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研究報告の概要

○ウエストナイルウイルス(WNV)核酸の血漿中からの消失後、全血中における数ヶ月の残存:輸血と移植の安全性に関する意義

背景: WNV RNAが血液中に残存するという既報により、WNVの輸血伝播リスクに関する懸念が高まっている。本研究では、54人のWNV感染供血者コホートにおいて縦断的に血液中のWNVウイルス血症動態を調査した。

研究デザインと方法: WNV RNAが陽性となった供血後1年間、定期的に血液サンプルを採取し、WNV IgMとIgG抗体及びWNV RNAについて検査を行った。血漿と全血サンプルのWNVウイルス量を比較し、血液型と臨床転帰を相関させた。

結果: 42%の供血者は、その後3カ月まで赤血球画分にWNV RNAが残存した。供血時に血漿中のWNV RNA量が最大であった供血者は、その後の3カ月間、全血中のWNV RNA量が多いまま持続した。血液型A型の供血者はO型の供血者と比べて、全血中のWNV RNA量が多かった(p=0.027)。WNV RNAは症候性被験者の全血中に持続的に存在する傾向が見られたものの、全血中のRNA量と疾患転帰の間に有意な関連性は認められなかった。

結論: 本研究は全血の赤血球画分にWNV RNAが残存することを証明し、さらに全血中の残存濃度は当初の血漿中のウイルス量を反映していることを示唆した。血液型との関連については、赤血球膜へWNVの結合を介在している分子が、O型よりもA型の赤血球表面により多く存在している可能性を示唆している。

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

WNV RNAが血漿中から消失してから数ヶ月後の全血中における残存について調べたところ、全血の赤血球画分にWNV RNAが持続的に存在することが証明され、また全血中における残存濃度は当初の血漿中のウイルス量を反映している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、ウエストナイル熱の既往がある場合、治癒後6カ月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。

West Nile virus nucleic acid persistence in whole blood months after clearance in plasma: implication for transfusion and transplantation safety

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BACKGROUND: Previous reports of West Nile virus (WNV) RNA persistence in blood compartments have raised concerns around the remaining risk of WNV transfusion transmission. This study characterized the dynamics of WNV viremia in blood compartments in a longitudinal cohort of 54 WNV-infected blood donors.
STUDY DESIGN AND METHODS: Blood samples were collected throughout the year after WNV RNA–positive blood donation (index) and characterized for WNV immunoglobulin (Ig)M and IgG antibodies and for WNV RNA by real-time reverse transcription–polymerase chain reaction. WNV viral loads were compared in plasma and whole blood samples and correlated with blood groups and clinical outcomes.
RESULTS: WNV RNA persisted in the red blood cell (RBC) compartment up to 3 months postindex in 42% of the donors. Donors with the highest WNV RNA levels in plasma at index maintained the highest WNV RNA levels in whole blood over the 3 months postindex. Blood group A donors maintained higher postindex WNV viral load in whole blood than blood group O individuals ($p = 0.027$). Despite a trend for WNV RNA to persist longer in whole blood from symptomatic subjects, no significant association was found between WNV RNA levels in whole blood and disease outcome.
CONCLUSION: This study confirmed that WNV RNA persists in the RBC fraction in whole blood and further suggested that the level of persistence in whole blood may be a reflection of initial viral burden in plasma. The association with blood groups suggests that WNV adherence to RBCs may be mediated by molecules overrepresented at the surface of blood group A RBCs.

West Nile virus (WNV), a mosquito-borne arbovirus and transfusion-transmitted flavivirus, was introduced into the United States in 1999 and has since been responsible for more than 39,000 reported clinical cases, of which 17,463 presented with neuroinvasive disease, 22,094 with West Nile fever, and 1668 with a fatal outcome.[1] However, WNV infection is asymptomatic in more than 80% of cases[2] and a recent study projected that more than 3 million persons have been infected with WNV in the United States from 1999 to 2010, resulting in approximately 780,000 illnesses.[3,4] With no treatment and no vaccine to prevent the development of symptomatic infections in humans,[5] it is essential to prevent transmission.

The demonstration of WNV transfusion transmission in 2002 alerted the blood banking community to the blood safety implications of large-scale arbovirus epidemics and to the need for expanded hemovigilance and focused research to proactively protect recipients from this class of

ABBREVIATIONS: ID = individual donation; LOD = limit of detection; MP = minipool; TMA = transcription-mediated amplification; WNV = West Nile virus.

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blood-borne agents.[6] In late 2002, the US Food and Drug Administration (FDA), US blood collection organizations, and test manufacturers began an accelerated program to develop and implement WNV nucleic acid amplification technology (NAT) assays to screen donors for WNV before the 2003 season. Assays were developed for use in a mini-pool (MP)-NAT format (plasma from six to 16 donations is pooled and tested), and by July 2003 essentially all blood donations in the United States were being tested for WNV using MP-NAT assays developed by Roche and Gen-Probe (San Diego, CA). Although MP-NAT of blood donations prevented hundreds of cases of WNV infection in 2003, it failed to detect units with a low level of viremia, some of which were antibody negative and infectious.[7] Documentation of MP-NAT "breakthrough" infections suggested that a significant rate of low-level viremic donations were missed by MP-NAT. Consequently, relatively cost-effective strategies for targeted NAT of individual donations (ID-NAT) in high-prevalence regions was developed and implemented successfully in 2004 with subsequent refinements over the past decade.[8]

Capitalizing on blood bank resources allowed for unparalleled access to WNV RNA–positive donors. Samples were collected from these otherwise inaccessible individuals in the a- or presymptomatic stage of acute WNV infection and systematic follow-up studies of viremic donors were conducted, which contributed to a better understanding of the natural history of viremia and immune responses.[9-14] Those studies provided insights into the dynamics of viremia and immune responses; the rates, determinants, and pathogenesis of symptomatic WNV disease; and refinement of screening strategies and deferral policies that have essentially eliminated the risk of WNV transfusion transmission in the United States. However, some questions remain unanswered and require further study, including the risk for transfusion transmission by units collected in the tail end of WNV viremia.[9,10,15]

WNV RNA compartmentalization (i.e., the association of viral nucleic acids and infectious virus with blood cells) was first reported in a cross-sectional cohort by Rios and colleagues[16] and was recently further characterized by Lai and coworkers[17] in a longitudinal cohort of 10 WNV-infected blood donors from Blood Systems. Notably, WNV RNA concentrations were consistently greater in whole blood than in plasma after seroconversion, and viral RNA was detected in whole blood up to 200 days after initial detection.[17] Further work was needed to more precisely characterize the dynamics of acute viremia in blood compartments and the association with WNV clinical disease. This study reports these findings based on laboratory and clinical characterization of a longitudinal cohort of 54 additional Blood Systems WNV-infected blood donors with symptom data collected around the time of initial donation and samples collected throughout the year postdonation. This study also explores the association between WNV RNA persistence in whole blood and blood groups, with potentially informative insights into WNV pathogenesis.

MATERIALS AND METHODS

Study population

WNV infected donors were enrolled by Blood Systems Research Institute between 2009 and 2011. Demographics such as age and sex were collected from all donors. All donors who tested positive for WNV RNA by routine donation screening (index; Procleix WNV transcription-mediated amplification [TMA] assay [Gen-Probe]) at United Blood Services collection facilities were eligible for enrollment. Infection was confirmed when index donation was repeat reactive by TMA and follow-up samples showed seroconversion to anti-WNV immunoglobulin (Ig)M. Confirmed infected donors were enrolled after signing an informed consent approved by the UCSF Committee on Human Research. Samples were successfully collected from 54 WNV-positive donors. Symptom questionnaires covering 12 possible WNV-related symptoms were administered at study enrollment and 2 weeks later for all donors.[11] As previously described,[11,13,18] a cutoff of four symptoms was used to categorize infected donors as asymptomatic (number of reported symptoms < 4, $n = 26$) or symptomatic (number of reported symptoms ≥ 4, $n = 28$). The mean age was 51 years for the WNV-positive cohort, 48 years for asymptomatic, and 53 years for symptomatic WNV-positive donors ($p = 0.19$).

Sample preparation

Whole blood, peripheral blood mononuclear cell (PBMNC), and plasma samples were prepared from anticoagulated blood specimens collected in ethylenediaminetetraacetate tubes. Blood was centrifuged at $872 \times g$ for 10 minutes before plasma was removed and aliquoted for long-term storage. The remaining white blood cells (WBCs), red blood cells (RBCs), and small-volume plasma, referred to here as "whole blood," were also aliquoted into cryovials for long-term storage at –80°C. PBMNCs were isolated on a Ficoll-Paque PLUS density gradient (GE Healthcare Life Sciences, Piscataway, NJ). Aliquots of 10×10^6 cells were frozen in medium containing 90% FBS (HyClone, Logan, UT) and 10% dimethyl sulfoxide (DMSO; Fisher BioReagents, ThermoFisher Scientific, San Diego, CA) and stored in liquid nitrogen.

WNV real-time reverse transcription–polymerase chain reaction assay

The WNV real-time reverse transcription–polymerase chain reaction (RT-PCR) assay in this study was used as previously described.[17] Briefly, RNA was extracted from undiluted thawed plasma and whole blood samples and washed PBMNCs (to remove any trace of DMSO) using a

viral RNA kit (Qiagen, Chatsworth, CA) with procedures slightly modified from the package insert. Viral RNA was extracted from 100 µL of plasma or whole blood samples and from 10×10^6 PBMNCs (thawed, washed with 500 µL of phosphate-buffered saline [PBS] and resuspended in 100 µL of PBS). Real-time RT-PCR used primers and probes that targeted highly conserved sequences within the capsid region or the NS1/NS2 region of the WNV genome.[19] After amplification, the mean cycle threshold values from two replicate tests were determined for whole blood and plasma-derived samples processed in parallel. WNV RNA–positive plasma with a known concentration, originally sourced from an FDA stock of WNV isolate (NY99) culture supernatant, was obtained from Center for Biologics Evaluation and Research/FDA and spiked into plasma as well as whole blood, which were then used as the standards for viral load extrapolation as previously described.[17]

WNV IgM and IgG antibody assay

Serologic testing of plasma for WNV IgM and IgG was performed using ELISA kits (Focus Diagnostics, Cypress, CA) in accordance with the manufacturer's instructions and as previously described.[20]

Statistical analysis

The t test (Excel, Microsoft Corp., Redmond, WA) was used to compare the age of asymptomatic and symptomatic WNV-positive donors. Computer software (GraphPad Prism, GraphPad, Inc., San Diego, CA) was used to compare differences in viral load between blood group A and blood group O WNV-positive donors and between asymptomatic and symptomatic WNV-positive donors by the nonparametric Mann-Whitney test. The nonparametric Wilcoxon signed rank test for matched pairs was used to compare viral load levels in plasma, whole blood, and PBMNC samples from the same 10 donors at a given time point. The nonparametric Mann-Whitney test was used to compare viral loads at index time points between groups of WNV-positive donors maintaining high versus low viral loads in whole blood at 60 days postindex. The method of generalized estimating equations was used to examine the difference between blood groups A and O over the time postindex and between asymptomatic and symptomatic WNV-positive blood donors in association with WNV viral load mean quantities per milliliter of whole blood. Significance was determined at p values of less than 0.05.

RESULTS

WNV RNA is maintained in whole blood at higher levels than in plasma for up to 3 months postindex

The 54 WNV-positive blood donors with available plasma and whole blood samples included in this study were enrolled between 2009 and 2011 as part of an intensive follow-up study that allowed for the collection of pedigreed biospecimens characterized for immune markers (Fig. 1A) and WNV viral load in plasma and whole blood (Fig. 1B). Frozen follow-up plasma and whole blood samples were available from these donors at 1 week, 2 weeks, 3 weeks, 4 weeks, 6 weeks, 2 months, 3 months, and 6 months postinitial blood donation (index). Specimens were thawed and characterized for WNV viral load by real-time RT-PCR (Figs. 1 and 2).

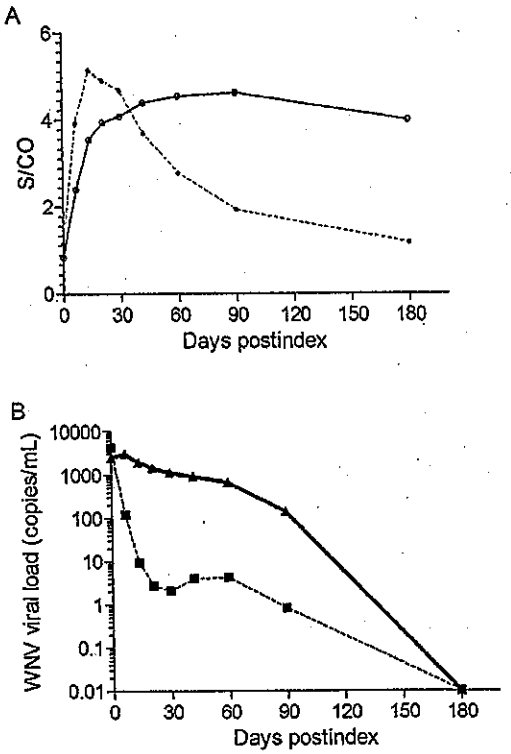

Fig. 1. Viral and immune variables of WNV infection over the 6 months postindex donation. (A) Mean anti-WNV IgM (–•–) and IgG (○) titers are shown for 54 WNV-positive donors over the 180 days after index donation and expressed as fold increase from cutoff (signal to cutoff [S/CO]). (B) Mean WNV viral load measured by real time RT-PCR in plasma (■) and whole blood samples (▲) from the same 54 WNV-positive donors over the same period are expressed in copies/mL.

At the time of index RNA-positive donations, when only six of 37 (16%) WNV-positive donors with viral load and antibody data had seroconverted to anti-WNV IgM (Table 1), there was no significant difference in the level of WNV viral load between plasma (4123 copies/mL) and whole blood (2488 copies/mL; $p = 0.36$; Fig. 1B). At follow-up visits, as an increasing number of WNV-positive donors seroconverted to anti-WNV IgM (on Day 7, 47 of 54 WNV-positive donors were IgM positive and by Day 14 all had seroconverted; Table 1), viral loads were significantly

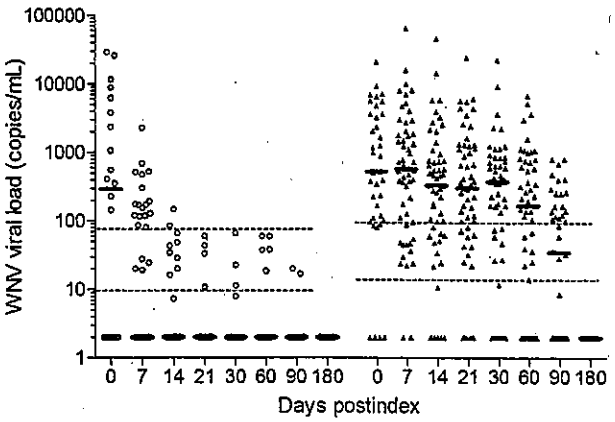

Fig. 2. WNV viral load in plasma (○) and whole blood (▲) samples from 54 WNV-positive blood donors over the year postindex donation. WNV viral load was measured by real-time RT-PCR in plasma and whole blood samples from 54 WNV-positive donors over the 3 months postindex donation and expressed in copies/mL. Bars = median viral loads. Dashed lines = 50 and 95% LOD of the assay, as previously characterized,[17] respectively, for plasma and whole blood.

higher in whole blood than in corresponding plasma samples (25-fold difference on Day 7; $p < 0.0001$). Viral loads persisted at higher levels in whole blood than in plasma throughout the first 3 months postindex (fold difference on Day 14, 210; on Day 21, 525; on Day 30, 532; on Day 60, 157; on Day 90, 167 times higher in whole blood than in plasma; $p < 0.0001$; Fig. 2). The mean WNV viral load was more than 1000 copies/mL over the first month (median, >300 copies/mL), then decreased over the second (mean, 666 copies/mL; median, 169 copies/mL) and third months (mean, 139 copies/mL; median, 35 copies/mL) postindex. While 100% of the WNV-positive donors had WNV RNA levels below the 95% upper limit of detection (LOD) of the assay in plasma (73.7 copies/mL) by the third week postindex, 42% of them maintained WNV RNA levels above the 95% LOD of the assay in whole blood (89 copies/mL) up to 3 months postindex (Fig. 2).

WNV RNA is associated with the RBC rather than the WBC compartment

To investigate whether the persistent, high levels of WNV RNA detected in whole blood relative to plasma are due to associations with the WBC and/or RBC compartments of the whole blood, samples from the 10 WNV-positive donors previously characterized by Lai and colleagues[17] for WNV viral loads in plasma and whole blood were further characterized for WNV viral load in plasma, PBMNCs, and whole blood by real time RT-PCR (Fig. 3). While WNV RNA was confirmed to be significantly higher in whole blood than in plasma on Day 7 ($p = 0.002$), Day 14 ($p = 0.004$), Day 21 ($p = 0.002$), and Day 30 ($p = 0.008$), it was also significantly higher in whole blood than in the corresponding concentrated PBMNC preparations on Day 14 ($p = 0.0039$), Day 21 ($p = 0.002$), and Day 60 ($p = 0.015$). WNV RNA levels were slightly higher in PBMNCs than in plasma but the difference was only significant at day 21 ($p = 0.03$). Overall we concluded that WNV RNA was associated with the whole blood but not the PBMNC compartment, and therefore we inferred that the WNV RNA was more likely associated with long-lived RBCs than with short-lived granulocytes and platelets (PLTs).

Correlation between the level of WNV RNA in whole blood and the level of WNV RNA in plasma at index donation

WNV viral load data in plasma and whole blood were only available for a limited number of index donations ($n = 22$). WNV-positive donors with the highest levels of WNV RNA in plasma (classified as "high" when index WNV RNA was more than 1000 copies/mL, compared to those classified as "low" when index WNV RNA was <1000 copies/mL) exhibited higher levels of WNV RNA in whole blood at index ($p = 0.034$) and also maintained higher levels of WNV RNA in whole blood at 60 days postindex compared to those who exhibited lower levels of WNV RNA in plasma at index ($p = 0.01$; Fig. 4A). Eleven of the 12 donors with WNV RNA in plasma of more than 100 copies/mL had detectable WNV RNA in whole blood, except for one donor who had a negative whole blood sample result but later had detectable WNV RNA in whole blood at 60 days postindex. All eight donors with the highest levels (>1000 copies/mL) of WNV RNA in plasma at index had WNV RNA in whole blood at 60 days postindex, whereas 60% (3/5) of those with WNV RNA in plasma between 100 and 1000 copies/mL maintained detectable WNV RNA levels in whole blood at 60 days postindex and eight of nine donors with WNV RNA in plasma of less than 100 copies/mL at index had undetectable WNV RNA levels in whole blood at 60 days postindex (Fig. 4B).

Overall these data suggest that WNV RNA was already present in the whole blood fraction at index (even though 84% of the WNV-positive donors had not yet seroconverted to anti-WNV IgM at that time), and donors with the highest WNV RNA levels in plasma during acute viremia were also those with the highest persistent levels of WNV RNA in whole blood.

Blood group A donors maintained higher WNV viral load in whole blood over the 3 months postindex than blood group O donors

Within the 54 WNV-positive donors included in this analysis, there were 23 blood group O, 23 blood group A, six blood group B, and two blood group AB individuals.

TABLE 1. Availability of samples and data over first three follow-up visits

Type of data available	Index	Follow-up visit		
		First	Second	Third
WNV-positive donors with TMA and IgM or IgG data				
Number	44	54	54	54
TMA positive*†	44 (100)	45 (83)	35 (65)	24 (44)
IgM positive*	10 (23)	45 (83)	54 (100)	54 (100)
IgG positive*	8 (18)	32 (59)	47 (87)	53 (98)
Days PI‡	0	8 (2-35)	16 (9-48)	23 (16-56)
WNV-positive donors with viral load data				
Number	37	54	54	54
TMA positive	37 (100)	45 (83)	35 (65)	24 (44)
IgM positive	6 (16)	45 (83)	54 (100)	54 (100)
IgG positive	4 (11)	32 (59)	47 (87)	53 (98)
Days PI	0	8 (2-35)	16 (9-48)	23 (16-56)

* TMA and IgM or IgG reactivity are listed as number (%).
† Samples were considered TMA-reactive (+) if at least one of the 2 or 3 replicates were reactive.
‡ Days postindex (PI) are listed as mean (range).

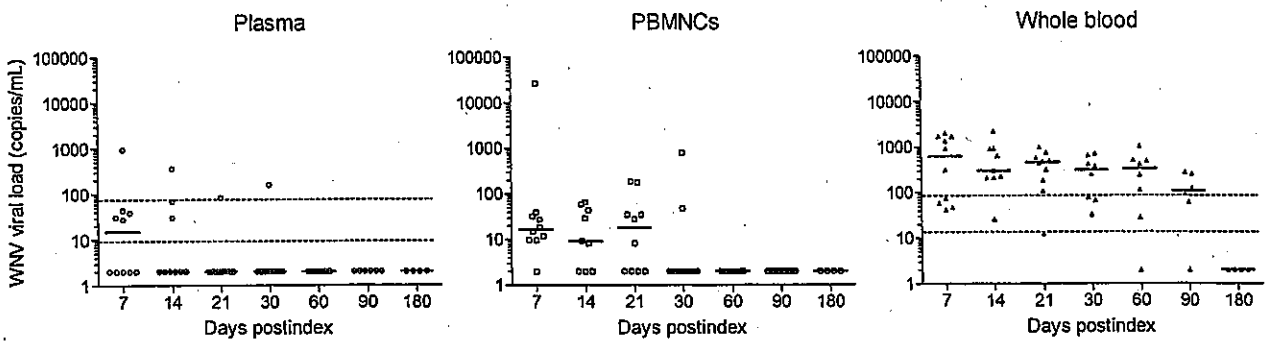

Fig. 3. WNV viral loads in plasma, PBMNCs, and whole blood samples from 10 WNV-positive blood donors over the 6 months postindex donation. Relative distribution of WNV RNA was investigated in plasma, PBMNCs, and whole blood samples from a longitudinal cohort of 10 WNV-positive donors enrolled in 2008.[17] WNV viral loads were measured in plasma, PBMNCs, and whole blood samples. WNV RNA concentrations are expressed in copies/mL. Bars = medians. Dashed lines = 50 and 95% LOD of the assay, as previously characterized,[17] respectively, for plasma and whole blood.

Examining the WNV viral load dynamics over time postindex in blood group O (n = 23) and blood group A (n = 23; excluding blood group B and AB individuals), it seemed that a slightly higher percentage of blood group A (70%) than blood group O donors (50%) maintained WNV RNA levels of higher than the 95% LOD of the assay in whole blood at 2 months postindex (Fig. 5A). It appeared that blood group A donors retained higher median WNV viral loads in whole blood than others during the 3 months after index donation (Fig. 5B). At 42 days postindex, blood group A donors had higher median viral loads than blood group O donors (medians A, 624; and O, 128 copies/mL; p = 0.028). Comparing the mean WNV viral load over time in whole blood from blood group O and A WNV-positive donors, it appeared that blood group A WNV-positive donors maintained higher mean viral loads over the 3 months postindex period than blood group O WNV-positive donors (generalized estimating equation p = 0.027; Fig. 5B).

WNV RNA persistence is not significantly associated with WNV disease outcome

The association between WNV persistence in whole blood and pathogenesis was investigated by comparing WNV RNA levels in asymptomatic (n = 26) and symptomatic WNV-positive donors (n = 28) over the 3-month period after index donation (Fig. 6). While at Day 7 postindex 79% of symptomatic and 76% of the asymptomatic WNV-positive donors had WNV RNA levels higher than the 95% LOD of the assay in whole blood, 52% of the symptomatic versus 35% of the asymptomatic WNV-positive donors with available samples had still detectable WNV RNA in whole blood at 90 days postindex (Fig. 6A), suggesting that asymptomatic WNV-positive donors may clear WNV RNA from whole blood faster than symptomatic WNV-positive donors. Despite a trend for the median viral load in whole blood to be 2.3 and 23 times higher in symptomatic than in asymptomatic WNV-positive donors at 2 and 3 months

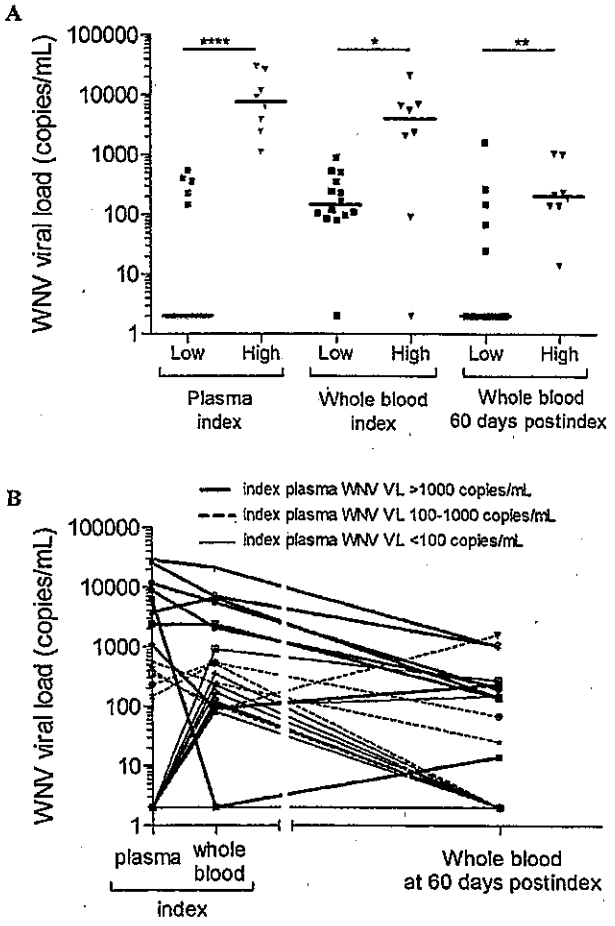

Fig. 4. Plasma WNV RNA levels in the first 2 weeks postindex predict persistent WNV RNA in whole blood at 2 months postindex. WNV viral load was measured by real-time RT-PCR in plasma and whole blood samples from 54 WNV-positive donors over the 3 months postindex donation and expressed in copies/mL for each individual donor over the 3 weeks postindex in plasma and over the 3 months postindex in whole blood. (A) WNV-positive donors with WNV viral load of fewer than 1000 copies/mL in plasma at index were classified as having "low" plasma WNV viral load and those with WNV viral load of more than 1000 copies/mL in plasma at index were classified as having "high" plasma WNV viral load. WNV viral load in whole blood for those classified as high or low WNV viral load in plasma at index are shown at index and at 60 days postindex. Middle bars = medians. *p < 0.05, **p < 0.01, and ****p < 0.0001 by Mann-Whitney test. (B) Individual WNV viral loads are shown for the 22 WNV-positive donors with available WNV RNA viral load data in plasma at index and for corresponding whole blood samples from the same donors at index and at 60 days postindex. Thicker lines are for WNV-positive donors with index plasma viral load of more than 1000 copies/mL. Dashed lines are for those with index plasma viral load between 100 and 1000 copies/mL. Thinner lines are for those with index plasma viral load of fewer than 100 copies/mL.

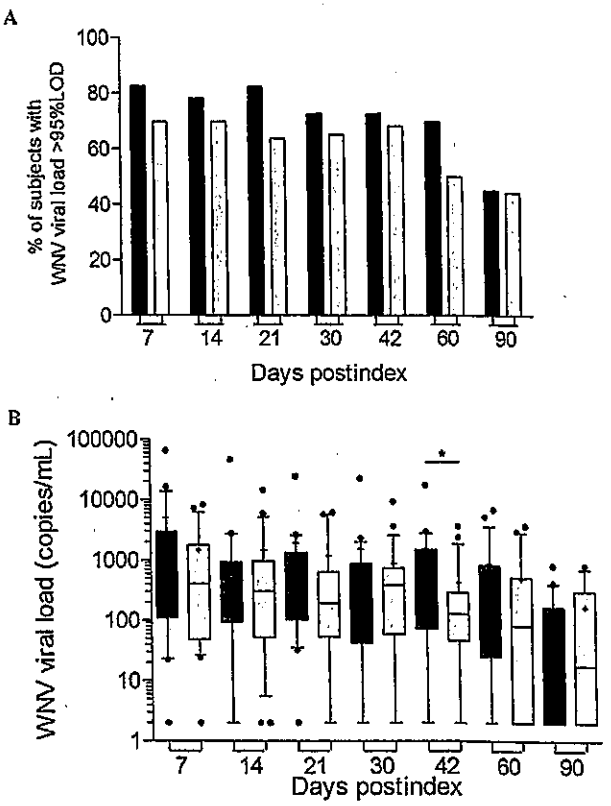

Fig. 5. Dynamics of WNV viral load in whole blood samples from WNV-positive blood donors with different blood groups. (A) The histograms represent the percentage of donors within blood group O (▭, n = 23) and blood group A (■, n = 23) WNV-positive donors with WNV viral load in whole blood above the 95% LOD of the WNV real time RT-PCR assay at different time points postindex. (B) Box and whiskers for 10th to 90th percentiles represents the distribution of WNV viral load in whole blood samples from blood group A (▬) and blood group O (▭) WNV-positive donors; middle bars = medians; + = means. *p < 0.05 by Mann-Whitney test to compare blood group A to blood group O WNV-positive donors on Day 42 postindex.

postindex, respectively, there was no significant difference between symptomatic and asymptomatic WNV-positive donors when mean or median WNV viral loads in whole blood were compared (Fig. 6B). Therefore, even though WNV RNA seemed to persist longer in symptomatic WNV-positive donors than in asymptomatic WNV-positive donors, there was no significant association between WNV RNA persistence in whole blood and WNV disease outcome.

DISCUSSION

This study was designed to measure WNV RNA levels in plasma and whole blood from a cohort of 54

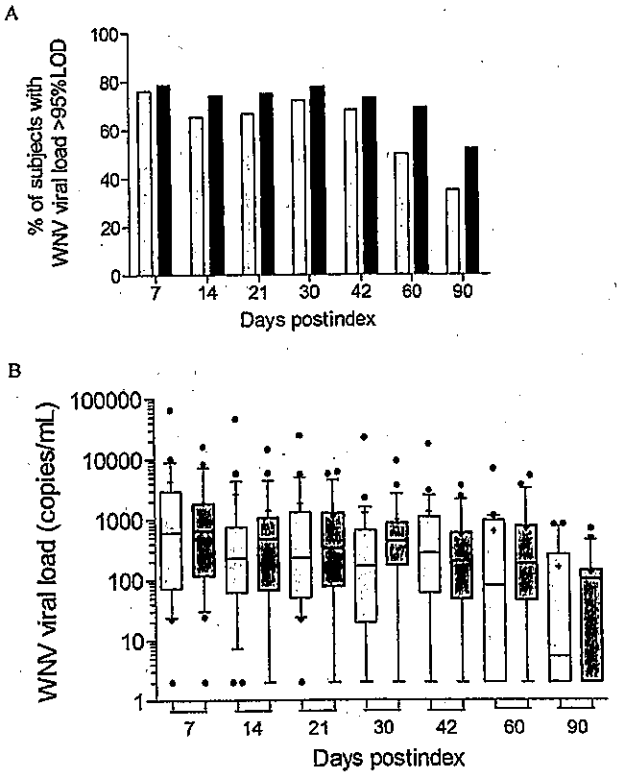

Fig. 6. Dynamics of WNV viral load in whole blood samples from asymptomatic (▭) and symptomatic (■) WNV-positive donors over the 3 months postindex donation. WNV viral load was measured by real-time RT-PCR in whole blood samples from asymptomatic (n = 26) and symptomatic (n = 28) WNV-positive donors over the 3 months postindex donation. (A) The histograms represent the percentage of donors within asymptomatic and symptomatic WNV-positive donors with WNV viral load in whole blood above the 95% LOD of the WNV real-time RT-PCR assay at different time points postindex. (B) Box and whiskers for 10th to 90th percentile represents the distribution of WNV viral load in whole blood samples from asymptomatic and symptomatic donors at 7, 14, 21, 30, 42, 60, and 90 days postindex; middle bars = medians, + = means.

WNV-infected blood donors enrolled from 2009 to 2011 who had demographic and symptom data collected around the time of donation and samples collected throughout the year after initial detection. Longitudinal samples were characterized for levels of WNV IgM and IgG antibodies and WNV RNA. The phenomenon of WNV blood compartmentalization[17] was confirmed: while 100% of the 54 donors had cleared WNV RNA from their plasma within the first 3 weeks postindex, 42% of the donors maintained substantial levels of WNV RNA in whole blood over the first 2 months postindex and in some cases up to 3 months postindex. As previously reported by others,[16] WNV RNA was found associated with whole blood and not the PBMNC fraction of whole blood. Interestingly those maintaining the highest levels of WNV RNA in whole blood were those with the highest levels of WNV RNA in plasma at index, suggesting that WNV RNA in whole blood could be the consequence of initial viral burden as reflected in the levels of acute viremia in plasma. Furthermore, WNV RNA persisted in whole blood from blood group A donors at higher levels than in whole blood from blood group O donors, suggesting binding to RBCs may be linked to blood group glycoproteins or mediated by molecules present at greater frequency on blood group A RBCs. Additionally, despite a trend for WNV RNA to persist longer in whole blood from symptomatic WNV-positive donors than in whole blood from asymptomatic WNV-positive donors, no strong association was found between WNV burden in whole blood and WNV disease course.

The persistence of WNV RNA in whole blood does not necessarily mean that infectious WNV is present. The persistence of infectious WNV in host tissues long after plasma clearance was demonstrated after investigating clusters of WNV organ transplant transmissions.[21] Indeed WNV was transmitted by organs collected from a donor without plasma viremia and with high titers of anti-WNV.[21] This suggests that low-level persistent viral particles are able to transmit WNV infection even after the development of humoral immune responses and clearance of detectable plasma WNV RNA. This is a concern as infectious diseases present particular risk to recipients of organ transplantations, who are often immunosuppressed and hence at greater risk of serious disease sequelae. From a transfusion transmission perspective, all cases of WNV transfusion transmissions have been linked to donations that tested WNV plasma RNA positive.[22-26] Even though a group at the FDA reported that infectious WNV could be amplified by cocultivating whole blood collected months after index donation with Vero cells and monocyte-derived cells despite the presence of anti-WNV,[16,27,28] only one out of thousands of units collected in the seropositive convalescent stage of WNV infection[9,29] has ever been linked to a breakthrough infection. Therefore, it is probably reasonable to assume that WNV particles persisting at low levels bound to RBCs in whole blood are neutralized after the development of anti-WNV. However, the recent exception is a case of WNV transfusion transmission that occurred in 2012 and had a fatal outcome despite donor seroconversion to WNV IgM antibodies.[30] This patient, who had non-Hodgkin's lymphoma, chemotherapy, and an autologous stem cell transplant, received allogeneic, leukoreduced, irradiated blood products collected from multiple donors including a donor who had tested nonreactive for WNV RNA by TMA and had seroconverted to WNV IgM antibodies.[30] Therefore, with several cases of transplantation transmission[21] and isolated cases of transfusion transmission despite universal blood screening,

WNV remains a concern for the blood bank and organ transplantation communities.[31]

The findings in this study could have implications for enhanced sensitivity screening of blood collected from donors in the seropositive phase of WNV infection in that a higher level of sensitivity could be achieved by screening whole blood instead of plasma. Improvement of the sensitivity for WNV detection assays might help in reducing WNV transfusion transmission from low-level viremia units, which may be particularly important for marrow and organ transplant recipients who are a more vulnerable population.[21] With better sensitivity, MP-NAT screening might be extended to reduce the period for ID-NAT screening, which could in turn decrease the cost for blood screening. Donors who tested positive for WNV RNA in plasma are deferred for a period of 120 days. This deferral period was defined based on evidence of WNV clearance in plasma after few weeks postindex donation; however, WNV RNA can now be identified in whole blood for several months postindex. Compromising between WNV MP- or ID-NAT testing and adjusting the deferral period during which blood donors who tested reactive for WNV are excluded from donation are key variables to ensure high quality, affordability, and availability of blood in the United States. This study suggests that an increased sensitivity could be reached by screening whole blood. However, the current screening platforms from Roche and Gen-Probe/Hologic, Inc., are optimized for plasma and it will require adaptation to be able to process whole blood.

The molecular basis underlying the persistence of WNV RNA in the RBC fraction of the whole blood, including whether the virus sticks to the RBC membrane or penetrates into the RBCs, remains unclear. The findings in this study corroborate the association of WNV with the RBC fraction of the whole blood previously reported by Rios and colleagues,[16] who suggested that WNV adheres to RBCs. Based on the finding that WNV RNA was associated with the whole blood but not the PBMNC compartment, we inferred that WNV RNA was more likely associated with long-lived RBCs than with short-lived granulocytes and PLTs. However, it would be interesting to further investigate the potential adherence to PLTs, and if detected the infectivity of PLT-associated WNV, as most of those are transfused to severely immunocompromised recipients who are at risk for the development of symptomatic WNV disease outcome.

Intriguingly, while WNV RNA is retrieved in the RBC fraction of all blood groups, blood group A donors seemed to maintain higher WNV RNA levels in whole blood than blood group O donors. This observation raises the possibility that the molecules mediating WNV attachment to the RBC membrane may be present at a higher concentration on the surface of RBCs from blood group A individuals than at the surface of RBCs from blood group O individuals. Similar mechanisms described for other pathogens such as *Plasmodium falciparum* could be at play during WNV infection. The utilization of blood group A antigen as a receptor or coreceptor for *P. falciparum* to enter the RBCs has been documented[32] and severe malaria outcomes are more frequent in blood group A individuals.[33] The longer persistence of the parasite in blood group A than in blood group O individuals was also explained by lower macrophage avidity for blood group A than for blood group O–infected RBCs.[34] Our finding of higher persistence of WNV RNA in the RBC compartment of blood group A individuals warrants further investigation as it could reveal mechanisms of viral entry and propagation, and potential genetic susceptibility that would have to be addressed in larger cohorts and ex vivo through spiking experiments investigating possible direct viral or antibody-mediated mechanisms. Characterizing the molecular basis for WNV adherence to RBCs is also important to understand whether WNV binds to the membrane at the surface of RBCs or penetrates into the cytoplasm of RBCs and whether the virus can be washed away during deglycerolyzation of frozen RBCs. Although not significant, the association between WNV RNA persistence and clinical disease outcome also suggests a link with pathogenesis. Indeed more symptomatic than asymptomatic WNV-positive donors exhibited persistence of WNV RNA up to 3 months postindex. This means that WNV infectious particles or WNV antigens may be persisting longer in symptomatic than in asymptomatic WNV-positive donors despite the development of the humoral and cellular immune response. RBCs have a life span of 120 days and represent potent vehicles to carry viruses and other pathogens throughout the body and the tissues, including the central nervous system, until they are eliminated. This may allow for infectious particles to reach otherwise inaccessible organs and tissues in a timely manner. This also poses a threat to organ recipients who are immunosuppressed and at risk for the development of symptomatic WNV disease after receiving transplants with remaining blood potentially carrying RBC-associated WNV. The persistence of WNV RNA in blood during the approximate life span of the RBCs in the circulation may be responsible for sustained immune responses that could translate into sustained tissue inflammation and immunopathogenesis. Further work in animal models may be required to address the correlation between levels of WNV RNA persisting in whole blood and initial viral burden in plasma as well as the potential association with pathogenesis in groups with different clinical disease outcomes.

In conclusion, this study confirmed the phenomenon of WNV RNA persistence for extended periods of time in whole blood compared to plasma in a cohort of 54 WNV-infected donors. The mechanisms underlying the persistence of WNV RNA in the RBC fraction remains unclear, but the association between the persistence of higher WNV RNA levels in whole blood from blood group A

donors is intriguing and requires further investigation as it could lead to new insights into the molecular basis of WNV attachment to host cells. This study examined the association between WNV RNA persistence and disease outcome with suggestive but not conclusive findings. Further studies will be required to determine whether longer WNV RNA persistence in whole blood from symptomatic WNV-positive donors is the cause or the consequence of immunopathogenesis. Finally, further studies addressing the persistence of infectivity in whole blood are needed to ascertain whether current screening strategies are sufficient for ensuring the highest quality of blood in the United States.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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医薬品 研究報告 調査報告書

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研究報告の概要

○アモトサレンとUVA照射による血漿中のデングウイルス(DENV)の不活化

背景: 輸血によるDENV感染は既に報告されており、デング熱の流行地域では、特にアウトブレイクの発生中には血液製剤がDENVに汚染されているリスクを考慮に入れる必要がある。アモトサレン/UVA処理などの血液製剤のウイルス不活化処理は、輸血伝播感染を削減するために開発された。本研究では、ヒト血漿中のDENVの不活化に用いるアモトサレン/UVA処理の有効性を明らかにする。

方法: 供血者の血漿ユニットにDENVを添加し、アモトサレン/UVA処理の前後に、ウイルス力価およびRNA量を測定した。

結果: 不活化前の血漿中の平均DENV価は5.61 log 50% 組織培養感染量($TCID_{50}$)/mL、平均RNA量は10.21 logコピー/mLであった。不活化後の血漿では、平均RNA量は9.37 logコピー/mLであったが、不活化した血漿を接種したウイルス培養の結果、細胞へのウイルス感染は確認されなかった。

結論: 本研究では、アモトサレンとUVA照射の組合せにより、血漿中のDENVが不活化された。同不活化処理は、血漿の輸血によるDENV伝播の効果的な予防法である。

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
アモトサレンとUVA照射の組合せにより、血漿中のDENVが不活化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週間献血不適としている。また、国内においてデング熱感染が確認された場合は、感染が確認された地区への訪問歴を確認し、最後に行かれてから4週間献血不適とするとともに、献血前後の発熱等に関する情報収集を強化することとしている。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。

Inactivation of dengue virus in plasma with amotosalen and ultraviolet A illumination

Didier Musso,[1] Vaea Richard,[1] Julien Broult,[2] and Van-Mai Cao-Lormeau[1]

BACKGROUND: Dengue virus (DENV) is the most prevalent arbovirus in tropical and subtropical regions. Transfusion-transmitted DENV infections have already been reported and the risk for blood products to be contaminated by DENV needs to be considered in dengue-endemic areas, especially during outbreaks. Blood product inactivation processes, including amotosalen and ultraviolet A (UVA) illumination, have been developed to reduce transfusion-transmitted infections. In this study we demonstrate the efficiency of using amotosalen and UVA illumination for DENV inactivation in human plasma.

STUDY DESIGN AND METHODS: Plasma units from volunteer blood donors were spiked with DENV. Viral titers and viral RNA loads were measured in plasma before and after amotosalen and UVA photochemical treatment.

RESULTS: The mean DENV titer in plasma before inactivation was 5.61 log 50% tissue culture infectious dose ($TCID_{50}$)/mL and the mean viral RNA load was 10.21 log copies/mL. In inactivated plasma, the mean DENV RNA load was 9.37 log copies/mL, but cell cultures inoculated with inactivated plasma did not result in infected cells and did not produce any replicative virus nor detectable viral RNA.

CONCLUSION: We report here that amotosalen combined with UVA light inactivated DENV in fresh-frozen plasma (5.61 log inactivation of viral titer). This inactivation process is an efficient method to prevent plasma transfusion-transmitted DENV infections.

Dengue fever is caused by four related enveloped single-stranded RNA viruses (dengue virus [DENV]-1, DENV-2, DENV-3 and DENV-4) belonging to the genus *Flavivirus*, family *Flaviviridae*.[1] DENV is the most prevalent arbovirus in tropical and subtropical regions, and this disease is a global public health problem. According to the World Health Organization, 50 to 100 million dengue infections occur annually in more than 100 countries and are responsible for 20,000 deaths.[2] The spectrum of the disease is large, ranging from asymptomatic to mild disease to severe dengue.[3] Natural transmission of DENV occurs through the bite of infected female mosquitoes of the genus *Aedes*.[4] Other modes of transmission have been reported including marrow[5] and renal[6] organ transplantation, intrapartum,[7] nosocomial,[8] mucocutaneous exposure to infected blood,[9] and blood transfusion.[10-12] DENV transfusion-transmitted cases have been reported after the use of blood components including fresh-frozen plasma (FFP), red blood cells (RBCs), and platelets (PLTs).[13] Evidence for transfusion-transmitted dengue requires documentation of the virus in the blood product or from blood recipient and blood donor; in addition, viral isolates identity must be demonstrated. This is rarely possible in most of the endemic areas, and the number of transfusion-transmitted DENV infections is probably underestimated.

ABBREVIATIONS: DENV = dengue virus; IFA(s) = indirect immunofluorescent assay(s); $TCID_{50}$ = 50% tissue culture infectious dose; WNV = West Nile virus.

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Screening of potential donors for blood transfusion usually includes medical questions, serologic screening and/or nucleic acid testing (NAT), and geographic exclusions based on potential exposures. Screening strategy based on medical questions does not detect asymptomatic viremic donors or donors before onset of symptoms.[14] Serologic screening and/or NAT cannot be performed for all pathogens. The option to minimize the risk of transfusion-transmitted DENV by deferral of at-risk blood donors (like persons who recently have traveled in a country where dengue is reported) is impossible in endemic areas because the entire population is at-risk for DENV infection. Because these screening strategies cannot totally guarantee the absence of any transfusion-transmissible agent, additional strategies such as pathogen inactivation have been developed. Pathogen inactivation consists of the addition of a compound to blood to reduce or abolish infectivity of pathogens including viruses, bacteria, and protozoa.[15]

Several inactivation processes have been developed including photochemical treatment of blood products by the use of psoralen combined with ultraviolet A (UVA) light illumination.[16] Amotosalen HCl (S-59), in combination with UVA light illumination, is a photoactive psoralen that has been demonstrated to inactivate a broad range of DNA and RNA-based single- and double-stranded pathogens.[17,18] The process occurs in three steps: 1) amotosalen intercalates into the double-helical structure of DNA or RNA; 2) after illumination with long-wavelength UVA light, the intercalated psoralens undergo a photoaddiction with a pyrimidine base to form a covalent monoadduct; and 3) the psoralen then undergoes another photoaddition with a pyrimidine base on the opposite strand forming a diadduct or interstrand cross-link. Cross-link formations in single-strand genomes of DNA and RNA viruses also occur. The high frequency of interaction between amotosalen and pyrimidine makes replication and transcription impossible and also inhibits DNA repair mechanisms. Amotosalen has no nucleotide sequence specificity so it can inactivate a large number of pathogens including viruses, bacteria, and parasites. Amotosalen is suitable for treatment of plasma and PLT components. Psoralen inactivation cannot be used for pathogen inactivation of RBC components due to poor penetration of UVA light in this dense material.[19]

The amotosalen photochemical treatment has been validated for DENV inactivation in apheresis PLTs suspended in plasma with up to 3.01[20] and 5.3[21] log reduction of DENV-2 as determined by plague assay. To our knowledge, the efficacy of amotosalen and UVA light to inactivate DENV in plasma has never been demonstrated.

To validate the use of amotosalen and UVA light for inactivation of DENV in plasma, we spiked DENV into pooled plasma units and determined viral titers and viral RNA loads before and after inactivation, in accordance with the recommendations for evaluation of pathogen reduction efficacy.[22]

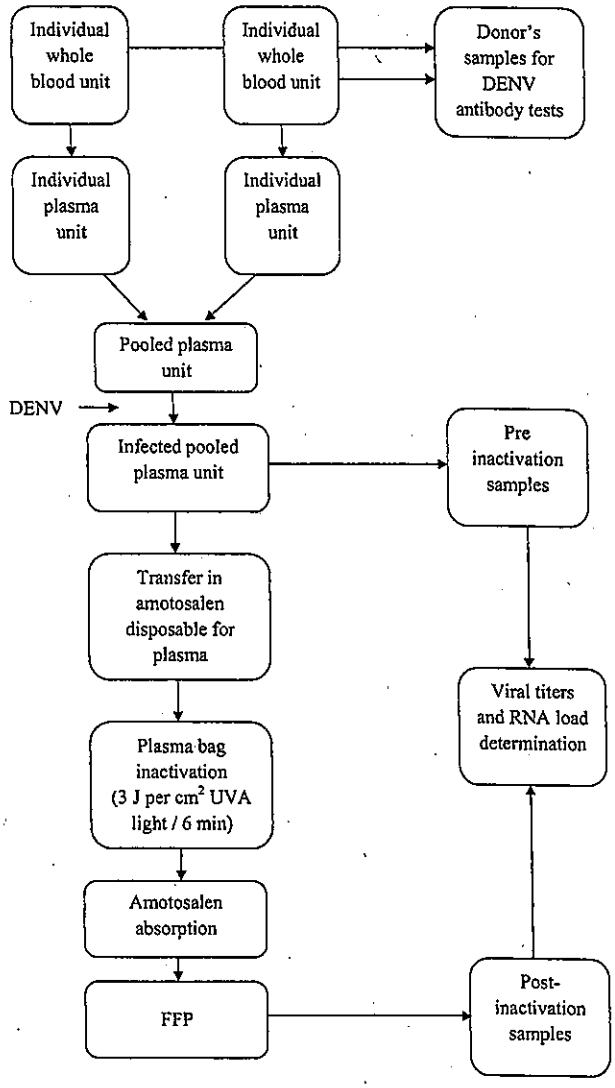

Fig. 1. Schematic flow diagram of the experimental design.

MATERIALS AND METHODS

Blood collection and inactivation of the contaminated plasma units were performed at the blood bank center of French Polynesia (Tahiti). The handling of infectious material (virus culture, infection of plasma bags, viral and RNA load determination) was performed at the "Institut Louis Malardé" (Tahiti, French Polynesia). The schematic flow diagram of the experimental design is illustrated in Fig. 1.

Virus

The DENV-1 strain (PF08/130208-76), initially isolated from the serum of a French Polynesian patient in 2008,

was propagated in larval *Aedes albopictus* C6/36 cells[23] as previously described.[24] The cell culture supernatants were concentrated by ultrafiltration using centrifugal filter devices (Centricon Plus-70, 100 K NMWL, Millipore, Schwalbach am Taunus, Germany). Four viral concentrates (approx. 2 mL each) were recovered and stored at −80°C with heat-inactivated fetal bovine serum (Life Technologies, Grand Island, NY) added at a dilution of 1:5.

Selection of blood donors

All plasma units were obtained from the blood bank center of French Polynesia. Blood donors without risk factors for previous DENV infection were selected during the medical questionnaire before blood donation. According to the French law, samples were collected after signed consent was obtained from the blood donors.

Whole blood and plasma collection

Whole blood samples were collected from venous puncture using whole blood collector devices (LQT6280L, MacoPharma, Tourcoing, France); the volume collected ranged from 450 to 500 mL. Whole blood units were stored at +4°C for 24 hours. During that time, all mandatory blood tests were performed (immunohematologic tests; human immunodeficiency virus [HIV], hepatitis B virus [HBV], hepatitis C virus [HCV], human T-lymphotropic virus, and *Treponema pallidum* detection by serology; HIV, HBV, and HCV detection by NAT) and, to confirm the absence of previous DENV infection, the detection of immunoglobulin (Ig)G antibodies against DENV was performed using IgG kit (DxSelect, EL1500G, Focus Diagnostics, Cypress, CA) and the detection of IgM antibodies against DENV was performed using anti-dengue IgM capture enzyme-linked immunosorbent assay (11EK20, Standard Diagnosis, Gyeonggi-Do, Korea). Any DENV IgG- and/or IgM-positive plasma units were discarded from the study to avoid interfering with the experimental infection of plasma with DENV. The selected plasma units were separated from whole blood according to the blood bank center procedure using a blood component extractor (T-ACE II+, Terumo, Somerset, NJ). Four pools of two plasma units each were prepared (A, B, C, and D).

Addition of DENV-1 into plasma units

Pooled plasma units A, B, C, and D were infected by injection of DENV concentrates in the plasma units with a 0.45-µm needle. After thorough mixing, a sample from each infected plasma unit (preinactivation sample) was collected with a new 0.45-µm needle. Preinactivation samples were stored at −80°C until the determination of viral titers and RNA loads.

Inactivation process

Infected pooled plasma units A, B, C, and D were transferred into Intercept plasma inactivation sets (Intercept disposable INT3102B, Cerus Corporation, Concord, CA) containing 15 mL of 6 mmol/L amotosalen HCl solution using a sterile-connect device. The final volume of the pooled plasma units ranged from 550 to 580 mL, in accordance with the volume recommended by the manufacturer (385 to 650 mL). A, B, and C infected pooled plasma units were exposed to 3 J/cm^2 UVA light for 6 minutes using an illuminator (Intercept, INT100, Cerus Corporation) according to manufacturer's instructions. Infected pooled Plasma Unit D was not inactivated and was the positive control. All samples were then transferred by gravity flow into a container with a compound absorption device to remove the residual amotosalen and the free photoproducts. A sample from each inactivated plasma unit (inactivated sample) and from the positive control (noninactivated sample) were then collected and stored at −80°C until the determination of viral titers and RNA loads.

Detection of replicative DENV and viral titration

For the detection of replicative DENV, all postinactivation and noninactivated samples were inoculated in duplicate on C6/36 cells on 24-well plates at a 1-in-40 dilution. After 30 minutes of incubation, inocula were removed and cells were rinsed twice with maintenance medium. The infected cells were maintained at 30°C for 7 days. Then, five successive passages of 7 days each were done by inoculation of 200 µL of the pure previous passage supernatant onto fresh C6/36 cells to amplify any trace of replicative viral particles. After each passage, indirect immunofluorescent assays (IFAs) were performed to detect DENV-infected cells.

For viral titration, triplicate 10-fold dilutions of all preinactivation and control samples were inoculated on C6/36 cells on 96-well plates. The infected cells were maintained at 30°C for 7 days before revelation by IFA. Infectious wells were counted for each dilution and viral titers were expressed as 50% tissue culture infectious dose ($TCID_{50}$/mL) using the method of Reed and Muench.[25]

IFA

For the 96-well plate titration assays, cells were directly fixed and immunostained on the plates. The cells from 24-well plates were scraped and then deposited on a glass slide before fixation and IFA. Cells were fixed in cold acetone for 10 minutes at room temperature and air-dried. Dengue Type 1 hyperimmune ascitic fluid (National Institute of Allergy and Infectious Diseases, Bethesda, MD) diluted 1:100 in phosphate-buffered saline (PBS) was applied for 30 minutes at 37°C in a humid chamber. Cells

TABLE 1. Detection of replicative DENV and DENV titration in log $TCID_{50}$/mL

Samples	Initial viral titers	Replicative DENV after First passage	Second passage	Third passage	Fourth passage	Fifth passage	Log reduction
Plasma A							
Preinactivation sample	5.74	+*	+	+	+	+	5.74
Inactivated sample	–†	–	–	–	–	–	
Plasma B							
Preinactivation sample	5.62	+	+	+	+	+	5.62
Inactivated sample	–	–	–	–	–	–	
Plasma C							
Preinactivation sample	5.46	+	+	+	+	+	5.46
Inactivated sample	–	–	–	–	–	–	
Plasma D (control)							
Preinactivation sample	6.15	+	+	+	+	+	
Noninactivated sample	6.06	+	+	+	+	+	

* Positive immunofluorescence.
† Negative immunofluorescence.

TABLE 2. DENV RNA quantification in log copies/mL

Samples	Initial RNA loads	RNA loads after First passage	Second passage	Third passage	Fourth passage	Fifth passage
Plasma A						
Preinactivation sample	10.19	10.81	10.64	10.63	10.31	10.16
Inactivated sample	9.39	—*	—	—	—	—
Plasma B						
Preinactivation sample	10.28	10.86	10.58	10.61	10.07	9.11
Inactivated sample	9.23	—	—	—	—	—
Plasma C						
Preinactivation sample	10.16	10.75	10.32	10.28	10.02	9.98
Inactivated sample	9.49	—	—	—	—	—
Plasma D (control)						
Preinactivation sample	10.01	10.74	10.52	10.59	10.16	9.96
Noninactivated sample	10.05	10.89	10.29	9.67	9.51	9.80

* DENV RNA not detected.

were washed twice in PBS and incubated in a solution of fluorescein isothiocyanate–conjugated anti-mouse IgG (Bio-Rad Laboratories, Steenvoorde, France) diluted 1:100 in PBS and Evans blue diluted at 1:10,000. After incubation for 30 minutes at 37°C, cells were washed in PBS and air-dried. Mounting medium (Fluoprep, bioMérieux, Marcy l'Etoile, France) was used for fixing coverslips.

DENV RNA quantification

For all plasma samples (preinactivation, noninactivated, and postinactivation), RNA extraction was performed from 140 µL of each sample using an automatic system for extraction of total nucleic acids from various specimens (NucliSENS miniMAG, bioMérieux), and real-time reverse transcription–polymerase chain reaction (RT-PCR) was performed in a thermocycler (Model CFX96, Bio-Rad) as previously described.[26,27] A standard curve using serial dilutions of a DENV RNA known concentration sample was included within the RT-PCR run to estimate the copy number in plasma samples. Results are expressed in log copies/mL.

RESULTS

Detection of replicative DENV and DENV titration

Viral titers in all samples collected before inactivation ranged from 5.46 to 5.74 log $TCID_{50}$/mL (mean, 5.61 log $TCID_{50}$/mL; Table 1). The culture of A, B, C preinactivation, and D noninactivated samples showed the presence of replicative viruses during the five successive passages. In contrast, no replicative virus was detected in inactivated samples, even after five passages.

DENV RNA quantification

Viral RNA loads in samples collected before the inactivation process ranged from 10.16 to 10.28 log copies/mL (mean, 10.21 log copies/mL; Table 2). Inactivated samples showed DENV RNA loads ranging from 9.23 to 9.49 log copies/mL (mean, 9.37 log copies/mL). From the first passage on C6/36 cells, DENV RNA was undetectable in all supernatants derived from inactivated samples, indicating that no viable virus was present after inactivation.

DISCUSSION

Transfusion-transmitted DENV should be considered in all endemic areas, especially during outbreaks. Members of the AABB reviewed information sources to identify infectious agents identified as being actual or potential causes of transfusion-transmitted diseases, and DENV was classified in the higher-risk category based on scientific and epidemiologic evidence of transfusion transmissibility.[28] The main documented transfusion-transmitted arbovirus is West Nile virus (WNV) with 33 confirmed cases in 2002 in the United States through transfused RBCs, PLTs, and FFP;[29] as a consequence WNV NAT was implemented in the United States in 2003.[30] Since then, approximately 3500 WNV-infected units have been removed from the blood supply in the United States and only 12 cases of transfusion-associated transmission of WNV have been identified.[31] It has been postulated that DENV could represent a greater blood safety threat worldwide than WNV, but it has been neglected because dengue occurs principally in developing countries.[32]

The true incidence of transfusion-transmitted dengue is probably underestimated for several reasons. Most dengue infections are asymptomatic or result in a mild febrile disease and posttransfusion dengue may not be reported as transfusion acquired or possibly misdiagnosed as another adverse transfusion affect. In most of the endemic countries specific dengue diagnosis tests are not available and case confirmation is not possible; in addition, those countries often lack hemovigilance tools to accurately investigate all potential posttransfusion dengue cases. There is no licensed test for dengue screening by NAT. In endemic areas it is difficult to prove transfusion transmission versus vector-borne transmission if culture or molecular testing of samples from the transfused blood products is not possible.

Duration of viremia in DENV infections correspond approximately to the duration of fever, typically 1 week. In addition, patients may be viremic from Day 2 before onset of symptoms according to studies conducted on human volunteers in the 1920s.[33] Blood donors are adults and the percentage of asymptomatic dengue in adults has been reported to be up to 76%.[34] The main risk is to collect blood donations from asymptomatic viremic patients. Retrospective studies performed to detect DENV RNA in samples from blood donors in endemic areas detected positive samples: 0.07% in blood donors during a dengue outbreak in Puerto Rico in 2005 (DENV-2 and DENV-3, viral RNA load ranging from 2×10^3 to 8.12×10^7 copies/mL);[35] 0.19% in Puerto Rico in 2007 (DENV-1, DENV-2, and DENV-3; viral RNA loads ranging from 1×10^5 to 1.12×10^9 copies/mL); 0.3% in Honduras in 2004 and 2005 (DENV-1, DENV-2, and DENV-4; viral RNA load ranging from 3×10^4 to 4.2×10^4 copies/mL);[36] 0.06% in Brazil in 2003 (DENV-1 and DENV-3; viral load ranging from 12 to 294 copies/mL); 0.4% in Brazil in 2010 (DENV Serotype 3);[37] and 1.5% in Madeira/Portugal in 2012 (DENV Serotype 1).[38] All DENV serotypes have been detected in blood donors. In Puerto Rico, a patient developed dengue hemorrhagic fever after transfusion of a blood unit containing 1.35×10^6 copies of DENV/mL; the donor did not report any dengue-related symptoms before, during, and after blood donation.

For arboviruses, transfusion risk models have been developed for WNV, DENV, and chikungunya virus.[39] For DENV, an average transfusion risk of 1.6 to 6 per 10,000 has been determined in Singapore in 2005.

Routine NAT could be implemented to identify viremic DENV blood donors, as was done in Madeira during the 2012 dengue outbreak. In Tahiti, we have implemented NAT for DENV as routine practice during outbreaks, but it is expensive and time-consuming. The cost and required equipment limit its use, especially in developing countries. In DENV-endemic areas, cost for pathogen inactivation versus NAT should be evaluated, especially in the area of cocirculation of arbovirus because, even if expensive, pathogen inactivation covers multiple pathogens compared to NAT and then is time- and cost-saving. On the other hand, pathogen inactivation by amotosalen and UVA light is suitable for PLT and plasma components but not for cell inactivation, highlighting the importance of NAT.

Theoretically the inactivation process is nonspecific and it can inactivate a wide range of pathogens, but there are some limitations. For example, some nonenveloped viruses with very tight nucleocapsids, such as porcine parvovirus, are resistant to inactivation by amotosalen and UVA light. However, the closely related virus, human erythrovirus B19, was inactivated.[40]

In addition, hepatitis E transmission by transfusion of plasma treated with amotosalen and UVA light inactivation has been reported.[41] For pathogens resistant to pathogen inactivation, the addition of NAT should be considered. Thus, it was important to demonstrate inactivation of DENV in plasma by amotosalen and UVA, even though inactivation of more than 6.8 logs of the closely related *Flaviviridae*, WNV, had already been reported.[42]

In our experiment, we monitored the DENV inactivation by both viral culture and RT-PCR. Immediately after inactivation we detected no replicative viruses. To ensure that we have no replicative forms we performed successive passages on C6/36 cells and we detected no replicative particles, even after five passages.

Immediately after inactivation, we detected a mean DENV RNA load of 9.37 log copies/mL. It was not surprising because NAT detects genomic material regardless of whether or not it is infectious and pathogens inactivated by amotosalen and UVA light can be detected by NAT. It was previously demonstrated that amotosalen and UVA light treatment did not impair PCR detection of alpha and poxviruses significantly when compared to untreated

samples;[43] a similar result was obtained with DENV. After the first passage on C6/36 cells, DENV RNA was no longer detected; it confirmed that there was no possible replication after inactivation treatment because all replicative particles were abolished.

Our study was conducted in accordance with the Food and Drug Administration, which recommends that, ideally, the pathogen inactivation process should be able to reduce the pathogen load by 6 to 10 logs. This level of efficacy was proposed in regard to the viral titers that can be up to 10^8 to 10^{10} copies/mL during the window period of detection of infected donors. In our experimental study, we demonstrated that amotosalen was able to inactivate a mean viral load of 5.61 log $TCID_{50}$/mL and a mean DENV RNA load of 10.21 log copies/mL, which achieves the goal recommended by the FDA.

French Polynesia is a high endemic area for DENV and the country is also at risk for the introduction of other arboviruses circulating in the Pacific. Amotosalen and UVA illumination have already been implemented in routine practice for PLT concentrate preparation during dengue outbreaks; based on our results we will also implement amotosalen inactivation of fresh plasma.

In this study we have demonstrated that DENV was inactivated in plasma below the limit of detection and was able to achieve a 5.61-log reduction of DENV viral titer. It should be kept in mind that amotosalen and UVA illumination are not suitable for RBC inactivation and that the risk of RBC transfusion-transmitted DENV persists.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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医薬品 研究報告 調査報告書

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一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名(企業名)	新鮮凍結血漿-LR「日赤」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)		Josephson CD, Caliendo AM, Easley KA, Knezevic A, Shenvi N, Hinkes MT, Patel RM, Hillyer CD, Roback JD. JAMA Pediatr. 2014 Nov 1;168(11):1054-62. doi: 10.1001/jamapediatrics.2014.1360	米国

研究報告の概要

○極低出生体重(VLBW)児における、輸血及び母乳によるサイトメガロウイルス(CMV)の伝播リスク

重要性: 出生後のCMV感染はVLBW児において重篤な疾患、死亡を来す恐れがある。これらの患者群における出生後CMV感染の主因は母乳と輸血であるが、現在、これらのリスクとCMV感染予防策の有効性については十分に解明されていない。

目的: CMV血清検査陰性かつ白血球除去を行った血液製剤の輸血及び母乳について、それぞれの出生後CMV伝播リスクを推定すること。

方法: 2010年1月～2013年6月、ジョージア州アトランタ市の3カ所の新生児集中治療室(大学病院2カ所と個人病院1カ所)において、前方視的・多施設出生コホート研究を行った。母親のCMV血清学的検査を行い、感染状況を調べた。CMVの感染源を特定するため、輸血した血液製剤と母乳に対してCMV NATを行った。出生から5日以内に、輸血を受けていない539人のVLBW児と、462人の母親を研究に登録した。新生児には出生時に血清と尿のCMV NATを行い先天性感染について調べ、さらに間隔を空けて5回及び出生後90日、退院あるいは死亡時にもCMV NATを実施した。

結果: 母親462人のCMV抗体陽性率は76.2%(n=352)であった。539人のVLBW児の12週間後のCMV感染累計発生率は6.9%(95%CI、4.2%～9.2%)であり、29人の出生後感染児のうち5人が発症するか、死亡した。57.5%(n=310)の新生児に計2061単位の輸血が行われたが、輸血に関連したCMV感染の発生はなかった。出生後CMV感染28例中27例がCMV NAT陽性の母乳を与えられていた。

結論: CMV抗体陰性・白血球除去血液製剤の輸血によるCMV伝播は見られなかった。同輸血製剤を受ける新生児の主な出生後CMV感染源は母乳である。

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
VLBW児におけるCMV感染源(CMV抗体陰性・白血球除去血液製剤の輸血、母乳)について前方視的・多施設出生コホート研究にて調査したところ、同血液製剤の輸血によるCMV伝播は見られず、主な出生後CMV感染源は母乳であることが分かった。	日本赤十字社では、保存前白血球除去した血液製剤のみを供給している。さらに、医療機関の要請に応じてCMV抗体(IgG及びIgM)が陰性であることを確認した血液製剤を供給している。今後もCMV感染に関する新たな知見等について情報の収集に努める。

Original Investigation

Blood Transfusion and Breast Milk Transmission of Cytomegalovirus in Very Low-Birth-Weight Infants
A Prospective Cohort Study

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Supplemental content at jamapediatrics.com

IMPORTANCE Postnatal cytomegalovirus (CMV) infection can cause serious morbidity and mortality in very low-birth-weight (VLBW) infants. The primary sources of postnatal CMV infection in this population are breast milk and blood transfusion. The current risks attributable to these vectors, as well as the efficacy of approaches to prevent CMV transmission, are poorly characterized.

OBJECTIVE To estimate the risk of postnatal CMV transmission from 2 sources: (1) transfusion of CMV-seronegative and leukoreduced blood and (2) maternal breast milk.

DESIGN, SETTING, AND PARTICIPANTS A prospective, multicenter birth-cohort study was conducted from January 2010 to June 2013 at 3 neonatal intensive care units (2 academically affiliated and 1 private) in Atlanta, Georgia. Cytomegalovirus serologic testing of enrolled mothers was performed to determine their status. Cytomegalovirus nucleic acid testing (NAT) of transfused blood components and breast milk was performed to identify sources of CMV transmission. A total of 539 VLBW infants (birth weight, ≤1500 g) who had not received a blood transfusion were enrolled, with their mothers (n = 462), within 5 days of birth. The infants underwent serum and urine CMV NAT at birth to evaluate congenital infection and surveillance CMV NAT at 5 additional intervals between birth and 90 days, discharge, or death.

EXPOSURES Blood transfusion and breast milk feeding.

MAIN OUTCOMES AND MEASURES Cumulative incidence of postnatal CMV infection, detected by serum or urine NAT.

RESULTS The seroprevalence of CMV among the 462 enrolled mothers was 76.2% (n = 352). Among the 539 VLBW infants, the cumulative incidence of postnatal CMV infection at 12 weeks was 6.9% (95% CI, 4.2%-9.2%); 5 of 29 infants (17.2%) with postnatal CMV infection developed symptomatic disease or died. A total of 2061 transfusions were administered among 57.5% (n = 310) of the infants; none of the CMV infections was linked to transfusion, resulting in a CMV infection incidence of 0.0% (95% CI, 0.0%-0.3%) per unit of CMV-seronegative and leukoreduced blood. Twenty-seven of 28 postnatal infections occurred among infants fed CMV-positive breast milk (12-week incidence, 15.3%; 95% CI, 9.3%-20.2%).

CONCLUSIONS AND RELEVANCE Transfusion of CMV-seronegative and leukoreduced blood products effectively prevents transmission of CMV to VLBW infants. Among infants whose care is managed with this transfusion approach, maternal breast milk is the primary source of postnatal CMV infection.

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Transfusion-transmitted cytomegalovirus (TT-CMV) and breast milk-transmitted CMV (BM-CMV) infections can cause serious morbidity and mortality in immunologically immature, very low-birth-weight (VLBW) infants (birth weight, ≤1500 g). Transfusion of CMV-seronegative and/or leukoreduced blood components is a common strategy used to prevent TT-CMV; however, studies[1-3] conducted to validate this approach were small and yielded imprecise estimates of TT-CMV risk. Many of these studies[1-3] did not address factors associated with breakthrough cases of TT-CMV including leukoreduction quality control (linked to white blood cell filter failures and CMV transmission) and donor window period infections (when immunologically based assays may not detect CMV viremia).[4] Additionally, studies of TT-CMV have not systematically evaluated BM-CMV, which may confound identification of the source of the infection. The burden of BM-CMV in VLBW infants has not been well quantified.[5] Other, less common, sources of CMV in this population are genital secretion from CMV-seropositive mothers and community-acquired transmission.[6,7]

We performed a multicenter, prospective, birth-cohort study to quantify the risk of CMV infection from transfusion CMV-seronegative and leukoreduced blood components. We also evaluated CMV transmission from maternal breast milk among infants who were fed breast milk and applied CMV nucleic acid testing (NAT) to transfused blood products and breast milk samples to determine the source of CMV in cases of postnatal transmission.

Methods

Infants born at 3 Atlanta-area hospitals (2 academically affiliated institutions [Emory University Hospital-Midtown and Grady Memorial Hospital] and 1 private hospital [Northside Hospital]) were screened (Figure 1). The institutional review boards of all centers approved the study. The participants did not receive financial compensation. Infants meeting the study criteria and whose parent or guardian gave written informed consent were enrolled and monitored from birth to 90 postnatal days, hospital discharge, or death. Infants transferred to Children's Healthcare of Atlanta hospitals were monitored at that hospital. Race and/or ethnicity, known to be associated with CMV infection, was determined by maternal report from options defined by federally funded study guidelines.[8]

CMV Surveillance

Maternal serum at study entry was tested with a CMV IgG/IgM assay. If the result of the serology test was positive, the sample was retested by an IgM-specific assay. For seronegative mothers, CMV NAT was performed on a maternal blood sample at study entry and conclusion to exclude infection that developed during the study.

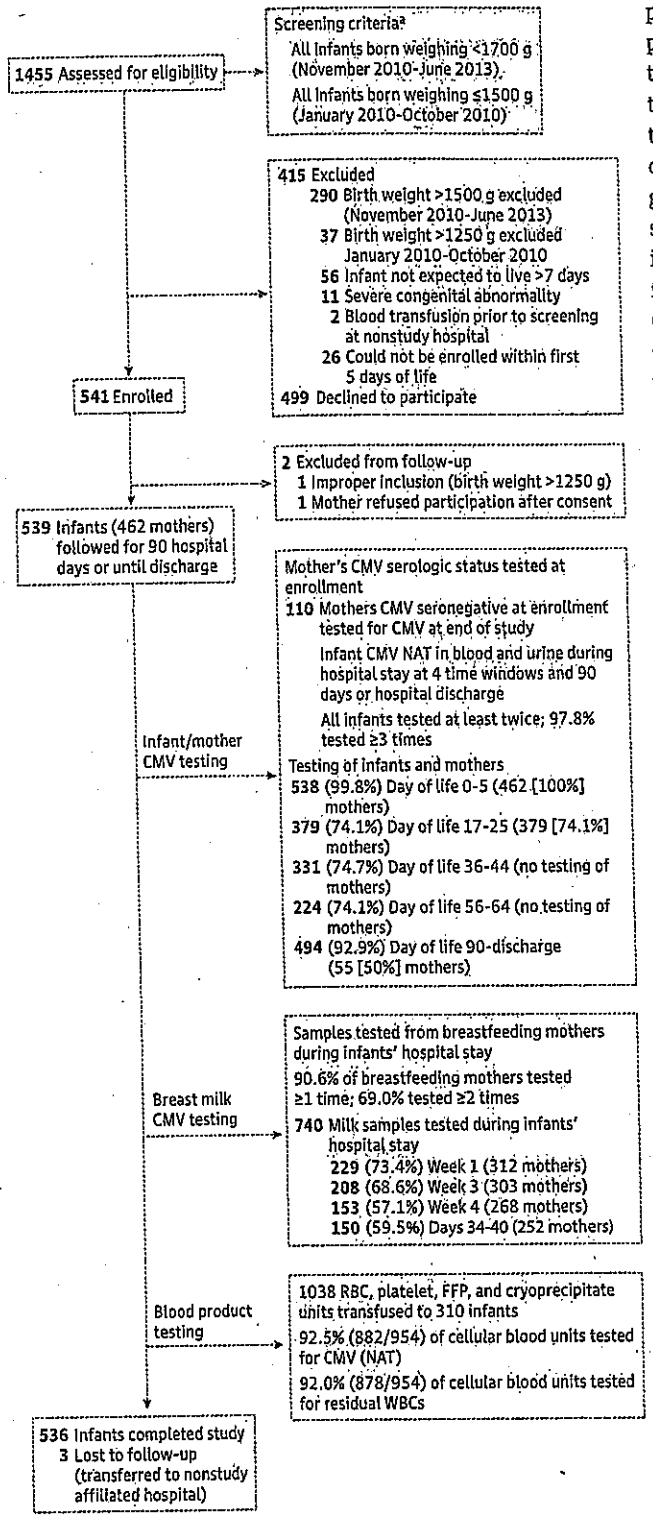
Cytomegalovirus infection was prospectively evaluated in all infants through CMV NAT of residual blood samples and urine. Congenital CMV infection was defined as a positive result of CMV NAT (or positive viral culture obtained from clinician-ordered testing) in blood or urine samples within 2 weeks of life. Postnatally acquired CMV infection was defined as a positive CMV NAT or viral culture result in blood or urine after 2 weeks of life with a previously documented negative result.[5] Transfusion-transmitted CMV was defined as a positive result of CMV NAT performed on blood products that were transfused to the infant combined with a positive result of CMV NAT detected in the infant's blood or urine after transfusion. Blood was tested on the day of birth and at 4 time windows (±4 days) near days of life 21, 40, 60, 90, and at discharge; urine was collected on the day of birth and at discharge if blood was not available. In the event of clinical or laboratory suspicion of CMV infection, results from clinician-initiated CMV testing were included in the study data. At least 2 times per week, screening for CMV-associated disease, defined as pneumonitis, hepatitis, abnormal hematologic indices, or fever in the setting of CMV infection, was performed by study personnel.

All transfused red blood cell and apheresis platelet units were CMV seronegative, leukoreduced before storage, and irradiated (some before and some after storage); residual leukocyte quantitation and CMV NAT were performed on samples from these products (Figure 1). Breast milk samples were obtained from lactating mothers during weeks 1, 3, and 4, as well as days 34 to 40.[9] If postnatal CMV infection was detected, CMV NAT was immediately performed on available milk samples. Otherwise, breast milk was stored and batch tested once an infant reached the study end point. Positive results of CMV tests were reviewed immediately by the study investigators (C.D.J. and A.M.C.) and reported to the patient's treating neonatologist who determined further evaluation and/or treatment.

Laboratory Methods

The presence of IgG/IgM-polyspecific CMV antibodies in maternal blood was determined by a US Food and Drug Administration–approved commercial serology assay (Immucor). Serum samples were tested for CMV IgM by enzyme-linked immunosorbent assay (Bio-Quant). Nucleic acid extraction for CMV NAT was performed using a commercial product (EZ1 Virus Mini Kit, version 2.0; Qiagen, Inc) All assays were performed following the manufacturers' protocols. Nucleic acid testing was performed with a polymerase chain reaction kit (Artus CMV TM, using the Roto-Gene instrument; Qiagen, Inc).[10] The polymerase chain reaction assay was validated on whole blood, urine, and breast milk samples, calibrated to the first World Health Organization international standard.[11] Newly diagnosed CMV in infants with a viral load of greater than 300 IU/mL was verified by repeating the first extraction as well as a new extraction. If there was an insufficient amount of a specimen for a second test, it was diluted 1:2. Any specimen that tested positive with a viral load of less than 300 IU/mL was repeated in duplicate and reported as *low positive* (<300 IU/mL). Specimens discordant on a second test were reported as *indeterminate*. Prior to testing breast milk samples were stored at 4°C for up to 7 days and at −20°C for long-term storage. Blood samples were stored at 4°C and tested within 7 days of collection. To quantify residual white blood cells in leukoreduced cellular blood products, a 100-µL volume of blood was added to 400 µL of propidium iodide/RNase reagent (Leucocount; BD Biosciences) and analyzed by flow cytometry (3Ti).

Figure 1. Study Flow Diagram and Laboratory Testing Schematic



CMV indicates cytomegalovirus; FFP, fresh frozen plasma; NAT, nucleic acid testing; RBC, red blood cell; and WBCs, white blood cells.

[a] The weight inclusion criterion was changed in November 2010 to increase enrollment.

Statistical Analysis

The onset of CMV infection time was estimated as the midpoint between the last negative result of CMV NAT and the first positive NAT result in blood or urine. The incidence of first-time CMV infection and death was estimated by the cumulative incidence function.[12] A competing risk analysis was done to estimate the cause-specific hazard ratio (CSHR) and the subdistribution hazard ratio for CMV and mortality using a Cox regression model. The 95% CIs were calculated using the Wilson score method and were 2-sided except in cases where the incidence was zero. In those cases a 1-sided upper limit confidence boundary is reported.[10] The cumulative incidence of CMV infection at given time points was estimated from the CMV cumulative incidence function. The CIs were estimated using bootstrapping by mother as the clustering unit (1000 bootstrap samples). The competing risk model for the CSHR was implemented with SAS PHReg, version 9.3 (SAS Institute Inc), using robust sandwich covariance estimates to account for within-mother correlation that may occur in outcomes of multiple-birth infants.[13] Additional methods are contained in the eMethods in the Supplement.

Results

Baseline Characteristics

From January 16, 2010, to June 11, 2013, a total of 541 VLBW infants born to 462 mothers were enrolled; 2 were excluded from follow-up (Figure 1). Three hundred fifty-two of the mothers (76.2%) tested positive for the CMV IgG/IgM combination test and, of these women, 11 (3.1%) tested positive for CMV IgM antibody. Infants born to CMV-seropositive or CMV-seronegative mothers did not differ significantly in baseline characteristics except for race and Apgar score (Table 1). The maternal groups did not differ significantly except in receipt of prenatal care and isolated spontaneous labor as an indication for premature delivery.[12] Three hundred seventy-one of the mothers (80.3%) fed breast milk to at least 1 of their infants, and the median duration of breastfeeding was 38 days (interquartile range, 19-56 days).

CMV Infection and Disease

Cytomegalovirus infection was detected in 29 infants (5.4% of the cohort) (Table 2). The cumulative incidence of postnatal CMV infection at 12 weeks was 6.9% (95% CI, 4.2%-9.2%) (Figure 2A). Five of 29 (17.2%) CMV-infected infants developed CMV disease and/or death (Figure 2B). All 29 infants with CMV infection had blood or urine CMV NAT performed within the first 5 days of life. Twenty-seven infants (93.1%) had CMV NAT performed on their blood and 25 infants (86.2%) had CMV NAT performed on their urine. One infant had positive blood and urine results, consistent with congenital infection, and the results of initial CMV NAT were negative in all remaining infants.

The percentage of longitudinal blood and urine samples from 539 VLBW infants with detectable CMV increased from 0.5% at 1 to 3 weeks to 3.2% at 4 to 6 weeks. By 10 to 12 weeks, 9.1% (95% CI, 4.9%-16.8%) of the samples had detectable virus (eFigure 1 and eMethods in the Supplement). Of 29 infants with

Table 1. Baseline Characteristics

Characteristic	No. (%)			
	Overall[a]	CMV Seronegative	CMV Seropositive	*P* Value[b]
Infants, No.	539	127	412	
Gestational age, mean (SD), wk	27.8 (2.6)	28.1 (2.3)	27.7 (2.7)	.14
Birth weight, mean (SD), g	1011 (273)	1032 (264)	1004 (276)	.33
Male sex	263 (48.8)	65 (51.2)	198 (48.1)	.54
Race/ethnicity				
Hispanic	44 (8.2)	6 (4.7)	38 (9.2)	.11
Black	313 (58.1)	47 (37.0)	266 (64.6)	
White	179 (33.2)	69 (54.3)	110 (26.7)	
Asian	23 (4.3)	4 (3.1)	19 (4.6)	<.001
>1 Race/ethnicity	20 (3.7)	6 (4.7)	14 (3.4)	
Other[c]	4 (0.7)	1 (0.8)	3 (0.7)	
Singleton birth	366 (67.9)	89 (70.1)	277 (67.2)	.55
Small for gestational age[d]	91 (16.9)	26 (20.5)	65 (15.8)	.28
Outborn	7 (1.3)	2 (1.6)	5 (1.2)	.75
Apgar score, median (IQR)[e]				
1-min	5 (3-7)	6 (4-8)	5 (2-7)	.005
5-min	8 (7-9)	9 (8-9)	8 (6-9)	<.001
SNAP, median (IQR)	11 (7-14)	12 (5-14)	11 (7-14)	.60
Time in study, median (IQR), d	64 (46-90)	71 (50-90)	63 (46-90)	.17
Mothers, No.	462	110	352	
Age, mean (SD), y	29.4 (6.5)	29.3 (5.8)	29.4 (6.7)	.97
≥1 Prenatal visit	429 (92.9)	108 (98.2)	321 (91.2)	.01
Premature rupture of membranes	173 (37.4)	41 (37.3)	132 (37.5)	.95
Rupture of membranes, >18 h	99 (21.4)	27 (24.5)	72 (20.5)	.20
Chorioamnionitis	68 (14.7)	10 (9.1)	58 (16.5)	.06
Cesarean delivery	349 (75.5)	88 (80.0)	261 (74.1)	.21
Receipt of antenatal corticosteroids	382 (82.7)	92 (83.6)	290 (82.4)	.76
Indications for premature delivery				
Isolated spontaneous labor	138 (29.9)	24 (21.8)	114 (32.4)	.03
Premature rupture of membranes, <37 wk	128 (27.7)	34 (30.9)	94 (26.7)	.39
Pregnancy-associated hypertension	109 (23.6)	29 (26.4)	80 (22.7)	.43
Fetal distress/poor biophysical profile	67 (14.5)	14 (12.7)	53 (15.1)	.54
Bleeding complication	44 (9.5)	10 (9.1)	34 (9.7)	.86

Abbreviations: CMV, cytomegalovirus; IQR, interquartile range; SNAP, Score for Neonatal Acute Physiology.

[a] Data are not included for 2 infant/mother pairs who were excluded from follow-up.

[b] Groups were compared with a 2-sample *t* test for continuous variables and a χ^2 test for categorical variables.

[c] Other race/ethnicity category included American Indian and Alaska Native, Native Hawaiian or other Pacific Islander, or other unidentified race/ethnicity.

[d] Based on previously reported[12] weight percentiles for small for gestational age.

[e] The 1-minute Apgar score was missing for 4 infants; the 5-minute Apgar score was missing for 2 infants.

CMV infection, virus was detected in blood samples of 26 infants and in urine samples of 16 infants (eFigure 1 in the Supplement). With mixed linear models used to account for multiple tests from each infant, the geometric mean viral load detected in the infants was estimated to be 2887 IU/mL (95% CI, 1462-5703) in blood and 133 783 IU/mL (95% CI, 23 922-748 170) in urine. Of the 27 mothers with infants who developed postnatal CMV infection, only 2 women (7.4%) had a positive CMV IgM test. Furthermore, of the 11 mothers who tested positive for IgM antibody, only 2 (18.2%) had an infant with CMV infection.

Five of 29 infants (17.2%) with CMV infection had abnormal laboratory values at the time of initial detection of CMV (details available in eResults in the Supplement). Among the 24 (82.8%) infants determined to have asymptomatic CMV infection, including 1 infant with a congenital infection, no laboratory abnormalities associated with CMV were detected up to 10 days before diagnosis of CMV infection (details available in eResults in the Supplement). Furthermore, no clinical suspicion of disease occurred for these 24 infants, and no further investigation or antiviral treatment was pursued. However, 5 CMV-infected infants developed disease or died. Infants with CMV disease or associated mortality had viral loads similar to those of infants with asymptomatic CMV infection. One infant died of pneumonia following the development of necrotizing enterocolitis (NEC). This infant had a maximum CMV viral load of 13 000 IU/mL. Two other infants died of NEC with viral loads at death of 8000 and 4000 IU/mL. The 2 surviving infants who developed CMV disease, one with punctate densities in the basal ganglia consistent with early signs of CMV infection and the other with a sepsis-like syndrome, were the only infants who received ganciclovir and/or valganciclovir treatment. Both patients had clinical improvement with treatment. All infants with CMV disease or associated mortality received only frozen/thawed breast milk and had negative initial CMV testing in the first 2 weeks of life.

Table 2. Study Outcomes

Characteristic	Incidence, % (No./Total No.) [95% CI][a]
CMV infection	5.4 (29/539) [3.8-7.6]
Cumulative incidence function, wk	
4	2.0 [0.8-3.3]
8	6.0 [3.7-8.2]
12	6.9 [4.2-9.2]
CMV infection in infants born to CMV-seronegative mothers	0 (0/127) [0.0-2.1]
CMV infection in infants born to CMV-seropositive mothers	7.0 (29/412) [4.9-9.9]
Cumulative incidence function, wk	
4	2.6 [1.0-4.3]
8	7.9 [4.9-10.5]
12	9.1 [5.6-12.3]
CMV disease	0.4 (2/539) [0.1-1.3]
CMV infection-related mortality	0.6 (3/539) [0.2-1.6]
All-cause mortality	5.6 (30/539) [3.9-7.8]
Source of CMV infection	
Breast milk[b]	12.2 (27/221) [8.5-17.1]
Cumulative incidence function, wk	
4	3.8 [1.4-4.5]
8	13.2 [8.4-17.6]
12	15.3 [9.3-20.2]
Transfusion[c]	0.0 (0/310) [0.0-0.9]
Vertical	0.2 (1/539) [0.0-1.0]
Unknown	0.2 (1/539) [0.0-1.0]
Breast milk	
CMV DNA lactia in CMV-seropositive mothers[d]	74.1 (189/255) [69.7-80.3]
CMV DNA lactia in CMV-seronegative mothers[e]	0.0 (0/81) [0.0-4.5]
Blood products	
TT-CMV rate from CMV-seronegative, leukoreduced, cellular blood components[f]	0.0 (0/880) [0.0-0.3]
Leukoreduction failure rate[g]	0.1 (1/878) [0.02-0.6]

Abbreviations: BM, breast milk transmitted; CMV, cytomegalovirus; TT, transfusion-transmitted.

[a] Incidence is reported as the relative frequency.

[b] BM-CMV was reported in 221 infants who received breast milk from 189 mothers (32 infants from multiple births) whose milk contained CMV according to nucleic acid testing.

[c] TT-CMV reported in infants who received transfusions.

[d] A total of 282 of 352 CMV-seropositive mothers fed breast milk to their infants (80.1%). Breast milk samples were obtained for testing from 255 feeding mothers (90.4%).

[e] A total of 89 of 110 CMV-seronegative mothers fed breast milk to their infants (80.9%). Breast milk samples were obtained for testing from 81 feeding mothers (91.0%).

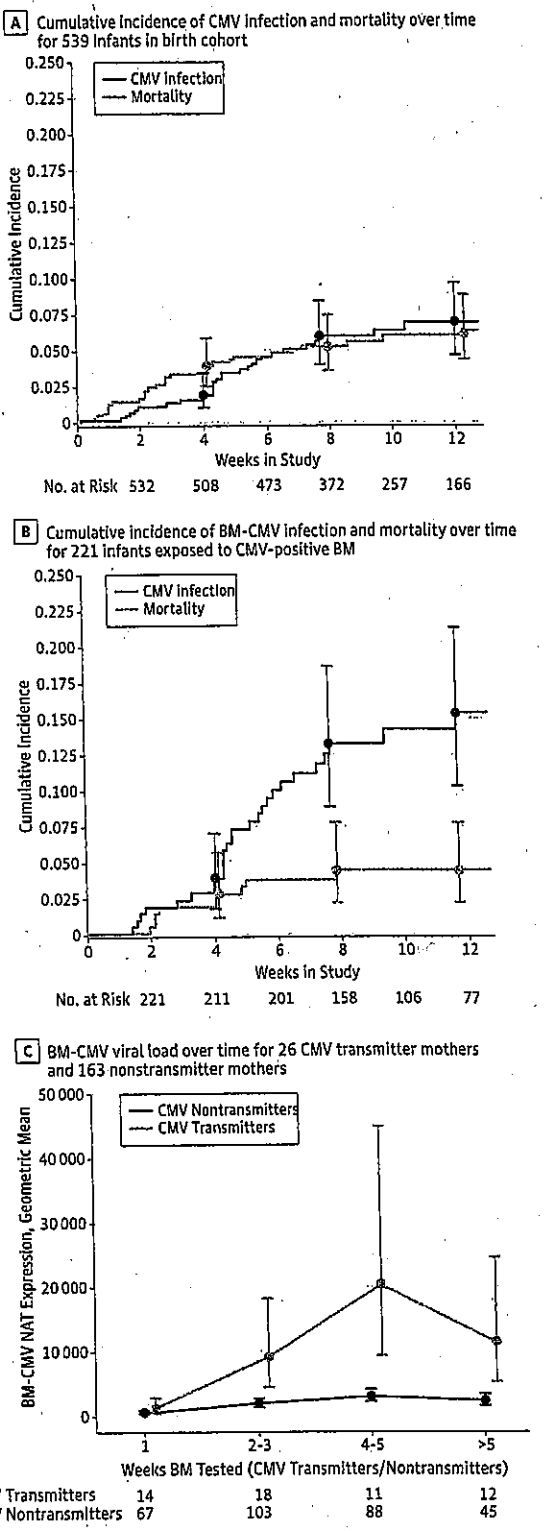
[f] A total of 882 of 954 (92.5%) cellular blood components were tested with CMV nucleic acid testing; the results were negative in 880 U and indeterminate in 2 U.

[g] A total of 878 of 954 (92.0%) cellular blood components were tested for residual white blood cells.

CMV and Blood Transfusions

A total of 310 infants (57.5%) received 1 or more transfusions. A total of 2061 transfusions were administered from 1038 cellular blood components during the study (1545 red blood cell

Figure 2. Cytomegalovirus (CMV) Incidence and Viral Load



A and B, The cumulative incidence of CMV infection and mortality during the study period, including 95% CIs at weeks 4, 8, and 12. C, Log_{10} CMV viral load values were analyzed with a repeated-measures model; mean estimates and their 95% CIs were back transformed to the original scale and are reported as the geometric mean with 95% CIs. BM indicates breast milk; NAT, nucleic acid testing; and limit lines, 95% CI.

Table 3. Risk Factors for CMV Infection and Mortality Using Univariable Cox Regression Model

Characteristic	No. of Infants	CMV		Mortality	
		CSHR (95% CI)[a]	P Value	CSHR (95% CI)[a]	P Value
CMV risk factor					
Birth weight, per 100-g increase	539	0.97 (0.85-1.11)	.62	0.58 (0.48-0.69)	<.001
Leukopenia at birth (WBC count <5000/μL)	539	1.54 (0.62-3.84)	.35	1.34 (0.50-3.53)	.56
Late-onset sepsis, >7 d[b]	539	0.58 (0.15-2.21)	.42	2.57 (0.79-8.36)	.12
Breast milk–feeding days, per 7-d increase[b]	539	1.68 (1.21-2.34)	.002	0.64 (0.41-0.99)	.05
Isolated spontaneous labor	539	0.53 (0.22-1.29)	.16	1.28 (0.58-2.80)	.54
Chorioamnionitis, clinical or histologic diagnosis	539	0.90 (0.31-2.62)	.84	1.71 (0.69-4.20)	.25
Premature rupture of membranes	538	3.14 (1.46-6.73)	.003	1.30 (0.61-2.78)	.50
Rupture of membranes, >18 h	539	1.83 (0.86-3.90)	.12	0.81 (0.31-2.12)	.66
Log_{10} CMV NAT expression in breast milk, per 1 log_{10} IU increase[b]	505[c]	2.71 (2.20-3.35)	<.0001	1.17 (0.88-1.56)	.30
Additional mortality risk factors					
Female sex	539	0.50 (0.23-1.07)	.07	1.05 (0.49-2.21)	.91
SNAP, per 1-U increase	539	1.02 (0.95-1.09)	.58	1.15 (1.07-1.23)	.001
Receipt of antenatal corticosteroids	539	0.96 (0.37-2.46)	.93	0.47 (0.21-1.08)	.07

Abbreviations:
CMV, cytomegalovirus;
CSHR, cause-specific hazard ratio;
NAT, nucleic acid testing; SNAP, Score for Neonatal Acute Physiology;
WBC, white blood cell.

SI conversion factor: to convert the WBC count to $\times 10^9$/L, multiply by 0.001.

[a] Competing risks: 29 infants with CMV infection and 30 total deaths; 3 infants with CMV infection died; and 27 deaths were used to estimate the CSHR for mortality.

[b] Time-dependent covariate.

[c] A total of 431 mothers with 28 infected infants (1 CMV source was unknown). Thirty-four infants who received breast milk from 31 mothers whose breast milk was not tested were not included in the analysis.

transfusions from 703 units, 379 platelet transfusions from 251 units, 129 fresh frozen plasma transfusions from 76 units, and 8 cryoprecipitate transfusions from 8 units). The overall TT-CMV incidence for infants was 0.0% (95% CI, 0.0%-0.9%); similarly, the TT-CMV incidence from 880 CMV seronegative and leukoreduced cellular blood components was 0.0% (95% CI, 0.0%-0.3%) (Table 2). One platelet unit had a leukoreduction failure (5.2×10^6 residual leukocytes), for an overall failure incidence of 0.11% (95% CI, 0.02%-0.6%). All blood components showed negative results on CMV NAT. The unit that failed leukoreduction was not associated with CMV transmission.

CMV and Maternal Breast-Milk Feeding

All 28 infants with postnatal CMV infection were fed maternal breast milk from CMV-seropositive mothers. Twenty-seven of these infants (96.4%) received maternal breast milk with positive CMV NAT before BM-CMV from 26 mothers (1 set of twins). The mean (SD) time from the first detection of CMV in maternal breast milk to the first detection of postnatal CMV infection in the infants was 36 (22) days. The source of CMV infection for the 28th infant, born to a CMV-seropositive mother, could not be identified. This infant's CMV infection was detected by NAT on day of life 25, prior to any blood transfusion and after receipt of breast milk with negative CMV NAT (tested in week 1). The 12-week incidence of CMV infection among infants fed CMV-positive breast milk was 15.3% (95% CI, 9.3%-20.2%) (n = 221) (Table 2 and Figure 2B).

Overall, 74.1% (95% CI, 69.7%-80.3%) of CMV-seropositive mothers had CMV DNA lactia in their expressed breast milk, compared with 0% (95% CI, 0.0%-4.5%) of CMV-seronegative mothers (Table 2). Once CMV was initially detected in breast milk, all subsequent breast milk samples contained CMV DNA. Of 189 mothers with CMV-positive breast milk, 26 women (13.8%) were CMV transmitters and 163 (86.2%) were CMV nontransmitters. Mean breast milk CMV viral loads according to NAT were similar for transmitting and nontransmitting mothers at week 1 (1306 vs 664 IU/mL; $P = .13$) but became significantly higher in CMV-transmitting mothers during postpartum weeks 2 to 3 (9129 vs 2033 IU/mL; $P < .001$) and in weeks 4 to 5 (20 421 IU/mL vs 3064 IU/mL; $P < .001$) (Figure 2C).

Most breast milk–fed infants (78.2%) received exclusively frozen/thawed milk. The CMV transmission rate from breast milk for 221 infants fed CMV-positive breast milk did not differ significantly between infants who were fed some fresh breast milk vs those fed exclusively frozen/thawed milk (12-week CMV incidence, 17.6% vs 11.6%; HR, 0.55; 95% CI, 0.19-1.56; $P = .26$).

Risk Factors for CMV Infection

Factors that increased the risk for postnatal CMV infection included a higher number of breast milk–feeding days, higher breast milk CMV viral load, and premature rupture of membranes (PROM) (Table 3). The adjusted hazard of CMV infection increased as the breast milk CMV viral load increased and the hazard was more than 3 times higher for infants born to mothers with PROM prior to delivery compared with infants born to mothers with other indications for preterm delivery (Table 4). PROM was also associated with an increase in the cumulative incidence of CMV infection (subdistribution hazard rate, 3.07; 95% CI, 1.31-7.18; $P = .01$).

Furthermore, PROM was an independent predictor of mother-to-infant CMV transmission among 189 CMV-seropositive mothers, whereas the mode of delivery was not associated with mother-to-infant transmission (eTable and eMethods in the Supplement). In addition, maximum log_{10} CMV expression in breast milk was associated with mother-to-infant CMV transmission among CMV-seropositive mothers, although the accuracy of CMV viral expression in breast milk to identify postnatal CMV infection was poor, as reflected by the receiver operating characteristic curve (eFigure 2 and eMethods in the Supplement).

Table 4. Risk Factors for CMV Infection and Mortality Using Multivariable Cox Regression Model

Risk Factor	CMV		Mortality	
	CSHR (95% CI)[a]	*P* Value (Reliability[b])	CSHR (95% CI)[a]	*P* Value (Reliability[b])
Birth weight, per 100-g increase	NA		0.58 (0.47-0.71)	<.001 (100)
Breast milk-feeding days, per 7-d increase[c]	NA		0.65 (0.43-1.00)	.05 (54)
Premature rupture of membranes	3.21 (1.43-7.23)	.005 (83)	NA	
Log_{10} CMV NAT expression in breast milk, per 1 log_{10} IU increase[c]	1.87 (1.48-2.35)	<.001 (100)	NA	

Abbreviations: CMV, cytomegalovirus; CSHR, cause-specific hazard ratio; NA, not applicable; NAT, nucleic acid testing.

[a] Competing risks: 29 infants with CMV infection and 30 total deaths; 3 infants with CMV infection died; and 27 deaths were used to estimate the CSHR for mortality.

[b] Percentage of times the risk factor appears in 1000 bootstrap models.[14,15] Covariates with reliability <50% were not included in the multivariable model.

[c] Time-dependent covariate.

Discussion

To our knowledge, the present prospective, multicenter, birth-cohort study is the largest reported evaluation of both blood transfusion and breast milk sources of postnatal CMV infection in VLBW infants. Prior to our study, the residual risks of TT-CMV with CMV-seronegative or leukoreduced transfusions were estimated to be 1% to 3%.[2-4,16] Furthermore, the efficacy of combining both approaches had not been rigorously examined.[17] The present results demonstrate that the exclusive use of blood components that are both CMV-seronegative and leukoreduced is effective in preventing TT-CMV. We believe that this approach should be adapted as a standard of care when administering transfusions to VLBW infants until the comparative effectiveness of alternative transfusion strategies to prevent TT-CMV can be evaluated.

Historically, failure to prevent TT-CMV with CMV-seronegative units of blood components was attributed to donors in the window phase of an infection,[14] whereas leukoreduced units were believed to transmit CMV if the leukoreduction filters failed.[15,18] In our study, only 1 unit had a filter failure and no donor-window-phase infections were identified. Thus, recent advances in serologic and leukoreduction methods may account for the effectiveness of the combined approach to prevent TT-CMV.

The American Academy of Pediatrics[19] states that, "the value of routinely feeding [fresh] human milk from [CMV] seropositive mothers to preterm infants outweighs the risks of clinical disease, especially because no long-term neurodevelopmental abnormalities have been reported." Given the benefits of breastfeeding, new strategies to prevent BM-CMV are needed, because freezing and thawing breast milk did not completely prevent transmission in the present study. Alternative approaches may include routine CMV-serologic testing of pregnant women to enable counseling regarding the risk of CMV infection and risk stratification of infants.[20] For feeding breast milk to VLBW infants born to seropositive mothers, pasteurization of breast milk until a corrected gestational age of 34 weeks, as is recommended by the Austrian Society of Pediatrics,[21,22] and routine screening for postnatal CMV infection may be warranted. Given the toxicity of antiviral therapy,[23] further research is needed to determine whether antiviral treatment in infants with asymptomatic CMV infection is beneficial, especially because it is unclear which infants will progress to CMV disease. Although we found an association between CMV DNA levels in breast milk and BM-CMV, we could not identify a viral load cut-off below which BM-CMV did not occur. Thus, any level of CMV DNA in breast milk should be considered potentially infectious until more detailed investigations can be performed. We also found that PROM and the amount of CMV virus in breast milk were independently associated with an increased risk of postnatal CMV infection. The role of PROM in postnatal CMV infection is unclear. Two studies[24,25] have reported that intrauterine CMV infection is not associated with PROM. Furthermore, vaginal delivery was not associated with postnatal infection in our study, making intrapartum acquisition an unlikely source of postnatal CMV infection.

Our study has several limitations. We did not compare the relative risk of TT-CMV between CMV-seronegative and CMV-leukoreduced units and blood components that were leukoreduced from only CMV untested donors. Therefore, we could not determine the relative safety of the latter approach in VLBW infants. Furthermore, we were unable to test all breast milk for enrolled infants since samples were not available or mothers were not breastfeeding during the evaluation period. Also, we were unable to ascertain with certainty whether CMV infection caused NEC or was simply a co-occurrence in the 3 infants with CMV infection who died, although CMV infection is a reported cause of NEC.[26] We were also unable to test genital tract secretions at delivery to identify this potential source of CMV infection owing to the complexity of our enrollment at sites involving numerous obstetrical practices. However, most infants in this study were delivered by cesarean section, and we did not detect an association between mode of delivery and mother-to-infant transmission of CMV infection. Furthermore, the results of all urine and blood CMV NAT of infants, with the exception of 1 infant with congenital CMV, were negative in the first 2 weeks of life. This makes the possibility that we misclassified infants with congenital CMV infection as having postnatal CMV infection unlikely. Finally, we did not perform systematic hearing assessments or long-term neurodevelopmental assessments.

The effect of asymptomatic postnatal CMV infection on long-term neurodevelopmental outcomes is unclear, with some

studies demonstrating an increased risk of adverse neurologic outcomes[27] and others revealing no difference in long-term outcomes[28] or suspected sensorineural hearing loss.[28,29]

Conclusions

Transfusion of CMV-seronegative and leukoreduced blood products effectively prevents transmission of CMV to VLBW infants. Among infants whose care is managed with this transfusion approach, maternal breast milk from CMV seropositive mothers, is the primary source of postnatal CMV infection. The frequency of CMV infection in our cohort raises significant concern regarding the potential burden of CMV infection among VLBW infants and potential sequelae. This concern necessitates large, long-term follow-up studies of neurodevelopmental outcomes in infants with postnatal CMV infection.

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医薬品 研究報告 調査報告書

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				2015. 1. 29	該当なし	

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研究報告の概要

○臍帯血ドナーのヒトヘルペスウイルス7(HHV-7)感染を検出する定量的PCR

目的: 臍帯血(UCB)は顆粒球コロニー刺激因子を用いた末梢血幹細胞や骨髄に代わる、移植片対宿主病のリスクが少ない造血幹細胞の供給源である。また、UCB移植は一般的な持続性ウイルス感染症(エプスタイン・バーウイルス、サイトメガロウイルス、HHV-6A・6Bなどのヘルペスウイルス)の伝播リスクも低いことが示されているが、HHV-7に関してはほとんど分かっていない。これらのウイルスは、移植後の免疫抑制状態の患者において潜伏感染から再活性化し、活動性感染に移行する可能性がある。そのため、ドナーの血漿及びバフィーコート中のHHV-7の存在とウイルス量を評価するためのlight-upon-extension(LUX)リアルタイムPCRが開発された。

方法: イランの病院において、825人のドナーからUCBサンプルを採取し、血漿及びバフィーコートからDNAを抽出し、LUXプライマーを用いた定量的リアルタイムPCRを行った。

結果: UCBドナーの3.64%(30/825)からHHV-7が検出された。HHV-7 DNAは26件(3.2%)のバフィーコートサンプルから検出され(潜伏感染)、血漿サンプルは4件(0.48%)のみがHHV-7 DNA陽性であり(活動性感染)、HHV-7の活動性及び潜伏感染を有するドナーが存在することが示された。HHV-7の平均ウイルス量は、潜伏感染で1.31×10コピー/mL、活動性感染で1.94×10^5コピー/mLであった。

結論: 血漿及びバフィーコートにおけるリアルタイムPCRは、UCBドナーにおける活動性及び潜伏HHV-7感染を検出し、伝播後の同ウイルスの役割を調べる方法として有用である。

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
UCBドナーの血漿及びバフィーコートにおけるHHV-7の存在とウイルス量を調べるリアルタイムPCRが開発され、825人のUCBドナーを調査したところ、3.64%(30/825)からHHV-7が検出された。本方法はUCBドナーのHHV-7感染を検出するために有用であるとの報告である。	今後も引き続き情報の収集に努める。

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Quantitative polymerase chain reaction for detection of human herpesvirus-7 infection in umbilical cord blood donors

E. Abedi, M. Kheirandish, Z. Sharifi, S. Samiee, P. Kokhaei, Z. Pourpak, M.J. Ashraf. Quantitative polymerase chain reaction for detection of human herpesvirus-7 infection in umbilical cord blood donors.
Transpl Infect Dis 2015: **17**: 21–24. All rights reserved

Abstract: *Objective.* Umbilical cord blood (UCB) has been a reasonable alternative to granulocyte colony-stimulating factor-mobilized peripheral blood or bone marrow, as a source of hematopoietic stem cells with a lower risk of graft-versus-host disease. In immunocompromised hosts after transplantation, the risk of viral infection in adults, especially with beta-herpesviruses such as human herpesvirus-7 (HHV-7), may be increased. This virus in immunocompromised patients can be reactivated from latency and converted to an active phase. Therefore, light-upon-extension real-time polymerase chain reaction (PCR) was developed to assess the prevalence and load of HHV-7 in the plasma and buffy coat of donors.
Methods. About 825 UCB samples under standard protocol from donors were collected. Then, DNA from plasma and buffy coat was extracted and quantitative real-time PCR was performed with light-upon-extension primers.
Results. Overall, HHV-7 was detected in 3.64% (30/825) of UCB donors. HHV-7 DNA was detected in 26 (3.2%) buffy coat samples (latent infection), and only 4 (0.48%) of them were positive for HHV-7 DNA in plasma samples (active infection); the mean HHV-7 viral load was 1.31×10^1 copies/mL in latent infection, and 1.94×10^5 copies/mL in active infection.
Conclusions. We suggest that real-time PCR in plasma and buffy coat could be a useful method to detect active and latent HHV-7 infection in UCB donors and determine its role in subsequent transmission events.

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Key words: umbilical cord blood transplantation; human herpesvirus-7; real-time polymerase chain reaction

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Since the first successful umbilical cord blood (UCB) transplantation for a patient with Fanconi anemia, UCB has been recommended as a reasonable alternative to granulocyte colony-stimulating factor-mobilized peripheral blood or bone marrow as a source of hematopoietic stem cells (HSC) (1–6). Although UCB is less available, it is a valuable source for earlier stage of HSC and progenitor cells to restore the hematopoietic system in transplant patients (7, 8). In addition, UCB transplantation is associated with lower rates of graft-versus-host disease, one of the main factors of transplant-related mortality.

Abbreviations: CMV, cytomegalovirus; HHV, human herpesvirus; HSC, hematopoietic stem cells; PCR, polymerase chain reaction; UCB, umbilical cord blood

Beta-herpesviruses are widespread pathogens with high seroprevalence in the adult population (9). UCB transplantation is associated with lower risk of transmission of highly prevalent persisting viral infections, such as Epstein–Barr viruses, cytomegalovirus (CMV), and human herpesvirus (HHV)-6A and -6B (10–12). However, little is known about HHV-7 (13–15). These viruses in immunocompromised patients can be reactivated from their latent state (10). Some authors have assumed a potential rise in virulence of HHV-7 during a simultaneous CMV reactivation, resulting in a greater danger of CMV illness after transplantation (14, 16). HHV-7 infection may impair the differentiation and survival of megakaryocytic cells (15) and also affect survival/differentiation of CD34+ hematopoietic progenitor cells (17).

The aim of this study was to investigate the prevalence of HHV-7 infections by using a light-upon-extension real-time polymerase chain reaction (PCR) (LUX Real-Time PCR) in UCB samples as a source of HSC transplantation.

Material and methods

Clinical specimens

In this study, 825 UCB samples from pregnant women with a mean age of 26.5 (range 17–35) years were included. Informed consent was obtained from all subjects, and UCB samples were gathered in Milad Hospital, Tehran, Iran and Shoshtari Hospital, Shiraz, Iran.

All mothers passed routine lab screening tests for pregnancy. The exclusion criteria were the existence of any of following conditions: (i) systemic diseases (e.g., diabetes mellitus and autoimmune conditions); (ii) infectious diseases (e.g., patients at high risk for human immunodeficiency virus infection or acquired immunodeficiency syndrome; existence of malaria fever in the past 3 years or taking anti-malaria treatment in the past 6 months; or hepatitis C virus antibody or hepatitis B surface-antigen positivity); (iii) any history of malignancy except for skin and cervix; (iv) history of tattooing in the past year; or (v) organ transplantation.

DNA extraction

In the K2EDTA tube, 10-mL samples of blood were collected from UCB, and DNA was extracted from buffy coat and plasma of the UCB samples using the High Pure Viral Nucleic Acid extraction kit (Roche, Mannheim, Germany) according to the manufacturer's instructions.

Preparation of plasmid standard DNA for HHV-7

An HHV-7 DNA fragment of approximately 312 base pair (bp) (nucleotides 138976-139287; GenBank accession No. AF037218) in the U95 gene region was selected and synthesized. Then, to construct a plasmid DNA containing the U95 gene region of HHV-7 as a reference for the quantitation of HHV-7, the amplified 312-bp product was cloned into the pCRII plasmid by using the TA cloning kit (Invitrogen Corp, San Diego, California, USA), according to the manufacturer's instructions and transformed into *Escherichia coli* TG1. To confirm cloning, recombinant plasmid pCRII-HHV-7 was purified and sequenced by using the BigDye Terminator version 3.1 Cycle sequencing kit (Bioneer Corp, Daejeon, South Korea). The concentration of the plasmid DNA containing the U95 gene region of HHV-7 was quantified by using a Nano-drop Spectrometer (Thermo Scientific, Waltham, Massachusetts, USA). Copy number of cloned plasmid was calculated according to a formula in the Qiagen QuantiFast Probe PCR Handbook (www.qiagen.com/resources/downdload.aspx?id).

The sensitivity of this method was determined with serial dilutions ($1 \times 10^1 - 1 \times 10^7$ copies/mL) of the plasmid DNA containing the U95 gene of HHV-7. The detection limit of this assay was 10 copies/mL.

Real-time PCR for HHV-7

Reaction mix consisted of 1X QuantiFast probe PCR Master Mix without Rox, Lux perimers (forward 139002-139023:5′TCCAACCACCAGTTAGCGTTGT3′) (reverse 139096-139078:5′ CGGGCACTGTCATTGTGATAATCC (FAM) G3′) (13); 5 μL extracted DNA was added to 15 μL Master Mix. Thermal profile was optimized for the 7500 Real-time PCR System (Applied Biosystems/Life Technologies, Grand Island, New York, USA) as follows: 1 cycle of denaturation at 95°C for 3 min, followed by 40X (cycle) of amplification at 95°C for 15 sec, and 60°C for 60 sec. Melting curve analysis was done according to the ABI Real-Time PCR default.

Results

Overall, HHV-7 was detected in 3.64% (30/825) of UCB donors. HHV-7 DNA was detected in 26 (3.2%) buffy

coat samples (latent infection), and only 4 (0.48%) of them were positive for HHV-7 DNA in plasma samples (active infection), which indicates the presence of latent and active HHV-7 infections in donors, respectively. In this study, the quantitative assay of HHV-7 viral load showed a mean of 1.31×10^1 copies/mL and 1.94×10^5 copies/mL in latent and active infections, respectively.

Discussion

UCB transplantation is increasingly used in children, because the risk of graft-versus-host disease is lower compared with unrelated bone marrow transplantation. Concern exists that a higher risk of opportunistic infections is transferred with UCB. Therefore, some common infections, such as HHV, are checked serologically.

In immunocompromised hosts and in primary infection, the pathogencity of HHV-7 remains unclear. Interestingly, a potential increase in virulence of both roseoloviruses (HHV-6, HHV-7) in the course of a simultaneous CMV reactivation has been postulated (16). Chapenko et al. (14) showed an association of latent HHV-7 with development of a febrile syndrome post kidney transplantation in 2 patients. This syndrome was independent to polyclonal anti-thymocyte globulin treatment. The risk of reactivation of CMV increased with co-infection of HHV-7 and CMV up to 2.2-fold for HHV-7 and 12-fold for CMV, compared with each of these infections alone (14). Chapenko et al. (14) also demonstrated that HHV-7 should be the co-factor for CMV disease progression, and they believed that dual (CMV and HHV-7) infection is a risk factor for CMV disease. Therefore, they suggested that "Screening diagnosis should include testing for both viral infections in transplant donors as well as in recipients before and after renal transplant" (14).

HHV-7 infection impairs the differentiation and survival of megakaryocytic cells (15). Studies by Mirandola et al. (17) indicate that HHV-7 infection may affect survival/differentiation of $CD34^+$ hematopoietic progenitor cells by inhibiting more progenitor cells and disturbing the maturation of myeloid cells. These findings may be important for groups at high risk of HHV-7 infection, such as UCB transplant patients, developing fetuses, or recipients of solid organ transplant or bone marrow transplant.

In 2005, Weinberg et al. (18) studied 362 mononuclear cells and sera from UCB. The incidence of HHV-7 in these samples was zero, which means that none of the samples were positive for HHV-7 DNA. However, among 825 UCB donor samples in our study, 26 (3.2%) were positive for HHV-7 DNA by real-time PCR in buffy coat as a latent infection, and 4 (0.48%) were positive for HHV-7 DNA by real-time PCR in plasma, representing active infection. The prevalence found in this study was different from that in other reports. These differences could be a result of the sample size, PCR sensitivity, or economic status and sanitary situation of the sample donors.

Based on our results, and considering the role of HHV-7 as an opportunistic infection in immunocompromised patients in inhibition of myeloid cell maturation and reactivation of CMV in these patients, we suggest that real-time PCR in plasma and buffy coat could be a useful marker to detect active and latent HHV-7 infection in UCB donors.

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化粧品

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一般的名称	①②抗 HBs 人免疫グロブリン ③④乾燥抗 HBs 人免疫グロブリン ⑤　ポリエチレングリコール処理抗 HBs 人免疫グロブリン	研究報告の公表状況		公表国 中国
販売名 （企業名）	①抗 HBs 人免疫グロブリン筋注 200 単位/1mL「JB」（日本血液製剤機構） ②抗 HBs 人免疫グロブリン筋注 1000 単位/5mL「JB」（日本血液製剤機構） ③ヘブスブリン筋注用 200 単位　（日本血液製剤機構） ④ヘブスブリン筋注用 1000 単位　（日本血液製剤機構） ⑤ヘブスブリン IH 静注 1000 単位（日本血液製剤機構）		Transfusion (Malden) 2015; 55(1): 154-163	

研究報告の概要

背景：ヒトボカウイルス（HBoV）は 2005 年に特定された新しいパルボウイルスである。これは、主に呼吸器感染および腸管感染において検出されており、輸血に関連した血液製剤の大規模な調査は行われていない。

調査デザインと方法：自家製定量的ポリメラーゼ連鎖反応（Q-PCR）が、血漿および血漿由来製剤中の HBoV DNA を検査するために開発された。健常ドナーから採取された血漿サンプル（n=6,096）、241 のプール血漿および 326 の血漿由来製剤が Q-PCR により HBoV DNA のスクリーニングされた。陽性サンプルは nested PCR によって確認され、更に配列分析および系統発生研究のために増幅された。HBoV 構造たん白質に特異的な免疫グロブリン（Ig）G および IgM の抗体陽性率は、ウイルス量に応じてグループ化した 209 のサンプルを酵素結合免疫吸着分析法によって測定した（1 群、HBoV DNA>10(4)コピー/mL；2 群、HBoV DNA>5×10(2)コピー/mL、10(4)コピー/mL 以下；3 群、HBoV DNA 陰性）。

結果：血漿ドナーにおける HBoV のゲノム有病率は、5.01×10(2)から 3.02×10(6)コピー/mL までの範囲で、9.06%であった。HBoV の特異的 IgG および IgM は、それぞれ 1 群で 20.00%と 7.50%、2 群で 20.29%と 2.90%、3 群で 13.00%と 34.0%検出された。系統発生解析は、HBoV 遺伝子型 1 が中国の血漿ドナーの流行遺伝子型であることを証明した。

結論：低値の HBoV DNA が、中国の血漿ドナーおよび血漿由来製剤において高い陽性率で検出された。更なる研究が、HBoV スクリーニングが必要であるかどうかを判断するために必要である。

使用上の注意記載状況・その他参考事項等

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。

報告企業の意見

ヒトボカウイルス（Human bocavirus：HboV）は、パルボウイルス科（Parvoviridae）パルボウイルス亜科（Parvovirinae）ボカウイルス属（Bocavirus）に分類され、ヒトパルボウイルス B19 と同じくヒトに病原性を持つウイルスで、エンベロープを持たない極めて小さな（約 18～24nm）1 本鎖 DNA ウイルスである。
これまで HBoV は、輸血用血液製剤および血漿分画製剤での感染事例の報告は無い。
国内の献血対象者となる 16 歳以上においては、高い抗体陽性率（90%以上）が報告され、逆に HBoV は呼吸器疾患等の患者においても検出されていない。また、欧米においては、血漿分画製剤の製造用プール血漿から HBoV は検出されず、凝固因子製剤からも検出されなかったと報告されている。今回の中国からの情報は、ドナーにおける抗体陽性率、HBoV 陽性率、プール血漿における HBoV 陽性率、製剤における HBoV 陽性率において、これら従来の情報とは大きく異なる報告となっている。現在のところ国内及び欧米における情報から特段の措置は必要でないと判断するが、今後も HBoV について情報監視に注力することとする。

今後の対応

ボカウイルスに関連する情報については、今後も注視することとする。

The genomic and seroprevalence of human bocavirus in healthy Chinese plasma donors and plasma derivatives

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BACKGROUND: Human bocavirus (HBoV) is a novel parvovirus identified in 2005. It has mostly been detected in respiratory and enteric infections and has not been studied large scale in blood products in relation to transfusion.

STUDY DESIGN AND METHODS: An in-house quantitative polymerase chain reaction (Q-PCR) was developed to test HBoV DNA in plasma and plasma derivatives. Plasma samples (n = 6096) collected from healthy donors, 241 plasma pools, and 326 plasma derivatives were screened for HBoV DNA by Q-PCR. Positive samples were confirmed by nested PCR and further amplified for sequence analysis and phylogenetic studies. The prevalence of immunoglobulin (Ig)G and IgM specific to HBoV structural proteins was measured by enzyme-linked immunosorbent assay in 209 samples grouped according to virus load (Group 1, HBoV DNA $>10^4$ copies/mL; Group 2, HBoV DNA $>5 \times 10^2$ copies/mL but below 10^4 copies/mL; Group 3,HBoV DNA negative).

RESULTS: The genomic prevalence of HBoV in the plasma donors was 9.06%, ranging from 5.01×10^2 to 3.02×10^6 copies/mL. HBoV-specific IgG and IgM were detected at 20.00 and 7.50% in Group 1, at 20.29 and 2.90% in Group 2, and at 13.00 and 4.0% in Group 3, respectively. Phylogenetic analyses proved that HBoV Genotype 1 was the prevalent genotype in Chinese plasma donors.

CONCLUSION: Low levels of HBoV DNA were detectable at high prevalence in Chinese plasma donors and plasma derivatives. Further study is needed to determine whether HBoV screening is necessary.

Human bocavirus (HBoV) is a member of the genus *Bocavirus* of the subfamily Parvovirus and was first described in 2005 by Allander and colleagues.[1] Since its discovery, the number of bocavirus genotypes isolated has increased rapidly. Four different species of HBoV have been identified (HBoVs 1, 2, 3, and 4). HBoV1 was first discovered in respiratory samples from pediatric patients with suspected acute respiratory tract infections and is frequently detected in these patients. HBoVs 2 through 4 are mainly detected in stool samples from patients with enteric infections.[1-4] As HBoVs are frequently codetected with other viruses in respiratory or stool samples,[2,5,6] the exact role of HBoVs in disease is still unclear and requires further study.

HBoVs are small, nonenveloped viruses with a linear single-stranded DNA genome approximately 5 kb in length. The HBoV genome consists of three open reading frames: one open reading frame encodes a nonstructural protein, the second encodes at least two capsid proteins (VP1 and VP2), and the third also encodes a nonstructural protein (NP-1).[1] The VP2 gene is nested within VP1. Thus, the sequences of VP1 and VP2 differ only in the

ABBREVIATIONS: B19V = parvovirus B19; HBoV(s) = human bocavirus(-es); MP = maximum parsimony; NJ = neighborhood joint; PARV4 = parvovirus 4; PCC = prothrombin complex concentrate; Q-PCR = quantitative polymerase chain reaction.

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N-terminal extension of VP1, which is deemed to be the VP1 unique region and plays a critical role in virus infectivity. NS1 protein is thought to be important in DNA replication. The function of NP-1 is unknown and it is absent in other parvoviruses.[1,7] Similar to other viruses of the Parvoviridae family, HBoVs are approximately 25 nm in diameter and have icosahedra symmetry with nonenveloped capsids.[8,9]

HBoVs have been found in many countries, suggesting a worldwide spread of the virus.[10] The prevalence of HBoV1 was between 1.5 and 19% in respiratory specimens from children hospitalized with acute respiratory diseases,[10] while the prevalences of HBoV 2, 3, and 4 have been reported as 1 to 26, 0.4 to 5, and 0% to 2% in fecal samples from patients with gastrointestinal illness, respectively.[11,12] HBoVs 1 and 2 have also been detected in the blood of patients with acute respiratory and gastrointestinal diseases, indicating that systemic dissemination of the virus is possible.[5,13] However, there is limited information about the prevalence of HBoV in blood. Recently, Bonvicini and coworkers[14] tested the prevalence of HBoV DNA in serum samples from healthy blood donors, and low virus loads and prevalence of the HBoV genome were indicated.

In this study, we describe the development of a quantitative polymerase chain reaction (Q-PCR) assay to detect and quantify HBoV. Assays were established for the detection of HBoV DNA and were used to screen individual donor plasma samples, manufactured plasma pools, and plasma derivatives to determine whether there is any evidence of HBoV contamination in these materials. Donor plasma samples were also screened for the seroprevalence of HBoV-immunoglobulin (Ig)G and HBoV-IgM antibodies. These data provide information about the genomic and seroepidemiology of HBoV in healthy Chinese plasma donors.

MATERIALS AND METHODS

Samples

Plasma samples screened in this study consisted of 6096 individual plasma samples collected from one Chinese blood product manufacturer between October 2012 and September 2013 (October 2012, 893 samples; November 2012, 90; December 2012, 714; January 2013, 719; February 2013, 711; March 2013, 630; April 2013, 90; May 2013, 630; June 2013, 449; July 2013, 450; August 2013, 360; September 2013, 360), 241 plasma pools, and 326 batches of blood products (including 65 batches of albumin, 197 batches of intravenous immunoglobulin [IVIG], 47 batches of antihemophilic Factor VIII [FVIII], 12 batches of fibrinogen, and five batches of prothrombin complex concentrate [PCC]) that had been collected in our laboratory during 2010 to 2012. All the samples tested negative for routine pathogens according to standards established by the Chinese Ministry of Health (routine pathogens were tested by the blood product manufacturers).[15]

DNA extraction and nucleic acid amplification technology

For individual plasma samples, aliquots of plasma (40 μL) from five plasma samples were pooled and subjected to nucleic acid extraction using a viral DNA isolation kit (QIAamp DNA blood Mini kit [250], Qiagen, Hilden, Germany) according to the manufacturer's instructions. The DNA extracts were stored at –80°C until PCR analysis. Screening of HBoV DNA in plasma was performed with primers located in conserved regions of the HBoV genome on the basis of our in-house–developed real-time PCR assay (Fig. 1A). The forward primer (5′-GTCTCCGG CGAGTGAACAT-3′) and reverse primer (5′-AGCAGGTTG AGAAAAAGCTCTAA-3′) target a conservative 100-bp sequence located in the NS1 gene. Real-time PCR was performed on a sequence detection system platform (ABI Prism 7900, Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. The amplification mixture of 20 μL consisted of 10 μL of 2× FastStart SybrGreen Master Mix (Faststart Universal SYBR Green Master [ROX], Roche, Mannheim, Germany), 5 pmol of each primer, and 5 μL (from a total eluted volume of 50 μL) of DNA extracted from 200 μL of pooled human plasma. Real-time quantitative amplification of HBoV DNA was performed under the following conditions: one cycle of 95°C for 10 minutes; 45 cycles of 95°C for 15 seconds, 60°C for 15 seconds, and 72°C for 30 seconds; and a final cycle of 95°C for 15 seconds, 60°C for 15 seconds, and a gradual increase to 95°C over 30 minutes at a ramp rate of 2%. The quantitative standards used in the real-time PCR assay were dilutions of plasmid pHBoV-1 containing an HBoV Genotype 1 genome (constructed in our laboratory). A quantitative standard curve was used to assign values (copies/mL) to the tested samples. Seven quantitative standards (5×10^6, 5×10^5, 5×10^4, 5×10^3, 5×10^2, 5×10^1, and 5×10^0 copies/mL) were included in each PCR procedure. With fluorescence measured at 2°C below the Tm (77°C), as few as five template copies could be distinguished from background levels. To achieve this sensitivity, it was necessary to measure fluorescence at several degrees below the Tm of the specific product. This avoided fluorescence from primer dimmers, which may be generated at high cycle numbers in samples with very few or no target sequences. If the HBoV DNA level in a reaction with the five pooled samples exceeded five copies, DNA was extracted from each of the five individual plasma samples (200 μL) and quantitated, separately. For plasma pools and plasma derivatives, HBoV DNA was extracted from each sample and measured by real-time PCR.

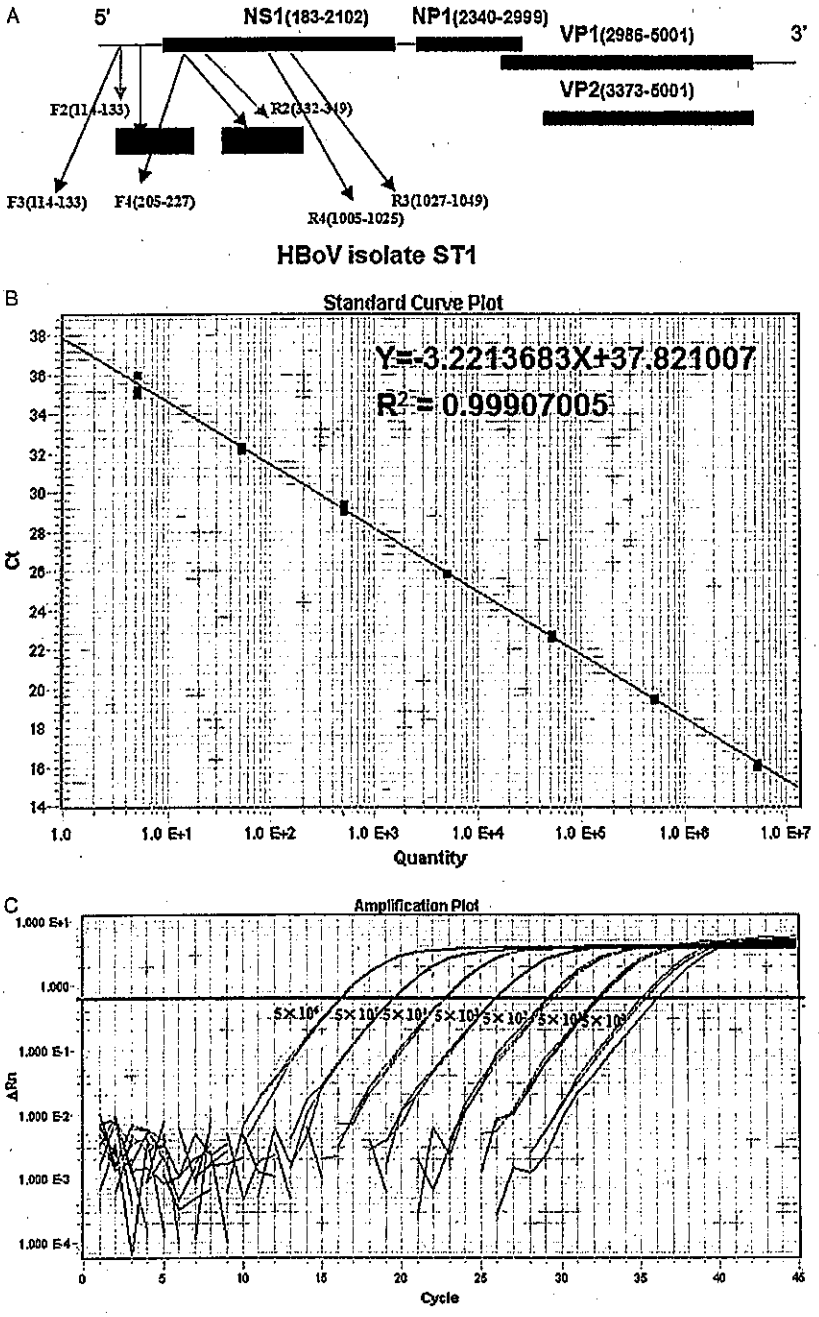

Fig. 1. The location of primers and real-time Q-PCR to determine the prevalence of HBoV DNA. (A) The schematic locations of Q-PCR, nested PCR, and sequencing primers in Bocavirus genome. The primers for real-time PCR—F1, R1, the primers for nested PCR; first round, F2, R2; second round, F1, R1; sequencing primers—first round, F3, R3; second round, F4, R4. All these primers are identical in four genotypes, except F4, R1, R2, R3, and R4 demonstrated 1- to 5-bp differences in Genotypes 3 and 4. Amplification of the conserved NS1 region of HBoV and titration of HBoV DNA by amplification of pHBoV; (B) the standard curve; (C) the amplification curve; (D) the melting curve; (E) agarose gel electrophoresis analysis of the HBoV genome fragment amplified by nested PCR was used to confirm the selected HBoV-positive samples.

Nested PCR

HBoV genome-positive individual samples were confirmed by nested PCR using two pairs of conserved primers located in the NS1 region (first round —F2 5′-TCAGACTGCATCCGGTCTCC-3′, R2 5′-GGATGAGGAGCGCAGTTT-3′; second round—F1 5′-GTCTCCGGCG AGTGAACAT-3′, R1 5′-AGCAGGTTGAGA AAAAGCTCTAA-3′). The location was indicated in Fig. 1A. The amplification was performed with 2× *Taq* PCR Master-Mix (Tiangen) in a total volume of 25 μL, containing 12.5 μL of 2× *Taq* PCR Master Mix, 10 pmol (each) of forward and reverse primers, and 5 μL of nucleic acids (first round) or 2 μL of the first-round PCR products (second round). Reaction conditions were as follows: predenaturation at 95°C for 5 minutes, followed by 35 cycles of 15 seconds of denaturation (95°C), 30 seconds of annealing (56°C), 30 seconds of extension (72°C), followed by a final extension at 72°C for 7 minutes. The cycling conditions of the second-round PCR were the same as the first-round PCR, except the annealing temperature was increased to 60°C. PCR cycling was performed on a thermal cycler (Veriti, Applied Biosystems).

Serologic assays

Testing for HBoV-specific antibodies was performed with a commercial assay kit (HBoV IgG or IgM enzyme-linked immunosorbent assay [ELISA] kit, Jinma, Shanghai, China) according to the manufacturer's recommendations. Plasma samples (n = 40) with HBoV genomic DNA titer higher than 10^4 copies/mL, genome-negative plasma (n = 100) and genome-positive plasma with HBoV genomic DNA titer below 10^4 copies/mL (n = 69) underwent serologic analysis. Testing was performed in duplicate. If results were ambiguous, the assay was repeated and the unambiguous result was taken as the final value for the specimen.

DNA sequencing and phylogenetic analysis

To obtain phylogenetic information, positive samples confirmed by nested

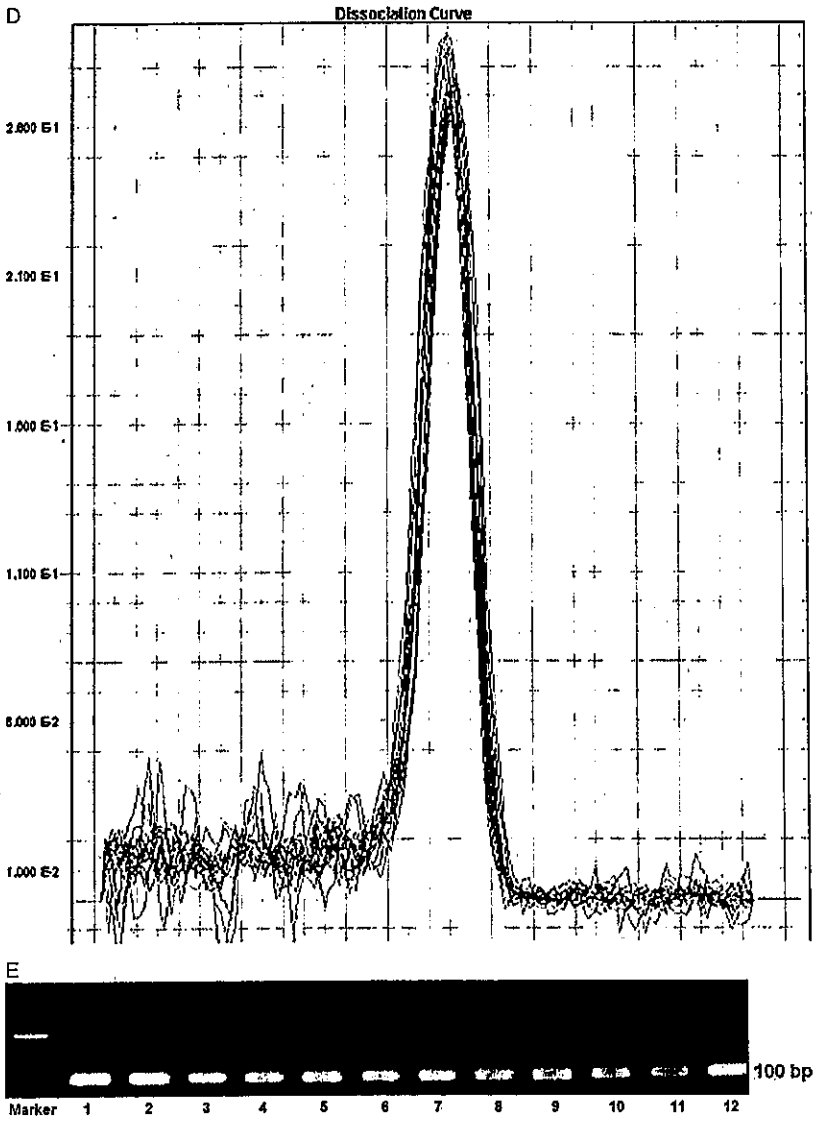

Fig. 1. *Continued*

PCR were amplified using another two pairs of primers (first round—F3 5′-TCAGACTGCATCCGGTCTCC-3′, R3 5′-TACCTCAGGAAGATGTTCCCACG-3′; second round —F4 5′-TTAGAGCTTTTTCTCAACCTGCT-3′, R4 5′- ACCA CGTCCTCCATAGGCCAT-3′) targeting an 820-bp sequence in the NS1 region. The location was indicated in Fig. 1A. PCR was performed in a total volume of 50 µL with 2× *Taq* PCR MasterMix (Tiangen). Amplification conditions were 95°C for 10 minutes; 35 cycles at 94°C for 15 seconds, 62°C for 30 seconds, and 72°C for 30 seconds; and a final extension of 72°C for 7 minutes. The conditions for the second-round PCR were the same as for the first-round PCR, except the annealing temperature was reduced to 60°C and used 2 µL of the first-round PCR product as template. Finally, 5 µL of the second-round PCR products was electrophoresed on 2% agarose gel and analyzed on a molecular imaging system (Image Quant 350, GE Healthcare, San Diego, CA). The positive samples were sent to the company (Invitrogen, AB, Carlsbad, CA) for sequencing, using the second-round primers from the PCR procedure as the sequencing primers. The resulting sequences were analyzed using computer software (ClustalX 1.83, http://www.clustal.org/).[16] Neighborhood joint (NJ) and maximum parsimony (MP) analyses were employed to detect the phylogenetic position of the samples in relation to reference sequences using computer software (MEGA 4, http://www.megasoftware.net/).[17] The GenBank accession numbers of the reference sequences are shown in Fig. 4. Node support was evaluated with 1000 bootstrap replicates.

Statistical analysis

Statistical analyses were conducted using statistics software (SPSS, SPSS Inc., Chicago, IL). The chi-square and Fisher's exact tests were applied to assess associations between categorical variants. A p value of less than 0.05 was used as the cutoff level for significance.

RESULTS

Development of a sensitive and specific PCR method to detect HBoV DNA and HBoV screening strategy

Using a sequence detection system platform (ABI 7500 Fast PCR system, Applied Biosystems), an in-house real-time Q-PCR amplification protocol for HBoV DNA screening was developed. To determine the sensitivity of the Q-PCR assay, 10 individual plasma samples with no detectable anti-HBoV specific IgM or IgG were selected as negative controls. When tested by Q-PCR, the 10 negative samples demonstrated no specific amplification after 45 PCR cycles. The detection limit of the HBoV DNA Q-PCR assay was determined by performing 10-fold serial dilutions of plasmid pHBoV into plasma negative for HBoV genomic DNA. The highest dilution at which all three replicates were positive was taken as the end point. The detection limit was 5 geq of HBoV DNA in each PCR reaction, which is equivalent to 500 copies/mL. For screening, reactive plasma or plasma pool samples

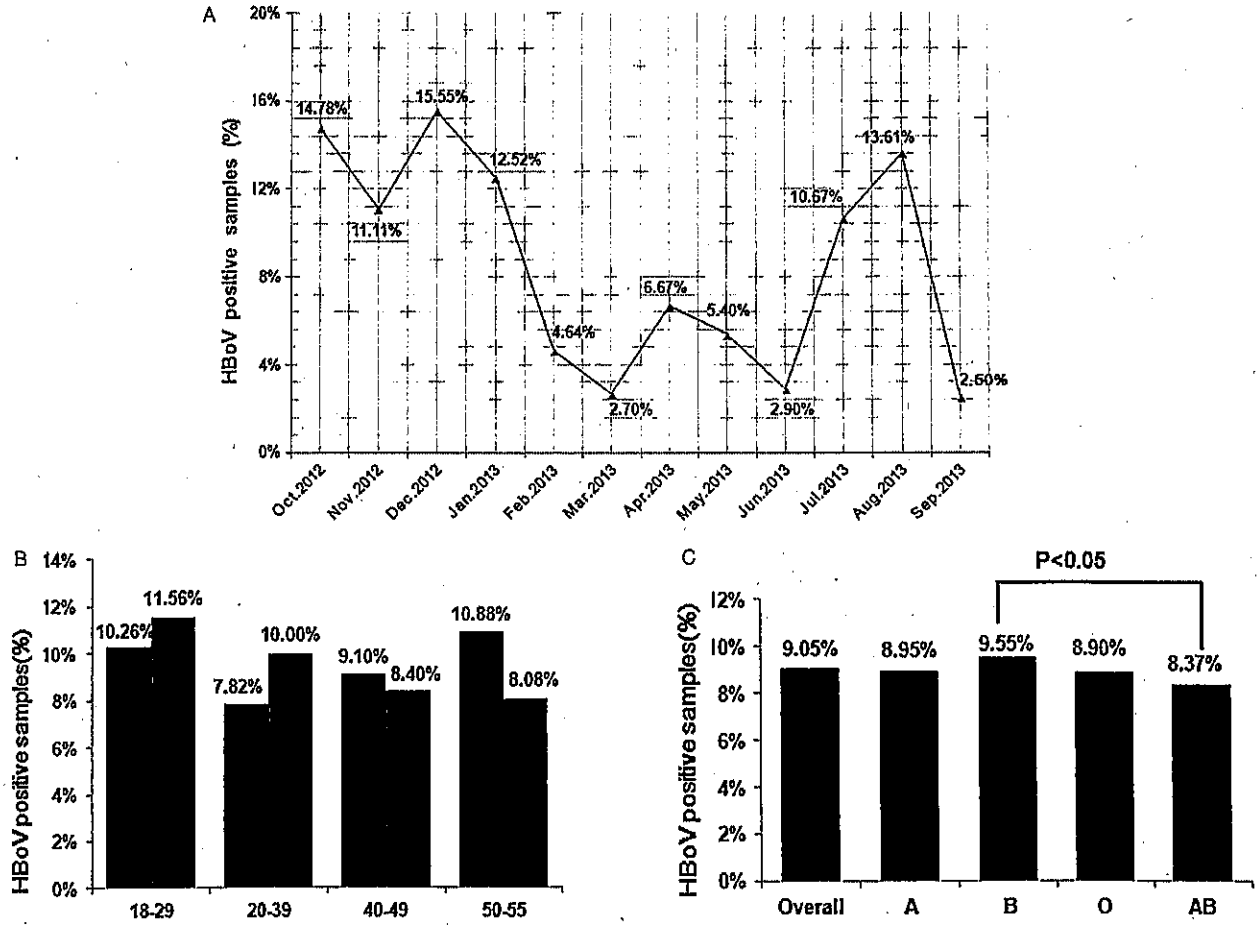

Fig. 2. Prevalence of HBoV DNA in Chinese plasmapheresis donors. (A) Prevalence of HBoV genome DNA from October 2012 to September 2013; (B) HBoV genome DNA prevalence in different age groups (■, male; ■, female); (C) HBoV DNA prevalence in different blood type groups.

were retested in duplicate and confirmed by nested PCR. If a signal of the highest dilution of the standard was detected at more than 40 cycles, the result was interpreted as invalid and the experiment was repeated. As indicated in Fig. 1B, a linear standard curve could routinely be generated in the range from 5 to 5×10^6 copies, and all the dilutions could be efficient amplified and distinguished from background levels (Fig. 1C). A melting curve (Fig. 1D) analysis demonstrated that a single predominant product with a distinct Tm was amplified. The predicted length of the products was confirmed by agarose gel electrophoresis (Fig. 1E) and sequencing (data not shown). Therefore, the Tm was used to identify specific products in subsequent analyses.

Real-time PCR and nested PCR assays were developed and used for both simultaneous screening and individual identification of HBoV genomes. Initial screening was performed by a minipool Q-PCR assay as described under Materials and Methods. Positive samples were then confirmed and quantitated by the single-virus real-time Q-PCR assay, with the positive samples further confirmed by nested PCR.

HBoV DNA and antibodies in plasmapheresis donors

Real-time PCR assays were performed to detect and quantify the HBoV genome copy number in individual plasma donors according to the protocol described under Materials and Methods. Of the 6096 individual plasma samples, 552 (9.06%, with a 95% confidence interval [CI] of 8.34% to 9.78%) were confirmed as HBoV genomic DNA positive. The genomic prevalence and trend over time of HBoV DNA in individual plasma from October 2012 to September 2013 is shown in Fig. 2A. The DNA levels ranged from 5.01×10^2 to 3.02×10^6 copies/mL, and 40 samples had a virus load greater than 10^4 copies/mL. The genome prevalence in plasma samples was also analyzed according to

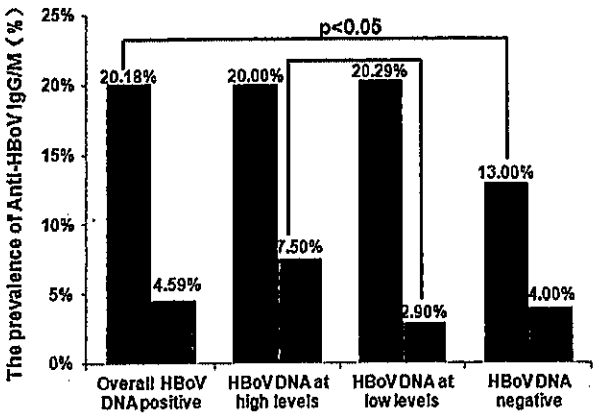

Fig. 3. Seroprevalence of the HBoV antibody in Chinese plasmapheresis donors. High-level and low-level HBoV DNA were defined as a level higher than 1×10^4 copies/mL and a level lower than 1×10^4 copies/mL, respectively. (■) IgG; (■) IgM.

age (Group 1, 18-29 years; Group 2, 30-39 years; Group 3, 40-49 years; Group 4, 50-55 years), sex, and blood type (A, B, O, AB). As indicated in Fig. 2B, there was no significant difference in HBoV prevalence between males (9.33%; 208/2229) and females (8.90%; 344/3866). The prevalence also did not differ according to age, which suggests HBoV persistence with no age bias in the plasma donor population. A significantly higher prevalence was observed in blood group B donors than in blood group AB (Fig. 2C), which suggests B blood group individuals are more susceptible to HBoV compared to individuals with blood group AB.

Considering a high load of HBoV may pose a threat to blood safety, seroprevalence of HBoV antibodies including IgM and IgG was investigated. The HBoV DNA–positive samples were divided into two groups, high or low, depending on whether the DNA concentration was higher or lower than 1×10^4 copies/mL. As indicated in Fig. 3, the seroprevalence of IgG was significantly higher in the HBoV DNA–positive samples than in the genome DNA–negative samples. The seroprevalence of IgM in the low-DNA-level group was significantly lower than that in the DNA high level group and was not significantly different from the HBoV DNA–negative group.

HBoV DNA in source plasma pool and plasma derivatives

To investigate HBoV DNA contamination in source plasma and plasma derivatives, we screened 241 plasma pools and 326 batches of blood products by individual Q-PCR. HBoV DNA was detected in 17.84% (43/241) of plasma pools, ranging from 5.90×10^2 to 8.70×10^4 copies/mL. Table 1 summarizes the HBoV DNA content in plasma products. Overall, 44 of 326 (13.50%) of these products were contaminated with HBoV DNA, ranging from 5.38×10^2 to 8.70×10^4 copies/mL. FVIII was highly contaminated, with 55.32% being recorded as positive with a relatively higher viral load. The rate of contamination of fibrinogen was lower (16.67%) and with a relatively lower viral load. The contamination rates of albumin and IVIG were below 10%, with a relative low genome content. No HBoV genome DNA was found in the five batches of PCC.

Phylogenetic relationships among different HBoV isolates

The positive samples confirmed by nested PCR were subjected to another nested PCR to amplify an 820-bp fragment. The samples that were successfully amplified were then sequenced. With deletion of ambiguous positions at both the beginning and the end of the gene segments, a total of 57 sequences with a final 753-bp DNA fragment (including one that was only 628 bp and one that was 698 bp) were provided by this study. The 57 sequences were then aligned with 25 reference sequences including four HBoV genotypes (all the reference sequences were downloaded from GenBank) using ClustalX 1.83. The alignment result was used to perform NJ and MP analyses, both of which provided identical and well-supported tree topologies. Only the NJ tree is shown (Fig. 4), with the MP and NJ bootstrap values (or NJ bootstrap values only) depicted on the branches. Four genotypes of HBoV were strongly supported by four main branches, with bootstrap values greater than 90%. As shown in the NJ tree, all the samples studied in our report strongly clustered in a monophyletic of HBoV Genotype 1 (bootstrap values = 100/99, Fig. 4).

DISCUSSION

Virus infections not only impose an enormous disease burden on humanity, but also cause potential threats to blood safety. Due to a multifocal approach to the collection, processing, and release of blood and blood components over the past decades, the incidence of clinically significant transfusion-transmitted diseases has markedly decreased. Regulatory oversight has been strengthened in the United States and hemovigilance systems have been established in many countries of the European Union, Canada, and Japan to identify new and emerging infectious and noninfectious transfusion risks. HBoV was first identified by Allander and coworkers[1] in 2005 and was proposed as a potential respiratory pathogen. Since its discovery, another three types of HBoVs have been identified in fecal specimens from children with acute gastroenteritis and acute flaccid paralysis[2-4] in a variety of countries, indicating a global circulation of the virus. Patients with HBoV infection present with a variety of signs and symptoms, including rhinitis, pharyngitis,

TABLE 1. Genomic DNA contamination rate and viral load of HBoV DNA detected in various blood derivatives

Sample types	Number of samples	HBoV DNA–positive number	Positive rate (%)	Viral load range (copies/mL)
Albumin	65	2	3.08	5.38×10^2, 5.58×10^2
IVIG	197	14	7.11	6.23×10^2-8.95×10^3
FVIII	47	26	55.32	6.82×10^2-1.09×10^4
Fibrinogen	12	2	16.67	8.16×10^2-1.67×10^3
PCC	5	0	0	0
Total	326	44	13.50	5.38×10^2-1.09×10^4

cough, dyspnea, wheezing, pneumonia, acute otitis media, fever, nausea, vomiting, and diarrhea.[18] However, there is still no clear evidence that the other three species are pathogenic except HBoV1. Evidence that HBoV1 can replicate autonomously in polarized primary human airway epithelia persistently and productively[19] alerts us to the potentially high pathogenicity of this new parvovirus. HBoV is frequently detected in nasopharyngeal swab and fecal samples. However, HBoV DNA is not restricted to the respiratory or digestive tracts; it has also been identified in acute- and convalescent-phase serum from children with acute respiratory tract infections, with viral loads in some cases higher than 10^4 copies/mL.[5,6,13,20-23] Another study detected HBoV DNA in healthy blood donors,[14] albeit at low levels, suggesting the possibility that the virus could be transmitted by blood transfusion and could pose a threat to blood safety.

Before HBoV was discovered, parvovirus B19 (B19V) and parvovirus 4 (PARV4) were the only two parvoviruses known to infect humans. Because of its small size (20-25 nm diameter) and nonenveloped nature, B19V is difficult to remove by filtration methods and is very resistant to many of the virus inactivation procedures used in the production of plasma derivatives, including solvent/detergent (S/D) and heat treatment. The transmission of B19V through the administration of S/D-treated[24] and certain dry heat–treated blood products has already been documented.[25-27] B19V can also be transmitted by blood components, at least by those with high concentrations of the virus.[28-30] As a result, the Food and Drug Administration has proposed a limit of 10^4 geq/mL B19V for manufacturing pools destined for all plasma derivatives and the European Pharmacopoeia has imposed a limit of 10^4 IU/mL for B19V in RhIG and pooled virus-inactivated plasma to reduce the potential risk of transmission.[31,32] PARV4 is a second pathogen of parvovirus family. Previous studies demonstrated it as a transfusion-transmissible agent that is resistant to viral inactivation and removal methods. PARV4 may still be regularly transmitted by plasma-derived blood products.[33,34] As a result, HBoV is similar to B19 and PARV4, with a small size (approx. 25 nm diameter) and noenveloped; therefore, its genome may also be resistant to removal by various virus inactivation and removal procedures, leading to concern about transmission by transfusion. However, epidemiologic information about HBoV in blood and blood products is currently sparse, including in China. Therefore, we tested the genome and seroprevalence of HBoV in healthy Chinese plasma donors, including individual plasma samples, plasma pools, and different kinds of plasma derivatives, to evaluate the risk posed by HBoV.

Past research on HBoV has focused more on its epidemiology in respiratory specimens and fecal samples than in serum, plasma, or blood. The proportion of respiratory specimens from symptomatic hospitalized children that contained HBoV sequences ranged from 1.5% to 19%, while a very low virus prevalence was reported in the respiratory tract of adults with symptomatic respiratory disease.[10] There has been little research on HBoV in blood and plasma donors. Recently, Italian healthy blood donors were screened for HBoV, with a prevalence of 5.51% and a load ranging from 1.79×10^2 to 3.33×10^3 copies/mL.[14] Our study indicated the genomic prevalence of HBoV DNA in healthy Chinese plasma donors was 9.06% (552/6096), with HBoV DNA titers ranging from 5.01×10^2 to 3.02×10^6 copies/mL, which were higher than those of Italian blood donors both in the prevalence and in the viral contents. Among the 552 nucleic acid testing–positive samples, 7.25% (40/552) had DNA levels of more than 1×10^4 copies/mL, including 0.54% (3/552) with more than 10^5 copies/mL and 0.18% (1/552) with more than 10^6 copies/mL. The highest HBoV DNA titer detected was 3.02×10^6 copies/mL, which was verified by DNA reextraction and requantification. We also kept track of the donor with this highest plasma viral load and were able to obtain two further plasma samples at intervals of 2 weeks and 1 month after the first plasma sample. Unexpectedly, these two plasma samples were both HBoV genome negative, which was verified by DNA reextraction and requantification and which suggests clearance by the immune system. As indicated in Fig. 2A, the HBoV DNA prevalence peaked in winter, which may reflect a temporal difference in HBoV infection. Since only 1 year was monitored in this study, whether it was representative demands further study.

Previous studies investigated the prevalence of HBoV in blood products or source plasma pools. In 2007, Fryer and coworkers[20] tested 351 manufacturing plasma pool samples for various parvoviruses, including HBoV, and in 2011 Modrow and coworkers[35] screened nonenveloped

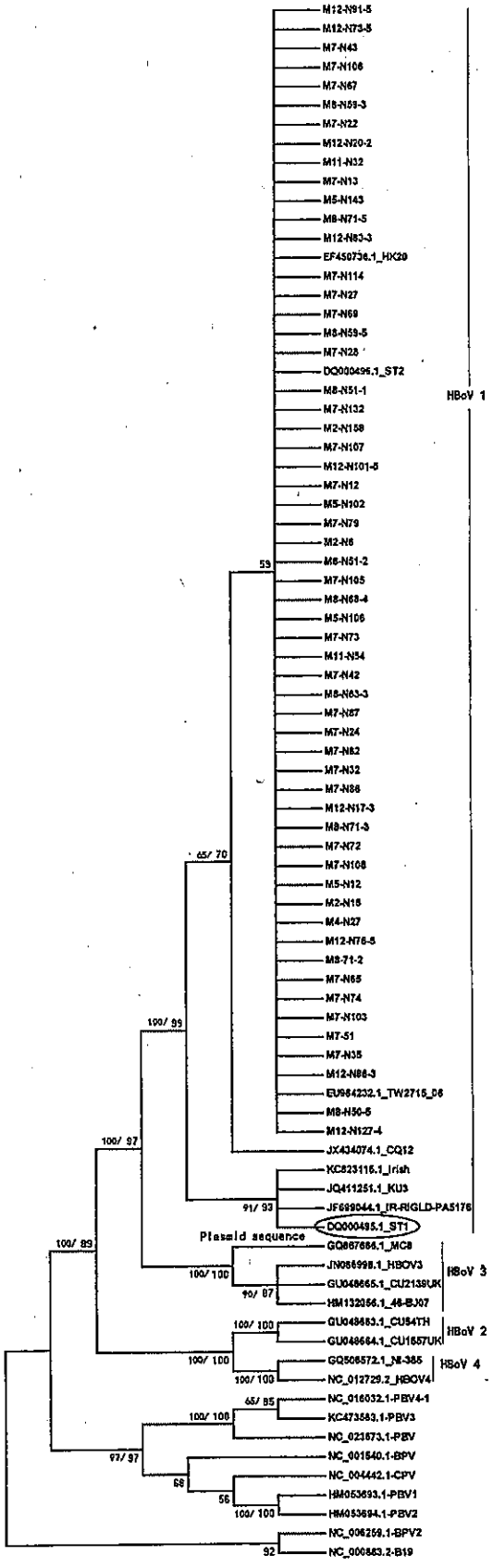

Fig. 4. Phylogenetic relationships of the NS1 region in different clones. The NJ tree was estimated using a 753-bp sequence. Values on the nodes indicate NJ (or MP/NJ) bootstrap values. The GenBank number of 25 reference sequence are shown as follows: B19, NC_000883.2; PBV, NC_023673.1; PBV1, HM053693.1; PBV2, HM053694.1; PBV3, KC473563.1; PBV4, NC_016032.1; CPV, NC_004442.1; BPV, NC_001540.1; BPV2, NC_006259.1; HBoV Genotype 1, DQ000495.1, DQ000496.1, KC823115.1, JQ411251.1, JX434074.1, EU984232.1, EF450736.1, JF699044.1; Genotype 2, CU54TH, CU1557UK; Genotype 3, NC_012564.1, HM132056.1, GU048665.1, GQ867666.1; Genotype 4, GQ506572.1, NC_012729.2.

viruses, including HBoV, in three batches of FVII product, three batches of two different FVIII products, four batches of FVIII/von Willebrand factor concentrate, and eight batches of activated PCC;[20,35] however, no HBoV sequences were detected in either study, which may result from the sensitivity of the assay they used. In this study, 241 source plasma pools and 326 plasma derivatives were screened, with the results showing a contamination rate of 17.84% (43/241) in plasma pools and 13.50% (44/326) in blood products, among which three plasma pools and two batches of FVIII contained HBoV DNA with titers above 1×10^4 copies/mL. However, it is not known whether the blood products produced from these HBoV viremic source plasma pools were also HBoV genome positive or what their HBoV DNA titers were. It is also not known whether the patients who accepted the HBoV-positive blood products (particularly those with HBoV DNA titers above 1×10^4 copies/mL) presented any side effects or developed viremia.

ELISAs designed to detect anti-HBoV IgG and IgM have mostly been based on virus-like particles generated from prokaryotic or eukaryotic expression systems and have been used in several studies, with seroprevalence ranging from 40.7% to 60% for children less than 4 years old, up to 85% for those 4 years old and older, and greater than 94% in healthy adults.[36] Antibodies specific for the different HBoV genotypes (HBoV1-4) were also analyzed in the studies by Kantola and colleagues[36] and Qiu and colleagues[12] to minimize the influence of cross-reactivity of anti-HBoV IgG among the four genotypes. Seroprevalence of HBoV-specific antibodies, including IgM and IgG, was measured in our study using a commercial ELISA kit, with the results showing a relatively low seropositive rate of 20.18% for IgG and 4.59% for IgM. These low values could be due to the low sensitivity of the ELISA kit or may reflect a regional difference. The higher seroprevalence of IgG in the HBoV genome–positive samples than in the genome-negative samples and the lower seroprevalence of IgM in the "low"-level DNA group than in the DNA "high" group were consistent with the

conclusions of Lin and other researchers. There is a limitation of this study, because the four known HBoVs are highly serologically cross-reactive[36] and the commercial assay was used with an antigen of unknown origin, so that it is difficult to know what species the serologic reactivity is really targeting that compromised these data.

Although four HBoV genotypes have been identified, only HBoV1 and 2 have been identified in the blood of patients with acute respiratory and gastrointestinal diseases.[5,37] The HBoV genotypes prevalent in blood samples from healthy people have not previously been determined. We attempted to identify the phylogenetic characteristics in healthy plasma donors, with our sequence analysis of 57 samples indicating that HBoV Genotype 1 was prevalent in healthy Chinese plasma donors. Larger sample sizes may be needed for phylogenetic analysis to clearly understand the epidemiologic characteristics of the four genotypes of HBoV in plasma donors.

Our study is the first to identify HBoV DNA in source plasma pools and plasma derivatives and is the first to demonstrate the existence of HBoV1 in Chinese plasma donors. Whether HBoV could be transmitted by blood transfusion and threaten blood safety demands further study of recipients using HBoV1-contaminated products.

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HL searched data, performed the experiments, collected and analyzed the data, and wrote the manuscript; WL designed the experiments and gave a critical view of data analysis and manuscript writing; and MH, PZ, ZG, GB, CY, and YZ provided help to HL during the process of experiments and data analysis. All the authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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No. 24

医薬品 研究報告 調査報告書

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研究報告の概要

○白血球除去血小板製剤が原因である可能性が高い、*Anaplasma phagocytophilum* (AP)の輸血伝播
背景:APは、ヒト顆粒球アナプラズマ症(HGA)の原因となる、ダニ媒介性の偏性細胞内寄生菌であり、これまでに7例の輸血感染HGAで赤血球製剤輸血の関与が指摘されている。本報告は、初の白血球除去血小板製剤によるAP感染の可能性が高い症例である。
症例:外傷で入院した41歳男性が、輸血25本を受けたのちAP陽性と判明した。関連する白血球除去赤血球及び血小板製剤の供血者12名を調査したところ、血小板製剤の供血者1名について、AP流行地域在住で、ダニ刺咬歴があり、AP-IgGが持続的に高値陽性、IgM陰性、PCR陽性であった。
結論:8例目の輸血感染HGAとなる本症例は、白血球除去血小板製剤がAP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	今後の対応
AP感染供血者由来の白血球除去血小板製剤による初の輸血感染症例の報告である。	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。

Probable transfusion-transmission of *Anaplasma phagocytophilum* by leukoreduced platelets

Rebecca L. Townsend,[1] Erin D. Moritz,[1] Lawrence B. Fialkow,[2] Victor Berardi,[3] and Susan L. Stramer[1]

BACKGROUND: *Anaplasma phagocytophilum* (AP), a tick-borne obligate intracellular bacterium, causes human granulocytic anaplasmosis (HGA) and has been implicated in seven transfusion-transmitted (TT)-HGA cases associated with red blood cells (RBCs). Here we report the first probable case of TT-HGA involving leukoreduced platelets (PLTs).

CASE REPORT: A hospitalized male received 25 blood components (November 2012) before his death from trauma. Hospital testing confirmed HGA by peripheral blood smears; samples were also sent to IMUGEN, Inc. (Norwood, MA), for AP-polymerase chain reaction (PCR) and AP-immunoglobulin (Ig)M and IgG enzyme immunoassay. All 12 potentially transmitting donors provided follow-up samples.

RESULTS: Recipient smears progressed from negative to predominantly positive 16 days posttransfusion; hospital-performed AP-PCR was positive on Day 22. IMUGEN sample testing was PCR positive and IgM and IgG negative 14 to 23 days posttransfusion. The recipient had no known AP risk factors. One of 12 donors of RBCs or PLTs (leukoreduced 5-day-old PLTs) provided six follow-up samples; all were strongly IgG positive and IgM negative; one was PCR-positive. The IgG-positive donor was a 52-year-old female from Hudson Valley, New York, an area endemic for AP. She reported tick bites in September to October 2012 with no travel outside New York. The donor remained asymptomatic and received no treatment. The cocomponent PLT unit was transfused to a 78-year-old male who died of causes unrelated to AP.

CONCLUSIONS: This eighth case of probable TT-HGA indicates that leukoreduced PLTs may be infectious. An antibody- and PCR-positive donor having prior tick exposure living in an endemic area was identified. PCR positivity and elevated IgG levels, which continue to exceed the assay's detectible range even in the absence of IgM, indicate active donor infection.

Anaplasma phagocytophilum (AP) is a tick-borne bacterial (rickettsial) obligate intracellular pathogen that preferentially infects neutrophils and causes human granulocytic anaplasmosis (HGA). Infections are primarily asymptomatic in healthy individuals, but disease can be severe in the immunocompromised and elderly. The most common signs and symptoms include unexplained fever, malaise, headache, and myalgia; hematologic abnormalities include leukopenia, thrombocytopenia, and increased serum transferases.[1,2] Infection is usually the result of a tick bite and is endemic in regions where the tick vector, *Ixodes scapularis*, is present (i.e., New England, the upper Midwest, and the northern Mid-Atlantic states). There are seven prior documented transfusion-transmitted (TT) cases of HGA: two involving nonleukoreduced red blood cells (RBCs), four involving leukoreduced RBCs, and one with no implicated product[3-8] (http://www.aabb.org/resources/bct/eid/Documents/anaplasma-phagocytophilum.pdf). Here we report the first probable case of TT-HGA involving leukoreduced platelets (PLTs).

CASE REPORT

In November 2012, a 41-year-old male presented to a Hartford, Connecticut, hospital with multiple gunshot

ABBREVIATIONS: AP = *Anaplasma phagocytophilum;* HGA = human granulocytic anaplasmosis; TT = transfusion transmitted.

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wounds. From November 11 to 25, he received 25 blood products, including 13 leukoreduced RBC units, three apheresis PLT units, and nine fresh-frozen plasma (FFP) units. Peripheral blood smears collected on November 11, 16, 24, and 27 were performed and read by the hospital; these demonstrated a progressively increasing number of intragranulocytic inclusions. A sample collected 22 days posttransfusion on December 3 was AP positive by a hospital-ordered polymerase chain reaction (PCR). The recipient died on December 5 from trauma unrelated to AP infection.

A peripheral blood smear from the recipient was considered suspicious due to granulocytic inclusions on November 16 (Day 5 posttransfusion); this triggered the hospital to begin ordering of AP and tick-borne disease PCR tests on November 25 (of which all were negative with the exception of AP from samples collected on December 3). The suspicious smear on November 16 also corresponded with decreased PLT counts and observed RBC abnormalities including microcytes and macrocytes, as well as polychromasia; also observed was white blood cell (WBC) toxic granulation. As time progressed (to December 3), additional RBC abnormalities were observed along with increasing numbers of WBC inclusions, vacuolated neutrophils, and decreased PLT counts including large and clumped PLTs.

Investigation

All 25 donors associated with the transfused components were investigated. Thirteen of 25 donors were excluded, including nine donors of FFP units (acellular) and four donors of leukoreduced RBCs whose units were transfused after AP-positive results were observed in the recipient. The 12 remaining donors, who provided nine leukoreduced RBCs and three leukoreduced PLT units, were investigated and provided follow-up samples that were sent for AP immunoglobulin (Ig)M and IgG antibody and DNA testing at IMUGEN, Inc. (Norwood, MA). The hospital was contacted and asked to provide residual volume from recipient samples collected on November 25, November 27, and December 4 (14, 16, and 23 days posttransfusion); these samples were sent to IMUGEN for testing as well. If a donor tested positive, (s)he was contacted by trained counselors at the American Red Cross Donor Client Support Center and interviewed about health and exposure history.

Assay characteristics

PCR and IgM and IgG enzyme immunoassay (EIA) characteristics have been reported elsewhere.[3] Briefly, the fast real-time PCR targets the *A. phagocytophilum* msp2 gene; detection of the product before 42 thermal cycles is considered positive.[3] IgM and IgG antibodies were detected with an indirect EIA using a recombinant fusion protein rErf-1 as a target.[9]

RESULTS

No history was available for prior health, travel, or other AP risks in the recipient. Eleven of 12 followed donors were negative by both AP-PCR and AP-IgM and IgG EIA in follow-up samples collected 46 to 192 days subsequent to their suspect donations collected from October 25 to November 7, 2012. The 12th donor, an apheresis PLT donor, whose PLT products were transfused 5 days after collection, provided a follow-up sample 70 days after her suspect collection. This sample was AP-IgG EIA positive and AP-IgM EIA negative, but not tested by PCR because the sample was not suitable for the assay. The donor subsequently provided five additional follow-up samples, the first (collected 105 days after collection of the implicated unit) was AP-PCR positive and AP-EIA (IgG) positive, followed by four AP-PCR–negative and AP-EIA (IgG)–positive follow-up samples (Table 1). All AP-IgM EIA results for the donor were negative. No subsequent units were collected during this time and the donor remains deferred. Recipient samples that were obtained between 14 and 23 days posttransfusion and tested at IMUGEN were all AP-PCR positive and AP-EIA (IgM and IgG)-negative. The timeline of events related to the AP transfusion transmission including all AP test results is included in Fig. 1.

The positive donor was a 52-year-old female from Albany County, New York, in the Hudson Valley, a reported

TABLE 1. Follow-up test results on the donor implicated in a case of probable TT-AP*

				S/CO	
Implicated donor f/u	Collect date	Days posttransfusion	PCR	IgM EIA	IgG EIA
1	January 16, 2013	70	Not tested	<1	>7.8†
2	February 20, 2013	105	Positive	<1	>7.5†
3	March 30, 2013	143	Negative	<1	>8.1†
4	April 15, 2013	159	Negative	<1	>8.0†
5	April 29, 2013	173	Negative	<1	>9.6†
6	August 2, 2013	268	Negative	<1	>8.5†

* All testing performed at IMUGEN, Inc.
† Exceeded the maximum absorbance of the test.
f/u = follow-up; S/CO = signal-to-cutoff ratio.

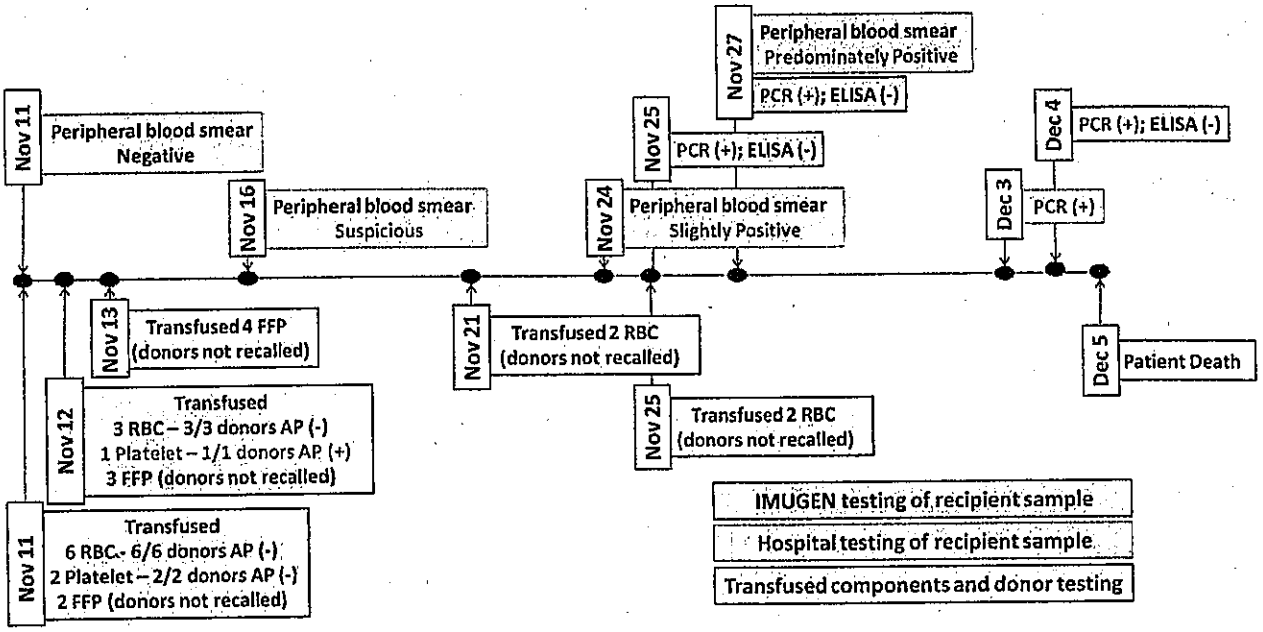

Fig. 1. Timeline of events related to the transfusion-transmission of AP. Donors were not recalled if the donated product was an FFP or if the RBC units were transfused after the diagnosis of possible AP infection (November 16, suspicious peripheral blood smear).

endemic region for AP. She was healthy and asymptomatic at the time of donation with no history of travel outside of New York, but reported tick bites in September to October 2012. The donor had remained asymptomatic throughout follow-up and had received no treatment for AP from her physician, due to resolution of active infection, as demonstrated by AP-PCR–negative results. The cocomponent implicated PLT product was transfused (also on Day 5) to a 78-year-old male acute myeloid leukemia patient with a history of chronic obstructive pulmonary disease, Parkinson's disease, and an upper gastrointestinal bleed. The patient was on a ventilator and received several RBC and PLT units. The family chose not to pursue further treatment and the patient then died of causes unrelated to AP.

DISCUSSION

This is the eighth reported probable case of TT-AP in a blood product recipient and the first implicating leukoreduced, apheresis PLTs as a transmission vehicle. The donor is considered the probable source since the donor had risk factors (e.g., reported tick bites), resided in an endemic area, was the only donor identified as positive (with levels of AP-IgG that uniformly exceeded the assay's detectable range throughout the follow-up period of approximately 9 months), and was AP-PCR positive indicating likely active infection. Previous TT-HGA cases involved RBCs, including leukoreduced units. RBC leukoreduction is a gravity-based filtration system designed to remove WBCs and, theoretically, any WBC-associated pathogens from blood products. While Mettille and colleagues[10] showed that leukoreduction removed up to 10^5 infectious units of *Orientia tsutsugamushi*, another rickettsial agent, from RBCs, this process does not appear to be completely effective in removing AP from RBCs.[3-5,7] Transmission via transfusion might occur because AP preferentially infects granulocytes, which are relatively numerous both before and after leukoreduction.[11] This unique case report suggests that apheresis PLTs are also not sufficiently leukoreduced to remove AP, providing additional insights into blood safety in general.

While one of the benefits of leukoreduction is to increase blood safety by removing cell-associated infectious disease agents,[12,13] the fact that TT-HGA cases have consistently occurred in recipients of leukoreduced units should serve as a reminder that a combination of screening, processing controls, and the future use of inactivation by pathogen reduction will likely be required to protect recipients from TT infectious agents.[14-16] The implicated PLT component was transfused on the last allowable day of storage (Day 5); it is unknown if AP can replicate in stored PLTs or merely survives the full length of PLT storage. Thus, it is possible that this length of storage contributed to the level of pathogen in the unit, although no estimates of bacterial load are available in this case and previous reports have indicated that development of clinical symptoms may be related to host response more than bacterial load.[1,17] Reduction of PLT storage time could serve as a potential intervention point, if aftergrowth is

believed to occur, particularly for recipients who may be at increased risk of severe AP disease or those receiving a high volume of product.

AP donation screening is a potential intervention to decrease the transmission of TT-HGA, but additional data are needed to determine its feasibility and clinical value. It is likely that cases of AP in the general population are underestimated due to a low index of suspicion by physicians and the fact that not all patients will develop morulae visible on blood smear.[13] The prevalence of AP in blood donors in endemic areas (based on three endemic US locations) is estimated to be between 0.5 and 11.3%,[18,19] but additional surveillance and case reporting are needed to determine the risk to recipients and therefore the utility of donor screening. Investigational donor screening has been implemented for another tick-borne agent (*Babesia microti*) due to an increasing number of TT cases reported in the same geographic areas as AP, likely due to the involvement of the same tick vector.[20,21] Reported cases of HGA in the United States have increased by approximately 50%, from 1761 cases in 2010 to 2575 in 2011, the largest reported increase since HGA became notifiable in 1998. The reported increase may be due to an increase in the tick population, expansion of the tick vector range, or increased use of diagnostic assays (http://www.cdc.gov/mmwr/PDF/wk/mm6053.pdf). Similarly, the state of Massachusetts, another babesia and anaplasma-endemic state, reported a 60% increase in reported cases of HGA in 2011 (and 22% increase in reported cases of babesiosis) (http://www.mass.gov/eohhs/docs/dph/hga-surveillance-2012.pdf; http://www.mass.gov/eohhs/docs/dph/babesiosis-surveillance-2012.pdf).

AP asymptomatically infects healthy individuals (e.g., blood donors) and can cause severe disease in the immunocompromised and the elderly (e.g., blood product recipients). The incidence of TT-HGA thus far has been low; however, the aforementioned AP characteristics and increased spread of its vectors and reservoirs make this a potentially important pathogen to monitor and a future target for consideration of donation screening or inactivation by pathogen reduction technologies.

CONFLICT OF INTEREST

RLT, EDM, LBE, and SLS have disclosed no conflicts of interest. VB is an employee of IMUGEN, Inc.

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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. 11. 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	Tolomelli G, Tazzari PL, Paolucci M, Arpinati M, Landini MP, Pagliaro P. Blood Transfus. 2014 Oct;12(4):611-4. doi: 10.2450/2014.0322-13. Epub 2014 Jun 5.	公表国
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研究報告の概要

○急性骨髄性白血病患者における輸血関連*Listeria monocytogenes*(*L.monocytogenes*)感染
36歳の白人女性が皮膚及び粘膜出血を伴う急性前骨髄性白血病と診断され入院した。アフェレーシス血小板製剤1本の輸血中に悪寒、頭痛、吐き気を呈し、輸血を中止した。その後38－38.5℃の発熱、血圧低下などが見られ、血液及び脳脊髄液培養の結果、*L.monocytogenes*血清型1/2aが検出された。抗生物質による15日間の治療により患者は回復した。患者に輸血された血小板製剤の残余を培養したところ、同菌が確認されたため、輸血による感染が考えられた。当該供血の採血時に細菌培養検査が行われたが、好気的、嫌気的状況下での細菌検査にもかかわらず、細菌は検出されていなかった。しかし血液センターへ当該供血者を呼び出し、血液を再検査した結果、31時間後に陽性となり、同菌が確認された。これらのことから、当該供血は供血から輸血までの4日間にバッグ内で同菌が増殖したと考えられる。供血者は無症状であり、菌血症となるリスク行動も見られなかった。
これは不顕性感染供血者由来のアフェレーシス血小板製剤による初の輸血関連*L.monocytogenes*感染症例である。

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
不顕性感染供血者由来のアフェレーシス血小板製剤による初の輸血関連*L.monocytogenes*感染症例の報告である。	日本赤十字社では全献血者に問診を実施しているほか、輸血による細菌感染予防対策として全輸血用血液製剤を対象に保存前白血球除去を行っている。また、輸血情報リーフレット等により、細菌感染やウイルス感染について医療機関へ情報提供し注意喚起しているほか、細菌感染が疑われる場合の対応を周知している。細菌やウイルスの検出や不活化する方策について検討している。

CASE REPORT

Transfusion-related *Listeria monocytogenes* infection in a patient with acute myeloid leukaemia

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Introduction

Transfusion-transmitted infections are feared complications of blood component administration, especially in Haematology Departments, where in-patients are severely immunocompromised and at a very high risk of opportunistic infections. The incidence of transfusion-transmitted viral infections has been greatly reduced (down to less than 1 in 200,000 transfusions) across the years due to increasing awareness and control[1]. The incidence of transfusion-transmitted bacterial infections (TTBI) is currently higher, there being up to 1:60,000 infections following single unit platelet transfusions[2].

Listeria monocytogenes is a Gram-positive bacterial pathogen, which is usually foodborne[3]. Immunocompromised hosts, pregnant women, neonates and elderly people are at higher risk of developing invasive listeriosis, including central nervous system infections such as meningo-encephalitis, cerebritis or meningitis, endocarditis and sepsis caused by uncontrolled bacteraemia. Haematological patients are an important subset of immunocompromised hosts, who are frequently transfused with multiple blood components because of the nature of their haematological malignancy and the chemotherapy that has been administered.

Listeriosis caused by transfusion has not been reported in the literature yet. However, *L. monocytogenes* contamination of an apheresis platelet product was previously reported: the contamination was detected with the BacT/ALERT automated system and confirmed by pulsed field gel electrophoresis[4,5]. Here we report the first case of transfusion-transmitted *L. monocytogenes* infection in a haematological patient after the transfusion of contaminated apheresis platelets, collected from an asymptomatic donor.

Case reports

A 36-year old Caucasian female was admitted to the Haematology Department of Sant'Orsola-Malpighi Hospital (Bologna, Italy) in July 2012 with a diagnosis of acute promyelocytic leukaemia, with cutaneous and mucosal haemorrhagic syndrome.

She had no fever and her blood pressure, heart rate and oxygen saturation were normal. Induction chemotherapy with all-trans-retinoic-acid (ATRA) at a dose of 45 mg/m^2 was started. Idarubucin was not started until later.

On the 25th of July, during the transfusion of a single unit of donor-derived apheresis platelets she developed chills, with headache and vomiting; the transfusion was stopped and hydrocortisone was administered based on the diagnosis of a transfusion reaction. The adverse event was reported to the Transfusion Centre of our hospital by filling in the appropriate form. The day after, the patient had persistent fever (range 38-38.5 °C), with low systolic blood pressure and normal heart rate. Blood cultures were performed, and empirical antibiotic therapy with piperacillin-tazobactam was started, in consideration of the patient's long-standing, severe neutropenia (<500 neutrophils/µL). We recorded an increase in liver enzymes with aspartate amino-transaminase 1,214 U/L (reference <32 U/L), alanine amino-transaminase 816 U/L (reference<31 U/L), lactate dehydrogenase 1,366 U/L (reference 135-214 U/L), total bilirubin 6.98 mg/dL (reference 0.2-1.1 mg/dL), suggesting damage to the liver caused by the low cardiac output during the febrile episode.

Three days later, the patient's headache worsened, with increased blood pressure (160/110 mmHg), vomiting and photophobia, without nuchal rigidity. She underwent computed tomography of the brain which did not show density alterations. Because of the persistence of symptoms, a lumbar puncture with culture of the cerebrospinal fluid (CSF) was performed. The protein concentration was 47 mg/dL (reference <50 mg/dL), while the glucose concentration was 71 mg/dL (reference 50-80 mg/dL). Cytological examination was positive, with 400 cells/µL, including mostly neutrophilic granulocytes and occasional lymphoid cells. No blast cells were seen. Computed tomography brain scanning was repeated, with an intravenous contrast agent, and confirmed the absence of density alterations.

Blood cultures were positive for *L. monocytogenes* serotype 1/2a, which was resistant to penicillin and trimethoprim-sulphamethoxazole and sensitive to ampicillin and erythromycin. The cultured CSF was also

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positive for *L. monocytogenes* serotype 1/2a, with the same antibiotic sensitivity spectrum (Table I). Piperacillin-tazobactam therapy was switched to intravenous ampicillin 3 g every 6 hours in combination with intravenous levofloxacin 500 mg every 12 hours (favourable kinetics in the CSF). Therapy was continued for 15 days. The fever had already resolved on July 26th, whereas the neurological symptoms subsided after a few days (on August 2nd) together with normalization of the laboratory examinations.

The single-donor apheresis platelets transfused on July 25th were subsequently cultured and resulted positive for *L. monocytogenes* serotype 1/2a. Interestingly, donor blood cultures had been performed on the day of the platelet apheresis harvest and tested aerobically and anaerobically for micro-organisms; however, no micro-organisms were detected in the blood culture bottles after 7 days of incubation at 35 °C. The healthy blood donor was recalled to the Transfusion Centre and his blood was again cultured in aerobic and anaerobic blood culture bottles. This time, positive results were obtained after 31 hours. Subculture on horse blood agar revealed *L. monocytogenes* serotype1/2a.

The asymptomatic donor was investigated for possible risk factors related to the bacteraemia, such as recent ingestion of contaminated food or water or exposure to farm animals. No relation with his recent health history, environment or dietary behaviour was found. The donor was suspended from making other donations for 3 months. As repeated blood cultures were negative, the donor was subsequently readmitted and donated without problems (a new platelet apheresis was negative for *L. monocytogenes* or other bacteria).

Discussion

Our patient is the first *definite* case of transfusion-transmitted *L. monocytogenes* infection, according to the definition given by Perez *et al.* in the French BACHTEM Case-Control Study[6], with the same bacteria being isolated from both the blood product and the transfusion recipient. Furthermore, the case fits the criteria defined by the "Assessment of the frequency of Blood Component Bacterial Contamination associated with Transfusion Reaction" (BaCon) study, i.e. the occurrence of the symptoms (any one among fever ≥39 °C, rigors, tachycardia >120 bpm or a rise or drop of ≥30 mmHg in systolic blood pressure) within 4 hours after transfusion in the blood product recipient, and confirmation by culture in both the blood component and the patient[7].

Only a few similar case reports have been described in the literature, but the contaminating organisms were always identified before transfusion of the platelet product. Listeria contamination was detected by an automated BacT/ALERT system following the implementation of screening for bacterial contamination of platelet products by the American Association of Blood Banks in 2004[5]. In the report by Guevara *et al.*, the donor was not interdicted from making other donations and in the subsequent month gave four other apheresis platelet donations all of which tested negative for *L. monocytogenes* contamination[4]. In our Transfusion Centre, platelets (from pools of buffy coats or from apheresis) are not routinely tested for bacterial pathogens, so we have no data regarding the frequency of *L. monocytogenes* contamination in platelet products. However, routine quality control of blood components shows that platelets may be contaminated at an expected rate (<1:1000) by exogenous environmental bacteria. However, the occurrence of a potentially life-threatening episode of TTBI has prompted us to re-evaluate the policy of screening blood products for bacterial contamination before transfusion.

The main concern related to this case is the asymptomatic *L. monocytogenes* infection in the donor, which led to a transfusion-transmitted infection in an immunocompromised patient. This is explained by transient bacteraemia (see Table I) in a donor not belonging to a high-risk group. Indeed, febrile gastroenteritis is the most common syndrome caused by listerial infection in healthy people, and it has an

Table I - *Listeria* monocytogenes isolates.

Date	Source	Cultures results	Drug sensitivity	Drug resistance
21st of July	Donor's PB (blood culture)	Negative	/	/
25th of July	Patient's PB (blood culture)	Serotype 1/2a	Ampicillin Erythromycin	Penicillin Sulpha/Trimeth
25th of July	Platelet apheresis	Serotype 1/2a	Ampicillin Erythromycin	Penicillin Sulpha/Trimeth
30th of July	Patient's CSF	Serotype 1/2a	Ampicillin Erythromycin	Penicillin Sulpha/Trimeth
1st of August	Donor PB (blood culture)	Serotype 1/2a	Ampicillin Erythromycin	Penicillin Sulpha/Trimeth

Positive cultures of the same *L. monocytogenes* serotype were obtained from the patient's and donor's blood cultures, the patient's CSF and the platelet apheresis. Sulpha/Trimeth: Sulphamethoxazole/trimethoprim: PB: peripheral blood; CSF: cerebrospinal fluid.

incubation period of about 24 hours. Invasive listeriosis with a longer incubation period can be excluded in a immunocompetent donor.

Prevention of bacterial contamination of blood products is based on donor history referral, donor examination and testing, diversion of the first flow, leucoreduction, component inspection and post-donation information[8]. Methods to improve tests for bacterial detection on platelet components before release are available, but not mandatory. Stringent screening of blood donors for TTBI is, therefore, crucial to ensure a safe supply of blood and blood products[9]. Donor questionnaires are not useful for screening out donors because *L. monocytogenes* infection in healthy individuals is normally asymptomatic. Indeed, in the BaCon study, screening 60-70% of blood banks in the United States over 3 years of active surveillance, cases of *L. monocytogenes* bacteraemia could not be identified. Risk factors for *L. monocytogenes* infection in healthy donors have not been established. Some Authors[10] have shown that iron overload is a risk factor for listeriosis. Adding questions about iron status during screening of donors could, theoretically, contribute to identifying donors at risk of bacterial infection, although the policy of people with iron overload donating blood is highly controversial[11]. It must be noted that a thorough review of the donor's behaviour did not reveal any known risk factors of exposure to *L. monocytogenes*, as also previously reported[4,5].

A bactericidal treatment such as exposure to ultraviolet light after psoralen sterilisation may be a promising method to prevent the occurrence of a TTBI[12]. In fact, photochemical treatment of platelet concentrates inactivates a broad spectrum of pathogenic bacteria, including *L. monocytogenes*[13], and 8-methoxypsoralen with long wavelength UV light (UVA) was found to be effective in reducing levels of bacteria without diminishing *in vitro* platelet function[14].

It is noteworthy that in our case the donor's blood cultures performed the same day as the platelet donation resulted negative, but when the single-donor apheresis platelet unit partially transfused to the patient was cultured, it was found to be positive for *L. monocytogenes* serotype 1/2a. This result seems to show a subsequent growth of *L. monocytogenes* in the apheresis product, occurring in the 4-day period between the day of the donation and the day of the transfusion, facilitated by the need to store apheresis platelets at room temperature.

Finally, the management of fever following transfusion is still controversial and requires cooperation between the clinician, blood bank, and microbiology services. As our case report has shown, the differentiation between an acute transfusion reaction and a TTBI in the early phase of a febrile episode during transfusion is the most difficult step. The recommendations include collecting blood from the opposite arm from that used for the transfusion and sending it for culture, performing a direct antiglobulin (Coombs') test and analysing the urine for free haemoglobin. If there is a high clinical suspicion of TTBI, empirical broad-spectrum antibiotic therapy should be started. The hospital blood bank and microbiology laboratory should also be alerted, and the blood product bag should be sent for Gram stain and culture. Moreover, if co-components from the same donation are present, they should be at least quarantined, and when leucocyte-depleted platelet concentrates obtained with the buffy coat method are transfused, all of the donors should be screened for a possible asymptomatic infection[15]. In our case, the transfusion was performed from a single donor platelets (apheresis). The plasma bag derived from the same apheresis was sent to the plasma derivation industry: a haemovigilance alert was transmitted from the Transfusion Centre to the pharmaceutical company to eliminate the plasma unit which was being held in quarantine. Improving the surveillance of the harvest, manipulation and delivery of blood products is necessary to improve transfusion safety, aided by the new pathogen inactivation techniques.

Keywords: *Listeria monocytogenes*, transfusion-transmitted infection, acute myeloid leukaemia

The Authors declare no conflict of interest.

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医薬品　研究報告　調査報告書

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販売名（企業名）	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）		ABC NewsLetter ,November.21, 2014 (#42)	米国

研究報告の概要

○感染症最新情報：シャーガス病
米国熱帯医学会は、11月4日に開催された年次大会で、米国人の多くにとって、シャーガス病が認識されていない公衆衛生上のリスクであることを発表した。*Trypanosoma cruzi*（*T. cruzi*）の感染を原因とするシャーガス病は、流行地域であるラテンアメリカでは公衆衛生上の問題として広く認識されているが、米国でも近年増えており、特にテキサス州では感染の増大が報告されている。
2008～2012年、ヒューストンで供血者のシャーガス病スクリーニングを行ったところ、確認された*T. cruzi*感染者17名のうち7名（41%）が、シャーガス病による心疾患と一致する症状を呈していた。感染者の多くは農村部在住、または長時間屋外で過ごしていた。6名はラテンアメリカの流行国への旅行歴はなく、また母親がこれらの国の出身者ではなかったことから、テキサス州の土着感染が示唆される。別の研究では、2008～2012年に調査した供血者の6,500名に1名が*T. cruzi*に感染していた。
米国では、シャーガス病については現在、国家的サーベイランスが存在しないため、これら供血者のスクリーニングデータにより、米国における感染実態についての貴重な知見が得られる。

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
米国人の多くにとって、シャーガス病が認識されていない公衆衛生上のリスクであるが、テキサス州では土着感染も示唆されるとの報告である。	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に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。

THE WORD IN WASHINGTON

Pictured left, ABC CEO Christine Zambricki, DNAP, CRNA, FAAN, (left) met with US Rep. Joyce Beatty (D-OH) (right) on Thursday morning to talk over tortillas about issues relevant to ABC's member blood centers. Dr. Zambricki provided Rep. Beatty with information about ABC and the life-saving mission of its member blood centers. She also reviewed the key advocacy issues that ABC is working to move forward on behalf of its member centers.

GLOBAL NEWS

The Biomedical Excellence for Safer Transfusion (BEST) Collaborative announced in a Nov. 13 press release its membership for the next four years (2014-2018). BEST is an international research organization that aims to improve the safety of transfusion and cell therapy and related services through standardization of analytic techniques, development of new procedures and execution of clinical trials in hematology and cell therapy. More than 80 scientific publications have resulted from the Collaborative's work. Thirteen of the 32 scientific BEST member positions have been filled by clinicians and scientists who are new to the Collaborative. BEST's work is conducted by four teams. A new Donor team has been established and the Clinical Studies and Transfusion Safety teams have been combined into a Clinical Transfusion Studies team. The full list of members can be viewed on the BEST website. BEST also announced that Professor Mike Murphy, MD, of Oxford in the UK, recently succeeded Larry Dumont, MD, as chair of the Collaborative. (Source: BEST press release, 11/13/14)

INFECTIOUS DISEASE UPDATES

CHAGAS DISEASE

Research presented at the American Society of Tropical Medicine and Hygiene (ASTMH) Annual Meeting on Nov. 4 in New Orleans suggests that Chagas disease poses a largely unrecognized public health risk to many Americans. While Chagas disease, caused by infection with *Trypanosoma cruzi* (T. cruzi), is well-recognized as a public health concern in endemic areas of Latin America, it is becoming more common in the US, particularly in Texas where higher levels of the infection have been reported in recent years. Among those infected are a significant number believed to have contracted the disease within the US borders, according to investigators from Baylor College of Medicine, whose research was presented in abstracts at the ASTMH's Annual Meeting. One abstract, presented by Melissa

(continued on page 13)

INFECTIOUS DISEASE UPDATES (continued from page 12)

N. Garcia, MPH, assessed blood donors screened for Chagas disease in Houston from 2008 to 2012. Of those with confirmed infection, 41 percent (7 out of 17) had heart disease symptoms consistent with those caused by Chagas disease. Most of these individuals lived in rural areas or spent a significant amount of time outside. At least six had neither traveled to endemic countries in Latin America nor have mothers from such areas, indicative of autochthonous infection in Texas. "Cardiologists should consider the changing transmission dynamics associated with Chagas disease in the southern US and should consider Chagas disease in patients who may have clinically-compatible electrocardiogram or cardiomyopathy, even if the patient has no history of residing in a Chagas-endemic country," concluded the authors. Previous research by this team, published in August in *Epidemiology & Infection*, found that one in every 6,500 blood donors tested between 2008 and 2012 tested positive for T. cruzi exposure. The researchers highlighted that because national surveillance data on Chagas disease is lacking, blood donor screening data provides valuable insight into its burden in the US. (Source: ASTMH press release, 11/4/14)

Citation: Garcia MN, *et al.* Chagas disease transmission and cardiac manifestations among Texas blood donors. 2014 ASTMH Annual Meeting Abstract.

Garcia MN, *et al.* Trypanosoma cruzi screening in Texas blood donors, 2008-2012. Epidemiol Infect. 2014 Aug. 29:1-4.

CHIKUNGUNYA VIRUS

The Centers for Disease Control and Prevention warned travelers in a Nov. 6 statement that despite fewer mosquitoes in the fall, the chikungunya outbreak in the Caribbean and Central and South American countries continues to spread. The outbreak, which began last December, has caused an estimated 795,000 chikungunya cases in 37 countries and territories in the Western Hemisphere as of the end of October. More than 1,600 travelers returning to the US with chikungunya have been reported. "The beginning of fall means that mosquito problems in the continental US will be decreasing. However, travelers to areas where the chikungunya outbreak continues are at risk of becoming infected. It is important that travelers understand these risks and take appropriate actions to prevent being bitten by mosquitoes," said Roger S. Nasci, PhD, chief of CDC's Arboviral Diseases Branch. This includes using insect repellent, wearing long-sleeved shirts and long pants during the day, and staying in air-conditioned or well-screened rooms at night. Travelers can check CDC's latest recommendations at www.cdc.gov/travel and can learn about ongoing chikungunya activity at www.cdc.gov/chikungunya. (Source: CDC press release, 11/6/14)

GRANT OPPORTUNITIES

The National Heart, Lung, and Blood Institute (NHLBI) has two ongoing funding opportunities called PARs in support of research on "Selected Topics in Transfusion Medicine (R01 and R21). These PARs encourage research grant applications from investigators who propose to study research topics in blood banking and transfusion medicine aimed at improving the safety and availability of the blood supply and the practice of transfusion medicine. These two funding opportunity announcements (FOAs) will expire on Jan. 8, 2017. Those interested can find more information about the first funding opportunity (R01) at http://1.usa.gov/1Hv9kBy; information on the second opportunity (R21) can be found at http://1.usa.gov/11lIQ4w. Questions or concerns may be directed to Shimian Zou, PhD, at shimian.zou@nih.gov or (301) 435-0065.

別紙様式第2-1

No. 12

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
				2015. 1. 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	ABC NewsLetter ,December 19, 2014 (#45)	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

○米国食品医薬品局(FDA)が血小板と血漿製剤の病原体低減化処理を行う初のシステムを承認
FDAは、輸血伝播感染症のリスクを削減するCerus社のIntercept Blood Systemを血漿に続き血小板製剤への使用に関しても承認した。本システムは血液製剤の病原体低減化処理を行うために米国で初めて認可されたシステムである。
血液事業者は、血小板の細菌汚染リスクを減らし、新興病原体(特にチクングニヤ、デング、エボラウイルス等、現在スクリーニング検査が存在しない病原体)から血液供給を保護するための新たな選択肢となる病原体低減化技術の承認を長く待ち望んでいた。本システムは、輸血後の感染症リスクの防止策と同時に、移植片対宿主病リスク削減のための放射線照射に替わる方法としても、血液事業者に、より積極的な活用を提案する。
本システムは2002年以降ヨーロッパにおける血小板処理のために承認されており、現在20カ国の100施設以上で使用されている。

報告企業の意見	今後の対応
FDAは、血小板と血漿製剤の病原体低減化処理を行うためのIntercept Blood Systemを承認したとの報告である。	今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」120
新鮮凍結血漿-LR「日赤」240
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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

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ABC NEWSLETTER

CURRENT EVENTS AND TRENDS IN BLOOD SERVICES

Visit ABC's Web site at: www.americasblood.org

2014 #45 December 19, 2014

INSIDE:

Please Note: The *ABC Newsletter* will take a two-week publication break for the holidays. Publication will resume on Jan. 9. ABC sends you warm wishes for a happy holiday season and a joyous new year!

FDA Approves First Pathogen Reduction System to Treat Platelets, Plasma

The Food and Drug Administration today approved Cerus Corp.'s Intercept Blood System for the treatment of platelets to reduce the risk of transfusion-transmitted infections (TTIs), after earlier in the week clearing the system for the treatment of plasma. Intercept is the first pathogen reduction system to be cleared in the US for the treatment of blood components to reduce the risk of TTIs.

Many in the blood community have long awaited the approval of pathogen reduction technology, adding to the number of interventions aimed at reducing the risk of bacterial contamination of platelets and protecting the blood supply from emerging pathogens, particularly those for which there are no available screening tests like chikungunya, dengue, and Ebola viruses. The Intercept system offers the blood community a more proactive approach to preventing both infectious risks, as well as an alternative to the use of radiation to mitigate the risk of graft-versus-host disease after transfusion.

Bacterial culture of platelets and other measures currently in place provide some protection from bacterial contamination of platelets, however it remains one of the most common causes of serious transfusion-related morbidity and mortality in the US. Careful donor selection, adherence to good manufacturing practices, and advanced blood donor screening tests have significantly decreased the risk of a number of TTIs. However, the blood community has long sought a more proactive approach to blood safety that will afford a margin of safety from emerging pathogens that may threaten the blood supply.

"We are pleased that we can now bring Intercept to the US … As a physician in San Francisco in the 1980s, we had to face our patients who had contracted HIV from transfusions of blood products. I am grateful to now be able to say to them that there is a proactive process to inactivate similarly virulent pathogens in the blood supply, even if we have not yet identified these newly emergent pathogens," Laurence Corash, MD, chief medical officer, senior vice president, and scientific officer of Cerus, said in a statement.

(continued on page 3)

FDA Approves Pathogen Reduction System (continued from page 1)

The Intercept Blood System has been shown to be effective in reducing a broad range of viral and bacterial pathogens using a photochemical process involving a controlled exposure to ultraviolet light and amotosalen, a chemical that interferes with nucleic acid synthesis in infectious pathogens and residual white blood cells in blood components.

Intercept has been approved for the treatment of platelets in Europe since 2002 and is currently used in more than 100 centers in 20 countries. It has recently been approved by FDA for use in two Investigational Device Exemption (IDE) studies. In the first study, Intercept is to be used to process platelets to reduce the risk of chikungunya and dengue viruses. In the second study, Intercept is to be used to prepare convalescent plasma collected from patients who have recovered from Ebola virus, which is used to treat patients with active Ebola infections.

Plasma prepared using the Intercept Blood System was evaluated in eight clinical trials consisting of 704 patients. The studies were conducted in various clinical settings, including acquired clotting disorders associated with liver disease and Thrombotic Thrombocytopenic Purpura (TTP). Adverse events experienced by patients receiving Intercept treated plasma were comparable to those experienced by patients receiving standard plasma.

"This is an important milestone for the US blood community," said America's Blood Centers Chief Medical Officer Louis Katz, MD. "Now, in absence of a mandate to use pathogen reduction, we will need to understand the value – in a very global sense – of what I believe is a safe and effective technology, which becomes available at a time of constrained resources. Do our clinicians find value? Do the payers recognize value, i.e., how much adoption of pathogen reduction will we be willing to support?"

More information on Intercept's approval in plasma can be found at http://1.usa.gov/1sOKsB0, and details on the approval in platelets can be found at http://1.usa.gov/1JghBKF. The package insert for both products is available at www.intercept-usa.com. (Sources: FDA press release, 12/16/14, 12/19/14; Cerus press release, 12/16/14, 12/19/14)

We Welcome Your Letters

The *ABC Newsletter* welcomes letters from its readers on any blood-related topic that might be of interest to ABC members. Letters should be kept relatively short and to the point, preferably about a topic that has recently been covered in the *ABC Newsletter*. Letters are subject to editing for brevity and good taste. Please send letters to ABC Publications Editor Betty Klinck at newsletter@americasblood.org or fax them to (202) 393-1282. Please include your correct title and organization as well as your phone number. The deadline for letters is Wednesday to make it into the next newsletter.

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	Journal of Virology 88.23 (Dec 2014): 13732-13736.	公表国 米国	
販売名(企業名)	－					

研究報告の概要

輸血によるvCJD感染リスクの予測は齧歯動物の実験データに大きく頼っているが、投与量（汚染された血液量）と反応（vCJDの感染）については未だ定量化されていない。本研究の目的は人間以外の霊長類に基づいた用量反応モデルを確立し、人におけるvCJDの輸血による感染の可能性を試算することである。
散発性と家族性CJDの患者の脳組織を脳内に注射した人間以外の霊長類から得た用量反応データをモデルとして使用した。ベータポワソンモデルを使用してデータを統計的に解析した。輸血量によるvCJD感染を予測するため、血液と脳組織を介した感染性、静注と脳内への注射による感染力の差の原因となるモデルパラメーターを調節した。潜伏期間終了間際の感染者から採血された全血１単位を輸血された人の平均感染率は 76%であると予測される。人間以外の霊長類のモデルによる予測は、齧歯類モデルによるこれまでの予測よりも、英国における白血球未除去の赤血球輸血によるリスクの分析よりと整合が高かった。
輸血によるvCJDの感染は、血液供給の安全性に影響を与える重要かつ緊急な脅威である。
英国の血液を用いて製造された、白血球未除去の赤血球と第Ⅷ凝固因子製剤が投与された患者においてvCJDの感染が報告されている。
異常プリオン（輸血によるvCJDの感染の原因となる因子）の量は血液製剤によって大きく異なるため、血液製剤や血漿分画製剤における広範囲なvCJD因子の投与量と用量反応に関する関係性を定量化する必要がある。
本報告は人以外の霊長類の実験から得られたデータを元に輸血によるvCJDの感染のメカニズム的な最初の用量反応モデルを示すものである。この新しいモデルは人への感染のリスクの可能性を予測することに貢献するだろう。

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
霊長類に基づいた用量反応モデルを用いたvCJD感染率に関する報告であるが、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vCJD伝播に関する情報等に留意していく。

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Development of Dose-Response Models of Creutzfeldt-Jakob Disease Infection in Nonhuman Primates for Assessing the Risk of Transfusion-Transmitted Variant Creutzfeldt-Jakob Disease

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ABSTRACT

Estimates for the risk of transmitting variant Creutzfeldt-Jakob disease (vCJD) via blood transfusion have relied largely on data from rodent experiments, but the relationship between dose (amount of infected blood) and response (vCJD infection) has never been well quantified. The goal of this study was to develop a dose-response model based on nonhuman primate data to better estimate the likelihood of transfusion-transmitted vCJD (TTvCJD) in humans. Our model used dose-response data from nonhuman primates inoculated intracerebrally (i.c.) with brain tissues of patients with sporadic and familial CJD. We analyzed the data statistically by using a beta-Poisson dose-response model. We further adjusted model parameters to account for the differences in infectivity between blood and brain tissue and in transmission efficiency between intravenous (i.v.) and i.c. routes to estimate dose-dependent TTvCJD infection. The model estimates a mean infection rate of 76% among recipients who receive one unit of whole blood collected from an infected donor near the end of the incubation period. The nonhuman primate model provides estimates that are more consistent with those derived from a risk analysis of transfused nonleukoreduced red blood cells in the United Kingdom than prior estimates based on rodent models.

IMPORTANCE

TTvCJD was recently identified as one of three emerging infectious diseases posing the greatest immediate threat to the safety of the blood supply. Cases of TTvCJD were reported in recipients of nonleukoreduced red blood cells and coagulation factor VIII manufactured from blood of United Kingdom donors. As the quantity of abnormal prions (the causative agent of TTvCJD) varies significantly in different blood components and products, it is necessary to quantify the dose-response relationship for a wide range of doses for the vCJD agent in transfused blood and plasma derivatives. In this paper, we suggest the first mechanistic dose-response model for TTvCJD infection based on data from experiments with nonhuman primates. This new model may improve estimates of the possible risk to humans.

Variant Creutzfeldt-Jakob disease (vCJD) is a human transmissible spongiform encephalopathy (TSE) first reported in the United Kingdom in 1996 (1). TSEs are slow neurodegenerative diseases affecting humans and animals with long asymptomatic incubation periods (IPs). TSEs are most reliably diagnosed postmortem based on typical histopathological changes and the detection, usually in the brain, of the abnormal, protease-resistant form of the prion protein (PrP^{TSE}) generated during this disease. Experimental and epidemiological evidence indicates that dietary exposure to the bovine spongiform encephalopathy (BSE) agent was responsible for most cases of vCJD in humans. Although foodborne vCJD cases are in decline, secondary transmissions of vCJD through blood transfusions, first reported in 2003, still pose a potential risk to public health. A better understanding of the relationship between the dose of the infectious agent that a person may receive and resulting infection is crucial to estimate the risk of vCJD transmission through blood transfusion.

The greatest challenge for studying the dose-response (DR) relationship for transfusion-transmitted vCJD (TTvCJD) is the scarcity of experimental data. Epidemiological studies typically provide limited information on the infectious doses. The United Kingdom Transfusion Medicine Epidemiology Review (TMER), a survey of human TTvCJD cases (2), reported information on the infection rate in recipients transfused with one unit of infected blood products, but no DR relationship could be derived from such data with a single data point or a narrow range of doses. Ideally, DR studies for intravenous (i.v.) exposure, the route of exposure for TTvCJD, should be based on well-controlled experiments with animals, possibly nonhuman primates, challenged intravenously with a range of doses of infected blood. However, such studies have not been reported. Nonhuman primates are preferred animal models because of their close phylogenetic relationship to humans and similar clinical signs, histopathological lesions, and PrP^{TSE} profiles following infections with either BSE or vCJD agents. Therefore, we considered data from a previously published review of TSE transmission studies conducted at the U.S. National Institutes of Health (NIH) over a number of years with nonhuman primates inoculated intracerebrally (i.c.) with TSE-infected brain tissues (3). We used mechanistic nonlinear DR models to characterize the animal response to different doses of

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TABLE 1 Dose-response data for 83 nonhuman primates exposed via the i.c. route to graded doses of brain tissues from patients with sCJD and fCJD[a]

Dose (g brain tissue)	No. of infected primates	No. of uninfected primates	Total no. of primates
1×10^{-2}	25	0	25
1×10^{-3}	6	0	6
1×10^{-4}	15	5	20
1×10^{-5}	4	8	12
1×10^{-6}	3	5	8
1×10^{-7}	0	6	6
1×10^{-8}	0	3	3
1×10^{-9}	0	3	3

[a] See reference 3.

infectious CJD agents. We further adjusted the model parameters using scaling factors to account for the differences in infectivity between blood and brain tissue and transmission efficiency between the i.v. and i.c. routes. The DR model developed in this study estimates the risk of vCJD infection by blood transfusion as a function of the volume transfused.

MATERIALS AND METHODS

Dose-response data. We searched PubMed and ISI Web of Science for available dose-response data for nonhuman primates exposed to TSE agents (prions) via the i.v. and i.c. routes (English-language original research articles published before March 2014; the search terms were "dose," "prion," "vCJD," "TSE," "primate," "intravenous," "intracerebral," and synonyms). We found a review article that summarized dose-response data from the NIH series of experiments with nonhuman primates infected with prions via the i.c. route (3). This large data set included eight graded doses causing infection rates ranging from 0 to 100% with intermediate responses, which presented a desirable pattern for dose-response modeling.

In these nonhuman primate experiments, diluted brain tissues from 27 patients with CJD and other human TSEs were inoculated into the animals via the i.c. route. Of the 27 patients, 22 were infected with CJD (21 with sporadic CJD [sCJD] and 1 with familial CJD [fCJD]) and 5 other individuals had Gerstmann-Sträussler-Scheinker syndrome (GSS) or kuru. Only data from nonhuman primates (total number = 83) inoculated with CJD-infected brains were used in our analysis to maintain homogeneity of the type of TSE inoculum. Most of the nonhuman primates were squirrel monkeys, with small numbers of chimpanzees, capuchin monkeys, and spider monkeys. The specific numbers of each species of primates were not reported. Linkage between CJD donors and recipient primates was also not described. TSE infection was diagnosed by postmortem neurohistopathological examinations of the brains of the animals. Table 1 shows the extracted data, including doses and numbers of infected/uninfected animals.

Dose-response modeling. Both exponential and beta-Poisson models have been widely used for DR analyses of infectious agents. Both models depend on a mechanistic DR relationship describing an estimated probability of a quantity of infectious agent surviving in the host and successfully initiating infection (4). The statistical programming language "R" (http://www.r-project.org/) was used to program the maximum likelihood estimation (MLE) computations for fitting models to DR data. Binomial MLEs were used to optimize the fit of the likelihood function by selecting the parameters with the best fit that minimized the deviances of the estimated function from the observed data. Confidence intervals (CIs) for best-fit models were determined by using bootstrap analyses with 5,000 bootstrap samples drawn from the DR data sets. To test the goodness-of-fit for a model's ability to predict the data outcomes, we compared the optimal minimum deviance of the model to the critical value of the chi-squared distribution at a 95% confidence level ($\chi^2_{0.95,df}$ where df is the degree of freedom, calculated by the number of dose levels minus the number of model parameters). The data in Table 1 were used to generate the DR model for i.c. inoculations of infected brains into primates. Because the goal of this study was to use the model to generate a DR curve for TTvCJD infection, we adjusted it by using a scaling factor that converted i.c. to i.v. transmissions based on the difference of their transmission efficiencies. In addition, as the infectious dose in the i.c. model was measured as grams of infected brain tissue, the model was adjusted to the infectious dose for the i.v. route and converted to milliliters of blood.

RESULTS

Dose-response models for i.c. exposure. DR models were fit to the primate data in Table 1. The beta-Poisson model (see the equation below) provided a statistically acceptable fit based on the goodness-of-fit test, while the exponential model did not (data not shown). In the equation below, d represents the infectious dose (grams of infected brain tissue or milliliters of infected blood) and $P(d)$ represents the probability of acquiring TSE infection at dose d. Optimized values for the parameters α and N_{50} were 0.456 and 9.6×10^{-6} (representing a 50% infective dose [ID_{50}] of 9.6 μg brain tissue), respectively, for brain inoculation i.c.; 0.456 and 13.7 (representing an ID_{50} of 13.7 ml blood), respectively, for blood inoculation i.c. (after adjustment with a factor accounting for the difference in infectivity between brain tissue and blood); and 0.456 and 75 (representing an ID_{50} of 75 ml blood), respectively, for blood transfusion (after adjustment with a factor accounting for the difference in efficiencies between the i.c. and i.v. routes of exposure). For brain inoculation, the parameter N_{50} can be interpreted as the amount (in grams) of brain tissue inoculum needed to cause a 50% chance of infection (equal to the ID_{50}). In this case, an N_{50} value equal to 9.6×10^{-6} means that 9.6 μg of infected brain tissue will cause a 50% chance of infection. α is the slope parameter describing the host-pathogen interaction. The experimental data and the beta-Poisson model (mean and 90th percentile values) are presented in Fig. 1.

$$P(d) = 1 - \left[1 + \left(\frac{d}{N_{50}}\right) \cdot \left(2^{1/\alpha} - 1\right)\right]^{-\alpha}$$

Adjustment for infectivity in blood versus brain tissue. We needed to convert the infectivity dose present in a gram of brain tissue to the equivalent infectivity dose in a volume of blood. To estimate this conversion, we analyzed data from studies using different methodologies and approaches and obtained a triangular distribution of scaling factors with the upper bound and most likely value from a transmission study by Douet et al. (5) and the lower bound based on other relevant studies.

Douet et al. (5) inoculated transgenic mice expressing bovine prion protein via the i.c. route using brain tissue and blood fractions (erythrocytes, plasma, and leukocytes) from a patient with confirmed vCJD. By comparing the infection rates, those researchers estimated that the quantity of infectivity present in 1 ml of whole blood was approximately equivalent to that present in 1.4×10^{-6} g of vCJD brain. To the best of our knowledge, this was the only study that quantified the difference in transmission efficiencies between vCJD-infected brain- and blood-derived inocula. In addition to transmission experiments, researchers have also developed laboratory techniques to measure the concentration of PrP^{TSE} in tissue samples directly. Wadsworth et al. (6) estimated

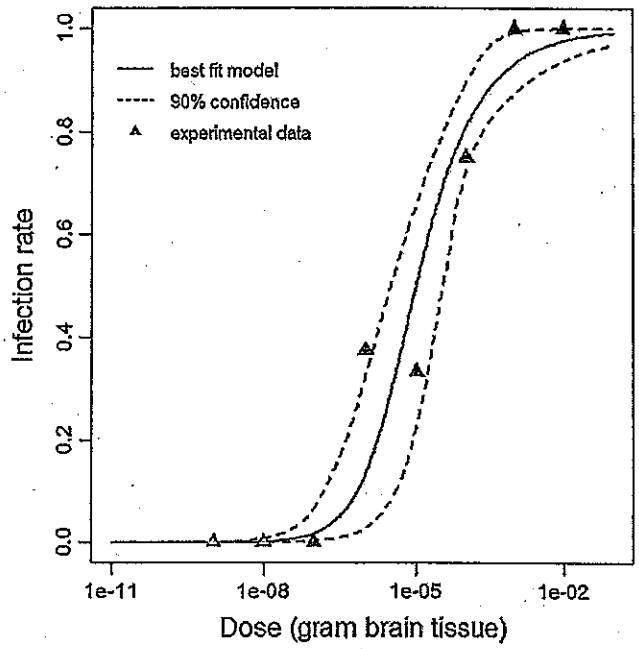

FIG 1 Beta-Poisson model fit to data from Table 1 for i.c. challenge of nonhuman primates (3) with confidence bounds around the optimized model ($\alpha = 0.456$; $N_{50} = 9.6 \times 10^{-6}$ [representing an ID_{50} of 9.6 μg brain tissue]).

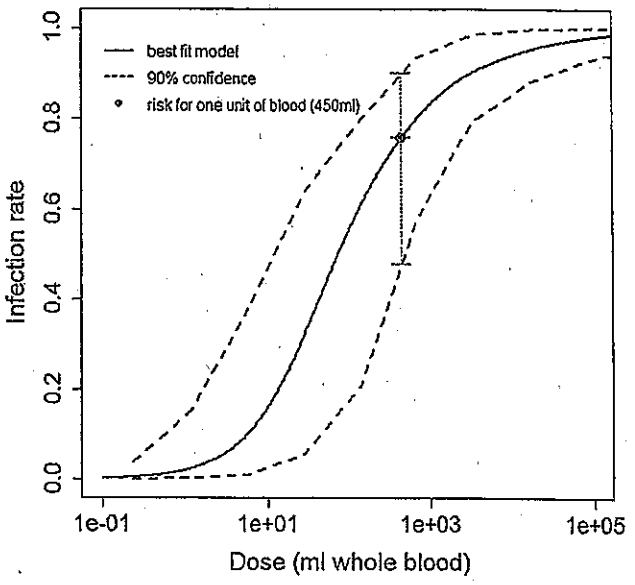

FIG 2 Beta-Poisson dose-response model for risk of transfusion-transmitted vCJD ($\alpha = 0.456$; $N_{50} = 75$ [representing an ID_{50} of 75 ml blood]). The estimated infection rate for one unit of transfused whole blood (450 ml) is 76% (90% CI, 48% to 90%).

PrP^{TSE} concentrations in different human tissues from patients with vCJD in the terminal stages of disease by using high-sensitivity Western blot analysis. Their data suggested that the amount of PrP^{TSE} in 1 ml blood was less than that in 10^{-7} g of human vCJD brain tissue. Both Gregori et al. (7), using a highly sensitive enzyme-linked immunosorbent assay (ELISA)-based assay, and Chen et al. (8), using protein misfolding cyclic amplification, estimated the concentration of PrP^{TSE} in plasma from scrapie-infected hamsters to be approximately 1×10^{-14} to 3×10^{-14} g per ml of plasma, and the PrP^{TSE} concentration in infected brain was estimated to be 2×10^{-5} to 6×10^{-5} g per g of brain tissue, which indicated that for hamsters, the amount of PrP^{TSE} in 1 ml of blood was equivalent to the amount present in $\sim 10^{-9}$ g of brain. These biochemical studies measured PrP^{TSE} present as mixtures with different levels of aggregation, but they did not distinguish between the PrP^{TSE} monomer and multimeric forms that might be more infectious (9).

To account for all these different values from the literature, we assumed that a reasonable scaling factor between the infectivities of 1 ml blood and 1 g brain tissue follows a triangular distribution, and we used 10^{-6} (from the transmission study using human tissues by Douet et al. [5]) as the upper bound as well as the most likely value of the distribution. While other biochemical studies reported lower ratios ($<10^{-7}$ for human by Wadsworth et al. [6] and 10^{-9} for hamster by Gregori et al. [7] and Chen et al. [8]) for the PrP^{TSE} concentrations in blood and brain tissues, the correlation between the reported concentrations and infectivity cannot be verified. Therefore, we used the lowest ratio (10^{-9}) from those studies to define the lower bound of the triangular distribution, which contributed much less to the final estimates than the most likely value (10^{-6}) from the study by Douet et al. The median of the triangular distribution (representing the center of the distribution) was used to convert the N_{50} from 9.6×10^{-6} (g of brain) to 13.7 (ml of blood). The parameter N_{50} for the adjusted model can be interpreted to mean that 13.7 ml of infected blood inoculated through the i.c. route has a 50% chance of transmitting infection.

Adjustment for efficiency of disease transmission by the i.v. versus the i.c. route. The DR model for blood inoculated by the i.c. route was further converted into a DR for the i.v. route by multiplying the parameter N_{50} with a scaling factor, which conceptually accounts for the difference in transmission efficiency between the two routes. Experiments with mice exposed to infectivity by the i.v. and i.c. routes suggested an i.c./i.v. ratio of 5 to 7 (10). Based on those experiments, in a previously reported vCJD risk assessment for blood clotting factors, the FDA suggested a uniform distribution, with 1 and 10 being the lower and upper bounds, respectively, for the ratio between the efficiencies of vCJD transmission via the i.c. and i.v. routes (11), which we adopted as the scaling factor for i.c.-to-i.v. conversion in the current study. Multiplied by the median of this uniform distribution, the parameter N_{50} (ID_{50}) was converted from 13.7 for the i.c. route to 75 for the i.v. route (see above).

Hence, the beta-Poisson DR model for blood transfusion has been derived with ml blood as the dose unit and with the optimized parameters of an N_{50} value of 75 and an α value of 0.456 (see above). The N_{50} value represents an ID_{50} equal to 75 ml blood. This model estimates a 76% infection rate (90% CI, 48% to 90% [CI generated by Monte Carlo simulation with 5,000 iterations]) for individuals receiving one unit (450 ml) of blood from a person with clinical vCJD (Fig. 2).

DISCUSSION

Linear DR models were previously adopted for vCJD and BSE risk assessments, (11–13), assuming a linear relationship between the exposure dose of the TSE agent and the probability of infection for

the entire range of doses. However, this linear relationship is certainly a simplification of the true dose-response interaction and does not account for the interactions between the agent and the host in the infection process, which may affect the final outcome. Nonlinear DR models were previously applied by Gravenor et al. (14) and by Huang (15) to data for hamsters orally exposed to scrapie agent (16), but no adjustments were made for the scenario of human transfusion-transmitted TSEs. In this study, we used a beta-Poisson DR model developed based on data for nonhuman primates to estimate the probability of vCJD infection, or TTvCJD risk, as a function of the volume of blood transfused. We believe this to be the first mechanistic DR model for TTvCJD generated by using data from experimental animal dosing studies.

Gregori et al. (17) analyzed TTvCJD data from the TMER study (2) and estimated that transfusion of a unit of infected nonleukoreduced red blood cells had a 75% infection rate (90% CI, 56% to 96%). The intervals between donation and clinical onset of vCJD in the implicated donors ranged from 1.4 years to 3.5 years. Given that the estimated median IP of food-borne vCJD is about 12 years, it seems that those donors whose blood transmitted the disease donated blood during the last one-third of the IP (17). Thus, the infection rate per unit transfused suggested previously by Gregori et al. represented the risk from blood of clinically normal donors during the later stage of the IP. Our model predicts an infection rate of 76% (90% CI, 48% to 90%) associated with one unit of whole blood transfused. If adjusted for a unit of nonleukoreduced red blood cells (equivalent to 24% to 30% of the infectious dose of whole blood [18, 19]), the rate would be 58% (90% CI, 20% to 78%). The DR model predictions based on nonhuman primate experiments and the results previously reported based on the human epidemiological study appear to be consistent. On the other hand, the ID_{50} (75 ml infected blood or equivalent blood products) given by this primate model is significantly higher than that based on rodent data (0.5 ml infected blood) (10, 11, 18). This implies that although studies of BSE and vCJD in rodents have provided important insights into host susceptibility, efficiency of transmission for different routes, and distribution of tissue infectivity, extrapolation of a dose-response model for humans from rodent data may overestimate the risk. A DR study using species more closely related to humans, e.g., a primate model, may be more relevant.

Nevertheless, due to the limited data available, our analysis necessarily incorporated the following assumptions. (i) The inocula used in the primate experiments were primarily infected brain tissues of sCJD patients, and we assumed that those inocula are a relevant surrogate model for vCJD-infected brains. Rubenstein and Chang (20) compared the tissue distributions and levels of PrP^{TSE} in patients with sCJD and vCJD; they reported no significant differences between the two TSEs in levels of PrP^{TSE} in both brain and other tissues (spleens, lymph nodes, and tonsils). While those findings support our assumption, uncertainties remain about whether infectivity titers in tissues and blood of patients with sCJD and vCJD are also similar. (ii) Different species of primates were used in the experiments. While we assumed all primate species tested followed the same dose-response pattern and could therefore be pooled into one model, such an assumption could not be validated. (iii) Extrapolation of the conversion rate of the i.c. versus the i.v. transmission route was based on imperfect and limited data and may not be precise. (iv) We assumed the ratio between PrP^{TSE} and infectivity in blood to be the same as that in brain. (v) Calculations to convert infectivity from weight of tissue to volume of blood were based on different studies, each with its own uncertainties. (vi) The current model ignores the impact of individual genotypes on susceptibility to vCJD. (vii) Early diagnosis, before symptoms of vCJD appear, is not yet possible because of the difficulty in identifying small quantities of abnormal prion protein in blood. While our model based on infected tissues from persons with overt disease is likely to apply to clinically healthy persons during the late IP of asymptomatic vCJD, the risk of TTvCJD from donors in an earlier stage of the disease will be lower.

Keeping these limitations in mind, this analysis establishes, for the first time, a plausible DR curve needed to quantify the risk of TTvCJD as a function of the volume of blood transfused. Based on these data, future vCJD risk assessment models for blood transfusion need no longer rely on a single-point estimate of infectivity titers. This dose-response curve can be used to better estimate the magnitude of risk, and the associated uncertainty, from exposures to low doses of a vCJD agent in transfused blood and plasma derivatives. This development should assist regulators to make better-informed science-based policy decisions to protect public health.

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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

2014/2/1 時点で vCJD と診断された 177 症例中、TMER(Transfusion Medicine Epidemiology Review)の研究で 15 症例が輸血を受けていたことが特定された。輸血歴は 1980 年代以前の 4 例は入手できず、1 例は医療履歴に輸血の記録がなかった。10 例において輸血歴が確認されたが、発症時点で輸血された 1 例は除外し、残り 9 例(女性 4 例、男性 5 例)における発症の平均年齢は 42.9 歳で、輸血による感染が確認された 3 例の平均年齢は 57.6 歳、関連が確認されない残りの 6 例の平均年齢は 35.5 歳であった。
これらの症例のうち、1 例は投与された製剤不明であり、残りの 5 例は全体として 116 人の供血者からの血液製剤が投与され、そのうち 112 人は特定されているが臨床的に vCJD を発症したとされる供血者はいない。今まで、特定されている 112 人の供血者のうち 5 人が亡くなっているが、vCJD が死因となった人はなく、また、その他の神経障害を呈した人もいなかった。
輸血で感染したとされる人の内 2 例は、生存中に vCJD の診断基準を満たさず、死体解剖で確認されている。いずれの症例も年齢層が高く（それぞれ 68 歳と 74 歳)、いずれも MRI 脳スキャンで陽性ではなかった。
残りの症例は生存中に vCJD の診断基準を満たしていたが、そのうち 2 例は非典型的な症状を呈しており、vCJD の発症の予測年齢より年齢が高かった。
結論として vCJD とは考えられていない供血者からの輸血を受けたことにより vCJD を罹患する症例が少なからずいることは考えられるが、証拠としては弱く、多くの vCJD 患者における過去の輸血歴が確認できないことから、輸血による感染と認識されていない症例は除外されている。

使用上の注意記載状況・その他参考事項等

重要な基本的注意
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
輸血による vCJD 患者は知られているより多くいる可能性があるとの報告であるが、筆者も証拠としては弱いと述べている。 今後の更なる情報に注意していきたい。	今後とも vCJD 伝播に関する情報等に留意していく。

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Variant CJD and blood transfusion: are there additional cases?

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Background and Objectives In this study, we compare variant Creutzfeldt-Jakob disease (vCJD) cases definitely linked to blood transfusion, those with a history of blood transfusion in which no donor has developed vCJD and primary cases with no history of blood transfusion. The aim is to determine whether there are any differences in the demographics or clinical phenotype in these groups that might suggest additional cases of transfusion transmission of vCJD.

Materials and Methods All cases of vCJD who are old enough to donate blood (i.e. >17 years old) are notified to the UKBTS at diagnosis, regardless of whether they are known to have a blood donation history. A search is then made for donor records and, if found, all components produced and issued to hospitals are identified and their fate determined. Recipient details are then checked against the NCJDRSU register to establish whether there is a match between these individuals and patients who have been diagnosed with vCJD. In the reverse study, attempts are made to trace the donors to all cases reported to have received a blood transfusion and donors' details are checked against the register to determine if any have developed vCJD.

Results Of the 177 cases of vCJD diagnosed in the UK as of 1 February 2014, the TMER study identified 15 cases reported to have received a blood transfusion. Transfusion records were unavailable for 4 of these cases, all pre-1980, and in one other case there was no transfusion recorded in the medical notes. Transfusion records were found for 10 cases. One case transfused at symptom onset was excluded from this analysis. The mean age at onset of symptoms of the remaining nine transfusion recipients (four female and five male) was 42·9 years; 57·6 years in the three known transfusion-transmitted cases and 35·5 years in the six not linked cases. In one of these cases, details of components transfused were unavailable, and the remaining five cases received a total of 116 donor exposures with 112 donors identified, none of whom is known to have developed clinical vCJD. To date, five of the 112 identified donors have died and none was certified as dying of vCJD or any other neurological disorder. Two of the transfusion-transmitted cases did not fulfil diagnostic criteria for probable vCJD during life but were confirmed at post-mortem. Both cases were in the older age range (68 and 74 years, respectively), and neither had a positive MRI brain scan. The remaining cases all fulfilled the criteria for the diagnosis of vCJD in life, but two of these had atypical features and were older than the expected age at onset for vCJD.

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Conclusion In conclusion, it is possible that one or more of the vCJD cases that received a blood transfusion derived from an individual not known to have vCJD were infected by the blood transfusion. However, the evidence for this is weak, and the absence of a past history of transfusion in most cases of vCJD excludes a large number of unrecognised transfusion-transmitted cases.

Key words: blood, transfusion, variant Creutzfeldt-Jakob disease.

Introduction

Variant Creutzfeldt-Jakob disease (vCJD) was identified in 1996 [1] as a zoonotic infection from the dietary transmission of Bovine Spongiform Encephalopathy (BSE) [2]. There have been 177 cases of vCJD diagnosed in the United Kingdom to date [3]. Although over recent years the annual incidence of clinical vCJD cases in the UK has declined, the total number of infected individuals is unknown. A growing body of evidence indicates that there may be a population of asymptomatic 'carriers' of vCJD who may or may not eventually develop symptoms. A retrospective study of lympho-reticular tissue estimated the prevalence of infection in the UK to be 237 per million [4], although a more recent survey suggests a higher prevalence of 1 in 2000 in the general population [5]. These estimates are higher than the number of confirmed cases and indicate that there may be vCJD infected individuals who may have a prolonged or permanent asymptomatic carrier state. This subclinical population poses a major concern for public health, particularly with regard to blood transfusion as, in contrast to other human prion diseases, the transmissible agent in vCJD is present at significant levels in tissues other than the central nervous system [6, 7], including lymphoid tissue.

In 1997, the Transfusion Medicine Epidemiology Review (TMER) was established. This collaborative study between the National CJD Research and Surveillance Unit (NCJDRSU) and the UK Blood Transfusion Services (UKBTS) had the principle aim of investigating whether vCJD was transmissible via blood transfusion. To date, the TMER study has identified three clinical cases of vCJD and one individual dying of intercurrent illness who were infected by non-leucodepleted blood derived from asymptomatic donors who later developed the condition [8]. The subclinical case was an MV heterozygote at codon 129, providing the first indication that individuals with *PRNP* genotypes other than methionine homozygous (MM) could be infected by the vCJD agent [9]. It is unknown whether this individual would have developed clinical disease, although infectivity has been demonstrated in the splenic tissue, but not the brain [10].

There is no validated screening test to identify vCJD infectivity in human blood, and the mainstay of risk reduction is to prevent those at greater risk from acting as blood donors [11]. Concern that the infectious agent may have adapted after cross-species transmission has been moderated by laboratory transmission studies which have suggested that there is no major change in agent properties between primary and secondary cases of vCJD [12].

Evidence of transfusion transmission of vCJD rests on identifying cases with a history of having received a blood transfusion derived from individuals who are also diagnosed with this condition. However, the potential for subclinical infection suggests that it is possible that an infected donor may never develop clinical disease but could nevertheless transmit infection, with the implication that a link between donor and recipient would then not be identifiable. The TMER study has identified seven cases of vCJD who received a blood transfusion, in which none of the linked donors is known to have developed vCJD, including one transfused at the time of onset of symptoms in whom transfusion can therefore be ruled out as the likely source of infection. In this study of vCJD cases, we compare those cases with a history of blood transfusion in which no donor is known to have developed vCJD, with primary cases who have no history of blood transfusion. The aim is to determine whether there are any differences in the demographics or clinical phenotype between these two groups that might suggest additional cases of transfusion transmission of vCJD.

Methodology

The methodology of the UK TMER study has been described previously [8]. All cases of vCJD who are old enough to donate blood (i.e. >17 years old) are notified to the UKBTS at diagnosis, regardless of whether they are known to have a blood donation history. A search is then made for donor records and, if found, all components produced and issued to hospitals are identified and their

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fate determined. Recipient details are then checked against the NCJDRSU register to establish whether there is a match between these individuals and patients who have been diagnosed with vCJD. In the reverse study, attempts are made to trace the donors to all cases reported to have received a blood transfusion and donors' details are checked against the register to determine if any have developed vCJD. Recipient and donor details are flagged with the Health and Social Care Information Centre to establish the date and certified cause of death. The study was granted ethical approval by the local Research Ethics Committee.

Results

Of the 177 cases of vCJD diagnosed in the UK to date, the TMER study has identified 15 cases reported to have received a blood transfusion (Fig. 1). Transfusion records were not available for four of these cases because the year of transfusion predated available records. In one case reported to have received a transfusion, no record of transfusion was found in their medical notes. Transfusion records were identified in 10 cases, including the three clinical cases of vCJD previously reported to have been infected through blood transfusion and 7 not linked cases, one of whom was excluded from this analysis as the blood transfusion occurred at symptom onset. The potential link to blood transfusion in two of the cases has previously been reported [13].

Of the 6 not linked cases of vCJD who received a blood transfusion, component details could not be traced in one case transfused before 1984 and thought to have had four donor exposures (Fig. 1).

In the remaining five cases, 116 components were traced with 112 donors identified, none of whom is known to have developed clinical vCJD. Names of all 112 identified donors were sent for flagging, and of these, 103 were positively identified; a possible trace was made in 4; a further 4 are currently not registered with a Health Authority doctor, and one was not identified. Five of the cohort of 103 flagged donors have died, and the causes of death are shown in Table 1. None was certified as dying of vCJD or indeed any other neurological disorder. The survival period for the donors since the time of transfusion is shown in Fig. 2.

Table 2 shows the age, gender and transfusion characteristics of each transfused case. Nine patients were included in the study, of whom 4 were female and five male. The mean age at onset of symptoms in the whole group was 42·9 years; in the three known transfusion-transmitted cases 57·6 years and in the 6 not linked cases 35·5 years. This compares with a mean age at onset overall in vCJD of 28·9 years (n = 177). The number of donor exposures was in single figures for 5/6 cases not linked to an affected donor and in one of the known cases of transfusion-transmitted vCJD. The other three cases had multiple donor exposures. The interval from transfusion to onset of clinical symptoms in the recipients ranged from 4 to 16 years (mean 9·3 years) with no clear distinction in this interval between cases linked or not linked to an affected donor. The duration of the clinical illness was similar in all cases, with the exception of one of the not linked cases, who received treatment with intraventricular pentosan polysulphate and survived for 45 months [14].

Table 3 details the clinical and investigative features of the cases that are included in the validated diagnostic

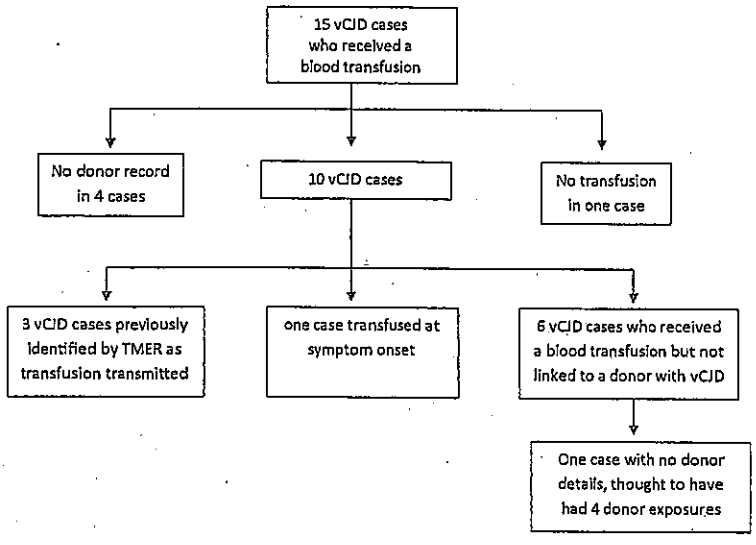

Fig. 1 Outcome of vCJD cases who received a transfusion.

Table 1 Cause of death among 112 donors to the 5 vCJD cases under review

Year of death	Interval from transfusion in index case to death in donor	Cause of death in donor
1994	1 year	Injury to abdominal aorta causing haemorrhage Verdict: Death by Misadventure
2001	8 years	Hypertensive heart disease (Coroner's post-mortem without inquest)
2006	13 years, 4 months	Pulmonary embolism/deep vein thrombosis/ischaemic heart disease (Coroner's post-mortem without inquest)
2008	15 years, 2 months	Bronchopneumonia/disseminated sigmoid colon carcinoma, pulmonary embolism
2012	18 years, 8 months	Complications of heart valve surgery

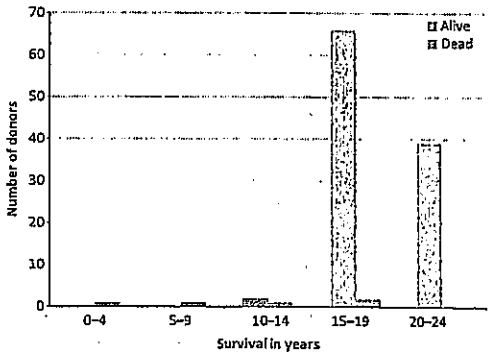

Fig. 2 Donor survival from transfusion in index case (n = 112).

criteria for vCJD. All were MM homozygous at codon 129 of the prion protein gene. Two of the three previously reported transfusion-transmitted cases did not fulfil diagnostic criteria for probable vCJD during life but were confirmed at post-mortem (cases 7 and 8 in Table 3). Both cases were in the older age range (68 and 74 years, respectively), and neither had a positive MRI brain scan. The remaining cases all fulfilled the criteria for the diagnosis of vCJD in life, but two of these had atypical features and were older than the expected age at onset for vCJD. In case 2, the clinical presentation was complicated by concurrent medical conditions that may have contributed to protracted early neurological symptoms. Case 3 had a prodrome of behavioural disturbance for a more extended period than that usually observed in vCJD. The other cases (1, 4, 5 and 6) had a typical clinical phenotype for vCJD including early psychiatric symptoms followed by progressive ataxia and cognitive decline associated with involuntary movements.

Discussion

The aim of this study was to investigate whether there may be unrecognised cases of transfusion-transmitted vCJD in the UK, as blood recipients with vCJD may have been infected by donors who have not yet developed clinical disease. Nine transfusion recipients with vCJD have been identified by the UK surveillance system, and only three have been linked to an affected donor. The six other recipients have not been linked to another case of vCJD, and the question is whether it is plausible that these cases may also have been infected by blood transfusion.

There are some differences between vCJD cases who have received a blood transfusion and those who did not, notably in relation to age at onset of symptoms. The mean age at onset in primary vCJD cases is 28·4 years (n = 174) and in the three transfusion-transmitted cases 57·6 years [8], perhaps reflecting the relatively high mean age of transfusion recipients and the incubation period of transfusion-transmitted vCJD of 6–8 years. In the six recipients with vCJD not linked to an affected donor, the mean age at clinical onset is 35·5 years, which is older than the mean age in primary cases, and raises the possibility that some of these cases may be caused by blood transfusion. Two of these cases were aged 47 and 50 years at onset of symptoms, and only 11% of vCJD cases have been aged over 45 years at death. However, the age differences between the groups are not statistically significant, perhaps reflecting the small numbers of transfusion recipients in this study.

The interval between transfusion and symptom onset in the 6 not linked transfusion recipients ranged from 4 to 16 years. Although only three of our cases have strong evidence to support transfusion–transmission, it is possible that some of the remaining six cases in this study could have been infected via blood transfusion, as the potential incubation periods in these cases were of the same order as the three cases known to be linked to transmission via blood transfusion [8]. Three of the six cases not linked to an infected donor had potential incubation periods which were more extended than the observed incubation periods in transfusion–transmission of vCJD, but more extended incubation periods may be expected, reflecting the incubation period distribution. On the other hand, the great majority of donors remain alive more than 20 years after the implicated donation (Fig. 2), which contrasts with the observed transfusion–transmission cases in which the donors died within 4 years. The recent population prevalence study indicates that all codon 129

Table 2. Characteristics of vCJD cases that received a blood transfusion

Cases	Age at symptom onset (Years)	Gender	Transfusion	Number of donor exposures	Transfusion to symptom onset	Duration of illness (Months)
1	32	Female	1 (12/1982)	2	13 years, 1 month	23
2	41	Male	1 (07/1993)	38	4 years, 9 months	7
			2 (10/1993)	65	4 years, 6 months	
3	50	Female	1 (09/1983)	2	15 years, 10 months	11
			2 (04/1993)	3	6 years, 3 months	
4	27	Male	1 (06/1994)	4	5 years, 4 months	11
5	16	Female	1 (02/1989)	4[a]	16 years, 9 months	16
6	47	Female	1 (02/2002)	2[b]	5 years, 11 months	45
7[c]	68	Male	1 (03/1996)	5	6 years, 6 months	13
8[d]	74	Male	1 (12/1997)	23	8 years, 4 months	11
9[c]	31	Male	1 (09/1997)	56	7 years, 10 months	10

[a]Component details not identified.
[b]This is the only case that received leucodepleted red cells.
[c,d,c]Cases of vCJD following transfusion from donors who later developed the condition.

Table 3. Clinical and investigative features of cases that received a blood transfusion

Case	Early psychiatric symptoms	Ataxia	Sensory symptoms	Involuntary movements	EEG	MRI	Post-mortem
1	Yes	Yes	No	Yes	Slow	+	Yes
2	Possibly[a]	Yes	No	1 episode	Slow	+	Yes
3	Yes but unusual[b]	Yes	Yes	Yes	Slow	+ (2nd)	No
4	Yes	Yes	No	Yes	Slow	+	Yes
5	Yes	Yes	No	Yes	Normal	+ (2nd)	Yes
6	Yes	Yes	Yes	Yes	Slow	+	Tonsil biopsy only
7[c]	Yes	No	Yes	Yes	Slow	–	Yes
8[d]	No	Yes	No	No	Slow	n	Yes
9[c]	Yes	Yes	Yes	Yes	Normal	+ (2nd)	Yes

[a]This case was older than expected for vCJD and has typical clinical features, but assessment was complicated by concurrent medical conditions.
[b]Unusual presentation with significant behavioural disturbance for 2 years followed by unexplained blackouts and falls. Progressive cognitive decline, ataxia, paranoid and aggressive behaviour followed 5 months later. There were no clear painful sensory symptoms until later in the illness. This patient's age and initial symptoms make this case an unusual presentation for vCJD.
[c,d,c]Cases of vCJD receiving transfusion from 2 donors who later developed the condition.

genotypes are represented in the presumed infected general population [5], and variation at this site may influence incubation period. The codon 129 genotypes of the blood donors in this study are unknown. It is, however, of note that five of these donors have died, between 1 and 18 years following blood donation, and all were certified as dying of non-neurological conditions. In conclusion, the interval between transfusion and onset of clinical symptoms in the recipients is not inconsistent with the known incubation periods in transfusion-transmitted vCJD and the extended survival following donation in the great majority of donors does not exclude the possibility of transfusion–transmission.

The clinical phenotype in the six cases not linked to a known infected donor is relatively typical for vCJD. Two cases had an unusually extended neuropsychiatric prodrome, but in one assessment was complicated by concurrent illness, and similar cases have been identified in the cohort of primary cases. The clinical and pathological phenotype in the three transfusion-transmitted cases is

also consistent with primary cases of vCJD, and this is perhaps not surprising as preliminary evidence from laboratory transmission studies does not suggest any major change in the agent characteristics after transfusion–transmission [12]. This suggests that scrutiny of clinical phenotype may not be useful in the determination of route of infection in vCJD.

In conclusion, it is possible that one or more of the vCJD cases that received a blood transfusion derived from donors not known to have developed vCJD were in fact infected by the blood transfusion. However, the evidence for this is weak and the epidemiological evidence from observed cases of vCJD excludes a large number of unrecognised transfusion-transmitted cases as the great majority of cases have no history of prior blood transfusion. The mismatch between the large cohort of presumed infected individuals in the general population, suggested by prevalence studies, a proportion of whom will be blood donors, and the very small number of identified cases of transfusion-transmitted vCJD emphasises the need for careful continued surveillance.

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研究報告の概要	使用上の注意記載状況・その他参考事項等
ヒツジスクレイピープリオンの潜在的人獣共通感染のエビデンス 牛海綿状脳症（BSE）はヒトにおける変異型クロイツフェルト-ヤコブ病（vCJD）の原因であるが、スクレイピープリオンの人獣共通感染の可能性は分かっていない。ヒトプリオンたん白質（tgHu）を過剰発現するように遺伝的に操作したマウスは、ヒトに伝播するプリオンの感染性を測るための現実に近いモデルとして登場した。これらのモデルは、あらゆる見かけの伝播障壁なしにヒトのプリオンを増殖することができ、BSE の人獣共通感染を確認するために使われている。ここでは、ヒツジスクレイピープリオンのパネルがウシ BSE のそれと同等の効率で、幾つかの tgHu マウスモデルに伝播することを示している。別のスクレイピーのシリアル伝播は、ヒトの孤発性 CJD（sCJD）を引き起こすものと表現型が同一であるプリオンに感染したこれらのマウスで分離した。これらの結果は、スクレイピープリオンが人獣共通感染の可能性があり、動物とヒトプリオンの間の結合の可能性についての新たな疑問を提起することを示している。	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)略 1)略 2)現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的な vCJD 等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

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Evidence for zoonotic potential of ovine scrapie prions

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Although Bovine Spongiform Encephalopathy (BSE) is the cause of variant Creutzfeldt Jakob disease (vCJD) in humans, the zoonotic potential of scrapie prions remains unknown. Mice genetically engineered to overexpress the human prion protein (tgHu) have emerged as highly relevant models for gauging the capacity of prions to transmit to humans. These models can propagate human prions without any apparent transmission barrier and have been used used to confirm the zoonotic ability of BSE. Here we show that a panel of sheep scrapie prions transmit to several tgHu mice models with an efficiency comparable to that of cattle BSE. The serial transmission of different scrapie isolates in these mice led to the propagation of prions that are phenotypically identical to those causing sporadic CJD (sCJD) in humans. These results demonstrate that scrapie prions have a zoonotic potential and raise new questions about the possible link between animal and human prions.

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Transmissible spongiform encephalopathies (TSE), or prion diseases, are fatal neurodegenerative disorders that affect a large spectrum of mammalian species. These conditions include for instance, scrapie in small ruminants, bovine spongiform encephalopathy (BSE) in cattle and chronic wasting disease (CWD) in wild cervids. In humans, the most common form of TSE is sporadic Creutzfeldt-Jakob disease (sCJD), which affects ~1–2 individuals per million of the population per year and is generally observed in people aged over 50 years. In the absence of identified external causes sCJD is considered to be a spontaneous disorder precipitated by mis-folding of the normal cellular prion protein (PrP^C)[1].

It has been known for several centuries that scrapie is endemic in small ruminant animals used for human food production[2]. Despite this dietary exposure, epidemiological studies have failed to identify any clear link between scrapie and TSE occurrence in humans[3,4]. This apparent lack of zoonotic transmission by scrapie is considered to be a consequence of the transmission barrier phenomenon that naturally limits the propagation of prions from one species to another[5]. In 1996, a new human prion disease, referred as variant CJD (vCJD), was observed in UK individuals. Several lines of evidence have indicated that vCJD is the probable consequence of dietary exposure of humans to the agent responsible for epizootic of bovine spongiform encephalopathy (BSE) in UK cattle[6]. The occurrence of vCJD has provided significant evidence to show that the transmission barrier does not constitute an absolute protection against the zoonotic risk of prions that circulate in animal populations. This suggests that the need for an in-depth assessment of the permeability of the human species barrier to animal TSE agents including those responsible for scrapie in sheep.

The potential diversity of scrapie prions has been investigated over many years by serial transmission of field isolates to the natural host of this condition, inbred mouse lines or as more recently to bank voles and mice with transgenic expression of different PrP sequences[5,7]. On the basis of the transmission features in conventional mice (incubation periods, distribution of the lesions in the brain), early transmission experiments described a considerable diversity of the scrapie agents with up to 20 prion strains reportedly identified[8]. However, more recent strain typing in transgenic mouse lines that express ovine PrP has identified a more restricted range that comprises at least four phenotypically distinct scrapie strains[5,9–12]. Despite converging evidence that scrapie is caused by a variety of prion strains, there is at this stage no comprehensive description of their diversity.

A fundamental event in prion propagation is the conversion of the normal cellular prion protein (PrP^C, which is encoded by the *PRNP* gene) into an abnormal disease-associated isoform (PrP^{Sc}) in tissues of infected individuals. PrP^C is completely degraded by digestion with proteinase K (PK), whereas PrP^{Sc} is N terminally truncated resulting in a PK resistant core, termed PrP^{res} (ref. 13). According to the prion concept, PrP^{Sc} is the principal if not sole component of the prion agent[14] and PrP^{res} is a disease marker for prion diseases[13,15]. Particular biochemical properties of PrP^{Sc}, such as detergent solubility, PK resistance and electromobility in western blotting (WB) can be used to distinguish between different prion agents or strains[16,17].

After identification of the gene encoding PrP, it was soon discovered that differences in amino-acid sequence between host PrP^C and donor PrP^{Sc} were the main driver for the transmission barrier. For example, the resistance of wild-type mice to clinical disease induced by hamster scrapie is abrogated by transgenic expression of hamster PrP^C in mice[18,19]. However, it is also established that strain properties have a significant impact on the ability of prions to cross the species barrier. This is evidenced by studies showing that human vCJD isolates can be transmitted readily to conventional mice but that it is extremely difficult for sCJD isolates to propagate in the same mouse lines[20,21]. Furthermore, the amino-acid sequence of PrP^{Sc} influences the efficacy of interspecies prion transmissions as tgHu models indicate that the human species barrier is more permeable to sheep-passaged BSE compared with its cattle counterpart[22].

Conceptually, the permeability of the prion transmission barrier is considered to be driven at the molecular level by the conformational compatibility between host PrP^C and the misfolded strain-specific protein assemblies of PrP^{Sc} that are present within infectious prion particles[23]. As a consequence, mice genetically engineered to express human PrP (tgHu), in the absence of endogenous mouse PrP, have emerged as relevant animal models to assess prion transmission across the human species barrier[24]. A major determinant of the susceptibility to human prion disease is Met/Val dimorphism at codon 129 of the *PRNP* gene[25,26], and tgHu mice lines exist that express these variants of human PrP[27]. These tgHu mouse lines propagate human prions without an apparent species barrier and have been successfully used to confirm the zoonotic ability of the BSE agent from cattle[6,22,28]. In addition, prion transmission studies in tgHu mouse lines have highlighted that L-type atypical BSE in cattle displays a higher capacity to cross the human species barrier than does classical BSE in cattle, the prion strain responsible for vCJD emergence[29,30]. In the context of the Met/Val dimorphism in human PrP^C and the potential diversity of sheep scrapie strains, it is clear that assessment of the permeability of human species barrier to ovine prions presents a considerable challenge[8,31].

In this study, we inoculated tgHu mice transgenic for expression of human PrP codon 129 variants intracerebrally with a panel of (i) biologically distinct ovine scrapie isolates, (ii) human prions and (iii) cattle BSE prions. The serial transmission of different scrapie isolates in tgHu mice led to the propagation of prions that were phenotypically identical to those that cause sporadic CJD (sCJD) in humans.

Results

Scrapie isolates transmit in tgHu mice. We initially performed serial transmission studies in three tgHu mouse lines. These mouse lines were homozygous for methionine (tg Met_{129}/ tg340) or valine (tgVal_{129}/tg361) at codon 129 of human PrP, or were their F1 cross (methionine/valine heterozygotes at codon 129; tgMet/Val_{129}). All of these mouse lines expressed human PrP approximately fourfold more compared with that seen in normal human brain tissue (Fig. 1). The three mouse lines were inoculated intracerebrally with a panel of six sheep scrapie isolates that had been collected between 1994 and 2002 from flocks naturally affected with this prion disease (Table 1). The scrapie isolates were selected on the basis of the different transmission profiles they showed when inoculated in murine (tga20), bovine (tgBov/ tg110) and ovine (tgShpXI and tg338) PrP transgenic mouse lines (Fig. 2), which were indicative that they contained different ovine prion strains[5,27].

At completion of the first passage, none of the challenged tgHu mice developed clinical disease (Table 1). However, western blot analysis showed weak but consistent disease-associated PK resistant PrP (PrP^{res}) accumulation in the brains of two tgMet/ Val_{129} mice inoculated with MF17 scrapie isolate (Table 1, Fig. 3a). We considered the PrP^{res} was not due to residual inoculum but rather to the conversion of tgHu PrP^C into PrP^{Sc}. Consequently, first passage mouse brains from each scrapie isolate transmission group were pooled and re-inoculated to the same tgHu line (Table 1). Following second passage of sheep scrapie in the tgHu mouse lines clinical disease was observed in a

proportion of the tgMet$_{129}$ and tgMet/Val$_{129}$ mice inoculated with four of the isolates (Table 1). Western blot analysis confirmed the accumulation of PrP^{res} in the brain of these second passage mice (Fig. 3b). There were no clinical signs of prion disease or PrP^{res} accumulation observed in control mice inoculated with PBS or prion-free control brain homogenate, which had also undergone two serial passages (Table 1). These controls confirmed that the prion disease observed in inoculated tgHu mice inoculated with the sheep scrapie isolates was not the consequence of a spontaneous PrP conversion and that cross-mouse contamination was unlikely.

Relative efficacy of scrapie and BSE transmission in tgHu. To determine the susceptibility of tgHu mice to a prion strain with known zoonotic potential, we inoculated human PrP mouse lines with two different cattle BSE isolates. At first passage there was either an absence or an inefficient transmission of bovine prions in tgMet$_{129}$ and tgMet/Val$_{129}$ mice (Table 1), although the attack rate significantly increased at second passage. No transmission or brain PrP^{res} accumulation was observed in tgVal$_{129}$ mice inoculated with either of the two BSE isolates (Table 1). These results concur with the commonly held view that a substantial species barrier limits the transmission of cattle BSE to humans[6,22,28]. The data also support the contention that the relative permeability of the human species barrier (as modelled here by the intracerebral transmission in tgHu mice) to cattle BSE and scrapie is not fundamentally different.

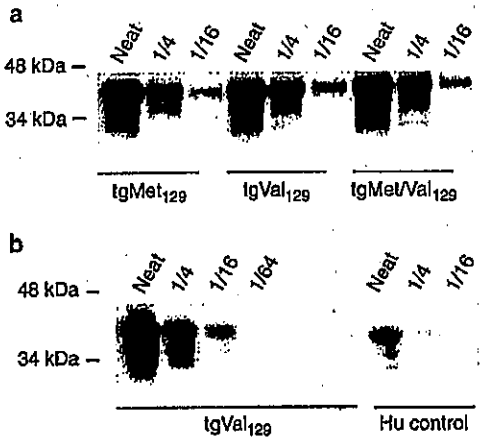

Figure 1 | PrP^{C} expression in tgHu mice. Brain homogenate dilutions (neat or 1/4, 1/8, 1/16 diluted in Laemmli's buffer) were analysed by SDS-PAGE and western blot using 12% acrylamide gel. Normal PrP was detected using the monoclonal antibody 3F4 (0.8 μg ml^{-1}). (a) tgMet$_{129}$, tgMet/Val$_{129}$ and tgVal$_{129}$ samples. (b) tgVal$_{129}$ and human control brain samples.

Impact of scrapie adaptation in bovine PrP transgenic mice. Sheep scrapie prions can be propagated experimentally in cattle and the hypothesis that BSE is derived from cattle exposure to scrapie remains a prominent theory[32–34]. Moreover, PrP^{Sc} amino-acid sequence can strongly influence cross-species prion transmission[22,23]. In this context, the isolates that we obtained after two passages of sheep scrapie isolates in bovine PrP transgenic mice (tgBov) (Fig. 2) were also inoculated into tgMet$_{129}$ tgHu mice (Table 2). On the first passage, clinical signs were observed in a proportion of tgMet$_{129}$ mice inoculated with each of the tgBov-adapted sheep scrapie isolates. Similar transmission properties were also observed with scrapie isolate PS48 that failed to propagate in tgHu mice after two passages (Table 1). These observations support the contention that the adaptation of ovine scrapie prions to the bovine PrP amino-acid sequence increases their capacity to propagate in tgHu mice.

Lack of transmission barrier for human prions in tgHu mice. In parallel to the inoculation with sheep scrapie isolates, we also inoculated the tgHu mice with human brain material from one vCJD and two sCJD patients. The sCJD cases were classified as Met/Met type 1 (MM1) and Val/Val type 2 (VV2), respectively [35], based on the patient's *PRNP* genotype at codon 129 and on the PrP^{res} western blot profiles of these samples. On first passage, 100% of the tgHu mice inoculated with the two sCJD isolates

Table 1 | Intracerebral inoculation of tgHu mice with a panel of human, bovine and ovine prion isolates.

Isolate	Origin	Tg Met/Met$_{129}$				TgMet/Val$_{129}$				TgVal/Val$_{129}$			
		Passage 1		Passage 2		Passage 1		Passage 2		Passage 1		Passage 2	
		Positive mice	Incubation (d.p.i. ± s.d.)	Positive mice	Incubation (d.p.i. ± s.d.)	Positive mice	Incubation (d.p.i. ± s.d.)	Positive mice	Incubation (d.p.i. ± s.d.)	Positive mice	Incubation (d.p.i. ± s.d.)	Positive mice	Incubation (d.p.i. ± s.d.)
sCJD MM1	Hu	6/6	219 ± 17	6/6	239 ± 8	6/6	243 ± 14	6/6	260 ± 13	6/6	327 ± 19	6/6	286 ± 16
sCJD VV2	Hu	6/6	618 ± 81	6/6	509 ± 41	6/6	588 ± 74	6/6	594 ± 86	6/6	168 ± 12	6/6	169 ± 12
vCJD	Hu	6/6	595 ± 25	6/6	581 ± 45	2/6	758, 801	6/6	615 ± 65	0/6	>750	0/6	>750
BSE	Bov	0/6	>750	3/6	572 ± 64	0/6	>750	NA		0/6	>750	0/6	>750
BSE	Bov	1/6	739	6/6	633 ± 32	0/6	>750	NA		0/6	>750	0/6	>750
MF17	Ov	0/6	>750	NA		2/6	743*, 760*	3/6	343, 374, 665	0/6	>750	0/6	>700
PS09	Ov	0/6	>750	1/6	432	0/6	>750	0/6	>600	0/6	>750	0/6	>700
PS21	Ov	0/6	>750	2/6	369,579	0/6	>750	0/6	>700	0/6	>750	0/6	>700
PS48	Ov	0/6	>750	0/6	>750	0/6	>750	0/6	>700	0/6	>750	0/5	>700
PS42	Ov	0/6	>750	1/6	475	0/6	>750	0/6	>700	0/6	>750	0/6	>700
PS310	Ov	0/6	>750	NA		0/6	>750	NA	-	0/6	>750	NA	
Negative brain	Hu	0/12	>750	0/12	>750	0/12	>750	0/6	>650	0/12	>750	0/6	>750
	Ov	0/12	>750	0/12	>750	0/12	>750	0/6	>650	0/12	>750	0/6	>750
PBS control		0/18	>800	0/12	>650	0/12	>750	0/6	>650	0/12	>750	0/6	>750

Transgenic mice that express the Met$_{129}$, Val$_{129}$ human PrP and their cross bred were inoculated intra-cerebrally (20 μl per mouse) with a 10% brain homogenate from (i) sporadic Creutzfeldt-Jakob (sCJD) variant Creutzfeldt Jakob (vCJD) methionine homozygous patients, (ii) bovine (Bov) spongiform encephalopathy-affected cattle, (iii) ovine (Ov) scrapie isolates (MF17, PS09, PS21, PS48, PS42 and PS310). After first-passage, clinically affected or asymptomatic mice that survived >500 d.p.i. were pooled and used for second passage in the same line. Mice were considered positive when abnormal PrP deposition was detected in the brain.
Incubation periods (days post inoculation: d.p.i.) are shown as mean ± s.d. except when <50% of mice were found to be PrP^{res}-positive. In that latter case, the incubation period of PrP^{res}-positive mice is individually presented. Control mice were inoculated with negative brain tissue or PBS controls. sCJD and scrapie isolates were inoculated into mice in different rooms of the animal facilities. (NA): still ongoing bioassay, results not available.
*Indicates PrP^{res} and found dead animals (asymptomatic).

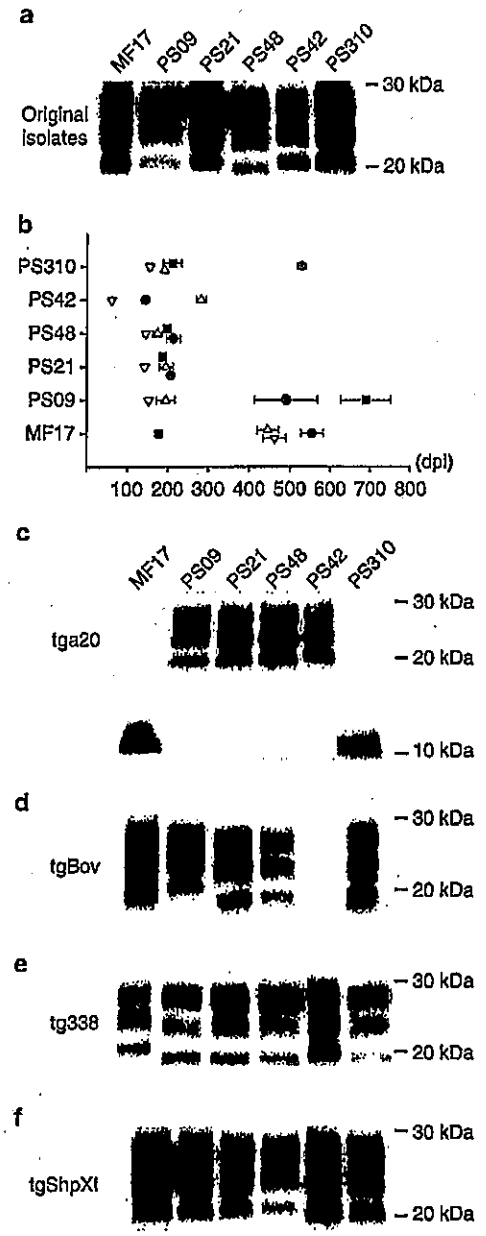

Figure 2 | Transmission properties of scrapie isolates in PrP transgenic mice. (**a**) The PrP^{res} western blot pattern of six different natural sheep scrapie isolates (MF17, PS09, PS21, PS48, PS42 and PS310) was established using a monoclonal antibody Sha31. This panel of scrapie isolates was inoculated in transgenic mice ($n=6$ per group) that express the murine (tg20-grey circle), the bovine (tgBov-black square), the ovine $A_{136}R_{154}Q_{171}$ (tgshpXI-triangle up) and the ovine $V_{136}R_{154}Q_{171}$ (tg338-triangle down) PrP variants. For each isolate two serial passages were carried out: (**b**) the incubation period (mean ± s.d.) as days post inoculation (dpi) of the second passage in the different mouse lines and (**c–f**) the PrP^{res} western blot profiles (10% brain homogenate) observed after two passages in each of the mouse lines.

developed clinical disease while the vCJD isolate was only propagated in $tgMet_{129}$ and $tgMet/Val_{129}$ mice (Table 1). Differences in incubation periods (Table 1) together with PrP^{res} molecular profile and PrP^{res} distribution patterns in the tgHu mouse brains concurred with the view that the vCJD, sCJD MM1 and sCJD VV2 isolates contained biologically distinct prions (Figs 3c and 4). As expected, there was no reduction in incubation period during the second passage of sCJD and vCJD isolates in tgHu mice, which indicated the absence of a measurable species barrier between these human prions and the human PrP transgenic mice.

Scrapie and sCJD display identical phenotypes in tgHu mice. The molecular profile of disease-associated PrP that accumulated in scrapie inoculated PrP^{res}-positive tgHu mice was distinct from that observed in the original sheep isolates. In contrast, the PrP^{res} that accumulated in tgHu mice after inoculation with either sCJD or scrapie shared a similar molecular profile (Fig. 3b). These data suggested that transmission of sCJD and sheep scrapie prions in mice that expressed human PrP might have resulted in the propagation of same prion strain. To confirm this, brain homogenates prepared from clinical prion diseased tgHu mice inoculated with either sheep scrapie (Table 3) or sheep scrapie adapted in tgBov (Table 2) were re-passaged in $tgMet_{129}$ and $tgVal_{129}$ mouse lines. All the inoculated samples induced a 100% transmission attack rate in both tgHu mouse lines. The incubation periods (Tables 2 and 3), together with the molecular profile (Fig. 3d) and distribution of PrP^{res} (Fig. 4) in the brains of these mice, were similar to those observed in similar animals inoculated with either VV2 or MM1 sCJD isolates. Strikingly, the transmission profiles of the majority of sheep scrapie prions in tgHu mice displayed a phenotype that was identical to MM1 sCJD. However, one of the sheep scrapie isolates that had been adapted in tgBov (PS310, Table 2) showed the same phenotype as the VV2 sCJD isolate (Figs 3d and 4).

Similar results are obtained in a different tgHu mouse line. To rule out the possibility that the phenomena we observed were a consequence of the particular tgHu mouse lines used, we inoculated a subset of the panel of *bona fide* sheep scrapie isolates into tg650 mice, which overexpress (approximately six fold) human PrP Met_{129} (ref. 29). In a manner similar to that seen in the Met_{129} tg340 mouse line, no clinical disease was observed on first passage of the sheep scrapie isolates in Met_{129} tg650 mice (Table 4). However, two tg650 mice (inoculated with two different scrapie isolates) were western blot-positive for PrP^{res} in the brain. The second passage in tg650 mice of isolate PS21 (using brain homogenate prepared from a PrP^{res} positive mouse on first passage) resulted in a 100% transmission attack rate. After third passage in tg650 mice, the incubation period of PS21 was 155 ± 3 days, which was identical to MM1 sCJD in this mouse line[36]. The molecular profile and distribution of PrP^{res} in the brain of tg650 mice inoculated with PS21 were identical to those observed in the same mouse line after inoculation with sCJD MM1 prions (Fig. 5).

Discussion

Historically, non-human primate models were considered as the reference model for testing the permeability of the human species barrier with regards to prion transmission[37,38]. The successful transmission of cattle BSE prions to marmosets[39] and cynomolgus macaques[40] was considered as a strong argument in support of its zoonotic potential. While data related to the transmission of ovine scrapie in primates remain very limited, the intracerebral challenge of marmosets with one sheep scrapie isolate (PG 85/02) induced a typical prion disease with a shorter incubation time than that observed after challenge with cattle BSE[39], suggesting an equivalent or higher ability of sheep scrapie

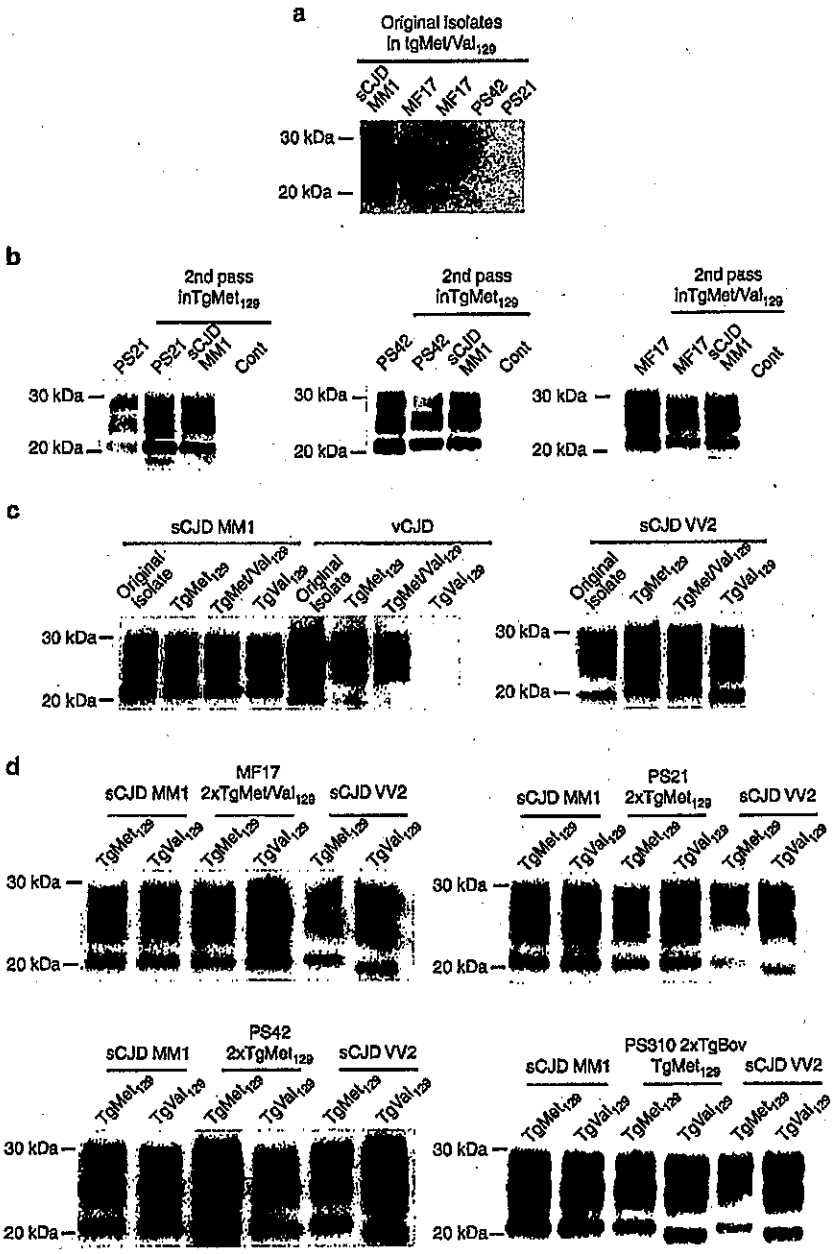

Figure 3 | PrP^{res} detection in scrapie-inoculated tgHu mice. $TgMet_{129}$, $tgVal_{129}$ and their F1 cross tgMet/Val_{129} mice were inoculated ($n=6$ per group) with (**a**) various natural sheep scrapie isolates (see Table 1- MF17, PS21, PS42 and PS310); and (**b**) the same scrapie isolates after primary passage in tgHu mice. (**c**) The same mouse lines were challenged with MM1 and VV2 sporadic Creutzfeldt-Jakob disease (sCJD) and variant CJD (vCJD) isolates. (**d**) $tgMet_{129}$ and $tgVal_{129}$ mice were inoculated with scrapie isolates that had been previously passaged twice in human PrP ($tgMet_{129}$ or tgMet/Val_{129}) or bovine PrP transgenic mice (tgBov, see Tables 3 and 4). PrP^{res} detection was carried out by SDS-PAGE and western blot with an anti-PrP monoclonal antibody Sha31.

to cross the species barrier. In contrast, other experiments failed to demonstrate the transmissibility of scrapie in non-human primates[41]. However, divergence exists between such these non-human primate and human PrP amino-acid sequences, which only show 96 to 99% homology. Furthermore, a number of experimental transmissions in PrP transgenic or wild-type animals indicate that one single amino-acid difference in the PrP sequence can markedly alter the issue of cross-species transmission[42,43]. In our view, these observations raise some concerns with regards to the relevance of primates as a model of human species barrier.

The transmission experiments reported here unambiguously show that sheep scrapie prions propagate in mice that express variants of human PrP. While the efficiency of transmission at primary passage was low, subsequent passages resulted in a highly virulent prion disease in tgHu Met_{129} and Val_{129} mice. Transmission of different scrapie isolates in tgHu mice leads to the emergence of prion strain phenotypes that showed similar

Table 2 | Intracerebral inoculation of transgenic mice that express human PrP with ovine scrapie isolates previously adapted in bovine PrP transgenic mice.

Isolate	Origin	First passage		Second passage			
		Tg Met$_{129}$		Tg Met$_{129}$		Tg Val$_{129}$	
		Positive mice	Incubation (d.p.i. ± s.d.)	Positive mice	Incubation (d.p.i. ± s.d.)	Positive mice	Incubation (d.p.i. ± s.d.)
sCJD MM1	Hu	6/6	219 ± 17	6/6	239 ± 8	6/6	298 ± 10
sCJD VV2	Hu	6/6	618 ± 81	6/6	509 ± 41	6/6	182 ± 9
vCJD	Hu	6/6	595 ± 25	6/6	581 ± 45	0/6	>750
PS48	TgBov	1/6	453	6/6	230 ± 16	6/6	305 ± 4
PS310	Tg Bov	1/6	630	5/5	492 ± 27	6/6	208 ± 5
PS09	Tg Bov	2/6	407, 700*	NA		NA	
PS21	Tg Bov	1/6	499	NA		NA	

Tg Met$_{129}$ mice were intracerebrally challenged with scrapie isolates (PS48, PS310, PS09, PS21) that had previously adapted into bovine PrP transgenic mice (two successive passages). In groups inoculated with PS48 and PS310 scrapie isolate one symptomatic TgMet$_{129}$ (showing abnormal PrP accumulation in its brain) was selected and its brain used for inoculating TgMet$_{129}$ and TgVal$_{129}$. Incubation periods (days post inoculation: d.p.i.) are shown as mean ± s.d. NA: still ongoing bioassay, results not available.
*Indicates PrP^{res}positive and found dead animals (asymptomatic).

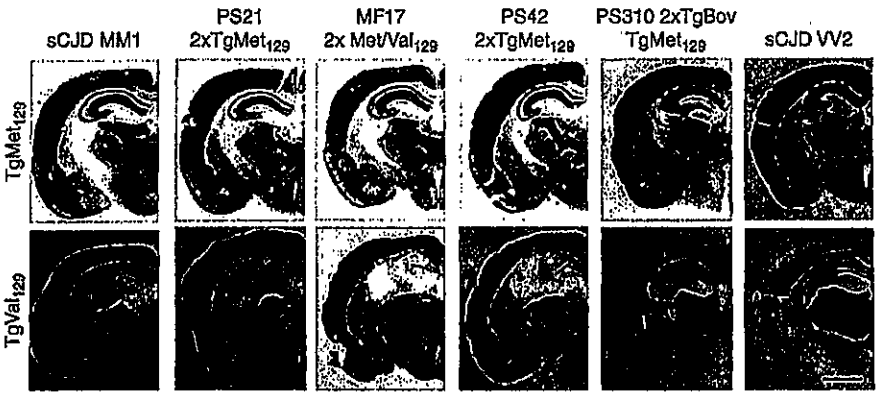

Figure 4 | Brain PrP^{res} deposition pattern in sCJD and scrapie inoculated tgHu mice. TgMet$_{129}$ or tgVal$_{129}$ mice were inoculated with MM1 and VV2 sCJD isolates. The same mouse lines were challenged with different natural sheep scrapie isolates (see Table 1-MF17, PS21, PS42 and PS310) that had been serially transmitted (two passages) in tgMet$_{129}$, tgMet/Val$_{129}$ (see Table 1) or in mice that expressed bovine PrP (tgBov, see Tables 2 and 3). PK-resistant PrP detection was carried out by PET blot using an anti-PrP monoclonal antibody Sha31. Scale bar, 150 µm.

Table 3 | Intracerebral inoculation of tgHu mice with human sCJD cases and sheep scrapie isolates adapted (two passages) in tgMet$_{129}$ mice or tgMet/Val$_{129}$.

Isolate	Origin	Tg Met$_{129}$		Tg Val$_{129}$	
		Positive mice	Incubation (d.p.i. ± s.d.)	Positive mice	Incubation (d.p.i. ± s.d.)
sCJD MM1	Met/Met$_{129}$ patient	6/6	219 ± 17	6/6	318 ± 20
	Tg Met$_{129}$	6/6	239 ± 8	6/6	298 ± 10
sCJD VV2	Val/Val $_{129}$ patient	6/6	618 ± 19	6/6	168 ± 12
	Tg Met$_{129}$	6/6	581 ± 45	6/6	182 ± 9
MF17	TgMet/Val$_{129}$	6/6	206 ± 3	6/6	283 ± 6
PS21	Tg Met$_{129}$	6/6	214 ± 2	6/6	283 ± 1
PS42	Tg Met$_{129}$	6/6	213 ± 10	6/6	291 ± 3

After two passages in tgMet$_{129}$ (scrapie isolates PS21 and PS42) or in tg Met/Val$_{129}$ (scrapie MF17) mice, the brain from one clinically affected mouse per isolate (showing PrP^{res} accumulation in the brain) was used to inoculate fresh groups of tgMet$_{129}$ and tgVal$_{129}$ mice. sCJD cases (Met/Met$_{129}$ type 1 and Val/Val$_{129}$ type 2) and the brain material obtained after their passage in tg Met$_{129}$ mice were used as controls. Incubation periods (days post inoculation: d.p.i.) are shown as mean ± s.d.

characteristics to those displayed by MM1 or VV2 sCJD prion isolates passaged in the same mouse lines. Change in phenotype is a common phenomenon associated with the transmission of prions across a species barrier[44,45]. Our observation that phenotypically similar prion strains emerged following transmission of different sheep scrapie isolates in tgHu mice is consistent with the concept that the range of PrP^{Sc} conformations, which are considered to reflect prion strain diversity, is limited for a given PrP sequence. This restriction on PrP^{Sc} conformations implies that prion propagation in hosts of a given PrP sequence results in a predefined range of prion strains[23,46,47].

An unexpected result of this study was the finding that transmission of sheep scrapie isolates in tgHu mice resulted in the emergence of prions with a similar phenotype to those associated with sCJD. As this result was obtained in two different tgHu

Table 4 | Intracerebral inoculation of tg650 transgenic mice with a panel of sheep scrapie isolates.

Isolate	Origin	Tg 650			
		Passage 1		Passage 2	
		Positive mice	Incubation (d.p.i. ± s.d.)	Positive mice	Incubation (d.p.i. ± s.d.)
sCJD MM1	Hu	6/6	177 ± 17	4/4	153 ± 3
sCJD VV2	Hu	6/6	566 ± 21	6/6	433 ± 18
vCJD	Hu	8/8	512 ± 15	7/7	581 ± 45
BSE	Bov	2/6	627; 842*	6/7	568 ± 65
PS21	ov	1/8	926*	11/11	180 ± 8
O100	ov	1/6	595*	NA	
PS310	ov	0/9	>750	0/15	>750
PS48	ov	0/6	>750	NA	
Healthy brain	Hu	0/9	>750	0/6	>750
	Ov	0/6	>750	0/6	>750

Tg650 mice that are transgenic for Met_{129} human PrP were inoculated intra-cerebrally (20 µl per mouse) with 10% brain homogenate from challenged with 10% brain homogenate from (i) sporadic Creutzfeldt-Jakob (sCJD) and variant Creutzfeldt Jakob (vCJD) patients, (ii) bovine (Bov) spongiform encephalopathy-affected cattle, (iii) ovine (Ov) scrapie isolates (PS21, O100, PS48 and PS310). After first passage, clinically affected or asymptomatic mice that survived >500 d.p.i. were pooled and used for second passage in the same mouse line. Mice were considered positive when abnormal PrP deposition was detected in the brain. Incubation periods (days post inoculation: d.p.i.) are shown as mean ± s.d., except when <50% of mice were found positive. In the latter case, the incubation periods of the positive mice are individually presented. Data in italics have been described in previous publications. NA: still ongoing bioassay, results not available.
*Indicates abnormal PrP positive and found dead animals (asymptomatic).

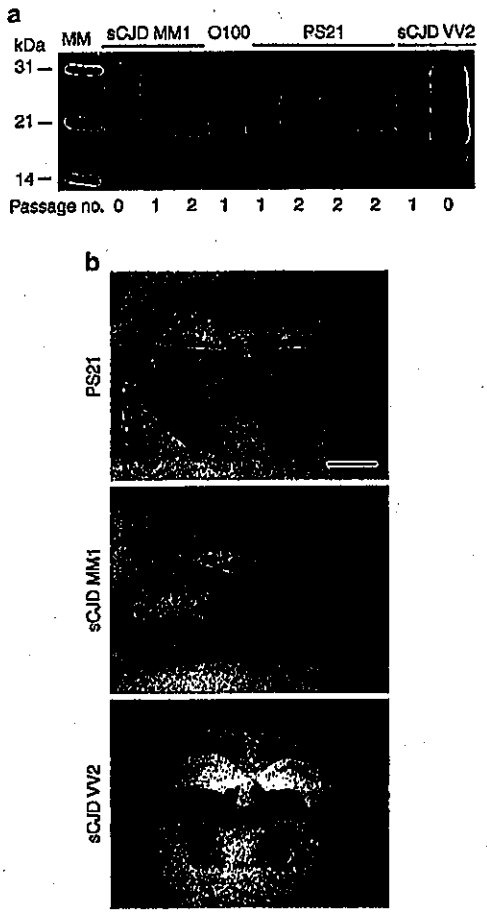

Figure 5 | PrP^{res} in the brains of prion-inoculated tg650 transgenic mice. Transgenic mice (tgMet129/tg650) that overexpress the methionine variant at codon 129 of human PrP were inoculated with different natural sheep scrapie isolates (ov), cattle BSE (bov), human (hu) vCJD and sCJD samples (MM1 and VV2). Brains from first and/or second passage tg650 mice were tested for the presence of PrP^{res} by (a) western blot (Sha31anti-PrP antibody) and (b) histo Blot (3F4 anti-PrP antibody). Scale bar, 160 µm.

mouse lines, we consider that our data support the view that passage of sheep scrapie prions across a human transmission barrier can lead to the emergence of sCJD prions.

Other recent studies have similarly reported that, after one passage, no clinical disease or PrP^{res} accumulation could be observed in tgHu mice overexpressing the human PrP following intracerebral challenge with a panel of scrapie isolates[48]. This observation concurs with our data here whereby propagation of sheep scrapie in tgHu mice was only clearly evident after second passage in these mouse lines. A similar phenomenon was also reported in a number of tgHu mouse models following the inoculation of cattle BSE[6,22,28]. This is a likely consequence of the strong transmission barrier limiting the propagation of both scrapie and BSE prions in mice that express human PrP. Our data were obtained by transmission studies in mice that overexpress human PrP and are in contrast with the reported lack of transmission of sheep scrapie isolates in human PrP knock-in mice[49]. However, while no species barrier is expected for transmission of human prions in tgHu mice, a significant proportion of sCJD isolates propagates with limited efficiency in human PrP knock-in mice[50]. This suggests that mice that express physiological levels of PrP might not be the most appropriate animal model to assess the permeability of the human species barrier to animal prions.

Do our transmission results in tgHu imply that sheep scrapie is the cause of sCJD cases in humans? This question challenges well-established dogma that sCJD is a spontaneous disorder unrelated to animal prion disease. In our opinion, our data on their own do not unequivocally establish a causative link between natural exposure to sheep scrapie and the subsequent appearance of sCJD in humans. However, our studies clearly point out the need to re-consider this possibility. Clarification on this topic will be aided by informed and modern epidemiological studies to up-date previous analysis that was performed at the end of the last century[3,4]. The value of such an approach is highlighted by the implementation in the year 2000 of large-scale active animal TSE surveillance programs around the world that provided an informed epidemiological-based view of the occurrence and geographical spread of prion disease in small ruminant populations[51]. The fact that both Australia and New-Zealand, two countries that had been considered for more than 50 years as TSE-free territories, were finally identified positive for atypical scrapie in their sheep flocks provides an example of how prion

dogma can be reversed[52]. However, the incubation period for prion disease in humans after exposure to prions via the peripheral route, such as in iatrogenic CJD transmission and Kuru, can exceed several decades[53,54]. In this context, it will be a challenge to combine epidemiological data collected contemporarily in animal populations and humans to investigate the existence of a causative link between prion disease occurrence in these different hosts. Furthermore, it is crucial to bear in mind that sporadic sCJD in humans is a rare disease (1–2 individuals per million of the population per year) and that scrapie has been circulating in small ruminants populations used for food purposes for centuries. Consequently, it is our opinion that even if a causative link was established between sheep scrapie exposure and the occurrence of certain sCJD cases, it would be wrong to consider small ruminant TSE agents as a new major threat for public health. Despite this, it remains clear that our data provide a new impetus to establish the true zoonotic potential of sheep scrapie prions.

Methods

TSE isolates. All the isolates used in this study were prepared as 10% (w/v) brain homogenates in 5% glucose. vCJD and sCJD isolates were obtained from the UK CJD reference centre in Edinburgh. The experimental protocol, including the use of human samples, was approved by the competent ethical committees (UK National CJD Research & Surveillance Unit Tissue bank: REC reference number 2000/4/157). All the sheep scrapie isolates, except one, were selected from the tissue archive of UMR INRA ENVT 1225. These scrapie isolates correspond to clinically suspect field cases that were collected between 1994 and 2004 from naturally affected commercial flocks in France. For each isolate, the exon 3 of the PrP encoding gene was sequenced[55,56]. All these sheep scrapie cases were confirmed positive by histopathology (identification of vacuolar changes in the brainstem). MF17 was a 5.5-year-old sheep, homozygous for ARQ at codons 136, 154 and 171 of the sheep *PrP* gene (ARQ/ARQ). PS09 was a 2.5-year-old ARQ/ARQ. PS21 was a 2.5-year-old ARQ/ARQ sheep. PS48 was a 5-year-old sheep, homozygous for VRQ at codons 136, 154 and 171 of the sheep *PrP* gene (VRQ/VRQ). PS42 was a 1.5-year-old VRQ/VRQ sheep. PS310 was a 7.5-year-old ARQ/ARQ sheep. The O100 scrapie isolate (inoculated in tg650 mice) was provided by the National French reference laboratory for scrapie diagnosis (ANSES, Lyon, France) and had been collected from a VRQ/VRQ sheep of unknown age.

Transgenic mouse lines. Transgenic mouse lines that express PrP^{C} of different species were used to propagate the selected panel of TSE isolates: tgBov (or tg110, bovine PrP), tg338 (ovine $V_{136}R_{154}Q_{171}$ PrP), tgShXI (ovine $A_{136}R_{154}Q_{171}$ PrP variant). Except for the bovine PrP expressing mice (tg110) these mice lines were established on the same PrP null background mouse line (PrPKo) (Zurich I)[57–59]. The relative PrP expression level in the brain of these mouse lines in comparison with the natural host species was of the order: tg338, six- to eightfold; tg110, eightfold,; tgShpXI, three- to fourfold[27]. Tg340 and tg361 mouse lines that express human PrP methionine at codon 129 or valine at codon 129, respectively, in a PrPKo background, were generated following the same procedure as described for tg340 mice[22]. Both tg340 and tg361 are homozygous for human *PRNP* gene and tgMet/Val_{129} mice used in our experiments were obtained by mating tg340 and tg361 mice (F1 generation).

Mouse bioassays. All animal experiments were performed in compliance with our institutional and French national guidelines in accordance with the European Community Council Directive 86/609/EEC. The experimental protocol was approved by the INRA Toulouse/ENVT ethics committee. Six- to ten-week-old female mice were anaesthetized and inoculated with 2 mg of brain equivalent (20 µl of a 10% brain homogenate) in the right parietal lobe using a 25-gauge disposable hypodermic needle. Mice were observed daily and their neurological status was assessed weekly. When clinically progressive TSE disease was evident, or at the end of their lifespan, animals were killed and their brains collected. Half of the isolated brain from those animals that had displayed TSE clinical signs was fixed by immersion in 10% formol and the other half was frozen at −20 °C. Tissues from found dead animals were frozen (no formalin fixation). Spleens from animals were not systematically collected (in particular in found dead animals) and data related to PrP^{Sc} accumulation in this tissue are not available. Survival time was expressed as the mean of the survival days post inoculation (d.p.i.) of all the mice scored positive for PrP^{res}, with its corresponding s.d. In cages where no clinical signs were observed, mice were killed at the end of their natural lifespan (650 to 800 days). In those cases, incubation periods reported in the table (>650 to 750 d.p.i.) corresponded to the survival time observed in at least three out of the six mice. Brain homogenates from PrP^{res} positive mice, when available, were used for serial tranmission. When all mice were scored negative for PrP^{res} on primary passage, PrP^{res}-negative brain homogenates of animals that survived for more than 500 d.p.i. were used for the next passage.

Anti-PrP monoclonal antibodies. The anti-PrP monoclonal antibodies used in this study (kindly provided by J.Grassi, CEA, France) were Sha31 (epitope: $_{145}$YEDRYYRE$_{152}$) and 3F4 anti-PrP (epitope: $_{111}$HMAGAAAA$_{118}$)[60].

PrP^{res} western blot detection. Frozen brain tissue (175 ± 20 mg) was homogenized in 5% glucose in distilled water in grinding tubes (Bio-Rad) adjusted to 10% (w/v) using a TeSeE™ Precess 48 homogenizer (Bio-Rad). A western blot kit (TeSeE WB kit Bio-rad) was used following the manufacturer's recommendations and PrP^{res} detection used a monoclonal antibody Sha31 at a dilution of 1/8,000. All the original western blot images are presented as Supplementary Data (Supplementary Figs 1–12).

Paraffin-embedded tissue blot and histoblot. Paraffin-embedded tissue sections were collected on a 0.45-µm nitrocellulose membrane, before drying and deparaffinization. PK digestion (250 µg ml^{-1}, 2 h per 55 °C; Roche 1373200) was performed before denaturation into isothiocyanate guanidium solution (3 M, 10 min, at room temperature). Immunodetection was carried out with a monoclonal antibody Sha31 (4 µg ml^{-1}) followed by an alkaline phosphatase coupled secondary antibody (Dako, Cat. No. D0314) at a dilution of 1/500). Alkaline phosphatase activity was revealed using NBT/BCIP substrate chromogen[61]. For the histoblotting procedure, brains ($n = 3$ per treatment group) were rapidly removed from killed mice and frozen on dry ice. Cryosections were cut at 8–10 mm, transferred onto Superfrost slides and kept at −20 °C until use. Sections were transferred to a nitrocellulose membrane (wetted in lysis buffer Nonidet P-40/0.5% sodium deoxycholate/100 mM NaCl/10 mM EDTA/10 mM Tris-HCl, pH 7.8); the glass slides were quickly and immediately pressed onto the membrane for 25 s. Membranes were then submitted to PK (Roche ref 1373200) digestion (250 µg ml^{-1}, 2H, 55 °C) before denaturation into isothiocyanate guanidium solution (3M, 10 min, at room temperature). PrP^{res} was probed using the anti-PrP monoclonal antibody 3F4 (concentration: 0.6 µg ml^{-1}), followed by an alkaline phosphatase coupled secondary antibody (Dako ref D0314 at a dilution of 1/500). Enzymatic activity was revealed using NBT/BCIP substrate chromogen[29].

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Author contributions

J.M.T., S.L.B., F.L., V.B. and O.A. designed the experiments. H.C., J.M.T., C.L., J.-Y.D., S.B., F.L., S.L., I.L., P.C., N.A., F.R., L.H., J.C.E., V.B. and O.A. performed in the experiments. H.C., J.M.T., S.B., F.L., V.B. and O.A. wrote the manuscript.

Additional information

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番号7

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識別番号・報告回数			報告日	第一報入手日 2015年02月03日	新医薬品等の区分 該当なし	厚生労働省処理欄

一般的名称	①②抗HBs人免疫グロブリン ③④乾燥抗HBs人免疫グロブリン ⑤　ポリエチレングリコール処理抗HBs人免疫グロブリン	研究報告の公表状況	www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=17023/2015/01/29	公表国 ノルウェー
販売名 (企業名)	①抗HBs人免疫グロブリン筋注200単位/1mL「JB」（日本血液製剤機構） ②抗HBs人免疫グロブリン筋注1000単位/5mL「JB」（日本血液製剤機構） ③ヘブスブリン筋注用200単位　（日本血液製剤機構） ④ヘブスブリン筋注用1000単位　（日本血液製剤機構） ⑤ヘブスブリンIH静注1000単位（日本血液製剤機構）			

研究報告の概要

ノルウェーでウシ海綿状脳症（非定型BSE H型）が確認された

報告の種類：即時報告
開始日：2015年1月16日
事象の確定日：2015年1月20日
報告日：2015年1月29日
病　因：プリオン（非定型BSE H型）
疫学的コメント
2015年1月29日の状態に基づいて：2015年1月20日、ノルウェーの獣医研究所は中枢神経系の材料で行われた初期のテストに基づき、ノルウェーのウシでBSEの疑いを報告した。この材料の一部は診断の検証のために、Weybridgeの欧州連合参照試験所に送られた（2015年1月21日）。感染した乳牛は15歳で、ノルウェーで生まれた。雌親はスウェーデンから輸入された。乳牛は高齢と怪我のために殺処分される（2015年1月12日）前に神経疾患の臨床徴候を示さなかった。BSE検査は、BSE監視プログラムの一部として行われた。ノルウェーの食品安全局（NFSA）は農場での移動に制限を入れ、疫学的調査を行った。NFSAは関連法令により、4つのリスク動物を特定している。これらの動物も公式の移動制限の下に置かれている。4頭の特定リスク動物は殺され、欧州連合法案に従って焼却処分された。起源となるウシと現在の疑われるウシからのリスク動物の追跡を含む疫学的調査は、(1)感染した乳牛の誕生日の前後12ヶ月以内に同じ群れで生まれた動物の一団、また（2)感染した乳牛の最初の一年間を一緒に飼育された動物の一団　の何れかに属していた2頭のウシに加えて、事例の前2年以内に生まれた2頭の子牛を特定した。事例の前2年以内に生まれた子牛とリスク動物の一団は移動制限下に置かれ、これらの動物の殺処分はできるだけ早く実施されるだろう。感染した乳牛の死体は完全に処理されていた。NFSAは、乳牛の死体はカテゴリー1処理工場において圧力滅菌により処理され、結果として生じた物質はは副産物規則に従って焼却/同時焼却のために送られたことを確認した。

使用上の注意記載状況・その他参考事項等

代表としてヘブスブリンIH静注1000単位の記載を示す。
2. 重要な基本的注意
(1)略
1)略
2)現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

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

抗HBs抗体

Bovine spongiform encephalopathy, Norway

Information received on 29/01/2015 from Dre Kristina Landsverk, Chief Veterinary Officer, Norwegian Food Safety Authority, Ministry of Agriculture and Food, Brumunddal, Norway

Summary

Report type	Immediate notification
Date of start of the event	16/01/2015
Date of pre-confirmation of the event	20/01/2015
Report date	29/01/2015
Date submitted to OIE	29/01/2015
Date event resolved	03/02/2015
Reason for notification	First occurrence of a listed disease
Manifestation of disease	Sub-clinical infection
Causal agent	Prion (atypical BSE type H)
Nature of diagnosis	Laboratory (advanced)
This event pertains to	the whole country
Related reports	Immediate notification (29/01/2015) Follow-up report No. 1 (06/02/2015) Follow-up report No. 2 (20/02/2015)

New outbreaks (1)

Outbreak 1	Verran, Tua, District office Innherred og Fosen, Region office Tr□delag og M□e og Romdal, NORD-TRONDELAG
Date of start of the outbreak	16/01/2015
Outbreak status	Resolved (03/02/2015)
Epidemiological unit	Farm

Affected animals

Species	Susceptible	Cases	Deaths	Destroyed	Slaughtered
Cattle	27	1	0	1	0

Affected population

The farm is a beef cattle farm with a herd of Scottish Highland cattle.

Summary of outbreaks

Total outbreaks: 1

Total animals affected

Species	Susceptible	Cases	Deaths	Destroyed	Slaughtered
Cattle	27	1	0	1	0

Outbreak statistics

Species	Apparent morbidity rate	Apparent mortality rate	Apparent case fatality rate	Proportion susceptible animals lost*
Cattle	3.70%	0.00%	0.00%	3.70%

*Removed from the susceptible population through death, destruction and/or slaughter

Epidemiology

Source of the outbreak(s) or origin of infection	Unknown or inconclusive
Epidemiological comments	Based on status on 29 January 2015: On 20 January 2015, the Norwegian Veterinary Institute reported suspicion of BSE on a cow in Norway, based on initial test done on CNS material. Part of this material was sent to European Union Reference Laboratory in Weybridge (21 January 2015) for verification of diagnosis. The affected cow was a 15-year-old and born in Norway. The dam was imported from Sweden. The cow did not show clinical signs of neurological disease before she was killed (12 January 2015) due to old age and injuries. The BSE test was taken as part of the BSE surveillance program. The Norwegian Food Safety Authority (NFSA) has put restrictions on movement on the farm, and performed epidemiological investigations. The NFSA has identified four risk animals, according to relevant legislation. These animals are also placed under official movement restrictions. The four identified risk animals will be killed and disposed by incineration according to European Union legislation. The epidemiological investigation including tracing of risk animals from the holding of origin as well as the present holding has identified 2 offspring borne within two years prior to the incident in addition to 2 cattle belonging either to (1) the cohort of animals born in the same herd as the affected animal within 12 months preceding or following the date of birth of the affected cow or (2) the cohort of animals which at any time during the first year of their lives were reared together with the affected cow during her first year of life. Progeny borne within two years prior to the incident and the cohort of risk animals are put under movement restrictions and the killing and destruction of these animals will be carried into effect as soon as possible. The affected cow□ carcass has been completely destroyed. The NFSA ensures that the cow□ carcass has been processed by pressure sterilisation in a Category 1 processing plant and that the resulting material has been sent for incineration/co-incineration in accordance with the By-Products Regulation.

Control measures

Measures applied	Movement control inside the country Screening No vaccination No treatment of affected animals
Measures to be applied	Modified stamping out

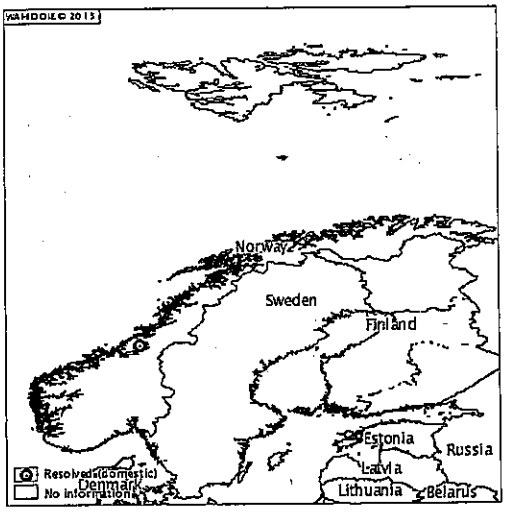
Diagnostic test results

Laboratory name and type	Species	Test	Test date	Result
Norwegian Veterinary Institute (National laboratory)	Cattle	enzyme-linked immunosorbent assay (ELISA)	20/01/2015	Positive
Norwegian Veterinary Institute (National laboratory)	Cattle	western blot	20/01/2015	Positive
EU Reference Laboratory, Animal and Plant Health Agency (APHA), Weybridge (United Kingdom) (OIE指 Reference Laboratory)	Cattle	immunohistochemical test	28/01/2015	Positive
EU Reference Laboratory, Animal and Plant Health Agency (APHA), Weybridge (United Kingdom) (OIE指 Reference Laboratory)	Cattle	western blot	28/01/2015	Positive

Future Reporting

The event is continuing. Weekly follow-up reports will be submitted.

Map of outbreak locations



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
				2015. 1. 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	El Shazly Y, Hemida K, Rafik M, Al Swaff R, Ali-Eldin ZA, GadAllah S. J Med Virol. 2015 Mar;87(3):424-7. doi: 10.1002/jmv.24074. Epub 2014 Sep 3.	公表国 エジプト
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

○HCV感染患者の健康な配偶者におけるオカルトHCVの検出
オカルトHCV感染の診断基準となるのは肝細胞中のHCV-RNA検出であるが、肝生検の侵襲性から、他の方法が模索されてきた。本研究は、リアルタイムPCRを用いて末梢血単核細胞(PBMC)中のHCV-RNAを検出することにより、慢性HCV-4感染患者の健康な性的パートナーにおけるオカルトHCV-4感染を特定することを目的とした。
慢性HCV-4感染エジプト人患者(血清HCV-RNA陽性、感染持続期間5～9年)の健康な配偶者(HCV抗体と血清HCV-RNA陰性、薬物乱用等のリスクのない者)50人を対象に、PBMC中のHCV-RNAを調査した。その結果、オカルトHCV-4感染の割合は4%であり、特に性感染症(淋病や性器ヘルペス)歴を有する者の陽性率は有意に高かった。婚姻期間、性交頻度及びALT値と、オカルト感染の陽性率の間には有意な関連は見られなかった。
この結果は、特に性感染症歴を有する者における、HCV-4感染の配偶者間伝播の重要性を示唆している。

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	今後の対応
慢性HCV-4感染患者の健康な配偶者50人において、リアルタイムPCRを用いてPBMC中のHCV-RNAを調査したところ、オカルトHCV感染が4%の被験者に見られ、特に性感染症歴を有する被験者における陽性率が有意に高いことが明らかとなったとの報告である。	日本赤十字社では、HCV抗体検査を行い、陰性の検体について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、陽性血液を排除している。HCV感染に関する新たな知見等について、今後も情報の収集につとめる。

Journal of Medical Virology 87:424–427 (2015)

Detection of Occult Hepatitis C Virus Among Healthy Spouses of Patients With HCV Infection

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The criterion standard for the diagnosis of occult hepatitis C virus (HCV) infection is detection of HCV-RNA in liver cells. However, because of the invasive nature of liver biopsy, other methods have been studied. The present study aimed to identify subjects with occult HCV-4 infection among healthy sexual partners of patients with chronic HCV-4 infection by detecting HCV-RNA in peripheral blood mononuclear cells (PBMCs) using real-time polymerase chain reaction (PCR). Fifty healthy Egyptian spouses of patients with chronic HCV-4 infection were included in this study. Real-time PCR was used to detect HCV-RNA in PBMCs in all the study subjects. The prevalence of occult HCV-4 infection was 4%, and a statistically significant higher prevalence was found among patients with a history of sexually transmitted infection. The results of the present study indicate the importance of intra-spousal transmission of HCV-4 infection, especially in subjects with a history of sexually transmitted infection. ***J. Med. Virol. 87:424–427, 2015.*** © 2014 Wiley Periodicals, Inc.

KEY WORDS: occult; HCV; intra-spousal transmission; STD

INTRODUCTION

Hepatitis C virus (HCV) infection is a blood borne infection transmitted mainly through injection drug users, blood transfusion, organ transplantation, accidental needle injuries, and other parenteral exposures [Tahome and Holmberg, 2010]. Intra-familial and sexual transmission of HCV infection is still controversial [Atome et al., 2009].

Epidemiological evidence indicates that HCV can be transmitted via the sexual route, though less efficiently than the hepatitis B or human immunodeficiency virus (HIV) [Caraballo et al., 2008].

Several high risk sexual factors may affect HCV transmission, which may include multiple sexual partners, history of sexually transmitted infection, and sexual intercourse with an intravenous drug user [Caraballo et al., 2008].

Occult HCV infection can be present in two different clinical situations, namely in anti-HCV and serum HCV-RNA negative patients with abnormal liver function test results, and in anti-HCV positive subjects with normal liver enzyme levels and without detectable serum HCV-RNA [Carreno, 2006].

The criterion standard for the diagnosis of occult HCV infection is detection of HCV-RNA in liver cells. However, because of the invasive nature of liver biopsy, other methods have been studied [Schmidt et al., 2000].

Using whole blood for HCV-RNA detection does not improve the accuracy of the diagnosis of occult HCV infection. Testing for HCV-RNA in peripheral blood mononuclear cells (PBMCs) is much more reliable in identifying patients with occult HCV infection [Carreno et al., 2004].

The primary aim of this study was to identify individuals with occult HCV-4 infection among healthy sexual partners of patients with chronic HCV-4 infection by detecting HCV-RNA in PBMCs using real-time polymerase chain reaction (PCR).

SUBJECTS AND METHODS

Fifty healthy spouses of patients with chronic HCV-4 infection were selected from the gastroenterology and hepatology outpatient clinic of Ain Shams

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Conflict of interest: The authors have no conflicts of interest to declare.

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University Hospitals. All the subjects had negative test results for HCV antibodies and serum HCV-RNA, while their infected spouses had positive qualitative and/or quantitative PCR results for serum HCV-RNA (duration of HCV infection ranged from 5 to 9 years).

This study was approved by the local ethical committee of Ain Shams University Hospitals, and written informed consent was obtained from each participant.

Subjects were excluded from the study if they met any of the following conditions: HIV infection, any autoimmune disorder, acute or chronic kidney disease, organ transplant recipient, current or past history of any malignancy, ultrasonographic evidence of liver disease, current or past history of heavy alcohol consumption or intravenous drug abuse, history of homosexuality or promiscuous heterosexuality, history of blood transfusion or a major operation, had undergone tattooing, had a history of traditional medicine use, received any form of immunosuppressive or antiviral therapy, pregnant or nursing an infant.

All the participants were subjected to detailed questioning, including sexual history (marital status, marriage duration, any sexual relationship beyond marital life, homosexuality or heterosexuality, safe sex practice, history of sexually transmitted infections, and average frequency of sexual intercourse per week); clinical examination; abdominal ultrasonography and laboratory investigations, including complete blood picture; liver functions tests; kidney function tests; and HIV and HCV antibody tests by enzyme-linked immunosorbent assay, HBV markers, and alpha-fetoprotein level measurement.

HCV genotyping was based on epidemiological assumption [Kamal and Nasser, 2008].

HCV-RNA detection in serum: HCV-RNA was detected by using a commercially available RT-PCR kit with a sensitivity limit of 50 IU/ml and 99% specificity. Briefly, viral RNA was extracted from plasma by lysis of viral particles with guanidinium thiocyanate (chaotropic agent), followed by alcohol precipitation. HCV-RNA was retrotranscribed to cDNA and amplified by the single tube RT-PCR primer set to amplify a sequence of 244 nucleotides within the conserved 5′UTR of the HCV genome. Amplified DNA was detected using target-specific oligonucleotide probes that permitted independent identification of HCV amplicons and internal control amplicons.

HCV-RNA detection in PBMCs: PBMCs from all the individuals under study were isolated from ACD-treated blood by Ficoll–Hypaque density gradient centrifugation. RNA was purified from 5 to 10×10^6 cells and stored at minus 80°C. RNA concentration was determined by spectophotometry. RNA was reverse transcribed into cDNA. The detection of the 5′UTR of both positive and negative HCV-RNA strands was performed. Two positive control samples from the blood of serum positive patients were processed along with the batch of test samples.

Sequences of the primers used for HCV strands detection (Applied Biosystems):

Sense primer: 5′-CGACACTCCACCATGAATCACT-3′

Antisense primer: 5′-GAGGCTGCACGACACTCATACT-3′

Statistical Analysis

Data were collected, coded, and entered to a personal computer (IBM compatible, 3 GHz). The collected data were analyzed with the program Statistical Package for Social Science version 16 for the Windows operating system. Mean values were calculated, and the chi-square (χ^2) test was performed. The probability of error (P) was interpreted as follows: $P > 0.05$, insignificant; $P \leq 0.05$, significant; $P < 0.01$, highly significant.

RESULTS

This study was conducted with 50 healthy spouses of Egyptian patients with chronic HCV-4 infection (25 men and 25 women). The mean age of the subjects was 46.8 ± 10.1 years, and the mean marriage duration was 21.4 ± 10.4 years (median 19.5 years). None of the study subjects practiced safe sex throughout their entire sex life.

Of the study subjects, 8% had a history of sexually transmitted diseases (STD) (gonorrhea and/or genital herpes). Patients with chronic HCV-4 infection also had a history of STD same to their healthy spouses. A significantly higher prevalence of STD was found among the male than among the female spouses (16% vs 0; $P = 0.03$).

The prevalence of occult HCV infection was 4%, with an insignificant difference between both genders. However, a significantly higher prevalence of occult HCV infection was found among the patients with a history of STD (Table I).

Neither marriage duration nor the frequency of sexual intercourse had any significant correlation with the prevalence of occult HCV infection (Tables II and III); the same finding was true for serum alanine aminotransferase (ALT) levels (Table IV).

DISCUSSION

Occult HCV infection is distributed worldwide and all HCV genotypes seem to be involved in this infection [Carreno et al., 2012].

Among people in so-called high-risk groups (gay men, prostitutes, people with multiple sex partners,

TABLE I. STD and Prevalence of Occult HCV

Occult HCV	Negative, No. (%)	Positive, No. (%)	χ^2	P
STD				
Negative (N = 46)	45 (97.8)	1 (2.2)	4.8	0.02
Positive (N = 4)	3 (75.0)	1 (25.0)		

TABLE II. Duration of Marriage and Prevalence of Occult HCV

Occult HCV	Negative No. (%)	Positive No. (%)	χ^2	P
Marriage duration				
<19.5years (N = 25)	25 (100.0)	0 (0)	2.8	0.09
>19.5 years (N = 25)	23 (92.0)	2 (8.0)		

TABLE III. Frequency of Sexual Intercourse and Prevalence of Occult HCV

Occult HCV	Negative No. (%)	Positive No. (%)	χ^2	P
Frequency of sexual intercourse per week				
Once (N = 13)	12 (92.3)	1 (7.7)	1.1	0.5
Twice (N = 22)	21 (95.5)	1 (4.5)		
Thrice (N = 15)	15 (100.0)	0 (0)		

TABLE IV. ALT Level and Prevalence of Occult HCV

Occult HCV	Negative No. (%)	Positive No. (%)	χ^2	P
ALT level				
Normal (N = 32)	31 (96.9)	1 (3.1)	0.1	0.6
High (N = 18)	17 (94.4)	1 (5.6)		

and people attending STD clinics), sexual transmission of HCV infection seems to be more common. The fact that the incidence rate of HCV infection is higher among people with more sex partners and other sexual risk factors, indicates that the disease can be sexually transmitted [Highleyman, 2010].

The present study aimed to identify individuals with occult HCV infection among healthy spouses of Egyptian patients with chronic HCV-4 infection by detecting HCV-RNA in PBMCs.

The present study revealed a 4% prevalence of occult HCV-4 infection among healthy sexual partners of the Egyptian patients with chronic HCV-4 infection. A higher prevalence of occult HCV infection was observed by Roque-Cuéllar et al. [2011], who investigated the incidence of occult HCV infection among 31 sexual partners of patients with chronic HCV infection. They studied the presence of HCV-RNA positive and RNA negative strands in PBMCs by performing strand-specific real-time PCR and found a 12.3% prevalence rate (4/31) of occult HCV infection among healthy sexual partners. This discrepancy in the prevalence rate is mainly related to differences in ethnicity, economic, social, and religious factors.

The present study revealed an insignificant difference in the prevalence of occult HCV infection between the male and female sexual partners of the Egyptian patients with chronic HCV-4 infection. Contrary to this finding, De Marco et al. [2009] found a higher incidence of occult HCV infection among women (3.6%) than among men (2.7%). Meanwhile, Youssef et al. [2012] reported a male predominance of occult HCV infection among 50 healthy persons. This contradiction seems to be related to differences in sample size and patient selection criteria; the study by De Marco et al. [2009] was conducted with 276 subjects who were selected from a healthy population, whereas the study by Youssef et al. [2012] included 100 subjects, of whom 50 had newly diagnosed different lymphoproliferative disorders and the other 50 were healthy volunteers.

Regarding the possible role of STD in increased risk of occult HCV infection, the present study revealed a significantly higher prevalence of occult HCV infection among the patients with a history of STD (25%). This finding agrees with those of Rai et al. [2012] and Highleyman [2010]. In these studies, occult HCV infection was found to be more common among subjects who had a history of STD.

Tanfer et al. [1995] showed that women are three times more likely to contract sexually transmitted infections than men, and that those who have two or three sexual partners are more likely to have STD. Moreover, Madkan et al. [2006] reported that women are more susceptible than men to acquire HIV infection and other STD. Once they have contracted STD, women often sustain more damage to their health than men do. In contrast to the previously mentioned studies, the present study revealed a significantly higher percentage of men with a history of STD (16%). Again, this contradiction is related mainly to differences in patient selection criteria between different studies, with special consideration of economic, social, and religious factors.

Regarding the relationship between age and occult HCV infection, the present study revealed an insignificant difference in prevalence of occult HCV infection between different age groups. The same finding was reported by Youssef et al. [2012], who found that occult HCV infection seems to most frequently occur during the age between 22 and 66 years. It seems logical to relate this finding mainly to increased risks of exposure to HCV infection, including sexual activity, during these ages. On the contrary, Abou Elazm et al. [2009] found a higher intra-spousal transmission rate of occult HCV infection with increasing age, but this was mainly related to longer marriage duration among older age groups.

The relationship between occult HCV infection and serum ALT levels is controversial. While some authors reported normal liver functions in patients with occult HCV infection [De Marco et al., 2009], others reported high serum ALT levels in such patients [Castillo et al., 2004; Saad et al., 2011]. Confirming this controversy, the present study revealed an insignificant difference in the prevalence of occult HCV infection between patients with normal or high liver function test results.

All the previous findings strongly confirm the need to screen all high-risk people (including healthy

spouses of patients with chronic HCV-4 infection) for occult HCV infection, especially those with a history of STD. Further studies are required to shed light on the pathogenic and epidemiological consequences of occult HCV infection.

The present clinical trial had few limitations. First, neither the presence of HCV-RNA in liver tissue nor the presence of HCV core antigen was evaluated in parallel with detection of HCV-RNA strands in PBMCs. However, the primary aim of the present study was to evaluate noninvasive alternatives to liver biopsy for detection of occult HCV infection. Moreover, the results of the present study warrant further studies to evaluate the role of liver tissue HCV-RNA and HCV core antigen detection in patients whose test results are negative for both HCV-RNA positive and RNA negative strands in PBMCs by performing strand-specific real-time PCR. Second, this study was limited by the relatively small sample size, which reduced the statistical significance of the results.

CONCLUSION

Intra-spousal transmission of HCV-4 infection is an important route of the spread of HCV-4 infection, especially in subjects with a history of sexually transmitted infection.

ACKNOWLEDGMENTS

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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. 10. 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		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)		Pavio N, Merbah T, Thébault A. Emerg Infect Dis. 2014 Nov;20(11):1925-7. doi: 10.3201/eid2011.140891.	フランス

研究報告の概要

○ブタの生レバーを含む食品のHEV汚染、フランス
フランスでは、2009年の調査で、養豚場の65%においてHEVに感染したブタの存在が明らかになっており、食品流通上にあるブタレバーの4%はHEVに汚染されているとの報告がある。ブタレバーを含む食品は土着HEV感染の原因である可能性がある。本研究では、生のブタレバーを含む食品におけるHEV RNA検出率を調査した。生のブタレバーを含む4つの異なる分類の食品394サンプルをリアルタイムRT-PCRを用いて分析したところ、68サンプルがHEV RNA陽性となった(フィガテル29例、塩漬けドライレバー1例、クネル及びクネルペースト10例、乾燥または新鮮レバーソーセージ28例)。食品分類毎のHEV RNA検出率は相対的な製品重量を用いて推定され、フィガテル(30%[95%CI 23%－38%])、レバーソーセージ(29%[95%CI 22%－36%])、クネル(25%[95%CI 15%－37%])で高かった。塩漬けドライレバーの汚染率は低かった(3%[95%CI 0%－10%])。ブタレバーソーセージのHEV RNAと土着HEV感染例との因果関係を評価するために、68サンプルの部分的なORF2配列を増幅し分析したところ、得られた全ての塩基配列はフランス及びヨーロッパの他の国において主に見られるジェノタイプ3であった。食品とヒトまたはブタHEV間の配列相同性をスクリーニングし、最も近縁の配列を検索したところ、食品33サンプルの配列がヒト及び/またはブタ株の配列と98%nt以上の相同性を有していた。

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
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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
フランスにおいて生のブタレバーを含む4つの異なる分類の食品394サンプルについてHEV RNA検出率を調査したところ、各分類の3～30%からHEV RNAが検出され、系統解析の結果、ヒトとブタのHEV配列には高度の相同性が見ら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。

Frequent Hepatitis E Virus Contamination in Food Containing Raw Pork Liver, France

Nicole Pavio, Thiziri Merbah, and Anne Thébault

Food products containing raw pork liver are suspected to be vehicles for transmission of hepatitis E virus. Four categories of food products, comprising 394 samples, were analyzed to determine hepatitis E virus prevalence. Virus was detected in 3%–30% of the different categories. Phylogenetic analysis showed high identity with human and swine sequences.

In humans, hepatitis E virus (HEV) is responsible for an acute, entero-transmissible form of hepatitis, similar to that caused by hepatitis A. In most cases, it is a self-limited infection with rapid viral clearance, but it can evolve into more severe forms, including fatal fulminant hepatitis. Chronic hepatitis E also has occurred in solid-organ transplant recipients and has progressed to more serious conditions, such as fibrosis or liver cirrhosis and liver failure (*1*).

HEV is the only hepatitis virus that can infect species other than primates. HEV infects many animal species, especially pigs, in which a very high prevalence has been described (*2*). Infections acquired in Western countries involve strains that are genetically similar to local swine strains, suggesting an autochthonous origin. Although water is the main vector of contamination in countries to which HEV is endemic, the origin of sporadic cases in other areas is more likely zoonotic. Direct contact with infected animals and consumption of infected meat are possible transmission pathways (*2*).

In France, the annual number of autochthonous cases appears to have increased, from 9 cases in 2002 to nearly 800 in 2012 (http://www.cnrvha-vhe.org/wp-content/uploads/2012/03/2012-Rapport-VHA-VHE.pdf). In a national survey in 2009, the presence of HEV in the swine reservoir was characterized and 65% of pig farms were found to have infected animals; 4% of pork livers entering the food chain were contaminated by the virus (*3*). Molecular analysis of HEV sequences in humans and pigs has shown high identity between the 2 populations (*4*). Food products containing pork liver have repeatedly been suspected of causing indigenous cases of HEV infection (*5*,*6*) and might be responsible for nearly 40% of the autochthonous HEV cases (*12*). Recent studies have confirmed the presence of HEV in the pork food chain, as well as in sausages (*7*,*8*). The objective of this study was to determine the apparent prevalence of HEV contamination in food products containing raw pork liver that were not marketed to be eaten without cooking.

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The Study

In 2011, four different categories of food products in France that were marketed by the food industry were identified as containing raw pork liver but sold to consumers to be eaten after cooking. These 4 categories were 1) figatellu and fitone, 2) dried salted liver, 3) quenelle and quenelle paste, and 4) dried or fresh liver sausages. HEV can be heat-inactivated by thorough cooking at 71°C for 20 min (*9*); however, consumers might not apply such precise thermal treatment. Thus, these food products might be able to transmit HEV. All 4 categories were local regional culinary specialties from eastern or southeastern France. The samples were collected, then frozen directly at the production step after packaging, just before distribution for commercial sale. The frozen samples were sent to the French Agency for Food, Environmental and Occupational Health and Safety laboratory and kept at –80°C until analysis. For each sample, HEV detection was performed on 20 g of product, which had been manually defatted and homogenized in 25 mL phosphate-buffered saline by using a blender. To avoid cross-contamination, each blender was autoclaved between samples. RNA extraction was performed on 500 µL suspension by using the RNeasy lipid Tissue Midi kit (QIAGEN, Hilden, Germany). Presence of inhibitors was assessed by addition of synthetic HEV RNA to the extracts (*9*).

Forty producers were randomly selected, and their relative contributions to total production within each food category were obtained. For each producer, 10 products were randomly selected. Six products were not included in the final analysis because the food category was uncertain. HEV RNA was detected by using real-time reverse transcription PCR as previously described (*9*). Of the 394 food samples analyzed, 68 were found positive (29 figatelli, 1 dried salted liver, 10 quenelle and quenelle paste, and 28 dried or fresh liver sausages). The HEV RNA quantification obtained was 10^2–10^6 copies of HEV RNA/g of food (Table). The prevalence for each food category was estimated by using the relative production weight for each food category, and the 95% CI was estimated by bootstrap. Statistical estimates were performed by using R 2.13.1

Table. Quantification and prevalence of HEV RNA in food containing raw pork liver, France, 2011

Food category	No. samples analyzed, N = 394	No. copies HEV RNA/g, range*	Prevalence (95% CI)
Figatelli and fitone	140	1.7×10^2 to 6.9×10^5	0.3 (0.23–0.38)
Dried salted liver	30	6.9×10^5	0.03 (0–0.10)
Quenelle and quenelle paste	55	2.6×10^2 to 2.83×10^5	0.25 (0.15–0.37)
Dried or fresh liver sausages	169	1×10^2 to 2.3×10^6	0.29 (0.22–0.36)

*Minimum and maximum numbers in each food product per category. Detection limit of the method used is 1×10^2 copies of HEV RNA/g. HEV, hepatitis E virus.

software (http://www.r-project.org). HEV RNA prevalence was high in figatelli (30% [95% CI 23%–38%]), liver sausages (29% [95% CI 22%–36%]), and quenelles (25% [95% CI 15%–37%]). The prevalence of HEV RNA was lower in dried salted liver: 3% (95% CI 0%–10%) (Table).

Four HEV RNA–positive food samples collected in the present study were tested for infectious virus in collaboration with 2 laboratories (Animal Health and Veterinary Laboratories Agency, Weybridge, UK, and Wageningen University and Research Centre Central Veterinary Institute, Lelystad, the Netherlands). Virus growth from 1 HEV-positive sample was observed in a 3-dimensional culture system developed by these laboratories (*10*). This analysis thus confirms that live viruses can be present in food products.

In preparation of these food products, a large quantity of liver (up to 750 livers per batch) was mixed with fat and spices. Therefore, even if only 4% of raw livers are infected, as shown in a previous study (*3*), the entire batch becomes contaminated; consequently, HEV prevalence is high in the food products. Because high quantities of virus can be present in liver (up to 10^8 copies of HEV RNA/g) (*9*), the dilution within a large batch will be limited and will not substantially reduce the risk for contamination. The oral infectious dose of HEV is still unknown. In contrast, dried liver is made from only 1 liver; thus the prevalence observed agrees with the prevalence of HEV in liver at the slaughterhouse (i.e., 4%) (*3*).

For further evaluating the link between HEV RNA in pork liver sausages and human autochthonous cases, 68 partial open reading frame 2 sequences were amplified (*3*). This sequence, although short (≈290 nt), reflects the diversity of the HEV full-length genome and is frequently used in phylogenic studies (*11*). All sequences obtained (GenBank accession nos. KJ558436–KJ558503) were of genotype 3, which is the major HEV genotype circulating in autochthonous cases in France and the rest of Europe. The overall mean distance of the sequences from the 68 food products was estimated to be 0.16 nt. To screen for high sequence identity between food products and human or swine HEV, each sequence was analyzed by using BLAST (http://blast.ncbi.nlm.gov/Blast.cgi) to identify the closest sequences. Thirty-three sequences had ≥98% nt identity with human and/or swine sequences. The human sequences with the highest identity originated in France, except for 2 sequences from the United Kingdom and 1 from Spain (Figure). This result confirms that most autochthonous cases might have a foodborne origin. Two food sequences had >99% nt identity with swine sequences previously described (Figure). Therefore, swine are also sources of autochthonous cases through foodborne transmission.

Conclusions

Our findings clearly demonstrate that some food products that contain raw pork liver and are marketed to be

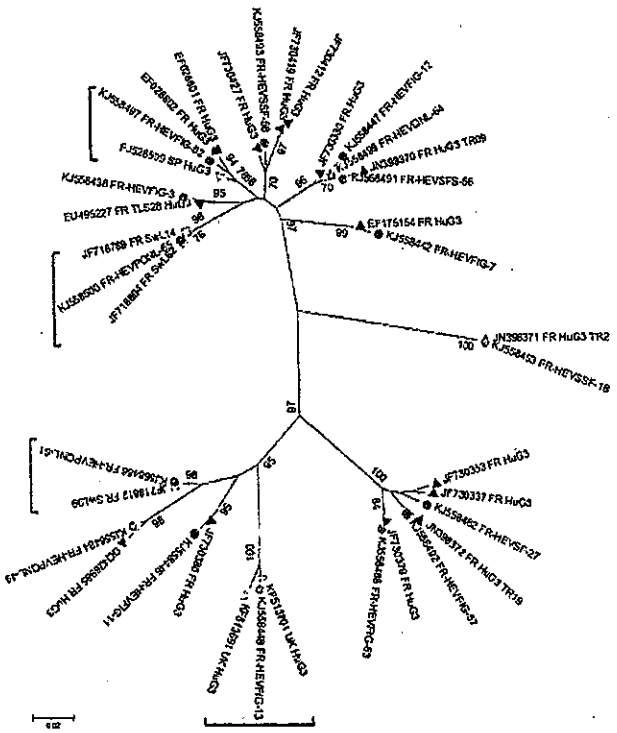

Figure. Phylogenetic tree of hepatitis E virus (HEV) sequences identified in food samples, France, 2011. Phylogenetic tree including 16 HEV sequences detected in food samples (gray circles) and the closest human (black triangles, French origin; white triangles, British or Spanish origin) or swine (white squares) sequences was constructed by using the neighbor-joining method with a bootstrap of 1,000 replicates based on the ClustalW alignment (MEGA4, http://www.megasoftware.net) on 290 nt sequences from open reading frame 2. HEV sequences retrieved from GenBank with ≥98% nt identity are indicated with their accession numbers. Bootstrap values of >70% are indicated on respective branches. Scale bar indicates nucleotide substitutions per site. Similar human and food sequences are shown in black brackets, similar swine and food sequences are shown in gray brackets.

cooked by the consumer can harbor HEV. The close sequence identity observed strongly suggests that foodborne transmission of HEV occurs frequently. Considering this high prevalence, consumers at risk for developing severe forms of HEV (e.g., solid-organ transplant recipients, person having underlying liver conditions, or pregnant women) should be informed about the HEV risk and should avoid eating such pork liver food products without thoroughly cooking them.

Acknowledgments

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Dr Pavio is a research director at the French Agency for Food, Environmental and Occupational Health and Safety. Her research interests include HEV zoonotic transmission and species barrier crossing.

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別紙様式第2-1
番号9

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 2015年02月24日	新医薬品等の区分 該当なし	厚生労働省処理欄

一般的名称	①②抗HBs人免疫グロブリン ③④乾燥抗HBs人免疫グロブリン ⑤　ポリエチレングリコール処理抗HBs人免疫グロブリン	研究報告の公表状況	www.upi.com/Health_News/2015/02/14/Aggressive-new-HIV-strain-detected-in-Cuba/2421423945549/2015/02/14	公表国 キューバ
販売名 (企業名)	①抗HBs人免疫グロブリン筋注200単位/1mL「JB」（日本血液製剤機構） ②抗HBs人免疫グロブリン筋注1000単位/5mL「JB」（日本血液製剤機構） ③ヘブスブリン筋注用200単位　（日本血液製剤機構） ④ヘブスブリン筋注用1000単位　（日本血液製剤機構） ⑤ヘブスブリンIH静注1000単位（日本血液製剤機構）			

研究報告の概要

ハバナ、2月14日（UPI）
キューバの一部の患者は、新たなHIV株により、発症期間が短いと思われる症例で、感染後3年以内にAIDSを発症した。研究者達は、発症は抗レトロウイルス薬による治療が手遅れになる程、非常に早く起こると語った。
ベルギーのLeuven大学の医学教授Anne-Mieke Vandammeによると、治療がなければ、HIV感染は通常、AIDSに罹るには5年から10年かかる。雑誌EBioMedicineに発表した研究によると、Vandammeは何が起こったかを知りたいキューバ保健当局にHIVの新たな攻撃的な株を警告した。
「このグループの患者は非常に早く発症したが、彼らは全員最近感染した。」とVandammeはVoice of Americaに説明した。「そして、我々は、彼らが1年、或いは最大2年前の検査でHIV陰性であったことを知っている。」
患者は誰もウイルスの治療を受けていなかった、そしてHIVの突然変異株に感染した患者全員が3年以内にAIDSを発症した。
AIDSへの早い進行は通常、HIVの特定の亜型よりも患者の免疫系が弱い結果であるとしても、キューバで起こっていることとは違った。
「我々は進行の早い患者群でのみHIVの変異株に感染していたことを発見した。他の2つのグループにはなかった。我々はこの変異株に焦点を当て、異なる検討をした。その結果、3つの異なる亜型の組換え体だと考えた。」
CRF19と名付けた新しい変異株は、HIV亜型A、DとGの組換え体である。
HIVは普通、補助受容体と呼ばれるものにそれ自身取り付くことにより細胞に感染し、そしてウイルスが一長い年月の後一補助受容体CCR5から補助受容体CXCR4に変わる時、AIDSへの移行が起こる。新しい株は変換がはるかに速くなる。
変異株はアフリカで観察されているが、あまりにも少ない症例のため十分に研究されていない。研究者達は株がキューバでより広範囲に広まっていると言う。
HIVの発症は殆んどの抗レトロウイルス薬に反応するが、彼らは薬を使う治療が手遅れになるまで、AIDSに罹ったことを理解できない可能性がある。複数のパートナーと無防備なセックスをする人々のために、HIVの検査を早期に、頻繁に受けることが不可欠だと、Vandammeは語った。

使用上の注意記載状況・その他参考事項等

代表としてヘブスブリンIH静注1000単位の記載を示す。
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。

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

報告企業の意見	今後の対応	
ヒト免疫不全ウイルス（Human Immunodeficiency Virus：HIV）は、レトロウイルス科（retrovirus）レンチウイルス属（Lentivirus）に属し、直径約 110nm のエンベロープを持つ一本鎖 RNA ウイルスである。血清学的に HIV-1 と HIV-2 に分類され、HIV-1 はは塩基配列により 4 群に分類され、グループ M（Major）、グループ O（Outlier）、グループ N（non-M/non-O）、グループ P（pending）に分けられるが、世界的に分布しているウイルスの多くがグループ M に属している。本剤の原料となる血液は抗 HIV-1 抗体，抗 HIV-2 抗体陰性であることを確認し、更に NAT スクリーニングを実施し、適合した血漿を使用している。万一、原料血漿に HIV が混入したとしても、HIV-1 を用い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るものではないと考えるので、特段の措置はとらない。	

抗 HBs

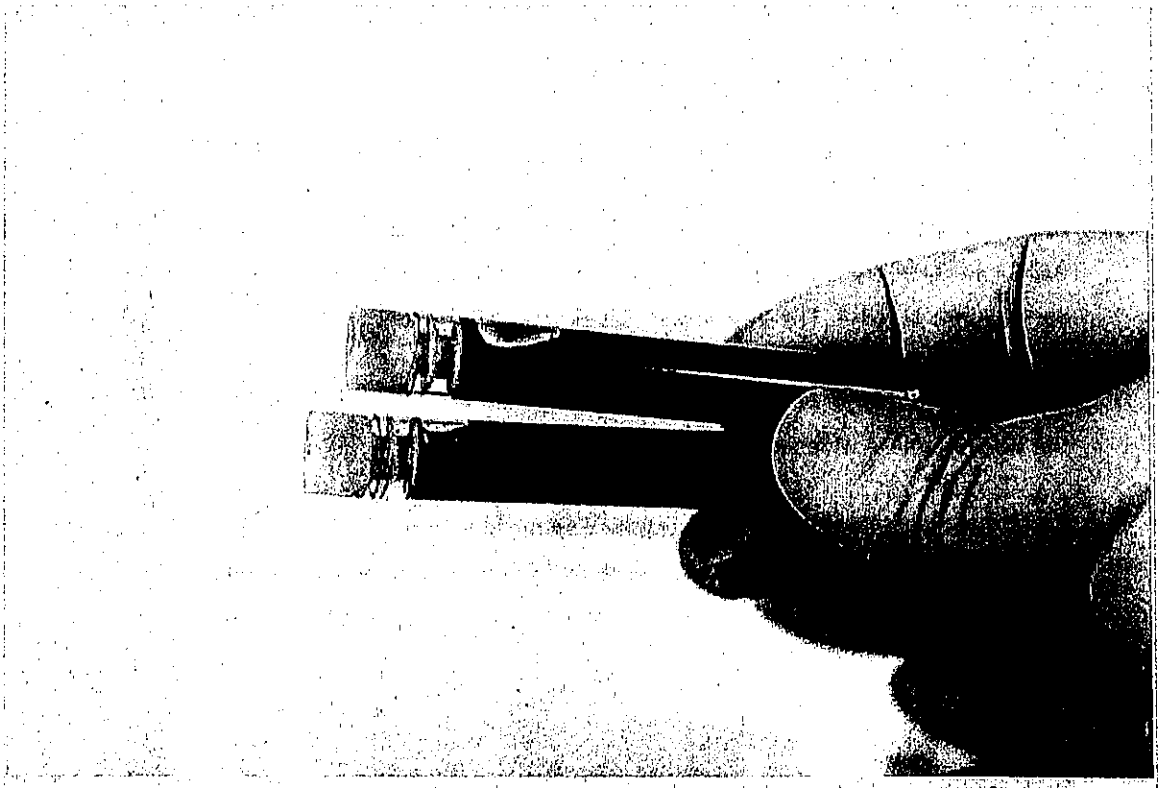
UPI

HOME / HEALTH NEWS

Aggressive new HIV strain detected in Cuba

Researchers said an aggressive HIV strain in Cuba progresses into AIDS so fast that treatment with antiretroviral drugs may come too late

By Denise Royal | Feb. 14, 2015 at 4:46 PM



An aggressive new strain of HIV has been detected in Cuba. Photo by Steve Collender/Shutterstock

HAVANA, Feb. 14 (UPI) -- A new HIV strain in some patients in Cuba appears to be much more aggressive and can develop into AIDS within three years of infection. Researchers said the progression happens so fast that treatment with antiretroviral drugs may come too late.

Without treatment, HIV infection usually takes 5 to 10 years to turn into AIDS, according to Anne-Mieke Vandamme, a medical professor at Belgium's University of Leuven. According to the study, published in the journal EBioMedicine, Vandamme was alerted to the new aggressive strain of HIV by Cuban health officials who wanted to find out what was happening.

"So this group of patients that progressed very fast, they were all recently infected," Vandamme explained to Voice of America. "And we know that because they had been HIV-negative tested one or a maximum two years before."

None of the patients had received treatment for the virus, and all of the patients infected with the mutated strain of HIV developed AIDS within three years.

While fast progression of HIV to AIDS is usually the result of the patient's weak immune system rather than the particular subtype of HIV, what's happening in Cuba is different.

"Here we had a variant of HIV that we found only in the group that was progressing fast. Not in the other two groups. We focused in on this variant [and] tried to find out what was different. And we saw it was a recombinant of three different subtypes."

The new variant, named CRF19, is a combination of HIV subtypes A, D and G.

HIV normally infects cells by attaching itself to what is called a co-receptor, and the transition to AIDS usually occurs when the virus switches -- after many years -- from co-receptor CCR5 to co-receptor CXCR4. The new strain makes the switch much faster.

The variant has been observed in Africa, but in too few cases to be fully studied. Researchers said the strain is more widespread in Cuba.

While the aggressive form of HIV responds to most antiretroviral drugs, people may not realize they have AIDS until it's too late for treatment to do any good. Vandamme said it's vital for people having unprotected sex with multiple partners to be tested for HIV early and often.

Read more:
http://www.upi.com/Health_News/2015/02/14/Aggressive-new-HIV-strain-detected-in-Cuba/2421423945549/#ixzz3SpDrxkXk

別紙様式第2-1

No. 19

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2014. 10. 20	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況	Roth A, Mercier A, Lepers C, Hoy D, Duituturaga S, Benyon E, Guillaumot L, Souares Y. Euro Surveill. 2014 Oct 16;19(41). pii: 20929.	公表国 ニューカレドニア
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

○デング、チクングニヤ、ジカウイルス感染の同時アウトブレイク－太平洋地域における蚊媒介性ウイルスの前例のない流行
2012年1月～2014年9月17日までの期間、太平洋地域で、デングウイルス(DENV)、チクングニヤウイルス(CHIKV)、ジカウイルス(ZIKV)の新規アウトブレイク28件(DENV1-4:18件、CHIKV:7件、ZIKV:3件)が報告された。2013年末にZIKVが拡がり始めてからはDENV1-3、CHIKV、ZIKVの同時流行も見られた。これらの蚊媒介性疾患の流行は以前よりも頻繁に、広範囲で発生しており、現在の流行はこの先数年間続く可能性がある。人々の免疫の問題や蚊の分布、大規模な人の移動や航空機による旅行などの理由から、太平洋地域で更なる拡散が起こるリスクも高いと考えられる。太平洋の島々の保健制度にとって既に大きな負担となっている疾患の流行を軽減し、他地域への波及を防ぐためには、サーベイランスシステムと対策を強化する必要がある。

報告企業の意見	今後の対応
2012年1月以降、太平洋地域ではDENV、CHIKV、ZIKV感染の新規アウトブレイクが28件あり、これらの蚊媒介性疾患の流行はこれまでより頻繁に広範囲で発生しており、流行はこの先数年間継続する可能性があるとの報告である。	日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

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

RAPID COMMUNICATIONS

Concurrent outbreaks of dengue, chikungunya and Zika virus infections – an unprecedented epidemic wave of mosquito-borne viruses in the Pacific 2012–2014

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Since January 2012, the Pacific Region has experienced 28 new documented outbreaks and circulation of dengue, chikungunya and Zika virus. These mosquito-borne disease epidemics seem to become more frequent and diverse, and it is likely that this is only the early stages of a wave that will continue for several years. Improved surveillance and response measures are needed to mitigate the already heavy burden on island health systems and limit further spread to other parts of the world.

Since January 2012, the Pacific is experiencing a high burden of mosquito-borne disease due to concurrent epidemics of dengue, chikungunya and Zika virus infections. So far over 120,000 people have been reported to be affected, a figure that is likely to substantially underestimate the real numbers due to underreporting. For as long as there has been data available from the Region (i.e. 40 years), this epidemic wave of mosquito-borne viruses with 28 new mosquito-borne viral outbreaks (n=25) and circulation (n=3) documented since January 2012 (18 Dengue virus (DENV) serotype 1–4, 7 chikungunya virus and 3 Zika virus infection outbreaks, respectively) is unprecedented (Table) [1-3]. We here present an overview of the surveillance and epidemiology of these mosquito-borne disease epidemics in the Pacific Region, to help facilitate response measures that are needed to mitigate the already heavy burden on island health systems and to limit further spread to other parts of the world.

Surveillance of mosquito-borne viruses in the Pacific Region

The Pacific Public Health Surveillance Network (PPHSN) is a voluntary network of countries, territories and organisations created in 1996. It is dedicated to the promotion of public health surveillance and response to health emergencies in the Pacific Region. It covers 22 Pacific Island countries and territories (hereafter referred to as the Pacific Region) with a population of 10.6 million inhabitants [4]. The network services include the timely exchange of information on outbreak-prone disease through PacNet, an email list with around 680 health professionals, and diagnostic support through a network of laboratories for identification and verification of pathogens.

In 2010, the Pacific Syndromic Surveillance System was introduced in the PPHSN. It monitors four syndromes and aims at improved early warning to complement routine notifiable disease notification systems that generally are not timely and seldom used for regional surveillance purposes in the Pacific Region. The Syndromic Surveillance system is under development and currently includes sentinel reporting from primary healthcare or hospital sites in all countries [5]. Manifest dengue, chikungunya and Zika virus infections have a similar initial clinical presentation and may be reported as any of the first three of the following four monitored syndromes: (i) acute fever and rash, (ii) prolonged fever, (iii) influenza-like illness and (iv) diarrhoea. Due to similar initial clinical features to the three mosquito-borne diseases, concurrent measles epidemics and leptospirosis pose diagnostic challenges in the Region.

There is a need for timely, reliable and detailed data on mosquito-borne virus outbreaks and circulation of the viruses in the Pacific Region. To obtain a comprehensible overview of the present epidemiological picture, several sources of information are used. Further to PacNet, syndromic and laboratory-based surveillance, event-based surveillance (mainly media and personal communications with health professionals) and surveillance by-proxy (reports of exported cases to neighbouring countries) [6] are also important. To facilitate better risk assessments and efficiency of data dissemination, this data is visualized in a recently launched interactive map available from: www.spc.int/phd/epidemics. The map, updated weekly, provides the region for the first time with a dynamic real-time picture of the current epidemic situation.

TABLE

Characteristics of new dengue, chikungunya and Zika virus infection outbreaks and circulation[a,b], Pacific Region, January 2012–17 September 2014[c] (n=28)

	Country	Month of onset	Latest information	Implicated mosquito borne virus	Summary	Sources
Dengue						
	Tuvalu	Mar-14	10/07/2014	DENV-2	408 suspected cases with 195 cases positive in rapid tests (NS1/IgM).	[32]
	Nauru	Mar-14	7/08/2014	DENV-3	251 suspected cases with 91 confirmed using IgM ELISA and/or rapid test. Samples sent for confirmatory serotyping.	[32, 33]
	Tonga	Feb-14	21/08/2014	DENV-3	Outbreak ongoing; 2 cases of dengue imported into New Zealand since 12 July 2014.	[32–34]
	New Caledonia	Feb-14	17/09/2014	DENV-3	In 2014, 338 cases of dengue recorded of which 55% were DENV-3. Virus circulation ongoing, with latest reported dengue case on 12 September 2014.	[35]
	Fiji	15/01/2014[c]	23/06/2014	DENV-2	Circulation of virus, no outbreak declared; 6 confirmed cases of DENV-2 imported into Queensland Australia from January to June 2014.	[33]
	Vanuatu	Dec-13	20/04/2014	DENV-3	1,561 dengue cases reported; 5 imported cases in Queensland Australia since January 2014 and 10 imported cases in New Caledonia from January to March 2014; 4 cases of DENV with serotype unknown imported into New Zealand since April 2014, of which 1 in July 2014.	[32–35]
	Kiribati	Nov-13	24/01/2014	DENV-3	As of 16 Jan 2014, 198 suspected dengue cases of which 85 were laboratory-confirmed. Outbreak over, only sporadic cases of fever.	[32], media: Radio New Zealand International
	Vanuatu	27/10/2012[c]	20/04/2014	DENV-1	Circulation of virus, no outbreak declared; 2 confirmed cases imported into Queensland Australia (1 case in 2013 and 1 case in 2014) and 6 cases into New Caledonia (5 cases in 2013 and 1 case in 2014); 4 cases of dengue serotype unknown imported into New Zealand since April 2014, of which 1 in July 2014.	[33, 35]
	Fiji	Oct-13	5/06/2014	DENV-3	25,300 suspected cases, 15 deaths. Outbreak is ongoing; 15 confirmed cases imported into Queensland Australia from December 2013 to May 2014.	[33], media: Radio Australia, Fiji Broadcasting Corporation
	French Polynesia	Feb-13	6/09/2014	DENV-1 DENV-3	As of 23 May 2014, 2188 positive cases since February 2013, and between 16 400 and 34 000 clinical visits estimated. 11 severe cases in March 2014 and 5 severe cases requiring hospitalisation in July 2014. DENV1 outbreak is still ongoing, but there are no cases of DENV3 reported since April 2014.	[36]
	Wallis & Futuna	Jan-13	28/03/2013	DENV-1	88 suspected cases and 16 confirmed of which 11 imported cases from New Caledonia.	[37]
	Solomon Islands	Dec-12	15/08/2014	DENV-3	7,697 reported cases as of 31 December 2013. As of June 2014, 1,762 suspected cases since January 2014, and 282 out of 1,500 samples tested positive in rapid tests. In March 2014 DENV-3 confirmed. Outbreak still ongoing.	[32, 33], media: Solomon Star
	Kosrae, Federated States of Micronesia	Sep-12	19/07/2013	DENV-4	729 reported clinical cases; 206 cases laboratory confirmed by rapid diagnostic tests. No deaths reported.	[9, 37]
	New Caledonia	Sep-12	17/09/2014	DENV-1	Largest ever recorded outbreak of dengue in New Caledonia with 10,978 cases and 5 deaths from September 2012 to September 2013; 338 cases of dengue recorded in 2014 of which 45% were DENV-1. Virus circulation ongoing, with latest reported dengue case on 128 September 2014.	[35]
	Fiji	15/07/2012[c]	31/12/2012	DENV-2	Circulation of virus, no outbreak declared. 2 imported cases in Queensland Australia;	[33]
	Kiribati	Mar-12	4/05/2012	DENV-1	243 clinical cases.	[32]
	Niue	Feb-12	20/07/2012	DENV-1	More than 100 cases.	Media: Radio New Zealand International

	Country	Month of onset	Latest information	Implicated mosquito borne virus	Summary	Sources
Chikungunya						
◉	Tokelau	Jul-14	11/09/2014	CHIKV	164 suspected cases reported. CHIKV confirmed.	[32]
◉	Samoa	Jul-14	1/09/2014	CHIKV	433 cases reported over 4 weeks. 21 RT-PCR positives out of 59 samples (as of 28 Aug 2014).	[37], media: Samoa Observer
◉	American Samoa	Jun-14	17/09/2014	CHIKV	823 probable cases reported, with 15 hospitalisations. CHIKV confirmed.	[37], media Radio New Zealand International
◉	Tonga	Feb-14	11/09/2014	CHIKV	Over 10,000 suspected cases reported. Ongoing circulation of CHIKV confirmed.	[32–34]
◉	Yap, Federated States of Micronesia	Aug-13	10/09/2014	CHIKV	A total of 1,711 suspected cases identified in Yap State. Circulation of CHIKV reconfirmed.	[37]
	New Caledonia	Jan-13	2/06/2014	CHIKV	A total of 32 confirmed cases from January to May 2013.	[35]
	Papua New Guinea	Jun-12	25/11/2013	CHIKV	A major outbreak spread over Papua New Guinea in 2013. Number of cases not reported, but estimated in media to be tens of thousands of cases.	[2], media: Australia Network News, Pacnews
Zika virus infections[d]						
	Cook Islands	Feb-14	29/05/2014	ZIKV	Outbreak is over. 932 suspected and 50 confirmed cases.	[32], media: Radio New Zealand International
◉	New Caledonia	Jan-14	17/09/2014	ZIKV	Imported cases reported in November 2013, first autochtonous case reported in January 2014; 1,400 confirmed cases of which 35 imported cases. Outbreak peaked in April 2014. Last case reported on 2nd August 2014.	[35]
	French Polynesia	Oct-13	4/05/2014	ZIKV	8,723 suspected cases reported and more than 30,000 estimated clinical visits due to Zika. Outbreak declared over but virus circulation may be ongoing.	[36]

◉ Cases reported are increasing or peaking

◉ Cases reported are decreasing or viral circulation is ongoing

Outbreak is reported to be over and/or no cases have been reported for one year.

CHIKV: chikungunya virus; DENV: dengue virus serotype 1-4; RT-PCR: reverse-transcriptase polymerase chain reaction; ZIKV: Zika virus.

[a] Only incident outbreaks and circulation notified during the reported period. Outbreaks first reported in 2011 (DENV-4 in Marshal Islands, DENV-2 in Yap and circulation of DENV in Papua New Guinea and Fiji) and still ongoing in 2012 are not presented.

[b] An outbreak is considered an outbreak when reported as such or when the first autochtonous cases are reported, and new circulation if there have been no events reported during one year.

[c] Month of start equals the month of first report, as this reflects circulation of virus.

[d] Easter Island experienced a Zika virus infection outbreak starting February 2014, but is not presented in the table as it is not part of the 22 countries and territories of the Pacific Public Health Surveillance Network .

The epidemiology of mosquito-borne viruses in the Pacific Region

Mosquito-borne virus diseases in the Pacific Region have a distinct epidemiology due to small populations scattered over thousands of tropical and sub-tropical islands on both sides of the equator in relative geographic isolation, together with (nowadays) significant people's mobility and thereby exposure to circulating arboviruses through the airline networks of the Asia-Pacific region (Figure 1).

Between January 2012 and 17 September 2014, a total of 28 new mosquito-borne viral outbreaks (n=25) and circulation (n=3) were documented: 18 DENV 1–4 outbreaks (2012: 7; 2013: 6; 2014: 5), 7 chikungunya virus (CHIKV) (2012: 1; 2013: 2; 2014: 4) and 3 Zika virus infection outbreaks (2012: 0; 2013: 1; 2014: 2), respectively.

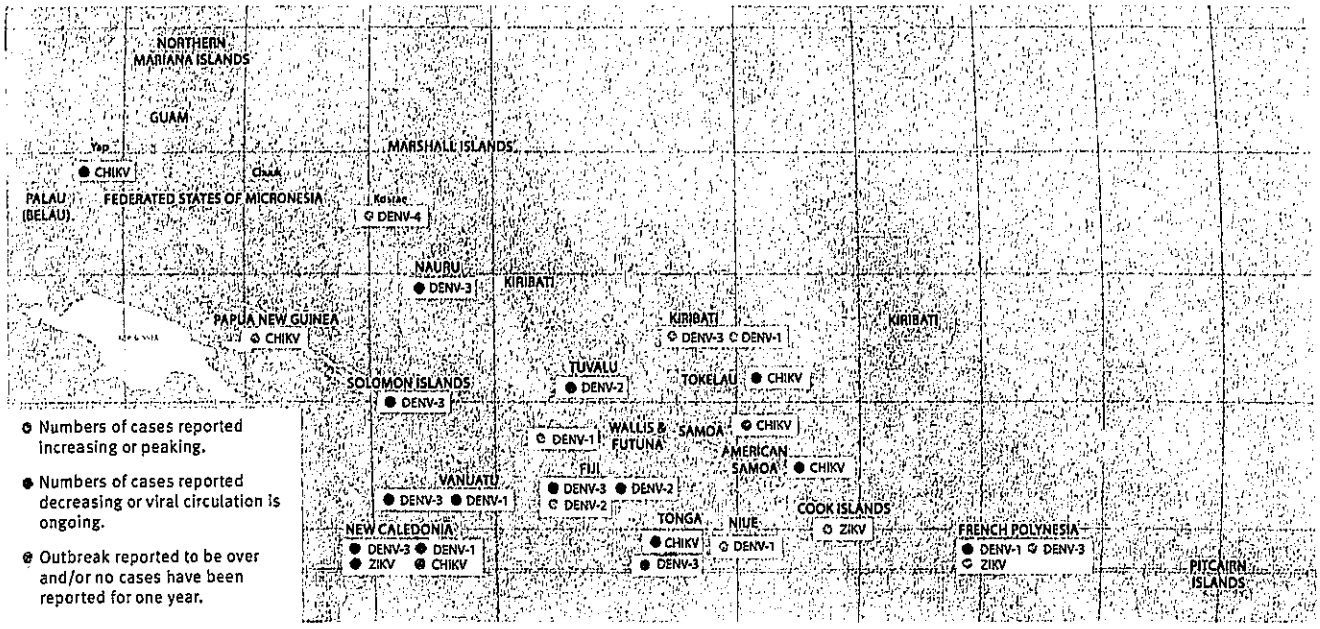
Looking at the first semester of 2014, the number of outbreaks and circulating mosquito-borne viruses seem to be increasing (Figure 2). During the same period, DENV-3 became the dominating dengue virus, and since Zika virus started to spread in the end of 2013, there was concurrent circulation of DENV-1,-2 and -3, CHIKV and Zika virus (Table, Figure 2)

Dengue

The epidemic pattern of dengue in the Pacific Region has typically presented in form of sporadic or rare epidemics rather than a hyperepidemic/endemic pattern, with one dominating serotype sweeping across the islands every 3 to 5 years, and with varying duration of circulation in different islands largely depending on population size [1,7- 8]. During 2012, there were outbreaks of all four serotypes of DENV documented for the first time during one year (Figure 2) [1]. DENV-1 was the dominating serotype in 2012 and beginning 2013, causing the largest documented dengue outbreak ever in New Caledonia, with 10,978 confirmed cases and 5 deaths from September 2012 to September 2013. Since 2012 there have only been reports of one outbreak with DENV-2 and -4 respectively: DENV- 2 recently caused an outbreak in Tuvalu with 408 suspected cases (4% of the population) and DENV-4 caused a large outbreak in Kosrae in September 2012 to March 2013 with 729 clinical cases (11% of the population) (Table) [9]. Furthermore there have been reports of new circulation of DENV-2 in Fiji. (Table) After having been absent in the region for 18 years, DENV- 3 has after the reintroduction in 2012, become the dominating DENV in the region with five ongoing outbreaks, one of them in Fiji, with 25,300 suspected cases and 15 deaths (Table, Figure 1) [1,10].

FIGURE 1

Map of newly reported dengue, chikungunya and Zika virus infection outbreaks or new virus circulation[a], Pacific Region[b], January 2012–17 September 2014[c] (n=28)



CHIKV: chikungunya virus; DENV: dengue virus serotype 1-4; ZIKV: Zika virus.

[a] Only incident outbreaks and virus circulation reported during the period. Outbreaks first reported in 2011 (DENV-4 in Marshal Islands, DENV-2 in Yap and circulation of DENV in Papua New Guinea and Fiji) and still ongoing in 2012 are not presented.

[b] The 22 Pacific Island countries and territories that are core members of the Pacific Public Health Surveillance Network and referred to as the Pacific Region.

[c] Real-time interactive map with current epidemiological situation and alerts is available from: www.spc.int?phd/epidemics

Chikungunya

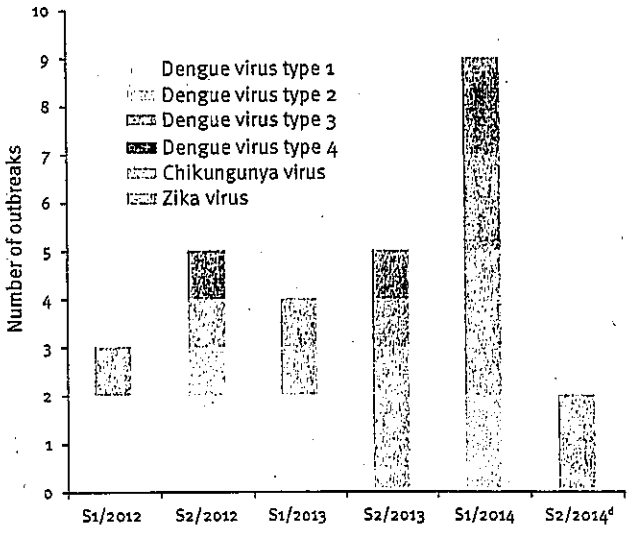
After being reported in the Pacific for the first time in a small tightly controlled outbreak in New Caledonia in 2011 [11], CHIKV is currently becoming established in the Region (Figure 1, Table) [2]. In Papua New-Guinea in 2012-13, the largest epidemic in the Region so far with estimated (though poorly documented) tens of thousands of cases, was caused by the East Central South African (ECSA) lineage of the virus [2]. The Asian lineage of the virus was responsible for the outbreak in Yap State (2013-14) [12] and also in New Caledonia (2013) where CHIKV re-emerged in the middle of a large DENV-1 epidemic and caused a small outbreak, similar to the 2011 outbreak (Table) [13]. Phylogenetic analyses of the CHIKV involved in the outbreaks in Tonga, Samoa and American Samoa are not yet available. Due to the on-going geographic expansion of *Aedes albopictus* in the Pacific region (Figure 3), virus genotype monitoring is a crucial aspect of surveillance.

Zika virus infections

After the first documented Pacific Zika outbreak in Yap in 2007 [14], the Asian lineage of the virus reappeared in French Polynesia in October 2013, and has since caused large outbreaks in New Caledonia (1,400 confirmed cases), Cook Islands (over 900 cases) and Easter Island that is not part of the PPHSN (Figure 1, Table) [3]. In French Polynesia, extrapolation of the 8,746 suspect cases reported by the sentinel surveillance network allows to infer that over 30,000 medical consultations were due to the spread of Zika virus throughout the archipelago. Between November 2013 and February 2014, increased incidence of neurological complications, including 42 cases of Guillain-Barré syndrome, was a unique and worrying feature of the French Polynesia outbreak that warrants further studies [3].

FIGURE 2

Incidence and aetiology of newly reported mosquito-borne virus outbreaks and circulation[a] by semester[b], Pacific Region, January 2012–17 September 2014[c] (n=28)



S: semester.

[a] An outbreak is considered an outbreak when reported as such, and new circulation of virus if there has been no event with the same virus reported during one year previously.

[b] S1 includes the months from January to June and S2 the months from July to December.

[c] Outbreaks or circulation that started before January 2012 or after 17 September 2014 are not presented in this graph to allow observation of a possible trend over time.

[d] Semester 2 in 2014 is not complete, and only includes reports from two full months out of six.

Discussion and Conclusions

Burden on the Pacific countries and territories

Mosquito-borne outbreaks are greatly exacerbating the pre-existing burden that Pacific Island primary healthcare systems face. If not managed well, the epidemic wave may threaten societies broadly, affecting trade, tourism and work force beyond the direct morbidity and mortality toll [2]. During the chikungunya outbreak in Reunion Island, one third of the around 800,000 inhabitants were infected, peaking at more than 47,000 estimated cases in one week, with estimated productivity loss of €17.4 million (range €6 to €28.9 million) and medical costs of €43.9 million that were met by the French state [15-17]. Much of the burden on the Pacific Region of the concurrent epidemics of all three diseases covered here is unknown and further studies are warranted, especially on co-infection and the effect of sequential infection with different viruses.

Zika virus disease, generally reported to have a mild clinical presentation, was associated with neurological complications during concurrent Zika virus disease and dengue epidemics in French Polynesia [3,18]. The Pacific Region may be particularly vulnerable to communicable diseases due to isolation and immunologically naive populations, but also due to rates of non-communicable disease, such as obesity, diabetes and cardiovascular disease, that are among the world's highest on some islands [19].

The risk for further spread

While there have been efforts to improve surveillance in the Pacific over the past two decades, it is not likely that the extent of the current increase in diversity and frequency of mosquito-borne virus outbreaks in the Pacific can be explained solely by improved surveillance systems. In the island setting of the Indian Ocean, the largest documented CHIKV outbreak lasted four years (2004–2007) [15]. Therefore, considering also the previous dengue outbreaks in the Pacific Region [1-2] and the diversity of the current outbreaks, it seems likely that the Pacific Region is in the early stages of an epidemic wave for the three mosquito-borne viruses that started in 2012 and is likely to continue for several years.

The risk for further spread in the Pacific Region is high for several reasons. Firstly, it is likely that there is little immunity to these diseases, as DENV-3 had not been

circulating in the Region since 1995 [1] and prior to the current wave, CHIKV and Zika virus occurrence in the Pacific was limited to two documented outbreaks [11, 14]. Secondly, competent vectors present in the Region, mainly *Ae. aegypti* and *Ae. albopictus*, but also other local mosquitoes such as *Ae. polynesiensis* or *Ae. hensilli* are known to transmit these viruses (Figure 3) [20]. These species have been incriminated in DENV transmission on epidemiological and/or experimental (laboratory infections) grounds. Several of them are confirmed or strongly suspected vectors of CHIKV and Zika viruses [21]. Thirdly, large population mobility and airline travel facilitate the spread [22].

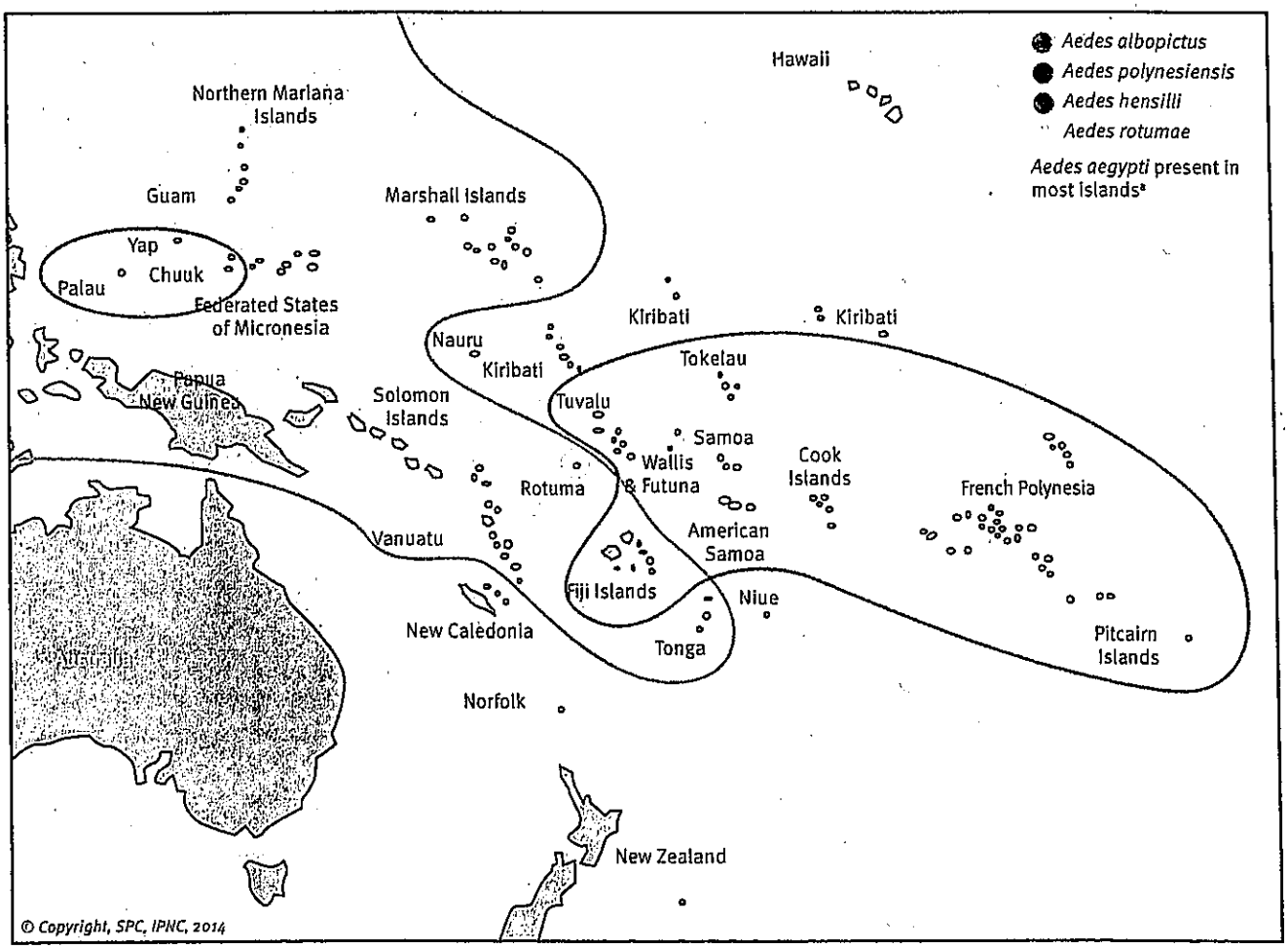
Vector control capacity in the Pacific Region is often limited or insufficient [11]. At present, there is no ongoing entomological surveillance system targeting vectors of dengue and other arboviruses established in the Region except in New Caledonia, Fiji and French Polynesia. The current knowledge about mosquito distribution in the other countries and territories is based on data collected during entomological investigations in surveys from the second half of the 20th century and from some more recent surveys [20]. Interestingly, the three viruses involved in this epidemic wave are not broadly mosquito-borne, but specifically *Aedes (Stegomyia)*-borne.

The cause of the recent increase in mosquito-borne disease in the Pacific Region is largely unknown, but is in line with a global increase of emerging diseases, and likely driven by a combination of socio-economic, environmental and ecological factors [23].

The continuous challenges of dengue and chikungunya [24] and more recently Zika virus infections [25]

FIGURE 3

Map of the known distribution of *Aedes (Stegomyia)* mosquitoes, vectors of dengue and possible vectors of chikungunya and Zika viruses, Pacific Region as of beginning October 2014



[a] *Aedes aegypti* (not represented on the map) is present throughout most of the region including North Queensland. It is absent from the rest of Australia, New Zealand, Hawaii, Futuna and some other remote islands, and it seems to be currently displaced by *Ae. albopictus* in many locations (e.g. Papua New Guinea and Solomon Islands).
The known or strongly suspected distributions of other vectors are as follows (not exhaustive): *Ae. scutellaris* (Indonesia; Northern Australia, Papua New Guinea); *Ae. marshallensis* (Marshall Islands; Western Kiribati; Kosrae; Pohnpei); *Ae. hebrideus* (Papua New Guinea; Solomon Islands; Vanuatu); *Ae. cooki* (Niue, Vava'u Group, Tonga); *Ae. tongae* (Ha'apai Group, Tonga); *Ae. tabu* (Tongatapu group, Tonga); *Ae. kesseli* (Niua group, Tonga); *Ae. pseudoscutellaris* (Fiji). [25]

for Europe, the re-emergence of dengue in Japan [26], and the first-time chikungunya transmission in the Americas [27], show that these viruses pose a threat to any country with competent vectors. The epidemiology of mosquito-borne viruses in the Pacific may be changing. There are close links between the several European overseas countries and territories in the Pacific Region and Europe and the United States [28]. Considering the extensive airline travel between the Pacific Region and other parts of the world where the viruses have not yet been established e.g. Europe and the United States, it should be of international interest to stay informed of the spread of the current Pacific Region wave of mosquito-borne viruses and to support surveillance and control efforts [2,23,29].

Examples of response from PPHSN partners to the epidemic situation include the provision of support and capacity building to Pacific Islands in surveillance, outbreak investigation and response, and mass-gathering surveillance. The Pacific Outbreak Manual is also being updated to include specific response guidelines for the three viruses [30].

To further enhance surveillance and response measures, Pacific Directors and Ministers of Health have shared the current risk assessment, and the upcoming Pacific International Health Regulations meeting will focus on mosquito-borne diseases. Island primary healthcare-based systems have difficulties to cope with high caseloads and there is a need for early multidisciplinary preparedness and response to face larger outbreaks adequately [2].

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Conflict of interest

None declared.

Authors' contributions

AR, YS, CL, DH conceived the idea of the paper. AR, AM, CL, SD and EB contributed to data gathering and cleaning. AR, CL and AM conducted the analysis. LG gathered and compiled all vector data and analysis. AR and AM drafted the first draft, and all
authors commented and agreed upon 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	新医薬品等の区分	総合機構処理欄
				2015. 1. 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		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)		Emerging Infectious Disease Journal, Vol.21 No.2; Available from: http://wwwnc.cdc.gov/eid/article/21/2/14-1433_article	フィリピン

研究報告の概要

○フィリピンのヘニパウイルス感染症アウトブレイク、2014年
2014年、フィリピン南部の2つの村において、ヘニパウイルス感染症による重症疾患がヒト及びウマに発生した。調査により確認された患者は17人であり、急性脳炎11人、重症インフルエンザ様疾患5人、髄膜炎1人で、急性脳炎症例の致死率は82%であった。17人のうち、7人(41%)はウマの屠殺への関与及び馬肉を摂取し、3人(18%)は屠殺には関わっていないが、馬肉は摂取していた。5人(29%)はウマとの接点はないが、他の患者との接触があり、そのうち2人は同村を訪れていない医療従事者であった。ヒト症例と同時期に、同村において10頭のウマが死亡し、1頭以外全てのウマに神経学的徴候が見られた。
臨床所見、疫学的調査結果及び血清学的検査結果は、このアウトブレイクの原因がヘニパウイルス属のウイルスであることを示唆し、最も可能性のあるウイルスはニパウイルスもしくはニパウイルスに血清学的、遺伝的に近縁なウイルスであると考えられた。今回のアウトブレイクで最も一般的な感染経路は感染したウマへの直接接触であったが、少なくとも5症例に関する臨床及び疫学的エビデンスはヒトーヒト感染を示唆している。ウマの感染源については不明であるが、ヘニパウイルスの生態より、最も可能性のある原因はオオコウモリである。

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
2014年、フィリピン南部の2つの村のヒト及びウマにおいて、ヘニパウイルス感染症アウトブレイクが発生したとの報告である。	日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。今後も引き続き情報の収集に努める。

DISPATCHES

Outbreak of Henipavirus Infection, Philippines, 2014

Paola Katrina G. Ching, Vikki Carr de los Reyes, Maria Nemia Sucaldito, Enrique Tayag, Alah Baby Columna-Vingno, Fedelino F. Malbas Jr., Gilbert C. Bolo Jr., James J. Sejvar, Debbie Eagles, Geoffrey Playford, Erica Dueger, Yoshihiro Kaku, Shigeru Morikawa, Makoto Kuroda, Glenn A. Marsh, Sam McCullough, A. Ruth Foxwell

During 2014, henipavirus infection caused severe illness among humans and horses in southern Philippines; fatality rates among humans were high. Horse-to-human and human-to-human transmission occurred. The most likely source of horse infection was fruit bats. Ongoing surveillance is needed for rapid diagnosis, risk factor investigation, control measure implementation, and further virus characterization.

Henipaviruses belong to a genus of recently emerging viruses within the family *Paramyxoviridae* (*1–3*) and include 2 zoonotic members: Hendra virus (HeV) and Nipah virus (NiV). HeV was first described in Australia in 1994, when it caused an outbreak of severe acute respiratory diseases that led to a high mortality rate among horses. Subsequently, several sporadic cases of HeV infection have occurred in horses in Australia; transmission to humans has occurred and the fatality rate was high (*4*,*5*). NiV was first recognized as a human pathogen in peninsular Malaysia in 1998. This outbreak among pig farmers and abattoir workers exposed to infected swine secretions (*6*) was associated with severe encephalitic illness and a high fatality rate. Subsequently, NiV emerged as a major public health problem in Bangladesh and India (*7–9*).

The natural reservoir of both viruses is pteropid bats, which harbor the viruses but do not show clinical illness (*3*). Virus transmission from bats to domestic animals is thought to be through pasture or feed contaminated by bat urine, feces, or other excretions (*10*). Transmission of HeV to humans has been invariably associated with close contact with ill horses (*4*), and transmission of NiV in Bangladesh is mainly through date palm sap contaminated with bat secretions (*11*). Human-to-human transmission of NiV also occurs (*12*,*13*).

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The Study

On April 2, 2014, the Philippine National Epidemiology Center received a report of human deaths in 2 villages, Tinalon and Midtungok, in the municipality of Senator Ninoy Aquino, province of Sultan Kudarat, island of Mindanao. The villages are ≈15 km apart, and the provincial referral hospital is in Isulan, 80 km away. An outbreak investigation led by the National Epidemiology Center identified additional human deaths and nonfatal infections and concurrent neurologic disease and sudden deaths in several horses, all of which were subsequently consumed by villagers. On May 12, 2014, the Philippine government asked the World Health Organization for further outbreak investigation assistance.

During May 22–24, 2014, a combined team from the Philippine Department of Health, Department of Agriculture, and the World Health Organization interviewed persons who survived, those with suspected cases, and family members of the deceased and conducted focus group interviews with other persons in affected villages. Key informants from local human and animal health agencies were also interviewed, and hospital records for persons with suspected cases were reviewed. We defined a human case as illness in any person with an epidemiologic link to the municipality of Senator Ninoy Aquino and who had experienced acute encephalitis syndrome, severe influenza-like illness (ILI), or meningitis during March 3–May 24, 2014.

The case definition was met by 17 persons (11 acute encephalitis syndrome, 5 ILI, 1 meningitis). Clinical signs developed for the index case-patient on March 10 and for the last case-patient on April 21 (Figure 1). The case-fatality rate among those with acute encephalitis syndrome was 82%; no patient with ILI or meningitis died. Of acute encephalitis syndrome survivors, 1 experienced residual severe cognitive impairment, motor weakness, and ataxia, and the other experienced persistent ophthalmoplegia. Median incubation period for case-patients with known exposure was 8 days.

Table 1. Exposure and infection profile of henipavirus case-patients, Sultan Kudurat, Mindanao, Philippines, March 3–May 24, 2014

Clinical presentation	Slaughter and meat consumption	Meat consumption alone	Exposure to probably infected human	Uncertain exposure	Total
Acute encephalitis syndrome					
No. patients	3	3	4	1	11
Sex, M:F	3:0	3:0	4:0	1:0	11:0
Age , y	21, 32, 60	30, 51, 54	24, 29, 35, and 46	28	32 (median)
Incubation period	6, 8, 8	7, 10, 20	3–8. 6, 7, 8	Unknown	3–20
No. deaths	3	2	3	1	9
Influenza-like illness (n = 5) or meningitis (n = 1)					
No. patients	4	0	1	1	6
Sex, M:F	4:0	NA	1:0	0:1	5:1
Age, y	21, 23, 26, 39	NA	46	26	26 (median)
Incubation period, d	7, 9, 15, 15	NA	4	Unknown	4–15
No. deaths	0	NA	0	0	0

*NA, not applicable.

Of the 17 case-patients, a total of 7 (41%) had participated in horse slaughtering and horse meat consumption, and 3 (18%) had only consumed horse meat and had no history of slaughtering or meat preparation (Table 1). Five (29%) case-patients had been exposed to other human case-patients but not to any horses. Of these, 2 were health care workers from Isulan who did not visit the villages, had no contact with sick horses, and did not consume horse meat (Figure 1); they reportedly wore minimal personal protective equipment (gloves, face mask) during patient procedures, 2 cared for case-patients in their homes, and 1 helped transport a case-patient (who was producing substantial respiratory secretions) to a hospital.

During March 3–May 11, ten horse deaths were reported in the 2 villages (Figure 1); 2 were found dead, and all but 1 of the others showed neurologic signs (head tilting, circling, ataxia). Progression of clinical signs was rapid. Among other domestic animals, 4 cats that had eaten horse meat died within 5 days of their probable exposure date; 3 were found dead and the other exhibited terminal bleeding from the nose and/or mouth. A dog was found dead after eating horse meat, but the epidemiologic link is unknown.

Blood was collected from surviving suspected case-patients, contacts of human or horse case-patients, and several domestic animals (cats, buffalo, dogs, horses, pigs, goats). Retrospectively collected cerebrospinal fluid (n = 2) and serum (n = 7) samples from persons with suspected cases underwent further testing. No samples were available from affected horses.

Testing for a range of neurotropic pathogens was conducted at the Australian Animal Health Laboratory and the National Institute of Infectious Diseases (Japan). Test results were negative for all agents except henipaviruses.

To detect neutralizing antibodies against HeV and NiV, we used neutralization assays with infectious HeV and NiV (*14*) and pseudotyped vesicular stomatitis virus possessing NiV envelope proteins (*15*) (Table 2). Samples with positive results were subsequently tested by ELISA

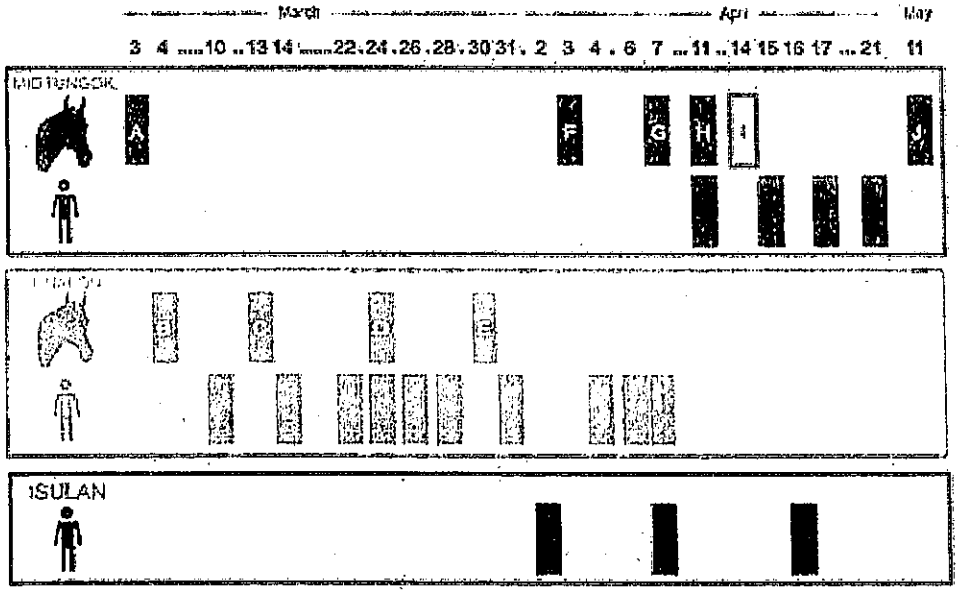

Figure 1. Temporal and geographic features of human and horse cases in 2 villages (Midtungok, Tinalon) and at the provincial referral hospital (Isulan), Philippines, 2014, by date of disease onset. Full rectangles represent cases based on case description. The empty rectangle (horse I) represents the horse death that did not fit the case description.

Table 2. Chronologic serologic test and nucleic acid detection results for 3 patients in NiV outbreak, Philippines, 2014*

Patient	Onset of clinical signs	Date of sample collection	IgM ELISA ratio	NiV SNT titer†	NiV SNT titer‡	Nucleic acid detection
1 (AES)	Apr 7	Apr 12	11.8	Neg	1:150	Pos (qPCR) from serum of Apr 12
		May 11	8.5	1:80	1:1,200	NA
		May 22	6.5	1:40	1:950	NA
2 (AES)	Apr 7	Apr 15	13.2 (6 AM), 12.9 (3 PM)	1:10	1:200	Pos (NGS) from CSF of Apr 12
		May 8	11.3	1:80	1:2,600	NA
		May 21	9.1	1:20	1:1,800	NA
3 (ILI)	Apr 2	May 21	5	1:40	1:420	NA

*The cutoff for the IgM NiV ELISA is a ratio of 2, for SNT using infectious NiV is ≥1:4, and for pseudotype-based SNT is 1:80. All samples were serum except for the sample tested by NGS, which was CSF. AES, acute encephalitis syndrome; CSF, cerebrospinal fluid; ILI, influenza-like illness; NA, not applicable; Neg, negative; NGS, next-generation sequencing; NiV, Nipah virus; pos, positive; qPCR, quantitative PCR for NiV; SNT, serum neutralization test.
†Test used infectious NiV.
‡Test used pseudotyped vesicular stomatitis virus.

for IgM against NiV. Neutralizing antibodies against NiV and correspondingly lower neutralizing antibody titers against HeV were found for 3 patients. IgM against NiV was also detectable in these same 3 patients. The pattern of neutralizing antibodies and IgM in acute-phase and convalescent-phase serum samples is evidence of recent exposure to a henipavirus. A serum sample from 1 of these patients (obtained 5 days after clinical sign onset) was also positive by real-time PCR for NiV, and a single-sequence read (71 bp) of the P gene of NiV was detected from a

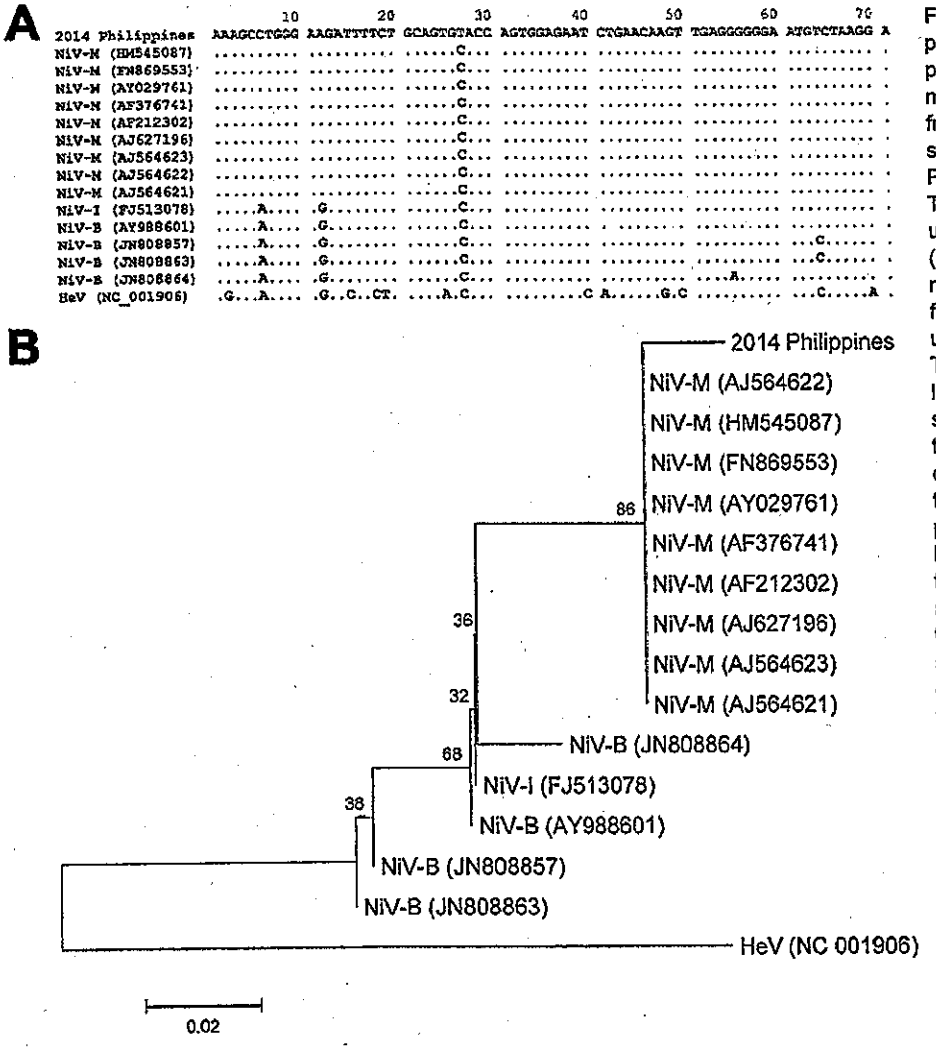

Figure 2. Alignment (A) and phylogenetic relationship (B) of partial phosphoprotein gene sequences (71 mer) of henipaviruses, including the fragment obtained by next-generation sequencing from a patient in Philippines (2014 Philippines). The alignment was conducted by using the MUSCLE program (http://www.ebi.ac.uk/Tools/msa/muscle/), and the phylogenetic tree from these data was constructed by using the neighbor-joining method. The optimal tree with sum of branch length equal to 0.23440320 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown. The phylogenetic tree is drawn to scale; branch lengths in the same units as those of the evolutionary distances are used to infer the phylogenetic tree. The scale bar represents 0.02 substitutions per site. The evolutionary distances were computed by using the Kimura 2-parameter method and are presented as number of base substitutions per site. The analysis involved 16-nt sequences. All positions containing gaps and missing data were eliminated. The final dataset contained 71 positions. Evolutionary analyses were conducted by using MEGA6 (http://www.megasoftware.net). The accession numbers of each sequence are shown for the viruses. HeV, Hendra virus. NiV-B, Nipah virus Bangladesh strain; NiV-I, Nipah virus Indian strain; NiV-M, Nipah virus Malaysian strain.

MiSeq (http://systems.illumina.com/systems/miseq.html) next-generation sequencing run of a cerebrospinal fluid sample from another of these patients (Figure 2). This short segment had 99% nt identity with NiV isolates from Malaysia and 94%–96% identity with NiV isolates from Bangladesh. Further attempts to amplify additional genome and isolate the virus were unsuccessful. The short-read archive has been deposited in the DNA Data Bank of Japan (accession no. DRA002637). All serum samples from 4 dogs were positive for neutralizing antibodies against NiV. NiV neutralizing antibodies were not detected in samples from animals of any other species.

Conclusions

Clinical presentations, epidemiologic findings, and serologic results suggest that the virus causing this outbreak was a henipavirus. It was most likely NiV or a virus that is antigenically and genetically closely related to NiV.

Epidemiologic data suggest that the most common route of virus transmission to humans was direct exposure to infected horses, contact with contaminated body fluids during slaughtering of sick horses, and/or consumption of undercooked meat from infected horses. However, for at least 5 cases, clinical and epidemiologic evidence suggest direct human-to-human virus transmission. No protective equipment was used by those who cared for case-patients in the home, and health care workers used gloves and a face mask but not eye protection. The evidence of human-to-human transmission in this outbreak confirms the need for preventative measures in home care and health care settings.

Although the source of the horse infections is unclear, on the basis of the known ecology of henipaviruses, the most likely source is fruit bats (family *Pteropodidae*) (*10*). Bats belonging to this family were reported near at least 1 of the 2 villages.

Ongoing surveillance in the area and neighboring regions is needed to help with prompt response to future outbreaks. Activities should include accurate and rapid diagnosis of new outbreaks, investigation of risk factors associated with spillover and virus transmission, implementation of control measures, and further characterization of the virus involved.

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Ms. Ching is a hospital staff nurse and a second-year fellow in the Philippine Field Epidemiology Training Program; she works at the Dr. Jose N. Rodriguez Memorial Hospital and the National Epidemiology Center, Department of Health, Philippines. Her research interests are outbreak investigations associated with vaccine-preventable diseases; foodborne illnesses; adverse events associated with fireworks use; and posttyphoon survival.

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別紙様式第2-1

No. 15

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2015. 1. 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	Abbott IJ, Papadakis G, Kaye M, Opdam H, Hutton H, Angus PW, Johnson PD, Kanellis J, Westall G, Druce J, Catton M. Am J Transplant. 2015 Feb;15(2):555-9. doi: 10.1111/ajt.12986. Epub 2015 Jan 12.	公表国 オーストラリア
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

○臓器移植ドナー由来のコクサッキーウイルスB3感染の検査による同定
臓器移植によるドナーからレシピエントへの予期せぬ感染症の伝播は、稀であるが重大な移植合併症である。本報告は、臓器移植後にコクサッキーウイルスB3(CVB3)のドナー由来伝播が確認された初の症例である。
生来健康なオーストラリア在住の若年成人ドナーが、10日間の頭痛を呈した後、くも膜下出血により2011年春に死亡した。脳死後に臓器移植のために肝臓、腎臓、膵臓、肺が摘出され、異なる州の4人のレシピエントに移植された。心臓弁は組織バンクに保存された。移植後、肝臓レシピエントにおいてALT値上昇が見られたため、保存されていた心臓弁の検査を行ったところ、顕著な好中球心膜炎、心筋炎及び血管周囲浸潤の所見が認められた。そこで、ドナー由来の感染症に関して調査が行われた。その結果、ドナー及び評価可能な3人のレシピエントのうち2人(肝臓、左腎/膵臓レシピエント)がCVB3のウイルス血症であることが示され、これらのレシピエントの糞便、直腸及び咽頭スワブからもウイルスが検出された。ドナーと2人のレシピエントから分離されたCVB3株は塩基配列的に同一であり、レシピエントの地域の他のCVB3株とは異なっていた。この結果、移植による感染であると考えられた。
これは、ドナー由来の感染症の可能性がある様々な病原体に対して、監視体制を維持する必要性を改めて強調する報告である。

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
臓器ドナーからレシピエントへCVB3が伝播した初の報告である。	今後も引き続き情報の収集に努める。

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Case Report

Laboratory Identification of Donor-Derived Coxsackievirus B3 Transmission

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Unexpected donor-to-recipient infectious disease transmission is an important, albeit rare, complication of solid organ transplantation. Greater work and understanding about the epidemiology of these donor-derived transmissions is continually required to further mitigate this risk. Herein we present the first reported case of proven donor-derived transmission of coxsackievirus serogroup-3, an enterovirus, following solid organ transplant. Swift and effective communication between the organ donation agency, treating physicians, laboratory testing and notification ensured a coordinated approach. The resulting clinical syndromes in the organ recipients were mild. This case highlights the requirement for ongoing surveillance over a broad range of infecting pathogens that may present as a donor-derived infection.

Abbreviations: ALT, alanine aminotransferase; BAL, bronchoalveolar lavage; CMV, cytomegalovirus; ct, cycle threshold; CVA, coxsackievirus group A; CVB, coxsackievirus group B; CVB3, coxsackievirus group B, serotype 3; DTAC, Disease Transmission Advisory Committee; EBV, Epstein-Barr virus; EV, enterovirus; HHV-8, human herpesvirus 8; HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; ICU, intensive care unit; ND, not detected; PCR, polymerase chain reaction; rt-PCR, real-time PCR; UNOS/OPTN, United Network for Organ Sharing/Organ Procurement and Transplantation Network; VF, ventricular fibrillation; VZV, varicella zoster virus

Introduction

Enteroviruses (EVs) are single stranded, positive sense RNA viruses in the family *Picornaviridae*. The EV genus is split into four distinct human species (human EV A, B, C and D). Within each species, based on molecular and biologic characteristics, individual serotypes maintain their traditional names such as coxsackievirus, divided into groups A and B, poliovirus and echovirus, as well as numbered strains, such as EV-A71 (1). These small RNA viruses are quite stable in liquid environments and can survive for many weeks in water, body fluids and sewage. They are ubiquitous agents found worldwide. Group A coxsackieviruses (CVA), which are split between the species EV-A, -B and -C, are the predominant EV subgroup associated with viral encephalitis, while group B coxsackieviruses (CVB), contained within the species EV-B, are an important cause of viral myocarditis, dilated cardiomyopathy and aseptic meningitis (2,3). There are six serotypes of CVB, and serotype 3 appears to have an additional tissue tropism for the liver and pancreas (4).

We describe the first report of donor-derived transmission of an EV by solid organ transplant, where the donor and two of the three evaluable organ recipients were all shown to be viremic with the same coxsackievirus serotype 3 (CVB3). Both recipients had detectable virus in other specimens, including feces, rectal and throat swabs. Clinical consequences for the infected organ recipients appear to have been relatively mild.

Case Report

The deceased donor, a young adult resident in Australia, without any significant past medical history, died in the springtime of 2011 following a subarachnoid hemorrhage associated with a ventricular fibrillation cardiac arrest after 10 days of headache culminating in collapse, but no fever. Other symptoms in the preceding weeks included nonspecific malaise and altered mood. The liver, kidneys, pancreas and lungs were procured for transplantation after development of brain death and with family consent. The heart was

not accepted for whole organ transplantation due to poor cardiac function. Transthoracic echocardiogram had revealed mild to moderate impairment of the left ventricle systolic function, with severe hypokinesis of the basal half, to two-thirds of the left ventricle, and akinetic septal, antero-septal and inferior segments. The procured heart valves were stored by the tissue bank and underwent routine histopathology and microbiological testing. Donation resulted in transplantation of the liver (recipient 1), left kidney/pancreas (recipient 2), lungs (recipient 3) and right kidney (recipient 4), which occurred in different Australian states from the donor.

Investigations for a possible donor-derived infection were prompted clinically, by the development of elevated transaminases in the liver recipient, and diagnostically, by the findings of a significant neutrophilic pericarditis, myocarditis and perivascular infiltration on the histopathological sections from the explanted heart valves. Stored plasma, serum, heart valve tissue and processing solutions from the donor were retrospectively recovered and tested. EV was detected by polymerase chain reaction (PCR) in two sera, one of the two plasma samples and in the initial heart collection solution (see Table 1). Herpes viruses (herpes simplex virus types 1 and 2 (HSV-1, HSV-2), cytomegalovirus (CMV), and varicella zoster virus and adenovirus were negative by PCR.

The liver recipient, transplanted for acute fulminant hepatic failure secondary to a presumed adverse drug reaction, developed a desquamating rash, markedly elevated alanine aminotransferase (ALT) greater than 2000 IU/L (normal range <45 IU/L), an elevated troponin I to 0.31 μg/L (normal range <0.04 μg/L) and a small pericardial effusion, in the first few days following transplant. Immunosuppressive medications included basiliximab induction, tacrolimus, azathioprine and steroids. A liver biopsy performed at day 5 posttransplant showed predominantly lobular changes with pan-acinar disarray, numerous acidophic hepatocytes and acidophilic bodies and small areas of confluent necrosis with a moderate lobular mononuclear inflammatory infiltrate. Eosinophils were not prominent. Immunohistochemical stains for HSV-1, HSV-2, CMV, adenovirus, Epstein–Barr virus and human herpesvirus 8 were negative. EV-specific stains were not available. The differential diagnosis included viral hepatitis, ischemia, drug reaction and antibody mediated rejection. Plasma exchange and intravenous gamma globulin were initially commenced as treatment for possible antibody mediated rejection. Antibiotics (doxycycline, vancomycin and ciprofloxacin) were also included to empirically cover bacterial pericarditis. Subsequent PCR testing on serum, throat swab and feces were positive for both EV and HSV-1. Intravenous acyclovir, 10 mg/kg 8 hourly, was commenced. The patient improved clinically and was discharged from the intensive care unit, 10 days after transplantation, with an ALT in the normal range.

Following the results generated from the first recipient and the donor, recipient 2, who received a combined kidney/pancreas transplant with immunosuppressant medications including basiliximab induction, tacrolimus, mycophenolate mofetil and steroids, underwent investigations where EV was detected by PCR in plasma, feces, rectal swab and throat swab. HSV-2 was also detected from the rectal swab. HSV-1 was not detected in any of the samples. Despite a subsequent brief episode of fever, vomiting and diarrhea 2 weeks posttransplant, for which the patient received intravenous immunoglobulin for its potential antiviral effects, repeat plasma, throat and rectal swab investigations at that time were negative for EV and the patient recovered quickly with excellent function of both organs. Recipient 3, who received a bilateral sequential lung transplant for end-stage lung disease secondary to cystic fibrosis, received basiliximab induction, then tacrolimus, azathioprine and steroids for immunosuppression, had an uneventful clinical course posttransplant, and throat swab, rectal swab, plasma and bronchoalveolar lavage (BAL) specimens were negative for both EV and HSV. She was discharged from hospital 2 weeks after transplantation. The treating team caring for recipient 4, who received the right kidney, was notified of the EV infection of the donor and recipient 1, but elected to not undertake further testing as the patient was asymptomatic, and no follow-up information was available. The stored heart valve tissue from the donor was not used. Pretransplant sera from the three evaluable recipients were negative for EV when retrospectively tested by PCR.

The laboratory used real-time (rt) TaqMan PCR for EV detection (primers and probe targeting 5′ UTR) (5) and HSV-1 and -2 detection (targeting glycoprotein-B, modified from Druce et al ([6])). PCR was run for 45 cycles. The cycle threshold (ct) values at which EV and HSV were detected in the individual samples are presented in Table 1. Where there was sufficient amplified product, positive EV detections were genotyped using conventional PCR and sequenced targeting VP1 (5). All sequenced EV PCR products from the donor and two recipients were identified as CVB3. Of the circulating EV strains that were sequenced in the same year, CVB3 accounted only for 8.7% of strains (10 out of 115 EV strains). In order to demonstrate the molecular relatedness of the CVB3 strains from the donor and the two recipients, a phylogenetic analysis compared these strains with all sequenced CVB3 strains from 2008–2013 (Figure 1). The CVB3 strains isolated from the donor and the two recipients were genetically identical and distinct from other circulating CVB3 strains. This analysis and the fact that the recipients were geographically separate from the donor, provides strong evidence for donor-derived infection rather than community-acquired infection.

Discussion

A wide range of infections have been recognized to be transmitted from donor to recipient through organ

Table 1: Investigation results of enterovirus and herpes simplex virus laboratory testing from the donor and recipients

	Timing of sample	Sample type	EV rt-PCR (ct)	HSV rt-PCR (ct)
Donor	Predonation	Serum	Detected (35)	Not detected
			Detected (40)	Not detected
		Plasma	Detected (40)	Not detected
			Not detected	Not detected
	Postmortem	Heart[1]	Detected (38)	Not detected
Recipient 1 (liver)	Pretransplant	Serum	Not detected	HSV-1 detected (41)
	5 days posttransplant	Serum	Detected (27)	HSV-1 detected (30)
		Liver biopsy[2]	Not detected	Not detected
	7 days posttransplant	Throat swab	Detected (43)	HSV-1 detected (20)
	8 days posttransplant	Feces	Detected (39)	HSV-1 detected (31)
	13 days posttransplant	Liver biopsy[3]	Not detected	Not detected
	17 days posttransplant	Serum	Inhibited	–
		Feces	Not detected	–
	18 days posttransplant	Throat swab	Not detected	–
	20 days posttransplant	Plasma	Not detected	–
Recipient 2 (kidney/pancreas)	Pretransplant	Serum	Not detected	Not detected
	7 days posttransplant	Plasma	Detected (36)	Not detected
		Throat swab	Not detected	Inhibited
		Rectal swab	Detected (36)	HSV-2 detected (24)
	8 days posttransplant	Plasma	Detected (37)	Not detected
		Feces	Detected (31)	Not detected
	17 days posttransplant	Plasma	Not detected	–
		Throat swab	Not detected	–
		Rectal swab	Not detected	–
	18 days posttransplant	Feces	Inhibited	–
Recipient 3 (lungs)	Pretransplant	Serum	Not detected	Not detected
	7 days posttransplant	Throat swab	Not detected	Not detected
		Rectal swab	Not detected	Not detected
	8 days posttransplant	Plasma	Not detected	Not detected
	10 days posttransplant	BAL	Not detected	Not detected

EV, enterovirus; HSV, herpes simplex virus; rt-PCR, real-time polymerase chain reaction; ct, cycle threshold; BAL, bronchoalveolar lavage; —, not tested.
[1]PCR positive from the collection solution; negative from aortic and pulmonary tissues and solutions, rinse solution, freeze solution and tissue trimmings (i.e. one positive out of nine samples tested).
[2]Paraffin-embedded tissue sample.
[3]Fresh tissue sample.

transplantation (7), although EV transmission has not been reported. Transplantation of organs from deceased donors who had fever or viral syndromes is controversial, prompting the need for improved microbiologic screening tools (8). Despite logistical constraints, the follow up and microbiological testing of the multiple organ recipients from the common donor was relatively swift and effective in this case.

Cases of donor-derived disease transmission are classified as either proven, probable or possible, based on the *ad hoc* United Network for Organ Sharing/Organ Procurement and Transplantation Network Disease Transmission Advisory Committee (7,9). Our case meets the classification as a proven case of donor-derived CVB3 transmission, namely meeting the following conditions:

- There was a suspected transmission event.
- Laboratory evidence of CVB3 in the organ recipient.
- Laboratory evidence that the same, genetically identical virus identified in another organ recipient and from the original common donor.
- Pretransplant laboratory evidence that the recipients did not have CVB3.

Cardiotropic viruses, especially CVB and adenoviruses, and to a lesser extent, human CMV and parvovirus B19, have been detected from a high proportion of myocardial tissue samples from heart (10) and heart valve donors (11), suggesting a significant risk for viral transmission by cardiovascular allografts. The clinical significance of such a transmission is suggested by the association of pediatric cardiac graft loss with the presence of viral genomes in the heart tissue (12) and reports of EV infection as an etiological cause for unexplained late severe adverse cardiac events in the adult heart-transplant population (13). EV viremia has also been reported in Scottish blood donors, at a frequency

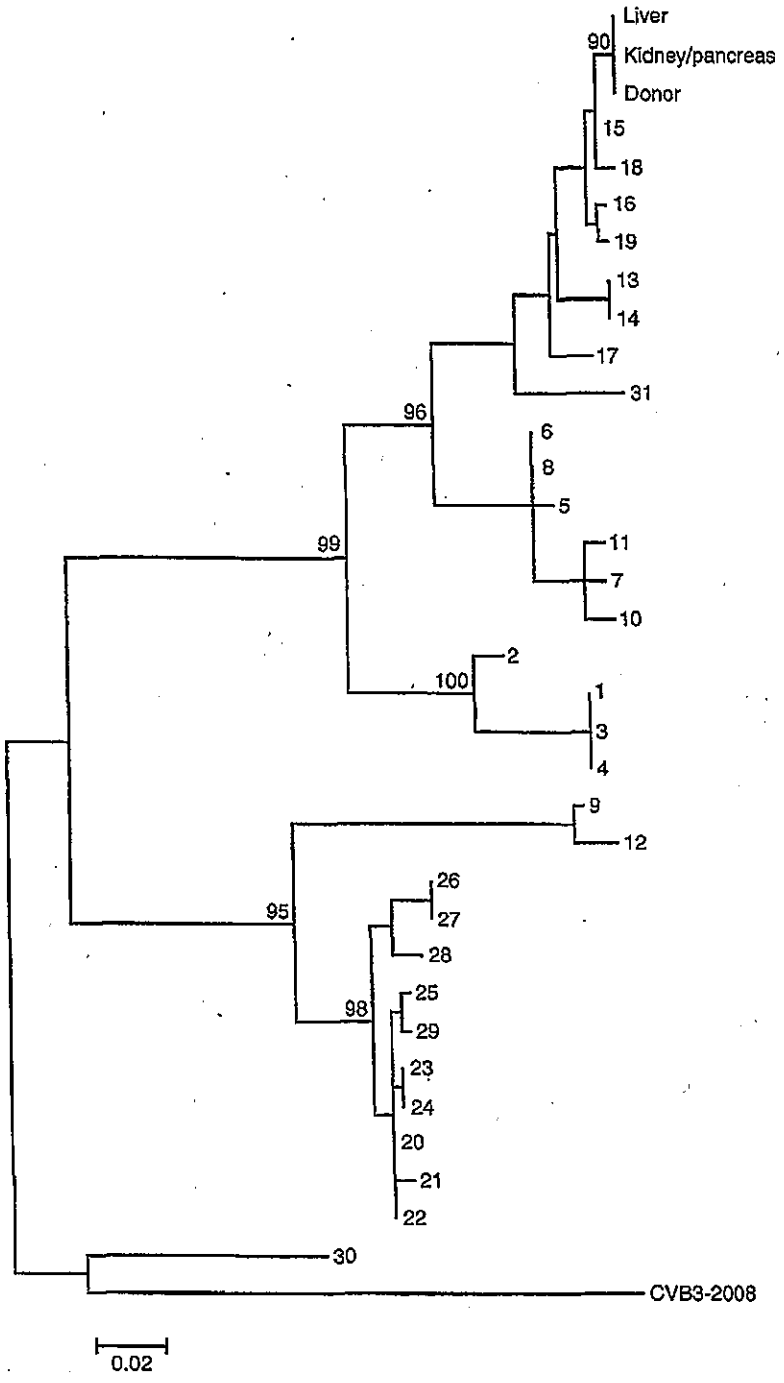

Figure 1: Phylogentic clustering of donor and recipient coxsackievirus B3. Maximum-likelihood tree depicting the relationships between CVB3 VP1 sequences from the donor, kidney/pancreas recipient and liver recipient, and 31 unrelated clinical strains collected over the period 2008–2013 (1–31). The tree was constructed by maximum-likelihood methods using the Tamura-3-parameter model of evolution based on analysis of 344 nucleotide VP1 sequences. The coxsackievirus-B3 prototype strain CVB3-2008 was included in the analysis and used as an out-group to root the tree. Bootstrap values greater than 90% are indicated. The clustering of the CVB3 strains from the donor and the two organ recipients indicates molecular evidence of genetic relatedness, distinct from other sequenced circulating CVB3 strains.

of approximately 1 in 4400 donors, which increases to 1 in 950 in the early winter months (October) (14).

Given that CVB3 is an important cause of viral myocarditis, it is plausible that the donor in this case had an unrecognized CVB3-related myocarditis, suggested by the reduced cardiac function premortem and subsequent demonstration of inflammation on the postmortem histopathology of the heart. This virus was then transmitted to the organ recipients. The liver recipient developed a severe hepatitis with a histological appearance in keeping with acute viral infection and most likely due to EV rather than HSV-1 reactivation given the negative HSV-1 immunohistochemistry. In contrast, there was a relatively mild clinical syndrome in the kidney/pancreas recipients, and incomplete transmission to the lung recipient. This variation in clinical severity of disease, is likely to be related to the inoculum and tissue tropism of CVB3 (4,15), the organ transplanted and the type of immune suppression used.

A coordinated and systematic approach between the laboratory and the different transplant units is required when investigating possible low frequency, "near miss" transmission events like this, especially given that the clinical consequences of a CVB3 donor-derived infection appears to be mild. A different clinical outcome, however, may occur in the setting of alternative organ procurement, especially heart transplantation. Furthermore, donor-derived transmission of another species of EV, with different tissue tropism, may have distinct and more severe clinical consequences. Given the impracticality of screening donors for all possible infections, transplant clinicians should consider and test for EVs when investigating cases of possible donor-derived infections.

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Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

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一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名(企業名)	新鮮凍結血漿-LR「日赤」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)		Park SW, Han MG, Yun SM, Park C, Lee WJ, Ryou J. Emerg Infect Dis. 2014 Nov;20(11):1880-2. doi: 10.3201/eid2011.140888.	韓国

研究報告の概要

○韓国の重症熱性血小板減少症候群(SFTS)
2013年、韓国で35人がSFTSと診断された。主な症状は高熱(100%)、胃腸症状(74%)、疲労(74%)、血小板減少(100%)、白血球減少(100%)であり、中国及び日本の患者と同様であった。韓国におけるSFTSの死亡率は47.2%であり、患者の約80%が50歳以上で、70%が農民であった。患者の多くが晩春から初夏にかけ発生し、また比較的温暖な南部の地域での報告が多く、環境温度が有病率に影響することが示唆された。
35症例中26例より重症熱性血小板減少症候群ウイルス(SFTSV)を分離し解析したところ、韓国分離株のM及びSセグメントの配列は中国と日本の分離株の配列と92%－100%一致し、密接に関連していた。系統発生解析では、本研究の韓国株の大半は日本の分離株と、また一部は中国の分離株と同じクラスターに属していることが示された。

使用上の注意記載状況・その他参考事項等

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

韓国では2013年に35人のSFTS患者が発生し、調査の結果、環境温度が有病率に影響することが示唆された。また系統発生解析により、今回の韓国のSFTSV分離株の多くは日本の分離株に近縁であることが示されたとの報告である。

今後の対応

日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また国内症例については、発熱などの体調不良者を献血不適とすることで対応している。今後も引き続き情報の収集に努める。

Severe Fever with Thrombocytopenia Syndrome Virus, South Korea, 2013

Sun-Whan Park, Myung-Guk Han, Seok-Min Yun, Chan Park, Won-Ja Lee, and Jungsang Ryou

During 2013, severe fever with thrombocytopenia syndrome was diagnosed in 35 persons in South Korea. Environmental temperature probably affected the monthly and regional distribution of case-patients within the country. Phylogenetic analysis indicated that the isolates from Korea were closely related to isolates from China and Japan.

Severe fever with thrombocytopenia syndrome (SFTS) is a newly emerging infectious disease. Symptoms and laboratory abnormalities are fever, thrombocytopenia, leukocytopenia, and elevated serum enzyme levels. Multiorgan failure occurs in severe cases, and 6%–30% of case-patients die. The syndrome is caused by the SFTS virus (SFTSV) (genus *Phlebovirus*, family *Bunyaviridae*). SFTS case-patients were first reported in China (*1*) and more recently were reported in Japan (*2*) and South Korea (*3*). Two case-patients with symptoms consistent with a similar virus, Heartland virus, were reported in the United States (*4*).

Ixodid tick species are implicated as vectors of SFTSV (*1*,*5*,*6*). One study described a SFTSV prevalence in *Haemaphysalis longicornis* ticks, a major vector of SFTSV, of 0.46% minimum infection rate in South Korea (*7*); in another study, SFTSV was detected in ticks that had bitten humans (*6*). From these studies, we realized that SFTSV was common throughout the country. We aimed to evaluate the prevalence of SFTS in South Korea and isolate the SFTSV to analyze its phylogenetic properties.

The Study

In March 2013, we established molecular diagnostic methods to detect SFTSV. During April–December 2013, from 125 hospitals throughout the country, we collected 301 serum samples from hospitalized persons who had SFTS signs and symptoms, such as high fever (temperatures ≥38°C), vomiting, diarrhea, and/or fatigue and showed laboratory parameters consistent with thrombocytopenia and/or leukocytopenia. We conducted reverse transcription PCR (RT-PCR) to detect the SFTSV medium (M) segment gene from acute-phase serum specimens with a previously described method (*6*). We also detected the SFTSV small (S) segment gene by RT-PCR with specific primers (SF3, 5′-GGGTCCCTGAAGGAGTTG-TAAA-3′; SR1, 5′-TGGTGAGCAGCAGCTCAATT-3′). The RT-PCR conditions were as follows: an initial step of 30 min at 50°C for reverse transcription and 15 min at 95°C for denaturation, followed by 35 cycles of 20 s at 95°C, 40 s at 58°C (for M segment) or 55°C (for S segment), and 30 s at 72°C and a final extension step of 5 min at 72°C.

From the 301 samples, we detected M and S segment genes from 34 and 29 samples, respectively. The nucleotide sequences were assembled by the SeqMan program implemented in DNASTAR software (version 5.06; Madison, WI, USA) to determine the consensus sequences. The nucleotide sequences of the Korea isolates showed 93%–98% homology to the China and Japan isolates.

To isolate SFTSV, we inoculated subconfluent monolayers of Vero E6 cells with the RT-PCR–positive serum. After the monolayers underwent 3 blind passages in new monolayers of Vero E6 cells (*8*), we examined the Vero E6 cells for SFTSV by RT-PCR. We considered the virus to be isolated when the specific genes were amplified by RT-PCR. The viruses did not cause cytopathic effects in Vero E6 cells during isolation. Isolation of SFTSV also was confirmed by indirect immunofluorescent assay (IFA) (Figure 1, panels A,B) and electron microscopy (Figure 1, panel C). For IFA, Vero E6 cells infected with SFTSV were incubated at 37°C in a CO_2 incubator. Cells were harvested, inoculated, and fixed with acetone on Teflon-coated well slides. IFA was conducted by using a monoclonal SFTSV nucleocapsid protein (N) antibody (manufactured in our laboratory) as the primary antibody. N proteins of SFTSV were distributed throughout the cytoplasm (Figure 1, panels A,B). By electron microscopy, Vero E6 cells infected with the SFTSV Korea isolate KAJJH showed bunyavirus-like particles, 80–100 nm in diameter, located in cytoplasmic vacuoles, presumably in the Golgi apparatus (Figure 1, panel C).

The amplified DNA products from the isolates were sequenced and compared with the sequences of other GenBank-registered SFTSV isolates. The sequences of partial M and S segments of the 26 Korea isolates (GenBank accession nos. KF282701, KF282702, and KJ739543–KJ739592) were closely related to those of the SFTSV isolates from China and Japan with 92%–100% identity. A phylogenetic tree was constructed by the neighbor-joining method on the basis of the partial M (Figure 2, panel A, http://wwwnccdc.gov/EID/article/20/11/14-0888-F2.htm) and S segment (Figure 2, panel B) sequences of the Korea SFTSVs in the study and 15 SFTSVs from China and

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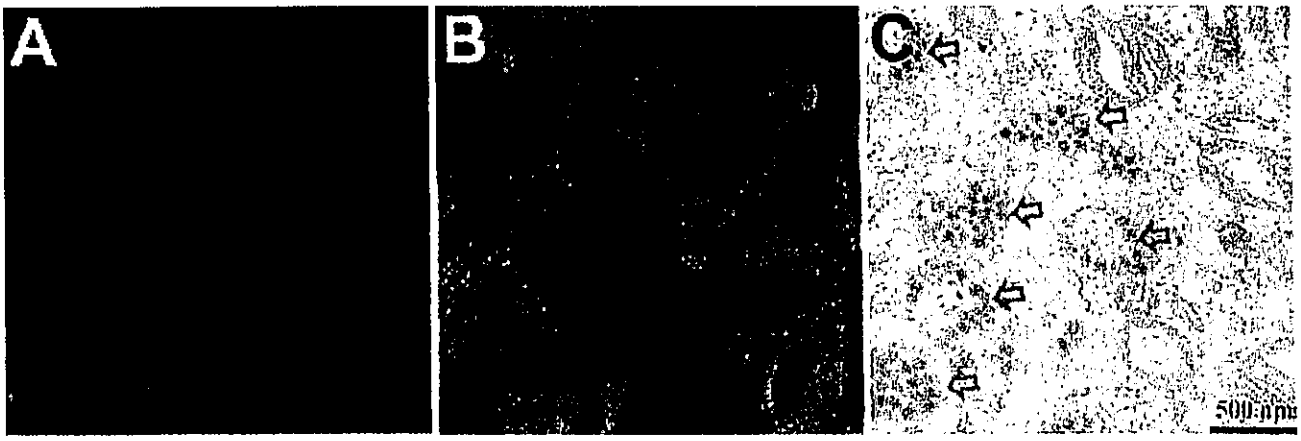

Figure 1. Isolation of severe fever with thrombocytopenia syndrome virus (SFTSV) from case-patients, South Korea, 2013. A, B) Indirect immunofluorescent features of Vero E6 cells primed with SFTSV N protein monoclonal antibody and reacted with fluoresce in isothiocyanate-conjugated anti-mouse IgG. B) Transmission electron microscopy image of Vero E6 cells infected with SFTSV. Scale bar indicates 500 nm.

Japan registered in GenBank. SFTSV isolates formed 2 major clusters in M and S segment sequences, and 1 other small group comprising only Korea isolates, KAGNH2 and KAUSH, was formed in M-segment sequences. Many Korea isolates formed the first cluster with the Japan isolates. Some Korea isolates clustered with the major group of China isolates, forming the second group.

Conclusions

We confirmed the SFTS in several localities around South Korea. We also isolated several SFTSVs from case-patient serum and analyzed the phylogenetic properties of the isolates. A total of 36 SFTS case-patients were reported in South Korea. The first SFTS case was identified in a retrospective study from 2012 (*3*). Subsequently, SFTS was diagnosed in 35 additional case-patients in South Korea. Another group diagnosed the first of the 35 cases in the country; we diagnosed the other 34 cases, from which we isolated the 26 SFTSVs. The major signs and symptoms of the 35 case-patients, including fever (100%), gastrointestinal symptoms (74%), fatigue (74%), thrombocytopenia (100%), and leukocytopenia (100%), were similar to those of case-patients in China and Japan (*9*).

The case–fatality rate for SFTS in South Korea was 47.2% (17/36), higher than that of the recent China cases (≈8.7%) (*10*). The low sensitivity of the detection method, the conventional 1-step RT-PCR, and the absence of a serologic diagnosis may have contributed to the relatively high case–fatality rate. Most cases occurred in older persons; ≈80% of patientswere >50 years of age. Approximately 70% were farmers, including persons who cultivated vegetable gardens (*9*). In many case-patients, the disease evolved during a relatively warm time of year, from late spring to early autumn (Figure 3, panel A). The geographic distribution of SFTS case-patients also indicated that environmental temperature affected the SFTS prevalence because many (86%, 30/35) SFTS cases evolved in relatively warm southern provinces and cities south of Chungcheongbuk, Chungcheongnam, and Gangwon Provinces (Figure 3, panel B). We have also observed that the tick density is high during May–August, a generally warm season in South Korea (*7*). SFTSV was also mainly detected during this season. These results indicate that the virus infection in humans is closely related to a high tick density and SFTSV infection in ticks in a warm climate.

As described in another report, Japan isolates formed an independent cluster from the China isolates (*2*). In our current study, SFTSV isolates formed 2 major clusters. Most of the Korea isolates formed a cluster with the Japan isolates, although some Korea and China isolates were included in the other group, perhaps not surprising given the geographic location of South Korea between China and Japan.

Acknowledgments

We thank all involved in SFTS diagnosis in 2013, especially the staff in 17 regional Institutes of Health and Environment. We also thank Young Eui Jeong and Ki Ju Choi for technical support with molecular evolution and the electron microscope.

This study was financially supported by the Korea National Institute of Health, Korea Centers for Disease Control and Prevention (program no. 4800-4837-300-210-13 and 4800-4861-304-210-13 [grant no. 2014-NG53002-00]).

Dr. Sun-Whan Park is a senior researcher at the Division of Arboviruses, National Institute of Health, Korea Centers for Disease Control and Prevention. His research focuses on bunyaviruses, including SFTSV and hantavirus.

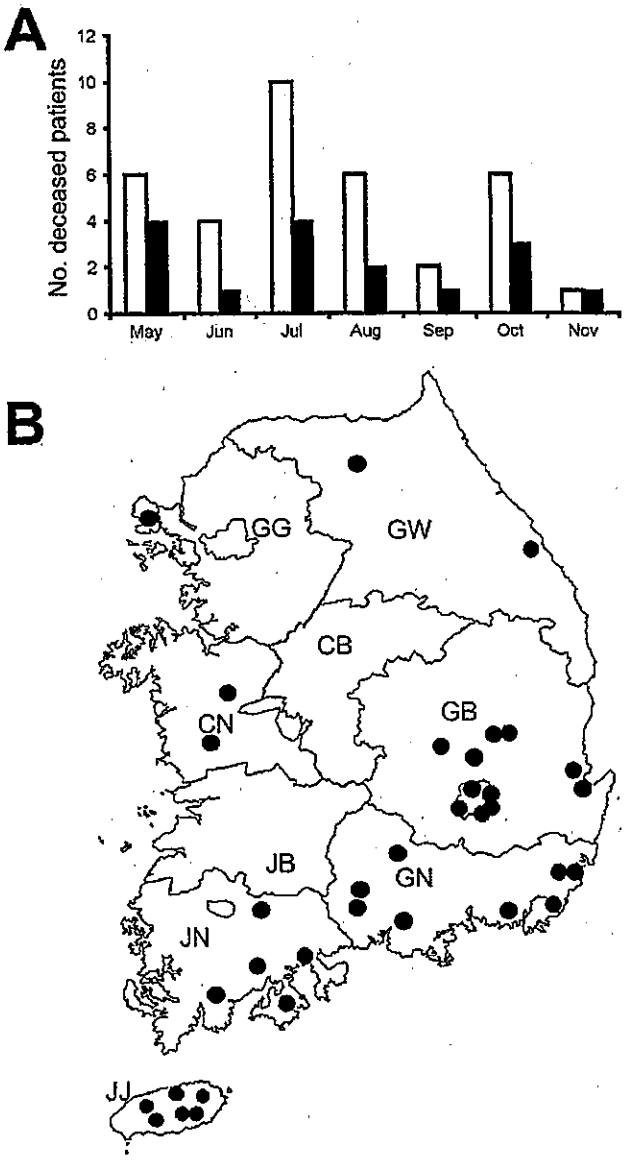

Figure 3. Seasonal (A) and geographic (B) distribution of case-patients with severe fever with thrombocytopenia syndrome (SFTS), South Korea, 2013. A) White and black bars indicate the numbers of total and deceased SFTS patients, respectively, in the indicated months. B) Black circles indicate the approximate residential regions of 35 SFTS case-patients in 2013 in South Korea. GG, Gyeonggi Province; GW, Gangwon Province; CB, Chungcheongbuk Province; CN, Chungcheongnam Province; GB, Gyeongsangbuk Province; GN, Gyeongsangnam Province; JB, Jeollabuk Province; JN, Jeollanam Province; JJ, Jeju special autonomous Province.

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別紙様式第２

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日 2015年2月23日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①フィブリノゲン加第XIII因子 ②人血液凝固第XIII因子		研究報告の公表状況	http://www.cdc.gov/media/releases/2015/a0220-newly-discovered-virus.html	公表国 米国	
販売名（企業名）	①ベリプラストPコンビセット組織接着用 ②フィブロガミンP静注用 （CSLベーリング株式会社）					

研究報告の概要	米CDC (the Center for Disease Control and Prevention)は，2014年晩春のKansas東部における，以前健康だった男性1例の死亡の要因となった可能性のある新しいウイルスの発見について報告している。CDCの報告は，男性の疾患の進行，本症例の治療および調査のためのCDC，Kansas Department of Health and Environment(KDHE)，University of Kansas Medical Center(UKMC)による対応を詳述している。このウイルスはBourbon virusといい，ソゴトウイルスと呼ばれるウイルス群に属する。今回初めて，この群のウイルスが米国においてヒト疾患を引き起こしたことが示された。ヒトにおいて症状を引き起こしたソゴトウイルスの既知症例は8例しかない。ソゴトウイルスは欧州，アジア，アフリカの一部でダニや蚊に関連しており，Bourbon virusもダニまたは他の虫の咬傷を介して広がる可能性がある。Kansasの症例は発症の数日前にダニに複数回咬まれた。CDCの検査でサンプル内に未知のウイルスのエビデンスが示され，CDCはこれを新しいウイルスであると決定した。CDCは，Bourbon virus疾患の他の症例を特定し，誰が発症し，どんな症状で，どのように感染するのか調べるためKDHEおよびUKMCと協力していることなどについて記載。

報告企業の意見	今後の対応
Bourbon virus は新たに発見されたウイルスである。製品の製造工程にはウイルス除去・不活化工程があり、各種モデルウイルスを用いたウイルスクリアランス試験で効果が認められていることから新型ウイルスに対しても安全対策は取られていると考えられる。また、原料となる血漿を得る段階において採血時の問診・診断、原料血漿の感染症に関する検査、原料血漿の貯留保管を経て製造工程に入るため、当社製品に本ウイルス感染者からの供血血漿が使用される可能性は低いと考えられる。	今後とも新しい感染症に関する情報収集に努める所存である。

使用上の注意記載状況・その他参考事項等

ABJ2015Z01

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™
(http://www.cdc.gov)

CDC A-Z INDEX

CDC Newsroom (/media)

CDC and Partners Investigate Newly Discovered Virus

Media Advisory

Contact: CDC Media Relations
(http://www.cdc.gov/media/)404-639-3286

What

Bourbon virus belongs to a group of viruses called thogotoviruses.

The Centers for Disease Control and Prevention (CDC) is today reporting on the discovery of a new virus that may have contributed to the death of a previously healthy man in eastern Kansas in late spring 2014. A CDC study published today details the progression of the man's illness and actions taken by CDC, the Kansas Department of Health and Environment (KDHE), and University of Kansas Medical Center (UKMC) to treat and investigate the case. The virus, named Bourbon virus for the county where the patient lived, is part of a group of viruses called thogotoviruses. This is the first time a virus in this group has been shown to cause human illness in the United States and only the eighth known case of thogotoviruses causing symptoms in people.

Where

The article was published today in CDC's *Emerging Infectious Diseases* journal and is available at: http://wwwnc.cdc.gov/eid/article/21/5/15-0150_article (http://wwwnc.cdc.gov/eid/article/21/5/15-0150_article).

Why

Since viruses in this group (thogotoviruses) have been linked to ticks or mosquitoes in parts of Europe, Asia, and Africa, Bourbon virus might also be spread through tick or other insect bites. The Kansas man had received multiple tick bites in the days before becoming ill. After test results for many infectious diseases came back negative, a sample of the patient's blood was sent to CDC for additional testing. Initial CDC testing showed evidence of an unidentified virus in the sample. CDC researchers then used Advanced Molecular Detection (AMD) and determined that it was a new virus.

CDC is collaborating with KDHE and UKMC to identify additional cases of Bourbon virus disease, determine who gets sick and with what symptoms, and how people are getting infected. CDC experts will also be working in the lab to better understand the virus itself, how it makes people sick, and what animals (if any) may play a role in its spread. This information will help determine the best ways to potentially prevent and control Bourbon virus.

The discovery of Bourbon virus, as well as the recent discoveries of Heartland virus in Missouri and severe fever with thrombocytopenia syndrome viruses in China, leads CDC researchers to believe that other undiscovered viruses are likely causing people to get sick. Use of AMD methods in laboratories across the world is an important tool for discovering and addressing new pathogens.

For more information on Bourbon virus, visit http://www.cdc.gov/ncezid/dvbd/bourbon/index.html (http://www.cdc.gov/ncezid/dvbd/bourbon/index.html).

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

Page last reviewed: February 20, 2015
Page last updated: February 20, 2015
Content source: Centers for Disease Control and Prevention (/)

eCards (http://tools.cdc.gov/ecards/)

YouTube (http://www.youtube.com/CDCstreamingHealth)

(http://www.hhs.gov/)

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  - FOIA (http://www.cdc.gov/od/foia/)
  - Accessibility (http://www.cdc.gov/contact/accessibility.html)
  - Privacy (http://www.cdc.gov/Other/privacy.html)
  - No FEAR Act (http://www.cdc.gov/diversity/nofearact/)
  - Inspector General (https://oig.hhs.gov/)
  - USA.gov (http://www.usa.gov/)
- Contact CDC

Centers for Disease Control and Prevention
1600 Clifton Road
Atlanta, GA 30329-4027 USA
800-CDC-INFO (800-232-4636)
Contact CDC-INFO (http://www.cdc.gov/cdc-info/requestform.html)

別紙様式第2-1

No. 11

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
				2015. 1. 5	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	ProMED 20141225.3053772	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

○バーボンウイルス－米国カンザス州
2014年12月22日、カンザス州健康環境局は、2014年夏にカンザス州の農業従事者の死亡原因となった新規ウイルスが患者の居住地にちなんでバーボンウイルスと名付けられたことを発表した。
オルトミクソウイルス科トゴトウイルス属に属する同ウイルスは、CDCの研究者により特定された。
2014年晩春、生来健康であった男性が高熱、筋肉痛と食欲不振のため病院に入院した。彼は屋外で労働した際しばしばダニに噛まれており、また血液検査で肝酵素値の上昇と血小板、白血球数の減少が見られたため、医師はダニ媒介性疾患であるロッキー山紅斑熱、エーリキア症またはハートランドウイルスによる疾患を疑い検査を行ったが、いずれも陰性であった。疾患は急性かつ重篤であり、肺障害と腎不全、ショックを引き起こし、入院後わずか10日で死亡した。
米国にさらなる症例が存在するかどうか、また、同ウイルスを媒介するのはダニなのか、あるいは蚊や他の動物なのかは現時点で不明である。

報告企業の意見

2014年夏に急性かつ重篤な症状で死亡した農業従事者から新たなウイルスが検出され、バーボンウイルスと名付けられたとの報告である。

今後の対応

日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、帰国（入国）後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」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

Published Date: 2014-12-25 02:37:53
Subject: PRO/EDR> Bourbon virus - USA (02): (KS)
Archive Number: 20141225.3053772

BOURBON VIRUS - USA (02): (KANSAS)
****
A ProMED-mail post
http://www.promedmail.org
ProMED-mail is a program of the
International Society for Infectious Diseases
http://www.isid.org

Date: Tue 23 Dec 2014
Source: New York Times [edited]
http://www.nytimes.com/2014/12/24/science/mysterious-virus-that-killed-a-farmer-in-kansas-is-identified.html

The newly discovered virus that led to the death of a farmer in Kansas last summer [2014] has been named the Bourbon virus, for the county where the patient lived, the Kansas Department of Health and Environment said in a statement released on Monday [22 Dec 2014]. The virus was identified by scientists at the federal Centers for Disease Control and Prevention [CDC]. Dr J Erin Staples, a medical epidemiologist at the CDC laboratory in Fort Collins, Colorado, said the virus was a type of thogotovirus, part of a larger family known as orthomyxoviruses.

The illness was fast-moving and severe, causing lung and kidney failure, and shock. The man, previously healthy, died after about only 10 days in the hospital, according to Dr Dana Hawkinson, an infectious disease specialist who treated the patient at the University of Kansas Hospital in Kansas City.

Researchers do not yet know whether there have been other cases in the United States. They hope to test stored blood samples from people who had similar illnesses in the past that could not be identified.

There is no treatment for the disease. The best defense is to avoid insect bites by wearing pants and long sleeves outdoors and applying bug spray that contains the repellent DEET.

The medical mystery began late last spring [2014], when the patient was admitted to the hospital with a high fever, muscle aches and loss of appetite. He worked outdoors and often had tick bites. That history and his symptoms, combined with abnormal results on blood tests -- his liver enzymes were too high, his platelets and white cells too low -- made doctors suspect tickborne diseases like Rocky Mountain spotted fever or ehrlichiosis. But tests for those illnesses came back negative, as did tests for another recently discovered tickborne illness caused by the Heartland virus.

But the researchers are not certain whether ticks or mosquitoes transmit the virus, or whether other animals might carry it. "We will be working with state and local health departments come springtime to do extensive field investigations," Dr Staples said.

For now, the risk to the public is low because ticks and mosquitoes are not active in cold weather. But ticks rebound earlier in the year than mosquitoes do, she said, once the temperature starts consistently reaching 55 degrees Fahrenheit [14 deg Celsius].

[byline: Denise Grady]

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communicated by:
ProMED-mail
<promed@promedmail.org>

[We look forward to learning more about this newly discovered orthomyxovirus. Although it has not yet been definitively shown to be tickborne, a number of other tickborne viruses have emerged recently including Heartland virus and severe fever with thrombocytopenia virus (Bunyaviridae: Phlebovirus) in the US and China, respectively. In addition, other tickborne viruses such as deer tick virus/Powassan virus (Flaviviridae: Flavivirus) appear to be increasing in incidence in regions of the US. - Mod.LK

A HealthMap/ProMED-mail map can be accessed at: http://healthmap.org/promed/p/219.]

See Also

Bourbon virus - USA: (KS) fatal, 1st report, vectorborne 20141223.3051132
Heartland virus - USA (02): (OK) 20140602.2513295
Heartland virus - USA: (MO) 20140330.2367026
Tick-borne encephalitis virus - USA: (NY) Powassan virus 20140823.2716624
2013

Heartland virus - USA: (MO) 20130724.1841984
Tick-borne encephalitis virus - USA (10): (MA, ME) Powassan virus 20131226.2137895
Tick-borne encephalitis virus - USA (09): (ME) Powassan virus 20131225.2136102
Tick-borne encephalitis virus - USA (08): (ME) Powassan virus 20131222.2130989
Tick-borne encephalitis - USA (07): (NJ) fatal Powassan virus 20131206.2097190
2012

Heartland virus - USA (MO) 2009 20120830.127400723
Heartland virus - USA (MO) 2009 20120830.1274007
.....lk/je/sh

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 2015年1月13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	The New York Times DEC. 23, 2014	公表国 米国	
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：米国で、重大な感染症を引き起こす新規のウイルス、バーボンウイルスが発表された。 2014年の夏、カンザスで農業従事者を死亡させた新種のウイルスを，患者が居住していた地区にちなんでバーボンウイルスと名付けたことをアメリカ疾病予防管理センターの科学者によって発表された。このウイルスはオルトミクソウイルス科に属するソゴトウイルス属の一種である。この疾患は進行が速く重度であり，肺不全，腎不全やショックを引き起こす。患者を治療したカンザス市のカンザス大学病院の感染症専門医によると，それまで健康であった男性患者は，入院後わずか10日ほどで死亡した。この疾患に対する治療法はない。ダニまたは蚊がウイルスを媒介するのか，あるいはその他の動物が媒介するのか，研究者にも分かっていない。	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。

MedDRA/J ver. 17.1

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献血ベニロンーⅠ静注用500mg、⑧献血ベニロンーⅠ静注用1000mg、⑨献血ベニロンーⅠ静注用2500mg、⑩献血ベニロンー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	米CDCの科学者によって同定されたバーボンウイルスは、オルソミクソウイルス科に属するトゴトウイルス属の新種の核酸は一本鎖RNAのエンベロープを有するウイルスであるが、現時点では感染動態等については不明である。 今回、報告された症例では、それまで健康であった男性が、入院から10日ほどで死亡しており、強い病原性が示唆される。 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。今回報告したバーボン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バーボンウイルスへの感染報告例は無い。 以上の点から、上記製剤はバーボンウイルス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

The New York Times http://nyti.ms/1zvJFa3

SCIENCE

Mysterious Virus That Killed a Farmer in Kansas Is Identified

By DENISE GRADY DEC. 23, 2014

Researchers have identified a previously unknown virus, thought to be transmitted by ticks or mosquitoes, that led to the death of a farmer in Kansas last summer.

The illness was fast-moving and severe, causing lung and kidney failure, and shock. The man, previously healthy, died after about only 10 days in the hospital, according to Dr. Dana Hawkinson, an infectious disease specialist who treated the patient at the University of Kansas Hospital in Kansas City.

The newly discovered microbe has been named the Bourbon virus, for the county where the patient lived, the Kansas Department of Health and Environment said in a statement released Monday. The virus was identified by scientists at the federal Centers for Disease Control and Prevention through a process that took several months, according to Dr. J. Erin Staples, a medical epidemiologist at the C.D.C. laboratory in Fort Collins, Colo.

She said the virus was a type of thogotovirus, part of a larger family known as orthomyxoviruses. Its nearest relatives are found in Eastern Europe, Africa and Asia, Dr. Hawkinson said. Those viruses are spread by ticks and mosquitoes.

Researchers do not yet know whether there have been other cases in the United States. They hope to test stored blood samples from people who had similar illnesses in the past that could not be identified.

"I think we have to assume this has been around for some time, and we haven't been able to diagnose it," Dr. Hawkinson said. He added, "We

suspect there have been milder cases and people have recovered from them, but we don't have a lot of information."

There is no treatment for the disease. The best defense is to avoid insect bites by wearing pants and long sleeves outdoors and applying bug spray that contains the repellent DEET.

The medical mystery began late last spring, when the patient was admitted to the hospital with a high fever, muscle aches and loss of appetite. He worked outdoors and often had tick bites. That history and his symptoms, combined with abnormal results on blood tests — his liver enzymes were too high, his platelets and white cells too low — made doctors suspect tick-borne diseases like Rocky Mountain spotted fever or ehrlichiosis. But tests for those illnesses came back negative.

Dr. Hawkinson suspected another, recently discovered tick-borne illness caused by the Heartland virus and sent blood samples to the C.D.C. for testing. But those tests also came back negative.

Researchers at the C.D.C. noticed that something else seemed to be growing in the samples that were tested for the Heartland virus, and they eventually identified the Bourbon virus.

But the researchers are not certain that ticks or mosquitoes transmit the virus, or whether other animals might carry it.

"We will be working with state and local health departments come springtime to do extensive field investigations," Dr. Staples said.

For now, the risk to the public is low because ticks and mosquitoes are not active in cold weather. But ticks rebound earlier in the year than mosquitoes do, she said, once the temperature starts consistently reaching 55 degrees Fahrenheit.

A version of this article appears in print on December 24, 2014, on page A13 of the New York edition with the headline: Mysterious Virus That Killed a Farmer in Kansas Is Identified.

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

報告企業の意見	今後の対応	
血漿分画製剤は理論的なvCJD伝播リスクを完全には排除できないため、投与の際には患者への説明が必要である旨を2003年5月から添付文書に記載している。2009年2月17日、英国健康保護庁(HPA)はvCJDに感染した供血者の血漿が含まれる原料から製造された第Ⅷ因子製剤の投与経験のある血友病患者一名から、vCJD異常プリオン蛋白が検出されたと発表したが、日本血液製剤機構の原料血漿採取国である日本及び米国では、欧州滞在歴のある献（供）血希望者を一定の基準で除外している。また、第81回 国際獣疫事務局（OIE）総会（2013年5月）により、我が国が「無視できるBSEリスク」の国に認定されたことからも、原料血漿中に異常型プリオン蛋白が混入するリスクは1999年以前の英国に比べて極めて低いと考える。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。	

医薬品　研究報告　調査報告書

<table>
<tr><td colspan="2">識別番号・報告回数</td><td></td><td>報告日</td><td>第一報入手日
2015年1月6日</td><td colspan="2">新医薬品等の区分
該当なし。</td><td rowspan="2">総合機構処理欄</td></tr>
<tr><td colspan="2">一般的名称</td><td>別紙のとおり。</td><td rowspan="2">研究報告の
公表状況</td><td rowspan="2">CID 2015;60(2):195-202</td><td rowspan="2">公表国
米国</td></tr>
<tr><td colspan="2">販売名（企業名）</td><td>別紙のとおり。</td></tr>
<tr><td rowspan="2">研究報告の概要</td><td colspan="5">問題点：米国で、人に感染する新しいポックスウイルスが報告された。

米国で、ウマと接触した2名が新しいポックスウイルス種によって皮膚感染を引き起こした。2つの分離株は遺伝子的に類似しており、地理的起源が共通している可能性がある。人畜共通感染症の可能性が考えられるが、接触動物及び環境からの大規模なサンプリングでも同じウイルスを検出できなかった。患者の臨床経過はパラポックスウイルスと似ており、このウイルスの起源は現時点では不明であるが人畜共通感染症が考えられる。</td><td>使用上の注意記載状況・
その他参考事項等
記載なし。</td></tr>
<tr><td colspan="3">報告企業の意見
別紙のとおり。</td><td colspan="3">今後の対応
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。</td></tr>
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MedDRA/J ver. 17.1

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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	ポックスウイルス科に属するウイルスは、全長が220～450nmの煉瓦状ないし卵形で、線状の2本鎖DNAをゲノムとして持つエンベロープを有するDNAウイルスである。ポックスウイルス科による病気の特徴は皮膚の発疹様斑紋とされており、ポックスウイルス科のウイルスによる疾患としては天然痘が知られている。 現時点では感染源は特定されていないが、ウマと接触した2名が新しいポックスウイルス種によって皮膚感染を引き起こしたとの報告であり、人獣共通感染症の可能性が考えられる。 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。今回報告したポックス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、仮性狂犬病ウイルス（PRV）が該当すると考えられるが、上記工程のPRV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ポックスウイルスへの感染報告例は無い。 以上の点から、上記製剤はポックスウイルス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

MAJOR ARTICLE

Novel Poxvirus Infection in 2 Patients From the United States

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Background. Some human poxvirus infections can be acquired through zoonotic transmission. We report a previously unknown poxvirus infection in 2 patients, 1 of whom was immunocompromised; both patients had known equine contact.

Methods. The patients were interviewed and clinical information was abstracted from the patients' medical files. Biopsies of the skin lesions were collected from both patients for histopathology, immunohistochemistry, and transmission electron microscopy analysis. Oral and skin swabs were collected from animals with frequent contact with the patients, and environmental sampling including rodent trapping was performed on the farm where the immunosuppressed patient was employed. "Pan-pox and high Guanine-cytosine" polymerase chain reaction assays were performed on patient, animal, and environmental isolates. Amplicon sequences of the viral DNA were used for agent identification and phylogenetic analysis.

Results. Specimens from both human cases revealed a novel poxvirus. The agent shares 88% similarity to viruses in the *Parapoxvirus* genus and 78% to those in the *Molluscipoxvirus* genus but is sufficiently divergent to resist classification as either. All animal and environmental specimens were negative for poxvirus and both patients had complete resolution of lesions.

Conclusions. This report serves as a reminder that poxviruses should be considered in cutaneous human infections, especially in individuals with known barnyard exposures. The clinical course of the patients was similar to that of parapoxvirus infections, and the source of this virus is currently unknown but is presumed to be zoonotic. This report also demonstrates the importance of a comprehensive approach to diagnosis of human infections caused by previously unknown pathogens.

Keywords. poxvirus; skin infection; parapoxvirus; immunocompromised; imiquimod.

Four genera of poxviruses contain species that infect humans: *Orthopoxvirus*, *Parapoxvirus*, *Yatapoxvirus*, and *Molluscipoxvirus* [1, 2]. Variola virus (the etiologic agent of smallpox) and molluscum contagiosum virus are primarily human pathogens, whereas the other poxviruses are zoonoses and humans are incidental hosts. The skin is the primary portal of entry for most poxviruses. These viruses are epitheliotropic and produce lesions that progress through well-described stages over several weeks [3]. Lesions may be restricted to specific body sites as in the case of parapoxvirus infections or generalized as seen in some orthopoxvirus infections such as smallpox and monkeypox.

Human infection with zoonotic poxviruses occurs as a result of direct or indirect contact (via fomites) with

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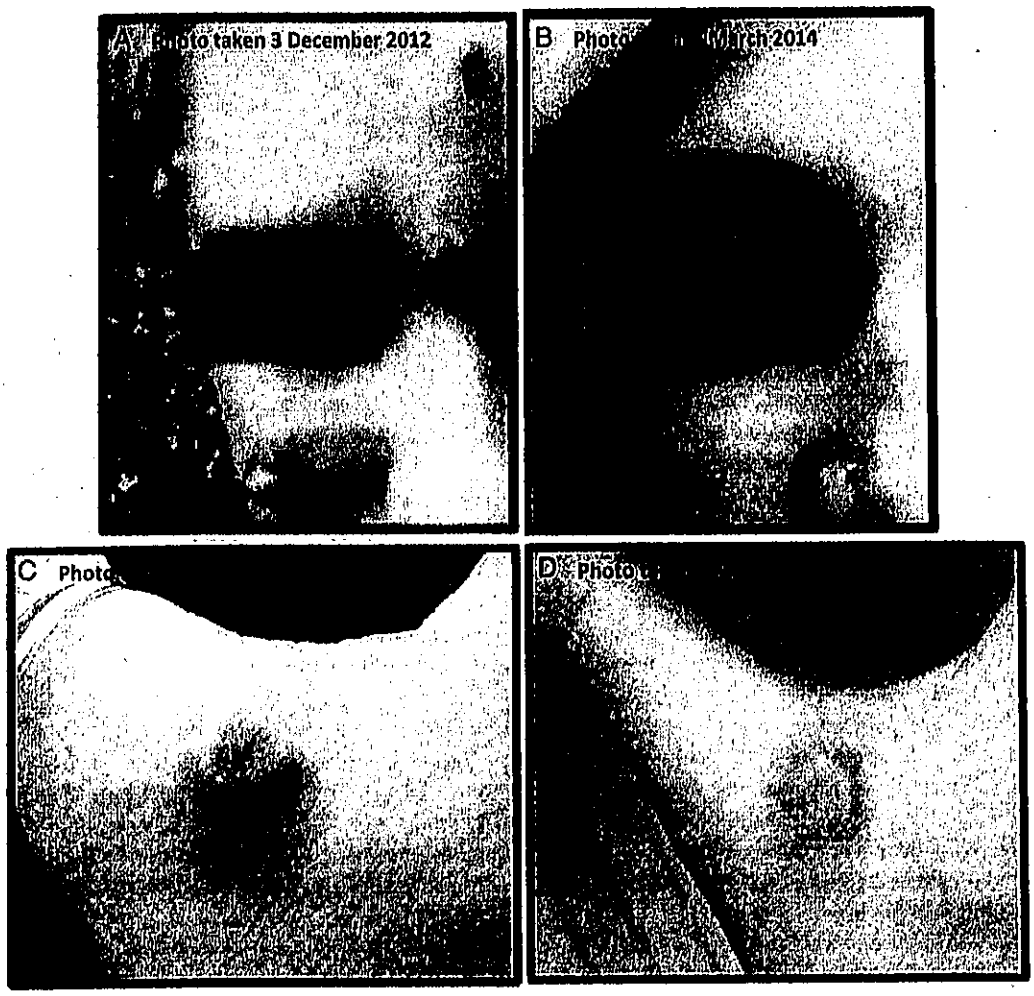

Figure 1. *A*, Large 1 to 1.5 cm facial nodules on patient 1 approximately 3 weeks after lesions developed. *B*, Patient 1's lesions 14 months after excision and cryotherapy. *C*, Marble-sized lesion in the web space between the right index finger and the thumb of patient 2 about 3 weeks after the lesion emerged. *D*, Patient 2's lesion 3 weeks after excision and cauterization.

infected animals. Such infections are typically self-limiting but may be protracted and may involve varying treatment modalities in immunocompromised individuals, as demonstrated in this case report.

Case Reports

Patient 1

On 16 November 2012, a 17-year-old woman from eastern Tennessee developed an erythematous macule on her right cheek. She had an orthotopic heart transplant in 2007 and was maintained on tacrolimus and mycophenolate-mofetil. On 18 November the lesion on her cheek grew in size and a new macule emerged on her right temple. Over the next 1–2 weeks, the lesions progressed from macules to papules to 1 to 1.5 cm brown nodules. The lesions were pruritic and painful. The patient had right cervical lymphadenopathy but no fever. On 20 November, the patient sought medical attention at a local children's hospital, where she was referred to a dermatologist. The dermatologist excised the right temple lesion and submitted it for dermatopathologic evaluation. Histopathology revealed ballooning keratinocytes with eosinophilic cytoplasmic inclusions suggestive of a poxvirus infection. The dermatologist prescribed topical and oral acyclovir and oral minocycline.

On 29 November, the patient returned to her dermatologist for recurrence of the right temple lesion and development of 4 additional facial lesions, which followed the same progression as the prior lesions. The dermatologist then referred her to a pediatric infectious disease specialist. The following day, at the consultation, 6 facial lesions at varying stages of progression were noted. The physician prescribed 5% imiquimod cream for thrice-weekly application and 2% mupirocin ointment for twice-daily application. The physician requested assistance from the Centers for Disease Control and Prevention (CDC)

Table 1. Sampling of Animals That Had Frequent Contact With Patients and List of Surfaces/Fomites Sampled on the Farm and the Horse Stable

Patient	Sampling Location/Site	Animal/Fomite (No.)	Type of Specimen	No. of Specimens	Comments
Patient 1	Farm	Horses (3)	Oral swabs, skin scabs, lesion swabs, and blood/serum	26	Clinical specimens were collected from all animals on the farm in December 2013 and May 2014.
		Hinny (1)	Oral and lesion swabs	3	
		Dogs (2)	Oral and lesion swabs	5	
		Indoor cats (2)	Oral swabs	2	
		Barn cat (1)	Oral and lesion swabs	3	The cat had frequent contact with rodents and would bring dead rodents into the animal barn.
		Chickens (3)	Oral swabs	3	The chickens were brought to the farm 1 month prior to the patient's symptom onset.
	Patient's home	Dogs (2)	Oral swabs	2	
		Cats (2)	Oral swabs	2	
	Friend's home	Dogs (2)	Oral swabs	2	
		Cats (2)	Oral swabs	2	
	Animal stall	Walls (3) Windows (4) Feeding and water troughs (9)	Swabs	20	Scratches were observed on the wall, which indicates frequent animal contact.
	Tack room	Bridles and harness (9)	Swabs	10	
Patient 2	Stable	Horse (1)	Oral swabs	2	The horse was housed in a stable with 20 other horses.
	Patient's home	Dogs (3)	Oral swabs	6	
		Cats (5)	Oral swabs	10	

for laboratory confirmation of a suspected poxvirus infection. On 4 December, examination of the patient revealed progression of the lesion on the glabella and 3 new lesions on the chin. The crust overlying the oldest nodule on the cheek was separating from the underlying skin (Figure 1*A*).

Despite the application of imiquimod for 2 weeks, the existing lesions progressed. On 13 December, the patient returned to the dermatologist, who excised the larger nodules and removed the smaller lesions with cryotherapy. The dermatologist discontinued imiquimod to lessen the risk of scarring. Four months after excision, the patient had atrophic scars and underwent laser therapy to mitigate scarring. Fourteen months later, only minimal scarring was evident (Figure 1*B*).

The patient owned and cared for a horse, which was kept at a stable with other horses, a hinny, dogs, cats, and chickens. The patient had been working at this stable daily for 4 years and regularly handled hay, animal manure, and animal feed and collected chicken eggs. None of these animals had mucus membrane or skin lesions. This history of long-term exposure to a stable environment coupled with the initial pathology result of a poxvirus infection led to the suspicion that the patient was infected with a parapoxvirus. The patient reported no contact with small ruminants, cattle, rodents, or other wild animals. She had no recent travel or exposure to individuals with proliferative or vesicular skin lesions.

Patient 2

On 8 April 2013, an immunocompetent 28-year-old woman from western Missouri presented to her physician with a marble-sized nodule in the web space between the right index finger and thumb. She had right axillary lymphadenopathy but was afebrile. On 10–17 March, the patient had traveled to northern Tanzania on a philanthropic mission providing care for donkeys and dogs. On 12 March, the patient sustained a rope burn on her right hand at the site of the lesion while restraining a donkey. On the following day, the patient rescued another donkey from an animal watering hole during which her hands were submerged in murky water. On 18 March, the patient returned to the United States and noticed a small papule at the site of the abrasion. By 30 March, the papule had developed into a nodule (Figure 1*C*). Given her travel history, she was referred to an infectious disease specialist and dermatologist. The dermatologist excised the lesion for dermatopathologic evaluation and the area was cauterized. Topical Neosporin cream as needed and 10 days of oral doxycycline and levofloxacin were prescribed for the patient. The day after the excision, the patient

experienced painful swelling of her right arm consistent with cellulitis; all symptoms resolved after 4–5 days. As of May 1, three weeks after the excision, the patient reported resolution of the skin lesion (Figure 1*D*). Histopathology showed eosinophilic inclusions suggestive of poxvirus infection and surface bacterial colonization. The dermatologist requested CDC assistance for laboratory confirmation.

While in Tanzania, the patient was primarily in contact with donkeys. The patient recalled seeing wounds on some of the donkeys' withers and legs. The patient was part of a group of 8 volunteers, and none of her colleagues developed lesions. In the United States, the patient owned dogs, cats, and a horse. The horse was kept in a nearby stable and the patient saw her horse 1 month prior to the trip. None of these animals had visible lesions.

METHODS

Clinical Specimens

One formalin-fixed, paraffin-embedded (FFPE) skin specimen from each patient was examined by histopathology, immunohistochemistry (IHC), transmission electron microscopy (TEM), and molecular assays.

Histopathology, Immunohistochemistry, and TEM

Sections, 3 micrometer in thickness, were cut from FFPE skin biopsy specimens and stained with hematoxylin and eosin. IHC for parapoxvirus was performed utilizing an immunoalkaline phosphatase technique. The antiparapoxvirus antibodies used were a polyclonal sheep anti–orf virus antibody and a polyclonal antibovine popular stomatitis virus antibody known to cross-react with orf. The FFPE sections from both patients were deparaffinized in xylene and embedded for thin-section TEM as previously described [4].

Polymerase Chain Reaction Assays

Extracted DNA was evaluated with the use of a pan-pox polymerase chain reaction (PCR) for general detection of poxvirus. The pan-pox PCR is a combination of 2 assays based on Guanine-cytosine (GC) content: a low-GC PCR for the detection of orthopoxvirus DNA, and a high-GC PCR for the specific detection of molluscipoxvirus and parapoxvirus DNA [5]. The amplicon sequences from the high-GC PCR assay were used for virus identification and phylogenetic analysis.

Animal and Environmental Sampling

On 26 December 2012, oral swabs were collected from 20 animals in contact with patient 1. The animals were inspected and no visible lesions consistent with poxvirus infections were present. In light of the second case, and because the both patients had contact with equines, a second extensive investigation was conducted in May 2013 at the farm where patient 1 worked. The following occurred during the field investigation: (1) interviews of the farm owner and veterinarian were conducted to determine the origin, movements, and health history of animals on the property; (2) a thorough physical examination was conducted of all domestic animals present in the barn environment, and oral swabs and swabs of skin irregularities (scabs, scar, and wounds) were obtained from 12 animals; (3) swab specimens of fomites (animal stall walls, surfaces, bridles) with likely contact with the oral mucosa of the equines were obtained; and (4) 180 traps were set for rodents over 2 successive nights. For patient 2, oral swabs were collected in May 2013 from the patient's 9 animals. Table 1 lists the animal and environmental specimens collected. DNA was extracted from all swabs using the

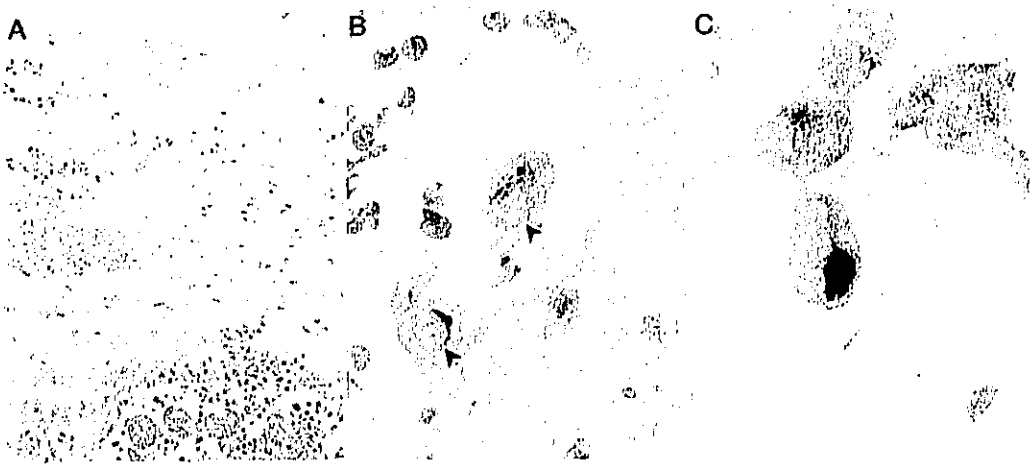

Figure 2. *A*, Photomicrograph of the lesion from patient 2 shows acantholysis, keratinocyte ballooning degeneration, and dermal capillary proliferation. *B*, Cytoplasmic inclusions are seen within degenerating keratinocytes (arrowheads). *C*, Immunohistochemistry staining shows parapoxvirus antigens in red within the keratinocytes.

BioRobot EZ1 system DNA tissue kit (Qiagen) according to the modified pretreatment protocol described previously, and specimens were tested using the pan-pox PCR assay [5].

RESULTS

Histopathology and Immunohistochemistry

The biopsies from both patients showed lesions typical of cutaneous poxvirus infection. The epidermis was acanthotic with a serocellular crust (Figure 2*A*). Keratinocyte ballooning degeneration and necrosis were prominent, and scattered, large, eosinophilic to amphophilic cytoplasmic inclusions were seen within the degenerating keratinocytes (Figure 2*B*). The dermis showed edema, capillary proliferation, and diffuse lymphocytic to mixed inflammatory infiltrates. Superficial colonization by bacteria was also noted. IHC demonstrated positive staining of keratinocyte cytoplasm using polyclonal sheep antisera generated against orf virus (Figure 2*C*). No staining was observed with the antisera generated against bovine popular stomatitis virus.

Transmission Electron Microscopy

The FFPE sections from both patients prepared for TEM revealed ovoid virions morphologically consistent with a poxvirus. Viral particles measured 200–300 nm by 100–150 nm (Figure 3).

Molecular Assays and DNA Sequence Analysis

The low-GC PCR assay revealed no amplification, whereas results of the high-GC PCR assay confirmed a poxvirus infection in both patients; however, the patients' isolates were negative for molluscipox and parapoxviruses using real-time PCR assays specific for these viruses. Phylogenetic analysis of the amplified

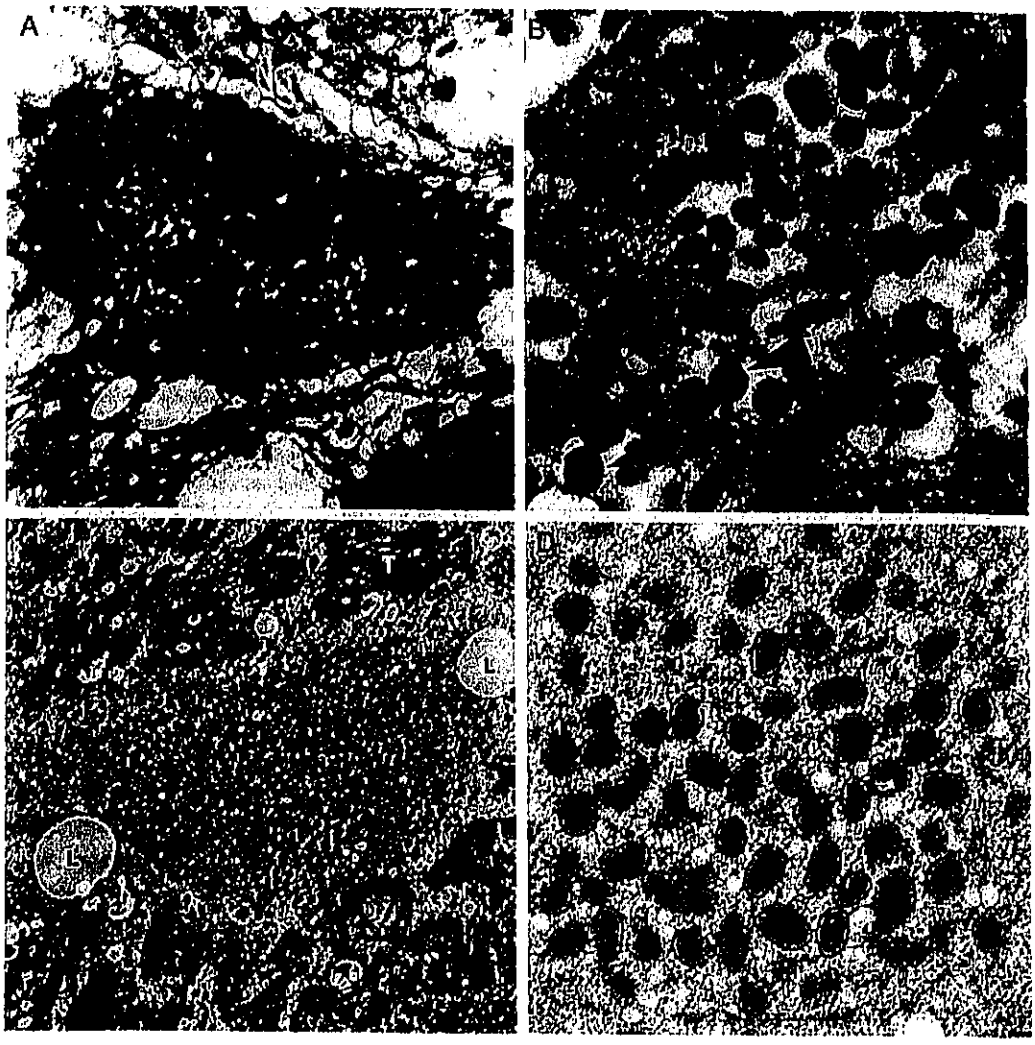

Figure 3. *A*, Tissue from patient 1 with intracellular poxvirus particles. *B*, Mature (arrow) and immature (arrowhead) viral particles at higher magnification. Similar findings were seen in micrographs taken of tissue from patient 2 (*C* and *D*). L denotes lipids; T denotes tonofilaments.

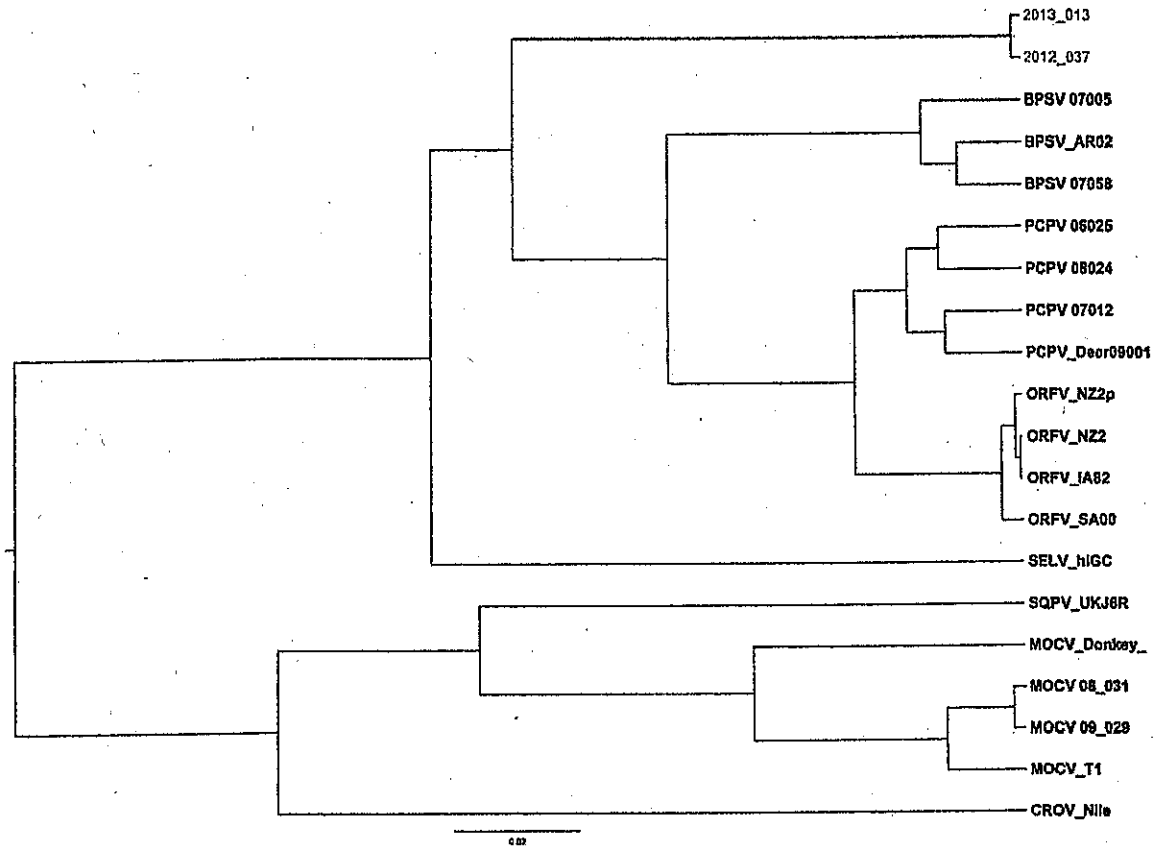

Figure 4. Phylogenetic tree showing the relationship of viruses isolated from patient 1 (2012_037) and patient 2 (2013_013) to other poxviruses. There was a single-nucleotide change between the patients' isolates. The phylogenetic tree was constructed from 620 nucleotide sequences of a high-GC polymerase chain reaction amplicon targeting the highly conserved viral RNA polymerase gene. GenBank accession numbers of individual amplicons are listed: patient 1 (KM491712), patient 2 (KM491713), 3 strains of bovine popular stomatitis virus (BPSV 07005, GQ902054.1; BPSV_AR02, AY386265.1; BPSV 07058, GQ902053.1), 4 strains of orf virus (ORFV_NZ2p, AX754989; ORFV_NZ2, DQ184476.1; ORFV_IA82, AY386263.1;ORFV_SA00, AY386264), a red squirrel pox from the United Kingdom (SQRV_UKHE601899), a molluscum contagiosum–like virus from a donkey (MOCV_Donkey JQ269324), 3 pseudocowpox virus strains (PCPV 06025, GQ902049.1; PCPV 08024, GQ902050.1; PCPV 07012, GQ902051.1), a pseudocowpox-like virus from a US deer hunter (PCPV_Deer09001) [6], a sealpox virus (SELV_hiGC,), 3 strains of molluscum contagiosum virus (MOCV_T1, U60315.1; MOCV 08_031, GQ902057; MOCV 09_029), and Nile crocodilepox virus (CROV_Nile, DQ356948.1). The DNA sequences were aligned with the use of the BioEdit and Clustal alignment programs. Phylogenetic analyses were performed with the use of the Bayesian analysis software packages BEAST and BEAUti, version 1.7.5. The analyses ran a Markov chain Monte Carlo chain length of 5 000 000, with a Hasegawa–Kishino–Yano nucleotide substitution model, strict molecular clock, and sampling of every 1000 states. To root the dendrogram, a myxoma virus, MYXV_wel (JX565582) not shown, is used as the outgroup. The 0.02 scale bar denotes the genetic distance in substitutions per site.

sequences from both patients' isolates indicated a novel, high-GC poxvirus (Figure 4). Results of a National Center for Biotechnology Information Basic Local Alignment Search Tool search indicated the amplicon sequence had an 88% similarity to viruses in the *Parapoxvirus* genus and 78% to molluscum contagiosum virus within the *Molluscipoxvirus* genus. Notably, known zoonotic parapoxviruses (orf, pseudocowpox virus, and bovine popular stomatitis virus) share 92% similarity to each other along the same amplicon sequenced from both patients' isolates. All 98 specimens (from 29 animals and 25 environmental sites) were negative for poxvirus using the pan-pox PCR assay, and no rodents were trapped.

DISCUSSION

We present 2 cases of a cutaneous poxvirus infection involving a previously unknown, currently unclassified virus. The DNA sequence from the highly conserved viral RNA polymerase gene (J6R) suggests that this virus is most closely related to the *Parapoxvirus* genus but belongs to a unique clade that is distinct and divergent. IHC data demonstrate antigenic similarities between the new virus and the orf virus but not bovine popular stomatitis virus. Studies such as that of Housawi et al suggest a link between cross-reactivity and genetic relatedness among parapoxviruses [7]. In that study, the authors detected

variations in the cross-reactivity of 27 monoclonal antibodies (mAbs) produced against orf virus for different parapoxvirus species and strains [7]. Notably, the number of cross-reactive mAbs decreased when strains were more distantly related. Only 2 mAbs reacted with the squirrel poxvirus (SQPV_UKJ6R in Figure 4), whereas 6 mAbs reacted with the seal parapoxvirus (SELV_hiGC in Figure 4). Thus, our IHC result may suggest a closer relationship between the novel poxvirus and the orf virus than bovine popular stomatitis virus, but due to the paucity of studies on the cross-reactivity of parapoxviruses, no further deductions can be made about the similarities of this new virus to other known parapoxviruses.

Based on clinical presentation, it is reasonable to compare this novel virus with parapoxvirus infections. Parapoxviruses are widespread and infect a wide range of mammals including ungulates and seals, and human infection occurs as a result of direct or indirect (via fomites) contact with infected animals [8–10]. The viruses are hardy and highly resistant to environmental degradation under ambient conditions [11]. Patients with parapoxvirus infections typically have a well-defined history of animal contact such as slaughtering, meat processing, or bottle feeding [6, 10, 12]. We were unable to identify the origin of this new virus for either patient. Despite this, we cannot rule out a zoonotic source, as poxviruses have a wide host range and both patients had habitual exposure to the species-rich environment of a horse stable. The high degree of genetic similarity between the 2 viruses could suggest a common geographic origin; however, patient 2 also had contact with donkeys in Tanzania, and the timing of lesion origination suggests that she acquired the virus in Tanzania. Having failed to identify a potential source of fomite contamination originating in the United States (ie, equipment transported from Missouri to Tanzania), we are unable to resolve this conundrum.

The appearance and progression of our patients' lesions was similar to those of the parapoxviruses. Persons infected with parapoxviruses generally present with solitary or regionally restricted lesions, usually on hands or arms rather than a disseminated rash, as is seen with some orthopoxvirus infections (smallpox, monkeypox). Furthermore, parapoxviruses replicate in regenerating epidermal keratinocytes; these cells are rich in nucleotide pools required for viral replication [13]. Both patients in this series had a compromised epidermal surface; patient 1 had mild acne and patient 2 sustained a rope burn at the site of the lesion 1 week prior to symptom onset.

These patients also highlight the differences in presentation between immunocompromised and immunocompetent persons. In immunocompetent patients, parapoxvirus lesions are generally self-limited, as was seen in patient 2. In contrast, immunocompromised patients often develop large, rapidly growing, exophytic lesions in atypical sites such as the face [14–17], as observed in patient 1.

Treatment strategies for cutaneous poxvirus infections depend on the patient's immune status and clinical course. In immunocompromised patients with orf virus lesions, application of imiquimod, an immunomodulatory agent, has been shown to result in the clearance of lesions within days to weeks [14–16], although in most of these cases, imiquimod was used in conjunction with other treatment modalities. Patient 1's lesions appeared to be refractory to imiquimod therapy, as the lesions continued to grow despite its application. Thus, the therapeutic effect of imiquimod in poxvirus infection remains unclear. Surgical excision and cryotherapy are also treatment options, which proved successful in both patients [17, 18]. Patient 2 experienced symptoms consistent with cellulitis, which is not uncommon in cutaneous infections.

Although we were unable to identify the source(s) of this novel poxvirus, we recommend the use of nonporous (rubber or latex) gloves for persons in contact with stable or barn environments, or involved in animal handling, particularly those individuals who are immunosuppressed or have open wounds on the hands. Also, all open wounds should be covered when handling animals, and skin should be immediately washed after contact with animals as poxviruses are known to infect damaged skin.

In summary, this is a report of a novel poxvirus infection in 2 patients with a common exposure to domestic animals, including equids. This report highlights the importance of a comprehensive approach to diagnosis. Collaboration between multiple specialists aided in dictating appropriate treatment modalities. In this instance, the identification of a novel poxvirus related to *Parapoxvirus* helped guide treatment options and possible outcomes.

Notes

Acknowledgments. We thank Dr Brett Peterson from the Division of High-Consequence Pathogens and Pathology, Centers for Disease Control and Prevention (CDC); Dr Charles Hatcher from the Tennessee Department of Agriculture; Glenis Moore from the Tennessee Department of Health; Dr Edward J. Primka III of Dermatology Associates of Knoxville; and Mr Randy Schillers and Ms Cindy Butler of the Missouri Department of Health for their participation.

Disclaimer. The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the CDC.

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研究報告の概要

○エチオピアのアファール州におけるシチリア型サシチョウバエ熱ウイルズ(SFSV)を原因とする急性熱性疾患(AFI)のアウトブレイク、2011年

マラリア流行地域の多くの医療機関では、マラリア以外のAFIの診断能力が限られている。その結果、AFIは発展途上国における主な疾患であるにもかかわらず、原因が特定されることは少ない。

2011年8月、エチオピアのアファール州で、発熱、悪寒、頭痛及び筋肉痛の急な出現を特徴とし、マラリア検査が陰性のAFIのアウトブレイクが報告された。調査チームは同地域の5カ所の医療施設のカルテを調べ、2011年8月7日～9月12日の間にAFI疑い症例を12,816例確認した。患者の症状は軽症で、発症3～4日以内に自然治癒し、死亡者はいなかった。AFI患者29人から血液サンプルを採取し、各種検査を行ったところ、マラリアを含む特定の病原体に関して全て陰性であった。これらの血液サンプルから抽出した核酸の大規模シークエンシングの結果、17サンプル(59%)がSFSV陽性であった。

SFSVは1943年、第二次世界大戦中のイタリアのシチリアにおいて、サシチョウバエ熱の原因として初めて特定された節足動物媒介性ウイルスである。SFSVは、主な媒介昆虫である*Phlebotomus papatasi*の活動時期である夏に流行する。

これは、エチオピアにおいて、AFIアウトブレイクの原因としてSFSVが特定された初の報告である。

報告企業の意見	今後の対応
エチオピアにおいて、AFIアウトブレイクの原因としてSFSVが特定された初の報告である。	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。

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

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An Outbreak of Acute Febrile Illness Caused by Sandfly Fever Sicilian Virus in the Afar Region of Ethiopia, 2011

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Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia; Global Disease Detection Program, Centers for Disease Control and Prevention–Kenya, Nairobi, Kenya; Departments of Molecular Microbiology and Pathology and Immunology, Washington University in St. Louis School of Medicine, St. Louis, Missouri; Division of Vector-Borne Disease, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado; Global Disease Detection Branch, Division of Global Health Protection, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, Georgia

Abstract. In malaria-endemic regions, many medical facilities have limited capacity to diagnose non-malarial etiologies of acute febrile illness (AFI). As a result, the etiology of AFI is seldom determined, although AFI remains a major cause of morbidity in developing countries. An outbreak of AFI was reported in the Afar region of Ethiopia in August of 2011. Retrospectively, 12,816 suspected AFI cases were identified by review of medical records. Symptoms were mild and self-limiting within 3 days after the date of onset; no fatalities were identified. All initial test results of AFI patient specimens were negative for selected pathogens using standard microbiological and molecular techniques. High-throughput sequencing of nucleic acid extracts of serum specimens from 29 AFI cases identified 17 (59%) of 29 samples as positive for Sandfly Fever Sicilian Virus (SFSV). These results were further confirmed by specific reverse transcription polymerase chain reaction. This is the first study implicating SFSV as an etiological agent for AFI in Ethiopia.

INTRODUCTION

Acute febrile illnesses (AFI) caused by a variety of pathogens pose a major public health challenge, in part because clinical examination cannot distinguish specific etiologies. Furthermore, practical and affordable diagnostic tests for the diagnosis of non-malarial etiologies of AFI are often not available in developing countries.[1–3] Consequently, the incidence and relative importance of the etiologic agents responsible for AFI remain unknown in many parts of the world.[2] This leads to potential misdiagnosis, inappropriate patient management, and an inability to effectively control or prevent additional cases. Sandfly fever, caused by infection with the Sandfly Fever Sicilian Virus (SFSV), is common in the Mediterranean region.[4] SFSV is an arthropod-borne virus that was first identified in Sicily, Italy in 1943 during World War II as the etiology of sandfly fever that was a cause of AFI in Allied armed forces.[4] The virus is spread during the summer season, which is the active period for *Phlebotomus papatasi*,[5] the main vector of SFSV. Other sandflies, such as *P. ariasi* and sandflies of Larroussius group, also transmit SFSV. Sandfly fever is a self-limited mild illness including fever, headache, and muscle and joint pain; patients usually recover fully within a few days.[5] Serologic evidence of sandfly fever indicated positive titers of SFSV from samples collected in Bangladesh, Djibouti, Ethiopia, Iraq, Morocco, Saudi Arabia, Somalia, Sudan, Tunisia, former republics of the Soviet Union, and Yugoslavia.[6] This investigation identified SFSV as the etiologic agent in an AFI outbreak that was initially assumed to be caused by malaria and later thought to be caused by dengue. To our knowledge, this is the first report of sandfly fever in Ethiopia.

METHODS AND MATERIALS

Samples were collected as a public health response under the direction of the Ethiopian Health and Nutrition Research Institute. As a public health response, this investigation was not categorized as research, and informed consent was not required.

In August of 2011, an outbreak of AFI was reported in Asayta District, Afar Regional State, Ethiopia. The outbreak later spread to two neighboring districts of Dubti and Afambo (Figure 1). The illness was characterized by acute onset of fever, chills, headache, and myalgia.

The regional health bureau (RHB) in the affected districts sought assistance from the Ethiopian Health and Nutrition Research Institute (EHNRI) when blood samples from patients with AFI tested negative for malaria. The national outbreak investigation team from the EHNRI initiated an investigation by examining medical records from five local health facilities. Using medical records, a suspect AFI case was defined as acute onset of fever, chills, headache, and myalagia from August 7 to September 12, 2011.

The national outbreak investigation team collected blood specimens from 29 acutely ill patients with AFI. These specimens were tested for malaria using a rapid diagnostic test (CareStart Malaria HRP2 [Pf] Test; Access Bio, Inc., Somerset, NJ) or microscopy on blood smears. In addition, samples were subcultured onto blood, chocolate, and MacConkey agar.

Sera, which had been stored at −80°C, from 29 acutely ill patients were transported to the Centers for Disease Control and Prevention (CDC) laboratories at the Kenya Medical Research Institute (KEMRI) in Kenya for real-time polymerase chain reaction (PCR) testing. Total nucleic acid extraction was performed using the MagMAX Viral RNA Isolation Kit (Life Technologies, Foster City, CA) as per the manufacturer's instructions. Amplification was done using the AgPath-ID One-Step RT-PCR Kit (Applied Biosystems, Carlsbad, CA) in individual PCR assays for dengue, Rift Valley fever (RVF), yellow fever (YF), filoviruses (Ebola zaire virus, Ebola bundibugyo virus, Ebola sudan virus, and Marburg virus), chikungunya, and Crimean-Congo Hemorrhagic Fever (CCHF) viruses. Primers and probes for the filoviruses were obtained from the CDC, Viral Special Pathogens Branch in Atlanta, Georgia. Primers and probes for YF, dengue, and RVF

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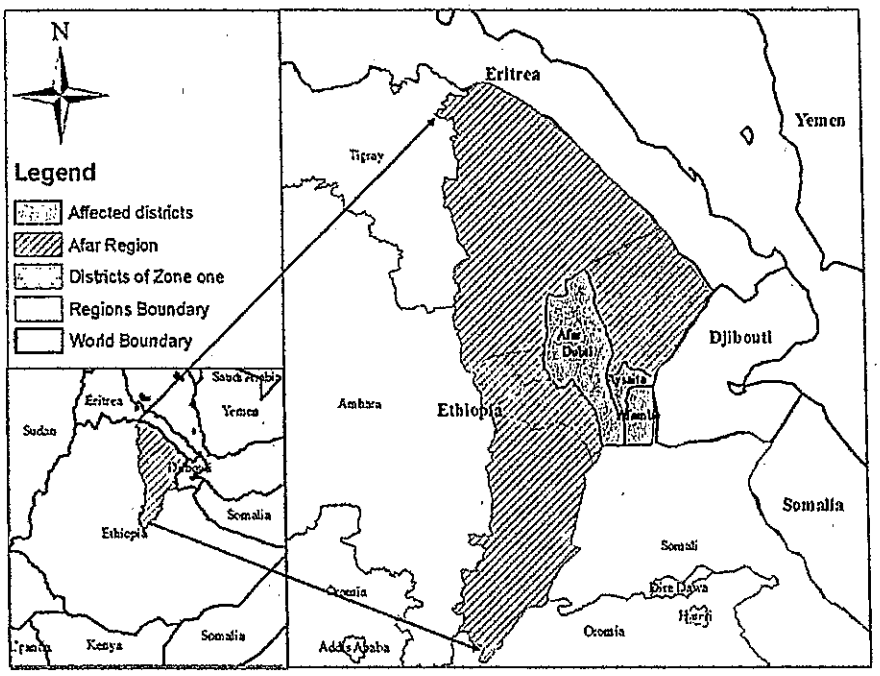

FIGURE 1. Acute febrile illness outbreak affected districts of Afar region, Ethiopia, 2011.

have previously been described, whereas those of chikungunya virus were obtained from the Division of Vector-Borne Diseases, CDC, in Fort Collins, Colorado.[7] Thermal cycling conditions included reverse transcription at 45°C for 10 minutes, AmpliTaq Polymerase activation at 95°C for 10 minutes, and then, 45 cycles of denaturation at 95°C for 15 seconds and primer annealing/extension at 55°C for 1 minute.

Total nucleic acids from 29 of the serum specimens were transported to Washington University in St. Louis for next-generation sequencing analysis. Nucleic acids were randomly amplified by sequence-independent PCR using barcoded primers, pooled, and sequenced in one run on the Roche Titanium/FLX platform as described previously.[8] Sequences were compared with publicly available sequence databases using a customized bioinformatics platform as previously described to identify microbial sequences present in the sample.[9] Contig assembly was performed with the Newbler assembler.

For result confirmation, RNA was extracted from specimens positive by next-generation sequencing and subjected to a phlebovirus-specific consensus reverse transcription PCR (RT-PCR) assay. Resultant amplicons were sequenced using previously described methods at the CDC Division of Vector-Borne Diseases in Fort Collins, Colorado.[10] All generated partial S-segment sequences were deemed identical in the amplified region. A phylogenetic analysis was conducted on a representative partial S-segment sequence (284 bp; GenBank accession no. KJ372529), which maps to nucleotides 139–422 of the GenBank reference SFSV-Turkey sequence (GenBank accession no. NC_015413.1) along with diverse SFSV sequences that are available in GenBank. Alignments were generated using the Clustal W function of MEGA, version 4 software.[11,12] A neighbor-joining tree was generated and analyzed with 2,000 replicates for bootstrap testing.[13]

RESULTS

In total, 12,816 suspect AFI cases were identified between August 7 and September 12, 2011 through the medical records review from five health centers in Afambo, Asayta, and Dubti Districts of the Afar region (Figure 2); 9,107 (71%) cases were male. For 29 patients from whom sera was collected, the age distribution ranged from 2 to 55 years of age, with a mean age of 25 years old. Patient symptoms were mild and self-limiting, with patients recovering within 3–4 days after date of symptom onset.

All 29 blood samples tested negative for malaria (*Plasmodium falciparum* and *P. vivax*) by microscopy. In addition, bacterial culture failed to identify any potential etiologies. The 29 serum specimens tested negative for dengue, RVF, YF, filoviruses, chikungunya, and CCHF viruses by real-time

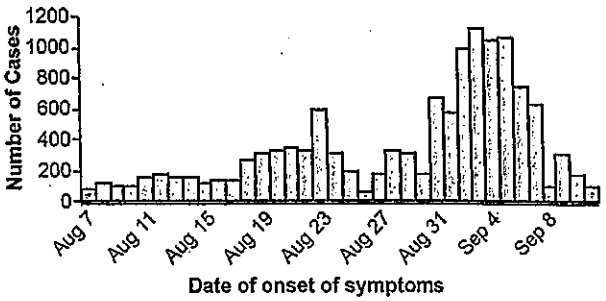

FIGURE 2. Suspect AFI cases by date of onset, Afar, August 7 to September 11, 2011.

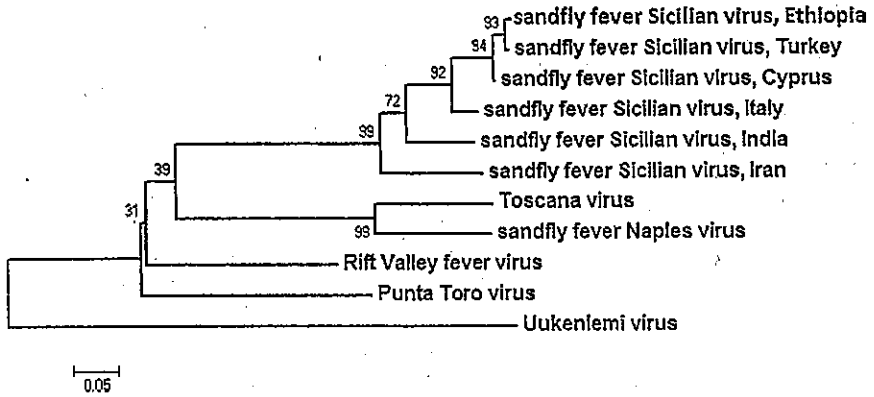

FIGURE 3. Phylogeny of the partial S segment of a geographic diversity of sandfly fever viruses. A neighbor-joining tree with bootstrap values determined by 2000 replicates is shown. The scale bar represents the number of nucleotide substitutions per site.

PCR. Attempts to culture virus from selected samples on Vero cells were unsuccessful.

After next-generation sequencing, computational analysis detected one or more nucleic acid sequences with ≥ 97% nucleotide identity to SFSV in 17 (59%) of 29 specimens. In addition, one specimen was positive for hepatitis B and SFSV; two specimens were positive for hepatitis GBV-C sequences, one of which was positive for SFSV.

To further analyze the SFSV-like sequence, data from one sample, where more than 1,000 of approximately 30,000 total reads derived from SFSV, were assembled. Contigs of 6,355 nucleotides (GenBank accession no. KM042102) and 4,349 nucleotides (GenBank accession no. KM042103) that correspond to the nearly complete L and M segments of SFSV, respectively, were generated. In addition, two shorter contigs of 790 and 767 nucleotides (GenBank accession nos. KM042104 and KM042105) were generated that aligned to different regions of the S segment of SFSV (with a gap of approximately 100 nucleotides). The four contigs shared 97–99% nucleotide identity to the reference SFSV-Turkey strain in GenBank (accession nos. NC_015411.1, NC_015412.1, and NC_015413.1).

Of 17 specimens sent to the CDC in Ft. Collins, Colorado for confirmatory testing, 7 (41%) specimens were SFSV-positive by RT-PCR, and 2 (12%) specimens were indeterminate. All seven positive samples yielded identical amplicons. The resultant tree reveals a strongly supported association between the SFSV sequences detected in Ethiopia and strains that were isolated in recent years from Cyprus (2002) and Turkey (2008), with Uukeniemi virus as the outgroup (Figure 3).

DISCUSSION

This is the first report of SFSV as the etiology of AFI in Ethiopia. The symptoms, which were mild and self-limiting within 3–4 days, are identical to those in previous reports of SFSV infection, also known as 3-day fever.[6,14]

Human case reports and seroprevalence studies have suggested that SFSV or an SFSV-like virus circulates in the Mediterranean region.[4] Recently, molecular evidence showed the presence of two distinct phleboviruses closely related to SFSV in Algeria and Tunisia.[15,16] Nucleic acids detected in this study within positive samples shared approximately 99% nucleotide sequence identity with strains that were associated with human illness in Cyprus and Turkey in 2008, implicating a possible Eastern Mediterranean origin of the Ethiopian strain of SFSV.

This large outbreak of AFI was initially identified through an extensive medical records review, and although only 29 specimens were ultimately collected, 17 specimens contained nucleic acid sequences that were ≥ 97% identical to SFSV using a next-generation sequencing-based assay. These results were independently corroborated by RT-PCR in 7 of 17 specimens in a different laboratory (CDC, Fort Collins, CO). Sequences obtained in these seven specimens were identical to those obtained in the Washington University laboratory.

There were a number of limitations to this study. An attempt to isolate a pathogen on Vero cells was unsuccessful (data not shown). Because of resource limitations, no vector assessment was conducted. Case identification was a challenge because of the pastoral nature of the affected community. Limited specimens were collected relative to the great size of the affected area, in part because cases were on the wane by the time that the investigative team arrived.

Although the epidemiologic investigation provided limited information on risk factors for infection or clues to potential etiologic agents, detection of SFSV in these specimens by high-throughput sequencing underscores the power of unbiased metagenomic strategies for pathogen detection. It is unclear at this point whether detection of sandfly fever reflects a recent expansion in the geographic range of the virus beyond the Mediterranean region or alternately, previously unrecognized endemicity uncovered by the increased diagnostic breadth of the unbiased next-generation sequencing assay. Broader studies are needed to determine the prevalence of sandfly fever in this geographic area. Furthermore, disease surveillance should be implemented in endemic regions to monitor the trends and quickly identify an outbreak. Surveillance would also provide data to the national level on disease burden and etiological agents of focus in resource-limited settings.

This outbreak highlights the diagnostic challenges in identifying etiologic agents responsible for AFI outbreaks in developing countries. AFI cases usually go uncharacterized in resource-limited settings and are mainly treated as malaria or typhoid. In our study, the outbreak etiological identification was inefficient primarily because of the lack of advanced molecular techniques, which necessitated international collaboration. Although etiological identification was successful in this instance, this approach is not cost-effective considering

the burden of AFI outbreaks in sub-Saharan Africa. There is a great need to develop laboratory capacity for both traditional microbiology and molecular techniques to improve identification of agents responsible for causing outbreaks of AFI in developing countries.

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