquots. Similarly unseeded controls (1 unseeded control for 5 seeded reactions) were included on each run.

A total of 68 PMCA runs were performed in the framework of this study. Contamination of some negative control reactions (false

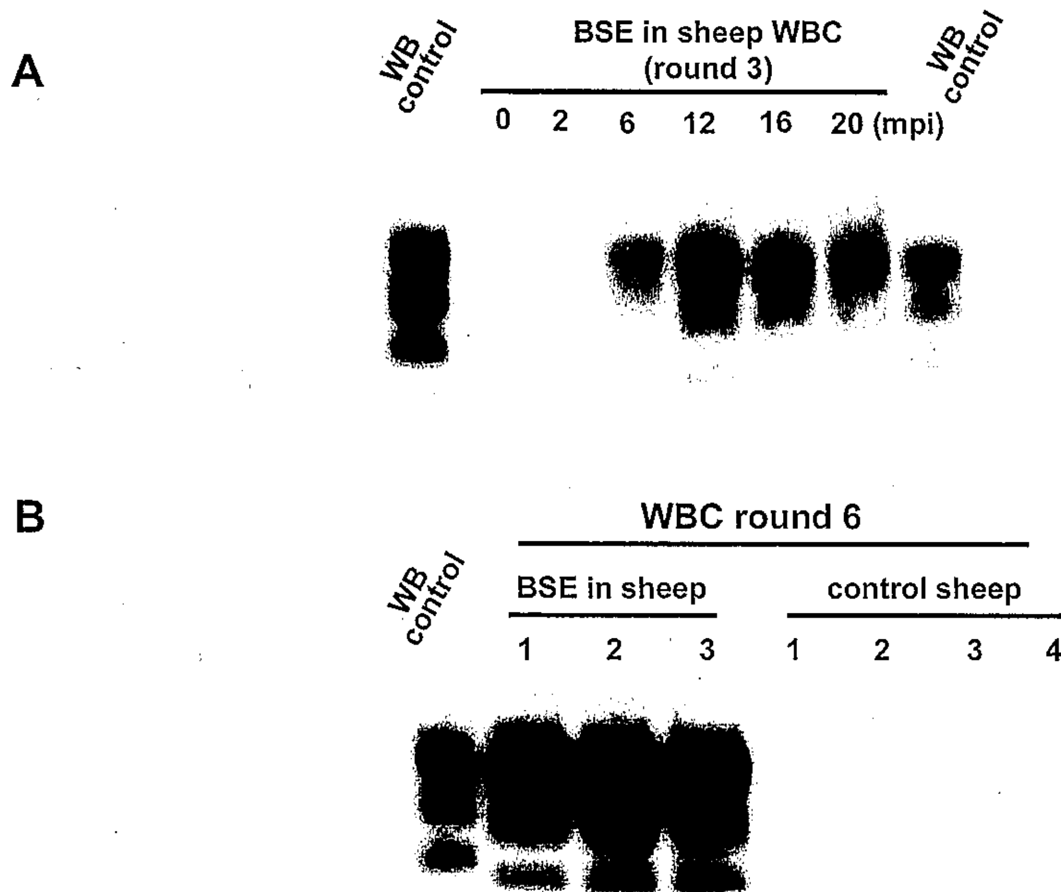

Figure 4. PrP^{res} detection in PMCA reactions seeded with white blood cells (WBC) from BSE infected and healthy sheep. WBC from BSE orally challenged and TSE free control ARQ/ARQ sheep were homogenised and used to seed PMCA reactions. Brain homogenate from ovine PrP transgenic mouse (ARQ variant) was used as PMCA substrate. Each sample was submitted to up to 6 rounds of amplification. Resulting PMCA products were analyzed by Western Blot (WB) for the presence of abnormal PK resistant PrP (PrP^{res} -antibody Sha31 epitope YEDRYYRE). On each gel a classical scrapie isolate (PK digested) was used as positive control (WB control). **(A)** In BSE orally challenged sheep (ARQ/ARQ), WBC prepared from blood collected at different time points (indicated as months post inoculation: mpi) of the incubation period were tested. The first clinical signs developed at 20 mpi. **(B)** BSE affected sheep (3 different individuals-20 mpi) and TSE free controls sheep (breed, genotype and age matched) were submitted to up to 6 PMCA rounds to check the specificity of the amplification.
doi:10.1371/journal.ppat.1004202.g004

Table 1. PrP^{res} detection in Protein Misfolding Cyclic Amplification (PMCA) reactions seeded with white blood cells from ARQ/ARQ sheep orally inoculated with BSE agent, collected at different time points of the incubation period.

	0 mpi				2 mpi				6 mpi				12 mpi				16 mpi				20 mpi			
	R2	R3	R4	R5	R2	R3	R4	R5	R2	R3	R4	R5	R2	R3	R4	R5	R2	R3	R4	R5	R2	R3	R4	R5
Sheep 1	-	-	-	-	-	-	-	-	-	-	-	-	-	2	4	4	3	4	4	4	4	4	4	4
Sheep 2	-	-	-	-	-	-	-	-	2	3	4	4	2	4	4	4	3	4	4	4	4	4	4	4
Sheep 3	-	-	-	-	-	-	-	-	-	2	4	4	1	4	4	4	4	4	4	4	4	4	4	4
Sheep 4	-	-	-	-	-	-	-	-	-	-	-	-	-	3	4	4	4	4	4	4	4	4	4	4

Four TSE free ARQ/ARQ sheep were orally challenged (before the age of 6 months) with 5 g of brain from BSE affected sheep. Blood was collected in these animals at 0, 2, 6, 12, 16 and 20 (clinical onset) months post inoculation (mpi). White blood cells (WBC) were obtained by osmotic lysis of the red blood cells. WBCs were then homogenized in PMCA buffer and homogenates were used to seed PMCA reactions in which the brain of transgenic mice that expressed the ARQ variant of the ovine PrP was used as substrate. Each sample was used to seed 4 independent reactions (two different runs onto 2 different sonicators). Five successive PMCA amplification rounds (R) were applied. After each amplification round, the number of PrP^{res} positive replicates (as assessed by Western blot) is indicated in the table. (-) indicated that all 4 replicates were negative.
(mpi): months post inoculation.
doi:10.1371/journal.ppat.1004202.t001

positives) was observed in 4 runs that had been performed in individual PCR tubes. In two of these runs, contamination was a likely consequence of a fault in the tube caps (obvious loss of reaction mixture in the tube). In two other cases the source of the contamination remained unclear, but the WB PrP^{res} pattern in false positive reactions was typical of a vCJD/BSE prion, making a cross contamination between tubes a likely explanation. No false positive reaction was observed in PMCA runs that were performed in 96 well PCR microplates. When a false positive was observed, the complete PMCA runs were discarded and restarted from the first amplification round.

Misonix 4000× Sonicator modifications

Modifications consisted of the enlargement (5 mm inner diameter) of existing holes and creation of new holes for water recirculation in the crown surrounding the plate horn. These holes allowed a closed water circulation system in the horn delivering over 1.5 liters per minute of water. Permanent water re-circulation was ensured by a peristaltic pump (Watson Marlow 520 U) and deflectors were added to the horn to avoid water projection. The water circuit consisted of 10 metres of flexible tygon tube (diameter 9.2 mm) placed in a water bath. This system allowed the temperature of the water in the horn to return to its nominal value (39.5°C) within 20–40 seconds following the sonication burst and also maintained the water level in the horn at a constant level.

The bottom of the reaction tubes or 96 well microplates were positioned at a height of 2 mm above the horn plate and the water level in the horn was adjusted (before each PMCA round) to be at the same level as the reaction mixture in the tubes. Finally the acoustic protection box containing the sonicator horn was placed in an environment (temperature regulated room or incubator) maintaining the air temperature between 35°C and 40°C (limit of condensation).

Western blot (WB) of abnormal PrP

PK resistant abnormal PrP extraction (PrP^{res}) and Western blot were performed as previously described [35], using a commercial extraction kit (Biorad, France). For PMCA products the equivalent of 20 µL of reaction product were loaded on to each lane. PrP immunodetection was performed using either Sha31 monoclonal antibody (0,06 µg per mL, epitope: YEDRYYRE, amino acid 145–152) or 12B2 (4 µg/mL) (epitope WGQGG, amino acid sequences 89–93). Both Sha31 and 12B2 antibodies have been described in previous studies to bind the mouse, ovine, bovine, porcine and human PrP^C and PrP^{res} in WB [27,36–39].

Results

The first goal of the study was to identify a substrate and experimental conditions that together would enable a highly efficient PMCA amplification of vCJD/BSE agent. For that purpose, brain material from different transgenic mouse lines expressing ovine ($A_{136}R_{154}Q_{171}$ and VRQ variants), bovine, human (Met_{129} variant) and murine PrP^C were used to prepare substrates. Reactions were then seeded with ten-fold dilution series of brain homogenate from vCJD/BSE-affected humans, primates, pigs, cows and sheep (figure 1).

After six PMCA rounds, no PK resistant abnormal PrP (PrP^{res}) could be detected by Western blot (WB) in un-seeded reactions (figure 1,2) or in those seeded with healthy brain material (data not shown). Whatever the substrate, no PrP^{res} was detected in reactions seeded with brain material from Alzheimer affected patients (figure 2A, B).

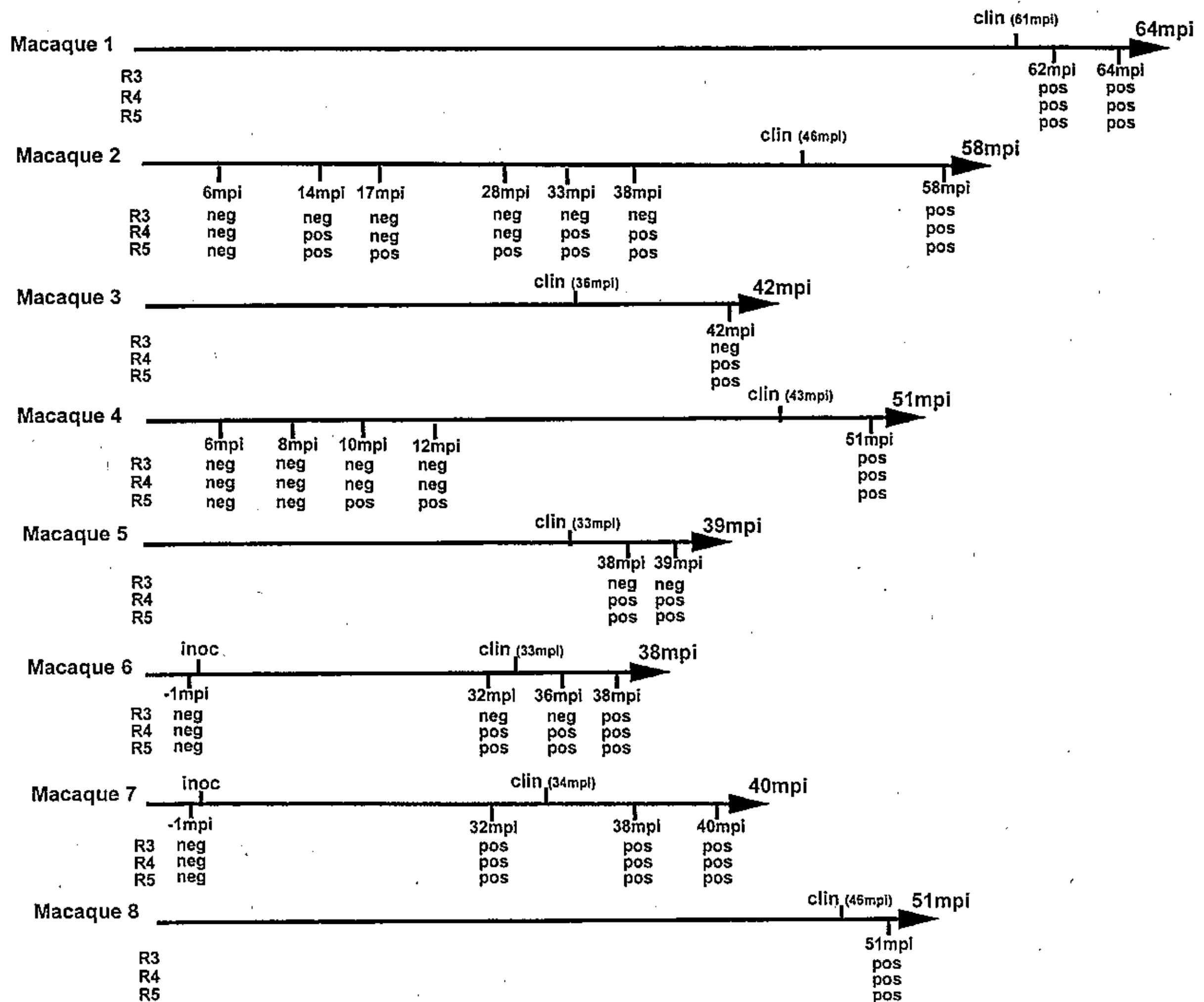

Figure 5. vCJD agent detection in the buffy coat of experimentally infected primates. Eight cynomologus macaques were intravenously challenged with vCJD brain homogenate or blood from a vCJD affected macaque (macaque 6). At different time points of the incubation period, blood was collected and buffy coat prepared. Clinical onset (clin) and time to euthanasia of the animals are indicated (upper label on arrows) as months post inoculation (mpi). The buffy coat samples were used (as homogenates 1/100 diluted in PMCA buffer) to seed PMCA reactions in which brain homogenate from ovine PrP transgenic mouse (ARQ variant) was used as substrate. Each sample was submitted to 6 rounds of amplification each comprising 96 cycles (30 s sonication-30 minutes incubation at 39.5°C) in a Misonix 4000 sonicator. PMCA products were analyzed by Western Blot (WB) for the presence of abnormal PK resistant PrP (PrP^{res} -antibody Sha31 epitope YEDRYYRE). Samples were received encoded and tested blind. The time point corresponding to blood samples (months post inoculation) that were tested and the results of PrP^{res} WB detection in PMCA reactions are indicated (under arrow). No positive WB result was observed before the third PMCA round. No additional positive result was observed after 5 PMCA rounds.

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All the tested substrates allowed the amplification of vCJD/BSE, but displayed dramatically different detection limits. Whatever the origin (species) of the BSE/vCJD agent, the ovine PrP substrates (ARQ and VRQ) provided the best detection performances, *i.e.* positive for reactions seeded with a 10^{-6} to 10^{-8} dilution of the original brain homogenates (figures 1, 2C). No amplification was observed in ovine substrate reactions seeded with sCJD brain homogenates (figure 2B). All these results support the view that the homology of PrP amino-acid sequence between the seed and the substrate may not be the crucial determinant for vCJD/BSE agent PMCA amplification.

Strikingly, the capacity of the human PrP substrate to amplify the vCJD/BSE agent varied greatly according the infectious source species (figure 2D). Human vCJD, porcine BSE and ovine BSE prions were amplified using human PrP as a substrate but in contrast vCJD/BSE from cattle or primates was barely or not amplified.

For all vCJD/BSE agent source/substrate combinations, the PrP^{res} WB pattern (glycoprofile and mobility) observed after PMCA amplification was indistinguishable from that observed in the brains of the transgenic mouse line used to prepare the PMCA substrate (figure 3A–D). In particular, the PrP^{res} obtained after PMCA displayed the same low/null immunoreactivity to 12B2 antibody (epitope WGQGG, amino acid sequences 89–93) as the original vCJD/BSE isolates (figure 3E). These results indicate that whatever the substrate, the amplified prion displays a PrP^{res} molecular signature consistent with BSE/vCJD.

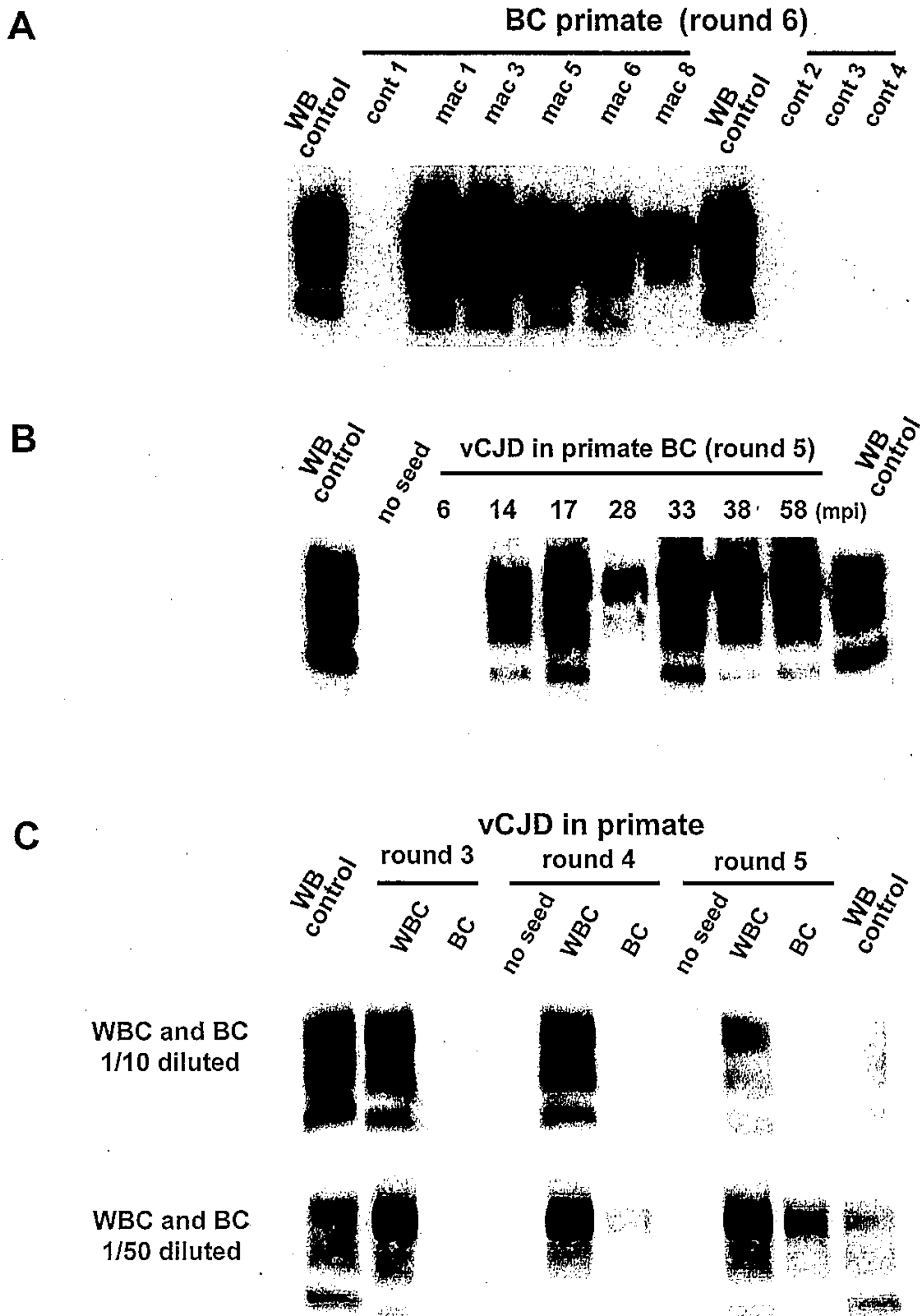

Figure 6. PrP^{res} in PMCA reactions seeded with blood samples from vCJD infected and healthy primates. Buffy coat samples (BC) or white blood cells samples (WBC) were prepared using blood volume collected from vCJD infected primates. Homogenized BC and WBC were used to seed PMCA reactions. In all PMCA brain homogenate from ovine PrP transgenic mouse (ARQ variant) was used as substrate. Each sample was

submitted to 6 rounds of amplification each comprising 96 cycles (30 s sonication-30 minutes incubation at 39.5°C) in a Misonix 4000 sonicator. The resulting PMCA products were analyzed by Western blot (WB) for the presence of abnormal PK resistant PrP (PrP^{res} -antibody Sha31 epitope YEDRYYRE). On each gel a classical scrapie isolate (PK digested) was used as a positive control (WB control). **(A)** BC from four different unchallenged cynomolgus macaques (cont) and 5 different vCJD affected primates (see figure 5) were blindly tested. The WB corresponds to the original one performed before decoding the samples after 6 PMCA rounds (3 controls and 4 infected animals). **(B)** BC samples (collected between 2005 and 2012) were prepared at different time points of the incubation period (indicated as mpi) in a vCJD inoculated primate (intravenous route, macaque 2 in figure 5). The animal developed clinical signs at 46 mpi and was euthanized at 58 mpi. **(C)** WBC and BC were prepared from the same blood sample collected in a clinically affected primate (macaque 6–38 mpi). Diluted WBC and BC were used to seed PMCA reactions (see table 2).
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In order to establish the capacity of the assay to detect endogenous vCJD/BSE agent in the blood, WBCs from 4 sheep orally challenged with BSE and 60 healthy control sheep were tested using the ovine ARQ substrate. In that experiment, the BSE infected sheep had developed disease 20 months post inoculation (mpi) (table 1). For all the symptomatic sheep, reactions seeded with WBC were shown to be positive after two PMCA rounds with a typical BSE PrP^{res} WB pattern. After four rounds, reactions seeded with WBCs collected at 6 mpi from some animals and at 12, 16 and 20 mpi from all animals were positive (table 1 and figure 4A). The WBC from the 60 TSE-free controls remained negative after 6 PMCA rounds (figure 4B).

These promising results enabled us to test blood samples collected from vCJD-infected primate experiments (figure 5). This model is considered to be the closest to infection in humans [2]. Buffy coat (BC-n = 33) and WBC (n = 14) obtained by red cell lysis of BC from vCJD-infected (n = 8) and control (n = 15) *cynomolgus* macaques were tested. The animals had been challenged by the intravenous route using either brain homogenate (n = 7) or blood from a vCJD-affected primate and developed the disease with incubation periods ranging from 33 to 61 months (figure 5). All samples were encoded before dispatch and were tested blind.

After 4 PMCA rounds, blood from all the clinically affected primates was positive (figures 5, 6A). All the reactions seeded with BC or WBC (n = 17) from unchallenged primates remained negative after 6 PMCA rounds (figure 6A).

In four vCJD-infected primates (macaques 2, 4, 6 and 7), BC had been collected at different times during the asymptomatic phase of the incubation period. The reactions seeded with BC collected from 10 mpi to 14 mpi (more than 32 months before clinical onset) were positive after five PMCA rounds (figures 5, 6B). These data indicate that vCJD infection can be detected in the early preclinical stage in primates.

The comparison of PMCA reactions seeded with BC and WBC prepared from the same blood samples suggested the presence of amplification inhibitor(s) in the BC (figure 6C and table 2). The negative effect of red blood cell presence on the vCJD amplification by PMCA was demonstrated by spiking a vCJD brain dilution series with red blood cell lysate (figure 7A). The addition of red blood cells resulted in a lack of amplification in reactions seeded with low dilutions of vCJD brain material (figure 7B, C). This loss of sensitivity in the vCJD amplification was not compensated by a higher number of PMCA rounds. However this inhibitory effect was compensated for/attenuated by diluting the red blood cell tainted seed in PMCA buffer prior to amplification. To limit inhibition, BC had to be diluted at least fifty-fold before being processed (figure 6C, table 2). This phenomenon could impact on the final sensitivity of the assay when applied to BC samples and could explain some of the negative results obtained in samples from asymptomatic but infected primates (figure 5 -macaque 2).

The results obtained in vCJD infected primates allowed access to a first panel of human blood samples that included WBCs from one French vCJD affected patient and 135 healthy controls. Samples were received encoded and tested blind. After 6 PMCA rounds, no PrP^{res} was detected in reactions seeded with WBC from human healthy controls (figure 8A). In contrast, two PMCA rounds (figure 8A) were sufficient to detect PrP^{res} in reactions seeded with the vCJD affected patient's WBC.

In order to test additional samples from vCJD infected patients we next contacted the MRC Prion Unit (London UK). They provided us with a panel of nine buffy coat samples that included three vCJD cases (confirmed by neuropathology and Western blot) and six healthy controls. The samples were received encoded and tested blind in two laboratories (UMR INRA ENVT 1225, Toulouse and UR 982 Jouy en Josas) using the same methodology.

In both laboratories, the PMCA results were identical. After six PMCA rounds no PrP^{res} was detected in reactions seeded with BC from the healthy controls. Two PMCA rounds were sufficient to detect PrP^{res} in PMCA reactions seeded with the buffy coat from two of the vCJD cases (figure 8B and 9A) However, even after these six PMCA rounds, no PrP^{res} was detected in reactions seeded with buffy coat prepared from the third vCJD affected patient.

The rarity of blood samples collected in vCJD affected patients and the lack of samples from infected patients at preclinical stage of the disease are two major limitations for the development and performance assessment of vCJD blood detection assays. To model the capacity of this assay to detect lower amounts of blood vCJD agent (as expected in patients at preclinical stage) a ten-fold dilution series of WBC and BC samples from the three positive vCJD patients was made.

Using the WBC sample from the French vCJD affected patient, three amplification rounds allowed PrP^{res} detection in one out of two PMCA reactions seeded with material equivalent to 0.05 µL of starting whole blood (figure 9A). Similarly, after three PMCA rounds, PrP^{res} was detected in reactions seeded with 10^{-3} to 10^{-5} diluted buffy coat homogenates from two UK vCJD affected patients (figure 9B). Under the assumption that 3.5 mL of whole blood were used to prepare these BC (see method), these results indicate that less than 0,5 nL of whole blood equivalent material was sufficient to detect endogenous vCJD agent in the blood of these two patients.

Finally, the WBC homogenate from the French vCJD affected patient were mixed with WBC from either eleven (8 different pools), twenty-three (4 different pools), forty-seven (2 different pools) or ninety-five (1 pool) healthy donors plus the WBC from the vCJD affected patient (figure 9C). After three PMCA rounds, reactions seeded using a pool constituted with up to forty-seven healthy donors plus the vCJD affected patient's WBC were PrP^{res} positive. All the reactions seeded with pools containing only WBC from healthy donors were negative.

Discussion

Cell free conversion assays have been extensively used to investigate PrP^{Sc} induced PrP^{C} to PrP^{Sc} conversion. Combinations of PrP^{Sc} and PrP^{C} from different species have provided insight into the molecular basis for barriers to the transmission of TSEs

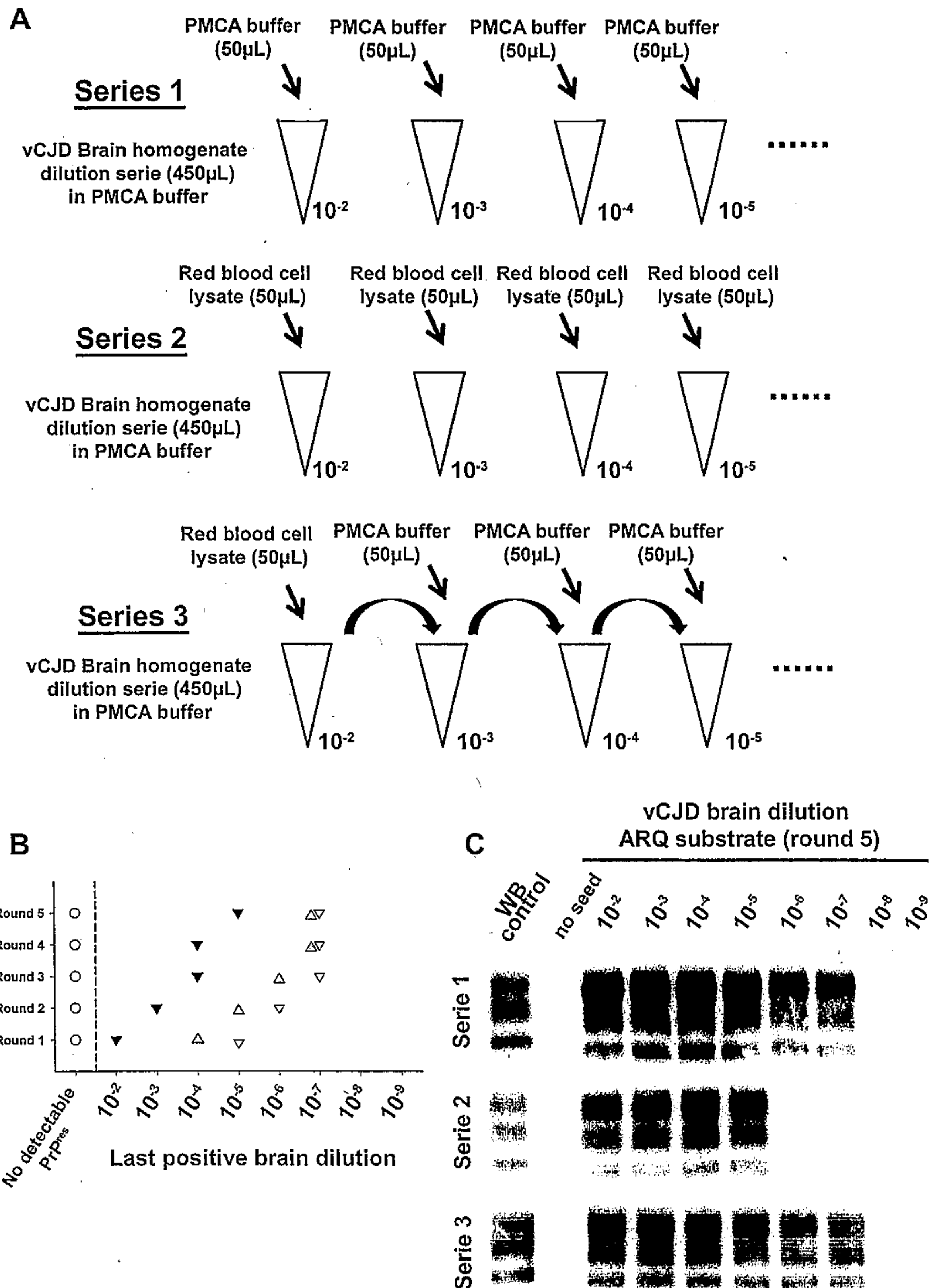

Figure 7. Red blood cell and inhibition of PMCA vCJD amplification. (A) Two vCJD brain homogenate (human) dilution series (1/10 dilution,

10^{-2} to 10^{-9}) were prepared (aliquots of 450 µL). Each aliquot also contained 50 µL of PMCA buffer (series 1: ▽) or Red blood cell lysate (human) (series 2:▼). A third series (series 3: ▲) was prepared starting from a 10^{-2} dilution of the vCJD brain homogenate (900 µL) in which 100 µL of red blood cell lysate have been added. In each aliquot (450 µL) of dilution series 3, 50 µL of PMCA buffer were added. The three dilution series were then used to seed PMCA reactions (7 µL of seed) in which ovine PrP expressing mice (ARQ variant) was used as substrate (63 µL). Five successive rounds of PMCA were performed. After each round PrP^{res} detection was carried out in PMCA reactions by Western blot (Sha31 anti PrP monoclonal antibody: epitope: YEDRYYRE, amino acid 145–152). Ten unseeded controls (O) were included in the experiment. The results of the PMCA amplification after each round are presented in graph (B). WB corresponding to the fifth round of amplification is presented as an illustration (C).
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between species (species barriers) and same-species hosts with different PrP genotypes (polymorphism barriers). Results obtained in this system, indicate that the reactions between PrP^{Sc} and PrP^{C} molecules of the same sequence are more efficient than heterologous sequence conversion. These results provided strong support for the concept that the sequence specificity in the conversion of PrP^{C} to PrP^{Sc} modulates the interspecies or intraspecies transmissibility of TSE agents [40–46].

The results obtained here when amplifying by PMCA vCJD/BSE agents originating from different species are not fully consistent with those findings. The observation that human PrP^{C} substrate support better PrP^{Sc} amplification when seeded with human vCJD agent than with any other source of vCJD/BSE agents, and that the murine PrP^{C} substrate was poorly efficient at amplifying non-murine vCJD/BSE agents, concur with the general conclusions derived from cell free conversion assay. However, the fact that whatever the considered source of vCJD/BSE agent (human, bovine, porcine etc...), the Q_{171} ovine PrP^{C} substrates provide better amplification than homologous PrP^{C} sequence substrates was unexpected.

PrP^{Sc} amplification levels in cell-free conversion assays remain very limited. This is a likely consequence of the fact that the newly formed PrP^{Sc} has either no or very limited seeding activity in this type of assay. In PMCA each sonication cycle is believed to create new seeding sites, including in the bulk of newly converted PrP^{Sc}. These new seeds have the same amino acid sequence as the PrP^{C} substrate and therefore the efficacy of the PrP^{C} conversion could be enhanced. These differences might explain the discrepancies between our results and those previously reported using cell free conversion assay.

In addition, it is worth noting that whereas conventional mice are poorly susceptible to sporadic Creutzfeldt Jakob, they propagate variant CJD isolates prepared from patients displaying identical (Methionine homozygous at codon 129) PrP^{C} sequence [47]. This illustrates that rather than depending solely on the donor/recipient host PrP sequence homology the capacity of a prion to propagate efficiently in a host and in PMCA is also directly dependant of its strain properties. Similarly while human PrP substrate supported amplification of BSE adapted in ARQ sheep in PMCA, it did not allowed the amplification of ARQ sheep scrapie [20,48]. This phenomenon could also contribute to an explanation for the results we obtained when amplifying vCJD/BSE by PMCA in different substrates.

Whether, at the molecular level, the species specificity of PMCA faithfully mimics the species barrier as observed in 'living hosts' remains to be thoroughly assessed. Interestingly prion strains amplified by PMCA using a homologous PrP amino acid sequence as the substrate share identical biological properties to the parental strain, e.g. in bioassay [49]. In addition, propagation of a prion by PMCA using a substrate with a heterologous PrP sequence, can result in an evolution of its strain properties identical to that observed after *in vivo* propagation of this strain in the heterologous host (*i.e.* PMCA can reproduce the transmission barrier phenomenon) [50]. Here, the vCJD/BSE agent amplification obtained with different PMCA PrP^{C} substrates paralleled to some extent the propagation efficiency already reported *in vivo*. For instance, ovine BSE propagates with an apparently similar efficiency to cattle BSE prions in bovine transgenic mice (tgBov) [36] and with an higher efficiency in human transgenic mice (tg650) [51]. BSE/vCJD agents propagate with little or no transmission barrier in transgenic mice expressing the ovine ARQ PrP [33,52,53] and can be passaged in those expressing the ovine VRQ PrP variant (tg338 mice) [54].

However, in our opinion, there are still missing elements to establishing whether the PMCA amplification efficiency of an isolate/substrate combination is systematically correlated to the corresponding bioassay sensitivity. In this context, a end-point titration of the vCJD/BSE isolates used in the different transgenic PrP mouse lines (tga20, tgBov, tg338 and tgShXI) has been initiated.

Despite the limited number of vCJD clinical cases observed so far (n = 177) in the United Kingdom, the most recent epidemiological studies indicate that, in this country, 1 out 2000 people could carry the vCJD agent. In the absence of validated vCJD screening assay, UK like most of the developed countries apply systematic measures aiming at mitigating the blood borne transmission risk of the disease. These measures represent a substantial cost and increase the difficulty met by the blood banking system to provide certain blood products. In that context the added value from a vCJD blood detection assay is obvious.

The absence of human blood samples that would have been collected in infected individuals at asymptomatic stage of the disease represents a major limitation for developing and validating such assay. Using the two animal models that are considered as the most relevant for vCJD agent infection (sheep and primates), our study demonstrates that an assay based on the *in vitro* amplification of BSE/vCJD by PMCA allows an early and specific detection of infected animals.

The blind testing of two sample panels, that included a limited number of vCJD cases (n = 4) and a substantial number of healthy controls (n = 141), provides evidence that PMCA can be used for detecting vCJD agent in blood in human. These results also demonstrate the very high sensitivity of the PMCA method for detecting the endogenous vCJD agent associated to WBC/buffy coat in three vCJD affected patients, as detection can be possible with the equivalent of 0.5 nL of infected whole blood. However, despite its sensitivity, our assay failed to amplify PrP^{res} in the reactions seeded with buffy coat from one of the vCJD affected patient.

This failure might be the consequence of several non-exclusive phenomena. First, it might be due to the sample processing. Indeed our experiments in vCJD infected primates clearly highlighted that buffy coat can contain PMCA inhibitors

Alternatively, this negative result might be due to a lower or absent prionemia in certain vCJD affected patients. This explanation would fit with the results reported by the MRC unit in the UK using a different vCJD blood detection assay. Rather than amplifying abnormal PrP, this assay is based on the capture of non PK digested disease-associated PrP on a solid-state binding matrix. Like our PMCA method, the MRC vCJD blood assay

Table 2. PrP^{res} detection results in PMCA reactions seeded with white blood cells (WBC) or buffy coat (BC) from *Cynomologus* macaques clinically affected with vCJD.

		1/10				1/50				1/100			
		R2	R3	R4	R5	R2	R3	R4	R5	R2	R3	R4	R5
Macaque 6	**WBC**	−	+	+	+	−	+	+	+	−	+	+	+
	BC	−	−	−	−	−	−	+	+	−	−	+	+
Macaque 8	**WBC**	−	+	+	+	−	+	+	+	−	−	+	+
	BC	−	−	−	+	−	−	+	+	−	−	+	+

Cynomologus macaques were intravenously challenged with (i) blood from a vCJD affected macaque (macaque 6) or (ii) human vCJD brain homogenate (macaque 8). WBC and BC were prepared from the same blood sample (5 mL citrate dextrose anticoagulant) collected from the clinically affected primate (just prior euthanasia); macaques 6 and 8 were respectively euthanased at 38 months post inoculation (mpi) and 51 mpi and sampled at that time (see figure 4).
WBC and BC were homogenized and then diluted 1/10, 1/50 and 1/100 in PMCA buffer before seeding PMCA reactions. Brain from transgenic mice that expressed the ARQ variant of the ovine PrP was used as substrate. Samples were submitted to 5 successive rounds (R) of amplification. PrP^{res} detection was carried in each PMCA reaction by Western blot (WB) using Sha31 anti PrP antibody (epitope epitope: YEDRYYRE, amino acid 145–152).
doi:10.1371/journal.ppat.1004202.t002

displayed an excellent analytical sensitivity and specificity. However about a third of the vCJD blood samples tested so far were score negative (6 out the 21 vCJD affected cases) [26,55,56]. The idea of a lower/absence of prionemia in certain vCJD cases is also indirectly supported by the observations recently reported by Mead et al. This author reported that in a vCJD affected patient that was negative using the MRC vCJD blood detection assay, the lympho-reticular tissues displayed unusually low PrP^{Sc} accumulation levels [57]. Beyond this, a low level or an absence of infectivity in the blood of certain vCJD infected patients could also explain the lack of disease transmission observed so far in certain patients who received blood from donors who later developed vCJD [58].

To date, the presence of vCJD endogenous infectivity in human blood has been formally established (bioassay) in a single affected patient [12]. In that context, measuring through bioassay the infectivity level in blood from a panel of vCJD affected patients (including if possible vCJD blood samples that were scored negative for PrP^{Sc} presence) would be highly valuable.

For more than a decade PMCA has been reputed to be a highly sensitive but unreliable technique [59]. Even if there is still a need for standardisation of protocols and for an optimisation of hardware, in our opinion, the reliability of the technique has now reach an acceptable level. Moreover, the recent progress in the miniaturisation of the method [60] and the demonstration that brain homogenate can be replaced by cell lysate [61] should further facilitate the use of this technique.

Over the last few years alternative methods to PMCA for *in vitro* amplification and detection of prions have been developed. The quaking induced conversion (QuIC) and the real time QuIC (RT-QuIC) are based on fibrillation of a recombinant PrP (rec-PrP) substrate triggered by the presence of a minute amount of PrP^{Sc} [62,63]. QuIC already allowed highly sensitive detection of abnormal PrP^{Sc} in various biological fluids and some studies reported its capacity to detect brain derived vCJD PrP^{Sc} in plasma [64–66]. The possibility of using bacterial rec-PrP and the apparent simplicity of these methods are quite attractive. However, at this stage, in case of a positive reaction, the assay does not offer the opportunity to confirm directly the nature of the TSE agent that triggered conversion. In contrast, since PrP^{Sc} amplified with PMCA has all the biochemical characteristics of the original seed (in our case BSE/vCJD) this method allows the direct identification of the vCJD agent signature in positive reactions.

Despite all the remaining difficulties, the results obtained so far by two different methodologies (PMCA as presented here and the abnormal PrP capture), and the rapid progress of QuIC derived technologies, allow potential new possibilities for vCJD screening and the prevention of its iatrogenic transmission.

Supporting Information

Figure S1 PrP^C in PMCA substrate prepared using brain from different mouse lines. Total proteins from an aliquot of each type of PMCA substrate were quantified and five µg of proteins were mixed with an equal volume of 2X Laemmli's buffer before Western blotting and PrP^C probing using Sha31 antibody (epitope YEDRYYRE).
(TIF)

Acknowledgments

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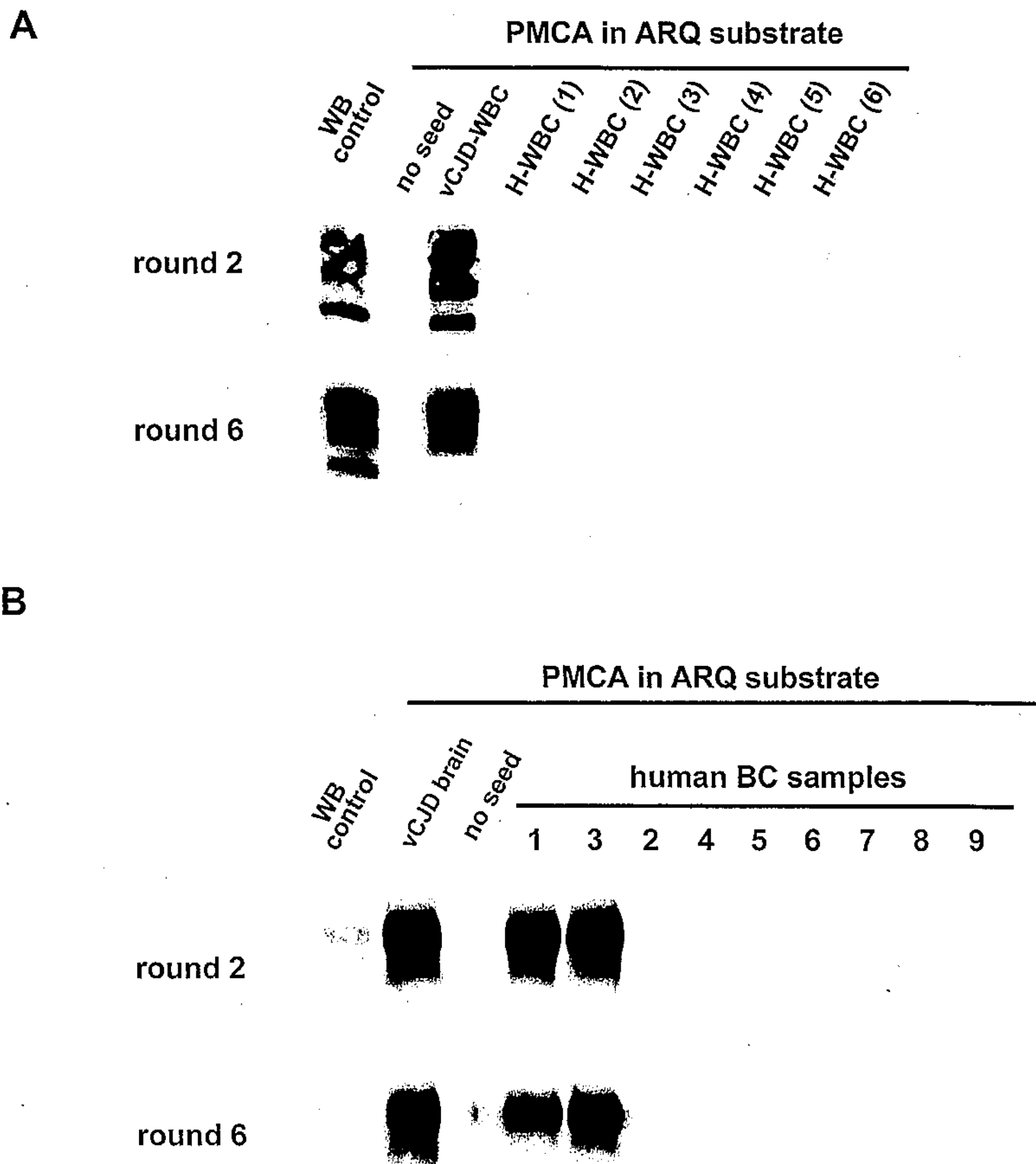

Figure 8. PrP^{res} in PMCA reactions seeded with WBC or BC from a vCJD affected patients and healthy controls. (A) WBCs from a French vCJD affected patient (vCJD-WBC) and healthy controls (H-WBC) were used to seed serial PMCA amplification (six rounds). PMCA controls included unseeded reactions (no seed). (B) Similarly, nine human buffy coat samples (received from the MRC Prion Unit (London, UK) were used to seed serial PMCA amplification (six rounds). The panel included three vCJD affected patients (sample 1, 3 and 8) and six healthy controls. A vCJD brain homogenate (10%, 10^{-8} diluted) was used as positive amplification control. In all cases brain homogenate from ovine PrP transgenic mice (ARQ variant) was used as substrate. PMCA products were analyzed by Western blot (WB) for the presence of abnormal PK resistant PrP (PrP^{res} -antibody Sha31 epitope YEDRYYRE). On each gel a classical scrapie isolate (PK digested) was used as positive control (WB control).
doi:10.1371/journal.ppat.1004202.g008

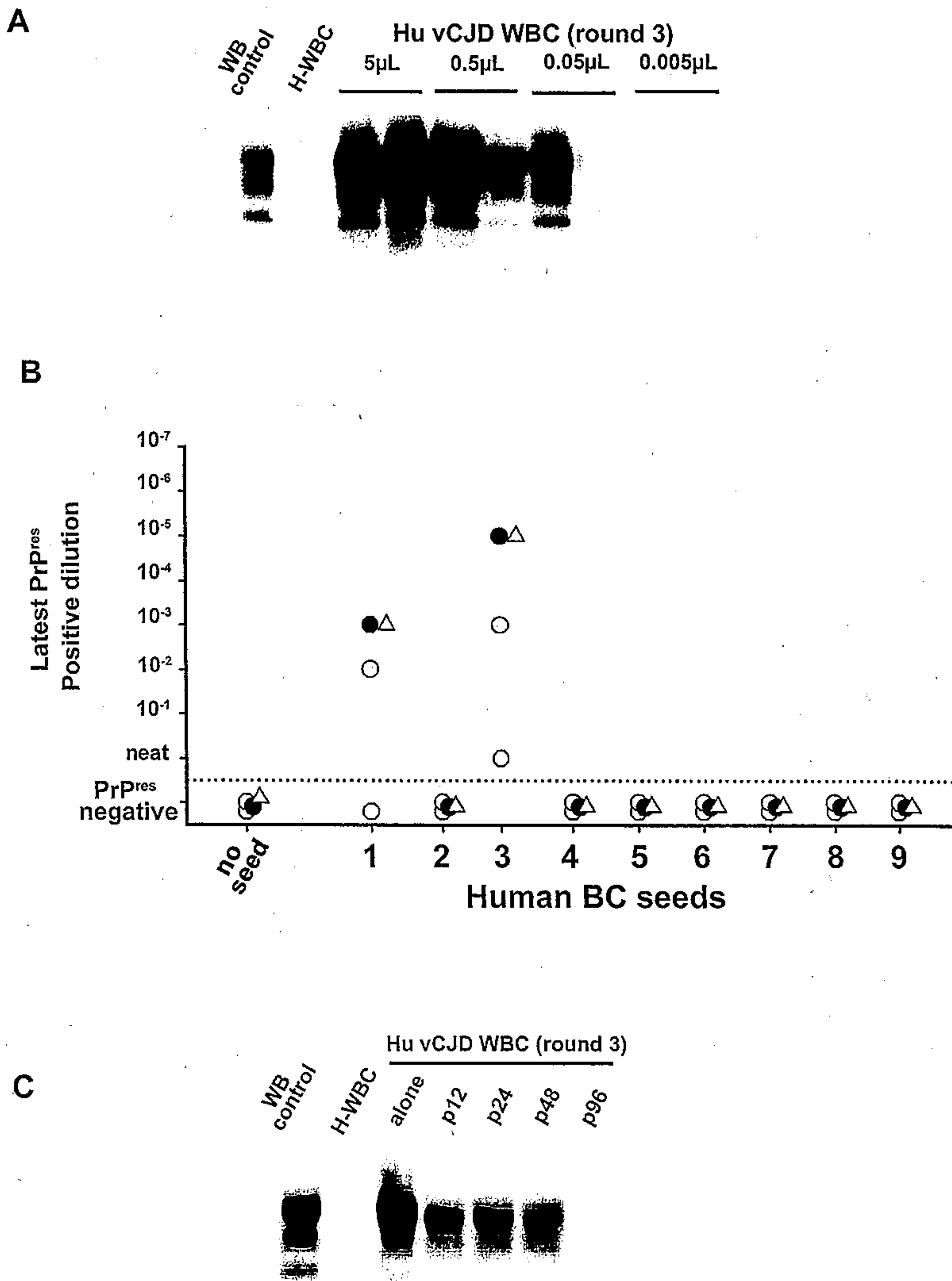

Figure 9. Analytical sensitivity of endogenous vCJD agent PMCA detection in the blood of affected patients. 1/10 dilution series (in PMCA buffer) were prepared using (A) WBC homogenate from one French vCJD affected patient and (B) buffy coat homogenates from two UK vCJD

affected patients. These dilutions series were used to seed serial PMCA amplifications (up to 6 rounds) using brain homogenate from ovine PrP transgenic mouse (ARQ variant) as substrate. After each round, PMCA products were analyzed by Western Blot (WB) for the presence of abnormal PK resistant PrP (PrP^{res} - antibody Sha31 epitope YEDRYYRE). On each gel a classical scrapie isolate (PK digested) was used as positive control (WB control). **(A)** WBC homogenate from the French vCJD affected patient (Hu vCJD WBC) and healthy patients (H-WBC) were submitted to three amplification rounds. Each dilution was tested in duplicate. The equivalent whole blood amount used to seed the reactions is indicated in the figure. **(B)** PrP^{res} WB detection after the first (white circle), the second (grey circle), the third (black circle) and the sixth (white triangle) round of PMCA. Reactions were unseeded (no seed) or seeded with serial 1/10 dilution of the nine BC samples provided by the MRC unit (London, UK). **(C)** WBC from the French vCJD affected patient was tested either alone or after pooling with WBC from 11 (p12), 23 (p24), 47 (p48) or 95 (p96) healthy controls. The WBC homogenates used to prepare pools were equivalent to 50 µL of starting whole blood. Reactions seeded with WBC from healthy controls (H-WBC) were included as controls.
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Author Contributions

Conceived and designed the experiments: EC APL HS VB DV OA. Performed the experiments: CLa MM SL CLi CJD IL VB MG GF PC NS FL JPD DV OA. Analyzed the data: EC VB DV OA. Contributed reagents/materials/analysis tools: EC APL HS MG JPD. Contributed to the writing of the manuscript: CLa EC HS VB DV OA.

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No. 3

医薬品 研究報告 調査報告書

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研究報告の概要

○三重県で発症した野生動物肉摂食歴のない4型E型肝炎ウイルス(HEV)愛知静岡株による急性E型肝炎の1例
静岡県西部から愛知県、岐阜県に及ぶ地域で野生動物肉摂取後にE型肝炎を発症した症例や、この地域の野生動物から塩基配列が極めて類似した遺伝子型4型のHEV株が多数分離され、4型愛知静岡株などと呼称されている。
三重県桑名市で農業に従事する69歳男性が、2013年10月初旬より全身倦怠感を自覚し受診したところ、急性肝障害を指摘され入院した。IgA-HEV抗体、IgM-HEV抗体、IgG-HEV抗体、HEV-RNA陽性と他のウイルスマーカーなどの結果から急性E型肝炎と診断し、治療により第29病日に肝機能障害は改善し退院した。患者から検出されたHEV-RNAの遺伝子配列解析を行ったところ、4型愛知静岡株と100%のbootstrap値でクラスターを形成した。このクラスターに属する株との塩基配列の相同性は97.8%以上であった。
患者に特記すべき既往歴、輸血歴、針治療歴はなく、発症約4カ月前の北欧諸国渡航時及び発症前の3カ月以内に野生動物肉の摂食歴もない。ブタ肉の喫食歴は頻繁にあったが、ブタレバー、ブタホルモンの喫食歴はなく、喫食したブタ肉は桑名市内のスーパーマーケット2軒で購入し自宅で調理して食べていた。
本症例は野生動物との関連が確認できず、発症前3カ月以内には愛知県、岐阜県、静岡県に行ったこともなく、三重県内で感染したことが強く疑われる。感染源、感染経路は今のところ不明であるが、4型愛知静岡株が野生動物以外にも広がり、その感染源が三重県にまで及んでいることは重大で、その同定にはさらなる症例の蓄積による情報収集が必要と考えられる。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
三重県で、野生動物の摂食歴がない者に4型E型肝炎ウイルス愛知静岡株による急性E型肝炎症例が発生したとの報告である。	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。今後もHEV感染の実態に関する情報の収集及び安全対策に努める。

12

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＜短　報＞

三重県で発症した野生動物肉摂食歴のない4型E型肝炎ウイルス愛知静岡株による急性E型肝炎の1例

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緒言：静岡県西部から愛知県，岐阜県に及ぶ地域で野生動物肉摂取後にE型肝炎を発症した症例や，この地域の野生動物から塩基配列が極めて類似した遺伝子型4型のE型肝炎ウイルス（HEV）株が多数分離されており，この地域の野生動物に特有な4型愛知静岡株などと呼称されている[1)~3)]．またこの株の感染では比較的重症化することも報告されている[2)]．今回我々はイノシシ，シカなどの野生動物肉を摂食していないにもかかわらず4型愛知静岡株によって急性E型肝炎を発症した症例を愛知県，岐阜県に隣接した三重県で経験した．

症例：69歳男性．三重県桑名市在住で農業に従事．既往歴に特記すべき事項なし．輸血歴，鍼治療歴なし．

Table　Laboratory data on admission

CBC		Chemistry		Viral Marker	
WBC	5,500 /μl	TP	7.7 g/dl	HBs Ag	(−)
neutro	54.6 %	Alb	4.2 g/dl	HBs Ab	(−)
lymph	27.1 %	T-Bil	7.6 mg/dl	HBc Ab	(−)
eosino	3.9 %	D-Bil	5.8 mg/dl	HBc-IgM Ab	(−)
baso	0.6 %	AST	3948 IU/l	HBV-DNA	(−)
mono	13.8 %	ALT	2726 IU/l	HCV Ab	(−)
aty-lym	0.0 %	LDH	3153 IU/l	HCV-RNA	(−)
RBC	522×10^4 /μl	ALP	429 IU/l	HAV-IgM Ab	(−)
Hb	16.9 g/dl	γ-GTP	1419 IU/l	CMV-IgM Ab	(+)
Ht	49.8 %	BUN	15.5 mg/dl	CMV-IgG Ab	(−)
PLT	16.6×10^4 /μl	Cre	0.74 mg/dl	CMV Ag (C7-HRP)	(−)
		Glu	316 mg/dl	EBV VCA-IgM Ab	(−)
Coagulation		HbA1c	9.3 %	EBV VCA-IgG Ab	(+)
PT%	90 %			HEV-IgA Ab	(+)
PT-INR	1.07	Immunochemistry		HEV-IgM Ab	(+)
		CRP	4.0 mg/dl	HEV-IgG Ab	(+)
		ANA	(−)	HEV-RNA	(+)
		AMA	(−)		

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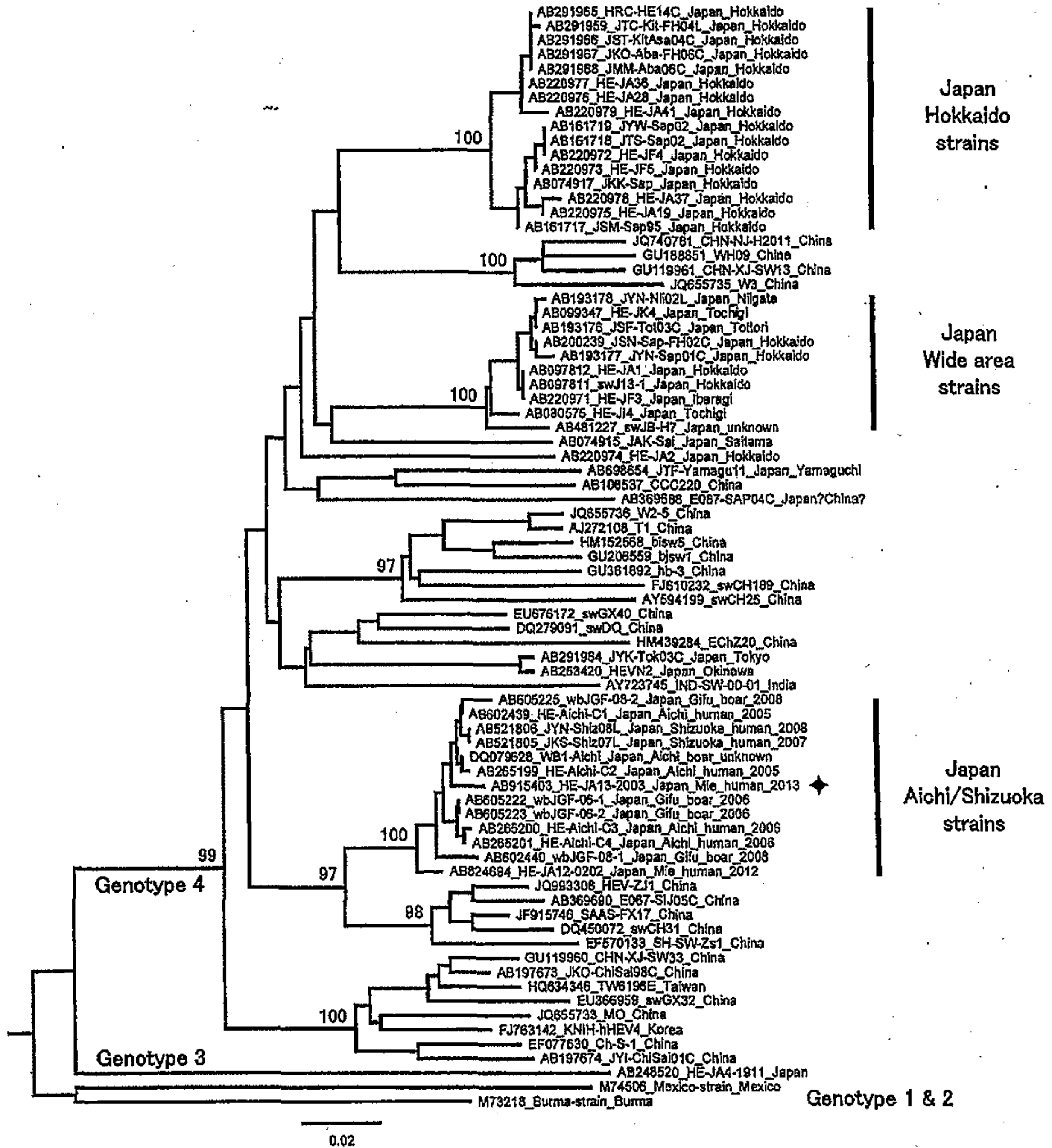

Fig. Phylogenetic tree constructed by the neighbor-joining method based on the partial nucleotide sequence (412 nt) of the ORF2 region of 77 human, wild animals and swine hepatitis E virus (HEV) isolates. In addition to the HEV isolate obtained in the present study, which are indicated by '✦', one each of the prototype HEV of genotype 1, 2 or 3, and 73 genotype 4 HEV isolates, and their accession numbers are shown, followed by isolates' names, countries of isolation, and the prefectures when they were isolated in Japan. Three major clusters of genotype 4 HEV in Japan, Hokkaido, wide area, and Aichi/Shizuoka strains are indicated. For Aichi/Shizuoka strains, the animal species from which they were isolated and year of isolation are also indicated. Bootstrap values are indicated for the major nodes as a percentage of the data obtained from 1,000 resamplings. The scale bar is in units of nucleotide substitutions per site.

発症の約4カ月前，2013年6月に北欧諸国への渡航歴あるもジビエ料理は食べていない．飲酒歴は80 g/day以上．発症前の3カ月以内にサプリメントや薬剤の服用歴なし．野生動物肉摂食歴なし．イノシシ，シカ，ブタとの接触歴なし．ブタ肉の喫食歴は頻繁にあったが，ブタレバー，ブタホルモンの喫食歴なし．喫食したブタ肉は自宅で調理して食べたもので，桑名市内のスーパーマーケット2軒で購入した．8月中旬，自宅近所で寿司を食べたが貝類は食べていない．9月下旬，桑名市内の農協にて購入したしじみを自宅で加熱して摂取した．井戸水，河川水を飲水したことなし．発症前3カ月に限れば，愛知県，岐阜県，静岡県で野生動物肉の喫食歴がないどころか，これらの県へ行ったこともなかった．

2013年10月初旬より全身倦怠感を自覚し近医を受診し急性肝障害を指摘され，精査加療目的にて10月8日当院へ紹介され入院となった．入院時意識は清明で，眼球結膜に黄疸を認めた．肝逸脱酵素の上昇，ビリルビンの上昇を認めたが凝固能は正常であった（Table）．腹部超音波検査，腹部CTでは肝辺縁の鈍化と脂肪肝の所見を認めた以外異常所見は認めなかった．IgA-HEV抗体，IgM-HEV抗体，IgG-HEV抗体，HEV-RNA陽性と他のウイルスマーカーなどの結果から急性E型肝炎と診断し（Table），補液とグリチルリチン製剤にて経過観察していたところ，第29病日には肝機能障害は改善し退院とした．入院時IgM-CMV抗体も陽性であったが，CMV抗原は陰性で，軽快後のIgM-CMV抗体価に変化がなかったため，非特異反応またはpersistent IgMと判断した．

HEV-RNAの検出とその遺伝子配列解析：患者の凍結保存血清から既報に従いHEVのORF2領域の412塩基長を増幅し塩基配列を決定し[4]，既報株の塩基配列とともに分子進化系統樹を作成した（Fig.）．本症例のHEV株HE-JA13-2003と最も近縁関係にあるHEV株をBLAST searchにて検索するとHE-Aichi-C1という遺伝子型4型株であり[2]，412塩基長の配列において99.0%の高い一致率を示した．HE-JA13-2003はHE-Aichi-C1など4型愛知静岡株と100%のbootstrap値でクラスターを形成した．このクラスターに属する株との塩基配列の相同性は97.8%以上であった．

考察：三重県では2012年に4型愛知静岡株による症例を鈴鹿市で確認しているが，その症例は愛知県内で捕獲されたイノシシ肉の喫食歴があり，愛知県で感染したと考えられる[5]．これまでの4型愛知静岡株はいずれも野生動物関連株であるが，本症例は野生動物との関連が確認できず，しかも発症前3カ月以内にはこれらの県へ行ったこともなく，三重県内で感染したことが強く疑われる．この調査結果から，4型愛知静岡株が野生動物以外にも広がり，その感染源が木曽三川を越えて三重県に侵入していることが疑われる．患者本人の詳細な生活歴の聴取を行ったが，これといった感染源は浮かび上がらず，本患者と生活，飲食を共にした者の中にも，検査はしていないものの明らかな肝炎を発症した者はいなかった．従って残念ながら今のところ感染源，感染経路は不明である．しかし，その不明の感染源が三重県にまで及んでいることは重大で，その同定にはさらなる症例の蓄積による情報収集が必要と考えられる．患者の記憶に無い，医師側にも思いもよらない感染理由があるのかもしれない．また，これまで三重，愛知，岐阜及び静岡西部などでHEV感染例を診療する際には，患者に野生動物肉摂取歴がなければ感染したHEV株が4型愛知静岡株の可能性は低く，従って重症化の懸念が少ないと推測できた．しかし本研究によって地域的に共有されたその認識が覆されたことも特筆すべきと思われる．

索引用語：E型肝炎ウイルス，遺伝子型4型，三重県

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本論文内容に関連する著者の利益相反：なし

英文要旨

A case of acute hepatitis E in Mie prefecture infected with genotype 4 hepatitis E virus strain endemic in Aichi and Shizuoka prefectures (Aichi / Shizuoka strain), without a history of eating wild animal meat

Shino Fujimoto[1], Satoshi Ishida[1], Tatsunori Nakano[2]*, Nagako Kitagawa[1], Kunihiro Higuchi[1], Michihiro Izumi[1], Naoki Nakagawa[1], Ryuichi Aikawa[1], Yukihiko Adachi[1,3], Masaharu Takahashi[4], Yoshiyuki Takei[5], Hiroaki Okamoto[4]

We experienced a case of hepatitis E in Mie prefecture infected with genotype 4 hepatitis E virus (HEV) strain endemic in Aichi and Shizuoka prefectures (Aichi/Shizuoka strain). The HEV isolate obtained from the patient clustered with other Aichi / Shizuoka strains with 100% of bootstrap value in the phylogenetic tree, and was more than 97.8% identical to other Aichi/Shizuoka strains, in the 412-nucleotide sequence within the ORF2 region. Interestingly, the patient had no history of consuming wild animal meat although all other reported Aichi/Shizuoka strains were recovered from meat from wild animals or humans after eating wild animal meat. The finding indicated that the origin of Aichi/Shizuoka strains is confined not only to wild animal meat but also to other unknown reservoirs.

Key words: hepatitis E virus, genotype 4, Mie prefecture

Kanzo 2014; 55: 405—408

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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年8月28日	新医薬品等の区分 該当なし。		総合機構処理欄
一般的名称	別紙のとおり。		研究報告の公表状況	デング熱の国内感染症例について(第一報) (厚生労働省、2014年8月27日)		公表国 日本	
販売名（企業名）	別紙のとおり。						

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
問題点：日本で1942年～1945年の発生後初めてデング熱の国内感染が確認された。 埼玉県在住の10代の女性がデング熱感染を疑われていたが、国立感染症研究所の検査により感染が確認された。 患者は東京都内の学校に在学中で、海外渡航歴が無く、8/20に突然の高熱により入院し、8/26に患者の血液検体について国立感染症研究所で検査されたところ、デング熱陽性の結果が得られた。 日本では、1942年～1945年に帰還兵を感染源とするデング熱の流行が見られ、最近では、海外で感染し、帰国後にデング熱と診断される例が増加傾向にある。	記載なし。

報告企業の意見	今後の対応
別紙のとおり。	今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。

別紙

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤人免役グロブリン、⑥乾燥ペプシン処理人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン、⑧乾燥スルホ化人免疫グロブリン、⑨乾燥スルホ化人免疫グロブリン、⑩乾燥スルホ化人免疫グロブリン、⑪乾燥スルホ化人免疫グロブリン*、⑫乾燥濃縮人活性化プロテインC、⑬乾燥濃縮人血液凝固第Ⅷ因子、⑭乾燥濃縮人血液凝固第Ⅷ因子、⑮乾燥濃縮人血液凝固第Ⅷ因子、⑯乾燥濃縮人血液凝固第Ⅸ因子、⑰乾燥濃縮人血液凝固第Ⅸ因子、⑱乾燥濃縮人血液凝固第Ⅸ因子、⑲乾燥抗破傷風人免疫グロブリン、⑳抗 HBs 人免疫グロブリン、㉑抗 HBs 人免疫グロブリン、㉒抗 HBs 人免疫グロブリン、㉓トロンビン、㉔トロンビン、㉕トロンビン、㉖フィブリノゲン加第XⅢ因子*、㉗フィブリノゲン加第XⅢ因子、㉘乾燥濃縮人アンチトロンビンⅢ、㉙ヒスタミン加人免疫グロブリン製剤、㉚人血清アルブミン*、㉛人血清アルブミン*、㉜乾燥ペプシン処理人免役グロブリン*、㉝乾燥濃縮人アンチトロンビンⅢ、㉞乾燥濃縮人血液凝固第X因子加活性化第Ⅶ因子
販売名（企業名）	①献血アルブミン 20 "化血研"、②献血アルブミン 25 "化血研"、③人血清アルブミン "化血研" *、④ガンマーグロブリン筋注 450mg/3mL「化血研」、⑤ガンマーグロブリン筋注 1500mg/10mL「化血研」、⑥献血グロブリン注射用 2500mg「化血研」、⑦献血ベニロン－Ⅰ静注用 500mg、⑧献血ベニロン－Ⅰ静注用 1000mg、⑨献血ベニロン－Ⅰ静注用 2500mg、⑩献血ベニロン－Ⅰ静注用 5000mg、⑪ベニロン*、⑫注射用アナクトC2,500 単位、⑬コンファクトF注射用 250、⑭コンファクトF注射用 500、⑮コンファクトF注射用 1000、⑯ノバクトM静注用 400 単位、⑰ノバクトM静注用 800 単位、⑱ノバクトM静注用 1600 単位、⑲テタノセーラ筋注用 250 単位、⑳ヘパトセーラ筋注 200 単位/mL、㉑ヘパトセーラ筋注 200 単位/1mL、㉒ヘパトセーラ筋注 1000 単位/5mL、㉓トロンビン "化血研"、㉔献血トロンビン経口・外用５千「化血研」、㉕献血トロンビン経口・外用１万「化血研」、㉖ボルヒール*、㉗ボルヒール組織接着用、㉘アンスロビンＰ500 注射用、㉙ヒスタグロビン皮下注用、㉚アルブミン 20%化血研*、㉛アルブミン 5%化血研*、㉜静注グロブリン*、㉝アンスロビンＰ1500 注射用、㉞バイクロット配合静注用
報告企業の意見	デング熱はデングウイルス感染を原因とする感染症である。デングウイルスは 40～60nm の球形、核酸は一本鎖 RNA、エンベロープを有するウイルスで、蚊によって媒介され、これまでに 1～4 型の 4 つの血清型が知られている。 今回は、1942 年～1945 年の流行以来、初めてデング熱の国内感染が確認された、との報告である。 上記製剤の製造工程には、冷エタノール分画工程、ウイルス除去膜ろ過工程、加熱工程といった原理の異なるウイルスクリアランス工程が複数導入されており、各工程のウイルスクリアランス効果は「血漿分画製剤のウイルスに対する安全性確保に関するガイドライン(医薬発第 1047 号、平成 11 年 8 月 30 日)」に基づく、モデルウイルスを用いたウイルスプロセスバリデーションにより確認されている。今回報告したデングウイルスのモデルウイルスには、エンベロープの有無、核酸の種類等から、ウシウイルス性下痢ウイルス（BVDV）が該当すると考えられるが、上記工程の BVDV クリアランス効果については上記バリデーションにより確認されている。また、これまでに上記製剤によるデングウイルスへの感染報告例は無い。 以上の点から、上記製剤はデング熱に対する安全性を確保していると考える。

*：現在製造を行っていない

INF2014-004

健感発 0827 第 1 号
平成 26 年 8 月 27 日

各 都道府県・保健所設置市・特別区 衛生主管部（局）長 殿

厚生労働省健康局結核感染症課長
（ 公 印 省 略 ）

デング熱の国内感染症例について（第一報）

日頃から感染症対策への御協力を賜り厚くお礼申し上げます。

今般、さいたま市内の医療機関から、さいたま市衛生主管部局を通じ、海外渡航歴がないにもかかわらず、デング熱（四類感染症）の感染が疑われる患者（別添１）について情報提供があったことから、国立感染症研究所において確認検査を実施したところ、デング熱の患者であることが確認されました。

患者には海外渡航歴がないことから、国内でデング熱に感染したと考えられます。現在、さいたま市は、厚生労働省及び関係自治体と協力して、疫学調査（患者の周辺者等における症例探索等）を実施しているところです。

つきましては、本事例（デング熱の国内感染事例）について、貴管内の医療機関等の関係者へ情報提供するとともに、海外渡航歴がない場合であっても、平成26年度厚生労働科学研究（※）が取りまとめたデング熱診療マニュアル案（別添２）等を参考の上、デング熱が疑われる症例については、検査の実施を検討するよう注意喚起をお願いします。また、デング熱の国内感染が疑われる事例については、速やかに保健所への情報提供を行っていただくよう協力要請をお願いします。

なお、本年１月、日本国内でデング熱に感染した可能性のあるドイツ人患者が報告されたことを受け、平成26年１月10日付け健感発0110第１号及び平成26年１月28日付け事務連絡を発出したところです。また、当該事例を踏まえ、平成25年度厚生労働科学研究（※※）において、デング熱の国内感染事例が発生した場合の対応・対策（感染地の特定や他の感染者の探索、媒介蚊対策等）について手引き案が取りまとめられましたので、業務の参考として配布いたします（別添３）。

※「国内侵入・流行が危惧される昆虫媒介性ウイルス感染症に対する総合的対策の確立に関する研究」（研究代表者：国立感染症研究所ウイルス第一部　高崎智彦室長）

※※「我が国への侵入が危惧される蚊媒介性ウイルス感染症に対する総合的対策の確立に関する研究」（研究代表者：国立感染症研究所ウイルス第一部　高崎智彦室長）

参考資料

別添１：患者に関する情報
別添２：デング熱診療マニュアル（案）
別添３：デング熱国内感染事例発生時の対応・対策の手引き　地方公共団体向け（案）
別添４：デング熱について（第2版）
別添５：デング熱に関するQ&A（第2版）

ホームページ
厚生労働省　デング熱について
（http://www.mhlw.go.jp/bunya/kenkou/kekkaku-kansenshou19/dengue_fever.html）
国立感染症研究所　デング熱とは
（http://www.nih.go.jp/niid/ja/encycropedia/392-encyclopedia/238-dengue-info.html）

別紙様式第2　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　別紙3

医薬品
医薬部外品　研究報告　調査報告書
化粧品

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2014 年 8 月 29 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称		研究報告の公表状況		Promed:Dengue – Japan (Saitama) http://www.promedmail.org/direct.php?id=2731079	公表国 日本	
販売名（企業名）						

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
日本保健当局は過去約 70 年間で初めての海外渡航歴のない患者 1 例（10 代の女性）におけるデング熱の地元感染症例を報告した。患者は埼玉県さいたま市の医療施設から報告された。検査後にデング熱の確定例として確認された。 デング熱はアジアや中央・南アメリカ、アフリカといった世界中で見られる、蚊によって媒介されるウイルス性熱性疾患である。ウイルスは人から人へは感染しない。日本ではヒトスジシマカがウイルスを媒介している可能性が最も考えられている。 国内での感染は 1940 年代以来である。しかし、最近は毎年約 200 例の輸入例が報告されており、その患者から蚊を媒介している可能性が考えられている。 2014 年 8 月 28 日、厚生労働省は日本においてさらに 2 例（東京在住の 20 代の男性および埼玉県在住の 20 代の女性）のデング熱の国内感染症例を確認した。最初に地元感染が確認された 10 代女性を含むこれら 3 例は同じ学校（東京都）へ通っており、海外への渡航歴はない。全員が最近、渋谷区の代々木公園を訪れ、蚊により感染したとみられる	BYL-2014-426 Promed:Dengue – Japan (Saitama) http://www.promedmail.org/direct.php?id=2731079

報告企業の意見	今後の対応
デング熱の日本での感染は 70 年ぶりである。デング熱は人から人への感染症を起さないが、蚊を介して感染が広がる。 コージネイト FS の製造工程における病原体除去・不活化処理は，ウイルス及び細菌に対して有効であることが報告されている。なお，2007 年 4 月以降，コージネイト FS の販売は行っていない。	現時点で新たな安全対策上の措置を講じる必要はないと考える。 今後も、新規人畜共通感染症や新たなウイルス感染症に関する情報収集に努める。

MedDRA バージョン 17.0

⑭

BYL-2014-0426

Published Date: 2014-08-28 19:37:14
Subject: PRO/EDR> Dengue - Japan: (ST)
Archive Number: 20140828.2731079

DENGUE - JAPAN: (SAITAMA)
****
A ProMED-mail post
http://www.promedmail.org
ProMED-mail is a program of the
International Society for Infectious Diseases
http://www.isid.org

[1]
Date: Tue 26 Aug 2014
From: Kazuko Fukushima <fukushima-kazuko@mhlw.go.jp> [edited]

Japan officially reports an autochthonous case of dengue fever in a patient (teenaged female) without any overseas travel history. The patient (currently hospitalized) was reported from a healthcare facility in Saitama City [Saitama Prefecture], and following confirmatory laboratory testing procedures at the National Institute of Infectious Diseases, the patient was verified as a confirmed case of dengue infection.

As the patient has no overseas travel history and is believed to have acquired the infection domestically, currently Saitama City is conducting epidemiologic investigations jointly with the Ministry of Health, Labour and Welfare (MHLW) and other local government counterparts.

In addition, regarding this event, MHLW is providing information and feedback to prefectural counterparts and has alerted healthcare facilities.

Dengue fever is a febrile viral illness. The virus is transmitted by mosquitoes, and is found widely throughout the world, such as in Asia, Central and South America, and Africa. The virus is not transmitted person-to-person. In Japan, the mosquito _Aedes albopictus_ (a mosquito species that is mostly active during daylight hours in outdoor environments) is the vector believed to be most likely to transmit the virus.

Domestic transmission of dengue virus was present in Japan during the early 1940s when many soldiers returned from the tropics, but a domestic case had not been confirmed since then. However, in recent years, approximately 200 imported cases (those that have onset after returning to Japan following overseas travel) are reported annually, and transmission from such patients (via mosquitoes) is a possibility.

As the investigation regarding this event is ongoing with Saitama City, updated information will be provided in a timely manner.

--
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[ProMED thanks Dr. Kazuko Fukushima for sending in this report. The receipt of official reports sent in so promptly, such as this one, is valued. - Mod.TY]

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[2]
Date: Wed 27 Aug 2014
Source: Japan Today [edited]

http://www.japantoday.com/category/national/view/japan-sees-1st-dengue-fever-case-in-nearly-70-years

Japanese health authorities have reported the 1st locally transmitted case of dengue fever in the country in nearly 70 years.

The ministry said the case occurred in Saitama Prefecture. Local media reports said the patient was a teenage girl who has since recovered.

Government spokesman Yoshihide Suga, in confirming the report, said Wednesday [27 Aug 2014] that the news was not cause for alarm because the illness is not transmitted directly from person to person.

Japan sees dozens of imported cases of dengue fever each year, mostly tourists who catch the illness while traveling in tropical regions. The disease, which is transmitted by mosquito, was common in Japan during World War II but was locally eradicated for decades.

Dengue causes symptoms including fever, severe joint pain and headaches. There is no treatment.

Dengue has been spreading in recent years, with 50 million to 100 million dengue infections a year in more than 100 countries. According to the World Health Organization, before 1970 only 9 countries had experienced dengue epidemics. Japan reported 249 cases last year.

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Communicated by:
ProMED-mail Rapporteur Kunihiko Iizuka

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[3]
Date: Thu 28 Aug 2014
Source: Jiji Press [edited]
http://jen.jiji.com/jc/eng?g=eco&k=2014082800632

The Ministry of Heath, Labor and Welfare said Thursday [28 Aug 2014] that 2 more domestic cases of dengue fever have been confirmed in Japan.

A man in his 20s from Tokyo and a woman in her 20s from Saitama Prefecture, north of Tokyo, have been confirmed to have contracted the disease, the ministry said.

The news came after the discovery on Wednesday [27 Aug 2014] that a woman in her teens had been infected with the tropical disease, the 1st locally transmitted dengue fever case in Japan in about 70 years.

The 3 go to the same school in Tokyo and have no history of overseas travel. As all of them went to Yoyogi Park in Tokyo's Shibuya Ward recently, they are believed to have been infected by mosquitos.

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Communicated by:
ProMED-mail Rapporteur Kunihiko Iizuka

[The occurrence of 3 cases occurring simultaneously in the same place, after a 70-year absence of dengue virus transmission in Japan, is remarkable. It seems likely that the 3 individuals were infected in the very same spot, and likely at the same time. It would be of interest to receive reports of any additional cases that might occur there and any related mosquito vector field studies that might be carried out, confirming the presence of _Aedes albopictus_.

There was a dengue case in Germany ex Japan that occurred in September 2013, reported in a ProMED-mail post of 9 Jan 2014 (see ProMED-mail archive no. 20140109.2162194). This case was reported again recently in Eurosurveillance (Schmidt-Chanasit J, Emmerich P, Tappe D, Gunther S, Schmidt S, Wolff D, Hentschel K, Sagebiel D, Schoneberg I, Stark K, Frank C. Autochthonous dengue virus infection in Japan imported into Germany, September 2013. Euro Surveill. 2014;19(3):pii=20681. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20681).

The summary of that report stated, "In September 2013, dengue virus (DENV) infection was diagnosed in a German traveller returning from Japan. DENV-specific IgM and IgG and DENV NS1 antigen were detected in the patient's blood, as were DENV serotype 2-specific antibodies. Public health authorities should be aware that autochthonous transmission of this emerging virus may occur in Japan. Our findings also highlight the importance of taking a full travel history, even from travellers not returning from tropical countries, to

assess potential infection risks of patients." This report was sent to ProMED by Roland Hubner. So perhaps the 3 cases above are not the 1st indications of local dengue virus transmission in nearly 70 years after all.

A HealthMap/ProMED-mail map showing the location of Saitama Prefecture can be accessed at http://healthmap.org/promed/p/41237. - Mod.TY]

See Also

Dengue/DHF update (03): Germany (Berlin) ex Japan, RFI 20140109.2162194
....jw/ty/je/jw

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医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2014 年 07 月 18 日	新医薬品等の区分	厚生労働省処理欄
一般的名称	①②③⑨⑩pH4 処理酸性人免疫グロブリン ④⑤⑥⑦⑧ポリエチレングリコール処理人免疫グロブリン ⑪⑫人免疫グロブリン		研究報告の公表状況	www.cdc.gov/media/releases/2014/p0717-chikungunya.html/2014/07/17	公表国 アメリカ	
販売名（企業名）	①日赤ポリグロビン N5%静注 0.5g/10mL　（日本血液製剤機構） ②日赤ポリグロビン N5%静注 2.5g/50mL　（日本血液製剤機構） ③日赤ポリグロビン N5%静注 5g/100mL　（日本血液製剤機構） ④献血ｳﾞｪﾉｸﾞﾛﾌﾞﾘﾝ IH5%静注 0.5g/10mL　（日本血液製剤機構） ⑤献血ｳﾞｪﾉｸﾞﾛﾌﾞﾘﾝ IH5%静注 1g/20mL　（日本血液製剤機構） ⑥献血ｳﾞｪﾉｸﾞﾛﾌﾞﾘﾝ IH5%静注 2.5g/50mL　（日本血液製剤機構） ⑦献血ｳﾞｪﾉｸﾞﾛﾌﾞﾘﾝ IH5%静注 5g/100mL　（日本血液製剤機構） ⑧献血ｳﾞｪﾉｸﾞﾛﾌﾞﾘﾝ IH5%静注 10g/200mL（日本血液製剤機構） ⑨献血ｳﾞｪﾉｸﾞﾛﾌﾞﾘﾝ-IH ﾖｼﾄﾐ　（日本血液製剤機構） ⑩日赤ポリグロビン N10%静注 5g/50mL（日本血液製剤機構） ⑪日赤ポリグロビン N10%静注 10g/100mL（日本血液製剤機構） ⑫ｸﾞﾛﾌﾞﾘﾝ筋注 450mg/3mL「ﾍﾞﾈｼｽ」　（日本血液製剤機構） ⑬ｸﾞﾛﾌﾞﾘﾝ筋注 1500mg/10mL「ﾍﾞﾈｼｽ」　（日本血液製剤機構）					

研究報告の概要

フロリダで報告された米国における初めてのチクングンヤ感染症例

蚊媒介性チクングンヤウイルスが西半球で認められてから 7 ヶ月後、本疾患の初めての地域伝播の症例が米国本土で明るみに出た。この症例は本日、フロリダで報告されたもので、最近、海外旅行をしていない男性の症例であった。疾病管理予防センター（CDC）はフロリダ保健局と緊密に連携し、この患者がどのようにこのウイルスに感染したのかを調査している。CDC は今後、数週間から数ヶ月間にわたって米国の他の地域伝播の症例についても監視していく予定である。
2006 年以降、米国ではこのウイルスの常在国（http://www.cdc.gov/chikungunya/geo/index.html）から帰国した旅行者の間でチクングンヤの輸入症例が年平均 28 例出ている。本年までに米国の 31 の州および 2 つの準州で旅行関連の症例 243 例が報告された（http://www.cdc.gov/chikungunya/geo/united-states.html）。しかし、この新たに報告された症例により、米国本土で初めて蚊が非旅行者にまでウイルスを伝播したと考えられることが示された。今年、プエルトリコおよび米国領バージン諸島はチクングンヤの地域伝播症例をそれぞれ 121 例および 2 例報告した。
CDC のアルボウイルス感染症部門の長である Roger Nasci, Ph.D. は、「チクングンヤウイルスは初めに熱帯アメリカに運び込まれてから、今では米国本土にも入っており、このウイルスやその他の外来病原体によってもたらされる危険性が浮き彫りにされている」と述べ、さらに「このことから、米国および全世界で効果的な調査ネットワークや診断研究所および蚊防除計画が維持されるよう策定された CDC の健康安全保障イニシアティブの重要性が強調される」と続けている。
チクングンヤウイルスはネッタイシマカおよびヒトスジシマカという 2 種類の蚊によってヒトに伝播される。この 2 種類の蚊はいずれも米国南東部および一部の南西部で認められており、ヒトスジシマカはさらに東海岸を北上し、中部大西洋沿岸地域全体でもみられるほか、中西部南部でも認められている。
CDC およびフロリダ保健局は他にも地域伝播した症例がないかどうかを評価しているほか、蚊を防除し、蚊に刺されないような個人および家庭での防護策について啓蒙することによってウイルスのさらなる蔓延を防ぐ方法について国民に助言している。CDC は米国におけるウイルス追跡

使用上の注意記載状況・その他参考事項等

代表として献血ヴェノグロブリン IH5%静注 0.5g/10mL の記載を示す。
2. 重要な基本的注意
(1) 本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-1 抗体陰性で、かつ ALT（GPT）値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分からポリエチレングリコール 4000 処理、DEAE セファデックス

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医薬部外品　　研究報告　調査報告書
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<table>
<tr><td colspan="2">に役立てるため、州の保健局にチクングンヤの症例を報告するよう求めている。地域伝播はこのウイルスの感染者を刺した蚊が別の人間を刺すことで発生する。
現在、チクングンヤが米国でどのような経過をとっているのかは不明である。CDC 職員は、米国におけるチクングンヤについてデングウイルス（http://www.cdc.gov/dengue）と類似する挙動を示すと考えている。即ち、輸入症例から散発的な地域伝播に至るが、広範囲に及ぶ発生には至っていないというものである。2006 年から 2013 年までの 200 例を上回るチクングンヤ輸入症例のうち、地域での発生を誘発したものはなかった。しかし、米国に入ってくるチクングンヤ感染旅行者が多いほど、チクングンヤの地域伝播が生じる可能性が高まる。
チクングンヤの発生はこれまで、アフリカ、アジア、欧州、インドおよび中東ならびにカリブ諸島セント・マーチン島のフランス領側で報告されている。2013 年 12 月、ウイルスはセント・マーチン島からカリブ諸島に迅速に蔓延し、南アメリカおよび中央アメリカに至った。米国の症例が認められるまでに、半球の 23 の国々で地域伝播が報告されている（http://www.cdc.gov/chikungunya/geo/americas.html）。
チクングンヤウイルスに感染すると、一般に発熱および関節痛を発症する。他にも、筋肉痛、頭痛、関節の腫脹または発疹がみられることもある。チクングンヤウイルスの活動性が高い地域から帰国した旅行者および米国でウイルスが報告された地域の居住者は、チクングンヤの症状がみられた場合、医師の診察を受ける必要がある。また、症例が報告された地域の医療従事者はチクングンヤの症例に備えて警戒する必要がある。チクングンヤウイルス感染者は虫除け剤を適用し、蚊が入らないように空調設備や窓およびドアの網戸を利用し、可能な場合は長ズボンおよび長袖シャツを着用して、自宅の外に水たまりがないようにしておくなど、自分自身を防御する必要がある。疾患発症後数日間、蚊に刺されないように自分自身および他者を防護することで、他の蚊が感染するのを防ぎ、さらなる蔓延のリスクを軽減することができる。
チクングンヤウイルス感染が致命的となることは稀であるが、関節痛は重度で身体を衰弱させることが多い。このウイルスはヒトからヒトへは感染しない。ワクチンはなく、感染に対する特別な治療法もないが、両分野で研究が進行中である。患者は約 1 週間で回復するが、関節痛が長期間にわたって続く患者もいる。CDC によれば、感染すると生涯にわたる免疫を獲得すると考えられている。</td><td rowspan="3">処理等により人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理、ウイルス除去膜によるろ過処理及び pH3.9～4.4 の条件下での液状インキュベーション処理を施しているが、投与に際しては、次の点に十分注意すること。</td></tr>
<tr><td>報告企業の意見</td><td>今後の対応</td></tr>
<tr><td>チクングニヤウイルス（Chikungunya virus：CHIKV）は、トガウイルス科（Togaviridae）アルファウイルス属（Alphavirus）に分類される直径 70nm のエンベロープを有する球状の 1 本鎖 RNA ウイルスで、蚊（ヤブカ属のネッタイシマカやヒトスジシマカ）によって媒介される。万一、原料血漿に CHIKV が混入したとしても、Bovine viral diarrhoea virus（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。</td><td>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。</td></tr>
</table>

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives. Protecting People.™

Press Release

For Immediate Release: Thursday, July 17, 2014
Contact: CDC Media Relations (mailto:media@cdc.gov)
(404) 639-3286

First Chikungunya case acquired in the United States reported in Florida

Seven months after the mosquito-borne virus chikungunya was recognized in the Western Hemisphere, the first locally acquired case of the disease has surfaced in the continental United States. The case was reported today in Florida in a male who had not recently traveled outside the United States. The Centers for Disease Control and Prevention is working closely with the Florida Department of Health to investigate how the patient contracted the virus; CDC will also monitor for additional locally acquired U.S. cases in the coming weeks and months.

Since 2006, the United States has averaged 28 imported cases of chikungunya (chik-un-GUHN-ya) per year in travelers returning from countries where the virus is common (http://www.cdc.gov/chikungunya/geo/index.html). To date this year, 243 travel-associated cases have been reported in 31 states and two territories (http://www.cdc.gov/chikungunya/geo/united-states.html). However, the newly reported case represents the first time that mosquitoes in the continental United States are thought to have spread the virus to a non-traveler. This year, Puerto Rico and the U.S. Virgin Islands reported 121 and two cases of locally acquired chikungunya respectively.

"The arrival of chikungunya virus, first in the tropical Americas and now in the United States, underscores the risks posed by this and other exotic pathogens," said Roger Nasci, Ph.D., chief of CDC's Arboviral Diseases Branch. "This emphasizes the importance of CDC's health security initiatives designed to maintain effective surveillance networks, diagnostic laboratories and mosquito control programs both in the United States and around the world."

Chikungunya virus is transmitted to people by two species of mosquitoes, Aedes aegypti and Aedes albopictus. Both species are found in the southeastern United States and limited parts of the southwest; Aedes albopictus is also found further north up the East Coast, through the Mid-Atlantic States and is also found in the lower Midwest.

CDC and the Florida Department of Health are assessing whether there are additional locally acquired cases and are providing consultation to the public on ways to prevent further spread of the virus by controlling mosquitoes and educating people about personal and household protection measures to avoid mosquito bites. CDC has asked state health departments to report cases of chikungunya to help track the virus in the United States. Local transmission occurs when a mosquito bites someone who is infected with the virus and then bites another person.

It is not known what course chikungunya will take now in the United States. CDC officials believe chikungunya will behave like dengue virus (http://www.cdc.gov/dengue) in the United States, where imported cases have resulted in sporadic local transmission but have not caused widespread outbreaks. None of the more than 200 imported chikungunya cases between 2006 and 2013 have triggered a local outbreak. However, more chikungunya-infected travelers coming into the United States increases the likelihood that local chikungunya transmission will occur.

Outbreaks of chikungunya have been previously reported from countries in Africa, Asia, Europe, India, and the Middle East, and on the French side of the Caribbean island of St. Martin. The virus spread quickly in St. Martin through the Caribbean in December 2013 and into South and Central America. Local transmission has been reported in 23 countries in the hemisphere (http://www.cdc.gov/chikungunya/geo/americas.html) prior to the U.S. case.

People infected with chikungunya virus typically develop fever and joint pain. Other symptoms can include muscle aches, headaches, joint swelling or rash. Travelers returning from areas with chikungunya activity and those living in areas where the virus has been reported in the United States should seek medical care if they experience chikungunya symptoms. Health care providers in areas with reported cases should be on the alert for possible cases. People infected with chikungunya should protect themselves by wearing insect repellents, using air conditioning or window and door screens to keep mosquitoes out, wearing long pants and long-sleeved shirts when possible, and emptying standing water outside your home. Protecting yourself and others from mosquito bites during the first few days of illness can help prevent other mosquitoes from becoming infected and reduce the risk of further spread.

Infection with chikungunya virus is rarely fatal, but the joint pain can often be severe and debilitating. This virus is not spread person to person. There is no vaccine and no specific treatment for infection, but research is underway in both areas. Patients recover in about a week, although long-term joint pain occurs in some people. According to CDC, infection is thought to provide lifelong immunity.

For more information, visit www.cdc.gov/chikungunya (http://www.cdc.gov/chikungunya).
For more information about preventing mosquito bites, visit www.cdc.gov/chikungunya/prevention/index.html (http://www.cdc.gov/chikungunya/prevention/index.html).

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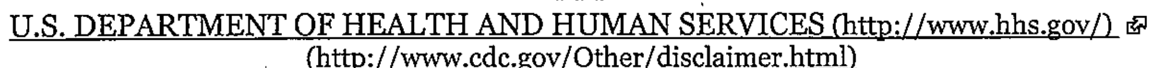
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(http://www.cdc.gov/Other/disclaimer.html)

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Content source: Centers for Disease Control and Prevention

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800-CDC-INFO (800-232-4636) TTY: (888) 232-6348 - Contact CDC–INFO

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. 7. 25	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	新鮮凍結人血漿		研究報告の公表状況	ABC NewsLetter ,July 25, 2014 (#28)	公表国 米国	
販売名(企業名)	新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)					

研究報告の概要	使用上の注意記載状況・その他参考事項等
○感染症最新情報:チクングニヤ 米国疾病管理予防センター(CDC)によると、西半球で蚊媒介性ウイルスであるチクングニヤウイルスが発見されてから数カ月後、フロリダ州において米国本土初の同ウイルスの地域内感染症例が報告された。 2014年7月17日、マイアミ・デイド郡とパームビーチ郡からそれぞれ1症例ずつ、最近国外に渡航していない者の感染が報告された。CDCは今後数カ月にわたり、地域内感染の報告がないか監視を行う。	新鮮凍結血漿-LR「日赤」120 新鮮凍結血漿-LR「日赤」240 新鮮凍結血漿-LR「日赤」480 血液を介するウイルス、細菌、原虫等の感染 vCJD等の伝播のリスク

報告企業の意見	今後の対応
米国本土における初の地域内チクングニヤ感染症例が、フロリダ州で報告されたとのことである。	日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

(16)

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– Byron Buhner
President and CEO, Indiana Blood Center

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INFECTIOUS DISEASE UPDATES

CHIKUNGUNYA

Seven months after the mosquito-borne virus chikungunya was recognized in the Western Hemisphere, the first locally transmitted cases in the US have been reported in Florida, according to the US Centers for Disease Control and Prevention. Two cases were reported on July 17, one in Miami Dade county and the other in a Palm Beach county; neither patient had traveled outside of the US recently. CDC will also monitor for additional locally acquired US cases in the coming weeks and months, reported the agency in a July 17 press release. "The Department has been conducting statewide monitoring for signs of any locally acquired cases of chikungunya," Anna Likos, MD, state epidemiologist in Florida and Disease Control and Health Protection director, said in a Florida Department of Health statement. Chikungunya is spread by the *Aedes aegypti* and *Aedes albopictus* mosquitoes, and symptoms can include sudden high fever, severe joint pain, headache, muscle pain, back pain, and rash. "It is not known what course chikungunya will take now in the US. CDC officials believe chikungunya will behave like dengue virus in the US, where imported cases have resulted in sporadic local transmission, but have not caused widespread outbreaks. None of the more than 200 imported chikungunya cases between 2006 and 2013 have triggered a local outbreak. However, more chikungunya-infected travelers coming into the US increases the likelihood that local chikungunya transmission will occur," said CDC in a

(continued on page 15)

INFECTIOUS DISEASE UPDATES (continued from page 14)

statement. Transfusion medicine experts have theorized that chikungunya virus could spread via blood transfusion, but no cases of transfusion-transmission have been recorded despite titanic outbreaks in Africa, Asia, Europe, India, the Middle East, and now the Caribbean. The US blood community is currently considering a range of mitigation strategies including observation and confirmation that there is any clinical risk of transfusion-transmission; donor deferrals for travel to epidemic regions; and the development of nucleic acid tests for use in the event of epidemic spread in the US. A chikungunya fact-sheet prepared by AABB's Transfusion Transmitted Diseases Committee can be viewed at http://bit.ly/1pkZk6Z. Donors with travel to epidemic areas are encouraged to report any illness consistent with chikungunya following donation. (Sources: CDC press release, 7/17/14; Florida Department of Health press release, 7/17/14)

HIV

A new report conducted by Centers for Disease Control and Prevention researchers shows that the annual diagnosis rate of HIV has dropped by one-third in the general population, but has climbed among young gay and bisexual males. The report, published in *JAMA: The Journal of the American Medical Association*, found that fewer heterosexuals, drug users, and women were diagnosed each year with HIV, however, the annual diagnosis rate more than doubled for young gay and bisexual males. CDC investigators, led by Anna Satcher Johnson, MPH, examined trends in HIV diagnoses from 2002 to 2011 in the US using data from CDC's National HIV Surveillance System. The report only examined people diagnosed with HIV, and health officials have said that many more are infected with the virus but are unaware of their infection. The data also does not offer any information on when these people were infected, making it difficult to pinpoint trends in efforts to prevent transmission of the virus. While almost 500,000 people were diagnosed during the study period, the annual rate of diagnoses fell 33 percent from 24 out of 100,000 people to 16. For women, the diagnosis rate dropped by about half and among men by more than a quarter. For blacks and Hispanics, the rates of diagnosis declined 37 percent and 41 percent, respectively. The report estimates that HIV infections due to injection drug use fell by roughly 70 percent and from heterosexual contact by more than one-third for men and women. The jump in the diagnosis rate among males aged 13 to 24 suggests that many gay males and bisexual men are not practicing safe sex, note the authors, who add that young people may fail to practice safe sex because the AIDS epidemic is not in the consciousness of this younger generation. "Among men who have sex with men, unprotected risk behaviors in the presence of high prevalence and unsuppressed viral load may continue to drive HIV transmission. Disparities in the rates among young men who have sex with men present prevention challenges and warrant expanded efforts," conclude the authors.

Citation: Johnson AS, *et al.* Trends in Diagnoses of HIV Infection in the US, 2002-2011. JAMA 2014 July 23;312(4):432-4. ♦

GLOBAL NEWS

The International Federation of Blood Donor Organizations (IFBDO) recently launched a newly redesigned website, available at www.fiods-ifbdo.org/. Founded in 1955 in Luxembourg and headquartered in Monaco, IFBDO consists of 72 blood donor organizations from around the world. The organization works to promote self-sufficient blood supplies supported by voluntary, unpaid blood donors in its member countries. The new website features information about the organization's history and events, relevant news, and World Blood Donor Day. (Source: IFBDO website, 7/17/14) ♦

別紙様式第2-1

No. 8

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. 6. 23	該当なし	
一般的名称	新鮮凍結人血漿		研究報告の公表状況	Lindsey NP, Lehman JA, Staples JE, Fischer M; Division of Vector-Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, CDC. MMWR Morb Mortal Wkly Rep. 2014 Jun 20;63(24):521-6.	公表国 米国	
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研究報告の概要

○ウエストナイルウイルス(WNV)及び他のアルボウイルス疾患－2013年、米国
米国疾病管理予防センター(CDC)が発表した2013年の米国におけるWNV及び他のアルボウイルス疾患の流行状況である。米国の3,141郡のうち830郡(26%)から2,605症例のアルボウイルス疾患が報告された(デング熱を除く)。
WNV疾患は2,469人で、米国の47州とワシントンD.C.の735郡から報告された。症例の90%が7月～9月に発症し、患者年齢の中央値は55歳で、58%は男性であった。1,267人(51%)が神経侵襲性疾患であり、1,494人(61%)が入院し、119人(5%)が死亡した(死亡例の年齢中央値は78歳)。神経侵襲性疾患患者のうち669人(53%)は脳炎、483人(38%)は髄膜炎、112人(9%)は急性弛緩性麻痺(うち76%は同時に脳炎あるいは髄膜炎を併発)を呈し、111人(9%)が死亡した。神経侵襲性疾患はカルフォルニア州、テキサス州、コロラド州、イリノイ州、ノースダコタ州及びオクラホマ州の6州から全体の半数以上の症例が報告され、発生率はノースダコタ州(8.9人/10万人)で最も高かった。
他は、ラクロスウイルス85人、ジェームズタウンキャニオンウイルス22人、ポワッサンウイルス15人、東部ウマ脳炎ウイルス8人、非特定のカルフォルニア血清群ウイルス5人、セントルイス脳炎ウイルス1人であった。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見

2013年、米国の830郡から2,605症例のアルボウイルス疾患がCDCに報告され、そのうちWNV疾患は2,469症例であったとの報告である。

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、ウエストナイルウイルス感染の国内発生に備え、平成17年10月25日付血液対策課発事務連絡に基づく緊急対応(献血制限、NAT検査)のほか、厚生労働科学研究「血液製剤の安全性確保と安定供給のための新興・再興感染症の研究」班と共同して対応について検討している。今後も引き続き情報の収集に努める。

17

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Morbidity and Mortality Weekly Report
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West Nile Virus and Other Arboviral Diseases — United States, 2013

Nicole P. Lindsey, MS[1], Jennifer A. Lehman[1], J. Erin Staples, MD[1], Marc Fischer, MD[1] (Author affiliations at end of text)

Arthropod-borne viruses (arboviruses) are transmitted to humans primarily through the bites of infected mosquitoes and ticks. West Nile virus (WNV) is the leading cause of domestically acquired arboviral disease in the United States (*1*). However, several other arboviruses also cause sporadic cases and seasonal outbreaks of neuroinvasive disease (i.e., meningitis, encephalitis, and acute flaccid paralysis) (*1*). This report summarizes surveillance data reported to CDC in 2013 for WNV and other nationally notifiable arboviruses, excluding dengue. Forty-seven states and the District of Columbia reported 2,469 cases of WNV disease. Of these, 1,267 (51%) were classified as WNV neuroinvasive disease, for a national incidence of 0.40 per 100,000 population. After WNV, the next most commonly reported cause of arboviral disease was La Crosse virus (LACV) (85 cases), followed by Jamestown Canyon virus (JCV) (22), Powassan virus (POWV) (15), and eastern equine encephalitis virus (EEEV) (eight). WNV and other arboviruses continue to cause serious illness in substantial numbers of persons annually. Maintaining surveillance remains important to help direct and promote prevention activities.

In the United States, most arboviruses are maintained in transmission cycles between arthropods and vertebrate hosts (typically birds or small mammals). Humans usually become infected when bitten by infected mosquitoes or ticks. Person-to-person transmission occurs rarely through blood transfusion and organ transplantation. Most human arboviral infections are asymptomatic. Symptomatic infections most often manifest as a systemic febrile illness and, less commonly, as neuroinvasive disease. Most endemic arboviral diseases are nationally notifiable and are reported to CDC through the ArboNET surveillance system (*2,3*). In addition to collecting data on human disease cases, ArboNET collects data on viremic blood donors, veterinary disease cases, and infections in mosquitoes, dead birds, and sentinel animals. Using standard definitions, human cases with laboratory evidence of recent arboviral infection are classified as neuroinvasive disease or nonneuroinvasive disease (*2*). Because of the substantial associated morbidity, detection and reporting of neuroinvasive disease cases is assumed to be more consistent and complete than for nonneuroinvasive disease cases. Therefore, incidence rates were calculated for neuroinvasive disease cases using U.S. Census Bureau 20[illegible] mid-year population estimates.

In 2013, CDC received reports of 2,605 cases of nationally notifiable arboviral diseases, including those caused by WNV (2,469 cases), LACV (85), JCV (22), POWV (15), EEEV (eight), unspecified California serogroup virus (five), and St. Louis encephalitis virus (SLEV) (one). Cases were reported from 830 (26%) of the 3,141 U.S. counties; no cases were reported from Alaska or Hawaii. Of the 2,605 arboviral disease cases, 1,383 (53%) were reported as neuroinvasive disease, for a national incidence of 0.44 per 100,000 population.

A total of 2,469 WNV disease cases, including 1,267 (51%) neuroinvasive cases, were reported from 725 counties in 47 states and the District of Columbia (Table 1). WNV disease cases peaked in early September; 90% of cases had illness

INSIDE

527 Use of MenACWY-CRM Vaccine in Children Aged 2 Through 23 Months at Increased Risk for Meningococcal Disease: Recommendations of the Advisory Committee on Immunization Practices, 2013
531 Notes from the Field: Increase in Fentanyl-Related Overdose Deaths — Rhode Island, November 2013–March 2014
532 Announcements
533 QuickStats

Continuing Education examination available at http://www.cdc.gov/mmwr/cme/conted_info.html#weekly.

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

onset during July–September. The median age of patients was 55 years (interquartile range [IQR] = 41–67 years); 1,425 (58%) were male. Overall, 1,494 (61%) patients were hospitalized, and 119 (5%) died. The median age of patients who died was 78 years (IQR = 67–83 years).

Of the 1,267 WNV neuroinvasive disease patients, 669 (53%) had encephalitis, 486 (38%) had meningitis, and 112 (9%) had acute flaccid paralysis. Among the 112 patients with acute flaccid paralysis, 85 (76%) also had encephalitis or meningitis. The national incidence of neuroinvasive WNV disease was 0.40 per 100,000 population (Table 2). States with the highest incidence rates included North Dakota (8.9 per 100,000), South Dakota (6.8), Nebraska (2.9), and Wyoming (2.8) (Figure). Six states reported approximately half (51%) of the WNV neuroinvasive disease cases: California (237 cases), Texas (113), Colorado (90), Illinois (86), North Dakota (64), and Oklahoma (60). Neuroinvasive WNV disease incidence increased with age, with the highest incidence among persons aged ≥70 years. Among patients with neuroinvasive disease, 111 (9%) died.

The 85 LACV disease cases were reported from 59 counties in 12 states; 77 (91%) were neuroinvasive (Table 1). Dates of illness onset for LACV disease cases ranged from June through October; 71 (84%) had onset during July–September. Forty-nine (58%) patients were male. The median age of patients was 7 years (IQR = 4–11 years); 76 (89%) were aged <18 years. LACV neuroinvasive disease incidence was highest in West Virginia (0.54 per 100,000), Tennessee (0.35), North Carolina (0.13), and Ohio (0.12) (Table 2). Those four states reported 60 (78%) LACV neuroinvasive disease cases. A total of 80 (94%) patients were hospitalized; two (2%) died.

Twenty-two JCV disease cases were reported from 20 counties in 10 states; 15 (68%) were neuroinvasive (Table 1). Eight states (Georgia, Idaho, Massachusetts, Minnesota, New Hampshire, Oregon, Pennsylvania, and Rhode Island) reported their first JCV disease cases. Dates of illness onset ranged from January through November, with 14 (64%) of the 22 cases occurring during July–September. The median age of patients was 46 years (IQR = 32–57 years); 17 (77%) were male. Twelve (55%) patients were hospitalized; none died. In addition to the LACV and JCV cases, five cases of California serogroup virus disease were reported for which the specific infecting virus was unknown.

Fifteen POWV disease cases were reported from 13 counties in seven states; 12 (80%) were neuroinvasive (Table 1). Three states (Massachusetts, New Hampshire, and New Jersey) reported their first POWV disease cases. Dates of illness onset ranged from May through November; five (33%) had onset during April–June, and six (40%) had onset during July–September. The median age of patients was 69 years (IQR = 45–75 years); 11 (73%) were male. Thirteen (87%) patients were hospitalized; two (13%) died.

Eight EEEV disease cases were reported from six states, including the first cases ever reported from Arkansas and Connecticut (Table 1). All eight were neuroinvasive. Dates of illness onset ranged from January through December. The

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TABLE 1. Number and percentage of reported cases of arboviral disease, by virus and selected characteristics — United States, 2013*

	Virus									
	West Nile (n = 2,469)		La Crosse (n = 85)		Jamestown Canyon (n = 22)		Powassan (n = 15)		Eastern equine encephalitis (n = 8)	
Characteristic	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Age group (yrs)										
<18	96	(4)	76	(89)	3	(14)	1	(7)	2	(25)
18–59	1,392	(56)	6	(7)	16	(73)	6	(40)	3	(38)
≥60	981	(40)	3	(4)	3	(14)	8	(53)	3	(38)
Sex										
Male	1,425	(58)	49	(58)	17	(77)	11	(73)	6	(75)
Female	1,044	(42)	36	(42)	5	(23)	4	(27)	2	(25)
Period of illness onset										
January–March	4	(<1)	0	(0)	1	(5)	0	(0)	2	(25)
April–June	49	(2)	9	(11)	5	(23)	5	(33)	1	(13)
July–September	2,223	(90)	71	(84)	14	(64)	6	(40)	2	(25)
October–December	193	(8)	5	(6)	2	(9)	4	(27)	3	(38)
Clinical syndrome										
Nonneuroinvasive	1,202	(49)	8	(9)	7	(32)	3	(20)	0	(0)
Neuroinvasive	1,267	(51)	77	(91)	15	(68)	12	(80)	8	(100)
Encephalitis	669	(27)	65	(76)	9	(41)	10	(67)	8	(100)
Meningitis	486	(20)	8	(9)	6	(27)	2	(13)	0	(0)
Acute flaccid paralysis†	112	(5)	4	(5)	0	(0)	0	(0)	0	(0)
Outcome										
Hospitalization	1,494	(61)	80	(94)	12	(55)	13	(87)	8	(100)
Death	119§	(5)	2	(2)	0	(0)	2	(13)	4	(50)

* Five unspecified California serogroup virus disease cases in addition to the La Crosse virus and Jamestown Canyon virus disease cases were reported.
† Of the 112 West Nile virus disease patients with acute flaccid paralysis, 85 (76%) also had encephalitis or meningitis. The four La Crosse virus disease patients with acute flaccid paralysis all also had encephalitis.
§ Of the 119 West Nile virus deaths, 111 (93%) occurred in patients with neuroinvasive disease and eight (7%) in patients with nonneuroinvasive disease.

median age of patients was 56 years (IQR = 33–74 years); six (75%) were male. All eight patients were hospitalized; four (50%) died. The median age of patients who died was 62 years (IQR = 33–86 years).

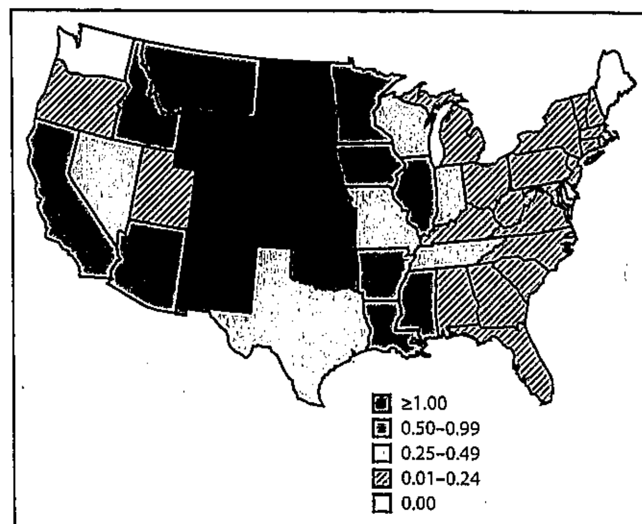
One SLEV neuroinvasive disease case was reported from Texas; the patient was hospitalized and survived.

Discussion

In 2013, WNV was the most common cause of neuroinvasive arboviral disease in the United States. However, LACV was the most common cause of neuroinvasive arboviral disease among children. More JCV cases were reported in 2013 than in any previous year and included the first cases reported from eight states. This increase is likely related to the initiation of routine immunoglobulin M testing for JCV at CDC in 2013 and suggests that the incidence of JCV infection in prior years might have been underestimated. EEEV disease, although rare, remained the most severe arboviral disease, with four deaths among eight patients. More than 90% of arboviral disease cases occurred during April–September, emphasizing the importance of focusing public health interventions during this period.

Reported numbers of arboviral disease cases vary from year to year. Weather (e.g., temperature and precipitation), zoonotic host and vector abundance, and human behavior (e.g., repellent use, outdoor activities, and use of air conditioning or screens in the home) are all factors that can influence when and where outbreaks occur. This complex ecology makes it difficult to predict how many cases of disease might occur in the future and where they will occur. Increased numbers of

FIGURE. Incidence* of reported cases of West Nile virus neuroinvasive disease, by state — United States, 2013



* Per 100,000 population, based on July 1, 2013 U.S. Census population estimates.

TABLE 2. Number and rate* of reported cases of arboviral neuroinvasive disease, by virus, U.S. Census region, and state — United States, 2013

	Virus									
	West Nile		La Crosse		Jamestown Canyon		Powassan		Eastern equine encephalitis	
U.S. Census region/State	No.	Rate	No.	Rate	No.	Rate	No.	Rate	No.	Rate
United States	1,267	0.40	77	0.02	15	<0.01	12	<0.01	8	<0.01
New England	11	0.08	—	—	3	0.02	3	0.02	2	0.01
Connecticut	1	0.03	—	—	—	—	—	—	1	0.03
Maine	—	—	—	—	—	—	1	0.08	—	—
Massachusetts	7	0.10	—	—	1	0.01	1	0.01	1	0.01
New Hampshire	1	0.08	—	—	1	0.08	1	0.08	—	—
Rhode Island	1	0.10	—	—	1	0.10	—	—	—	—
Vermont	1	0.16	—	—	—	—	—	—	—	—
Middle Atlantic	34	0.08	—	—	3	0.01	5	0.01	—	—
New Jersey	10	0.11	—	—	—	—	1	0.01	—	—
New York	18	0.09	—	—	3	0.02	4	0.02	—	—
Pennsylvania	6	0.05	—	—	—	—	—	—	—	—
East North Central	167	0.36	20	0.04	7	0.02	3	0.01	—	—
Illinois	86	0.67	—	—	—	—	—	—	—	—
Indiana	19	0.29	1	0.02	—	—	—	—	—	—
Michigan	24	0.24	—	—	—	—	—	—	—	—
Ohio	21	0.18	14	0.12	—	—	—	—	—	—
Wisconsin	17	0.30	5	0.09	7	0.12	3	0.05	—	—
West North Central	288	1.38	4	0.02	1	0.00	1	0.00	—	—
Iowa	24	0.78	—	—	—	—	—	—	—	—
Kansas	34	1.17	—	—	—	—	—	—	—	—
Minnesota	31	0.57	4	0.07	1	0.02	1	0.02	—	—
Missouri	24	0.40	—	—	—	—	—	—	—	—
Nebraska	54	2.89	—	—	—	—	—	—	—	—
North Dakota	64	8.85	—	—	—	—	—	—	—	—
South Dakota	57	6.75	—	—	—	—	—	—	—	—
South Atlantic	36	0.06	27	0.04	—	—	—	—	5	0.01
Delaware	3	0.32	—	—	—	—	—	—	—	—
District of Columbia	—	—	—	—	—	—	—	—	—	—
Florida	5	0.03	—	—	—	—	—	—	3	0.02
Georgia	4	0.04	1	0.01	—	—	—	—	1	0.01
Maryland	11	0.19	—	—	—	—	—	—	—	—
North Carolina	3	0.03	13	0.13	—	—	—	—	1	0.01
South Carolina	3	0.06	1	0.02	—	—	—	—	—	—
Virginia	6	0.07	2	0.02	—	—	—	—	—	—
West Virginia	1	0.05	10	0.54	—	—	—	—	—	—

See table footnotes on page 525.

reported cases and the identification of cases in new locations might reflect actual changes in incidence and epidemiology or increased disease awareness.

The incidence of WNV neuroinvasive disease declined substantially in 2013 (incidence of 0.40 per 100,000 population) compared with 2012 (0.92 per 100,000 population), when a large multistate outbreak occurred, with incidence nearing the levels observed in 2002 and 2003 (*4*). However, the incidence in 2013 was similar to that during 2004–2007 (median = 0.43; range = 0.39–0.50) and was higher than that during 2008–2011 (median = 0.18; range: 0.13–0.23) (*3–5*). WNV activity remained focalized in 2013, with more than half of the neuroinvasive disease cases being reported from just six states.

The findings in this report are subject to at least two limitations. First, ArboNET is a passive surveillance system that relies on clinicians to consider the diagnosis of an arboviral disease and obtain appropriate diagnostic tests, and on health-care providers and laboratories to report laboratory-confirmed cases to public health authorities. Second, testing and reporting are incomplete, leading to a substantial underestimate of the actual number of cases (*6*). For example, data from previous studies suggest there are 30–70 nonneuroinvasive disease cases for every reported case of WNV neuroinvasive disease (*7–9*). Extrapolating from the 1,267 WNV neuroinvasive disease cases reported, an estimated 38,000–88,500 nonneuroinvasive disease cases might have occurred in 2013. However, only 1,202 (1%–3%) were diagnosed and reported.

TABLE 2. (*Continued*) Number and rate* of reported cases of arboviral neuroinvasive disease, by virus, U.S. Census region, and state — United States, 2013

	Virus									
	West Nile		La Crosse		Jamestown Canyon		Powassan		Eastern equine encephalitis	
U.S. Census region/State	No.	Rate	No.	Rate	No.	Rate	No.	Rate	No.	Rate
East South Central	48	0.26	26	0.14	—	—	—	—	—	—
Alabama	3	0.06	1	0.02	—	—	—	—	—	—
Kentucky	1	0.02	—	—	—	—	—	—	—	—
Mississippi	27	0.90	2	0.07	—	—	—	—	—	—
Tennessee	17	0.26	23	0.35	—	—	—	—	—	—
West South Central	223	0.59	—	—	—	—	—	—	1	<0.01
Arkansas	16	0.54	—	—	—	—	—	—	1	0.03
Louisiana	34	0.74	—	—	—	—	—	—	—	—
Oklahoma	60	1.56	—	—	—	—	—	—	—	—
Texas	113	0.43	—	—	—	—	—	—	—	—
Mountain	216	0.94	—	—	—	—	—	—	—	—
Arizona	50	0.75	—	—	—	—	—	—	—	—
Colorado	90	1.71	—	—	—	—	—	—	—	—
Idaho	14	0.87	—	—	—	—	—	—	—	—
Montana	10	1.00	—	—	—	—	—	—	—	—
Nevada	8	0.29	—	—	—	—	—	—	—	—
New Mexico	24	1.15	—	—	—	—	—	—	—	—
Utah	4	0.14	—	—	—	—	—	—	—	—
Wyoming	16	2.75	—	—	—	—	—	—	—	—
Pacific	244	0.47	—	—	1	<0.01	—	—	—	—
Alaska	—	—	—	—	—	—	—	—	—	—
California	237	0.62	—	—	—	—	—	—	—	—
Hawaii	—	—	—	—	—	—	—	—	—	—
Oregon	7	0.18	—	—	1	0.03	—	—	—	—
Washington	—	—	—	—	—	—	—	—	—	—

* Per 100,000 population, based on July 1, 2013 U.S. Census population estimates.

What is already known on this topic?

West Nile virus (WNV) is the leading cause of domestically acquired arboviral disease in the United States. However, several other arboviruses can cause sporadic cases and outbreaks of neuroinvasive disease, mainly in the summer.

What is added by this report?

In 2013, WNV was the most common cause of neuroinvasive arboviral disease in the United States (1,267 cases). However, La Crosse virus was the most common cause of neuroinvasive arboviral disease among children. More Jamestown Canyon virus disease cases (22) were reported in 2013 than in any previous year and included the first cases reported from eight states. Eastern equine encephalitis virus disease, although rare, remained the most severe arboviral disease, with a 50% case-fatality ratio.

What are the implications for public health practice?

WNV and other arboviruses continue to be a source of severe illness each year for substantial numbers of persons in the United States. Maintaining surveillance remains important to identify outbreaks and guide prevention efforts. Prevention efforts depend upon applying insecticides, reducing mosquito breeding grounds, use of repellents, and wearing protective clothing.

Arboviruses continue to cause substantial morbidity in the United States. However, cases occur sporadically, and the epidemiology varies by virus and geographic area. Surveillance is essential to identify outbreaks and guide prevention efforts aimed at reducing the incidence of these diseases. Health-care providers should consider arboviral infections in the differential diagnosis of cases of aseptic meningitis and encephalitis, obtain appropriate specimens for laboratory testing, and promptly report cases to public health authorities (*2*). Because human vaccines against domestic arboviruses are not available, prevention of arboviral disease depends on community and household efforts to reduce vector populations (e.g., applying insecticides and reducing mosquito breeding sites), personal protective measures to decrease exposure to mosquitoes and ticks (e.g., use of repellents and wearing protective clothing), and screening blood donors.

Acknowledgment

ArboNET surveillance coordinators in state and local health departments.

別紙様式第２－１　　4

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	第88回日本感染症学会学術講演会，第62回日本化学療法学会総会合同学会(2014.6.18,19,20)/福岡県福岡市 62(SA)269/(2014.5)	公表国 日本	
販売名(企業名)	—					

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
観光のため 12/2～12/7 に仏領ポリネシアのボラボラ島に滞在した 27 歳の男性。37.2℃の発熱と顔面、体幹、四肢に斑状発疹を認めた以外は正常であった。国立感染症研究所における Real-time PCR にて Zika ウイルス RNA が検出され、日本における輸入 Zika 熱の初めての例と判断した。1 日後には発熱および他の症状は静まり、発疹も徐々に消退した。	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
血漿分画製剤でのジカウイルス感染伝播の報告はなく、製造工程中にモデルウイルスであるウシ下痢症ウイルス(BVDV)の不活化除去が確認された工程を設けているが、今後とも関連情報に注意していく。	今後ともジカウイルスに関連する情報に留意していく。

18

G1413878

O2-065 B型肝炎合併HIV感染者における治療経過
感

東京医科大学病院臨床検査医学科
○村松　崇，山元泰之，近澤悠志，備後真登，清田育男，
四本美保子，大瀧　学，尾形享一，萩原　剛，鈴木隆史，
天野景裕，福武勝幸

【背景】HIV感染者においてHBVの合併は高頻度で見られ，将来的に肝関連合併症を発症する可能性が高いとされる．HIV，HBV両者に有効な抗ウイルス療法によりウイルスは抑制されるが長期的経過に関する情報は少ない．
【対象・方法】2012年に当科外来を受診したHIV感染者でHIV治療開始時にHBs抗原が陽性であった症例のうち，2004年以降に治療開始し治療期間が2年以上継続している症例を対象とした．診療録を参照し後方視的に検討を行った．
【結果】48例が該当した．年齢の中央値は35（22-65）歳であった．男性が47例，女性1例であった．日本人が45例（94%）を占めていた．治療開始時のCD4数の中央値は212.2（4.6-532.8）/μLであり，HIV-RNAの中央値は52,000（230-620,000）copies/mLであった．HBVジェノタイプは32例（67%）で判明しており A 19例（59%），B 4例（13%），C 9例（28%）であった．治療を開始してからの観察期間の中央値は4.28（2.2-9.1）年であった．全例でテノホビルに加えてラミブジンあるいはエムトリシタビンを含めた治療が行われ，HBV-DNAは47例（98%）で2.1log-copies/mL未満に達していた．HBs抗原の陰性化は12例（25%）で認められ，HBs抗原が治療開始1年以内に陰性化した症例は4例（8%），2年以内に陰性化した症例は10例（21%）であった．
【考察】B型肝炎を合併したHIV感染者では抗ウイルス療法によりHBV-DNAは抑制されていた．HBs抗原消失にはより長期な治療期間を要する可能性が示唆された．

G1413879

O2-066 当院のリウマチ性疾患患者におけるB型肝炎ウイルス（HBV）血清学的プロファイルおよびB型肝炎再活性化患者の臨床的な特徴について
感

国家公務員共済組合連合会横浜南共済病院リウマチ感染症内科[1]，国家公務員共済組合連合会横浜南共済病院膠原病リウマチ内科[2]
井畑　淳[1,2]，小林幸司[2]，長田　侑[2]，○長岡章平[2]

【はじめに】近年リウマチ性疾患の治療においてHBVの再活性化が問題となっている．ガイドラインでもステロイドを含む免疫抑制療法を行う際のスクリーニングが推奨されている．厚生労働省研究班で前向き研究が既に行われリスクも検討されているが，市中病院では患者層が違うためリスクも異なる可能性がある．
【目的】当施設におけるリウマチ性疾患患者のHBV血清学的プロファイルと再活性化のリスク因子を解析すること．
【方法】当院通院中の患者2012例中HBVのスクリーニングを行った1090例のHBs抗原，HBs抗体，HBc抗体を後方視的に調査し，エンテカビル投与患者について臨床的なデータを他の患者と比較解析した．
【結果】年齢は67.3歳（18-95），男女比は1:3.7であった．HBs抗原陽性率は1.18%であり，HBs抗体陽性率は19.4%，HBc抗体陽性率は15.3%であった．年代ごとの陽性率は20，30，40，50，60，70歳以上でそれぞれHBs抗原（0/0/0/0.8/1.5/1.5%），抗HBs抗体（0/11.7/8.8/21.5/17.8/23.8%），抗HBc抗体（0/5.0/2.1/16.0/15.3/19.5%）であった．エンテカビル投与患者は7例で，6例が女性．年齢は69.9歳，うち6例でメトトレキサート（MTX）を，3例でステロイドを使用していた．生物学的製剤は3例で使用されていた．
【結論】HBVの血清学的陽性率，特に抗HBc抗体は年齢が上がるにしたがって上昇する傾向がみられた．エンテカビル投与患者の中にはMTXを使用していない患者も含まれていた．

X1480017

O2-067 わが国におけるE型肝炎の最近の流行状況
感

国立感染症研究所実地疫学専門家養成コース[1]，防衛医科大学校国際感染症学講座[2]，国立感染症研究所感染症疫学センター[3]
○金山敦宏[1,2]，山岸拓也[3]，砂川富正[3]，八幡裕一郎[3]，
松井珠乃[3]，大石和徳[3]

【背景】E型肝炎は，2003年11月の感染症法改正で「E型肝炎」として独立した4類感染症に分類され，診断後直ちに届出が必要な全数把握疾患となった．E型肝炎はA型肝炎より致死率が高いといわれており，その疫学情報は感染リスクを知るうえで重要である．【方法】感染症発生動向調査において2005年以降2013年11月までにE型肝炎と報告された患者626例についての疫学的解析を行った．【結果】2005~2011年は年間42-71例の報告であったが，2012年以降は年間100例を超えた．国内例の割合は，2005~2008年の間は71~79%であったが，2009年以降に86~94%と増加した．2005年~2013年11月の患者は，男性が約8割を占めた．国内例は中高年が多いのに対し，国外例は幅広い年齢から報告された．国内例の報告は，北海道が全国の34%，東京都が15%を占めた．国外例の主な推定感染地はアジアであった．記載のあった国内例の主な推定感染経路は，ブタやイノシシなどの肉類の喫食であったが，国外例には飲水などの記載もみられた．死亡者は3人（致死率0.5%）であった．【考察】最近のE型肝炎感染者の著しい増加は，E型肝炎の検査診断薬が保険適応されたことが一因と考えられたが，国民全体に感染のリスクについて改めて周知徹底することが重要であることを示唆している．謝辞：E型肝炎の報告に携わった関係者の皆様に感謝致します．

X1480018

O2-068 The first imported case of Zika fever in Japan
感

国立国際医療研究センター国際感染症センター[1]，国立感染症研究所ウイルス第一部第2室[2]
○忽那賢志[1]，高崎智彦[2]，藤谷好弘[1]，馬渡桃子[1]，
竹下　望[1]，早川佳代子[1]，加藤康幸[1]，金川修造[1]，
大曲貴夫[1]

A previously healthy 27-year-old man presented to our hospital on December 13, 2013 after four days of fever, headache, arthralgia and one day of rash. He had visited Bora Bora Island, an island in French Polynesia, from 2 to 7 December for sightseeing. He did not use insect repellent during the travel. His body temperature was 37.2℃ and he had maculopapular rash on his face, trunk, and extremities. Other clinical examination was normal. Zika virus RNA was detected by real-time PCR performed at National Institute of Infectious Diseases, and thus we made the diagnosis of Zika fever. His fever and other symptoms subsided one day after the first presentation and his rash had gradually disappeared. This is the first case of imported Zika fever in Japan.Zika fever is a dengue-like illness caused by Zika virus that spread through mosquito bites. The Centers for Disease Control and Prevention recently announced that the French Polynesian Department of Health has confirmed an outbreak of several hundred cases of Zika fever in French Polynesia, including Bora Bora.Zika fever should be considered in febrile patients with rash returned from French Polynesia.

別紙3

番号1

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 ：平成26年8月20日	新医薬品等の区分 ：該当なし	総合機構処理欄
一般的名称	－		研究報告の公表状況	－	公表国： WHO	
販売名（企業名）	－					
研究報告の概要	問題点（エボラウィルス疾患の増加） 2014年8月17日～18日，ギニア，リベリア，ナイジェリアおよびシエラレオネからエボラウイルス疾患（EVD）の新規症例計221例（検査確定例，可能性例，疑い例）および死亡106例が報告された．2014年8月18日時点の症例数の表（更新情報）が掲載されており，これら4ヵ国におけるEVDの累計は死亡1350例を含む2473例（確定例1460例，可能性例694例，疑い例319例）となっている．					使用上の注意記載状況等・その他参考事項等
報告企業の意見			今後の対応			
本報告は，当該生物由来製品による感染症情報ではない． 当該生物由来製品の原材料は「生物学的製剤基準」人血清アルブミンであり，本報告は人から人への感染と示唆されたものであったため，安全対策上報告することにした．			今後も感染症情報の収集に努め，当該生物由来製品に係る情報を入手した場合には速やかに調査・報告を行い安全性の確保に努める．			

19

NMP2014-0001

Global Alert and Response (GAR)

Ebola virus disease update - west Africa

Disease outbreak news
20 August 2014

Epidemiology and surveillance

Between 17 and 18 August 2014, a total of 221 new cases of Ebola virus disease (laboratory-confirmed, probable, and suspect cases) as well as 106 deaths were reported from Guinea, Liberia, Nigeria, and Sierra Leone.

Health sector response

WHO continues to engage in high-level communication efforts with affected countries, companies and organizations doing business in and from Africa, and national and global leaders. Currently, some companies have taken the decision to suspend services to the affected countries. This includes airlines and shipping companies. As a result of these decisions, countries are beginning to experience supply shortages, including fuel, food, and basic supplies. WHO is working with the UN World Food Programme to ensure adequate food and supplies, but calls on companies to make business decisions based on scientific evidence with regard to the transmission of Ebola virus.

Related links

— Ebola virus disease
— Fact sheet: Ebola virus disease
— Features: Ebola virus disease
— Disease outbreak news: Ebola virus disease (archive)
— Advice for travellers

Country information

— Guinea
— Liberia
— Nigeria

In the current outbreak, the majority of Ebola virus disease cases are a result of human-to-human transmission and failure to apply appropriate infection prevention and control measures in home care, some clinical settings, and in burial rituals. It is important to understand that EVD is not an airborne disease. Individuals may become infected as a result of contact with the bodily fluids (vomit, diarrhoea, sputum, blood, etc.) from persons who are confirmed to have EVD or who have died from EVD. Companies bringing goods and services to the affected countries are at low risk for exposure to EVD and WHO, under the International Health Regulations, encourages companies and organizations to continue providing these necessary supplies.

Countries around the world continue to engage in active surveillance for cases of EVD. Reports have been received by WHO of suspected cases and systematic verification is underway in a number of countries to confirm whether these are actual EVD cases. Overall, these reports are a positive sign that surveillance is working and countries are stepping up their preparedness to respond. As of today, no cases have been confirmed outside Guinea, Liberia, Nigeria, or Sierra Leone.

A high-level delegation from WHO is currently in the affected countries, working with the national authorities and partners to adapt strategic operations response plans. Meetings are planned with leaders in Liberia and Sierra Leone, where transmission continues to be high.

WHO does not recommend any travel or trade restrictions be applied except in cases where individuals have been confirmed or are suspected of being infected with EVD or where individuals have had contact with cases of EVD. (Contacts do not include properly-protected health-care workers and laboratory staff.) Temporary recommendations from the Emergency Committee with regard to actions to be taken by countries can be found at:

IHR Emergency Committee on Ebola outbreak in west Africa

Disease update

Confirmed, probable, and suspect cases and deaths from Ebola virus disease in Guinea, Liberia, Nigeria, and Sierra Leone, as of 18 August 2014

		New (1)	Confirmed	Probable	Suspect	Totals
Guinea						
	Cases	36	423	140	16	579
	Deaths	2	254	140	2	396
Liberia						
	Cases	126	242	502	228	972
	Deaths	95	212	239	125	576
Nigeria						
	Cases	0	12	0	3	15
	Deaths	0	4	0	0	4
Sierra Leone						
	Cases	59	783	52	72	907
	Deaths	9	335	34	5	374
Totals						
	Cases	221	1460	694	319	2473
	Deaths	106	805	413	132	1350

1. New cases were reported between 17 and 18 August 2014.

The total number of cases is subject to change due to ongoing reclassification. retrospective investigation, and availability of laboratory results. Data reported in the Disease Outbreak News are based on official information reported by Ministries of Health.

別紙様式第2　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　別紙3

医薬品
医薬部外品　研究報告　調査報告書
化粧品

識別番号・報告回数	回	報告日 年　月　日	第一報入手日 2014年8月29日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称		研究報告の公表状況	Disease Outbreak News:Ebola virus disease update – west Africa http://www.who.int/csr/don/2014_08_28_ebola/en/	公表国 ギニア、リベリア、ナイジェリア、シエラレオネ	
販売名（企業名）					

研究報告の概要

エボラウイルス疾患（EVD）の感染国4ヵ国（ギニア，リベリア，ナイジェリアおよびシエラレオネ）の各Ministry of Healthにより報告された進行中のEVDのアウトブレイクにおける可能性例および確定例数の合計は3069例（死亡1552例）である。本アウトブレイクは引き続き加速しており，過去21日以内に総症例数の40%以上が発生した。大半の症例が少数の地域のみに集中している。全体的な症例死亡率は52%（シエラレオネ：42%，ギニア：66%）である。

継続中の分析によると，暫定結果では症例は，引き続き本アウトブレイクのエピセンターであるGueckedou（ギニア），症例が増加し続けているLofa（リベリア），およびKenemaとKailahun（シエラレオネ）において集中している。

WHOとパートナーはエボラの治療拠点を作り、検査体制・接触者の追跡・社会的動員・安全な埋葬・エボラ以外の医療を強化している。
WHOは世界各地の疑い例について引き続きモニターを続け、系統的な確定診断を進めている。
WHOはEVDが確定している、あるいは疑われる人、EVD患者に接触した人（適切に防護している医療従事者や検査スタッフの接触は除く）を除いて旅行や貿易を制限は推奨していない。

使用上の注意記載状況・その他参考事項等

BYL-2014-425
Disease Outbreak News:Ebola virus disease update – west Africa

http://www.who.int/csr/don/2014_08_28_ebola/en/

報告企業の意見	今後の対応
エボラウイルス感染は人から人への感染症を起こす。現在、本ウイルスによる感染の報告は増加し続けている。 コージネイトFS及びコージネイトFSバイオセットの製造工程における病原体除去・不活化処理は，ウイルス及び細菌に対して有効であることが報告されている。なお，2007年4月以降，コージネイトFSの販売は行っていない。	現時点で新たな安全対策上の措置を講じる必要はないと考える。 今後も、新規人畜共通感染症や新たなウイルス感染症に関する情報収集に努める。

MedDRAバージョン 17.0

BYL-2014-0425

Global Alert and Response (GAR)

Ebola virus disease update - west Africa

Disease outbreak news
28 August 2014

Epidemiology and surveillance

- The total number of probable and confirmed cases in the current outbreak of Ebola virus disease (EVD) in the four affected countries as reported by the respective Ministries of Health of Guinea, Liberia, Nigeria, and Sierra Leone is 3069, with 1552 deaths.
- The outbreak continues to accelerate. More than 40% of the total number of cases have occurred within the past 21 days. However, most cases are concentrated in only a few localities.
- The overall case fatality rate is 52%. It ranges from 42% in Sierra Leone to 66% in Guinea.
- A separate outbreak of Ebola virus disease, which is not related to the outbreak in West Africa, was laboratory-confirmed on 26 August by the Democratic Republic of Congo (DRC) and is detailed in a separate edition of the Disease Outbreak News.

Health sector response

A full understanding of the outbreak that will lead to improved response requires detailed analysis of exactly where transmission is occurring (by district level) and of time trends. This analysis is ongoing. Preliminary results show that cases are still concentrated (62% of all reported cases since the beginning of the outbreak) in the epicentre of the outbreak in Gueckedou (Guinea); Lofa (Liberia), where cases continue to rise; and Kenema and Kailahun (Sierra Leone). Capital cities are of particular concern, owing to their population density and repercussions for travel and trade.

WHO and its partners are on the ground establishing Ebola treatment centres and strengthening capacity for laboratory testing, contact tracing, social mobilization, safe burials, and non-Ebola health care.

WHO continues to monitor for reports of rumoured or suspected cases from countries around the world and systematic verification of these cases is ongoing. Countries are encouraged to continue engaging in active surveillance and preparedness activities. Cases of EVD have been reported from the Democratic Republic of Congo. The cases in DRC are not related to the outbreak in West Africa. Outside of the four affected countries in West Africa and DRC, no new cases have been confirmed in other countries.

WHO does not recommend any travel or trade restrictions be applied except in cases where individuals have been confirmed or are suspected of being infected with EVD or where individuals have had contact with cases of EVD. (Contacts do not include properly-protected health-care workers and laboratory staff.) Temporary recommendations from the Emergency Committee with regard to actions to be taken by countries

can be found at:

IHR Emergency Committee on Ebola outbreak in west Africa

Disease update

As of 26 August 2014, the cumulative number of cases attributed to EVD in the four countries stands at 3069, including 1552 deaths. The distribution and classification of the cases are as follows: Guinea, 647 cases (482 confirmed, 141 probable, and 25 suspected), including 430 deaths; Liberia, 1378 cases (322 confirmed, 674 probable, and 382 suspected), including 694 deaths; Nigeria, 17 cases (13 confirmed, 1 probable, and 3 suspected), including 6 deaths; and Sierra Leone, 1026 cases (935 confirmed, 37 probable, and 54 suspected), including 422 deaths.

Confirmed, probable, and suspect cases and deaths from Ebola virus disease in Guinea, Liberia, Nigeria, and Sierra Leone

	Confirmed	Probable	Suspect	Totals
Guinea				
Cases	482	141	25	648
Deaths	287	141	2	430
Liberia				
Cases	322	674	382	1 378
Deaths	225	301	168	694
Nigeria				
Cases	13	1	3	17
Deaths	5	1	0	6
Sierra Leone				
Cases	935	37	54	1 026
Deaths	380	34	8	422
Totals				
Cases	1 752	853	464	3 069
Deaths	897	477	178	1 552

Note: Cases are classified as confirmed (any suspected or probable cases with a positive laboratory result); probable (any suspected case evaluated by a clinician, or any deceased suspected case having an epidemiological link with a confirmed case where it has not been possible to collect specimens for laboratory confirmation); or suspected (any person, alive or dead, suffering or having suffered from sudden onset of high fever and having had contact with: a suspected, probable or confirmed Ebola case, or a dead or sick animal; or any person with sudden onset of high fever and at least three of the following symptoms: headache, vomiting, anorexia/loss of appetite, diarrhoea, lethargy, stomach pain, aching muscles or joints, difficulty swallowing, breathing

difficulties, or hiccup; or any person with unexplained bleeding; or any sudden, unexplained death).

The total number of cases is subject to change due to ongoing reclassification, retrospective investigation, and availability of laboratory results. Data reported in the Disease Outbreak News are based on official information reported by Ministries of Health.

Related links

Ebola virus disease
Fact sheet: Ebola virus disease
Features: Ebola virus disease
Disease outbreak news: Ebola virus disease (archive)
Travel guidance for health authorities and the transport sector

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Country information

Guinea
Liberia
Nigeria
Sierra Leone

別紙様式第2-1

No. 10

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. 8. 29	該当なし	
一般的名称	新鮮凍結人血漿		研究報告の公表状況	WHO/Global Alert and Response (GAR); 29 August 2014.	公表国 WHO	
販売名(企業名)	新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)					

研究報告の概要

○WHO:エボラ対応　状況報告　2014年8月29日
2014年8月29日までの西アフリカにおけるエボラウイルス疾患(EVD)の確定症例、可能性の高い症例、疑い症例の合計数は3,052例であり、うち死亡例は1,546例である。
感染が蔓延、深刻な状況にある国々(ギニア、リベリア、シエラレオネ)について、症例数は直近の6週間、持続的に増加している。ギニアとシエラレオネでは新規報告症例数は比較的安定していたが、3カ国の先週の症例数増加はこれまでで最多であった。
アウトブレイクの完全な把握のためには、感染が実際に起きている場所と経時動向の詳細な分析が必要であり、現在そのような分析が行われている。予備的な結果から、症例は依然としてアウトブレイクの発生地であり現在も増加しているゲケドウ(ギニア)とロファ(リベリア)及びケネマとカイフラン(シエラレオネ)に集中し、アウトブレイク発生以降の全症例数の62%を占める。また人口が密集し、往来・交易の影響が強い首都は特に懸念される。
全体的な死亡率は51%であり、シエラレオネでは41%、ギニアでは66%である。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
WHOによる西アフリカのエボラウイルス疾患アウトブレイクの状況報告である。	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。

WHO: Ebola Response Roadmap Situation Report 1
29 August 2014

This is the first in a series of regular updates on the Ebola Response Roadmap. The update contains a review of the epidemiological situation and response monitoring. This first update provides a baseline against which progress on the full implementation of the roadmap can be measured against core Roadmap indicators over time. Additional indicators will be reported as data are consolidated.

The data contained in this report are based on the best information currently available. Substantial efforts are being made to improve the availability and accuracy of information about both the epidemiological situation and the response implementation.

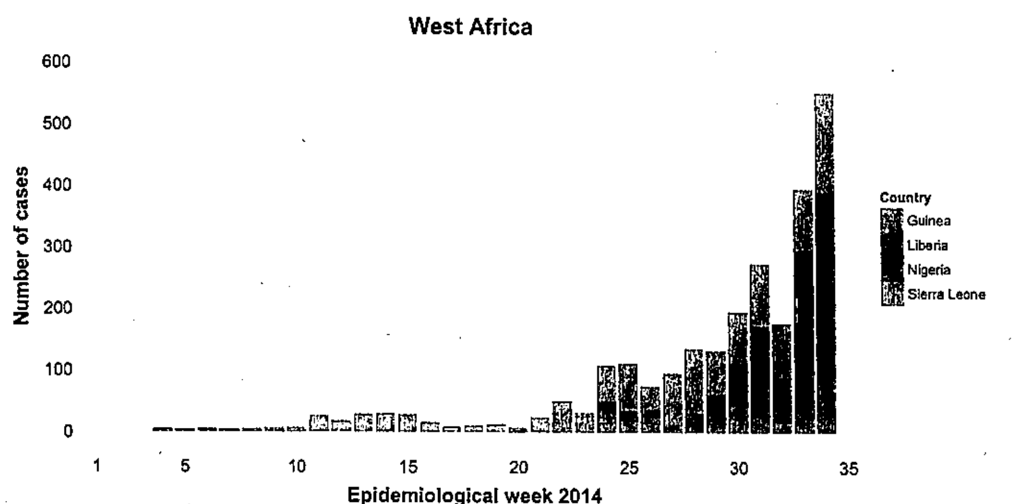
Following the roadmap structure, country reports fall into three categories: those with widespread and intense transmission (Guinea, Liberia, and Sierra Leone); those with an initial case or cases, or with localized transmission (Nigeria); and those sharing land borders with areas of active transmission (Benin, Burkina Faso, Côte d'Ivoire, Guinea-Bissau, Mali, Senegal) and those with international transportation hubs.

The total number of probable, confirmed and suspect cases in the current outbreak of Ebola virus disease (EVD) in West Africa is 3052, with 1546 deaths. Countries affected are Guinea, Liberia, Nigeria, and Sierra Leone.[1] The figure below shows the number of cases by country that have been reported between the beginning of January 2014 (epidemiological week 1) and 25 August 2014 (epidemiological week 34).

COUNTRIES WITH WIDESPREAD AND INTENSE TRANSMISSION

In the past six weeks, cases have continued to increase. Although the numbers of new cases reported in Guinea and Sierra Leone had been relatively stable, last week saw the highest weekly increase yet in all three affected countries. This highlights the urgent need to reinforce control measures and increase capacity for case management.

Combined epidemiological curves

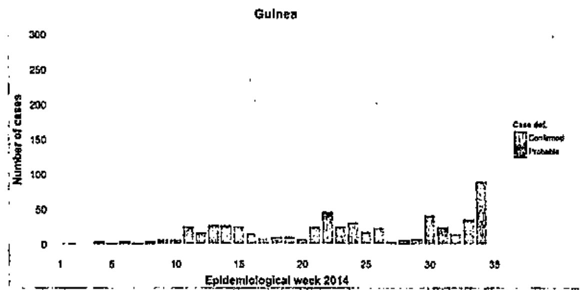


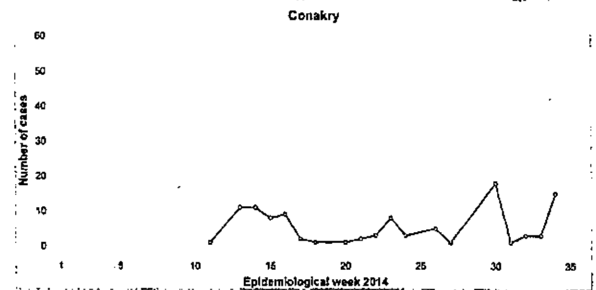
[1] A separate outbreak of Ebola virus disease, which is not related to the outbreak in West Africa, was reported on 24 August by the Democratic Republic of the Congo.

The figures below show the distribution of confirmed and probable cases in each of these countries, accompanied by numbers of cases over time in capital cities.

GUINEA

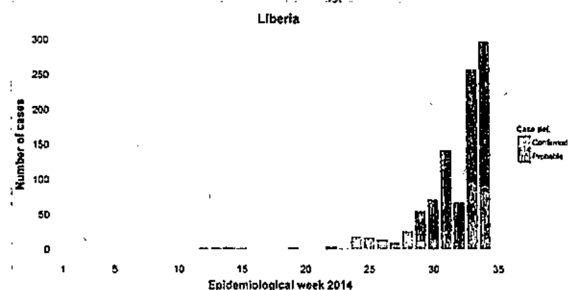
These data indicate that the reporting of cases in Guinea appears to have been relatively stable, but with a marked increase in the recent week. Priorities continue to be to reduce incidence in the epicentre (Gueckedou), and to address threatening foci in Conakry.

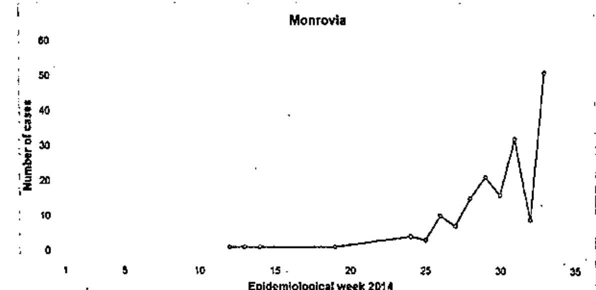




LIBERIA

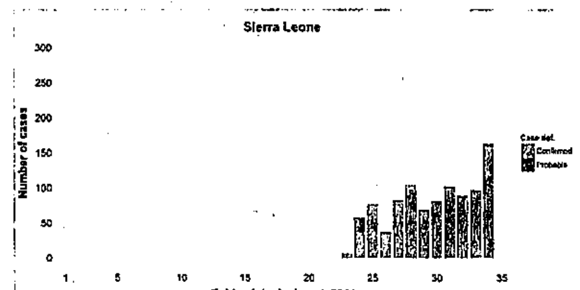
By contrast, in Liberia, cases are increasing in the epicentre (Lofa) and in the capital, Monrovia.

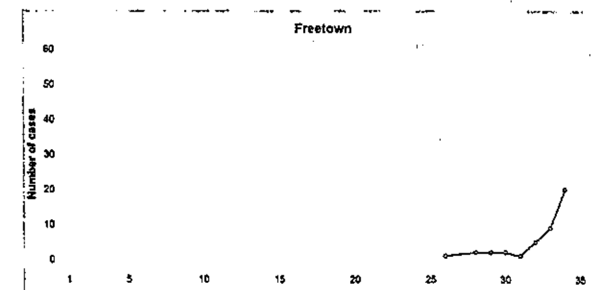




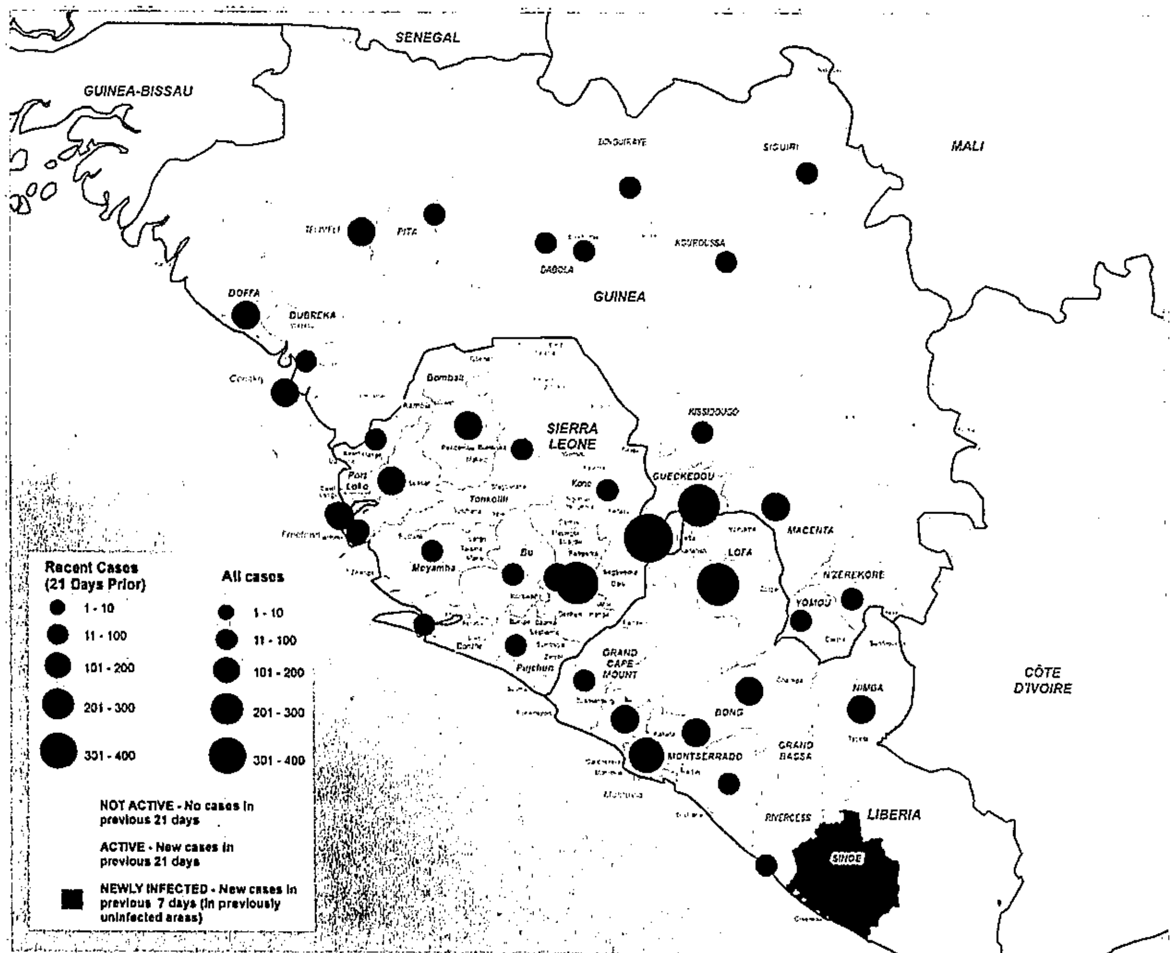
SIERRA LEONE

The incidence of cases in Sierra Leone has been relatively flat, although with increases in the past week. Problems in scaling up response measures persist, notably in two districts, Kenema and Kailahun. Numbers of cases increased in the capital, Freetown.





The map below shows the location of cases throughout the countries with most intense transmission, differentiating the cumulative number of cases to date in each area, and the number occurring within the past 21 days (i.e. corresponding to the incubation period for Ebola).



A full understanding of the outbreak that will lead to improved response requires detailed analysis of exactly where transmission is occurring (by district level), and of time trends. This analysis is ongoing. Preliminary results show that cases are still concentrated (62% of all reported cases since the beginning of the outbreak) in the epicentre of the outbreak, in Gueckedou (Guinea); Lofa (Liberia), where cases continue to rise; and Kenema and Kailahun (Sierra Leone). Capital cities are of particular concern, owing to their population density and repercussions for travel and trade.

The outbreak continues to escalate. Over 40% of the total number of cases have occurred within the past 21 days. However, most cases are concentrated in only a few places.

The overall case fatality rate is 51%. It ranges from 41% in Sierra Leone to 66% in Guinea.

Total number of probable, confirmed and suspected cases and deaths in Guinea, Liberia and Sierra Leone

Country	Case def.	Cases			Deaths			
		Total	21 days	21 days (%)	Total	21 days	21 days (%)	CFR (%)
Guinea	Confirmed	482	131	27	287	8	3	60
	Probable	141	7	5	141	0	0	100
	Suspected	25	20	80	2	0	0	8
	All	648	158	24	430	8	2	66
Liberia	Confirmed	322	192	60	225	16	7	70
	Probable	674	423	63	301	24	8	45
	Suspected	382	251	66	168	14	8	44
	All	1378	866	63	694	54	8	50
Sierra Leone	Confirmed	935	308	33	380	35	9	41
	Probable	37	4	11	34	2	6	92
	Suspected	54	19	35	8	2	25	15
	All	1026	331	32	422	39	9	41
All	All	3052	1355	44	1546	101	6	51

Note: Cases are classified as ***confirmed*** *(any suspected or probable cases with a positive laboratory result);* ***probable*** *(any suspected case evaluated by a clinician, or any deceased suspected case having an epidemiological link with a confirmed case where it has not been possible to collect specimens for laboratory confirmation); or* ***suspected*** *(any person, alive or dead, suffering or having suffered from sudden onset of high fever and having had contact with: a suspected, probable or confirmed Ebola case, or a dead or sick animal; or any person with sudden onset of high fever and at least three of the following symptoms: headache, vomiting, anorexia/ loss of appetite, diarrhoea, lethargy, stomach pain, aching muscles or joints, difficulty swallowing, breathing difficulties, or hiccup; or any person with unexplained bleeding; or any sudden, unexplained death).*

COUNTRIES WITH AN INITIAL CASE OR CASES, OR WITH LOCALIZED TRANSMISSION

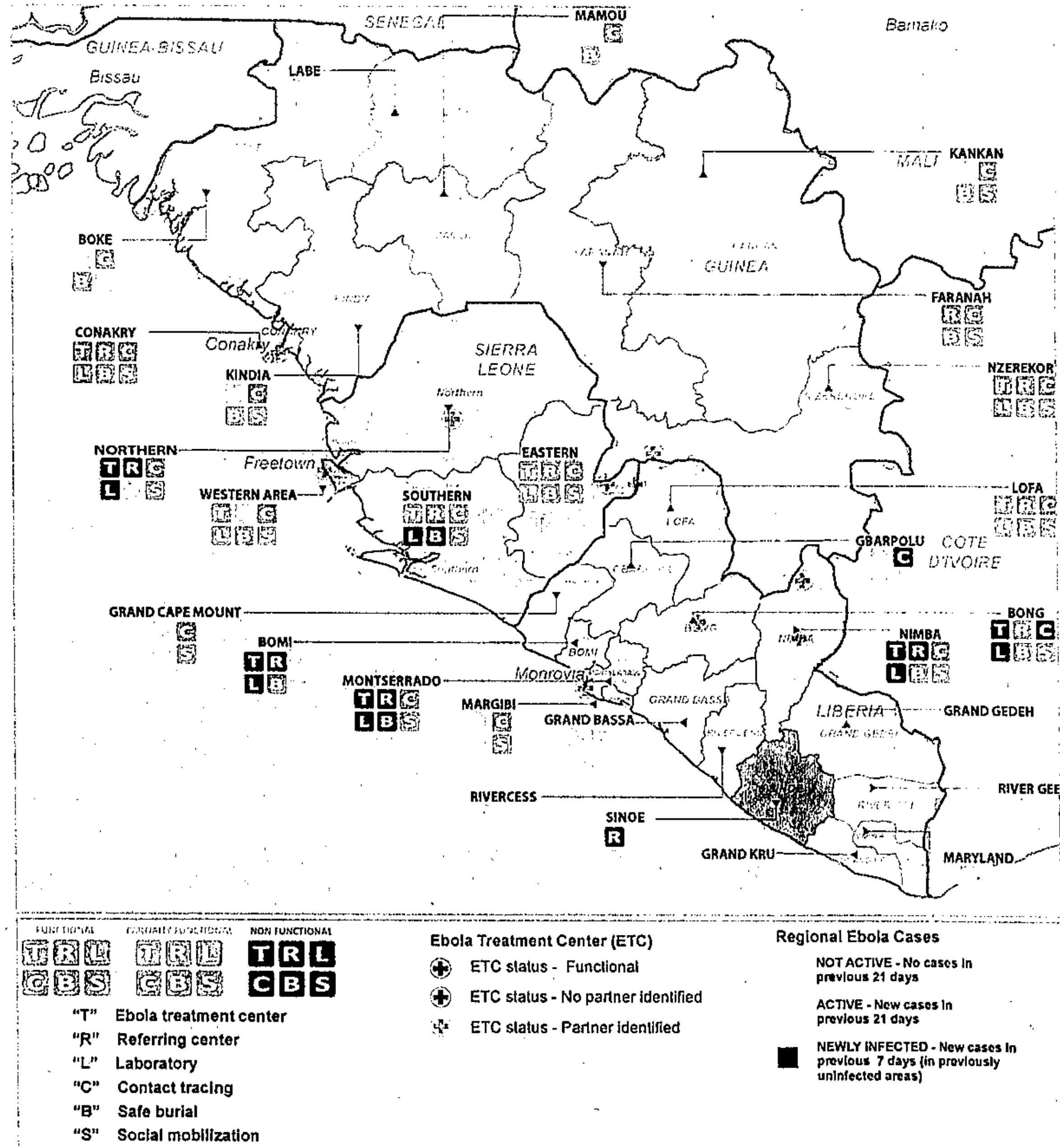
To date, the only country with cases linked to a case imported from a country with widespread and intense transmission is Nigeria. The table below shows the distribution of cases in that country.

Case definition	Cases			Deaths			
	Total	21 days	21 days %	Total	21 days	21 days %	CFR
Confirmed	15	6	40%	6	4	67%	40%
Probable	1	1	100%	1	0	0%	100%
Suspected	3	3	100%	0	0	-	0%
Total	19	10	53%	7	4	57%	37%

The first 14 confirmed cases were all linked to persons, including health care workers, in close contact with an air traveller from Liberia, who entered Lagos on 20 July and died five days later. On 27 August, 1 additional case was confirmed in Port Harcourt by the Ministry of Health. A Ministry of Health and WHO team is in Port Harcourt supporting contact tracing and further investigation of the incident.

RESPONSE MONITORING

The map below shows the availability of the six interventions that are necessary to control Ebola in the countries with most intense transmission. Work is ongoing to fully assess the coverage and quality of each intervention in the affected areas.



Disclaimer: Most of the analysis contained in this report is based on secondary data analysis from multiple sources. It has not yet been possible to verify all of this information.[2] Updates will be provided as more information becomes available.

[2] *Sources of information include: WHO and Ministries of Health reports, OCHA 3 W matrix and maps, reports from UNICEF Conakry and Geneva, situation reports from NGOs (IMC) and communications with other partners and foreign medical teams.*

Case management and infection prevention and control

There are serious problems with case management and infection prevention and control. The situation is worsening in Liberia and Sierra Leone.

- In Guinea, the capacity to manage the current load of EVD cases is currently adequate in Gueckedou and in Conakry.
- In Liberia, the capacity to cope with the increasing caseload remains dramatically low, especially in the capital, Monrovia, as well as in Bong and Nimba counties.
- In Nigeria, a 40-bed isolation unit has been set up in the Mainland Hospital, and is sufficient to accommodate the patients currently isolated. The Ministry of Health has set up an isolation unit in Lagos town to care for cases.
- In Sierra Leone, there is inadequate capacity to accommodate patients in Freetown. Patients must be transferred to Kenema, which is already overwhelmed by local demand.
- Health care workers continue to be seriously affected in all countries, especially in Liberia and Nigeria.

Laboratory capacity

- In Guinea, laboratory capacity currently appears to be sufficient. Support is being provided by the Pasteur Institute Dakar in Conakry, the European Union Mobile Laboratory in Gueckedou, and WHO.
- In Liberia, specimens from Lofa county are tested in Guinea. Additional laboratory support is needed in Lofa to alleviate this burden. Specimens from other counties far from Lofa are sent to Monrovia, where laboratory capacity, supported by the United States Army Medical Research Institute of Infectious Diseases, US National Institutes of Health, and US Centers for Disease Control and Prevention, is stretched. The need for more laboratory support is being assessed in Bong (Pheebe hospital), Nimba and Bomi counties.
- In Sierra Leone, additional laboratory support is needed in addition to the Kenema laboratory (supported by Metabiota and the US Department of Defense Critical Reagent Team) to cope with the increasing disease burden. A mobile laboratory from South Africa has been deployed to Freetown, where Ebola treatment centres are being constructed to care for patients locally and in better conditions, rather than referring them to Kenema.
- In Nigeria, the Lagos University Teaching Hospital virology lab and the Lagos University Laboratory are being supported by WHO and an EU mobile team from the WHO Collaborating Centre in Hamburg, Germany.

COUNTRIES SHARING LAND BORDERS WITH AREAS OF ACTIVE TRANSMISSION AND THOSE WITH INTERNATIONAL TRANSPORTATION HUBS

The following countries share land borders or major transportation connections with the affected countries and are therefore at risk for spread of the Ebola outbreak: Benin, Burkina Faso, Côte d'Ivoire, Guinea-Bissau, Mali, and Senegal.

WHO and a range of partners are working with countries to ensure that full Ebola surveillance, preparedness and response plans are in place in these countries. Priority activities include activation of active surveillance for clusters of unexplained death due to fever, provision of appropriate information and advice to the general public and travellers; identification of an isolation unit; verified access to a WHO-recognized laboratory; and the establishment of a strategy for identifying and monitoring contacts of any suspected case.

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. 7. 3	該当なし	
一般的名称	新鮮凍結人血漿		研究報告の公表状況	Azhar EI1, El-Kafrawy SA, Farraj SA, Hassan AM, Al-Saeed MS, Hashem AM, Madani TA. N Engl J Med. 2014 Jun 26;370(26):2499-505. doi: 10.1056/NEJMoa1401505. Epub 2014 Jun 4.	公表国 サウジアラビア	
販売名（企業名）	新鮮凍結血漿-LR「日赤」120（日本赤十字社） 新鮮凍結血漿-LR「日赤」240（日本赤十字社） 新鮮凍結血漿-LR「日赤」480（日本赤十字社）					

研究報告の概要

○ラクダからヒトへ中東呼吸器症候群コロナウイルス（MERS-CoV）が伝播した証拠
生来健康な43歳のサウジアラビア人男性が、発熱、鼻漏、咳、倦怠感及び息切れを呈し、2013年11月3日に入院したが、その後状態は悪化し続け、2013年11月18日に死亡した。患者はジッダの南約75kmにある小屋に9頭のヒトコブラクダを所有し、入院3日前まで3人の友人とともに毎日訪問していた。患者が発症する前週に4頭のラクダが鼻漏を呈し、患者は発症7日前に、症状のあるラクダのうち1頭の鼻に薬を塗布した。友人たちはラクダの分泌物や粘膜に直接接触せず、患者の発症後60日経っても健康であり、MERS-CoV抗体も検出されなかった。患者（入院1、4、14、16日目）及び薬を塗布されたラクダ（患者の入院5日目）から採取された鼻腔スワブ検体は、リアルタイムRT-PCRによりMERS-CoVのupE、ORF1a及びORF1b領域の全てが陽性であったが、28日後に同ラクダから採取された鼻腔スワブ検体はMERS-CoV RNA陰性であった。患者とラクダから得られたウイルス分離株のRNA配列は一致した。また、血清学的データの比較で、ヒトでのMERS-CoV感染が発生する前に、ラクダが感染していたことが示された。
これらのデータは、このヒトMERS-CoV感染致死症例が、感染したラクダとの接触を通して感染したことを示唆する。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見

サウジアラビアの43歳男性が鼻漏のあるラクダに接触した後にMERSで死亡した。患者とラクダから得られたMERS-CoV分離株が一致したため、ラクダからヒトへMERS-CoVが伝播したことが示唆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ほか、発熱などの体調不良者を献血不適としている。また、中東呼吸器症候群（MERS）が指定感染症として定められたことから、問診を徹底するよう関係職員に周知している。今後も引き続き情報の収集に努める。

22

BRIEF REPORT

Evidence for Camel-to-Human Transmission of MERS Coronavirus

Esam I. Azhar, Ph.D., Sherif A. El-Kafrawy, Ph.D., Suha A. Farraj, M.Sc., Ahmed M. Hassan, M.Sc., Muneera S. Al-Saeed, B.Sc., Anwar M. Hashem, Ph.D., and Tariq A. Madani, M.D.

SUMMARY

We describe the isolation and sequencing of Middle East respiratory syndrome coronavirus (MERS-CoV) obtained from a dromedary camel and from a patient who died of laboratory-confirmed MERS-CoV infection after close contact with camels that had rhinorrhea. Nasal swabs collected from the patient and from one of his nine camels were positive for MERS-CoV RNA. In addition, MERS-CoV was isolated from the patient and the camel. The full genome sequences of the two isolates were identical. Serologic data indicated that MERS-CoV was circulating in the camels but not in the patient before the human infection occurred. These data suggest that this fatal case of human MERS-CoV infection was transmitted through close contact with an infected camel.

MERS-COV IS A NEWLY IDENTIFIED HUMAN CORONAVIRUS THAT HAS RE-cently emerged in the Middle East region.[1-3] Current epidemiologic data suggest multiple zoonotic transmissions from an animal reservoir leading to human infection, sometimes with secondary transmission events in humans.[4] In this study, we describe reverse-transcriptase–polymerase-chain-reaction (RT-PCR) detection, isolation, and sequencing of MERS-CoV from a camel and from a patient who died of laboratory-confirmed MERS-CoV infection in Jeddah, Saudi Arabia.

From the Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences (E.I.A.), Special Infectious Agents Unit, King Fahd Medical Research Center (E.I.A., S.A.E., S.A.F., A.M. Hassan, M.S.A., A.M. Hashem), and the Departments of Medical Microbiology and Parasitology (A.M. Hashem) and Medicine (T.A.M.), Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia. Address reprint requests to Dr. Madani at the Department of Medicine, King Abdulaziz University, PO Box 80215, Jeddah 21589, Saudi Arabia, or at tmadani@kau.edu.sa.

This article was published on June 4, 2014, and updated on June 9, 2014, at NEJM.org.

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CASE REPORT

A 43-year-old previously healthy Saudi man who had retired from the military was admitted to the intensive care unit at King Abdulaziz University Hospital, Jeddah, on November 3, 2013, with severe shortness of breath. Eight days before admission, fever, rhinorrhea, cough, and malaise developed, followed 5 days later by shortness of breath that gradually worsened. The patient owned a herd of nine camels that he kept in a barn about 75 km south of Jeddah. The patient and three of his friends had been visiting the camels daily until 3 days before his admission. The patient's friends reported that four of the animals (Camels B, F, G, and I) had been ill with nasal discharge during the week before the onset of the patient's illness (Table 1). As reported by his friends, the patient had applied a topical medicine in the nose of one of the ill camels (Camel B) 7 days before the patient's onset of illness. None of the patient's friends had had direct contact with the camels' secretions or mucous membranes. They all remained well during the 60 days that followed the onset of illness in the patient.

Five days after the patient's hospitalization, symptoms of upper respiratory tract

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Table 1. Identification of MERS-CoV RNA in Nasal Swabs and MERS-CoV Antibodies in Human and Dromedary Samples.*

Host	Age	Real-Time RT-PCR				IFA	
		Original Nasal Samples†			Culture Isolates‡	Serum Sample 1§	Serum Sample 2¶
		upE	ORF1a	ORF1b			
	yr	*cycle threshold*					
Patient	43	27.5	34.7	33.3	13.2	<1:10	1:1,280
Camel A	9	ND	ND	ND	ND	1:51,200	1:51,200
Camel B‖	<1	36.3	36.9	37.5	11.1	1:160	1:1,280
Camel C	10	ND	ND	ND	ND	1:12,800	1:12,800
Camel D	12	ND	ND	ND	ND	1:1,280	1:1,280
Camel E	12	ND	ND	ND	ND	1:640	1:12,800
Camel F‖	<1	ND	ND	ND	ND	1:80	1:1,280
Camel G‖	<1	ND	ND	ND	ND	1:320	1:640
Camel H	12	ND	ND	ND	ND	1:10	1:640
Camel I‖ **	2	ND	ND	ND	ND	1:10	1:1,280

* IFA denotes immunofluorescence assay, MERS-CoV Middle East respiratory syndrome coronavirus, and ND not detected.

† Results of a real-time reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay are shown for the first samples collected from the patient and the camels. Eluted RNA was screened for the MERS-CoV upstream region of the E gene (upE region) and confirmed by targeting the open reading frame ORF1a and ORF1b regions. Samples with a cycle threshold below 40 were considered to be positive.

‡ The culture isolates tested for the upE region were procured from Vero cells inoculated with the first nasal samples collected from the patient and the camels.

§ Serum sample 1 was obtained on hospital admission from the patient and 5 days later from the camels.

¶ Serum sample 2 was collected on hospital day 14 from the patient and 28 days after the collection of sample 1 (i.e., 33 days after the patient's hospital admission) from the camels.

‖ The presence of rhinorrhea was reported during the week preceding the onset of the patient's illness.

** The first nasal sample from this camel was collected 33 days after the patient's hospital admission.

infection developed in his 18-year-old daughter and resolved spontaneously within 3 days without any complications. After admission, the patient's condition continued to deteriorate, and he died on November 18, 2013. Figure 1 shows a timeline of the main events. Details of the clinical assessment and hospital course are provided in the Supplementary Appendix (available with the full text of this article at NEJM.org), including laboratory investigations (Table S1), chest radiography (Fig. S1), and computed tomography (Fig. S2).

METHODS

FIELD INVESTIGATIONS

Five days after the patient was admitted to the hospital, a veterinarian examined the nine camels, a procedure that was repeated for the following 27 days. During these examinations, the camels were found to be completely healthy, with no nasal discharge.

SAMPLE COLLECTION

Nasal swabs were obtained from the patient on hospital days 1, 4, 14, and 16. Blood samples were also collected from the patient on days 1 and 14. In addition, a nasal swab was obtained from the patient's daughter 1 day after the onset of her illness. Six days after the patient's admission, blood samples were collected from his three friends, who were asymptomatic. Nasal swabs, blood, milk, urine, and rectal swabs were collected from the nine camels 5 days after the patient's admission. Blood and nasal samples were collected again from the camels 28 days later (i.e., 33 days after the patient's hospital admission). All nasal swabs, which were immersed in viral transport medium, and other samples were transported in a cold container to the Special Infectious Agents Unit, a biosafety level 3 laboratory at King Fahd Medical Research Center, King Abdulaziz University, Jeddah, for analysis. Blood samples were centrifuged and serum samples were collected for analysis.

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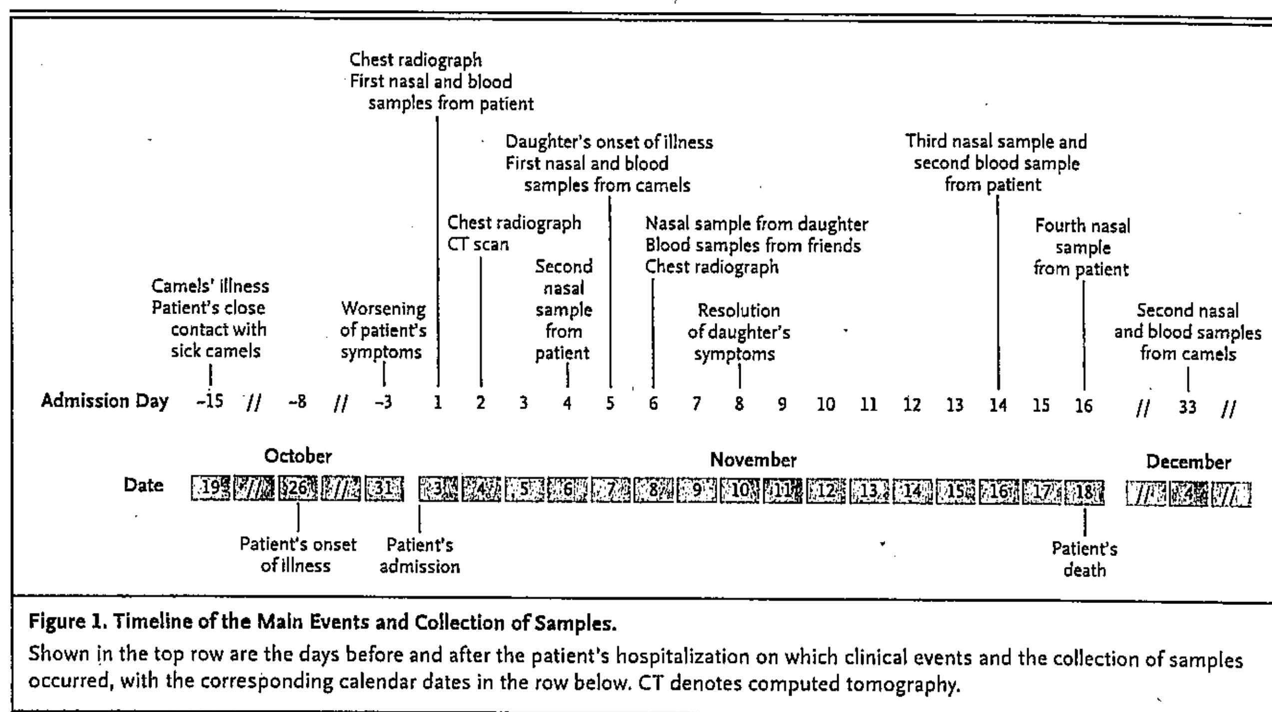

Figure 1. Timeline of the Main Events and Collection of Samples.

Shown in the top row are the days before and after the patient's hospitalization on which clinical events and the collection of samples occurred, with the corresponding calendar dates in the row below. CT denotes computed tomography.

CELL CULTURE

Vero cells (ATCC CCL-81) were inoculated with 100 μl of the nasal swab medium and maintained in complete Dulbecco's Modified Eagle's Medium, as described previously.[4] The cells were incubated in a humidified atmosphere at 37°C in 5% carbon dioxide and examined daily for a cytopathic effect. Cell-culture supernatants were collected when a cytopathic effect was observed and were analyzed by means of real-time RT-PCR. The MERS-CoV isolate that was generated from the first culture passage was used for whole viral genome sequencing.

MOLECULAR DETECTION

RNA was extracted from the nasal swabs or culture supernatants with the use of the QIAamp Viral RNA Mini Kit (Qiagen), according to the manufacturer's instructions. Eluted RNA was screened for the MERS-CoV upstream region of the E gene (upE region) and confirmed by targeting the open reading frame region ORF1a and ORF1b with the use of real-time RT-PCR, as described previously.[2] Further confirmation was performed by partially sequencing the RNA-dependent RNA polymerase (RdRp) and nucleocapsid (N) regions of the viral genome, as recommended by the World Health Organization.[5] Both assays were conducted on the original samples collected from the patient and from Camel B. (Details regarding the RT-PCR assay and sequencing are provided in the Supplementary Appendix.)

VIRAL GENOME SEQUENCING

Viral RNA extracted from culture supernatants that had been inoculated with samples from the patient and from Camel B were subjected to RT-PCR amplification with an ABI Veriti thermal cycler (Applied Biosystems) with the use of primer pairs covering the whole length of the viral genome. The RT-PCR fragments were then sequenced, as described in the Supplementary Appendix. Sequences were deposited in GenBank and given accession numbers (KF958702 MERS-CoV-Jeddah-human-1 for the patient's isolate and KF917527 MERS-CoV-Jeddah-camel-1 for the camel's isolate). Genomic regions containing unique mutations were also partially resequenced from the original samples obtained from both the patient and the camel.

PHYLOGENETIC ANALYSIS

Sequences were aligned with all MERS-CoV reference strains retrieved from GenBank. Phylogenetic analysis and distance calculations were performed with the use of Molecular Evolution-

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ary Genetics Analysis (MEGA) software, version 5, by means of the neighbor-joining method with 1000 bootstrap replicates.

CONVENTIONAL IMMUNOFLUORESCENCE ASSAY

Serum samples that were collected from the patient and the camels were serially diluted up to 1:51,200. A conventional immunofluorescence assay to detect anti–MERS-CoV antibodies was performed in Vero cells infected with MERS-CoV, with the use of a commercially available test kit (Anti-MERS-CoV IFT, EUROIMMUN), as described previously.[2]

RESULTS

Nasal swabs that were collected from the patient on hospital days 1, 4, 14, and 16 were all positive for MERS-CoV upE, ORF1a, and ORF1b regions on real-time RT-PCR (Table 1). The first nasal sample collected from one camel (Camel B) was also positive for the three regions (Table 1). The second sample collected from this camel 28 days later was negative. Nasal samples that were collected from the other camels on day 1 (seven camels) and on day 28 (eight camels) were negative for MERS-CoV RNA (Table 1). The milk, urine, and rectal samples collected from all camels were negative for MERS-CoV RNA. The nasal sample collected from the patient's daughter, who had symptoms of upper respiratory tract infection, was negative for MERS-CoV and H1N1 influenza virus RNA.

Vero cells that had been inoculated with the first samples obtained from the patient and from Camel B showed a cytopathic effect in the form of detachment of cells 3 days after inoculation. Culture supernatants collected 3 days after inoculation with both samples were positive on real-time RT-PCR for the upE, ORF1a, and ORF1b regions. (Table 1 shows the results for the upE region.)

To further confirm these results and to exclude the possibility of cross-contamination between the cultures for the patient and Camel B, RNA samples that had been extracted from the original nasal swabs obtained from the patient and from Camel B were subjected to partial genome sequencing of 242-bp fragments (nucleotides 15049 to 15290) and 312-bp fragments (nucleotides 29549 to 29860) in the RdRp and N regions of the viral genome, respectively. The presence of MERS-CoV–specific sequences in these samples was confirmed. Alignment of these two fragments from the two samples confirmed that they were identical.

Figure 2 (facing page). Phylogenetic Analysis of the Full Spike-Protein Coding Gene and the Full Genome in Human and Camel Samples.

Shown are the phylogenetic trees that were constructed for the gene encoding the full spike protein (nucleotides 21450 to 25511) (Panel A) and the full genome (Panel B). Trees were generated from nucleotide alignments of sequences from culture isolates obtained from both the patient and Camel B and from the Middle East respiratory syndrome (MERS) coronavirus sequences available in the GenBank database. The sequences of the samples obtained from the patient and Camel B are indicated by red squares. The abbreviation cg denotes complete genome sequence, and pg denotes partial genome sequence.

Full genome sequencing of culture isolates obtained from the patient and from Camel B showed that the two samples were 100% identical. Alignment of the sequences that were obtained in this study with others reported in GenBank showed unique mutations in 14 nucleotide positions (Table S2 in the Supplementary Appendix). Partial genome sequencing of the regions containing these mutations from the original samples obtained from the patient and from Camel B showed the same mutations except for a T-to-C substitution at position 10154 and a T-to-G transversion at position 25800 (Fig. S3 in the Supplementary Appendix). Apart from these differences, there was complete concordance between the two sets of partial sequences obtained directly from the original samples and those obtained from cultures.

Phylogenetic analysis of the gene encoding the full spike protein (nucleotides 21450 to 25511) and the whole genome indicated that the MERS-CoV isolates obtained from the patient and from Camel B were closely related to the Riyadh 3/2013 isolate (KF600613.1), the MERS coronavirus isolate (known as the Munich/Abu Dhabi isolate) (KF192507.1), the betacoronavirus England 1 isolate (KC164505.2), and the human betacoronavirus 2c England-Qatar/2012 isolate (KC667074.1), with 99.8% similarity in sequence identity matrix (Fig. 2). Similar topology was also observed for other viral genes obtained in this study.

The serum sample collected from the patient

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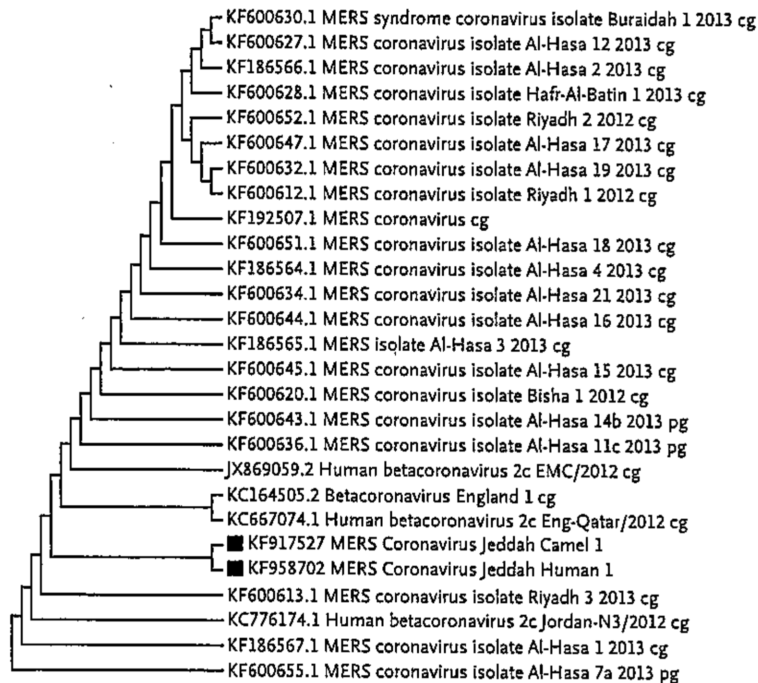

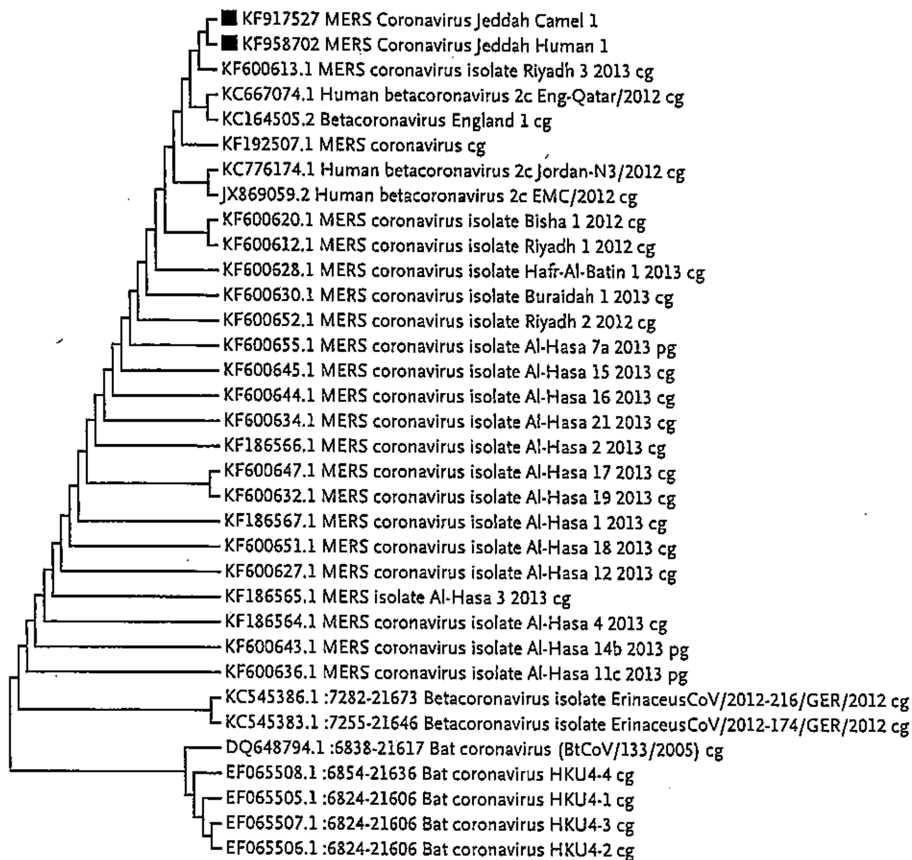

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on day 1 was negative for MERS-CoV antibodies (<1:10) on immunofluorescence assay, whereas the sample collected on day 14 showed quadrupling of the antibody titer (1:1280) (Table 1, and Fig. S4 in the Supplementary Appendix). Paired serum samples that were collected from Camel B also showed an increase in the antibody titer by a factor of more than 4, an increase that also occurred in four other camels (Camels E, F, H, and I) (Table 1). The remaining four camels (Camels A, C, D, and G) had high antibody titers in both samples (Table 1). No MERS-CoV antibodies were detected in serum samples obtained from the patient's three friends.

DISCUSSION

The epidemiologic features of the MERS-CoV infections suggest zoonotic transmission from an animal reservoir to humans, perhaps through an intermediate animal host.[4,6-9] Limited secondary transmission from human to human has also been confirmed in both health care and household settings.[6,7,10] Analysis of short genomic sequences indicated that MERS-CoV might have an ancestor in bats.[8,11,12] This hypothesis was further supported by the detection of a small fragment of genomic sequence identical to that of EMC/2012 MERS-CoV Essen isolate (KC875821) in an Egyptian tomb bat (*Taphozous perforatus*) that had been captured in Saudi Arabia.[13] In addition, serologic studies have shown the presence of cross-reactive antibodies to MERS-CoV in dromedary camels in Oman, the Canary Islands, and Egypt.[4,14]

However, the mere presence of antibodies that are cross-reactive to MERS-CoV without simultaneous isolation or PCR detection of the virus from camels in these studies could not confirm their role as reservoirs or intermediate hosts for transmitting the virus to humans. In a recent study, the presence of MERS-CoV RNA was confirmed on real-time RT-PCR assay and partial genome sequencing of the viral RNA in 3 of 14 nasal samples collected from 14 camels on a farm in Qatar and in 2 nasal swabs collected from two patients on the same farm.[15] However, conclusive evidence of transmission from camels to humans or vice versa could not be established.[15]

In our study, the evidence suggests that a dromedary camel was the source of MERS-CoV that infected a patient who had had close contact with the camel's nasal secretions. The presence of identical sequences in the two MERS-CoV isolates recovered from the patient and from Camel B suggests that direct cross-species transmission had probably occurred between the two without any intermediate host. Although cross-contamination between the two samples could be a concern, it is unlikely, owing to the independent times and locations of collection and processing of the two samples. We concluded that the camels had been infected before the patient, since the first serum samples collected from four of the nine camels showed high MERS-CoV antibody titers that did not subsequently increase, as compared with negative MERS-CoV antibody (<1:10) in the patient's first serum sample and the subsequent quadrupling (1:1280) in the patient's second sample, collected 2 weeks later. In addition, active transmission of MERS-CoV among the nine dromedary camels was suggested by a rising MERS-CoV antibody titer and the detection of MERS-CoV RNA and viral isolation from the nasal secretions of Camel B, along with evidence of MERS-CoV antibody titers in the other eight camels that were either high (in four camels) or rising (in four camels). Although it is possible that camels shed the virus intermittently, the clearance of MERS-CoV from the nasal secretions of Camel B and the absence of any detectable MERS-CoV RNA from the secretions of the other camels, despite the serologic evidence of recent infection, suggest that these camels were transiently infected and did not shed the virus subsequently. These two isolates showed close similarity (99.8%) to the Munich/Abu Dhabi isolate (KF192507.1), betacoronavirus England 1 isolate (KC164505.2), human betacoronavirus 2c England-Qatar/2012 isolate (KC667074.1), and Riyadh 3/2013 isolate (KF600613.1). However, unique mutations were identified in the sequences that differentiated them from other, previously reported sequences (Table S2 in the Supplementary Appendix). The nucleotide differences between sequences of the original samples and those of the culture isolates at positions 10154 and 25800 were probably caused by cell-culture adaptive mutations.

In conclusion, these data suggest that MERS-CoV causes a zoonotic infection that can infect dromedary camels and can be transmitted from them to humans through close contact. These data also suggest that the camels were transiently infected, since the virus seemed to be

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cleared after the acute infection. Camels may act as intermediate hosts that transmit the virus from its reservoir to humans. The exact reservoir that maintains the virus in its ecologic niche has yet to be identified.

Editor's note: The patient and camels discussed in this article are also described in Memish ZA, Cotten M, Meyer B, et al. Human infection with MERS coronavirus after exposure to infected camels, Saudi Arabia, 2013. Emerg Infect Dis 2014;20:1012-5.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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一般的名称	①②③⑨⑩pH4 処理酸性人免疫グロブリン ④⑤⑥⑦⑧ポリエチレングリコール処理人免疫グロブリン ⑪⑫人免疫グロブリン	研究報告の公表状況	ww.nhs.uk/news/2014/07July/Pages/Deadly-MERS-camel-flu-may-now-be-airborne.aspx/2014/07/24	公表国 サウジアラビア	
販売名（企業名）	①日赤ポリグロビン N5%静注 0.5g/10mL （日本血液製剤機構） ②日赤ポリグロビン N5%静注 2.5g/50mL （日本血液製剤機構） ③日赤ポリグロビン N5%静注 5g/100mL （日本血液製剤機構） ④献血ｳﾞｪﾉｸﾞﾛﾌﾞﾘﾝ IH5%静注 0.5g/10mL （日本血液製剤機構） ⑤献血ｳﾞｪﾉｸﾞﾛﾌﾞﾘﾝ IH5%静注 1g/20mL （日本血液製剤機構） ⑥献血ｳﾞｪﾉｸﾞﾛﾌﾞﾘﾝ IH5%静注 2.5g/50mL （日本血液製剤機構） ⑦献血ｳﾞｪﾉｸﾞﾛﾌﾞﾘﾝ IH5%静注 5g/100mL （日本血液製剤機構） ⑧献血ｳﾞｪﾉｸﾞﾛﾌﾞﾘﾝ IH5%静注 10g/200mL （日本血液製剤機構） ⑨献血ｳﾞｪﾉｸﾞﾛﾌﾞﾘﾝ-IH ﾖｼﾄﾐ （日本血液製剤機構） ⑩日赤ポリグロビン N10%静注 5g/50mL （日本血液製剤機構） ⑪日赤ポリグロビン N10%静注 10g/100mL （日本血液製剤機構） ⑫ｸﾞﾛﾌﾞﾘﾝ筋注 450mg/3mL「ﾍﾞﾈｼｽ」 （日本血液製剤機構） ⑬ｸﾞﾛﾌﾞﾘﾝ筋注 1500mg/10mL「ﾍﾞﾈｼｽ」 （日本血液製剤機構）				

研究報告の概要

致命的な MERS「ラクダインフルエンザ」は現在、空気感染性と考えられている
見出しの裏に隠された真実
2014 年 7 月 24 日木曜日

2012 年中東において MERS が出現
「致命的な MERS ウイルスは”現在、空気感染性の可能性がある”と考えられている」と、インデペンデント紙は報告している。サウジアラビアのラクダ飼育小屋で採取された大気サンプルから、致死率 30%と推定される中東呼吸器症候群（MERS）ウイルスが検出された。これによって、このウイルスがインフルエンザと同じように空気を介して伝播する可能性が出ている。
MERS は 2012 年に出現し、極めて深刻な状況と考えられ、重度の呼吸困難、腎不全および死に至る（中には無症候性のウイルスキャリアとなる場合もあるようである）。
世界保健機関（WHO）は、2012 年以降、研究施設が確認した感染症例 837 例が報告され、少なくとも 291 例がこの疾患に関連して死亡していると発表した。
2013 年、ラクダがこのウイルスの主たる感染源であるエビデンスが認められた（別称の由来）。
この話題の裏で行われた研究によって、サウジアラビアに居住するある男性と彼が飼育するラクダの例が浮かび上がった。この男性と彼のラクダのうち数頭が MERS に感染し、不幸にも男性はその後、死亡した。
調査の結果、感染ラクダが飼育されていた小屋で採取された大気サンプルから MERS ウイルスの遺伝子断片が検出された。
男性が空気を介して感染したという具体的なエビデンスはなく、男性がラクダと直接接触していたことも指摘しておかなければならない。
しかし、空気感染の可能性があり、懸念されている。
このウイルスがどのようにヒトに伝播するのかについて深く理解するためにはこの新たなウイルスの研究を続け、その伝播を追跡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

代表として献血ヴェノグロブリン IH5%静注 0.5g/10mL の記載を示す。
2. 重要な基本的注意
(1) 本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-1 抗体陰性で、かつ ALT（GPT）値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分からポリエチレングリコール 4000 処理、DEAE セファデックス

23

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旅行者へのアドバイス 現在、MERS 感染を防ぐための薬剤などはない。 中東に旅行する場合は、風邪やインフルエンザなどの空中浮遊ウイルスに対する標準的な注意事項を守ることが危険性を抑えるのに役立つと思われる。これには次のようなものがある。 ・必ず石鹸と水で定期的に手を洗う ・動物に触れる前および触れた後は手を洗う ・病気の動物との接触を避ける ・病原菌を除去するため、キーボード、電話器およびドアの取っ手などの表面を定期的に清浄な状態にする ・咳やくしゃみをする場合はティッシュを用いて口や鼻を押さえる ・使用済みのティッシュはできるだけすぐにゴミ箱に捨てる WHO はより具体的に、ウイルスが蔓延していることがわかっている地域の農場、市場または飼育小屋エリアを訪れる場合は、動物、特にラクダとの密接な接触を避けるよう助言している。更に、生のラクダの乳または尿を飲まないようにするほか、適切に調理されていないラクダの肉も摂取してはならない。 **この話題はどこから生じたものか？** サウジアラビア王国 Jeddah の King Abdulaziz 大学の研究者らによって試験が行われた。試験の研究資金はこの大学から援助されたものである。 試験は米国微生物学会のピアレビュー誌で発表された。 このジャーナル全体はオープンアクセスであり、この最新の研究を含め、誰でも無料でオンライン上の発表論文を読むことができる。 この試験によって、重度呼吸器症状および腎不全の原因となる新規ヒトウイルスである中東呼吸器症候群コロナウイルス（MERS-CoV）の起源および伝播の可能性に関する新たな研究が浮き彫りにされた。 WHO は、この疾患が認められてから、研究室で確認されたヒトでの感染症例 837 例と少なくとも死亡 291 例があったと報告している。 著者らは、これらの症例がアジア、アフリカ、欧州および北米の 17 カ国以上でみられ、多くはサウジアラビアを含むアラビア半島の国々であると報告している。 更に、このウイルスは家族および医療従事者など濃厚接触者間で感染能力があり、罹患者の多くの死亡原因となると述べている。このことはこのウイルスが世界的に公衆衛生を脅かす可能性があることを示している。30%弱という死亡率は呼吸器系ウイルスにしては稀な高さであり、懸念が高まっている統計値である。 著者らは、MERS の起源および伝播については十分に理解されていないが、感染したラクダに直接接触することでヒトが罹患する可能性があるように思われると述べている。しかし、空気感染と同様にこの過程における中間段階も示唆されている。 この最新の研究の目的は、MERS コロナウイルス（MERS-CoV）がラクダからヒトへ空気を介して伝播するのかどうかを調べることであった。 **これはどのような種類の研究か？** これはサウジアラビアのラクダ飼育小屋から採取された大気サンプルの実験室分析であった。 その結果、ある男性と彼の 9 頭のラクダのうち数頭がまさに同じ MERS ウイルス（実験室で検査したところ 100%一致）に感染していたことが判明したが、これはラクダからその男性に感染したことを示唆するものであった。 しかし、その男性がラクダに触れたことでウイルスに感染したのか、ラクダ由来のウイルスを含む空気を呼吸したことで感染したのかは不明であった。 **この研究ではどのようなことが行われたか？**	処理等により人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理、ウイルス除去膜によるろ過処理及び pH3.9～4.4 の条件下での液状インキュベーション処理を施しているが、投与に際しては、次の点に十分注意すること。

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医薬部外品　研究報告　調査報告書
化粧品

研究者らは MERS ウイルスの痕跡について、感染ラクダが収容されていた飼育小屋の大気サンプルを検査し、少なくとも理論上、ウイルスが空気を介して伝播する可能性があるのかどうかを調べた。
2013 年 10 月 26 日、男性は体調を崩し、また彼のラクダのうち 4 頭が 10 月 19 日から呼吸器疾患にかかっていると報告された。2013 年 11 月 3 日、男性は集中治療室（ICU）に入院した。11 月 7 日から 3 日間連続して、ラクダの飼育小屋から大気サンプル 3 件が採取された。3 件のサンプルはすべて、MERS ウイルス遺伝物質の有無について検査され、ラクダは MERS-CoV 感染の検査を受けた。
2013 年 11 月 18 日、男性は死亡した。彼のラクダは生き延びたようである。

基本的結果は何か？
空気中 MERS 遺伝物質について初日の大気サンプルのみ陽性結果であった。続く 2 日間に採取された残るサンプル 2 件は陰性であった。著者らは、初日のサンプルは 9 頭のラクダのうち 1 頭が MERS 感染について陽性結果が出たその同じ日に採取されたものであるが、9 頭のうち 4 頭は何週間も呼吸器疾患の徴候を示していたと指摘している。
著者らは、空中から採取された遺伝子断片が感染した男性および感染したラクダから見つかった断片と 100%一致したことを確認した。著者らはこのことについて、空気中の遺伝物質が感染ラクダを起源としていることを示唆していると述べている。
実験室で大気サンプル中のウイルス物質によって細胞を感染させることはできなかった。これは大気サンプルからウイルスの感染性が喪失した可能性を示している。

研究者らはこの結果をどのように解釈したか？
著者らは、データから「ラクダは MERS-CoV の感染源になりうると考えられ、閉鎖された空間内でヒトに伝播する可能性がある」ことが示唆され、また「以上の結果は、大気サンプルが MERS-CoV 伝播および排出における空気感染の役割を調査する有用な方法となる可能性も示唆している」と述べている。
加えて、「この致命的なウイルスの伝播を防ぐために規制対策や予防措置を実行できるよう、MERS-CoV の伝播におけるラクダの役割や、MERS-CoV の伝播に空中感染が何らかの役割を果たしているのかどうかを十分に理解するため、至急、詳細な研究が必要である」と述べた。

結　　論
この遺伝子研究で、MERS 感染ラクダが収容された飼育小屋の大気サンプル中に MERS ウイルスの遺伝子断片が認められた。これにより、これまでに予測されたとおり、飼い主は直接的な接触が原因ではなく空気感染によって MERS ウイルスに感染した可能性が生じた。
しかし、この研究のみでは、空気感染を示唆する具体的なエビデンスはなく、今後、調査すべき可能性が示されたにすぎないことに気づくことが重要である。
疾患発症後 12 日目、男性が ICU に入院してから 4 日目に採取された大気サンプル 3 件のうち 1 件でのみウイルスが検出されたことも稀なことであった。これについては飼育小屋の換気など多くの説明が考えられる。
いずれにせよ、MERS ウイルスまたは少なくともそのいくつかのウイルス株が、通常、空中に浮遊しているのかどうか、またどのくらいの期間、空中に留まっているのかを立証するためにはさらなる調査が必要であることが浮き彫りにされた。
研究によって、MERS 感染がラクダとヒトの間をどのように伝播するのか、この感染は空気を介して行われるのかどうかを立証する必要がある。空気感染が可能であるなら、このウイルスはインフルエンザウイルスなどの他の空気感染性疾患と同様、より迅速に、かつ広範囲に伝播する可能性があることになる。また、ヒトが接触した表面が MERS ウイルスの間接的な感染経路となった場合に感染性が存続するのかどうかも研究で立証する必要がある。
本研究は、MERS ウイルスが未だ詳細に理解されていない新たなウイルスであるために重要である。このウイルスに関する研究を続け、その伝播を追跡して、どのようにヒトに感染するのかを十分に理解することが重要である。これにより、最終的により良い予防対策や規制措置に関する情報を提供する助けとなり、この致命的となりうる感染症からヒトを守ることになる。
一方で、WHO は感染者をケアする際に、手袋、マスクおよび保護メガネなどの厳重な感染対策をとることを医療従事者に推奨している。未だ、

医薬品
医薬部外品　研究報告　調査報告書
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旅行制限も貿易制限もなく、また、他国への入国に際してスクリーニング検査の勧告もない。	

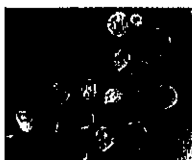
報告企業の意見	今後の対応
「Middle east respiratory syndrome coronavirus : MERS-CoV (マーズコロナウイルス)」と命名された新種のコロナウイルスである。コロナウイルスは、コロナウイルス科 (Coronaviridae) コロナウイルス亜科 (Coronavirinae) に属する直径 80～220nm の円形或いは楕円形のエンベロープを有する 1 本鎖 RNA ウイルスで、万一原料血漿にコロナウイルスが混入したとしても、Bovine viral diarrhoea virus (BVDV) をモデルウイルスとしたウイルスクリアランス試験成績から、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。	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。

NHS choices
Your health, your choices

Deadly MERS 'camel flu' may now be airborne

Behind the Headlines

Thursday July 24 2014



MERS emerged in the Middle East during 2012

"Deadly Mers virus 'could now be airborne'," The Independent reports. The Middle East Respiratory Syndrome (MERS) virus, which has an estimated case fatality rate of 30%, has been detected in an air sample in a camel barn in Saudi Arabia. This raises the possibility the virus could be spread through the air in the same way as flu.

MERS emerged in 2012 and can be extremely serious, leading to severe breathing difficulties, kidney failure and death (though it appears some people may become unaffected carriers of the virus).

The World Health Organization (WHO) states that 837 laboratory-confirmed cases of infection have been reported since 2012, including at least 291 related deaths.

In 2013, evidence emerged that camels could be the main source of the virus (hence the nickname).

The research behind the story highlights the case of a man and his camels living in Saudi Arabia. The man and some of his camels were infected with MERS, and the man sadly died as a result.

On investigation, genetic fragments from the MERS virus were detected in an air sample from the barn housing the infected camels.

There is no concrete evidence that the man was infected through the air, and it should also be noted that he had direct contact with the camels. However, the possibility of airborne transmission has raised concerns.

It is important to keep researching this new virus and tracking its spread, to gain a better understanding of how it is transmitted to people.

Advice for travellers

There are currently no medications, or similar, to protect against the MERS infection.

If you are travelling to the Middle East, taking standard precautions against airborne viruses, such as the common cold and flu, could help reduce your risk. These include:

- making sure you wash your hands regularly with soap and water
- washing your hands before or after touching animals
- avoiding contact with sick animals
- cleaning surfaces such as keyboards, telephones and door handles regularly to get rid of germs
- using tissues to cover your mouth and nose when you cough or sneeze
- putting used tissues in a bin as soon as possible

More specifically, the WHO advises that people avoid close contact with animals - particularly camels - when visiting farms, markets or barn areas where the virus is known to be circulating. People should also avoid drinking raw camel milk or camel urine, or eating meat that has not been properly cooked.

Where did the story come from?

The study was carried out by researchers from King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia, and was funded by the same institution.

The study was published in the American Society for Microbiology, a peer-reviewed science journal.

This whole journal is open-access, meaning that anyone can read the publications for free online, including this latest research.

The study highlights new research into the origins and possible transmission of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) – a new human virus that causes severe respiratory symptoms and kidney failure.

Since it was recognised, the WHO reports there have been 837 laboratory confirmed cases in humans, and at least 291 deaths.

The authors report that these have been in at least 17 countries in Asia, Africa, Europe and North America – most originated from countries in the Arabian Peninsula, including Saudi Arabia.

They say the virus has the ability to infect close contacts such as family members and healthcare workers, and that it causes the death of many of those affected. This means the virus is a potential global public health threat. A case death rate of just under 30% is unusually high for a respiratory virus, a statistic that has sparked concern.

The authors say the origin and transmission of MERS is not fully understood, but it seems humans could contract it through direct contact with infected camels. However, intermediate steps in this process have been suggested, as well as airborne transmission.

This latest research aimed to investigate whether MERS-CoV might be transmitted from camels to humans via the air.

What kind of research was this?

This was a laboratory analysis of air samples collected from a camel barn in Saudi Arabia.

The researchers knew of a man and some of his nine camels that had contracted exactly the same MERS virus (100% identical when tested in the lab), suggesting that the camels had infected the man.

However, it was not clear whether the man contracted the virus from touching the camels or from breathing in air containing the virus originating from the camels.

What did the research involve?

The researchers tested the air from the barn where the infected camels were housed for traces of the MERS virus to see if, at least in theory, it was possible for the virus to be transmitted through the air.

The man became unwell on October 26 2013 and reported that four of his camels had suffered respiratory illness since October 19. He was admitted to an intensive care unit (ICU) on November 3 2013. Three air samples were collected from the camel barn on three consecutive days from November 7. All three samples were screened for the presence of the MERS virus genetic material, and the camels were tested for the MERS-CoV infection.

The man died on November 18 2013; his camels appear to have survived.

What were the basic results?

Only the first air sample tested positive for airborne MERS genetic material. The other two samples, collected in the next two days, were negative. The authors note that the first sample was collected on the same day as one of the nine camels also tested positive for the MERS infection, although four of the nine camels had been showing signs of respiratory illness for many weeks.

They confirmed that the genetic fragments from the air were 100% identical to fragments found in the infected man and the infected camel. This, they said, suggested that the genetic material in the air had originated from the infected camel.

The virus material from the air samples was not able to infect cells in the laboratory, indicating there may have been loss of viral infectivity from the air sample.

How did the researchers interpret the results?

The authors say the data suggests that, "camels may be a source of infectious MERS-CoV, which can be transmitted to humans within confined spaces" and that, "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."

They added that, "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."

Conclusion

This genetic research found genetic fragments from the MERS virus in an air sample of a barn housing MERS-infected camels. This raises the possibility that the owner contracted the MERS virus from airborne transmission, rather than through direct contact, as had previously been assumed.

However, it is important to realise that, based on this research alone, there is no concrete evidence to suggest the transmission was airborne, only that it is a possibility to investigate further.

It was also unusual that the virus was only detected in one of the three air samples, taken 12 days after the onset of illness and four days after the man had been admitted to an ICU. There could be many explanations for this – such as ventilation in the barn.

Either way, it highlights that more investigation is needed to establish whether the MERS virus, or at least some strains of it, is regularly airborne and how long it may stay airborne.

Research needs to establish how MERS infection is spread between camels and humans, and whether this can happen through the air. If it can, this makes the virus more likely to spread quicker and more widely, like other airborne diseases, such as flu. Research also needs to establish whether the MERS virus can remain infectious when on surfaces, which may be touched by people, providing further indirect infection routes.

This study is important because the MERS virus is new, so is not yet fully understood. It will be important to keep researching this virus and tracking its spread to better understand how it is transmitted to humans. This will, ultimately, help inform better prevention or control measures, thereby protecting people from this potentially fatal infection.

In the meantime, the WHO recommends strict control measures such as gloves, mask and eye protection for healthcare workers when caring for affected individuals. There are as yet no travel or trade restrictions, and no screening recommendations for entry into other countries.

Read the latest WHO Disease outbreak news here.

Analysis by Bazian. Edited by NHS Choices. Follow Behind the Headlines on Twitter. Join the Healthy Evidence forum.

Links to the headlines

Deadly Mers virus 'could now be airborne', warn scientists in study on Saudi Arabian barn. The Independent, July 22 2014

Deadly MERS virus 'may be airborne', sparking fears it may spread quicker. Mail Online, July 23 2014

Links to the science

Azhar EI, Hashem AM, El-Kafraw SA, et al. Detection of the Middle East Respiratory Syndrome Coronavirus Genome in an Air Sample Originating from a Camel Barn Owned by an Infected Patient. mBio. Published online July 22 2014

Analysis by Bazian.

Edited by **NHS Choices**

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医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分		総合機構処理欄
一般的名称	－		研究報告の公表状況	感染症学雑誌/88(2)155-159/(2014.3)		公表国	
販売名（企業名）	－					日本	

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
2013年1月～3月までオーストラリアのメルボルン（1週間のみタスマニア）に滞在していた症例で、左足背の疼痛と腫脹、右膝の疼痛、関節稼働域制限が出現し、現地で家庭医を受診したが、原因ははっきりしなかった。 4月上旬に疼痛が悪化し、5月には疼痛が徐々に改善し歩けるようになった。また、この頃ロスリバーウイルス（RRV）の検査が陽性であったと告げられた。 5月中旬に帰国し本邦で受診した。経過中、発熱、皮疹などの関節痛以外の症状はなかった。症状、オーストラリアでの検査結果よりロスリバーウイルス感染を疑い、国立感染研究所による検査を依頼し、ロスリバーウイルスIgG陽性、IgM陽性となり、再検査においても同様の結果であり、本邦における初のロスリバーウイルスの急性感染と判定された。	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
本邦初の症例との報告であることから、報告対象としたが、血漿分画製剤からの感染報告は入手していない。	今後とも本邦初の感染症に関連する情報に留意していく。

24

NIHONSEIYAKU
2008-044

○ 0610 Z1480007

症　例

オーストラリア渡航中に発症したロスリバーウイルス感染症の本邦初報告

[1]京都市立病院感染症科，[2]国立感染症研究所ウイルス第一部第２室
杤谷健太郎[1]　清水　恒広[1]　篠原　浩[1]
土戸　康弘[1]　モイメンリン[2]　高崎　智彦[2]

（平成25年６月21日受付）
（平成25年９月17日受理）

Key words：Ross River virus, imported infectious disease, Australia, travel

序　文

ロスリバーウイルス（Ross River virus；RRV）感染症はオーストラリアをはじめとしたオセアニアで見られる，RRVによって引き起こされる感染症である．これまで日本国内において確定診断された症例はなく，今回本邦初の症例を経験したため報告する．

症　例

患者：31歳，女性．

主訴：関節痛．

既往歴：特記事項なし．

現病歴：2013年１月からワーキングホリデーを利用して，オーストラリアに渡航していた．２月末から１週間タスマニアに旅行している以外はメルボルンに滞在していた．

３月中旬の起床時に左足背の疼痛と腫脹，右膝の疼痛を自覚し，歩くのも困難なほどであった．翌日には疼痛が悪化し関節可動域制限も出現したため，現地で家庭医（general practitioner）を受診した．血液検査を施行されたところ，WBC 4,600/μL，Hb 13.9g/dL，Plt 180,000/μL，ESR 7mm/hr，AST 20IU/L，ALT 14IU/L，CRP 0.03mg/dL，その他検査でも特記異常なく，原因ははっきりせず消炎鎮痛薬処方となった．その後，疼痛は悪化こそないものの，改善も徐々にしかない状態であった．３月末には左手母指の起始部にも疼痛が出現した．

４月上旬に疼痛が悪化し，発症時と同程度の疼痛となった．発症時のような腫脹はみられなかった．その後も疼痛は続き，４月中旬に現地の整形外科を受診した．血液検査を施行され，WBC 3,800/μL，Hb 12.3g/dL，Plt 180,000/μL，ESR 5mm/hr，その他の検査でも特記すべき異常は見られなかった．RRV，バーマ森林ウイルスといったウイルス疾患も考慮され，ウイルス検査提出され消炎鎮痛薬にて経過をみることとなった．

５月初旬から疼痛は徐々に改善し，歩けるようになってきた．またこの頃，RRVの検査が陽性であったと医師より告げられた（現地検査の結果：Ross River virus serology：IgG antibody：low positive，IgM antibody：positive 2013/4/19，IgG antibody：positive，IgM antibody：positive 2013/5/9）．

５月７日頃から両手指に痛みがあり動かしにくかった．症状が続くため５月中旬に帰国し，帰国３日後に当院受診となった．

経過中，発熱，皮疹など関節痛以外の症状はなかった．

内服：インドメタシン，セレコキシブ．

初診時現症：意識清明，血圧109/59mmHg，脈拍76回/分，体温37.4℃．

頭頸部・胸部・腹部に特記所見なし，皮疹なし．

左足関節，足背に圧痛あり，腫脹熱感発赤なし．

右膝に自発痛あり，腫脹熱感可動域制限といった関節炎所見はなし．

その他の関節にも関節炎所見はなし．

初診時検査（Table 1）：炎症反応の上昇はなく，特記すべき所見を認めなかった．

初診後経過：オーストラリアで発症した関節炎を主体とした症状，現地での検査結果より，RRV感染症を疑い，国立感染症研究所に検査を依頼した．初診時の血液検査にて，Ross River virus IgG陽性，IgM陽

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Table 1 Laboratory findings

WBC	5,300 /μL	AST	14 IU/L	ANA	<40 ×
Neutro	64.0 %	ALT	11 IU/L	anti-dsDNA	<7 IU/mL
Lympho	26.2 %	LD	147 IU/L	RF	3.4 IU/mL
Mono	6.5 %	ALP	138 IU/L	anti-CCP	0.6 U/mL
Eosino	2.7 %	γ-GT	10 IU/L		
Baso	0.6 %	Amy	94 IU/L	Urinalysis	
Hb	12.5 g/dL	Crea	0.59 mg/dL	glucose	(−)
Plt	19.3×10⁴ /μL	BUN	10.0 mg/dL	protein	(−)
PT-INR	0.96	Na	139 mEq/L	blood	(−)
APTT	28.5 s	K	4.5 mEq/L	ketone	(−)
ESR	4 mm/hr	Cl	107 mEq/L	RBC	1-4 /HF
TP	7.1 g/dL	T-Bil	0.4 mg/dL	WBC	<1 /HF
Alb	4.2 g/dL	CRP	0.01 mg/dL		
CK	102 IU/L				

Table 2 Ross River virus serology test

	Day of first visit	2 weeks after
IgG absorbed IgM ELISA (panbio)	index=2.84 positive	Index=2.82 positive
IgM capture ELISA (in house)	P/N ratio=2.8 endpoint titer: 1,600x positive	P/N ratio=2.4 endpoint titer: 400x positive
IgG ELISA (panbio)	index=2.84 endpoint titer: 800x positive	index=2.82 endpoint titer: 800x positive
Neutralizing antibody	titer: 1,280x positive	titer: 640x positive

性（Table 2, Fig. 1）であり，RRVの急性感染と考えられた．その後初診時から2週間後に再度検査を行ったが，やはりRoss River virus IgGは陽性であり，IgMはcapture ELISAにおいて，1,600xから400xと抗体価の低下を認め，RRVの急性感染として矛盾しない所見であった．そのためRRV感染症と診断した．

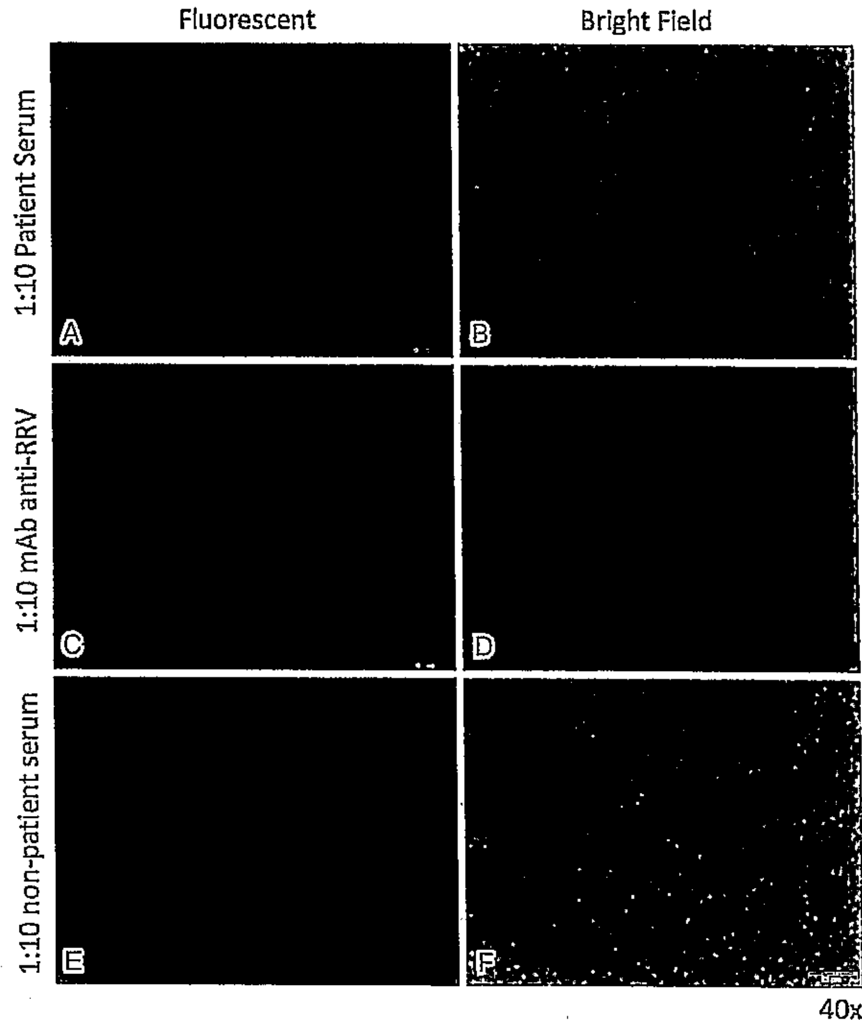
考　察

RRVは蚊によって媒介されるアルボウイルスの一種であり，トガウイルス科，アルファウイルス属に分類される．1959年にオーストラリア，クイーンズランド州のRoss Riverで，*Aedes vigilax*から初めて同定された[1]．*Aedes vigilax*以外に*Aedes camptorhynchus*, *Culex annulirostris*などの蚊も媒介するとされている．オーストラリアでは毎年約4,000人の患者が発生しており，主に北部，西部を中心に，雨期（12月から2月頃）に流行する．オーストラリア以外でも，パプアニューギニア，ニューカレドニア，フィジー，サモア，クック諸島といった近隣の国でも発生が報告されている．

RRVの宿主はカンガルーやワラビーであり，そこから蚊が媒介することによってヒトに感染が起こる．感染しても半数以上は無症状で，一度感染すると産生された中和抗体によりその免疫は生涯続くとされ，これまで再感染の報告はない[2]．

RRV感染症の潜伏期間は通常7～9日であるが，3～21日に及ぶこともある[2]．関節炎・関節痛，皮疹や倦怠感，筋肉痛，発熱，リンパ節腫脹といった全身症状が主な症状である．関節炎・関節痛はほぼ全ての患者に生じ，主として小関節，多発性で，手関節，膝関節，足関節，指関節，肘関節などが対称性に侵される．関節痛が長期間続くことが特徴で，通常3～6カ月続くが，それ以上続く場合もある．症状が遷延する患者の中には，後にリウマチ性疾患やうつ病を合併していたと診断される患者も多く，これらの疾患が症状遷延の原因となっているという報告もある[3]．症状が遷延する患者においては，他疾患の合併も念頭に入れて診療していく必要がある．皮疹は1～5mmの紅色斑状丘疹がおよそ50%の患者にみられる．関節症状や全身症状から数日遅れて出現し，5～10日で消退することが多いが，持続あるいは再燃することもある．分布は特徴的ではなく，多くは体幹，四肢にみられ，顔には

Fig. 1 Detection of Ross River virus antibodies using the immunofluorescence method.
Immunodetection using 1: 10 diluted patient serum sample (A, B), 1: 10 diluted polyclonal RRV antibody (C, D), and non-RRV human serum (E, F). RRV infected cells were stained with the primary antibody or a serum sample and the secondary green-fluorescent goat-anti human IgG antibody. Both patient serum sample and positive control (anti-RRV polyclonal antibody) detected the RRV antigen (bright green, figure 1A and C). Images were obtained using fluorescent (A, C, D) and bright field microscopy (B, D, F). Scale indicates 50μm.



少なく，手掌・足底に見られることもある．倦怠感は50% 以上の患者で見られ，筋肉痛は58%，発熱は33～50% の患者でみられる[1]．全身症状は通常1週間程度で軽快する．

診断には流行地への渡航歴と，蚊への暴露を問診することが重要となる．検査所見で異常がみられることは少なく，時にわずかな白血球の上昇，赤沈の亢進がみられる．CRP は正常なことが多い[2]．血清学的診断として，ELISA による抗体検査が流行地であるオーストラリアでは利用できる．日本においては一般の検査会社では抗体検査はできないが，国立感染症研究所ウイルス第一部第2室に依頼することが可能である．ウイルス血症は感染後数日しか持続せず，その時期にPCR で RNA を検出できることもあるが，感度は高くない．抗体はバーマ森林ウイルス，チクングニアウイルス，シンドビスウイルスといった他のアルファウイルスと交差反応することもあり，解釈には注意が必要だが，特異度は IgG，IgM ともに，97.6%，96.5% と高く診断に有用である[1]．IgM は感染後数カ月持続するので，IgM の検出は最近の感染を示している．また IgG のペア血清を測定し，陽転あるいは有意な上昇みられれば最近の感染と考える．

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治療はNSAIDによる対症療法を行う．安静，水泳やマッサージのような理学療法が良いという報告もある[4]．ワクチンはなく，防蚊対策が予防には重要である．

RRV感染症の重要な鑑別疾患として，同じトガウイルス科のウイルスが原因となる，バーマ森林ウイルス感染症，チクングニア熱が挙げられる．バーマ森林ウイルス感染症の報告はオーストラリアからのみで，その症状はRRV感染症とほぼ同様であるが，関節炎は30%程度と少ない[5]．また患者数も年間約2,000人弱と少なく[6]，本邦での報告もない．一方チクングニア熱はアジア，アフリカで流行しており，日本において国内発症は確認されていない．2006年に海外からの輸入症例が報告され，2011年に感染症法上の四類感染症に指定されたことで全数届出疾患となった．2013年8月まで国内では39例の症例が報告されている[7]．症状はRRV感染症と同様で，特に関節痛の症状が強く，長期間に及ぶ点は類似しており臨床的には鑑別が困難である．ただし，チクングニア熱はオーストラリアでの発生は非常に稀である点が異なる[6]．これらのウイルスはPCR，抗体検査によって鑑別が可能であり，今症例においては，バーマ森林ウイルスは現地の抗体検査で陰性であり，チクングニアは帰国後に行った検査において，IgM補足ELISA法がP/N ratio＝1.33（2.0以上が陽性）と陰性であり，ともに感染は否定された．

本症例が現地でも診断に時間を要した理由として，第一にRRV感染症の流行はオーストラリア北部，西部が中心で，2011年にビクトリア州で流行があったものの，近年は患者数の少ない地域で患者が罹患したこと，第二に症状が関節痛のみで発熱や皮疹が見られない非典型例であったことがあげられる．しかし罹患後長期に関節痛が続いている点は典型的経過であり，オーストラリア渡航歴のある関節痛患者では常にRRV感染症を念頭に置く必要がある．

これまで日本で確定したRRV患者の報告はない．疾患の認知度が低いこと，類縁のチクングニア熱と異なり感染症法上の届出対象疾患ではないこと，検査可能な施設が限られていることなどが要因と言える．しかし，南オーストラリアでの流行状況調査結果が，国立感染症研究所感染症情報センター2005年発行のInfectious Agents Suveillance Report（IASR）で報告され[8]，また厚生労働省検疫所のホームページでも，2011年1月から複数回にわたり，オーストラリアでのRRV感染症の流行が注意喚起されるなど，以前から輸入症例の日本での発生が危惧されていた[9]．ドイツ，シンガポールなどでは既に渡航者におけるRRV感染症が報告されており[10][11]，日本からオーストラリアへの渡航者の多さを考えると，今後日本でも輸入症例の診断が増加するものと思われる．

今回オーストラリアで罹患したRRV感染症の症例を経験した．発熱，関節痛，皮疹を主訴とする患者を診療する際には，渡航歴を確認し，オーストラリアへの渡航歴があればRRV感染症も鑑別として考えるべきである．

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The First Case Report of Ross River Virus Disease in a Japanese Patient who Returned from Australia

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A 31-year-old Japanese female had stayed in Australia from January to May 2013. She presented with a sudden onset of left ankle and right knee arthralgia in March but neither fever nor rash was present. As her arthralgia persisted, she visited our hospital upon her return to Japan in May. When she came to our hospital, she complained of left ankle and right knee pain, but no arthritis findings. Laboratory findings were also within normal ranges. Ross River virus (RRV) antibody levels were examined as she was suspected of having contracted the disease in Australia. RRV IgG antibody and IgM antibody were positive, and the patient was confirmed as a case of acute RRV disease. RRV disease is endemic in Australia, but there are no prior reports of the disease in Japan. This is the first case of RRV disease confirmed in Japan. Typical symptoms of RRV disease include arthralgia, fever, and rash. Our patient had only arthralgia. With the increase in the number of travelers and length of stay in RRV endemic regions, health care providers need to consider the disease in their differential diagnosis, among returning travelers with arthralgia, fever, rash and a travel history to RRV-endemic regions.

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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

○仏領ギアナからドイツに輸入されたマヤロウイルス(MAYV)のゲノム分析
MAYVはトガウイルス科に属する蚊媒介性の新世界アルファウイルスであり、南米の熱帯雨林において非ヒト霊長類と *Haemagogus* 属の蚊の間で循環が維持されている。感染した蚊による刺咬後、7〜12日の潜伏期を経て発疹、発熱、頭痛、関節痛などのデング熱やチクングニヤ熱に類似した症状を引き起こし、数週間で自然治癒する。
2013年8月、仏領ギアナへの2.5週間の滞在から4日前に帰国した44歳の女性が、発熱、悪寒、頭痛、疲労感、手指関節の腫れと両足の痛みを来して受診した。患者は旅行中、複数カ所を蚊に刺されていた。検査ではCRP値の上昇とLDH値のわずかな上昇及び白血球数の減少が認められたが、白血球数は発症後8日後に基準値に戻り、患者は完全に回復した。
第16病日の追跡検査でチクングニヤウイルスに対するIgG抗体価の上昇が示されたが、IgM抗体は陰性であった。同サンプルにおいてアルファウイルスに対する追加検査を行ったところ、間接免疫蛍光アッセイによりMAYV IgM抗体価は2,560、IgG抗体価(基準値20以下)は10,240を示した。急性MAYV感染が強く疑われ、第2病日の血清サンプルにおいてRT-PCRを行ったところ、アルファウイルスの陽性結果を示し、直接配列決定によりMAYVに特異的な配列が示された。同サンプル中には蚊がウイルスを媒介するのに十分なウイルス量(1.24×10^7コピー/mL)が含まれていた。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
仏領ギアナに滞在していたドイツ人女性が帰国後に発熱、頭痛、疲労感、手指関節の腫脹や両足の痛みを来して受診したところ、患者の血清サンプルからMAYVが検出されたとの報告である。	日本赤十字社では、輸血感染症対策として受付時に海外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。

25

and G1P[6] along with G2P[4] and G9P[8]. Our study results are similar to those of recent studies conducted in other African countries (*5–8*) and confirm results of studies that found that the same genotypes circulated in western Cameroon in 2003, albeit at different percentages (*4,9*).

Our study provides relevant data about the genotypes of rotavirus-A from children in the Central African Republic, 25 years after the most recent study (*2*). These data represent baseline information that will help with monitoring for potential changes in genotype prevalence after the introduction of rotavirus-A vaccine in the Central African Republic.

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Genome Analysis of Mayaro Virus Imported to Germany from French Guiana

To the Editor: Mayaro virus (MAYV), a mosquito-borne New World alphavirus of the family *Togaviridae*, causes a febrile arthralgia syndrome resembling dengue and chikungunya fever. The virus is maintained in a natural cycle involving nonhuman primates and *Haemagogus* spp. mosquitoes in tropical rainforest areas of South America (*1*). After a incubation time of 7–12 days following an infectious mosquito bite, rash, fever, headache, and arthralgia develop in patients, followed by restoration to their original conditions after several weeks (*1*).

Outbreaks of Mayaro fever have been reported from the Amazon region (*1,2*). There are increasing reports of travel-related infections imported from South America to Europe and the United States (*3–7*). We describe an acute MAYV infection in a German traveler who returned from French Guiana. Full-length MAYV genome amplification was performed on virus obtained from a serum sample of the patient.

In August 2013, a 44-year-old woman (bookkeeper) came to an ou patient clinic with fever (temperature ≤38.7°C), chills, a mild headache, severe fatigue, highly painful swelling of small finger joints, and pain in both feet. Symptoms appeared 2 days before when she experienced aches in her wrists and left forefoot. Four days before, the patient had returned from a 2.5-week visit to French Guiana, where she traveled with her partner and caught butterflies. She had conducted these activities during her holidays for the past 5 years, mostly in spring or autumn. In July 2013 at the end of the rainy season, she had many mosquito bites, especially on her hands, despite use of repellents and bed nets.

Physical examination showed a body temperature of 38°C, throat enanthema, generalized macular exanthema, and slightly swollen and tender interphalangeal joints of the hands and feet. Her medical history was unremarkable, and her partner was asymptomatic. Laboratory tests showed reference values for hemoglobin concentration; platelet count; and levels of liver enzymes, creatinine, and antinuclear and anti–citrulline peptide antibodies. C-reactive protein level was increased (24.2 mg/L; reference value <5 mg/L), and serum lactate dehydrogenase level was slightly increased (4.4 µkat/L; reference value <4.12 µkat/L). Leukopenia (2.4 G/L; reference value 4.0–10.0 G/L) was present, which intensified the next day (2.0 g/L). The leukocyte count returned to a reference value 8 days after disease onset and the patient fully recovered.

Malaria, dengue fever, and rickettsiosis were excluded by using several tests. Blood cultures obtained on day 2 after disease onset remained sterile, and a viral infection was suspected. Follow-up investigation on day 16 of illness showed an increased IgG titer (80) against chikungunya virus (by indirect immunofluorescence assay; reference value <1:20) (*6*) but no IgM titer. Additional tests for alphaviruses were then performed on the same sample, and indirect immunofluorescence assay showed an IgM titer of 2,560 and an IgG titer of 10,240 (reference value <20) (*6*) against MAYV. Results of serologic tests were negative for Venezuelan equine encephalitis virus, Eastern equine encephalitis virus, and Oropouche virus. IgM (80) and IgG (160) titers for antibodies against Ross River virus were low.

An acute MAYV infection was strongly suspected and a stored serum sample from day 2 underwent generic reverse transcription PCR (RT-PCR) for alphaviruses with primers VIR2052F (5′-TGGCGCTATGATGAAATCTGGAATGTT-3′) and VIR2052R (5′-TACGATGTTGTCGTCGCCGATGAA-3′) (*8*) and quantitative MAYV real-time RT-PCR (in-house) with primers MayaroF (5′-CCTTCACACAGATCAGAC-3′), MayaroR (5′-GCCTGGAAGTACAAAGAA-3′), probe labeled with 6-carboxyfluorescein (FAM) and black hole quencher 1 (BHQ-1) MayaroP (5′-FAM-CATAGACATCCTGATAGACTGCCACC-BHQ1 3′) by using the AgPath-ID One-Step RT-PCR Kit (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. The generic RT-PCR for alphaviruses showed a positive result, and direct sequencing of the amplicon showed a MAYV-specific sequence. The serum sample had an MAYV viral load of 1.24 × 10^7 copies/mL when in vitro–transcribed RNA from a reference plasmid was used as a quantification standard.

Attempts to isolate MAYV in cell culture were not successful. Therefore, the serum sample was used to obtain the complete MAYV genome sequence by using primers designed from multiple alignments of the MAYV genomes obtained from databases. (Primer sequences used are available on request.) The complete MAYV genome (strain BNI-1, KJ013266) was amplified from the serum sample, and phylogenetic analysis of a 2-kb genomic fragment showed that strain BNI-1 belonged to genotype D (*9*) and is closely related to strains circulating in Brazil (Figure, http://wwwnc.cdc.gov/EID/article/20/7/14-0043-F1.htm).

In 2 clinic-based syndromic surveillance studies in South America, 0.8%–3% of febrile episodes were caused by MAYV infection (*2,10*). In travelers, MAYV infections were acquired in tropical rainforest or wildlife conservation areas (*7*) and were sometimes associated with insect-hunting activities (*5*). Successful complete genome amplification of MAYV strain BNI-1 from a clinical sample might help identify regions in the MAYV genome that undergo rapid mutations caused by the isolation process in cell culture and improve phylogenetic and functional genome analysis. Moreover, the viral load in our patient was high enough for efficient transmission of MAYV to a susceptible mosquito vector (S. Becker, pers. comm.). Thus, in disease-endemic regions, patients with an acute MAYV infection should be protected from mosquito bites during the first week of disease to prevent spread of the virus.

Acknowledgment

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

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EMERGING INFECTIOUS DISEASES

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Diphtheria-like Disease Caused by Toxigenic *Corynebacterium ulcerans* Strain

To the Editor: Toxigenic *Corynebacterium ulcerans* is an increasingly reported cause of diphtheria in the United Kingdom and is often associated with a zoonotic origin (*1*,*2*). Here, we report a case of diphtheria caused by toxigenic *C. ulcerans* in a woman, 51 years of age, from Scotland, UK, who was admitted to a hospital in August 2013 with a swollen, sore throat and a gray-white membrane over the pharyngeal surface. The patient had returned from a 2-week family holiday in the state of Florida, United States, before the admission and also reported recent treatment of a pet dog for pharyngitis. The patient was believed to have been vaccinated against diphtheria during childhood. She was immediately admitted to an isolation ward and treated with a combination of clindamycin, penicillin, and metronidazole.

Microscopic examination of the throat biofilm (collected by using a swab) showed gram-positive bacilli; swab samples from the exudative membrane and throat produced small, black colonies indicative of *Corynebacterium* spp. on Hoyle medium. Further efforts to identify the strain by using VITEK MS and VITEK2 ANC card systems (bioMérieux, Marcy l'Etoile, France) to evaluate the swab samples suggested that the infection was caused by either *C. ulcerans* or *C. pseudotuberculosis* (50% CI). The isolate detected from this process was sent to the *Streptococcus* and Diphtheria Reference Unit, Public Health England, Colindale, UK, and was confirmed to be a toxigenic *C. ulcerans* strain that we designated RAH1. Throat swab samples were collected from family members of the patient and were negative for *C. ulcerans*. The family dog was not tested for presence of the organism, although it is known that *C. ulcerans* infections are often of a zoonotic nature (*1*,*2*). After treatment, the patient made a full recovery.

Toxigenic *C. ulcerans* can produce both diphtheria-like and Shiga-like toxins (*3*); to identify the genetic basis of toxin production and other potential virulence factors in this strain, a whole genome sequencing approach was applied to the isolate. The genome was sequenced by using an Ion PGM System (Thermo Fischer Scientific, Loughborough, Leicestershire, UK) and resulting reads (2,965,044 reads, ≈90× coverage. Data are available on GenBank SRA: high-throughput DNA and RNA sequence read archive (http://www.ncbi.nlm.nih.gov/Traces/sra/sra.cgi?view=search_obj, accession no.: SRR1145126) and were mapped onto the published genome sequences of a Shiga-like toxin–producing clinical isolate 809, asymptomatic canine strain BR-AD22 (*3*), and diphtheria-like toxin–producing strain 0102 (*4*). Most of the previously identified virulence genes (*3*,*4*) were present in the patient isolate (Table). The *tox* gene, encoding diphtheria toxin, was present, which verified the diphtheria-like disease in the patient. The *rbp* gene, responsible for Shiga toxin–like ribosome-binding protein, was absent. However, strain RAH1 also possessed the venom serine protease gene (*vsp2*), which, in *C. ulcerans* strain 809, has been implicated in the increased virulence in humans. The *tox* gene was present in a prophage that showed similarities to ΦCULC809I (*3*) and ΦCULC0102-I (*4*). Genome-based phylogenetic analysis of the RAH1 strain (ClonalFrame analysis [*5*]) and strains 809, BR-AD22, and 0102 indicates a much wider phylogenetic diversity of *C. ulcerans* strains than previously appreciated (data not shown).

This case raises the issue of waning vaccine protection in older patients and suggests that toxin-mediated corynebacterial disease remains a threat to public health. The declining costs of next-generation sequencing and availability

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識別番号・報告回数			報告日	第一報入手日 2014 年 07 月 03 日	新医薬品等の区分	厚生労働省処理欄
一般的名称	①②③⑨⑩pH4 処理酸性人免疫グロブリン ④⑤⑥⑦⑧ポリエチレングリコール処理人免疫グロブリン ⑪⑫人免疫グロブリン		研究報告の公表状況	第 88 回日本感染症学会学術講演会, 第 62 回日本化学療法学会総会合同学会 (2014. 6. 18, 19, 20)/福岡県福岡市	公表国 日本	
販売名 (企業名)	①日赤ポリグロビン N5%静注 0.5g/10mL　(日本血液製剤機構) ②日赤ポリグロビン N5%静注 2.5g/50mL　(日本血液製剤機構) ③日赤ポリグロビン N5%静注 5g/100mL　(日本血液製剤機構) ④献血ｳﾞｪﾉｸﾞﾛﾌﾞﾘﾝ IH5%静注 0.5g/10mL　(日本血液製剤機構) ⑤献血ｳﾞｪﾉｸﾞﾛﾌﾞﾘﾝ IH5%静注 1g/20mL　(日本血液製剤機構) ⑥献血ｳﾞｪﾉｸﾞﾛﾌﾞﾘﾝ IH5%静注 2.5g/50mL　(日本血液製剤機構) ⑦献血ｳﾞｪﾉｸﾞﾛﾌﾞﾘﾝ IH5%静注 5g/100mL　(日本血液製剤機構) ⑧献血ｳﾞｪﾉｸﾞﾛﾌﾞﾘﾝ IH5%静注 10g/200mL (日本血液製剤機構) ⑨献血ｳﾞｪﾉｸﾞﾛﾌﾞﾘﾝ-IH ﾖｼﾄﾐ　(日本血液製剤機構) ⑩日赤ポリグロビン N10%静注 5g/50mL　(日本血液製剤機構) ⑪日赤ポリグロビン N10%静注 10g/100mL (日本血液製剤機構) ⑫ｸﾞﾛﾌﾞﾘﾝ筋注 450mg/3mL「ﾍﾞﾈｼｽ」　(日本血液製剤機構) ⑬ｸﾞﾛﾌﾞﾘﾝ筋注 1500mg/10mL「ﾍﾞﾈｼｽ」　(日本血液製剤機構)					

研究報告の概要

【背景】近年日本では性感染症が減少傾向にあると言われるが、梅毒に関しては違う傾向が見られるとの情報があることから、近年の梅毒発生動向を探った。

【方法】感染症発生動向調査で 2009～2013 年に梅毒と診断され報告された症例（2013 年 12 月 19 日現在）を記述的に分析した。

【結果】2013 年の報告総数は 1,176 例（男 945, 女 231）で、2009 年より 1. 70 倍（男 1. 78 倍, 女 1. 38 倍）増加していた。感染経路が判明した 1,011 例（報告総数の 94. 0%）の内、ほぼ全例を占める 1,004 例が性的接触によるものであり、その内訳は、男性の 10～20 代で同性間性的接触（同性間, 異性間・同性間）感染が、2009 年 58 例に対し 2013 年 118 例と 2. 03 倍に増加した。また、30～40 代も 85 例から 258 例（3. 04 倍）に増加した。異性間性的接触感染は 10～20 代で 49 例から 83 例（1. 69 倍）と増加が認められた。女性は 10～20 代の異性間性的接触感染が 2009 年 56 例に対し 2013 年は 73 例（1. 30 倍）であった。早期梅毒の届出は、男性が 2009 年 315 例に対し 2013 年 573 例と 1. 82 倍に増えていた。

【結論】近年、梅毒は 10～40 代の男性同性間性的接触感染が急増している。これは性器クラミジア感染症や淋菌感染症が増加していないことと対照的であり、流行している集団が異なることを示唆している。梅毒に対しては、10～40 代の男性同性間を中心とする感染予防などに関する情報提供と注意喚起が必要である。

使用上の注意記載状況・その他参考事項等

代表として献血ヴェノグロブリン IH5%静注 0.5g/10mL の記載を示す。

2. 重要な基本的注意

(1) 本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-1 抗体陰性で、かつ ALT (GPT) 値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分からポリエチレングリコール 4000 処理、DEAE セファデックス

26

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報告企業の意見	今後の対応	
梅毒トレポネーマ（Treponema pallidum）はスピロヘータ科(Spirochaetaceae)トレポネーマ属(Treponema)の一種で、直径：0.1～0.2μm、長さ：6～20μmの6～14旋回の螺旋状をした細菌である。低温保管や凍結乾燥、加熱処理により死滅するとされている。そのため、万一原料血漿に梅毒トレポネーマが混入したとしても、製造工程において滅菌または除菌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。	処理等により人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理、ウイルス除去膜によるろ過処理及び pH3.9～4.4 の条件下での液状インキュベーション処理を施しているが、投与に際しては、次の点に十分注意すること。

O1-077 感 「健常小児の上気道細菌叢の研究」—肺炎球菌・インフルエンザ菌を中心に—

新潟大学大学院医歯学総合研究科生体機能調節医学専攻内部環境医学講座小児科学分野[1]，佐藤小児科医院[2]，中野こども病院[3]，川崎医科大学小児科[4]，慶應義塾大学感染症学[5]，産業医科大学泌尿器科[6]，株式会社キューリン[7]，中浜医院[8]
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岩田　敏[5]，松本哲朗[6]，村谷哲郎[7]，中浜　力[8]

【目的】上気道は無菌部位ではないため，培養結果の解釈には健常時の状態を把握する必要がある．そこで，健常小児の咽頭，鼻咽頭について調査を行い，検討した．
【方法】2013年4月～11月に，3か所の医院を受診した0～14歳の気道症状のない健常小児に対し，本人か保護者の同意取得後，滅菌スワブを用い，鼻咽頭か咽頭より検体を採取した．
【成績】183例の鼻咽頭と173例の咽頭由来の検体を採取し，*S. pneumonia* (SP) は15.3%，6.9%，*H.influenzae* (HI) は26.5%，16.8% 検出された．以下，鼻咽頭検体で検討すると，年齢群別では，SPとHIいずれも5歳以下までの検出率が多い傾向にあった．5歳以下において，本人か兄弟の集団生活がある群と，いずれの集団生活もない群とのSPとHIの検出率は24.7%と9.5%，27.9%と13.6%で，いずれも本人か兄弟の集団生活がある群で高い傾向にあった．SP検出例の37%ではHIも，HI検出例の25%ではSPも検出されていた．SPまたはHIが検出されていた群では，いずれも検出されていない群に比べ，*M.catarrhalis* が検出される率が高く，反対に，*S. aureus* が検出される率は低い傾向にあった．ワクチン接種との関連はなかった．
【考察】今後，ワクチンの普及や抗菌薬の使用実態により状況が変化する可能性があり，経時的な調査が重要である．

O1-078 感 感染症発生動向調査からみた国内の梅毒報告の増加，2009～2013年

国立感染症研究所感染症疫学センター[1]，国立感染症研究所細菌第一部[2]，川崎市健康安全研究所[3]
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牧野友彦[1]，大石和徳[1]，中山周一[2]，大西　真[2]，岡部信彦[3]

【背景】近年日本では性感染症が減少傾向にあると言われるが，梅毒に関しては違う傾向が見られるとの情報があることから，近年の梅毒発生動向を探った．【方法】感染症発生動向調査で2009～2013年に梅毒と診断され報告された症例（2013年12月19日現在）を記述的に分析した．【結果】2013年の報告総数は1176例（男945，女231）で，2009年より1.70倍（男1.78倍，女1.38倍）増加していた．感染経路が判明した1011例（報告総数の94.0%）の内，ほぼ全例を占める1004例が性的接触によるものであり，その内訳は，男性の10～20代で同性間性的接触（同性間，異性間・同性間）感染が，2009年58例に対し2013年118例と2.03倍に増加した．また，30～40代も85例から258例（3.04倍）に増加した．異性間性的接触感染は10～20代で49例から83例（1.69倍）と増加が認められた．女性は10～20代の異性間性的接触感染が2009年56例に対し2013年は73例（1.30倍）であった．早期梅毒の届出は，男性が2009年315例に対し2013年573例と1.82倍に増えていた．【結論】近年，梅毒は10～40代の男性同性間性的接触感染が急増している．これは性器クラミジア感染症や淋菌感染症が増加していないことと対照的であり，流行している集団が異なることを示唆している．梅毒に対しては，10～40代の男性同性間を中心とする感染予防などに関する情報提供と注意喚起が必要である．謝辞：梅毒の報告に携わった関係者の皆様に感謝致します．

O1-079 化 メロペネム低感受性株に対するメロペネムと各種抗菌薬の併用効果～2012年度メロペン全国感受性調査菌株を用いた追加検討～

大日本住友製薬株式会社創薬開発研究所[1]，大日本住友製薬株式会社製品企画部[2]，東邦大学医学部微生物・感染症学講座[3]，東邦大学医学部感染症高度統合解析講座[4]
○藤本孝一[1]，竹本浩司[1]，金澤勝則[2]，石井良和[3]，
舘田一博[3]，山口惠三[4]

(目的) 2012年度メロペン全国感受性調査にてメロペネム (ME) は，優れた抗菌活性を示し，過去の調査結果に比して顕著な耐性化傾向も認められず，重症感染症治療薬としての有用性を保持していることが示された．一方，緑膿菌や *B. fragilis* group で ME 低感受性株が少数例分離されており，これら菌株に対する治療対策の検討は重要である．対策の一つとして ME と他抗菌薬の併用療法が考慮され，海外では緑膿菌及び嫌気性菌感染症にそれぞれ国内未承認薬であるコリスチン (CL) やメトロニダゾール (MNZ) などが併用されることが多い．これらの抗菌薬は国内で開発中であることから，今回，ME と CL 及び MNZ を始めとする各種抗菌薬の in vitro 併用効果を検討した．(方法) 上記調査で収集された ME 低感受性の緑膿菌52株及び *B. fragilis* group 7株を使用．寒天平板希釈法によるチェッカーボード法で併用効果を検討．(結果及び考察) 緑膿菌に対して ME と CL との併用は，52株中27株で相加効果，25株で不関であった．ME とトブラマイシンとの併用は，19株で相乗効果，31株で相加効果を認め，優れた併用効果を示した．*B. fragilis* group に対して ME と MNZ またはクリンダマイシンとの併用では，7株中5株以上の菌株で相乗及び相加効果を示した．上記の何れの併用においても拮抗は認められなかった．国内で分離された ME 低感受性株に対して ME と上記抗菌薬の併用療法は有用と考えられた．会員外共同研究者　小早川千衣，大滝真理子

O1-080 化 臨床，家畜，飼育者から分離されたセフェム系薬耐性菌の特性

帝京大学医学部微生物学講座
○中野竜一，中野章代，斧　康雄

【目的】薬剤耐性菌は，医療現場において難治化や院内感染などの問題となるが，食品や家畜，環境などからも多く検出される．ヒトは食品などを通じてこれら耐性菌の保菌者となっていることがその一因と推測されるが，具体的な因果関係を解明するには数多くの疫学研究を必要としている．本邦における耐性菌の疫学データは不明な点が多いため，家畜とその飼育者，臨床現場から分離されるセフェム系薬耐性菌に注目し，その関連性を解析した．【方法】家畜について，国内大規模生産地の牛と豚それぞれ50頭以上から検体として糞便を収集した．家畜飼育者と病院患者の糞便も同様に収集した．DHL寒天培地を用いて検体より菌株を分離培養し，質量分析装置にて菌種同定を行った．薬剤感受性試験をCLSI法に準じて行い，薬剤耐性遺伝子をPCRなど遺伝学的手法により明らかにした．【結果，考察】家畜の糞便から分離されたセフェム系薬耐性菌について，大腸菌，肺炎桿菌，緑膿菌，アシネトバクター，エンテロバクターなどが多く検出された．大腸菌について耐性遺伝子を解析したところ，CTX-M1型とM9型が多く分離された．家畜飼育者と病院患者についても同様の耐性遺伝子が確認された．耐性プラスミドのIncなど共通性が確認されたものの家畜由来株と臨床由来株との関連性は不明であった．今後さらに詳細に検討する必要があると考えられた．会員外共同研究者　西寒水隆治，西寒水香織，浅原美和，田中孝志，古川泰司

別紙様式第2-1

No. 25

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. 4. 30	該当なし	
一般的名称	新鮮凍結人血漿		研究報告の公表状況	IASR 35: 79-80, 2014; Available from: http://www.nih.go.jp/niid/ja/syphilis-m/syphilis-iasrs/4582-pr4112.html	公表国 日本	
販売名(企業名)	新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)					

研究報告の概要

○東京都における梅毒の発生状況(2007～2013年)
日本において梅毒は減少傾向にあったが、近年多くの先進諸国同様、男性と性交をする男性(MSM)を中心に感染が拡がっていることが明らかとなっている。今回、東京都における2007～2013年の梅毒の発生動向についてサーベイランス情報から記述疫学を実施した。
2013年の総報告数は417人(人口10万対3.2人)であり、2010年を境に増加が見られた。2013年の男女比は7:1(男365人:女52人)、人口10万対報告数は男性5.6人、女性0.8人であった。2010年と2013年を比較すると、男性では2.4倍、女性では2.9倍に増加した。感染経路では、男性では2013年に346例(94.8%)が性的接触と報告されており、そのうち同性間性的接触248例(71.7%)、異性間性的接触60例(17.3%)であった。男性の同性間性的接触による感染の報告数は増加しており、2013年は2007年に対して11.3倍に増加した。女性は40例(76.9%)が性的接触と報告された。女性の異性間性的接触では、2013年は2010年に対して3.7倍に増加した。
2013年の総報告数は、過去5年平均＋2SDの値(=322)を大きく超えており、アウトブレイクと捉えることができる。全国の人口10万対報告数(2013年:男1.6、女0.4)と比べると、男女ともにその報告数は多く、特に男性で顕著である。都内ではMSM間での感染が報告数増加の主体と考えられる。増加している梅毒の動向を把握し、流行の周知、検査案内、患者や接触者への介入等の対策を進める必要がある。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
東京都における2013年の梅毒総報告数は、過去5年平均＋2SDの値を大きく超えており、アウトブレイクと捉えることができるとの報告である。	日本赤十字社では、輸血感染症対策として問診時に梅毒を含む性感染症の既往歴を確認し、梅毒の既往歴がある場合は献血不適としている。また、献血血液に対して梅毒抗体検査を実施し、陽性血液を排除している。今後も引き続き情報の収集に努める。

27

＜速報＞東京都における梅毒の発生状況(2007～2013年)

(掲載日　2014/4/21)

はじめに:わが国において梅毒は減少傾向にあったが、近年、多くの先進諸国同様、男性と性交をする男性(men who have sex with men: MSM)を中心に感染が広がっていることが明らかとなっている。今回、東京都における2007～2013年の梅毒の発生動向についてサーベイランス情報から記述疫学を実施したので報告する。

方法:日本の感染症サーベイランスでは、全国統一のシステムである感染症発生動向調査システム(NESID)によって国が全体のデータを集約している。東京都の人口は2013年現在、約1,300万人で、都内の約12,000の医療機関から保健所(31カ所)を経由して、サーベイランス情報が国まで報告されている。すべての医師は、梅毒の診断例(無症状者を含む)を指定された個票で保健所に報告することが求められており、報告症例は保健所担当者によってNESIDへ登録され、東京都感染症情報センターで届出内容が確認される。NESIDに登録された梅毒症例のうち、2007～2013年に都内で診断された症例を抽出し解析を行った(2013年3月3日現在)。人口当たりの報告数には人口動態統計による各年の東京都の推定人口を使用した。

結果:2013年の総報告数は417人(人口10万対3.2人)であった(図1)。2010年を境に増加が見られ、2011年は2010年に対して1.4倍、2012年は2011年に対して1.2倍、2013年は2012年に対して1.4倍に増加していた。

2013年の病期別報告数は早期顕症Ⅱ期が183人(43.9%)と一番多く、次いで無症候160人(38.4%)、早期顕症Ⅰ期62人(14.9%)の順であった。2007年に64人だった早期顕症Ⅱ期はその後一貫して増加し、晩期顕症は10人前後、先天梅毒は0～3人で推移した。無症候と早期顕症Ⅰ期は、2010年にそれぞれ53人、17人の報告であったが、この年を境に増加に転じた。

性別では、2013年の男女比は7:1(男365人:女52人)、人口10万対報告数は男性5.6人、女性0.8人であった(図1)。2010年と2013年を比較すると、男性では2.4倍、女性では2.9倍に増加した。年齢群別の人口10万対報告数を2007年と2013年で比較すると、男性では20~50代で増加し、特に20～30代の増加が顕著であり、女性では、20～24歳で増加が見られた(図2)。2013年の男性では30～34歳が12.1で最も高く、女性では20～24歳が4.2で最も高かった。

感染経路では、男性では2013年に346例(94.8%)が性的接触と報告されており、そのうち同性間性的接触248例 (71.7%)、異性間性的接触60例(17.3%)であった(図3)。男性の同性間性的接触の報告数は増加しており、2013年は2007年に対して11.3倍に増加した。女性は40例(76.9%)が性的接触と報告され、そのうち異性間性的接触が33例(82.5%)と多くを占めた。女性の異性間性的接触では、2013年は2010年に対して3.7倍に増加した。

保健所別の人口10万対報告数は、2013年に特別区保健所(n=23)では4.3人、多摩・島しょ地区保健所(n=8)では0.6人であった。前者は2007～2010年に1.7から2.1で推移していたが、その後年々増加し、2013年は2010年に対して2.5倍に増加した。後者は2007～2012年に0.3から0.4で推移していたが、2013年は2012年に対して1.8倍に増加した。特別区保健所には2013年に392人の報告があり、そのうちの225人(57.4%)が1つの保健所(A区保健所)に報告されたものだった(図4)。A区保健所での2013年の人口10万対報告数は68.2であり、2013年は2007年に対して7.4倍に増加していた。

考察:2013年の総報告数は、過去5年平均+2SDの値(=322)を大きく超えており、アウトブレイクと捉えることができる。全国の人口10万対報告数(2013年:男1.6、女0.4)と比べると、男女ともにその報告数は高く、特に男性で顕著である。都内ではMSM間での感染が報告数増加の主体と考えられる。特にA区保健所管内で集積しているが、A区での増加は、MSM人口が多く、ケアする医療機関が複数あることが要因の一つと考えられる。男女とも異性間性的接触が増加傾向にあるほかA区以外からの報告も増えており、一般住民への広がりも危惧される。また、早期顕症Ⅰ期の増加は直近の梅毒感染が増えていることを示しており、感染拡大が現在も進行していると考えられる。この都市部でのMSMによる梅毒の流行について公衆衛生として感染拡大予防の取り組みを

行う必要がある。具体的には、増加している梅毒の動向を把握し、流行の周知、検査案内(都内の保健所等では性感染症検査を匿名・無料で実施中)、患者や接触者への介入等の対策を進める必要がある。また、ハイリスク層、20代女性層への疫学調査を実施し、より詳細な実態を把握していかなければならない。

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別紙様式第2-1

No. 24

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. 6. 2	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	新鮮凍結人血漿		研究報告の公表状況	Tappe D1, Stich A, Langeheinecke A, von Sonnenburg F, Muntau B, Schäfer J, Slesak G. Euro Surveill. 2014 May 29;19(21). pii: 20816.	公表国 ドイツ	
販売名(企業名)	新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)					

研究報告の概要	使用上の注意記載状況・その他参考事項等
○マレーシアのティオマン島から帰国した旅行者における肉胞子虫症(サルコシスチス症) 肉胞子虫症は、120種以上が知られている肉胞子虫属の細胞内寄生原虫によって引き起こされる、世界的な人獣共通感染症である。 2014年5月、マレーシアのティオマン島からドイツに帰国した6人が、肉胞子虫症による発熱を伴う筋炎を発症した。全員がティオマン島の北西部に滞在し、離島後1～18日(平均10日)後に発症した。患者の旅行歴や島での滞在先、推定潜伏期間、臨床像及び検査結果は、2011年と2012年にティオマン島から欧州各国に帰国した旅行者における肉胞子虫症関連の筋炎症例と一致した。ティオマン島での肉胞子虫感染に関与する原虫種、感染源及び動物宿主は現在調査中である。2011年と2012年の流行は7月～10月に発生したが今回の発生は春であり、今夏の流行が示唆される。	新鮮凍結血漿-LR「日赤」120 新鮮凍結血漿-LR「日赤」240 新鮮凍結血漿-LR「日赤」480 血液を介するウイルス、細菌、原虫等の感染 vCJD等の伝播のリスク

報告企業の意見	今後の対応
マレーシアのティオマン島から帰国したドイツ人旅行者6人が、肉胞子虫症を発症したとの報告である。	日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

28

Suspected new wave of muscular sarcocystosis in travellers returning from Tioman Island, Malaysia, May 2014

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In May 2014, six patients presented in Germany with a *Sarcocystis*-associated febrile myositis syndrome after returning from Tioman Island, Malaysia. During two earlier waves of infections, in 2011 and 2012, about 100 travellers returning to various European countries from the island were affected. While the first two waves were associated with travel to Tioman Island mostly during the summer months, this current series of infections is associated with travel in early spring, possibly indicating an upcoming new epidemic.

Here we report the clinical and laboratory findings of a new, third wave of *Sarcocystis*-associated febrile myositis syndrome in travellers returning to Germany from Tioman Island, Malaysia, in spring 2014.

Case series

Six previously healthy German patients, aged 15–44 years were seen in early May 2014 in travel clinics in Tübingen, Saarbrücken and Munich, with a febrile myositis syndrome after travel. The patients (three female, three male) complained of current or very recent episodes of fever of up to 40 °C, headache and myalgia. All had returned at the end of March to the end of April from Tioman Island, Malaysia. Laboratory investigations revealed eosinophilia in all but one and elevated muscle enzyme levels in half of the patients (Table). There were no clinically relevant electrocardiogram abnormalities but mild splenomegaly was seen in some. Serological tests for trichinellosis, toxoplasmosis and dengue virus infection were negative in all patients. Tests for chikungunya virus antibodies were not carried out for one patient, but negative in all others. All patients had stayed in the north-west of Tioman Island and developed symptoms 1–18 days (mean: 10) after leaving the island. Their travel history, including locality of lodging on the island, estimated incubation time (a few days to three weeks), clinical picture and laboratory results were consistent with the *Sarcocystis*-associated febrile myositis syndrome seen in travellers returning to various European countries from Tioman Island in 2011 and 2012 [1-4]. No such series of infections were seen in 2013.

Patients 1–3

In three patients who sought medical attention 1–6 days after first onset of fever, headache and slight myalgia (i.e. in the early, rather non-specific phase of clinical disease) and who had no elevated creatine kinase (CK) levels, therapy with trimethoprim/sulfamethoxazole (cotrimoxazole) (2 × 960 mg/day) [4] was started within a few days. All three improved clinically but developed higher eosinophilia after 7–20 days, yet there was no elevation of CK level.

Patients 4–5

A couple presented in a later phase of the disease. One patient was currently asymptomatic with moderate CK elevation 23 days after a previous short-lived febrile episode. The other had fever and severe myalgia plus high eosinophilia and CK elevation 28 days after onset of a short-lived episode with high fever, headaches and night sweats. Both showed a further increase of eosinophil counts (maximum count of 500/µl and 5,260/µl, respectively) after the start of cotrimoxazole treatment. The previously symptom-free patient developed moderate myalgia together with increasing CK levels thereafter; the other had to be started on high-dose steroids four days later (prednisolone starting dose 100 mg/day) because of intensifying severe myalgia.

Patient 6

The patient with the longest interval (43 days) since onset of first symptoms (fever, headache, myalgia) was treated with steroids (prednisolone starting dose 40 mg/day).

Administration of steroids resulted in rapid clinical improvement in both patients (Patients 5 and 6), similar to observations during the first two waves of

Table

Characteristics of patients returning from Tioman Island, Malaysia, with *Sarcocystis*-associated febrile myositis syndrome, May 2014 (n=6)

Characteristic	Patient number						Norm
	1	2	3	4	5	6	
Sex	M	F	M	F	M	F	–
Days from departure from Tioman Island, Malaysia, until symptom onset	9	12	7	18	13	1	–
Myalgia (yes/no)	yes	yes	yes	yes	yes	yes	–
Maximum pain (score 0=none to 10=maximum)	2	4	4	6	9.5	8	–
Arthralgia (yes/no)	yes	yes	yes	no	no	yes	–
Headache (yes/no)	yes	yes	yes	yes	yes	yes	–
Neck pain (yes/no)	no	yes	yes	no	yes	yes	–
Body temperature (°C)	No data	38	38	38	40	40	–
Laboratory tests							
Creatine kinase (U/L)	138	80	133	159	207	450	<170 males; <145 females
Creatine kinase MB fraction (U/L)	21	15	14	22	35	9	<25 or <6% of total creatine kinase
Cardiac troponin (troponin I/ troponin T, µg/L)	<0.014	<0.014	<0.014	Negative	Negative	Not done	<0.4
Lactate dehydrogenase (U/L)	326	342	383	201	324	307	<240
Aspartate amino transferase (U/L)	49	56	46	18	39	23	<50 males; <35 females
Eosinophil count per µl	430	620	260	170	4,490	1,150	<350
Eosinophils (%)	9	8	4	8	41	12	<7
Spleen length (cm)	13.2	9.2	13.0	No data	12.1	12.5	<11
Treatment	Co-trimoxazole[a]	Co-trimoxazole[a]	Co-trimoxazole[a]	Co-trimoxazole[a]	Cotrimoxazole[a] plus prednisolone	Prednisolone	–
Days from symptom onset to start of treatment	6	3	8	23	28	43	–

F: female; M: male.

[a] Trimethoprim/sulfamethoxazole.

Sarcocystis-associated febrile myositis syndrome in travellers returning from Tioman Island in 2011 and 2012 [3,4].

In the current cluster, no muscle biopsies were taken and the presumptive diagnosis was based on the travel history, symptoms and blood test results of increasing eosinophil count and CK level and the exclusion of other infectious causes.

Background

Sarcocystosis is a cosmopolitan zoonotic disease that is caused by intracellular apicomplexan/coccidian parasites of the genus *Sarcocystis*, with more than 120 recognised species [5]. These protozoal parasites are maintained in a two-host life cycle involving a carnivore predator final host and its 'prey' as intermediate host, such as snake–rodent or human–cattle relationships, for example. In the intermediate host, invasive muscular sarcocystosis develops after infective *Sarcocystis* oocysts shed in faeces of an infected final host are ingested. Sporozoites are released from the oocysts, which invade muscular tissue of the intermediate host after several cycles of replication. In the myocytes, tube- or sac-like sarcocysts are eventually formed, which contain numerous single-celled bradyzoites. When the intermediate host falls prey to a carnivore or omnivore, the bradyzoites can complete their life cycle sexually in the intestine of the final host. Humans are final hosts for two *Sarcocystis* species, *S. hominis* and *S. suihominis*, which cause non-invasive self-limiting diarrhoeal symptoms [5]. However, humans can serve as accidental intermediate hosts after incidental ingestion of food faecally contaminated with oocysts for a presumed number of several *Sarcocystis* species, among them *S. nesbitti*, [6,7] and develop the invasive muscular form. Invasive muscular sarcocystosis causes fever and myalgia, but not diarrhoea, in contrast to the intestinal form.

Already in 1991, sarcocystosis was regarded as a possible emerging food-borne zoonosis in Malaysia, as high human seroprevalence [8] and high positive autopsy rates [9] were found. In 1993, the first cluster of patients with symptomatic muscular sarcocystosis was seen in United States service personnel in rural Malaysia [10]. In 2011 and 2012, in a two-wave outbreak, the largest series of symptomatic muscular sarcocystosis in humans worldwide was noted in travellers returning to Europe from Tioman Island, Malaysia [1-4]. The course of disease was typically biphasic, with a prodromal stage of one week characterised by fever, myalgia and headache, followed by a two-week asymptomatic period and later by a long-lasting feverish episode with severe myalgia with eosinophilia and CK level elevation [4]. An environmental survey for *Sarcocystis* oocysts conducted in November 2011 on Tioman Island could not detect the source of infection [11].

Definitive diagnosis is achieved after muscle biopsy with histological demonstration of typical sarcocysts or by molecular methods [3,6]. However, despite severe myalgia, parasite density in the muscle is apparently low and sarcocysts have thus been detected in a few patients only [3,4,6,10]. |

Conclusions

This cluster of travellers with a febrile myositis syndrome returning from Tioman Island indicates the beginning of a third wave of a presumably *Sarcocystis*-associated invasive illness. In the first two waves, in 2011 and 2012, patients acquired the disease mainly in the summer months (July to October) [1-4]. In contrast, symptom onset in patients of this current new cluster took place in spring, possibly indicating a larger upcoming epidemic in returning travellers in the summer months of this year.

The source of the infection on the island has not been determined so far, but is obviously persisting or re-emerging. The nearly simultaneous outbreak of invasive sarcocystosis among Malaysian students and teachers on a different Malaysian island, Pangkor [6], is intriguing. The snake-associated *S. nesbitti* [6,7,12] was molecularly determined to be the causative agent on Pangkor. The quest for the *Sarcocystis* species involved, the source of infection and the animal reservoir on Tioman Island is currently ongoing. It remains to be determined whether environmental factors, such as climate change or increasing reptile populations (i.e. possible final hosts) [13], play a role in this disease (re-) emergence.

Physicians should be aware of this unusual re-emerging outbreak and pre-travel advice should be given regarding individual prevention measures, such as the consumption of cooked food, well-peeled fruit and pre-packed or boiled/filtered water only. Treatment with cotrimoxazole may be a therapeutic approach in the early phase of disease to prevent muscle invasion, whereas steroids seem effective to treat severe myalgia/myositis in the later phase.

Conflict of interest

None declared.

Authors' contributions

Wrote the manuscript: DT, AS, AL, FvS, JS, GS; performed laboratory or epidemiological investigations: DT, BM; performed data analysis: DT, AS, AL, FvS, JS, GS; performed patient examinations: JS, GS, AL, FvS.

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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）

感染症定期報告に関する今後の対応について

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。

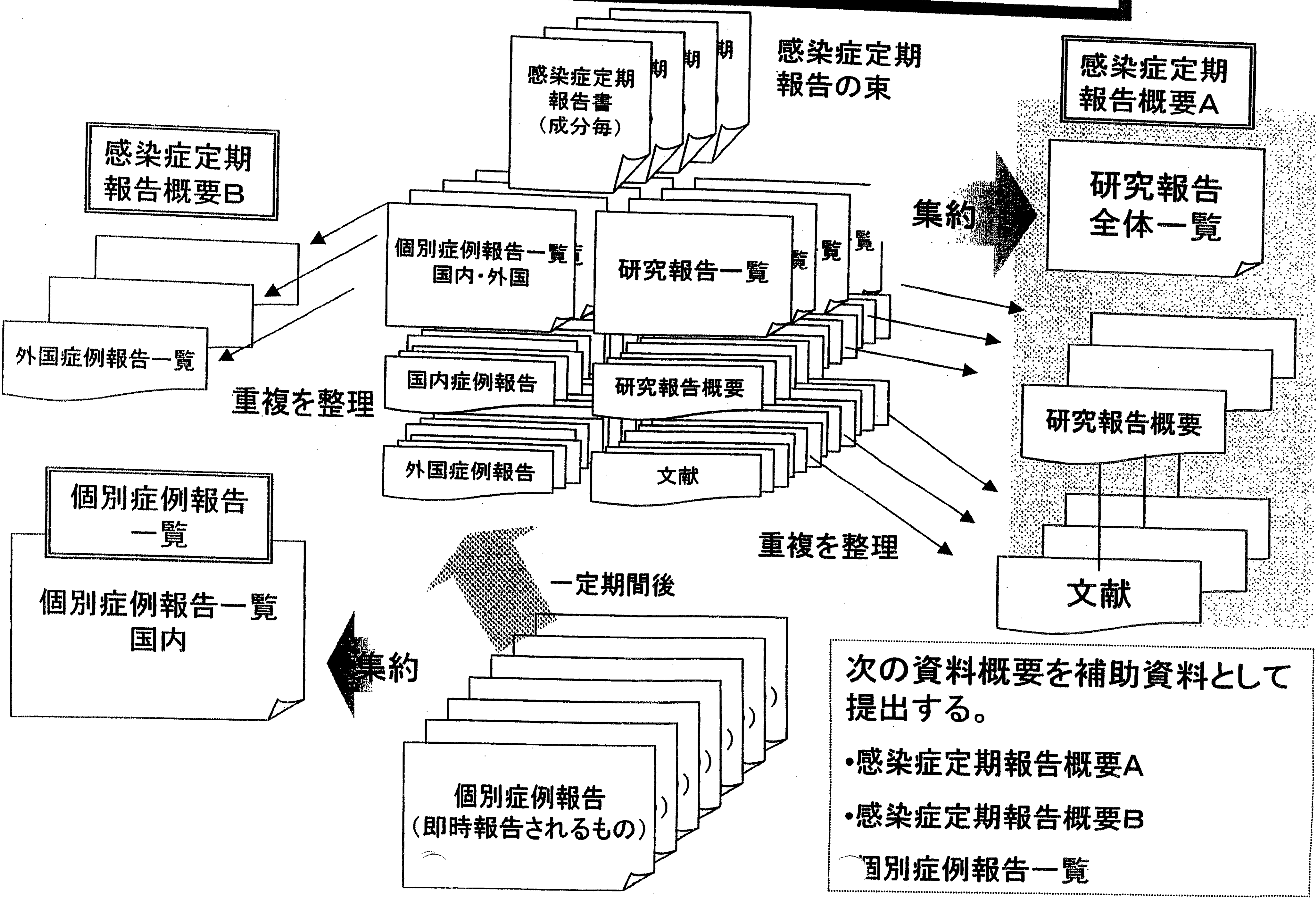
① 研究報告は、同一文献による重複を廃した別紙のような形式の一覧表を作成し、当該一覧表に代表的なものの報告様式（別紙様式第2）及び該当文献を添付した**「資料概要A」**を事務局が作成し、送付する。

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