資料２－２

Ａ　研究報告（詳細版）

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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)			

研究報告の概要

○有機溶媒/界面活性剤(SD)で不活化した血漿によるE型肝炎ウイルス(HEV)の輸血感染
HEVは非エンベロープウイルスであるため、フランスで病原体不活化のために100アフェレーシス血漿を1ロットとして行われているSD処理は無効である。
2012年、異なる2ロットのSD処理血漿を輸血された2人の患者のHEV感染が報告された。SD処理血漿に用いられた2人の供血者の血漿からRNAが検出され、ウイルス量は145,000及び3,060コピー/mLで、対応するロットのウイルス量はそれぞれ541及び62コピー/mLであった。ジェノタイプはいずれも3fであり、供血者のウイルスの遺伝子配列は、対応する患者のウイルスと一致した。2ロットのウイルス量がいずれも低かったのは、希釈効果と中和抗体の存在によるものと考えられる。HEV感染は不顕性であることが多く、SD処理血漿による輸血感染の多くが診断されずに治癒した可能性もある。

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
SD処理されたプール血漿による輸血HEV感染が発生したとの報告である。	日本赤十字社では、厚生労働科学研究「経口感染によるウイルス性肝炎(A型及びE型)の感染防止、病態解明、遺伝的多様性及び治療に関する研究」班と共同して、献血者におけるHEV感染の疫学調査を行っている。今後もHEV感染の実態に関する情報の収集及び安全対策に努める。

SP403

Searching for Non-Primate Hepacivirus (NHPV) Related Genomes in HCV-Reactive Donors with Indeterminate Blot Patterns

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Background/Case Studies: Hepatitis C virus (HCV) belongs to the genus Hepacivirus, one of the four genera in the family Flaviviridae along two established genera (Flavivirus and Pestivirus,) and one proposed genus, Pegivirus. Among the Flaviviruses and Pestiviruses there are important human pathogens, known to be transmitted to humans by arthropods and bats, depicting the importance of the behavior of human blood sucking for cross-species transmission of infectious agents. Surprisingly, no primate homologue to HCV has ever been found, neither has any arthropod been demonstrated to transmit HCV to humans. The search for a zoonotic origin for HCV has been renewed recently when Kapoor and co-workers (PNAS 2011; 108:11608-11613) found a canine virus with a high homology to HCV, now named as Non-Primate Hepacivirus (NHPV). A variable proportion of anti-HCV reactive donors submitted to the immunoblot (IB) to confirm their HCV status, present indeterminate results, meaning that it was verified some reactivity, in general weak, to one or two antigenic fractions, that does not allow the assignment of a positive result but neither to discard it. The degree of homology between HCV and NPHV suggests that humans may be infected by NPHV or NPHV-like viruses. Maximum aminoacid similarity between NHPV and HCV is observed in the non-structural regions 3 and 5. Peptides representing both domains are present in IB assays, so we reasoned that blood donors reactive for anti-HCV in screening and further reacting to these antigenic fractions in IB may potentially harbor such viruses. This study searched for NPHV sequences in the plasma of blood donors found anti-HCV reactive but not confirmed by neither IB or RT-PCR analysis. **Study Design/Methods:** Fifty-seven plasma samples from blood donors found reactive for anti-HCV second or third generation assays and presenting immunoblot indeterminate results were submitted to five distinct PCR reactions under low-stringency conditions, employing primers targeting GBV-C 5'UTR and NS3, Flavivirus-genus NS5, and NHPV 5'UTR and NS3. **Results/Findings:** No amplification was obtained with all primer pairs tested except for three samples that amplified both 5'UTR and NS3 fragments from GBV-C. No other sample presented reproducible amplification of fragments within the expected size with any of the primer sets. **Conclusion:** The presence of any NHPV or Flavivirus related virus did not appear among well-selected blood donors with IB indeterminate patterns. Certainly, now-available metagenomics tools such as next-generation sequencing may provide or rule out definitely the existence of HCV-related viruses in immunoblot indeterminate samples.

Disclosure of Commercial Conflict of Interest

S. N. Cabral: No Answer; S. C. Ferreira: Nothing to disclose; J. M. Kutner: No Answer; J. E. Levi: Nothing to disclose; M. A. Mota: Nothing to disclose; A. S. Nishiya: Nothing to disclose; R. A. Pereira: Nothing to disclose; M. B. Polite: Nothing to disclose; C. M. Romano: Nothing to disclose

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SP404

Case Report: A Case of Hepatitis E Virus (HEV) in a Blood Donor

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Background/Case Studies: Hepatitis E (HEV) is a liver disease caused by a non-enveloped, single stranded RNA hepatitis E virus. The virus is transmitted enterically, but parental transmissions via transfusions have been reported since 2004. The seroprevalence in blood donors reported from various countries ranges from 1% to 32%. **Study Design/Methods:** An apparently healthy, 27-year-old male donor passed all eligibility criteria tests and donated a triple blood bag at a tertiary cancer hospital. After 20 days, donor informed the blood bank that he had developed vomiting and jaundice 1 day post donation for which he had visited a physician. He was investigated by a local laboratory 1 day post donation for liver function tests which were elevated. (Bilirubin: total: 5.2, direct: 2.5, indirect: 2.7), AST: 688, ALT: 660 (n = upto 40I U/L), Urine: bile pigment present, bile salt: present, Widal and Malaria test: negative, CBC: normal. **Results/Findings:** Donor contacted this blood bank and advised about an outbreak of similar symptoms in his residential community during the same period. Accordingly, he was investigated 20 days post donation for HAV and HEV in the hospital Microbiology laboratory. The HAV IgM antibody by chemiluminiscent microparticle immunoassay was nonreactive. The HEV IgM antibody by ELISA was positive. The liver profile had improved (total bilirubin: 2.68, Direct: 1.07, indirect: 1.61, AST: 47, ALT: 68, Total proteins: 8.2, S.Albumin:4.6, S.globulin: 3.6). The donor's packed cells had already been issued during this interim period of 20 days as all mandatory TTI tests were negative. However, the cryoprecipitate and Factor VIII deficient plasma were immediately discarded. The patient receiving the implicated packed cells was symptom free 35 days post transfusion. He could not be investigated for HEV as he was discharged from hospital and was a resident of another state. The incubation period following exposure to the HEV ranges from 3-8 weeks (mean~40 days). This donor was in incubation phase during blood donation and was detected as anti HEV IgM positive. The donor initiated call back was pivotal to this case. Even though the disease is self limiting and resolves within 4-6 weeks, a fulminant hepatitis may develop which can lead to death. **Conclusion:** HEV is being considered as a re-emerging infectious disease across the world. Routine screening for the virus is presently not performed in blood banks. Studies indicate that HEV seroprevalence exists in blood donors, especially in endemic areas. Screening donor blood for this virus may be worthwhile to safeguard patients as severity is higher in high risk patients. Being a referral cancer center, donors in this setup are representative of the nation's population. A pilot project for HEV RNA donor testing would help in understanding the incidence and facilitate in HEV screening policy decisions.

Disclosure of Commercial Conflict of Interest

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SP405

Transfusion Transmission of Hepatitis E Virus (HEV) by Solvent-Detergent Inactivated Plasma

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Background/Case Studies: Hepatitis E Virus (HEV) belongs to hepeviridae family. This RNA virus is non-enveloped. Thus solvent detergent (SD) process used in France to inactivate pathogens in lots of 100 apheresis plasmas is ineffective on this virus. In 2012, two cases of HEV contamination (genotype 3) were identified in two recipients of SD-inactivated plasma. The first case (case A) was detected in a patient presenting elevated alanine aminotransferase (ALT) after treatment by plasma exchange. In the second case (case B), the patient who underwent a liver transplant and received SD-plasma showed high ALT level, 3 months after transplantation. The plasmas transfused to these patients were issued from two different lots A and B, respectively In both patients HEV RNA was detected and quantified by Real-Time (RT) polymerase chain reaction (PCR) and genotype determined by nucleotide sequencing. **Study Design/Methods:** Haemovigilance alerts from hospitals about recipient contaminations were notified to the SD-plasma production site. The corresponding contaminated lots (A and B) were then identified and screened positive for HEV ribonucleic acid (RNA). Donors' RNA positive plasmas (from A and B lots) were retrieved by testing crossing pools on the archive plates containing samples entering in the composition of the two contaminated lots. HEV RNA was extracted on Easy mag device (BioMérieux) and then amplified and detected by a RT PCR using HEV PCR realStar kit (Eurobio) on CFX instrument (Biorad). Sequencing of HEV RNA was performed in HEV positive donors, and then sequences compared to those isolated from patients. **Results/Findings:** RNA was detected in the two donor plasmas entering in the production of two different SD-plasma lots. The viral loads in the donors contaminated plasmas were 145,000 copies /mL and 3,060 copies/mL, respectively. The viral loads in corresponding lots A and B were respectively 541 and 62 copies/mL. In both cases the genotype was 3f and donor virus sequences were respectively identical to those isolated from patients. **Conclusion:** Recipient contamination by HEV has been evidenced for SD-plasma transfusion. The low viral load values in both lots can be explained by a dilution effect as well as the probable presence of anti-HEV neutralizing antibodies. Contamination by

HEV is often asymptomatic and it is probable that in number of cases transfusion of contaminated SD-plasma resulted in undiagnosed resolved infection. The protective role of anti-VHE antibodies in recipients, as well as in the pooled plasma lots, is being further investigated. Transfusion transmission of HEV is a matter of concern for the French Blood Transfusion Establishment (EFS). Hence HEV screening was implemented in December 2012 on pools of 96 samples, as it was already done for Parvovirus B19 and Hepatitis A virus. Infectivity of HEV-RNA containing SD-plasma lots and HEV transmission by other labile components are currently being investigated.

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Disclosure of Grants Conflict of Interest

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√ SP406

Photochemical Inactivation of Hepatitis E Virus (HEV) in Platelet Samples (PCs) using Mirasol Pathogen Reduction Technology (PRT) system

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Background/Case Studies: Hepatitis E Virus (HEV) had previously been considered to be transmitted perorally and to be particularly localized to developing regions, but has recently been found to be spreading throughout the world, including in industrialized nations. It has also been demonstrated that HEV can be transmitted via blood transfusion. Thus, the risk of HEV transmission via transfusion is now a major global concern. The Japanese Red Cross Society has become focused on this virus as a blood-borne infectious disease, and is the first manufacturer in the world of blood-related pharmaceuticals to introduce nucleic acid amplification test (NAT) screening for HEV tentatively. An assay system for the infectious titer (tissue culture infectious dose; [TCID]) of HEV cultured from human HEV-RNA-positive plasma and serum was recently successfully demonstrated. This methodology was applied to investigate the ability of the Mirasol PRT system (Terumo BCT, Lakewood, CO) to inactivate live HEV in contaminated PC. **Study Design/Methods:** PCs were spiked with cultured HEV genotype (G) 3 or G4 and treated with riboflavin and ultraviolet (UV) light using the Mirasol PRT system. PCs were examined before and after treatment for HEV load via infectivity titration (TCID) against A549, an HEV-infectible cell line. In addition, the quantitative real time reverse transcription-polymerase chain reaction (PCR) was performed to determine the load of HEV interacting with A549 cells based on a quantitative change in a continuous process of virus binding and entry, to clarify the effects of the Mirasol PRT system on the initial step of HEV infection. **Results/Findings:** A significant reduction in HEV G3 infectious titer of more than 3 log 10 was recorded after the treatment of PCs with the Mirasol PRT system. In addition, an inactivation of more than 2 log 10 was confirmed when HEV G4 was used. However, it was demonstrated that no alteration of the efficiency of HEV binding and entry into host cells had been observed. **Conclusion:** The current data indicated that the Mirasol PRT system efficiently inactivated live HEV in PCs, and could therefore potentially be used to lower the possibility of HEV transmission through the blood products. The limited load (infectious titer, RNA copies) of HEV obtained from in vitro cultivation precluded an evaluation of the robustness of the measured log reduction in HEV infectivity. Hence, a more reliable log reduction value might be obtained if a higher load of the virus could be applied. The inactivation mechanism expressed by this system may be attributed to an inhibition of HEV replication during the post-viral entry process. In summary, the Mirasol PRT system clearly reduced the risk of HEV transmission due to blood transfusion.

Disclosure of Commercial Conflict of Interest

M. Igarashi: No Answer; M. Kaneko: Nothing to disclose; C. Matsumoto: Nothing to disclose; T. Owada: Nothing to disclose; M. Satake: Nothing to disclose; K. Tadokoro: No Answer; S. Uchida: Nothing to disclose

Disclosure of Grants Conflict of Interest

M. Igarashi: No Answer; M. Kaneko: Nothing to disclose; C. Matsumoto: Nothing to disclose; T. Owada: Nothing to disclose; M. Satake: Nothing to disclose; K. Tadokoro: No Answer; S. Uchida: Nothing to disclose

√ SP407

Development of an Effective Prevention Method for T-cell Lymphotropic Virus Type I (HTLV-1) Infection Using HTLV-1 Sero-Positive Serum In Vitro

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Background/Case Studies: Human T-lymphotropic Virus Type I (HTLV-1) is a human RNA retrovirus that causes adult T-cell leukemia. The route of viral transmission is from mother to infant via breast milk. Thus, many countries recommend HTLV-1 sero-positive mothers not to breastfeed. In a Japanese endemic area, Nagasaki, this recommendation was more effective in reducing the number of vertical infections from mother to infant. However, new prevention methods and adequate treatment options apart from preventing HTLV-1 sero-positive mothers from breastfeeding are required. To develop effective prevention methods, immunoglobulin (IgG) was isolated from HTLV-1 sero-positive healthy carriers serologically excluded from blood donation by the Japanese Red Cross, and tested for its ability to prevent cell-to-cell infection. **Study Design/Methods:** An in vitro assay was developed to screen the inhibitory effect of IgGs on HTLV-1 infection using HTLV-1 infected cell lines MT-2 and SLB-1. Secondly, 30 HTLV-1 positive serums were prepared from excluded blood donations by CLEIA (confirmed by immunofluorescence assay in Japan in 2008), according to pro-viral load (PVL) and immunoreactivity to HTLV-1 antigens (gp46, p24, pep180 [gp46 amino acids 180-204]) to assess the prevention of HTLV-1 infection in vitro. **Results/Findings:** An in vitro HTLV-1 infection system was established using HTLV-1 infected cell lines MT2 and SLB-1 with the immortalized T lymphocyte cell line, Jurkat. During co-culture of Mitomycin C treated MT-2 or SLB-1 cells with Jurkat cells, HTLV-1 was successfully transmitted to Jurkat cells. When SLB-1 cells were donors, syncytium formation typical of HTLV-1 infected cells was observed. Next, 30 HTLV-1 sero-positive serums were tested using the co-culture system, and it was demonstrated that HTLV-1 sero-positive serum effectively inhibited HTLV-1 infection from MT-2 or SLB-1 cells to the Jurkat cells ($P < 0.0001$). In addition, syncytium formation was also dramatically inhibited by HTLV-1 sero-positive serum treatment. These effects were more prominent when HTLV-1 sero-positive serum isolated from higher PVL carriers (PVL > 4%) was used. **Conclusion:** An assessment method was successfully developed for in vitro HTLV-1 infection using HTLV-1 infected cell lines. This method may be a powerful tool to screen anti-viral agents in vitro. In addition, it was found that HTLV-1 positive serum taken from asymptomatic donors with high PVL strongly inhibited HTLV-1 infection in the in vitro assay. Hyperimmune globulins are now being prepared from each donor's plasma to identify key epitopes that prevent HTLV-1 infection more effectively, both in vitro and in vivo using a humanized mouse model.

Disclosure of Commercial Conflict of Interest

I. Hamaguchi: Nothing to disclose; M. Kuramitsu: Nothing to disclose; C. Matsumoto: Nothing to disclose; T. Mizukami: Nothing to disclose; K. Nojima: Nothing to disclose; K. Okuma: Nothing to disclose; M. Satake: Nothing to disclose; R. Sobata: Nothing to disclose; K. Tadokoro: No Answer; K. Yamaguchi: Nothing to disclose

Disclosure of Grants Conflict of Interest

I. Hamaguchi: Nothing to disclose; M. Kuramitsu: Nothing to disclose; C. Matsumoto: Nothing to disclose; T. Mizukami: Nothing to disclose; K. Nojima: Nothing to disclose; K. Okuma: Nothing to disclose; M. Satake: Nothing to disclose; R. Sobata: Nothing to disclose; K. Tadokoro: No Answer; K. Yamaguchi: Nothing to disclose

√ SP408

Humana T-Lymphotropic Virus (HTLV)-Screening of blood donors: Follow-Up of Infected Donors for 16 Years

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Background/Case Studies: Blood centers in Brazil began screening blood donors for HTLV antibodies in late 1993 and today these centers routinely screen for HTLV I/II. Infection occurs in certain geographic locations around the world and in Brazil the prevalence in blood donors ranges around 0.46%. The aim of this study was to evaluate the frequency of discarded blood donations over the past 16 years, due to positive HTLV I/II screening tests in

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. 2. 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	Hauser L, Roque-Afonso AM, Beylouné A, Simonet M, Deau Fischer B, Burin des Roziers N, Mallet V, Tiberghien P, Bierling P. Blood. 2014 Jan 30;123(5):796-7. doi: 10.1182/blood-2013-09-524348.	公表国 フランス
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

○Intercept blood systemで処理した血漿製剤の輸血によるE型肝炎感染
同じ供血者からのIntercept法(DNA及びRNAの複製を阻害するためにソラレン化合物であるアモトサレン処理とUVA照射による血液製剤の病原体不活化法)処理血漿によるHEV感染2症例の報告。
1人目の患者は慢性腎不全のため腎移植を受けた36歳男性であり、2012年3～6月に計59ユニットの血液製剤を投与され、HEV RNA及びIgM抗体の検出により2012年10月にE型肝炎と診断された。2人目の患者はアルコール性肝硬変のために肝移植を受けた61歳男性で、計72ユニットの血液製剤が投与され、2013年2月にHEV RNAの検出によりE型肝炎感染が認められた。HEV RNAは臓器移植日までにいずれの患者及び臓器提供者からは検出されていなかったが、輸血されたIntercept処理新鮮凍結血漿(FFP)において検出された。更なる調査で、原因とされたFFPは分割される前にアモトサレン/UVA処理された同じアフェレーシス供血に起因することが明らかとなった。FFP受血者2人と当該供血者はHEVジェノタイプ3株に感染しており、オープンリーディングフレーム(ORF)1及びORF2領域の部分的な配列において相同性を示した。
Intercept処理FFPを介した上記HEV感染は、Intercept病原体不活化技術に対するHEVの抵抗性を立証する。なお、フランスではHEVスクリーニングについて検討を開始している。

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
Intercept blood systemで病原体不活化処理された血漿製剤を輸血された患者2人に輸血HEV感染が発生したとの報告である。	日本赤十字社では、厚生労働科学研究「経口感染によるウイルス性肝炎(A型及びE型)の感染防止、病態解明、遺伝的多様性及び治療に関する研究」班と共同して、献血者におけるHEV感染の疫学調査を行っている。今後もHEV感染の実態に関する情報の収集及び安全対策に努める。

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To the editor:

Hepatitis E transmission by transfusion of Intercept blood system–treated plasma

Hepatitis E virus (HEV) is a small nonenveloped RNA virus usually transmitted by the enteric route, although transmission by blood transfusion has also been reported.[1-3] HEV infection usually leads to benign acute hepatitis. It can sometimes be fulminant, particularly among pregnant women and patients with preexisting liver disease, or evolve to a chronic state, especially in immunosuppressed subjects.[1,4]

Pathogen reduction (PR) of blood products (BPs) has demonstrated its effectiveness with regard to a large number of pathogens.[5,6] Among PR methods, the Intercept method combines a synthetic psoralene amotosalen HCl treatment with ultraviolet A (UVA) light illumination to block the replication of DNA and RNA.

We report 2 cases of HEV transmission by 2 units of Intercept-treated plasma originating from the same donor. The first patient is a 36-year-old man with chronic renal failure. He underwent a kidney transplantation, which was followed by acute humoral rejection and was treated by plasma exchanges from March/June 2012, during which 59 BPs were transfused. Liver cytolysis was observed since June 2012. The diagnosis of hepatitis E was reached in October 2012 with detectable HEV RNA and weakly reactive anti-HEV immunoglobulin M. As of June 2013, the patient remained viremic, and ribavirin was introduced. HEV RNA was undetectable on the day of transplantation, as well as on the graft donor, but was detected in apheresis donation leading to transfused Intercept-treated fresh frozen plasma (FFP). The second patient is a 61-year-old man who underwent a liver transplantation for alcoholic liver cirrhosis in August 2012. Hepatitis E infection was detected in February 2013 with detectable HEV RNA and negative HEV serology. As of April 2013, the patient remained viremic, and ribavirin was introduced. He had received 72 BPs; HEV RNA was undetectable on the day of transplantation and in the graft donor but was detected in apheresis donation leading to transfused Intercept-treated FFP. All other blood donations for these patients tested negative for HEV RNA (using cryopreserved plasma samples collected on donation day). Further investigations revealed that the incriminated FFPs resulted from the same apheresis donation that was amotosalen/UVA light treated before segmentation in 3 units. Two of the 3 units were transfused to the 2 patients described above; the third was transfused to a patient who died 2 days following transfusion. The 2 FFP recipients and the donor were infected by a genotype 3f strain presenting a strict homology on partial sequences of the open reading frame 1 (ORF1) and ORF2 regions as previously described (Figure 1).[7] Such strain identity demonstrates that both amotosalen and UVA light-treated FFPs provided by a unique donor transmitted HEV to ≥2 transfusion recipients. The involved donor was a 32-year-old woman who did not reveal any factor that could suggest that she was infected by HEV during the period of donation.

Such novel HEV transmission through Intercept-treated FFP establishes resistance of HEV to Intercept PR technology. Nonenveloped viruses such as HEV are also known to be resistant to solvent/detergent treatment. In vitro, assessment has established that hepatitis A virus, a similar nonenveloped virus, as well as feline calicivirus, a model for HEV, are poorly sensitive to amotosalen/UVA light.[5,6] Hepatitis E has recently emerged as a significant cause of transfusion-induced viral hepatitis. Generalizing HEV screening for all blood donations, or alternatively for a fraction of BPs to be transfused in high-risk patients, is being considered in France.

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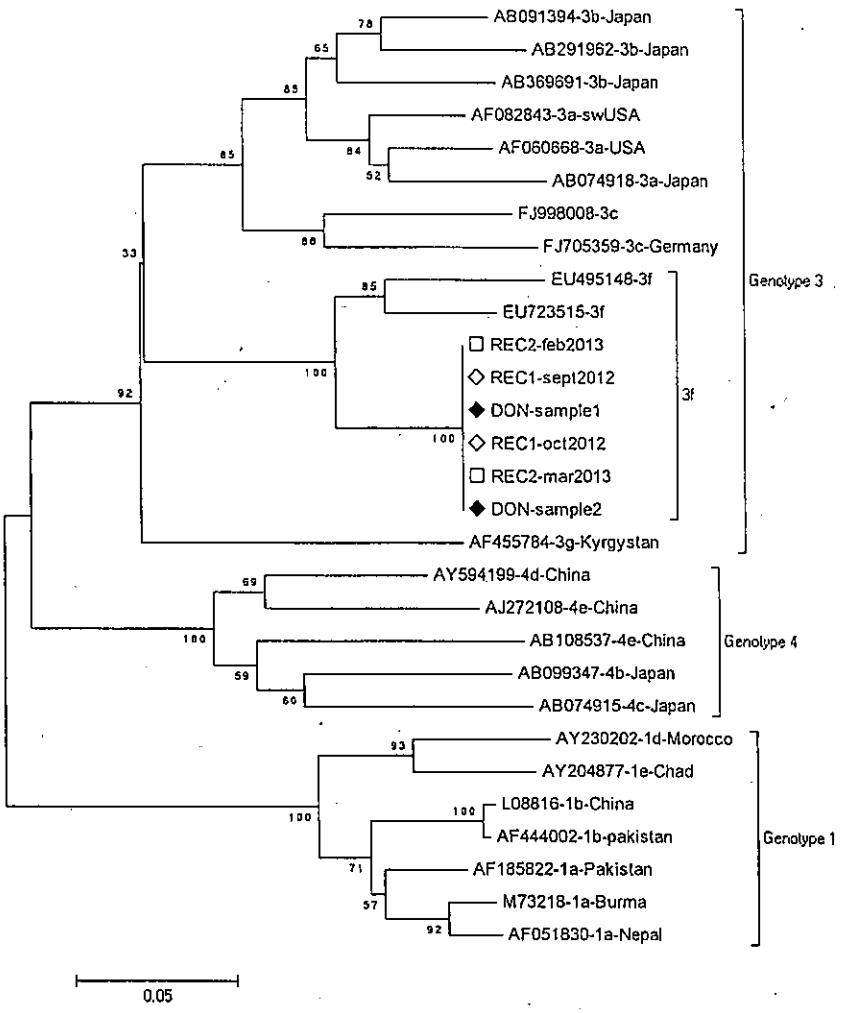

Figure 1. Phylogenetic analysis of partial ORF1 sequence. Phylogenetic tree was constructed on the MEGA4 software using the neighbor-joining method from a Kimura 2-parameter distance matrix based on partial nucleotide sequences of ORF1 encoding for RdRp, as previously described (4). Bootstrap values obtained from 500 resamplings are shown. A 100% homology is observed between blood donor sequences obtained from 2 aliquots of the same donation (DON sample 1 and sample 2) and recipient's sequences (REC1 and REC2) obtained from 2 different samples. GenBank reference sequences are indicated by their accession number.

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別紙様式第2-1

No. 5

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. 2. 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	Féray C, Pawlotsky JM, Roque-Afonso AM, Samuel D, Dhumeaux D. Lancet. 2014 Jan 18;383(9913):218. doi: 10.1016/S0140-6736(14)60072-6.	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)			フランス

研究報告の概要

○血液製剤にHEV RNA検査を実施すべきか？

近年、肝硬変や神経学的合併症を引き起こす慢性E型肝炎感染症が、西欧諸国の免疫不全患者に確認された。慢性E型肝炎は、臓器移植や造血幹細胞移植を受けた患者、化学療法後の患者、HIV感染者やステロイドが投与されている患者において報告されている。

スウェーデンとドイツではそれぞれ1/7986及び1/4525の血漿供血がHEV RNA陽性であり、またドイツの血漿分画製剤のプール血漿のうち10%がHEV RNA陽性であった。2012年以降、輸血を介した慢性E型肝炎5症例(367例の移植中)がフランスの2カ所の医療機関から報告された。

E型肝炎検査未実施の血液製剤の輸血は、免疫不全患者において慢性E型肝炎のリスクに関連する。E型肝炎流行国において、潜在的供血者である一般集団の罹患率及び免疫不全状態患者における症状の重篤性から考えると、E型肝炎感染マーカーについて血液製剤の体系的なスクリーニング(核酸検査)を行わなくてはならないだろう。

報告企業の意見	今後の対応
近年、免疫不全患者において慢性E型肝炎が報告されており、潜在的供血者である一般集団のE型肝炎罹患率や免疫不全状態患者におけるE型肝炎症状の重篤性を考慮すると、血液製剤における体系的なHEVスクリーニング検査が必要であるとの報告である。	日本赤十字社では、厚生労働科学研究「経口感染によるウイルス性肝炎(A型及びE型)の感染防止、病態解明、遺伝的多様性及び治療に関する研究」班と共同して、献血者におけるHEV感染の疫学調査を行っている。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。

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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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

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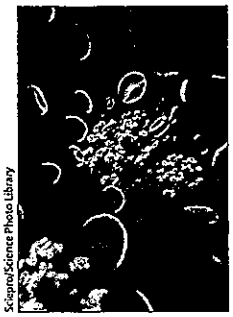

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Should we screen blood products for hepatitis E virus RNA?

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Hepatitis E was first identified during an outbreak of acute hepatitis in the Kashmir Valley in 1978. The causal agent, an enterically-transmitted non-enveloped RNA virus, was identified in developing countries where the most prevalent genotypes are genotypes 1 and 2.[1] Hepatitis E virus is endemic in many developing countries; animals such as swine, boar, and deer are reservoirs of hepatitis E virus genotypes 3 and 4. Human infections occur after ingestion of undercooked meat or liver from infected animals. Very high seroprevalences, up to 16%, have been described in some regions.[2]

Recently, chronic hepatitis E infections leading to cirrhosis or neurological complications were described in patients with immunodeficiencies in west European countries.[3] Chronic hepatitis E infection has been described in patients receiving liver, kidney, pancreas, lung, or heart transplants, after haemopoietic stem cell transplantation, after chemotherapy, in HIV patients, and in patients taking steroids.[3]

Importantly, hepatitis E virus can be transmitted by blood-derived products. A high prevalence of IgM against hepatitis E virus was observed after transfusions in Asia and in European countries. In Sweden and Germany 1 of 7986 and 1 of 4525 plasma donations tested positive for hepatitis E virus RNA, respectively. 10% of plasma pools tested positive for HEV RNA in Germany.[2]

Since 2012, five cases of chronic hepatitis E transmitted through blood transfusions were diagnosed (out of 367 transplantations) in the Paul Brousse Centre (Villejuif, France)[4] and Créteil liver transplant centre (Créteil, France). Treatment of chronic hepatitis E infection in liver transplant recipients is decreasing immunosuppression and ribavirin. In these patients, eradication of hepatitis E is not always obtained by antiviral drugs, and substantial liver damage might persist, even after viral clearance.

Transfusion of blood products not screened for hepatitis E is associated with a risk of chronic hepatitis E infection in immunocompromised patients. Two recombinant hepatitis E vaccines have successfully gone through phase 3 trials,[5] but they are not yet available and their efficacy on hepatitis E genotype 3 is unknown.

In view of the prevalence of hepatitis E infection in the general population (and therefore in potential blood donors) and the severe consequences of hepatitis E infection in immunocompromised patients, we believe that systematic screening of blood products for markers of hepatitis E infection should be implemented in countries where hepatitis E is endemic, including Germany, Sweden, and France. Because serological testing is poorly sensitive, hepatitis E nucleic acid testing should be considered.

We declare that we have no conflicts of interest.

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Department of Error

Tyrer P, Cooper S, Salkovskis P, et al. Clinical and cost-effectiveness of cognitive behaviour therapy for health anxiety in medical patients: a multicentre randomised controlled trial. Lancet 2014; 383: 219–25—In figure 1 of this Article, the number of patients who declined to participate or did not complete baseline assessments should have been 3935. The figure has been reformatted for clarification. This correction has been made to the online version as of Oct 22, 2013, and to the printed Article.

Feigin VL, Forouzanfar MH, Krishnamurthi R, et al, on behalf of the Global Burden of Diseases, Injuries, and Risk Factors Study 2010 (GBD 2010) and the GBD Stroke Experts Group. Global and regional burden of stroke during 1990–2010: findings from the Global Burden of Disease Study 2010. Lancet 2014; 383: 245–55—In this Article, on line 7 of the Findings section of the Summary, the sentence should have read "...had significantly increased since 1990 (68%, 84%, 26%, and 12% increase, respectively)". This correction has been made to the online version as of Oct 25, 2013, and to the printed Article.

Jamison DT, Summers LH, Alleyne G, et al. Global health 2035: a world converging within a generation. Lancet 2013; 382: 1898–955—In this Commission (Dec 7, 2013), Karen H Ulltveit-Moe's name was spelt incorrectly. In the second paragraph of the section "Macroeconomics studies", the following phrase should have read "...although health improvements do lead to income growth, they also lead to more than compensatory increases in fertility..." In table 10, in column 2, row 3, the text should have read "bans on transfats and regulation of salt in processed food". In figure 16, the text label in the bottom right dark blue box should have read "expanded cancer package". These corrections have been made to the online version as of Jan 17, 2014.

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No. 6

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
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一般的名称	新鮮凍結人血漿	研究報告の公表状況	Hyams C, Mabayoje DA, Copping R, Maranao D, Patel M, Labbett W, Haque T, Webster DP. J Med Virol. 2014 Mar;86(3):478-83. doi: 10.1002/jmv.23827. Epub 2013 Nov 8.	公表国 英国
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

○サイトメガロウイルス（CMV）及びエプスタインバーウイルス（EBV）との交差反応は急性E型肝炎ウイルス（HEV）感染の診断の障害となる

HEV感染はウイルス血症期間が短く、急性感染ではHEV IgM抗体とIgG抗体は同時に上昇する。これまでの研究でHEVは、EBV、CMVとの交差反応によりHEV IgM抗体の偽陽性反応が発生することが報告されており、A型肝炎ウイルス（HAV）との交差反応も示唆されている。

ロンドンの三次医療機関で、3年間にわたるHEV血清学的検査の後方視的分析が行われ、血清サンプル1,423例中HEV IgM抗体33例が陽性、28例が不確定であった。111例がHEV IgG抗体陽性かつIgM抗体陰性であり、過去の感染を示唆していた。HEV IgM抗体陽性患者はいずれもHAVに対する偽陽性反応を示さなかったが、EBV及びCMVとの交差反応は頻繁に確認され、HEV IgM抗体陽性サンプルの33.3%及び24.2%がそれぞれEBV、CMV IgM抗体陽性となった。HEV IgM抗体陽性サンプルのうち13.3%のみがHEV PCRでも陽性になると予測され、血清学的検査の陽性的中率の低さを強く示している。ウイルス性肝炎の診断は、臨床的特徴、トランスアミナーゼ値の上昇、血清学的検査、PCRによる確認検査に基づいて行うべきである。

使用上の注意記載状況・その他参考事項等

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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
血清学的検査においてHEVとEBV、CMV IgM抗体の交差反応性が示されたとの報告である。	日本赤十字社では、厚生労働科学研究「経口感染によるウイルス性肝炎（A型及びE型）の感染防止、病態解明、遺伝的多様性及び治療に関する研究」班と共同して、献血者におけるHEV感染の疫学調査を行っている。今後もHEV感染の実態に関する情報の収集及び安全対策に努める。

Journal of Medical Virology 86:478–483 (2014)

Serological Cross Reactivity to CMV and EBV Causes Problems in the Diagnosis of Acute Hepatitis E Virus Infection

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Hepatitis E virus (HEV) infection is an important public health concern as a major cause of enterically-transmitted hepatitis worldwide. The detectable window of viraemia is narrow, and HEV IgM and IgG rise simultaneously in acute infection. Furthermore, previous investigators have shown HEV IgM false positive reactions occur against EBV, CMV and potentially hepatitis A. A retrospective analysis of HEV serology testing was performed at a London tertiary referral hospital over a 3-year period. A thousand four hundred and twenty three serum samples were tested for HEV serology, with 33 samples HEV IgM positive and 28 HEV IgM equivocal. One hundred and eleven samples were HEV IgG positive but IgM negative suggesting past infection. No patients with HEV IgM positivity had false positive reactions against hepatitis A. A high degree of EBV and CMV cross reactivity was noted, with 33.3% and 24.2% of HEV IgM positive samples also testing positive for EBV and CMV IgM, respectively. HEV RNA was detected in four HEV IgM positive samples, indicating true positivity, although three demonstrated cross reactivity against EBV. Only 13.3% of samples with positive HEV IgM were HEV PCR positive, highlighting a low positive predictive value of serology testing. Overall a high level of HEV, EBV and CMV IgM cross reactivity was demonstrated, indicating that serology is unreliable in the diagnosis of acute viral hepatitis. It is concluded that that the diagnosis of viral hepatitis should be based on clinical features, raised transaminases, serology, and confirmatory PCR testing. ***J. Med. Virol. 86:478–483, 2014.*** © 2013 Wiley Periodicals, Inc.

KEY WORDS: hepatitis E virus; Epstein–Barr virus; cytomegalovirus; serology

INTRODUCTION

Hepatitis E virus (Family *Hepeviridae*, genus *Hepevirus*, species *Hepatitis E virus*) infection is an important public health concern as a major cause of enterically transmitted hepatitis worldwide. Transmission of Hepatitis E virus (HEV) is usually waterborne via the feco-oral route, however, the mammalian virus has a reservoir in pigs and zoonotic foodborne transmission of genotype-3 virus has been shown [Meng et al., 1998; Aggarwal, 2010]. It is endemic in developing countries with poor sanitation and public health structures, accounting for over 50% of acute viral hepatitis [Dalton et al., 2008a; Aggarwal, 2010]. In developed countries, HEV was traditionally considered an imported infection [Schwartz et al., 1999], but with newer molecular tests and increased surveillance, autochthonous (locally-acquired) infections have been identified, establishing HEV as an important clinical problem [Ijaz et al., 2005; Dalton et al., 2008a]. While there are four genotypes of hepatitis E, genotype 1 is usually seen in developing countries while genotype 3 is usually seen in developed countries and does not generally lead to epidemics. Genotype 4 is mainly found in Asia, with swine believed to be an important reservoir of infection [Meng et al., 1998; Schwartz et al., 1999; Ijaz et al., 2005; Lu et al., 2006; Dalton et al., 2008a; Aggarwal, 2010]. The clinical manifestations vary from asymptomatic infection to uncomplicated acute viral hepatitis and fulminant hepatic failure. The disease pathogenesis and reason for

Conflict of interest: none.

All authors contributed to the preparation of this manuscript.

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varying disease severity largely remain unknown [Srivastava et al., 2011]. Prognosis can be poor in individuals with underlying chronic liver disease, with mortality approaching 70%, as well as during pregnancy [Dalton et al., 2007; Peron et al., 2007].

In developed countries, HEV seroprevalence is estimated to be 4–25% [Aggarwal and Naik, 1997; Dalton et al., 2008a]. In England, a prevalence of 13.0% in 1991 and 13.5% in 2004 has been found [Ijaz et al., 2009]. However, acute HEV infection incidence is low, raising questions of HEV underdiagnosis, clinically silent presentations or non-specific HEV IgG testing. Diagnosis is based on combining clinical features, elevated liver transaminases, serology, and specific HEV PCR. While various protein constructs have been used in diagnostic assays to detect HEV specific antibodies, the major antigenic target remains the ORF2-encoded protein [Khudyakov and Kamili, 2011]. All four HEV genotypes arise from a single serotype, therefore diagnostic antigens from a single HEV genotype should detect antibody against HEV strains from different genotypes [Emerson and Purcell, 2003]. A classic serological pattern of antibody response to HEV has been found, in which anti-HEV IgM appears during acute illness, is detectable 4 days after the onset of jaundice and persists for up to 5 months [Favorov et al., 1992], with strongly positive reactions rare after 3 months [Dalton et al., 2008b]. Over 90% of HEV infected patients have detectable anti-HEV IgM within 2 weeks of illness onset [Favorov et al., 1992]. The serological appearance of anti-HEV IgM is followed shortly by anti-HEV IgG, so that both may appear to develop simultaneously in acute infection, thereby complicating the serological diagnosis of acute HEV infection. However, HEV IgG persists for longer than anti-HEV IgM and is detectable 1–14 years post infection [Dawson et al., 1992; Khuroo et al., 1993; Bryan et al., 1994]. HEV RNA can be detected in serum using RT-PCR but may be of reduced diagnostic value due to a brief 28-day viraemia period (range 17–48 days) after the onset of symptoms [Dalton et al., 2008b].

Serological testing is hindered by the potential inability to detect HEV IgM and in addition false-positive results have been reported to occur in Herpesvirus infections including Epstein–Barr virus (EBV; Family *Herpesviridae*, genus *Lymphocryptovirus*, species *Human Herpesvirus 4*) and Cytomegalovirus (CMV; Family *Herpesviridae*, genus *Cytomegalovirus*, species *Human cytomegalovirus*) [Fogeda et al., 2009]. Furthermore, potential false-positive reactions have been described in hepatitis A virus (HAV; Family *Picornaviridae*, genus *Hepatovirus*, species *Hepatitis A virus*) infection [Gray et al., 1992]. HEV IgM cross-reactivity between HAV, EBV and CMV is clinically problematic as these viruses form the differential diagnosis for acute hepatitis in such patients. HEV serology testing at a large tertiary referral hospital was therefore evaluated, processing samples from both hospitalized and primary care patients.

MATERIALS AND METHODS

A retrospective analysis over a 3-year period (July 2009 to August 2012) of patients who underwent HEV serology testing at a large tertiary referral hospital was performed. Serum samples were reflexly tested for HEV serology if the patient serum tested negative for hepatitis A, B and C in the presence of elevated transaminases (ALT > 80 IU/L). Alternatively, patients were also tested under clinical discretion on the basis of a significant travel history or if HEV testing was specifically requested by clinicians. Patients who had undergone laboratory HEV IgM/IgG testing were identified using the pathology patient database.

HEV serology testing was performed using *recom*Well HEV IgM and *recom*Well HEV IgG enzyme immunoassay (MIKROGEN Diagnostik, Neuried, Germany), with equivocal and positive results confirmed using *recom*Line HEV IgG/IgM immunoblot (MIKROGEN Diagnostik). In cases where there was discordance between *recom*Well HEV IgM/HEV IgG and confirmatory *recom*Line HEV IgG/IgM immunoblot testing, results from the latter assay superseded the initial result due to the fact that the immunoblot testing has a higher sensitivity and specificity. HEV IgM positive was classed as >24 U/ml, equivocal as between ≥20 and ≤24 U/ml, and negative being <20 U/ml. EBV IgM EIA was performed on the Liaison platform (DiaSorin, Saluggia, Vicenza, Italy), while HAV and CMV IgM was analysed using EIA on the Abbott Architect i2000SR (Abbott Park, IL). HAV IgM positive was defined as >1.6 U/ml, equivocal ≥0.8 and ≤1.6 U/ml, with negative HAV IgM being <0.8 U/ml. EBV IgM positive was defined as >40 U/ml, equivocal ≥20 and ≤40 U/ml, and negative EBV IgM <20 U/ml. CMV IgM positive was defined as >1.7 U/ml, equivocal ≥0.85 and ≤1.7 U/ml, with negative CMV IgM <0.85 U/ml. Samples which were found to test positive for HEV IgM or for EBV IgM from individuals with elevated transaminases during the last 12 months of the study underwent further serology testing in a reference laboratory at the Health Protection Association at Colindale, using Wantai ELISA (Fortress Diagnostics, Antrim, UK) to evaluate the performance of the laboratory assay.

An in-house reverse transcription polymerase chain reaction (RT-PCR) testing for HEV was started midway through the study period, using Superscript III RT PCR (Invitrogen, Carlsbad, CA) and HEV forward primer GCC CGG TCA GCC GTC TGG, reverse primer CTG AGA ATC AAC CCG GTC AC, HEV probe FAM-CGG TTC CGG TGG TTT CT-TAMRA. Statistical analysis was performed using GraphPad Software Inc. (San Diego, CA).

RESULTS

Overall, 1,423 serum samples were tested for HEV IgG and IgM. Of these, 33 (2.3%) samples were IgM

positive and 28 (1.9%) samples were equivocal for IgM. In contrast, 133(9.3%) samples were IgG positive and 13 (0.9%) samples were equivocal for HEV IgG. Of the IgG positive samples, 111(83.4%) were negative for IgM, suggesting previous exposure to hepatitis E (Table I) and giving an overall population seroprevalence of 7.8% (111/1,423). About 39.3% of patients with serum which was positive for HEV IgM also had serum which tested positive for HEV IgG (13/33). Of the 17 HEV IgM positive samples which were HEV PCR tested concurrently, four (23.5%) individuals had HEV infection confirmed by PCR. Of note, all of these individuals had significantly deranged liver function tests, with levels of transaminases being at least five times greater than the upper limit of normal. Additionally, a relevant travel history was documented in three out of four of these patients.

Hepatitis A virus (HAV), EBV, and CMV IgM reactivity in patients with reactive HEV IgM serum was investigated. There were no patients who had reactive HEV IgM who simultaneously demonstrated reactivity to HAV IgM laboratory testing (Table II). EBV and CMV IgM testing were not performed in individuals who had previously tested EBV EBNA IgG or CMV IgG positive. Overall a high degree of EBV and CMV IgM cross-reactivity to HEV IgM was demonstrated, with 33.3% (11/33) and 24.2% (8/33) of samples which were HEV IgM positive also showing reactivity to EBV and CMV, respectively (Table II). Similar cross reactivity levels were found in samples which were HEV IgM equivocal, 32.1% (9/28) demonstrated EBV IgM cross-reactivity and 17.9% (5/28) demonstrating CMV IgM cross-reactivity on laboratory testing (Table II).

HEV IgM serology was found to predict HEV PCR positivity in 13.3% of cases, highlighting the unreliability of HEV IgM testing. HAV IgM cross-reactivity with the HEV IgM EIA assay was not demonstrated, suggesting that false positives reactions using immunofluorescence testing may not be relevant using EIA analysis.

Given the high rates of cross reactivity that were found, all patient samples which had tested negative for EBV VCA IgG and EBNA IgG but positive for EBV IgM over the preceding year were identified. Fifteen samples had concomitant elevated liver enzymes, and these samples were tested for reactivity to CMV and HEV IgM, with HEV PCR also performed on these samples. Fourteen (93.3%) of samples tested positive for CMV IgM, and four (26.7%) for HEV IgM of which all were HEV PCR negative (Table III). Of the four samples which tested positive for both HEV and EBV IgM, three were negative for HEV IgG. No samples which were positive for EBV IgM and had elevated transaminases tested positive using HEV PCR, although this is perhaps not surprising given the fragility of HEV RNA. HEV RNA may have degraded in the ensuing period of time between initial sampling and subsequent PCR testing given that HEV PCR was performed after samples were frozen and defrosted; this would affect the yield of PCR performed retrospectively.

TABLE I. HEV Serology Results

	HEV IgG positive (>24 U/ml)	HEV IgG equivocal (≥20 U/ml, ≤24 U/ml)	HEV IgG negative (<20 U/ml)	Total
HEV IgM positive (>24 U/ml)	13 (10%)	1 (7.7%)	19 (1.5%)	33
HEV IgM equivocal (≥20 U/ml, ≤24 U/ml)	9 (6.9%)	2 (15.4%)	17 (1.3%)	28
HEV IgM negative (≥20 U/ml)	111(83.4%)	10 (76.9%)	1,241 (97.2%)	1,362
Total	133	13	1,277	1,423

Percentages given represent the proportion of samples based on HEV IgG serology result.

TABLE II. Cross Reactivity of HEV IgM Reactive Samples to HAV, EBV, and CMV IgM Assays

	HEV IgM positive			HEV IgM equivocal		
	HAV	EBV	CMV	HAV	EBV	CMV
IgM positive	0	9 (26.5%)	7 (21.2%)	0	3 (10.7%)	4 (14.3%)
IgM equivocal	0	2 (6.0%)	1 (3.0%)	0	6 (21.4%)	1 (3.6%)
IgM negative	0	12 (35.3%)	16 (48.4%)	0	7 (25.0%)	11 (39.3%)
IgM not tested	0	10 (30.3%)	9 (26.5%)	0	12 (42.9%)	12 (42.9%)
Totals	0	33	33	0	28	28

HAV IgM positive was defined as >1.6 U/ml, equivocal ≥0.8 and ≤1.6 U/ml, with negative HAV IgM being <0.8 U/ml. EBV IgM positive was defined as >40 U/ml, equivocal ≥20 and ≤40 U/ml, and negative EBV IgM <20 U/ml. CMV IgM positive was defined as >1.7 U/ml, equivocal ≥0.85 and ≤1.7 U/ml, with negative CMV IgM <0.85 U/ml. HEV IgM positive was classed as >24U/ml, with equivocal results being ≥20 and ≤24 U/ml.

TABLE III. Cross Reactivity of Samples That Were EBV IgM Positive With Elevated Liver Enzymes Against HEV and CMV IgG

	CMV IgG	HEV IgG
IgG positive	1 (6.7%)	4 (26.7%)
IgG equivocal	5 (33.3%)	0
IgG negative	9 (60%)	11 (73.3%)
Total	15	15
HEV PCR Positive	—	0

HEV IgM positive was classed as >24 U/ml, equivocal as ≥20 and ≤24 U/ml, and negative being <20 U/ml. CMV IgM positive was defined as >1.7 U/ml, equivocal ≥0.85 and ≤1.7 U/ml, with negative CMV IgM <0.85 U/ml.

Although there is no current universally accepted "gold standard" for hepatitis E serology testing, in order to demonstrate the reproducibility of the HEV serology testing performed at the investigating center, samples were sent to a reference laboratory for further HEV serology profiling. The reference laboratory in question employed the use of a Wantai ELISA which has been compared to Western blots and other commercially available assays in population-based studies and has been found to be highly sensitive. [Zhang et al., 2003; Li et al., 2006; Bendall et al., 2010]. Zhang et al. [2003] propose that the peptides used in the Wantai ELISA may form dimers which have a stronger reaction with HEV-reactive sera than the monomeric antigens used in other assays, thereby conferring an increased sensitivity. Samples which tested negative for both HEV IgM and IgG demonstrated 100% agreement between the original laboratory testing result and the reference laboratory result. However, it was found that two samples which tested positive for HEV IgM (22.2%) and four which tested positive for HEV IgG (40%) tested negative using the reference laboratory assay. As mentioned in the methods, all samples underwent HEV serology testing with enzyme immunoassay testing which was then confirmed using immunoblot testing if initial testing demonstrated an equivocal or positive result. Of 269 samples that underwent confirmatory testing for HEV IgM or IgG antibody 13 (4.8%) had discordant results between the initial enzyme immunoassay testing and confirmatory immunoblot testing, indicating a rate of concordance of 95.2% between the initial and confirmatory assays used in this study.

It was then investigated whether elevated transaminases were more likely to be found in patients who had HEV IgM reactive serum. There was no statistical difference in the value of transaminases between patients who were strongly reactive or those whose serum tested equivocal. However, higher levels of both ALT and AST were more likely to be found in patients whose serum was reactive for both HEV IgM and IgG in comparison to patients with serum which was reactive for only HEV IgM (Fig. 1). Patients who tested positive for both HEV IgM and IgG had a median ALT of 446 U/L and AST of 205 U/L, whereas individuals whose serum was reactive only to HEV IgM had a median ALT of 84 U/L and AST of 66 U/L.

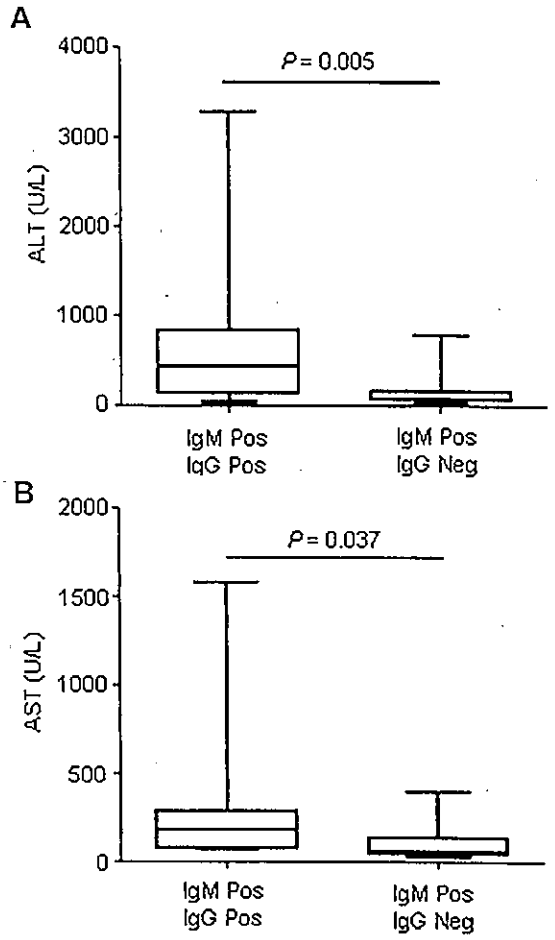

Fig. 1. Level of transaminases detected in patient serum Levels of (A) ALT and (B) AST recorded at the time of presentation in patients who tested positive for HEV IgM and IgG in comparison to patients who tested positive for HEV IgM alone. -values represent the results of Mann–Whitney *U* tests.

DISCUSSION

Hepatitis E is both an imported infection and an autochthonous disease in developed countries [Aggarwal and Naik, 1997; Schwartz et al., 1999; Ijaz et al., 2005; Dalton et al., 2008a]. Diagnosis is based upon combining clinical features with laboratory testing, which includes identifying elevated transaminases, testing for specific HEV IgM in serum or by detecting HEV genome sequences by RT-PCR. Performing HEV RNA testing is generally unavailable in most hospitals worldwide, and hence the accuracy of laboratory-based diagnosis depends on the specificity of enzyme-immunoassay or recombinant immunoblot against HEV IgM.

Previous investigators have shown that acute infection with EBV and CMV cause false reactivity to anti-HEV IgM in laboratory tests [Fogeda et al., 2009; Ghinoiu et al., 2009]. This study demonstrates a similar level of cross-reactivity against EBV IgM as that found by Fogeda et al. [2009], although it detected a significantly higher level of false-positive reactions with CMV IgM. Even with samples reactive enough to be termed positive for HEV IgM, it was found that 33.3% of samples also tested positive for EBV IgM and 24.2% for CMV IgM. Since all four genotypes of HEV belong to a single serotype, diagnostic antigens from a single serotype should detect antibody against HEV strains of different genotypes. Proteins derived from serotype 1 and 2 may be differently immunoreactive with sera from anti-HEV positive serum [Dawson et al., 1992], indicating that the variation in protein structure of HEV strains does indeed affect detection of anti-HEV activity in serum samples [Khudyakov and Kamili, 2011]. Differences in HEV strain and therefore sequence heterogeneity may account for the difference in results between this study and that of previous researchers [Fogeda et al., 2009; Ghinoiu et al., 2009].

This study found a level of cross-reactivity in serology testing for HEV, CMV, and EBV specific IgM that is concerning, given that in a jaundiced patient the presence of anti-HEV IgM along with rising titres of anti-HEV IgG would be considered diagnostic of acute HEV infection. Furthermore, polyclonal stimulation of memory B-cell clones and immunological cross-reactivity are known problems for the diagnosis of acute viral disease involving human herpes viruses [Karner and Bauer, 1994; Thomas et al., 1999]. This would be expected to be clinically relevant, given that HEV, CMV, and EBV constitute a large component of the differential diagnosis of acute viral hepatitis once HAV is excluded. Since liver involvement is not rare in EBV and CMV infection, HEV-induced acute hepatitis may be incorrectly diagnosed as being due to CMV or EBV infection if the diagnosis is not confirmed by molecular tests. Given the narrow period of viraemia in HEV infection, the diagnostic value of RT-PCR against HEV RNA is reduced, and this may further compound the difficulty in making an accurate laboratory diagnosis. Additionally, of the four samples which tested HEV positive using PCR, three were reactive for EBV IgM, demonstrating that HEV infection can result in false positive acute EBV serology. Hence EBV and possibly CMV serology alone may result in missed instances of HEV infection. This may provide an explanation for the high seroprevalence of HEV IgG but low clinical incidence of HEV infection, with cases of HEV being misdiagnosed as EBV or CMV infection, in addition to cases of subclinical infection.

Antibody avidity has been used in the diagnosis of many infections including hepatitis E [Prince and Wilson, 2001; Zhang et al., 2002; Kneitz et al., 2004]. Low avidity IgG antibodies are usually produced early in primary infection, and are then replaced by IgG antibodies with a higher avidity during convalescence. Antibodies produced during re-infection are typically of high avidity. IgG avidity could therefore be of use in determining the cause of an acute hepatitis in a patient who had serum which tested negative for HEV IgM, or which tested positive against multiple viruses. Antibody avidity testing was not performed on samples that tested positive for HEV, CMV, or EBV IgG. Previous evaluations of serological assays specific to IgM antibodies against HEV have been performed, some using serum samples from patients infected from different HEV genotypes [Seriwatana et al., 2002; Yu et al., 2003; Drobeniuc et al., 2010.]. Different immunoassays have been shown to have varying diagnostic sensitivity values [Drobeniuc et al., 2010]. When compared with the assay used at a UK reference laboratory, the local assay demonstrated a strong negative predictive value, with no discordance seen in negative samples. However a discordance rate of 22.2% was found for HEV IgM positive and 40% for HEV IgG positive samples when taking the reference laboratory assay into account, raising the possibility that these results were false positive. It is important to note that although the assay used by the reference laboratory is known to have a high specificity and sensitivity for HEV IgG in population-wide seroprevalence studies, its applicability as a reference test for diagnosis of acute hepatitis E is less clear [Zhang et al., 2003; Li et al., 2006; Bendall et al., 2010]. This result is in keeping with those of previous investigators who have demonstrated that there is some variability in the HEV positivity rates found with different serological tests [Seriwatana et al., 2002; Yu et al., 2003; Drobeniuc et al., 2010.]. These results highlight the need for standardisation of HEV serological diagnosis in light of the various commercial assays in use.

A statistically significant elevation of both ALT ($=0.0005$) and AST ($=0.037$) was demonstrated in individuals who tested positive for both HEV IgM and IgG in comparison to those who tested positive for HEV IgM alone. The median ALT level found in patients testing positive for HEV IgM and IgG was five times greater than those who tested positive for IgM alone, with AST being three times greater. This suggests that individuals who test positive for HEV IgM and IgG are more likely to have a significant increase in their level of serum transaminases, representing a more severe hepatitis. It is therefore suggested that HEV serology testing should be interpreted in combination with elevation of transaminases in patients with suspected HEV hepatitis.

In conclusion HEV IgM serology was found to be of limited value given high false-positive reaction rates with EBV and CMV IgM, low levels of associated HEV PCR confirmed infections, and the inability of HEV IgG to exclude acute infection. One approach

would be performing EBV, CMV, and HEV PCR to confirm positive serology in all patients with possible viral hepatitis. This may be limited by the additional cost of performing PCR, and given the self-limiting nature of CMV, EBV, and HEV infection PCR testing would not necessarily alter clinical outcome. Overall the authors suggest that HEV diagnosis remains a significant virological challenge, and should combine clinical features, travel history, elevated liver enzymes, serology, and PCR analysis where appropriate.

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一般的名称	①②③⑩⑪pH4 処理酸性人免疫グロブリン ④⑤⑥⑦⑧⑨ポリエチレングリコール処理人免疫グロブリン ⑫⑬人免疫グロブリン	研究報告の公表状況	Emerging Infectious Diseases・www.cdc.gov/eid 2014; 20(1): 114-117	公表国 フランス; スペイン; ドイツ
販売名 （企業名）	①日赤ポリグロビン N5%静注 0.5g/10mL（日本血液製剤機構） ②日赤ポリグロビン N5%静注 2.5g/50mL（日本血液製剤機構） ③日赤ポリグロビン N5%静注 5g/100mL（日本血液製剤機構） ④献血ヴェノグロブリン IH5%静注 0.5g/10mL（日本血液製剤機構） ⑤献血ヴェノグロブリン IH5%静注 1g/20mL（日本血液製剤機構） ⑥献血ヴェノグロブリン IH5%静注 2.5g/50mL（日本血液製剤機構） ⑦献血ヴェノグロブリン IH5%静注 5g/100mL（日本血液製剤機構） ⑧献血ヴェノグロブリン IH5%静注 10g/200mL（日本血液製剤機構） ⑨献血ヴェノグロブリン-IH ヨシトミ（日本血液製剤機構） ⑩日赤ポリグロビン N10%静注 5g/50mL（日本血液製剤機構） ⑪日赤ポリグロビン N10%静注 10g/100mL（日本血液製剤機構） ⑫グロブリン筋注 450mg/3mL「ベネシス」（日本血液製剤機構） ⑬グロブリン筋注 1500mg/10mL「ベネシス」（日本血液製剤機構）			

研究報告の概要

ヒトのクロイツフェルト･ヤコブ病（CJD）の発症率は低く（年間 100 万人当たり 1 人程度）、孤発性（sCJD）または家族性/遺伝性（fCJD）がある。家族性 CJD はプリオン蛋白遺伝子（Prnp）の変異が関与しているが、孤発性 CJD の明確な疫学リスク因子は特定されていない。sCJD は臨床的および神経病理的に一律的な表現型を示す疾患ではない。sCJD は、プロテアーゼ耐性プリオン蛋白（PrP）配列のコドン 129 の多型（メチオニン/バリン）および異常 PrP（PrPres）のプロテイナーゼ K 耐性コアの電気泳動パターンに応じて、タイプ 1 またはタイプ 2 のいずれかに分類される。sCJD のタイプ 1 およびタイプ 2 は、別の伝達性海綿状脳症（TSE）の感染物質が関与していると考えられている。

医原性 CJD は比較的稀であるものの、過去 60 年間で数百件が特定されている。患者の血液分画をげっ歯類の脳内に接種したところ、sCJD 患者の血液中に感染因子が存在したとのデータがいくつか報告されている。こうした観察は、他の研究で確認されていないため、確実なものではない。

1996 年には、新たなタイプの CJD がヒトで特定され、変異型 CJD（vCJD）と命名された。vCJD は、ウシの牛海綿状脳症を起こす原因物質に起因することが示された。英国では、後に vCJD 検査で陽性であることが判明したドナーによる非白血球除去赤血球濃縮製剤の輸血で、vCJD 感染 4 件（臨床例 3 人、無症候例 1 人）が起きた可能性が報告された。さらに最近では、vCJD 診断があったドナーによる複数のユニットを含めて、血漿分画製剤を輸血された血友病患者で、vCJD 感染したと推定される症例がさらに英国で報告された。vCJD の血液感染を示す疫学エビデンスはあるものの、従来のげっ歯類モデルを用いたバイオアッセイでは、血液中の感染因子の存在を突き止めることはできなかった。従来のげっ歯類モデルで TSE 伝播が認められないことは、vCJD および sCJD 患者の血液中では感染因子濃度が低いためであるか（ヒツジおよびげっ歯類の TSE モデルで記載される通り）、種間バリアがあるために動物モデルへのヒトプリオンの伝播が制限されていることが原因であろうと思われる。他の動物種の PrP を発現する種間バリアがないトランスジェニックマウスモデルを用いた過去 10 年間の研究から、現在では、低濃度の感染因子が検出できる可能性が示されている。

本研究では、vCJD または sCJD の TSE 感染因子への感受性が高い 2 種類のトランスジェニックマウスモデルを用いて、vCJD および sCJD 患者の血液分画に含まれる感染価を推定した。英国、ドイツ、フランスの法律に従い、ヒト検体の利用を含む実験プロトコルが英国では

使用上の注意記載状況・その他参考事項等

代表として献血ヴェノグロブリン IH5%静注 0.5g/10mL の記載を示す。

2. 重要な基本的注意

(1)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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National CJD Research & Surveillance Unit tissue bank、ドイツではREC reference number 2000/4/157-German TSE reference center、フランスではRef Nr 11/11/93, PHRC ref2004-D50-353で承認された。

本研究の詳細

ウシPrP（tgBov）を過剰発現するトランスジェニックマウスは、牛海綿状脳症の原因物質の検出に高感受性であることが、過去の研究で報告されている。tgBovはvCJD感染にも高い感受性を有することを示すため、本モデル（Tg110）を用いて、脳内接種によりvCJD単離物（10%脳ホモジネート）をエンドポイントまで希釈した。sCJDの原因と考えられているTSE感染因子は多様である可能性を考え、PRP遺伝子のコドン129がメチオニンのホモ接合であるタイプ1（MM1）のみを対象とした。ヒトPrP遺伝子の129メチオニン型を発現するマウスモデル（tgHu：Tg340）を用いて、sCJD MM1の10%脳ホモジネートをエンドポイント希釈した。これにより、tgBovおよびtgHuモデルにおいて、それぞれvCJDおよびsCJD MM1の感染因子について、基準脳ホモジネートの10-6倍希釈までの検出能が確認できた（表1；13）。この値は、種々のTSE動物モデルで報告されている脳/血液の相対的感染性の範囲内である。

次の手順として、vCJD確定患者1人の血液分画（赤血球、血漿、白血球）をtgBovマウスの脳内に注射した。同じく、sCJD MM1患者4人の血漿サンプルをtgHuに接種した（表2）。血液分画製剤は、研究室規模の血液学的プロトコル（online Technical Appendix、wwwnc.cdc.gov/EID/article/20/1/13-0353-Techappl.pdf）で準備し、血液銀行が採用する手順は採用しなかった。この手法では、vCJDの血液感染リスクを抑制するために大半の国で血液変性製品に採用される白血球除去を実施しないことになる。sCJD患者4人の脳組織サンプルもtgHuに接種した。培養期間（表2）およびパラフィン包埋組織ブロットで評価した場合の脳におけるPrPres分布パターンを基準とすると、こうした単離物のTSE感染因子は、エンドポイント希釈したsCJD MM1患者のものとは識別できなかった（図、パネルA）。

リン酸緩衝食塩水または健常人対照の脳および血漿を接種したtgBovまたはtgHuマウスでは、TSEの臨床徴候またはPrPres蓄積は観察されなかった。vCJD患者の血液分画3品は陽性であったが、tgBovマウスでは出現率は低かった（表2）。こうした結果から、赤血球および血漿中の感染性は、接種量の2.12感染量（ID）/mLであると推定された。白血球では、感染価は全血の2.23 ID/mLであると推定された。こうした値とサンプルのヘマトクリット（online Technical Appendix）から、患者の全血の総感染価は4.45ID/mL程度であると思われた。こうした感染性レベルは、エンドポイント希釈した基準vCJD脳サンプルの1.4μgにほぼ等しい（表1）。

tgHuマウスでは、血漿サンプル4品中2品を接種したマウスで伝播があった（表2）。伝播があった両血漿サンプルの感染価は、それぞれ2.12ID/mLおよび3.7ID/mLであると推定され、これはエンドポイント希釈した基準sCJD MM1脳サンプルの0.3～0.5μgに相当する（表1）。しかしながら、接種マウス数は少なく（n＝24）、アッセイの全体的感受性も限定的（上限CI限界、6.24ID/mL）であったため、他の血漿サンプル2品を接種されたマウスでは伝播がなかった、と確実に結論付けられるものではない。

vCJDを接種したtgBov、sCJDを接種したtgHuでは、脳ホモジネートおよび血液成分で誘発した動物のウエスタンブロットで観察されたPrPresバンドパターンは、同じであった（図、パネルC、D）。これらの結果から、tgBovマウスとtgHuマウスに伝播されたTSE感染因子は、それぞれvCJDおよびsCJDの感染因子であったとの主張が支持される。

結　　論

本稿で報告したデータから、vCJD患者から得た赤血球、白血球、血漿中に感染因子の存在が確認され、すべてではないが一部のsCJD患者から得た血漿中に感染因子の存在が明確に示されている。vCJDおよびsCJDの血液成分で測定された感染性レベルは、種々のTSE動物モデルで報告されたレベルと一致していた。本研究の症例数は限定的であり、新たな実験では、これよりも多くの症例を対象として、sCJD患者の種々の血液分画を用いて、本データを洗練する必要があろう。しかしながら、以上の結果からは、sCJDおよびvCJDのヒト間血液伝播リスクの評価に大きな知見が示されている。

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報告企業の意見	今後の対応	
従来、sCJD の血液を介した伝播の可能性は低いと見られてきたが、sCJD 患者の血液にも感染因子の存在が明確に示されたとする報告である。 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。なお、本剤の製造工程においてプリオンが低減される可能性を検討するための実験を継続して進めているところである。	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。 今後とも同様の情報収集に努める。	

Detection of Infectivity in Blood of Persons with Variant and Sporadic Creutzfeldt-Jakob Disease

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We report the presence of infectivity in erythrocytes, leukocytes, and plasma of 1 person with variant Creutzfeldt-Jakob disease and in the plasma of 2 in 4 persons whose tests were positive for sporadic Creutzfeldt-Jakob disease. The measured infectivity levels were comparable to those reported in various animals with transmissible spongiform encephalopathies.

Among humans, Creutzfeldt-Jakob disease (CJD) is a low incidence disease (≈1 case per million per year) that occurs as either a sporadic (sCJD) or a familial/genetic (fCJD) form. Whereas familial disease forms are linked to a mutation in the prion protein gene (*Prnp*), no clear epidemiologic risk factors have been identified for sporadic disease forms. sCJD is not a uniform disorder in terms of clinical and neuropathological phenotype. sCJD cases are classified as type 1 or 2 according to the polymorphism at codon 129 of the protease-resistant prion protein (PrP) sequence (methionine/valine) and to the electromobility of the proteinase K–resistant core of the abnormal PrP (PrP^{res}) (*1*). Type 1 and type 2 isoforms in sCJD are believed to correspond to different transmissible spongiform encephalopathy (TSE) agents

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Despite their relative rarity, several hundred iatrogenically transmitted CJD cases were identified during the past 60 years (*2*). Some data supporting the presence of infectivity in the blood of sCJD-affected patients were reported following the intracerebral inoculation of blood fractions from affected patients into rodents. These observations remain ambiguous because other studies did not confirm them (*3*,*4*).

In 1996, a new form of CJD, named variant CJD (vCJD), was identified in humans. Variant CJD was demonstrated to be caused by the agent that causes bovine spongiform encephalopathy in cattle (*5*). In the United Kingdom, 4 vCJD transmissions (3 clinical cases and 1 asymptomatic infection) were probably caused by the transfusion of non–leuco-depleted erythrocyte concentrates prepared from donors who later had positive test results for vCJD (*6*). More recently, a presumed additional case of vCJD infection was reported in the United Kingdom in a hemophilic patient who had received fractionated plasma products, including some units linked to a donor who had vCJD diagnosed (*7*). Despite the epidemiologic evidence of bloodborne transmission in vCJD, bioassays performed on conventional rodent models failed to demonstrate the presence of infectivity in the blood (*8*). The lack of TSE transmission in conventional rodent models could be a consequence of a low infectivity level in blood from vCJD- and sCJD-affected patients (as described in sheep and rodent TSE models) (*9*) or of the existence of the species barrier phenomenon that limits the transmission of human prions to these animal models. The development during the last decade of transgenic mice models expressing PrP from others species that abrogate the species barrier now offers the potential to detect low level of infectivity (*10*).

In this study, we used 2 transgenic mouse models that displayed a high sensitivity to the vCJD or sCJD TSE agents to estimate the infectious titer in certain blood fractions from vCJD- and sCJD-affected patients. According to legislation of the United Kingdom, Germany, and France, the experimental protocol, including the use of human samples, was approved by UK National CJD Research & Surveillance Unit tissue bank: REC reference number 2000/4/157-German TSE reference center: Ref Nr 11/11/93, PHRC ref 2004-D50-353 for patient from France.

The Study

Previous studies reported a high sensitivity in transgenic mice overexpressing bovine PrP (tgBov) for the detection of the bovine spongiform encephalopathy agent. To demonstrate that tgBov also displays a high sensitivity to vCJD infection, we titrated to endpoint a vCJD isolate (10% brain homogenate) by intracerebral inoculation in this model (Tg110) (*11*). Considering the potential diversity

Table 1. Titration of sCJD and vCJD isolates in transgenic mice expressing the human or bovine prion protein*†

Dilution	sCJD MM1 in tgHu		vCJD in tgBov	
	Positive transmission in mice	Incubation period, d	Positive transmission in mice	Incubation period, d
Not diluted	6/6	186 ± 10	6/6	249 ± 2
10^{-1}	6/6	213 ± 15	6/6	283 ± 15
10^{-2}	6/6	240 ± 13	6/6	316 ± 21
10^{-3}	6/6	263 ± 24	6/6	342 ± 10
10^{-4}	6/6	296 ± 26	6/6	453 ± 66
10^{-5}	6/6	323 ± 29	4/6	499 ± 17
10^{-6}	1/6	316	1/6	502
10^{-7}	0/6	>650	0/6	>700
Infectious titer, ID_{50}/g of brain (95% CI)	$10^{6.67}$ ($10^{6.33}$–$10^{6.97}$)		$10^{6.33}$ ($10^{5.84}$ –$10^{6.82}$)	

*sCJD, sporadic Creutzfeld-Jakob Disease; tgHu, human PrP gene ;PrP, protease-resistant prion protein; vCJD, variant CJD; tgBov transgenic mice overexpressing bovine PrP, ID, infectious dose.
†Successive 1/10 dilutions of 10% brain homogenate (frontal cortex) from patients affected by vCJD and sCJD were injected intracerebrally to tgHu (n = 6) and tgBov (n = 6) mice, respectively. Those 2 patients were different from the 1 whose blood was tested in bioassay (Table 2). Mice were euthanized when they showed clinical signs of infection or after 650 days postinfection. Mice were considered infected when abnormal prion protein deposition was detected in the brain by western blot by using Sha31 monoclonal antibody, which recognizes amino acids 145–152 (YEDRYYRE) of the sheep prion protein. Infectious titers were estimated by the Spearman-Karber method (*14*).

of TSE agents that may cause sCJD, we decided to focus only on type 1 homozygous for methionine at codon 129 of the PRP gene (MM1) sCJD cases. An endpoint titration of a MM1 sCJD 10% brain homogenate was performed in a mouse model that express the methionine 129 variant of the human PrP gene (tgHu:Tg340) (*12*). This enabled confirmation of the capacity of the tgBov and tgHu models to detect the vCJD and sCJD MM1 agent, respectively, up to a 10^{-6} dilution of the reference brain homogenates (Table 1; *13*). This value was within the range of the brain/blood relative infectivity reported in various TSE animal models (*9*,*14*).

In the next step of our experiment, blood fractions (erythrocytes, plasma, and leukocytes) from 1 vCJD-confirmed patient were injected intracerebrally in tgBov mice. Similarly, plasma samples from 4 sCJD MM1 patients were inoculated with tgHu (Table 2). The blood fraction

Table 2. Intracerebral inoculation of blood components collected from 1 vCJD and 4 sCJD cases (MM1) in transgenic mice expressing the bovine or human prion protein gene*†

Mouse model	Donor	Specimen	Inoculated mice	Positive mice	Incubation period, d	ID/mL (95%CI)‡
tgBov	vCJD	Leukocyte	24	3	476, 567, 576	2.23 (0–4.87)
		Plasma	24	1	453	2.12 (0–6.52)
		Erythrocyte	24	1	433	2.12 (0–6.52)
tgHu	sCJD case 1	Plasma	14§	1	338	3.70 (0–11.65)
		Brain	6	6	216 ± 2	NA
	sCJD case 2	Plasma	24	0	>700	0 (0- 6.24)
		brain	6	6	217 ± 5	NA
	sCJD case 3	Plasma	24	1	233	2.12 (0–6.52)
		Brain	6	6	205 ± 5	NA
	sCJD case 4	Plasma	24	0	>700	0 (0–6.24)
		Brain	6	6	207 ± 3	NA
tgHu	Control human	Plasma	12	0	>650	NA
tgBov	Control human	Plasma	12	0	>650	NA
tgHu	Control human	PBS	12	0	>700	NA
tgBov	Control human	PBS	12	0	>700	NA
tgHu	Control human	Brain	24	0	>700	NA
tgBov	Control human	Brain	24	0	>700	NA
tgHu	Control human	None	24	0	>750	NA
tgBov	Control human	None	24	0	>750	NA

*vCJD, variant Creutzfeld-Jakob disease; sCJD, sporadic Creutzfeld-Jakob disease; dpi, days postinfection; ID, infectious dose; tgBov, bovine prion protein; tgHu, human prion protein;; PBS, phosphate-buffered saline.
†The leukocyte(s) from a single vCJD case corresponding to a starting volume of 3 mL of blood were suspended in 1 mL of 5% glucose solution. The leukocyte suspension and the crude erythrocytes were homogenized by using a high speed cell disrupter. The leukocyte and erythrocyte homogenates (vCJD case) and crude plasma (vCJD and sCJD cases) were intracerebrally injected into mice (20 µL per mouse). For the 4 sCJD MM1 cases, brain homogenate (10%, temporal cortex) were also inoculated in tgHu. Mice were euthanized when they showed clinical signs of infection or after 650 or 750 dpi. Mice were considered infected when abnormal protease-resistant prion protein; deposition was detected in brain tissue by using Western blot analysis with Sha31 monoclonal antibody: epitope amino acids 145–152 (YEDRYYRE) of the sheep PrP sequence. For samples showing 100% attack rate, incubation periods are reported as mean (± SD). For other samples, individual incubation period of CJD-positive mice are presented; their infectious titers were estimated by using limiting dilution titration method (application of Poisson model) described by Brown et al (*13*).
‡Leukocyte titer is expressed as ID/mL of the starting whole blood. Plasma and erythrocyte titers are expressed as ID/mL of inoculum.
§24 mice were inoculated; 10 died because of the acute toxicity of the sample.

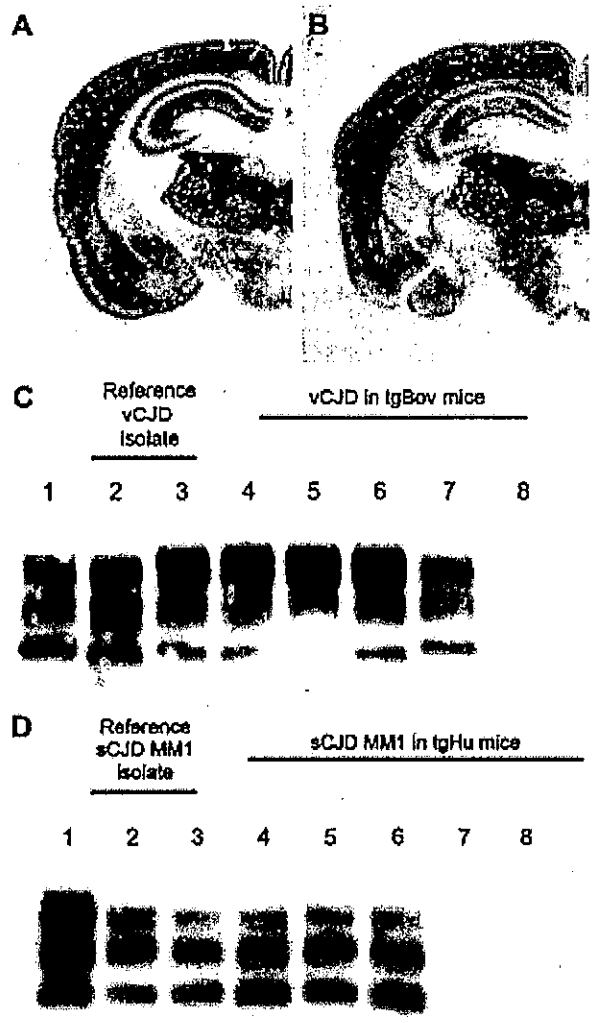

Figure. Abnormal prion protein (PrP^{res}) detection by using Western blot (WB) and paraffin-embedded tissue (PET) blot in the brain of transgenic mice expressing the methionine 129 variant of the human prion protein (PrP) (tgHu) or bovine PrP (tgBov). A, B) PET blot PrP^{res} distribution in coronal section (thalamus level) of tgHu mice inoculated with sporadic Creutzfeldt-Jakob disease (sCJD) MM1 isolates (10% brain homogenate): A) reference isolate used for the endpoint titration in Table 1; B) sCJD case 1 (Table 2). C) PrP^{res} WB of variant Creutzfeldt-Jakob disease (vCJD) reference isolate (used for endpoint titration in Table 1) and tgBov mice inoculated with the same vCJD reference isolate or vCJD blood fractions. Lane 1, WB-positive control; lanes 2 and 3, reference vCJD isolate; lane 4, leukocytes; lane 5, erythrocytes; lane 6, plasma; lane 7, WB-positive control; lane 8, healthy human plasma in tgBov. D) (PrPres Western blot of the sCJD reference isolate (used for endpoint titration in Table 1) and tgHu mice inoculated with the same sCJD reference isolate and plasma from sCJD cases. A proteinase K–digested classical scrapie isolate in sheep was used as positive control for the blots in panels C and D. (PrPres immunodetection in PET and Western blots was performed by using Sha31 monoclonal antibody (epitope: 145YEDRYYRE152 of the human PrP). Lane 1, WB-positive control; lanes 2 and 3, reference sCJD MM1 isolate; lane 4, brain tissue from case 1; lane 5, plasma from case 1; lane 6, plasma from case 3; lane 7, plasma from case 2; lane 8, plasma from case 4.

preparation was performed by using laboratory scale hematologic protocols (online Technical Appendix, wwwnc.cdc.gov/EID/article/20/1/13-0353-Techapp1.pdf), not by following the procedure applied by blood banking services. This method implies that the leucodepletion that is applied to blood labile products in most countries to reduce the vCJD bloodborne transmission risk was not performed. Brain tissue samples from each of the 4 sCJD cases were also inoculated with tgHu. On the basis of the incubation period (Table 2) and PrP^{res} distribution pattern in the brain as assessed by using paraffin-embedded tissue blot, the TSE agents in those isolates were indistinguishable from those in the MM1 sCJD case that was used for endpoint titration (Figure, panel A).

No TSE clinical signs or PrP^{res} accumulation were observed in the tgBov or tgHu mice inoculated with phosphate-buffered saline or brain and plasma from healthy human controls. The 3 blood fractions from the vCJD-affected patient caused a positive result but low attack rate among tgBov mice (Table 2). On the basis of these results, infectivity in erythrocytes and plasma was estimated to be 2.12 infectious dose (ID)/mL of inoculum. In leukocytes, the infectious titer was estimated to be 2.23 ID/mL of whole blood. According to these values and the hematocrit of the sample (online Technical Appendix), the global infectious titer whole blood in the tested patient would be ≈4.45 ID/mL. Such infectious level is approximately equivalent to 1.4 μg of the reference vCJD brain sample that was endpoint-titrated (Table 1).

In tgHu mice, positive transmission was observed among mice inoculated with 2 of 4 plasma samples (Table 2). The infectious titers in both positive plasma samples were estimated to be 2.12 and 3.7 ID/mL of plasma, which is equivalent to 0.3–0.5 μg of the reference sCJD MM1 brain sample that was endpoint titrated (Table 1). However, because of the limited number of mice inoculated (n = 24) and the overall sensitivity of the assay (upper CI limit 6.24 ID/mL), the absence of transmission in mice inoculated with the 2 other plasma samples cannot be interpreted conclusively

In tgBov inoculated with vCJD and tgHu inoculated with sCJD, the PrP^{res} banding patterns observed by Western blot in animals challenged with brain homogenate and blood components were identical (Figure, panels C, D). These results support the contention that the TSE agent propagated in tgBov mice and tgHu were vCJD and sCJD agents, respectively.

Conclusions

The data reported here confirm the presence of infectivity in erythrocytes, leukocytes, and plasma from vCJD-affected patients and demonstrate unambiguously the presence of infectivity in the plasma of some, but not all, sCJD-affected patients. The infectivity levels that we

measured in the tested vCJD and sCJD blood components were comparable to those reported in various TSE animal models. The number of cases included in our study was limited; a new experiment that would include a larger number of cases and different blood fractions from sCJD cases will be necessary to refine the data. However, these results represent a substantial input for assessing the risk for interindividual bloodborne transmission of sCJD and vCJD.

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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
				2013. 11. 8	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		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)		P Saa, O Yakovleva, J de Castro, I A Vasilyeva, L Cervenakova. AABB Annual Meeting&CTTXPO 2013; October 12-15, 2013, Denver, CO.	米国

研究報告の概要

○in vitroでの異常プリオン蛋白の増幅により、伝達性海綿状脳症(TSE)感染マウスの循環血中エキソソームにおけるPrP^{TSE}の存在を示した初の証拠

TSEの原因とされているプリオンの血中分布についてはほとんど明らかになっていない。TSE感染培養細胞から抽出したエキソソームがマウスにおいて臨床症状を引き起こすことが示されている。血液成分から分離したエキソソームにはPrP^{c}が含まれているが、TSEの原因となり得るPrP^{TSE}が含まれている可能性もある。本研究では、ヒトTSEに感染させ、臨床症状を呈するマウスと対照マウスの血漿からエキソソームを分離し、未感染マウスの脳ホモジネートに加え、saPMCA法により増幅した後、ウェスタンブロット法でPrP^{TSE}検出を行った。その結果、TSE感染マウスの血漿エキソソームからPrP^{TSE}が特異的に検出された。これは、血漿エキソソーム中に含まれるPrP^{TSE}を生化学的に検出した初の報告である。

報告企業の意見	今後の対応
臨床疾患を呈するTSE感染マウスの血漿エキソソームからPrP^{TSE}を生化学的に検出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。

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

Donor Follow-up Testing

Collect Date	Days Post Donation	PCR	ELISA Signal/cutoff (where ≥1=+) IgM	IgG
1/16/2013	70	Not Tested	<1	>7.8
2/20/2013	105	Positive	<1	>7.5
3/30/2013	143	Negative	<1	>8.1
4/15/2013	159	Negative	<1	>8.0

SP381

First Proof of PrP^{TSE} Presence in Blood-Circulating Exosomes from Transmissable Spongeform Encephalopathies (TSE)-Infected Mice by In Vitro Amplification of Misfolded Prion Protein

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Background/Case Studies: Exosomes (membrane vesicles of 30-120 nm in diameter) were originally considered to be a cellular mechanism to eliminate unwanted proteins. Recent investigations however, have attributed new roles to these vesicles including intercellular communication and agent dissemination. Little is known about the distribution of prions (the agents implicated in transmissible spongiform encephalopathies (TSEs) in blood. Cellular prion protein, PrP^{C}, and its misfolded counterpart, PrP^{TSE}, have been identified in exosomes from various TSE models; and exosomes obtained from TSE-infected cell cultures have caused clinical disease in mice. Exosomes isolated from blood components contain PrP^{C}. This finding has raised the possibility that they may also contain PrP^{TSE} and serve as vehicles for the transfusion transmission of TSEs. In this study we isolated exosomes from plasma samples of mice infected with human TSE agents and demonstrated the presence of PrP^{TSE} by biochemical methods. These findings set the ground for the design of novel blood-based diagnostic tests for TSEs. **Study Design/Methods:** Exosomes were isolated with ExoQuick reagent (System Biosciences) from 250 µl of platelet poor plasma collected from clinically sick mice infected with human TSEs, and uninfected controls. Exosome pellets were mixed with uninfected mouse brain homogenates and amplified by saPMCA method (which allows the multiplication of minute amounts of PrP^{TSE} to levels detectable by standard biochemical assays). After proteinase K digestion, PrP^{TSE} was detected by Western blot using the anti-PrP monoclonal antibody 6D11. **Results/Findings:** Exosomal preparations were positive for exosomal markers Hsp70 and Flotillin by Western blotting. saPMCA allowed the specific detection of PrP^{TSE} in samples from TSE-infected animals. Different numbers of saPMCA rounds were necessary to amplify PrP^{TSE} to detectable levels in individual exosomal samples. These findings suggest that different quantities of PrP^{TSE} are present in plasma at the terminal stage. **Conclusion:** The biochemical detection of PrP^{TSE} in plasma exosomes, obtained from clinically sick animals, is reported here for the first time. These experiments provide an invaluable foundation for the development of new diagnostics and potential targets for TSEs treatment. Further characterization of PrP^{TSE}-containing microvesicles may lead to the identification of the cellular origin of prion-containing exosomes and the site(s) of prion replication in blood. Support. The study was partially supported by Fondation Alliance BioSecure, France.

Disclosure of Commercial Conflict of Interest

L. Cervenakova: Nothing to disclose; J. de Castro: No Answer; P. Saa: Nothing to disclose; I. A. Vasilyeva: Nothing to disclose; O. Yakovleva: Nothing to disclose

Disclosure of Grants Conflict of Interest

L. Cervenakova: Nothing to disclose; J. de Castro: No Answer; P. Saa: Nothing to disclose; I. A. Vasilyeva: Nothing to disclose; O. Yakovleva: Nothing to disclose

SP382

Prevalence of *T. Cruzi* Antibodies in Blood Donors from a Blood Bank Placed in a Non Endemic Area with High Migration Rates

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Background/Case Studies: Chagas disease, an infection caused by *Trypanosoma cruzi* (*T. Cruzi*), is endemic in several regions in Central and South America. Increasing migration rates have extended the number of cases to non-endemic regions such Europe and the US. Several mitigation strategies have been implemented in recent years for preventing transfusion-associated cases in these regions. In Colombia, serologic screening for *T. Cruzi* antibodies to all collected blood became mandatory in 1993. During the last 10 years, the capital city of Bogotá, a non endemic region, has experienced substantial migrations from the countryside caused by the internal social conflict. The prevalence of *T. cruzi* antibodies in blood donors from a reference blood bank placed in a non-endemic area (Bogotá, 8530 ft AMSL), during the last 8 years is reported here. **Study Design/Methods:** Results of screening tests for *T. cruzi* were retrospectively reviewed for all blood donations from January 2005-December 2012 at the National Blood Bank Colombian Red Cross in Bogotá, Colombia. Various ELISA-based assays were used for donor screening until January 2012, when a chemiluminescence method was introduced (ARCHITECT Chagas assay, Abbott GmbH & Co. KG). From 2005-2007 and 2009: ELISA cruzi test (bioMérieux, Mercy-l'Etoile, France). From 2008-2009: Chagatest (Wiener Laboratorios S.A.I.C. Argentina). From 2010-December 2011: ORTHO ELISA Test System (Johnson & Johnson, HighWycombe, UK). All reactive samples were confirmed by Indirect Immunofluorescence. **Results/Findings:** In total, 191,964 donations were tested during the time of the study, resulting in 645 samples being reactive. Only 280 samples were confirmed to be positive. The reactivity rate for *T. cruzi* was 0.37%, although, as the table shows, in 2007 and 2008 there was an unexpected rise in the detection rate, explained by transitory problems with the cut off values assigned during that time. The positivity rate was 0.14% (1 confirmed case every 686 units). **Conclusion:** The reactivity and positivity rates found in this bank are much higher than those reported recently in the US or Europe. Based on these data, Bogotá has comparable positive rates to other endemic places in the world.

Disclosure of Commercial Conflict of Interest

J. D. Muñoz: Nothing to disclose; G. A. Orjuela: Nothing to disclose

Disclosure of Grants Conflict of Interest

J. D. Muñoz: Nothing to disclose; G. A. Orjuela: Nothing to disclose

Year	2005	2006	2007	2008	2009	2010	2011
Total Donors	22053	23862	23494	24270	25497	25970	21650
Reactive donors	65	60	167	175	80	43	55
Confirmed cases	32	35	45	35	36	31	35

Transfusion-Transmitted Infectious Diseases: Viruses

SP383

Eleven-Year Experience of Parental and Non-Parental Whole Blood Donor Infectious Disease Testing at a Pediatric Hospital Blood-Donor Center

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Background/Case Studies: Directed donation is well known to be associated with increased infectious disease rates. However, little is known of the infectious disease risk of parental donation as a special subcategory of directed donations. It was hypothesized that: 1) parental donors would have higher infectious disease risk than homologous donors because of internal parental incentives to donate in order to help their child; and 2) have similar or greater infectious disease risk than non-parental directed donors because of internal pressure to donate. **Study Design/Methods:** An 11 year retrospective study (January 1997-December 2008) of whole blood donation at a pediatric hospital blood donor center was undertaken. Review of blood donor deferral records, including infectious disease testing positivity, was performed to obtain transfusion transmitted risk. Total yearly blood donations were stratified by donor relationship (parental, non-parental directed, and homologous) and first time versus repeat donation. Median (5th and 95th percentile) yearly incidence of positive infectious disease testing was determined for all groups. Mann-Whitney U test was used to compare median incidence rates between each group. **Results/Findings:** Over an 11-year period, a total of 23,865 first time and repeat donors attempted whole blood donation. Although there was a trend towards higher median yearly incidence of positive infectious disease testing among parental donors

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	Jackson GS, Burk-Rafel J, Edgeworth JA, Sicilia A, Abdilahi S, Korteweg J, Mackey J, Thomas C, Wang G, Mead S, Collinge J. Blood. 2014 Jan 16;123(3):452-3. doi: 10.1182/blood-2013-11-539239.	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)			英国

研究報告の概要

○極めて特異性の高い変異型クロイツフェルト・ヤコブ病(vCJD)血液検査
vCJDには、無症候性キャリアからの血液を輸血することによる二次的な感染リスクが存在する。虫垂検体を調査した英国の健康保護局による最近の研究では、現在英国には2000人に1人の割合で無症候性キャリアが存在すると推定している(95%信頼区間[CI]:1/1248～3546人)。ステンレス鋼粉末を用いて全血から疾患関連プリオン蛋白質を捕捉、濃縮し、優れた解析力と診断感度が証明された検査について、特異性及び曝露歴とリスクを有する集団のスクリーニングとして適しているかどうかを調査するため、BSEへの曝露が最低限であり真の陽性と想定されない米国赤十字社の血液検体5,000件を検査したところ、陽性となった検体はなかった(特異性100%;95%CI:99.93-100%)。より小規模の英国の健常者集団でも高い特異性が反映された。このアッセイの陽性尤度比から真の陽性率は偽陽性率と比べて7,000倍高く、また、陰性尤度比から真の陰性率は偽陰性率と比べて3倍高いことが示された。また、vCJD感染患者と非感染者の小規模パネルを盲検的に調査した結果、10人のvCJD患者検体のうち7検体が陽性(感度70%)であり、以前の評価と同等であった。
種々の限界や不確実性はあるものの、この検査はプリオンに曝露した集団と非曝露集団を比較する有病率試験の正当性を裏付けるには十分な性能を有している。

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
疾患関連プリオンタンパク質を検出するためのステンレス鋼粉末を用いた血液検査は、種々の限界や不確実性はあるものの、プリオン曝露集団と非曝露集団を比較する有病率試験の正当性を裏付けるのに十分な性能を有していることが分かったとの報告である。	日本赤十字社は、vCJDの血液を介する感染防止の目的から、受付時に過去の海外滞在歴を確認し、欧州等38カ国に一定期間滞在したドナーを無期限に献血延期としている。また、英国滞在歴を有するvCJD患者が国内で発生したことから、1980～96年に1カ月以上の英国滞在歴のある人の献血を制限している。今後もプリオン除去フィルター等の技術を含め、CJD等プリオン病に関する新たな知見及び情報の収集に努める。

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

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To the editor:

A highly specific blood test for vCJD

Variant Creutzfeldt-Jakob disease (vCJD) is a fatal neurodegenerative disease originating from exposure to bovine spongiform encephalopathy (BSE). Despite low clinical incidence, the risk of secondary vCJD infection via blood transfusions from subclinical carriers persists. A recent study of appendix samples by the United Kingdom (UK) Health Protection Agency estimates 1 in 2000 silent prion infections in the UK population (95% confidence interval [CI]: 1 in 1248-3546).[1] This estimate is in sharp contrast to the small number of recognized clinical cases that may be explained at least in part by the extremely prolonged incubation periods associated with human prion disease and the possibility that the barrier to infection is lower in lymphoreticular tissue than in brain,[2] leading to subclinical infection.

A sensitive and specific blood-based assay for vCJD infection, though urgently needed, has been elusive because abnormal prion protein (PrP) levels are extremely low in blood and chemically identical normal PrP is in large excess. Previously, we demonstrated a prototype assay that captured and enriched disease-associated PrP from whole blood using stainless steel powder, achieving impressive analytical (10^{-10} dilution of vCJD-affected brain) and diagnostic (71% of vCJD-infected patient blood samples) sensitivity.[3]

To explore the assay's specificity and hence suitability for screening exposed and at-risk populations, we tested 5000 blood samples from the American Red Cross in which, because of minimal BSE exposure, no true positives would be expected (US normals). No samples tested positive (100% specificity; 95% CI: 99.93%-100%) (Table 1). The high specificity was reflected in a smaller, healthy UK cohort (UK normals). The assay's positive likelihood ratio indicates true positives are more than 7000 times as likely as false positives; the negative likelihood ratio indicates true negatives are more than 3 times as likely as false negatives. Finally, we tested a small blind panel of unaffected and vCJD patient samples. Seven of 10 vCJD patient samples tested positive (70% sensitivity), reconfirming but not refining our previous sensitivity estimate.

A remaining question is whether the assay can detect sub- or preclinical vCJD-affected individuals. Although it has been suggested that asymptomatic individuals would have particularly low concentrations of abnormal PrP, preclinical blood involvement[4]

Table 1. vCJD infection blood test performance

vCJD-negative cohorts	Samples	Positive	Specificity	95% CI
US normals	5000	0	100%	99.9-100%
UK normals	200	0	100%	98.2-100%
vCJD-positive cohorts	**Samples**	**Positive**	**Sensitivity**	**95% CI**
vCJD spikes	192	192	100%	98.1-100%
vCJD patients	21	17	71.4%	47.8-88.7%
US normals + vCJD patients			**Value**	**95% CI**
Positive likelihood ratio			7047	435-114 146
Negative likelihood ratio			0.30	0.16-0.56

Whole blood samples were incubated overnight in buffer containing stainless steel powder, as previously described.[3] The powder was heat treated and sequentially incubated with biotinylated anti-PrP antibody, NeutrAvidin-HRP, and chemiluminescent substrate. Samples were scored reactive if the mean signal from 3 replicates exceeded an on-plate negative control cutoff. Repeat-reactive samples were considered positive for vCJD; nonreactive and single-reactive samples were considered negative for vCJD.

Anonymous blood samples from unaffected blood donors were obtained from the American Red Cross (US normals) and the National Health Service Blood and Transplant service of England and Wales (UK normals). Positive control samples consisted of 0.1% wt/vol vCJD-infected brain homogenate in whole unaffected human blood (vCJD spikes) and were used to preserve scarce stocks of endogenous vCJD patient samples (vCJD patients). Clopper and Pearson's "exact" method was used for confidence intervals of proportions. Likelihood ratios and their confidence intervals were calculated using Haldane's correction and a derived approximation for risk ratios.

HRP, horseradish peroxidase.

and transmission[5] have been demonstrated in animal models. Despite some limitations and uncertainties, the prototype vCJD assay has sufficient performance to justify a prevalence study comparing prion-exposed and prion-unexposed populations, which would require 20 000 samples from each cohort ($1-\beta = 80\%$, $\alpha = 5\%$). A blood prevalence study would provide essential information for deciding if routine vCJD screening is needed for blood, tissue, and organ donations and for patients before high-risk surgical procedures.

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Contribution: G.S.J. and J.C. conceived the study; J.A.E., A.S., S.A., J.K., J.M., C.T., and G.W. performed assays; S.M. provided clinical expertise and samples; G.S.J. and J.B.-R. analyzed results and made the figures; and G.S.J., J.B.-R., and J.C. wrote the letter and all authors provided input.

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To the editor:

Application of CIBMTR risk score to NIH chronic GVHD at individual centers

A new risk score to predict mortality in patients with chronic graft-versus-host disease (GVHD) was recently reported by Arora et al by analyzing a large amount of data between 1995 and 2004 from the Center for International Blood and Marrow Transplant Registry (CIBMTR).[1] The risk score consists of 10 variables defined at transplantation or at onset of chronic GVHD that are objective and

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識別番号・報告回数			報告日	第一報入手日 2014 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	Health Protection Report/Vol. 8 Nos. 5/6 - 7 and 14 February 2014	公表国 イギリス
販売名 （企業名）	①抗 HBs 人免疫グロブリン筋注 200 単位/1mL「日赤」（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「日赤」（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

感染症報告書
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新たな感染症/CJD
年 2 回の CJD に関する最新情報（2014 年 1 月）－新たなヒトプリオン病の概況
半年に 1 回の本報告書は、潜在的な医原性（医療による感染）クロイツフェルト・ヤコブ病（CJD）感染に対して行われた強化監視活動の最新情報を提供するものである。データは 2013 年 12 月 31 日の時点で正確である。CJD 症例報告書の件数については National CJD Research and Surveillance Unit（NCJDRSU, http://www.cjd.ed.ac.uk/data/html）から提供されているデータを参考とされたい。また、最近確認されたヒトプリオン病の可変プロテアーゼ感受性プリオン症に関する概況も以下に示す。

CJD の‘感染リスクが高い’患者のモニタリング
その人物が受けた医療の結果、CJD 感染リスクが高いことが確認されている人物にはそれぞれの曝露状況を知らせ、他者への感染を避けるために公衆衛生上の注意に従うよう求めている。また、このような患者をフォローアップして、種々の経路で患者に伝播する CJD 感染リスクを確認すると共に、高度の CJD 感染リスクに曝露された可能性のある人物が CJD を発症することになるのかどうかを確認する一助としている。公衆衛生のフォローアップ活動には、臨床モニタリング、一般開業医（GP）からの最新情報の取得のほか、このような高リスク群の無症候性の人物が CJD 病原物質に感染していたのかどうかを確認するために行う検視も含まれる。研究目的のために血液や組織試料が提供される場合もある。このような活動には以下のとおり、多くの異なる機関が与している：Public Health England（旧 Health Protection Agency）、Health Protection Scotland（HPS）、UCL Institute of Child Health/Great Ormond Street Hospital（ICH）、NHS Blood and Transplant（NHSBT）、National CJD Research and Surveillance Unit（NCJDRSU）、National Prion Clinic（NPC）、UK Haemophilia Centre Doctors’ Organisation（UKHCDO）。
PHE（Public Health England）の CJD 班は、CJD 感染リスクが高いことが確認され、これについて通知を受けている人物のデータ収集をまとめている。このような人物には公衆衛生のモニタリングや種々の機関による研究活動を通してフォローアップが行われている。

PHE の CJD 班は現在、感染リスクのある以下の患者群についてデータを保有している。
・献血後に vCJD を発症したドナーから血液成分の輸血を受けたレシピエント
・輸血後に vCJD を発症した人物に献血した血液ドナー

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。

・このような血液ドナーから血液成分の輸血を受けたその他のレシピエント
・1990年から2001年までに特定の血漿製剤の投与を受けたレシピエント（非出血性疾患の患者）
・CJDと診断された患者との特定の外科的な接触
・輸血が多いレシピエント

以下のリスク群に関するデータはPHE以外の機関が保有している。
・1990年から2001年までに血漿製剤の投与を受けた出血性疾患の患者（UKHCDO）
・1985年以前にヒト由来成長ホルモンの投与を受けたレシピエント（ICH）
・1992年8月以前に硬膜移植を受けた可能性のある患者（現在、データは収集されていない）
・1973年以前にヒト由来ゴナドトロピンの投与を受けたことがある人物（現在、データは収集されていない）
・遺伝性プリオン病（NPC）のリスクがある家族

UKHCDOのデータは、UK原料由来の血液凝固因子を投与された‘感染リスクのある’出血性疾患患者の実際の数を過小評価している可能性があり、それはリスクがあると確認された患者に関して血友病センターからUKHCDOデータベースへの報告が不完全であったためである。リスクがあると通知を受けた患者はUKHCDOデータベースから詳細を削除する選択肢が与えられており、リスクがある患者合計から削除される。ヒト由来ヒト成長ホルモンの投与を受けた感染リスクのある患者に関してICHが保有しているデータは、実際の総数よりわずかに下回っている。これはこれらのうち少数の患者がICHのフォローアップに含まれていないためである。

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可変プロテアーゼ感受性プリオン症

可変プロテアーゼ感受性プリオン症（VPSPr）は、ごく最近同定されたヒトプリオン病であり、2008年に「プロテアーゼ感受性の異常なプリオンタンパク質による新たなヒト疾患」としてGambettiらが米国で初めて報告した。それ以来、他の諸国でも同様の症例が確認されており、National CJD Research and Surveillance Unitも英国で9例を確認した。そのうち3例は回顧的に確認されたものであり、残りの症例は1991年以降に収集された検体およびデータから推定されたものである。他の対象例は現在、調査中である。
VPSPr患者には後天性ヒトプリオン病の特定のリスク因子がなく、またプリオンタンパク質遺伝子（PRNP）のコード配列に関連突然変異が認められていない。当初の報告で、一部の患者にはっきりしない認知症の家族歴がみられたが、これはごく最近同定された症例の特徴ではなかった。VPSPrは、散発性クロイツフェルト・ヤコブ病（sCJD）の場合と同じ年齢層の患者が罹患し、ほとんどは60歳以降の患者が発症する。臨床的特徴はsCJDより変化に富んでおり、運動異常、認知機能低下および歩行不安定がある。臨床症状を呈する期間はsCJDより長く、ほとんどの患者がこの疾患で死亡するまで1年以上生存する。そのため、臨床診断の基準を設定することが困難であり、この疾患については解明の途上と考えられ、この問題に関するさらなる研究が望まれる。
sCJDと同様、VPSPrもPRNP遺伝子のコドン129の多型、すなわちMM、MVおよびMVによって定義されるすべての遺伝子群で発生する。sCJDとは異なり、コドン129-Vのハプロタイプが優勢である。VPSPrは顕著な神経病理学的特徴があり、最も典型的な特徴は、標的様に並んだ微小なプラークで、特に小脳に多い。この微小なプラークは様々な抗PrP抗体で異なる染色性を示し、一般的なsCJD VV2および稀なsCJD VV1サブタイプの両者と区別することが可能である。VPSPrの最も顕著かつ明白な特徴は脳における異常なプリオンタンパク質の生化学的性状であり、タンパク分解に対してほとんど抵抗性がなく、Western blotアッセイでは低含量の切断された8kDa（約）のバンドがみられる。このフラグメントは18～30kDaの範囲まで延長した淡い梯子状のバンドを付随することが多い。VPSPrの中には、異常なプリオンタンパク質のWestern blot解析でしばしば小脳にsCJD様のパターンを示す症例もあり、VPSPrとsCJDとの間で分子の重複があることが示唆される。

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	VPSPr の疫学、臨床的および病理学的な診断基準ならびに伝播の特性を十分に確立するにはさらなる研究が必要である。Dangerous Pathogens Transmissible Spongiform Encephalopathy の諮問委員会（ACDP TSE）のサブグループは、今後の研究で VPSPr がどのように伝播するのかを明らかにするまで、CJD およびその他の関連疾患に関する感染対策の指針にこの新たなヒトプリオン病を追加することが望ましいと結論した。	

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。なお、本剤の製造工程においてプリオンが低減される可能性を検討するための実験を継続して進めているところである。	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。	

Infection reports
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Emerging infections/CJD

CJD biannual update (2014/1), with briefing on novel human prion disease

This six-monthly report provides an update on the enhanced surveillance of potential iatrogenic (healthcare-acquired) exposures to Creutzfeldt-Jakob Disease (CJD). The data is correct as at 31 December 2013. For numbers of CJD case reports, readers should consult data provided by the National CJD Research and Surveillance Unit (NCJDRSU, http://www.cjd.ed.ac.uk/data.html). A briefing on a recently-identified human prion disease – Variably Protease-Sensitive Prionopathy – is also presented below.

Monitoring of patients 'at increased risk' of CJD

Individuals who have been identified as at increased risk of CJD as a consequence of their medical care are informed of their exposure and asked to follow public health precautions to avoid potentially transmitting the infection to others. They are also followed-up to help determine the risks of CJD transmission to patients through different routes and to ascertain whether any people who may have been exposed to increased CJD risks go on to develop CJD.

Public health follow-up activities include clinical monitoring, general practitioner (GP) updates, and post mortem investigations to determine whether asymptomatic individuals in these groups have been infected with the CJD agent. Some individuals also provide blood or tissue specimens for research purposes. A number of different organisations are involved in these activities: Public Health England (formerly the Health Protection Agency), Health Protection Scotland (HPS), UCL Institute of Child Health/Great Ormond Street Hospital (ICH), NHS Blood and Transplant (NHSBT), National CJD Research and Surveillance Unit (NCJDRSU), National Prion Clinic (NPC), and the UK Haemophilia Centre Doctors' Organisation (UKHCDO).

The PHE CJD Section coordinates the collation of data on individuals identified as at increased risk of CJD, and who have been informed of this. These individuals are followed up through public health monitoring and research activities by different organisations (table 1).

The PHE CJD Section currently holds data on the following groups of 'at risk' patients:

- recipients of blood components from donors who subsequently developed vCJD
- blood donors to individuals who later developed vCJD
- other recipients of blood components from these blood donors
- recipients of certain plasma products between 1990 and 2001 (non-bleeding disorder patients)
- certain surgical contacts of patients diagnosed with CJD
- highly transfused recipients.

Data on the following risk groups are not held by PHE, but are held by other organisations:

- bleeding disorder patients who received plasma products between 1990 and 2001 (UKHCDO)
- recipients of human derived growth hormone before 1985 (ICH)
- patients who could have received a dura mater graft before August 1992 (data not currently collected)
- people who have been treated with gonadotrophin sourced from humans before 1973 (data not currently collected)
- family risk of genetic prion disease (NPC).

The data from the UKHCDO are likely to be an underestimate of the true number of 'at risk' patients with bleeding disorders who received UK-sourced clotting factors, as there was incomplete reporting of identified 'at risk' patients by haemophilia centres to the UKHCDO database. Notified 'at risk' patients are given the option of removing their details from the UKHCDO database, and are then removed from the 'at risk' totals.

The data on 'at risk' patients who received human-derived human growth hormone held by the ICH is a slight underestimate of the total as a small number of these patients are not included in the ICH follow-up.

Table 1. Summary of all 'at risk' groups on which data are collected (as at 31 December 2013)

'At risk' Group	Identified as 'at risk'	Number notified as being 'at risk'		Cases	Asymptomatic infections [b]
		All	Alive		
Recipients of blood from who later developed vCJD	67	27	15	3	1
Blood donors to who later developed vCJD	112	107	104	–	–
Other recipients of blood components from these donors	34	32 [c]	19 [c]	–	–
Plasma product recipients (non-bleeding disorders) who received UK sourced plasmsa products 1980-2001	11	10	4	–	–
Certain surgical contacts of patients diagnosed witih CJD	154	129 [d]	113 [e]	–	–
Highly transfused patients	11	10	6	–	–
Total for 'at risk' groups where PHE holds data	**389**	**315** [f]	**261** [f]	**3**	**1**
Patients with bleeding disorders who received UK-sourced plasma products 1980-2001 [a]	3,875	National information incomplete	National information incomplete	–	1
Recipients of human-derived growth hormone [a]	1,883	1,883	1,504	75	–
Total for all 'at risk' groups [a]	**6147**	**At least 2198**	**At least 1765**	**78**	**2**

a. These are minimum figures. Central reporting for bleeding disorder patients is incomplete, and seven patients have opted out of the central UK Haemophilia Centre Doctors' Organisation database. A small number of 'at risk' growth hormone recipients are not included in the Institute of Child Health study. Not all of 'at risk' growth hormone recipients have been notified. There is no central record of who has been informed.
b. An unsymptomatic infection is when an individual does not exhibit any of the signs and symptoms of CJD in life but abnormal prion protein indicative of CJD infection has been found in tissue obtained from them. In these cases the abnormal prion protein was identified during post mortem after the individuals had died of other causes.
c. One patient notified by proxy. d. Four of these notified by proxy. e. Two of these notified by proxy. f. Includes patients notified by proxy.

Variably Protease-Sensitive Prionopathy

Professor James W Ironside and Dr Mark W Head,

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Variably protease-sensitive prionopathy (VPSPr) is the most recently identified human prion disease, first described in the USA by Gambetti *et al.* in 2008 as "a novel human disease with abnormal prion protein sensitive to protease" [1]. Since then, similar cases have been identified in other countries; the National CJD Research and Surveillance Unit has identified nine cases in the UK, three of which have been identified retrospectively and the others prospectively from samples and data collected since 1991 [2-6]. Other candidate cases are currently under investigation.

Patients with VPSPr have no identified risk factors for acquired human prion disease and no associated mutations in the prion protein gene (PRNP) coding sequence have been found. In the original description a proportion of the patients had family histories of ill-defined dementia, but this has not been a feature in more recently identified cases [1,2,6]. VPSPr affects patients in the same age range as sporadic Creutzfeldt-Jakob disease (sCJD), occurring mostly in patients over the age of 60. The clinical features are more varied than in sCJD and include movement abnormalities, cognitive decline and unsteadiness while walking. The clinical illness is longer than for sCJD; most patients survive for over a year before succumbing to the illness. Diagnostic clinical criteria are therefore difficult to establish, and further work is required on this topic since this disease is likely to be under-ascertained [2,6].

Like sCJD, VPSPr occurs in all genetic groups defined by the polymorphism at codon 129 in the PRNP gene, ie MM, MV and MV. Unlike sCJD, there is a preponderance of the codon 129-V haplotype. VPSPr has distinctive neuropathological features, the most typical of which are microplaques that occur in a target-like arrangement and are particularly common in the cerebellum. These microplaques show differential staining with a panel of different anti-PrP antibodies, allowing a distinction from both the common sCJD VV2 and the rare sCJD VV1 subtypes [1,2,5,6]. The most distinctive and defining feature of VPSPr is the biochemistry of the abnormal prion protein in the brain, which is only poorly resistant to proteolytic digestion, yielding a low abundance, truncated 8kDa (approx) band in Western blot assays [1]. This fragment is often accompanied by a faint ladder of bands extending into the 18-30kDa range [1,2]. Some cases of VPSPr also show a sCJD-like pattern on Western blot analysis for abnormal prion protein, often in the cerebellum, suggesting molecular overlaps between VPSPr and sCJD [6,7].

Further work is required to fully establish the epidemiology, clinical and pathological diagnostic criteria and transmission characteristics of VPSPr. The Advisory Committee on Dangerous Pathogens Transmissible Spongiform Encephalopathy (ACDP TSE) Subgroup concluded that until further research can demonstrate how transmissible VPSPr may be, it would be advisable to add this novel form of human prion disease to the infection control guidance for CJD and other related disorders.

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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)	－					

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
E型肝炎のプライマリホストは豚で、人獣共通感染症であると考えられている。 未調理もしくは簡易調理された豚肉製品が主要感染経路と考えられており、他にE型肝炎ウイルスに汚染された血液製剤やHEV感染ドナーの肝臓移植があるが、ほとんどの場合、感染源と感染経路は不明である。 2011年5月、42歳の女性が、倦怠感、下痢、吐き気、嘔吐が1週間つづき、受診したところ、肝機能検査で肝炎を示した。 患者のHEV IgMおよびIgG血清学的検査結果は陽性であり、HEV遺伝子型3と逆転写PCRおよび配列決定によって同定された。 検査によりエプスタイン-バーウイルス、肝炎ウイルスA、BおよびCを含むウイルス性肝炎および肝細胞性黄疸、自己免疫性肝炎は除外された。 多くのHEV患者と同じように、12週間後には臨床的に回復し、肝機能検査は8週間後に正常化した。 この症例における感染源、感染経路は不明であるが、発症4週間前に腹腔鏡下虫垂切除術を受けており、血液製剤の投与は受けていなかったが、血栓塞栓予防として低分子ヘパリンの投与を受けていた。 ヨーロッパや北米で使用されるヘパリンはブタ腸粘膜から生成されるが、製造工程の詳細が開示されていないため、ヘパリンの製造業者によるHEVの除去または不活性化が十分であるかどうかを評価することは困難である。著者らの知る限り、臨床グレードのヘパリンのウイルス汚染についての検討はなされていないことから、ヘパリンがHEV感染の原因との仮説を立てた。 この可能性を調べるために、虫垂炎の治療時に使用されていたダルテパリンナトリウムのバッチを含む、ヘパリンの複数のバッチをスクリーニングした。 分析した全ての試料が試験された3つのウイルス(HEV、PCV2、PPV)について陰性であったことを考えると、ヘパリンの製造プロセスは、ウイルスを除去するのに十分であると推察される。	

報告企業の意見	今後の対応
術後にHEV感染がみられた患者の感染源についてヘパリンが原因との仮説を立てたが、ヘパリンの製造プロセスはウイルスを除去するのに十分であると推察されると結論づけていることから、現時点で安全性に関する懸念はないものと考える。	今後とも安全性情報等に留意していく。

Powassan Virus Encephalitis, Minnesota, USA

To the Editor: Birge and Sonnesyn report the first death of a Minnesota resident caused by Powassan virus (POWV) (*1*). However, they provide an inaccurate description of several critical diagnostic and surveillance issues concerning POWV.

The 17 POWV infections detected in Minnesota residents from 2008 through 2011 (6 cases were identified through 2010, not 8 as reported by Birge and Sonnesyn) (Minnesota Department of Health [MDH], unpub. data) were found through enhanced surveillance. Health alerts to Minnesota medical providers described POWV as a possible etiologic agent for viral meningitis and encephalitis. Providers consulted with MDH on suspected cases and submitted serum and cerebrospinal fluid specimens to MDH. MDH conducted serologic testing for endemic arboviruses (including POWV) and reverse transcription PCR (RT-PCR) for flaviviruses and POWV. MDH would not have detected any POWV infections without enhanced surveillance. Limited field studies also identified POWV-infected ticks in 4 Minnesota counties (not 2 as reported [*1*]) (MDH, unpub. data).

Commercial laboratories do not provide testing for POWV, and only a few state health department laboratories and the Centers for Disease Control and Prevention offer testing. Serologic testing (enzyme immunoassay with plaque-reduction neutralization testing confirmation) is preferred (*2*) because POWV RT-PCRs are not validated, and the short viremic periods of flaviviruses limit their usefulness (*3*).

Few POWV infections are identified by lineage (prototype vs. deer tick virus); Minnesota's first case in 2008 was identified as a deer tick virus infection, but the lineage was unknown for the other 16 cases. However, many case-patients had likely exposure to *Ixodes scapularis* ticks (blacklegged ticks), the tick species associated with deer tick virus transmission, and viruses from all POWV-positive tick pools were confirmed as deer tick virus by sequencing. The distribution of the 2 lineages in North America is poorly understood, and most cases likely go undetected without specific POWV surveillance efforts.

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Hepatitis E Virus and Porcine-derived Heparin

To the Editor: Cases of sporadic, locally acquired hepatitis E have been increasingly identified in industrialized countries over the last few years (*1*). In this setting, hepatitis E is thought to be a zoonotic infection, with pigs as the primary host. Consumption of uncooked or lightly cooked pork meat products is thought to be a key route of infection, but other routes of transmission have been documented (*2*). For example, there have been several iatrogenic cases after transfusion of hepatitis E virus (HEV)–contaminated blood products (*3*) and transplantation of an HEV-infected donor liver (*4*). However, in most cases the source and route of infection are uncertain.

In May 2011, a 42-year-old woman sought care at the Royal Cornwall Hospital in Truro, United Kingdom, for a 1-week history of malaise, diarrhea, nausea, and vomiting. Physical examination results were normal. Her liver function test results, however, indicated hepatitis: alanine aminotransferase 2,785 IU/L (reference range 10–36 IU/L), alkaline phosphatase 319 IU/L (reference range 30–130 IU/L), and bilirubin 30 μmol/L (reference range <21 μmol/L). HEV IgM and IgG serologic test results for the patient were positive, and HEV genotype 3 was identified in her blood by reverse transcription PCR and sequencing. Other causes of viral hepatitis and hepatocellular jaundice, including hepatitis viruses A, B, and C; Epstein-Barr virus; and autoimmune hepatitis, were excluded by testing. As with most immunocompetent persons with HEV, the patient made an uneventful clinical recovery after 12 weeks, and her liver function tests returned to normal after 8 weeks.

Table. Heparin samples tested for hepatitis E virus, porcine circovirus 2, and porcine parvovirus*

Producer, proprietary name/other names, batch or lot no.	Use	Excipient	Concentration	Quantity tested, IU	95% upper CL, /IU†
Sanofi‡					
Clexhane/enoxaparin	Injection	H_2O			
ILA01			20 mg/0.2 mL	6,000	0.0006
34751			40 mg/0.4 mL	4,000	0.0009
OLC56			80 mg/0.8 mL	8,000	0.0005
ILA53			60 mg/0.6 mL	6,000	0.0006
OLC07			100 mg/ mL	10,000	0.0004
12255			120 mg/0.8 mL	12,000	0.0003
Pfizer§					
Fragmin/dalteparin sodium	Injection	H2O pH adjusted with HCl or NaOH			
12339A01			5,000 IU/0.2 mL	15,000	0.0002
12338A01			5,000 IU/0.2 mL	15,000	0.0002
12327B01			5,000 IU/0.2 mL	15,000	0.0002
12257A01			5,000 IU/0.2 mL	15,000	0.0002
12444A01			5,000 IU/0.2 mL	10,000	0.0004
12122C01			7,500 IU/0.3 mL	7,500	0.0005
74774D51			10,000 IU/0.4 mL	10,000	0.0004
74871B51			12,500 IU/0.5 mL	25,000	0.0001
74779G51			12,500 IU/0.5 mL	12,500	0.0003
74871B51			12,500 IU/0.5 mL	12,500	0.0003
74743C52			15,000 IU/0.6 mL	30,000	0.0001
74755A51			15,000 IU/0.6 mL	30,000	0.0001
74832A52			15,000 IU/0.6 mL	15,000	0.0002
74832A01			15,000 IU/0.6 mL	15,000	0.0002
X08580	¶	¶	100,000 IU/4 mL	100,000	0.00004
Wockhart#					
Monoparin	Injection	H2O pH adjusted with HCl or NaOH			
PK40319			1,000 IU/mL	20,000	0.0002
3090			1,000 IU/mL	10,000	0.0004
Hepsal	Flushing	NaCl, H2O, HCl, and NaOH			
5000090			10 IU/mL	120	0.03
91180			50 IU/mL	50	0.07
1069			200 IU/mL	200	0.02
Leo**					
Heparin sodium	Intravenous flushing	Benzyl alcohol, methyl parahydroxybenzoate, propyl parahydroxybenzoate, sodium citrate, NaCl, and H_2O			
DD7314			100 IU/mL	200	0.02
CC4338			100 IU/mL	200	0.02
Celgene††,					
Refludan/Lepirudin, 25561611A‡‡	Powder used for solution for injection/infusion	Mannitol, NaOH, and H_2O	12.5 mg/mL	NA	NA
Total quantity tested	NA	NA	NA	404,270	0.000009

*NA, not applicable.
†The 95% upper confidence limit of the probability of a virus-positive result per IU was calculated on the basis of the quantity tested for each batch. This was estimated, assuming perfect detection of a Poisson process, by using Fisher exact test. For the pooled result, the upper 95% estimate is ≈1 per 100,000 IU.
‡Sanofi (Guilford, UK).
§Pfizer (Sandwich, UK).
¶Multidose vials used for injection, Excipients: Benzyl alcohol and H_2O.
#Wockhart (Wrexham, UK).
**Leo (Buckinghamshire, UK).
††Celgene (Uxbridge, UK).
‡‡Non-porcine–derived anticoagulant alternative.

The source and route of infection in this case was uncertain. A detailed in-person assessment of potential risk factors was undertaken with the patient. She had not traveled outside the United Kingdom in the previous 3 months. She rarely ate pork products (well cooked bacon only); ate no shellfish; and had no workplace, domestic, or recreational exposure to pigs or their effluent. However, 4 weeks before symptom onset, the patient had

acute appendicitis for which she underwent an uneventful laparoscopic appendectomy and was hospitalized for 2 days. During hospitalization she received no blood products, but, as prophylaxis for thromboembolic disease, she received 2 doses (5,000 IU each) of low–molecular weight heparin (Fragmin [dalteparin sodium]; Pfizer, Sandwich, UK) by subcutaneous injection. All heparins used in Europe and North America are isolated from porcine intestinal mucosa (*5*). The exact purification methods used by heparin manufacturers are deemed commercially sensitive and not in the public domain, so it is impossible to evaluate whether the isolation process would be sufficient to remove or inactivate any contaminating HEV. The virus is known to be acid and alkaline stable; heat sensitivity varies, depending on strain and heating conditions, although heating at 60°C for 1 hour is generally sufficient to achieve 96% inactivation (*6*). To our knowledge, no investigation has determined whether clinical-grade heparin could contain viral contaminants. Thus, we hypothesized that the heparin the patient received might have been the source of her HEV infection.

To examine this possibility, we screened multiple batches of hospital pharmacy–grade heparin for the presence of HEV, including batches of dalteparin sodium that were in use at the hospital when the patient received treatment for appendicitis. Before testing, the samples were ultracentrifuged to concentrate any contaminating virus and enable the removal of excipients, which could inhibit the assay. We tested samples by quantitative reverse transcription PCR (*7*) in parallel with positive World Health Organization HEV RNA standard spiked controls, which showed the limit of detection (LOD) to be 500 IU/mL, regardless of the heparin's excipient or concentration. This LOD is within the range used by collaborating laboratories in the establishment of the World Health Organization HEV RNA standard (http://whqlibdoc.who.int/hq/2011/WHO_BS_2011.2175_eng.pdf). In addition, we tested the heparin samples for porcine circovirus 2 (PCV2), an identified adventitious agent of several rotavirus vaccines (*8*) and porcine parvovirus (PPV) (*9*), a known contaminate of porcine clotting factor hyate:C (*10*). Although samples were tested in parallel with PCV2- and PPV- positive spiked controls, we were unable to calculate the LOD for these assays because international standards are not available for these viruses.

All samples tested negative for HEV, PCV2, and PPV (Table), which would indicate the patient's source of HEV infection is unlikely to have been the heparin. However, we cannot rule out low-level viral contamination below the sensitivity of the assay. We also cannot exclude that the negative test results were related to the Poisson effect. Given that all samples analyzed were negative for all 3 viruses tested, it seems likely that the heparin manufacturing process is sufficient to remove viral contaminants. However, this may not necessarily be the case for other porcine-derived products, such as porcine insulin, factor VIII C, pancreatin, and poractant alfa. Further investigation is warranted to exclude these products as potential sources of HEV infection.

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医薬品 研究報告 調査報告書

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				2013. 11. 8	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況	Geurtsvankessel CH, Islam Z, Mohammad QD, Jacobs BC, Endtz HP, Osterhaus AD. Clin Infect Dis. 2013 Nov;57(9):1369-70. doi: 10.1093/cid/cit512. Epub 2013 Jul 30.	公表国
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研究報告の概要

○E型肝炎とギランバレー症候群(GBS)
HEV感染は肝疾患の他に、GBSや上腕神経炎のような神経症状を呈することがある。GBS患者には高い頻度で*Campylobacter jejuni*、サイトメガロウイルスなどの先行感染が認められる。多くの発展途上国ではGBSに先行する感染症について調査されていなかったことから、HEVジェノタイプ1感染及びGBSの両方がよく見られるバングラデシュにおいて、GBS患者の症例対照研究を行った。
2006年7月～2007年6月の間、ダッカの病院でGBS患者を連続して100人登録し、1患者につき2人の対照者(患者と同じ家庭で生活する家族[FC]及び年齢、性別、入院期間が適合した同じ病棟の他の神経疾患患者[ONDC])を設定した。GBS患者の平均年齢は24歳、ONDCは24歳、FCは33歳であった。GBS患者のHEV IgG抗体の陽性率(44%)は、対照と同程度であった(ONDC;46%及びFC;41%)が、対照的にHEV IgM抗体の陽性率は、対照(ONDC;2%及びFC;0%)と比較しGBS患者(11%)で有意に高かった。IgM抗体陽性者1人からHEV RNAが検出され、ジェノタイプ1と分類された。
本研究により発展途上国におけるGBSと、それに先行するHEV感染の関連が初めて示された。2000年にバングラデシュからポリオが根絶されて以来、GBSは急性弛緩性麻痺の最も一般的な原因となっており、輸入ポリオ症例が未だに報告される現在、HEV関連GBSと区別するための適切な診断法が必要である。

報告企業の意見	今後の対応
バングラデシュにおいてギランバレー症候群患者のHEV IgM及びIgG抗体の陽性率を調査したところ、対照群に比べてIgM抗体陽性率が有意に高く、GBSとHEV感染の関連が示されたとの報告である。	日本赤十字社では、厚生労働科学研究「経口感染によるウイルス性肝炎(A型及びE型)の感染防止、病態解明、遺伝的多様性及び治療に関する研究」班と共同して、献血者におけるHEV感染の疫学調査を行っている。今後もHEV感染の実態に関する情報の収集及び安全対策に努める。

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

Hepatitis E and Guillain-Barré Syndrome

To the Editor—Hepatitis E virus (HEV) infection is the most common cause of acute hepatitis worldwide. Whereas in developed countries it usually presents as a self-limiting disease caused by genotype 3, genotype 1 and 2 infections in resource-limited countries are associated with considerable morbidity and mortality [1]. Besides liver disease, neurologic manifestations may occur, such as Guillain-Barré syndrome (GBS) and brachial neuritis [2]. GBS is the most common cause of acute neuromuscular paralysis in countries where poliomyelitis has been eliminated [3]. GBS patients frequently report preceding gastrointestinal or respiratory illnesses, such as those caused by *Campylobacter jejuni*, cytomegalovirus, Epstein-Barr virus, and *Mycoplasma pneumoniae* [4], but in many developing countries antecedent infections have not been investigated. Recent reports on the global burden of HEV infection prompted us to perform a case-control study among GBS patients in Bangladesh, where both HEV genotype 1 infection and GBS are commonly diagnosed [3, 5].

A prospective case-control study was conducted between July 2006 and June 2007 enrolling 100 consecutive GBS cases from Dhaka Medical College Hospital, Bangabandhu Sheikh Mujib Medical University, and Dhaka Central Hospital in Dhaka, Bangladesh. Two controls per case were recruited: one among the family members of the patient living in the same household (family control [FC]); and one was an age-, sex-, and day-matched patient hospitalized in the same ward with another neurologic disease not related to recent infections (other neurological disease control [ONDC]). Written informed consent was obtained from all patients and controls. The project protocol was reviewed and approved by the institutional

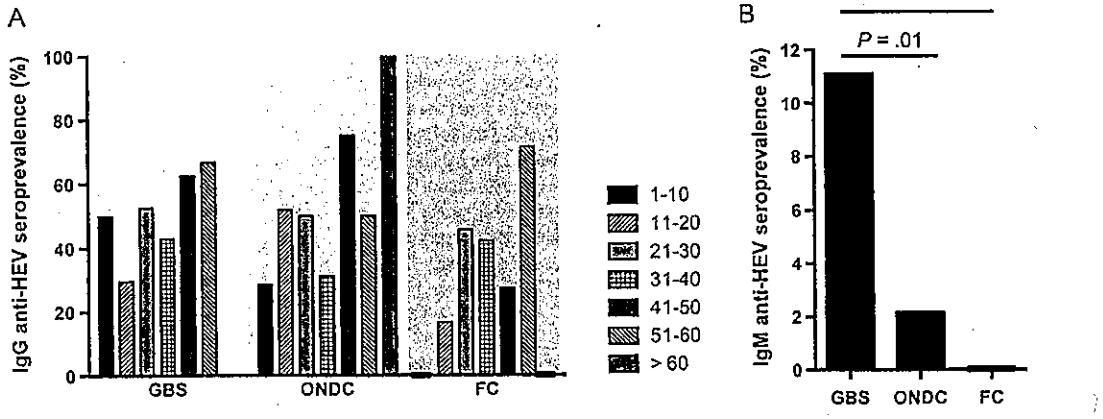

Figure 1. Anti–hepatitis E virus (HEV) immunoglobulin G (IgG) and immunoglobulin M (IgM) seroprevalence. *A*, Percentage of patients and controls within each age group with anti-HEV IgG serum antibodies. *B*, Percentage of patients and controls with anti-HEV IgM serum antibodies. Abbreviations: FC, family control; GBS, Guillain-Barré syndrome; HEV, hepatitis E virus; IgG, immunoglobulin G; IgM, immunoglobulin M; ONDC, other neurological disease control.

review board and the ethical com-mittees at Dhaka Medical College and Hos-pital, Bangladesh.

Ages of GBS patients ranged from 2 to 65 years (mean, 24 [standard deviation {SD}, 14]), and of ONDC from 4 to 65 years (mean, 24 [SD, 14]). FCs were significantly older ($P < .001$) as their ages ranged from 11 to 57 years (mean, 33 [SD, 10]). Seventy-two percent of GBS patients and 74% of ONDCs were male, whereas 47% of the FCs were male. HEV-specific serum immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies were detected with a commercial enzyme-linked immunosorbent assay (ELISA; Wantai, Beijing, China). The IgG seroprevalence is depicted by age group in Figure 1*A*. It is in line with earlier reports [5] and illustrates the high prevalence of HEV infection. The mean IgG seroprevalence among GBS patients (44%), ONDCs (46%), and FCs (41%) was similar between patients and controls (data not shown). In contrast, anti-HEV IgM seroprevalence (Figure 1*B*) was significantly higher among GBS patients as compared to ONDCs ($P < .01$) and FCs ($P < .001$). IgM levels directed against other viral pathogens and *Mycoplasma* were measured as well to control for cross-reactivity (data not shown). IgM seropositive individuals for HEV RNA [6], yielded 1 positive serum sample classified as HEV genotype 1, with a viral load of 6.29 log IU/mL. The sequence identified was deposited into GenBank (accession number KF192078).

These data for the first time show an association between GBS and antecedent HEV infection in a unique case-control study in a developing country. Additional prospective case-control studies should confirm this association, which would add GBS to the disease burden associated with HEV infection. Since poliomyelitis was eradicated from Bangladesh in 2000, GBS has been the most prevalent cause of acute flaccid paralysis. Sporadic cases of imported poliomyelitis are still described and may be clinically misdiagnosed as HEV-associated GBS, emphasizing the need for adequate diagnostic methods to distinguish between these disease entities.

Notes

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Potential conflicts of interest. A. D. M. E. O. is chief science officer for Viroclinics Biosciences BV, a contract research organization that collaborates with pharmaceutical companies. All other authors declare no potential conflicts.

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

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別紙様式第2-1

No. 16

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2014. 1. 14	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況	ProMED 20140109.2162194	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)			ドイツ

研究報告の概要

○日本におけるデングウイルス(DENV)感染
2013年9月9日、2週間の日本旅行から帰国した51歳ドイツ人女性が、40℃の発熱、嘔気、斑点状丘疹を呈してベルリンの病院に入院した。患者は笛吹市においてブドウ狩りをした際、複数箇所蚊に刺されたことを申告している。発症7日後の血清サンプルにおいてDENV IgMとIgG抗体及びNS1抗原がすべて陽性であったことから、急性DENV感染が示唆された。DENV RNAのリアルタイムRT-PCR及びフラビウイルス共通遺伝子のRT-PCRは陰性であった。入院一週間後、患者は回復して退院した。発症110日後の血清サンプルにおいて、DENV IgG抗体価の有意な減少とNS1抗原及びIgM抗体の陰性結果が示された。
患者の行動やDENV潜伏期間を考慮すると、日本で感染した可能性が高く、日本からドイツに持ち込まれた初のDENV感染症検査確定症例となる。従って、2013年晩夏に日本から帰国した発熱患者における鑑別診断には、デング熱を考慮すべきである。さらに、日本のDENV感染について適切な予防措置を早期に実施するために、詳細な調査が必要である。

報告企業の意見	今後の対応
日本から帰国したドイツ人旅行者がデング熱を発症し、日本においてDENVに感染した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

Published Date: 2014-01-09 17:02:15
Subject: PRO/EDR> Dengue/DHF update (03): Germany (Berlin) ex Japan, RFI
Archive Number: 20140109.2162194

DENGUE/DHF UPDATE (03): GERMANY (BERLIN) ex JAPAN, REQUEST FOR INFORMATION
****

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: Thu 9 Jan 2014
From: Jonas Schmidt-Chanasit <jonassi@gmx.de> [edited]

Autochthonous dengue virus infection in Japan

A previously healthy 51-year-old woman sought treatment in a hospital in Berlin on 9 Sep 2013 after returning from travel to Japan (Honshu). Since 3 Sep 2013 she suffered from fever up to 40 deg C [104 deg F] and nausea, followed by a maculopapular rash. 9 days before admission she had returned from a 2 week round trip (19-31 Aug [2013]) from Japan [with sites visited on the following dates in August 2013]:

19-21 Ueda
21-24 Fuefuki
24-25 Hiroshima
25-28 Kyoto
28-31 Tokyo

She reported several mosquito bites while grape picking in Fuefuki. She flew nonstop from Frankfurt (International Airport) to Tokyo (Narita International Airport) and [back the same way]. Among several other diseases, dengue fever was suspected, because of the clinical picture. Initially, the 1st serum sample collected 7 days after disease onset gave a positive result in the dengue virus (DENV) IgM and IgG antibody tests (IFA [indirect fluorescent antibody] and rapid test), as well as for DENV NS1 antigen (ELISA and rapid test) demonstrating an acute DENV infection of the patient. Real-Time RT-PCR for DENV RNA and generic flavivirus RT-PCR were negative. After one week in hospital the patient was discharged with a characterization of restitutio ad integrum [total recovery]. A follow-up serum sample was collected in December 2013, because this acute case of dengue fever imported from Japan was considered very unusual. This 2nd serum sample collected 110 days after disease onset revealed a significant DENV IgG titer decrease (IFA) and negative results for DENV NS1 antigen (ELISA and rapid test) and DENV IgM (IFA and rapid test), respectively.

This is the 1st laboratory confirmed case of DENV infection imported from Japan to Germany. Most likely, according to the patient's activities and DENV incubation period, the infection was acquired in Japan. Thus, differential diagnosis in febrile returning travelers from Japan (Honshu) in late summer [2013] should include dengue fever. In addition, the autochthonous transmission of DENV in Japan should be further investigated to take adequate prevention measures early.

--
Jonas Schmidt-Chanasit, Petra Emmerich, Dennis Tappe, Stephan Gunther
Bernhard Nocht Institute for Tropical Medicine, WHO Collaborating Centre for Arbovirus and Haemorrhagic Fever Reference and Research, National Reference Centre for Tropical Infectious Diseases, Hamburg, Germany
<jonassi@gmx.de>

Daniel Sagebiel, Kathrin Hentsche
State Office of Health and Social Affairs, Berlin, Germany

Christina Frank, Irene Schuneberg, Klaus Stark
Robert Koch Institute, Department for Infectious Disease Epidemiology, Berlin, Germany

[This case is, indeed, highly unusual because it implicates a dengue virus infection acquired locally in Japan, and indicates that there must have been other infected individuals in the area where she became infected. This is the 1st

case of locally acquired dengue virus infection in Japan that ProMED-mail has ever posted.

There have been several examples of individuals who have become infected with dengue viruses in Southeast Asia and Africa and subsequently become ill in Japan, indicating that viremic individuals have been bringing dengue virus into the Japanese islands.

It would be of interest to know if there have been other cases of dengue virus infection acquired locally in Japan during the summer of 2013, and to know about the status of populations of dengue virus vector mosquitoes (_Aedes aegypti_ and _Ae. albopictus_) in the areas that the patient visited.

ProMED-mail thanks Dr Jonas Schmidt-Chanasit and colleagues for sending in this 1st-hand report.

A HealthMap/ProMED-mail map of Japan can be accessed at http://healthmap.org/r/62Cn. - Mod.TY]

See Also

2012

Dengue/DHF update 2012 (12) 20120319.1074013
2010

Dengue/DHF update 2010 (44) 20100826.3010
2008

Dengue/DHF update 2008 (35): Japan ex Cote d'Ivoire 20080818.2573
.....ml/ty/mj/ml

医薬品　研究報告　調査報告書

<table>
<tr><td>識別番号・報告回数</td><td colspan="2"></td><td>報告日</td><td>第一報入手日
2014年1月10日</td><td>新医薬品等の区分
該当なし。</td><td>総合機構処理欄</td></tr>
<tr><td>一　般　的　名　称</td><td colspan="2">別紙のとおり。</td><td rowspan="2">研究報告の
公表状況</td><td rowspan="2">デング熱の国内感染疑い例の症例について
（厚生労働省、2014年1月10日）</td><td rowspan="2">公表国
日本</td><td rowspan="2"></td></tr>
<tr><td>販売名（企業名）</td><td colspan="2">別紙のとおり。</td></tr>
<tr><td>研究報告の概要</td><td colspan="5">問題点：60年以上ぶりにデング熱の日本国内感染を否定できない症例が確認された。

ドイツのロベルト・コッホ研究所から、2013年8月下旬に日本を周遊して帰国した51歳の生来健康なドイツ人女性で、デング熱の感染が確認されたとの情報提供がなされた。患者は、フランクフルト-日本間を直行便で往復しており、発症7日後の血清でデングウイルスIgM、同IgG及びデングウイルスNS1抗原が陽性であったことからデングウイルスの急性感染であることが示された。日本の専門家による検討の結果、当該患者が感染した場所の特定には至らなかったが、日本国内で感染した可能性は否定できないとの結論が得られた。</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</td><td colspan="3">今後の対応</td><td></td></tr>
<tr><td colspan="3">別紙のとおり。</td><td colspan="3">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><td></td></tr>
</table>

別紙

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤人免役グロブリン、⑥乾燥ペプシン処理人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン、⑧乾燥スルホ化人免疫グロブリン、⑨乾燥スルホ化人免疫グロブリン、⑩乾燥スルホ化人免疫グロブリン、⑪乾燥スルホ化人免疫グロブリン*、⑫乾燥濃縮人活性化プロテインC、⑬乾燥濃縮人血液凝固第Ⅷ因子、⑭乾燥濃縮人血液凝固第Ⅷ因子、⑮乾燥濃縮人血液凝固第Ⅷ因子、⑯乾燥濃縮人血液凝固第Ⅸ因子、⑰乾燥濃縮人血液凝固第Ⅸ因子、⑱乾燥濃縮人血液凝固第Ⅸ因子、⑲乾燥抗破傷風人免疫グロブリン、⑳抗HBs人免疫グロブリン、㉑抗HBs人免疫グロブリン、㉒抗HBs人免疫グロブリン、㉓トロンビン、㉔フィブリノゲン加第ⅩⅢ因子*、㉕フィブリノゲン加第ⅩⅢ因子、㉖乾燥濃縮人アンチトロンビンⅢ、㉗ヒスタミン加人免疫グロブリン製剤、㉘人血清アルブミン*、㉙人血清アルブミン*、㉚乾燥ペプシン処理人免役グロブリン*、㉛乾燥濃縮人アンチトロンビンⅢ
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ボルヒール*、㉕ボルヒール組織接着用、㉖アンスロビンP500注射用、㉗ヒスタグロビン皮下注用、㉘アルブミン20%化血研*、㉙アルブミン5%化血研*、㉚静注グロブリン*、㉛アンスロビンP1500注射用
報告企業の意見	デング熱はデングウイルス感染を原因とする感染症である。デングウイルスは40～60nmの球形、核酸は一本鎖RNA、エンベロープを有するウイルスで、蚊によって媒介されるが、人から人への直接感染はない。 今回の報告は、ドイツ-日本間を直行便で往復した患者が、ドイツ国内でデング熱を発症し、日本でのデングウイルス感染を否定できない、との報告である。 上記製剤の製造工程には、冷エタノール分画工程、ウイルス除去膜ろ過工程及びペプシン処理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導入されており、各工程のウイルスクリアランス効果は「血漿分画製剤のウイルスに対する安全性確保に関するガイドライン（医薬発第1047号、平成11年8月30日）」に基づく、モデルウイルスを用いたウイルスプロセスバリデーションにより確認されている。今回報告したデングウイルスのモデルウイルスには、エンベロープの有無、核酸の種類等から、ウシウイルス性下痢ウイルス（BVDV）が該当すると考えられるが、上記工程のBVDVクリアランス効果については上記バリデーションにより確認されている。また、これまでに上記製剤による新規デングウイルスへの感染報告例は無い。 以上の点から、上記製剤はデング熱に対する安全性を確保していると考える。

*：現在製造を行っていない

INF2013-010

健感発 0110 第 1 号
平成 26 年 1 月 10 日

各 { 都道府県 / 保健所設置市 / 特別区 } 衛生主管部（局）長 殿

厚生労働省健康局結核感染症課長

デング熱の国内感染疑いの症例について
（情報提供及び協力依頼）

日頃より感染症対策へのご協力を賜り厚くお礼申し上げます。

デング熱（四類感染症）については、東南アジア諸国等を旅行した際に、現地で感染し、帰国後発症した輸入症例が、昨今では、年間200例前後報告されています。今般、ドイツのロベルト・コッホ研究所より、昨年８月下旬に日本を周遊して帰国した後、発熱、皮疹等の症状を呈したドイツ人について検査を実施したところ、デング熱に感染していたことが確認された旨、一報がありました。

ドイツから提供された情報に基づき、専門家による検討を行った結果、当該患者が、日本国内においてデング熱に感染した可能性は否定できないとの結論に至りましたので、情報提供します（事例の概要は別添１のとおり。日本への一報後、ProMedに公表されたもの。）。

デング熱は、ヒトからヒトへの直接的な感染はなく、主として、ネッタイシマカ（日本国内での生息は確認されていない）やヒトスジシマカを介して、ヒトー蚊ーヒトという経路で感染が成立します。日本国内での感染例は過去60年以上にわたり認められていませんが、ヒトスジシマカは北海道と青森県を除く全国に分布しています。わが国においても、急性期の患者（輸入症例）の血液を吸血したヒトスジシマカに刺されることによりデング熱に感染するといった散発事例が発生する可能性は皆無ではなく、今回のドイツ人患者についても、仮に、日本国内で感染したとすれば、そのような感染経路が示唆されるところです（なお、国内で行われている捕集蚊のサーベイランスにおいては、これまでデングウイルスが検出されたという報告はありません。）。

つきましては、本事例について、貴管内の医療機関等関係者への情報提供をお願いします。また、引き続き、海外渡航者の注意を喚起するとともに、海外からの帰国者に本疾病の患者が発生した場合は、患者が媒介蚊に刺咬されないように注意し、万一、患者家族等から発症する者があった場合には、速やかに医療機関の受診と保健所への報告を行っていただくよう助言をお願いします。

なお、別添２・３のとおり本疾患に関するQ&Aなど、資料を取りまとめましたの

でご活用ください。

参考資料
別添１：ProMed概要
別添２：デング熱について（ファクトシート）
別添３：デング熱に関するQ&A

デング熱に関する技術的な問い合わせ先：
国立感染症研究所ウイルス第一部第二室長　高崎智彦
電 話：03－5285－1111

別添1

ProMed（2014年1月10日）概要

デングウイルス感染ー日本（本州）から帰国したドイツ人旅行者におけるデングウイルス感染

日本（本州）旅行から帰国した生来健康な女性（51歳）が、2013年9月9日にドイツ（ベルリン）の病院を受診。9月3日より、40度の熱、嘔気、続いて、斑状丘疹状皮疹が出現。入院9日前に、2週間の日本旅行（8月19～31日）から帰国。旅程は以下のとおり。

8/19-21　長野県上田市
8/21-24　山梨県笛吹市
8/24-25　広島県
8/25-28　京都府
8/28-31　東京都

患者は、笛吹市において、複数個所、蚊に刺されたと申告している。フランクフルトー東京間の往復は直行便を利用。鑑別診断の結果、臨床像より、デング熱を疑った。発症後7日目に採取された、第1回目の血清サンプルにおいて、デングウイルスIgM及びIgG抗体（間接蛍光抗体法、迅速試験）及びデングウイルスNS1抗原（ELISA法、迅速試験）ともに陽性であったことから、患者はデングウイルス急性感染であることが示された。デングウイルスRNA（リアルタイムRT-PCR法）及びフラビウイルス共通遺伝子（RT-PCR法）は陰性であった。入院1週間後、患者は回復して退院した。日本からのデング熱の輸入症例は極めて珍しい[1]ことから、2013年12月（発症後110日目）に第2回目の血清サンプルを採取し、デングウイルスIgG抗体（間接蛍光抗体法）が有意に減少、デングウイルスNS1抗原（ELISA法、迅速試験）及びIgM抗体（間接蛍光抗体法、迅速試験）が陰性との結果が得られた。

これは、日本からドイツに輸入され、実験室診断されたデングウイルス感染症の第一例目である。患者の行動履歴によれば、患者の行動やデングウイルスの潜伏期間を考慮すると、当該患者は日本でデング熱に感染した可能性が高い。以上より、2013年夏に日本（本州）から帰国した発熱を有する旅行者に対する鑑別診断では、デング熱が含まれることになる。さらに、日本におけるデングウイルス感染に対しては、早期に十分な予防法がとられるよう、より詳細な調査がなされるべきである。

投稿者：
Jonas Schmidt-Chanasit *et al.* Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany
Christiana Frank *et al.* Robert Koch Institute, Berlin, Germany

原典：http://www.promedmail.org/direct.php?id=2162194

[1]厚生労働省による注：過去60年以上、デング熱の日本国内における感染例は報告されていない。

別添２

デング熱について

1　疾病名

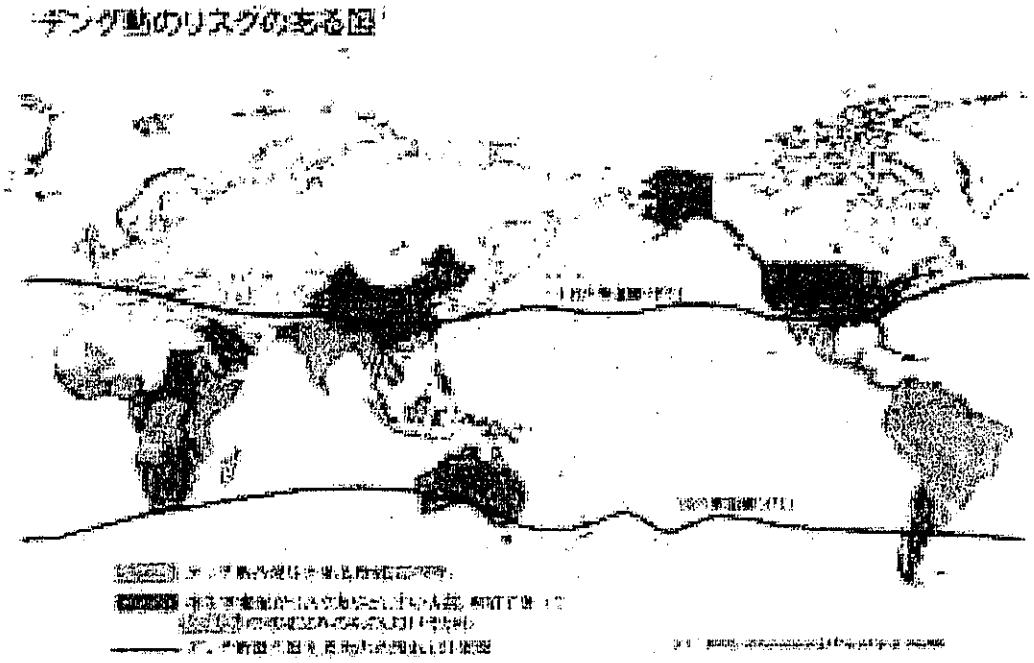
デング熱

2　病原体

デングウイルス（フラビウイルス科フラビウイルス属）

3　発生状況

・　アジア、中南米、アフリカなど熱帯・亜熱帯地域に広くみられる。
・　世界中で 25 億人以上が感染するリスクがあり、毎年約 5,000 万～１億人の患者が発生していると考えられている。
・　日本では、海外において感染し帰国後発症するいわゆる輸入症例が、近年は年間約 200 例報告されている。2012 年は 222 例、2013 年は 12 月 22 日までに 244 例報告されている。（※2012 年、2013 年ともに暫定値）
・　かつて国内流行がみられたものの、過去 60 年以上国内における感染報告はない。



（出典：FORTH）

4 感染経路

- ウイルスを保有した蚊に吸血された際に感染する。
- 媒介蚊は日中、屋外の幅広い地域に生息するヤブカ類である。
- 人-蚊-人の経路で感染が伝播するが、人から人への直接的な感染はない。

5 症状

- 突然の発熱、激しい頭痛、関節痛、筋肉痛、発疹など。
- 潜伏期間は２～15 日（多くは３～７日）
- デング熱患者の一部は重症化して出血やショック症状を発症することがある。

6 病原診断

- 血液等のサンプルからのウイルスの分離・同定及び RT-PCR によるウイルス遺伝子の検出
- 非構造蛋白抗原（NS1）の検出
- 特異的 IgM 抗体の IgM 捕捉 ELISA 法による検出
- 急性期及び回復期におけるウイルスに対する血清中 IgG 抗体価、中和抗体価の有意な上昇の確認

7 治療

- 特異的な治療法はなく、対症療法が主体となる。
- 有効な抗ウイルス薬はない。

8 予防法

- （特に感染リスクのある地域では）蚊との接触をさけること。具体的には、①長袖、長ズボンを着用し、素足でのサンダル履き等は避ける。②虫除け剤の使用等によって、屋外だけではなく屋内でも蚊に刺されないように注意する。③室内の蚊の駆除を心がける。④蚊幼虫の発生源を作らないように注意する。
- 実用化されたワクチンはない。

別添３

デング熱に関するQ&A

（第１版　平成26年１月10日作成）

１．一般の方向け

Q1　デング熱とは、どのような病気ですか？

デングウイルスが感染しておこる急性の熱性感染症で、発熱、頭痛、筋肉痛や皮膚の発疹などが主な症状です。

Q2　どのようにして感染するのですか？

ウイルスに感染した患者を蚊が吸血すると、蚊の体内でウイルスが増殖し、その蚊が他者を吸血することでウイルスが感染します（蚊媒介性）。ヒトからヒトに直接感染するような病気ではありません。また、感染しても発症しないことも多くみられます。

Q3　世界のどの地域が流行地ですか？

熱帯や亜熱帯の全域で流行しており、東南アジア、南アジア、中南米で患者の報告が多く、その他、アフリカ、オーストラリア、南太平洋の島でも発生があります。最も日本に近い流行地は台湾です。

Q4　日本国内での発生はありますか？

日本国内で感染した症例は、過去60年以上報告されていません。ただし、海外の流行地で感染し帰国した症例が近年では毎年200名前後報告されています。

Q5　感染を媒介する蚊は日本にもいますか？

主たる媒介蚊はネッタイシマカ（日本には常在していません）です。ただし、日本のほとんどの地域でみられるヒトスジシマカも媒介できます。

Q6　治療薬はありますか？

デングウイルスに対する特有の薬はありませんので、対症療法となります。

Q7　罹ると重い病気ですか？

デング熱は、体内からウイルスが消失すると症状が消失する、予後は比較的良好な感染症です。しかし、患者の一部に出血症状を発症することがあり、その場合は適切な治療がなされないと、致死性の病気になります。

Q8　どのように予防すればよいですか？

流行地にでかける際は、蚊に刺されないように注意しましょう。長袖、長ズボンの着用が推奨されます。また蚊の忌避剤なども現地では利用されています。

Q9　予防接種はありますか？

デング熱に有効なワクチンはありません。

Q10　海外旅行中（流行地域）に蚊に刺された場合はどこに相談すればよいですか？

すべての蚊がデングウイルスを保有している訳ではないので、蚊にさされたことだけで過分に心配する必要はありません。

ご心配な場合は、帰国された際に、空港等の検疫所でご相談ください。また、帰国後に心配なことがある場合は、最寄りの保健所等にご相談ください。なお、発熱などの症状がある場合には、医療機関を受診ください。

Q11　今回のドイツ人旅行者はどこで感染したと考えられますか？

ドイツ政府からの情報をもとに専門家と検討した結果、感染した場所の可能性として、日本国内の旅行地、旅行者が使った国際航空機の機内、若しくは母国と日本で利用した国際空港が可能性として考えられます。

Q12　日本国内でデング熱に感染する可能性はあるのでしょうか？

日本にはデング熱の主たる媒介蚊のネッタイシマカは常在していませんが、媒介能力があるヒトスジシマカは国内に広く生息しています。このことから、仮に流行地でウイルスに感染した発症期の人（日本人帰国者ないしは外国人旅行者）が国内で蚊にさされ、その蚊がたまたま他者を吸血した場合に、感染する可能性は低いながらもあり得ます。ただし、仮にそのようなことが起きたとしても、その蚊は冬を越えて生息できず、また卵を介してウイルスが次世代の蚊に伝わることも報告されたことがないため、限定された場所での一過性の感染と考えられます。

2．医療機関・検査機関の方向け

Q1　デング熱の病原体は何ですか？

フラビウイルス科フラビウイルス属に属するデングウイルスです。
ウイルスには１～４までの４つの型がありますが、どの型によっても同様の病気がおこり、症状からは感染したウイルスの型はわかりません。

Q2　潜伏期間はどのくらいですか？

２～15 日（多くは３～７日）です。

Q3　どのような症状が出ますか？

突然の高熱で発症し、頭痛、眼（か）痛、顔面紅潮、結膜充血を伴い、発熱は２～７日間持続します（二峰性であることが多い）。初期症状に続き、全身の筋肉痛、骨関節痛、全身倦怠感を呈します。発症後３～４日後、胸部、体幹から始まる発疹が出現し、四肢、顔面に広がります。症状は１週間程度で回復します。

なお、ごくまれに一部の患者において、発熱２～７日後、血漿漏出と出血傾向を主な症状とする重篤な致死的病態が出現することがあります。

Q4　検査はどのように行うのですか？

血液所見では、発症後数日で高度の白血球減少、血小板減少がみられます。

診断のための検査は、血液からの病原体の検出、PCR 法による病原体遺伝子の検出、ELISA 法による病原体タンパク NS1 の検出、IgM 抗体の検出、中和試験等による抗体の検出などで、確定検査を行います。

なお、届出におけるデング出血熱の場合には、出血傾向、血小板減少、血管透過性亢進による血漿漏出も含めて、上記の確定検査をともに行います。

Q5　鑑別が必要な疾病はありますか？

発疹を有するウイルス性疾患（麻疹、風疹、チクングニア、エンテロウイルス感染症）、チフス、マラリア、猩紅熱、A型肝炎、レプトスピラ症などとの鑑別が必要です。デング熱でも時に呼吸器症状が見られることがあり、呼吸器感染症との鑑別も必要になることがあります。

Q6　治療法はありますか？

対症療法となります。痛みと発熱に対してのアスピリンの投与は、出血傾向増悪やライ症候群発症の可能性があるので禁忌です。血漿漏出などの症状が出現した場合は、血漿漏出による循環血液量の減少を輸液により補うことが治療の中心になります。

Q7　患者の経過と予後はどうでしょうか？

デング熱の予後は比較的良好です。血漿漏出と出血傾向が主症状の場合は適切な治療がなされないと致死性が高いですが、症状が回復し始めると迅速に回復するのが特徴です。

Q8　確定患者の管理はどのように行えばよいでしょうか？

本病は、蚊を介しないヒトからヒトへの直接的な感染はありません。ただし、発熱中の患者が蚊に刺されることがないように指導することは必要です（日本にいるヒトスジシマカでもウイルス血症期の患者を吸血すれば他者にウイルスを伝播する可能性があります）。

Q9　感染症法上の取り扱いはどうなっていますか？

４類感染症に指定されており、医師が患者を診断した場合は、最寄りの保健所に直ちに届出が必要です。

Q10　ヒトスジシマカについて教えてください。

ヒトスジシマカは、北海道と青森県を除く国内全域に分布しています。その活動時期は５月から 10 月です。ヒトスジシマカの幼虫は、例えば、ベランダにある植木鉢の受け皿や空き缶・ペットボトルに溜まった水、放置されたブルーシートや古タイヤに溜まった水などによく発生します。人がよく刺されるのは、墓地、竹林の周辺、茂みのある公園や庭の木陰などとされています。

(参考)

国立感染症研究所昆虫医科学部ホームページ

http://www.nih.go.jp/niid/ja/from-lab/478-ent/3466-ent-photos.html

ヒトスジシマカの写真

http://www0.nih.go.jp/niid/entomology/pictures/albopictus/albopictus.html

Q11　ヒトスジシマカの体内でデングウイルスは増えますか？

ヒトスジシマカの体内でウイルスは増え、デング熱流行を起こす能力がありますが、ネッタイシマカに比べるとその増殖は低いとされています。

Q12　ヒトスジシマカは越冬しますか？

ヒトスジシマカは卵で越冬します（卵越冬）。なお、その卵を通じてデングウイルスが次世代の蚊に伝播した報告は国内外でありません。

Q13　ネッタイシマカの特徴等について教えてください。

現在、ネッタイシマカは国内には生息していません。かつては国内でも沖縄や小笠原諸島に生息し、熊本県牛深町には 1944～1947 年に一時的に生息していたことが記録されていますが、1955 年以降は国内から消滅したとされています。ただ今日では、航空機によって国内に運ばれる例も確認されており、定着の可能性は皆無ではありません。

なお、ネッタイシマカとヒトスジシマカが同所的に分布しているような熱帯・亜熱帯地域においては、ネッタイシマカのウイルス媒介能はヒトスジシマカよりも高いとされています(ネッタイシマカからのウイルスの検出率が高く、ヒトを吸血対象とする依存性が圧倒的に強いことがその理由です)。

(参考)

国立感染症研究所昆虫医科学部ホームページ

http://www.nih.go.jp/niid/ja/from-lab/478-ent/3466-ent-photos.html

ネッタイシマカの写真
http://www0.nih.go.jp/niid/entomology/pictures/aegypti/aegypti.html

Q14　ネッタイシマカは国内に定着できますか？

ネッタイシマカの分布の北限は台湾の台中市周辺とされています。従って、国内では沖縄県の南方（石垣島・西表島など）以北の野外では定着できないと考えられます。しかし、空港ターミナルなど、一定の温度が維持されているような特別な場所では定着できるかもしれません。

なお、ネッタイシマカにおいて、デングウイルスの経卵巣伝搬の可能性を示唆した報告はありますが(インドの乾季に捕集されたオス蚊や幼虫からウイルス遺伝子が検出された例)、その割合は非常に低く、次の流行を引き起こすことは極めて難しいと結論されています。

Q15　蚊に刺されないようにするにはどうしたらよいでしょうか？

ヒトスジシマカやネッタイシマカは日中に活動し、ヤブや木陰などでよく刺されます。その時間帯に屋外で活動する場合は、長袖・長ズボンの着用に留意し、忌避剤の使用も推奨します。

Q16　日本でデング熱に感染する可能性はありますか？

日本に生息するヒトスジシマカもデングウイルスを媒介することができますので、流行地で感染した人が帰国し、症状がある期間は蚊に吸血されることにより、その蚊が周囲の方にウイルスを伝播する可能性は低いながらもあり得ます。帰国者（患者）の周囲の方でデング熱を疑うような症状があれば、渡航歴の有無にかかわらず検査を行うことも、場合によっては必要です。

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
				2013. 12. 16	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	Marchand E, Prat C, Jeannin C, Lafont E, Bergmann T, Flusin O, Rizzi J, Roux N, Busso V, Deniau J, Noel H, Vaillant V, Leparc-Goffart I, Six C, Paty MC. Euro Surveill. 2013 Dec 12;18(50):20661.	公表国 フランス
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

○フランスの土着性デング熱症例
2013年10月、ヒトスジシマカ(*Aedes albopictus*)の生息地である南フランスのブーシュ=デュ=ローヌ県で、50歳代女性検査技師がデング熱と診断された。患者は発症15日前からその地域を出ていないことや、職業感染の可能性が除外されたことから、当該地域の昆虫媒介による伝播の可能性が高い。これは、2010年にアルプ=マリティーム県で発生した2件の症例に続く、フランス本土における2番目の土着性デング熱症例の可能性がある。

報告企業の意見	今後の対応
フランスにおいて2番目となる土着性デング熱症例の報告である。	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

Autochthonous case of dengue in France, October 2013

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5. Laboratory Network Bioactif, Bouches-du-Rhône, France
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Marchand E, Prat C, Jeannin C, Lafont E, Bergmann T, Flusin O, Rizzi J, Roux N, Busso V, Deniau J, Noel H, Vaillant V, Leparc-Goffart I, Six C, Paty MC. Autochthonous case of dengue in France, October 2013. Euro Surveill. 2013;18(50):pii=20661. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20661

Article submitted on 3 December 2013 / published on 12 December 2013

In October 2013, autochthonous dengue fever was diagnosed in a laboratory technician in Bouches-du-Rhône, southern France, a department colonised by *Aedes albopictus* since 2010. After ruling out occupational contamination, we identified the likely chain of local vector-borne transmission from which the autochthonous case arose. Though limited, this second occurrence of autochthonous dengue transmission in France highlights that efforts should be continued to rapidly detect dengue virus introduction and prevent its further dissemination in France.

In October 2013, the French National Reference Laboratory for arboviruses (NRL) – hosted at the Institut de Recherche Biomedicale des Armées, Marseille – reported an autochthonous case of dengue fever to the Regional Health Authority of Provence-Alpes-Côte d'Azur. The case lived in the department of Bouches-du-Rhône, France. The national and regional health authorities initiated a multidisciplinary investigation to determine the source of infection of the case and the extent of possible dissemination of dengue virus (DENV).

Case report

On 11 October 2013, a female laboratory technician in her early fifties, residing and working in the area of Aix-en-Provence, Bouches-du-Rhône department, developed sudden fever with incapacitating myalgia, predominantly in her legs. She had not left the department in the 15 days before onset of symptoms. Four days later, she developed a rash on her legs and consulted her general practitioner, who prescribed symptomatic treatment of fever and aches. As the symptoms persisted, she was taken to a hospital's emergency department on two days later. On admission to hospital, she was normotensive, with a body temperature of 38.4 °C. Laboratory analyses showed a normal white blood cell (7,200/mL; norm: 4,000–10,000 /mL) and platelet count (197,000/mL; norm: 150,000–450,000/mL) and an elevated C-reactive protein level (145 mg/L; norm: <7.5 mg/L). As her condition had improved, despite the rash having expanded to her arms and back, she was discharged after 24 hours, with a diagnosis of 'probable viral infection'. She consulted a dermatologist three days later, who suspected an arboviral infection and sent blood samples to the NRL.

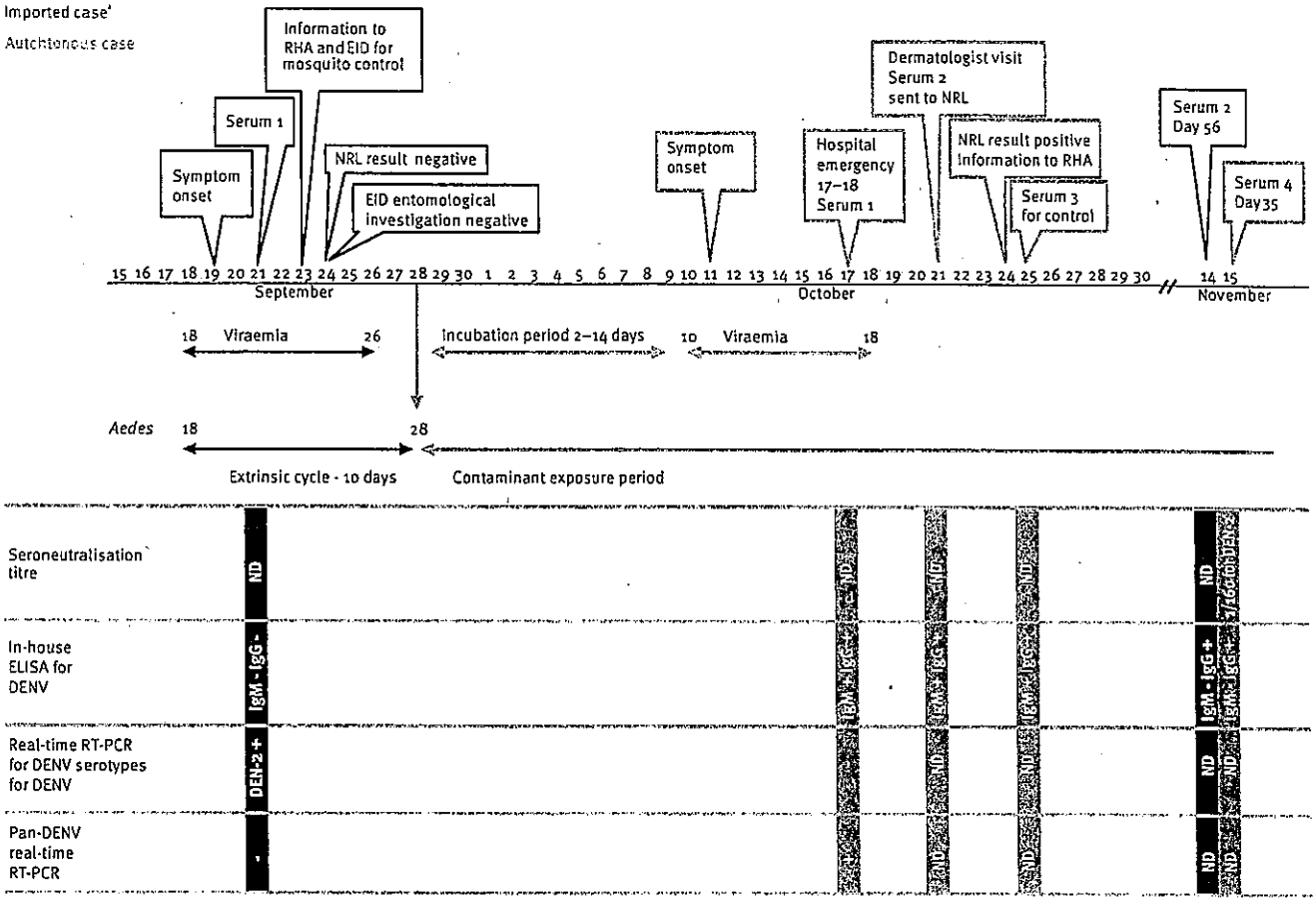
A panel of sera obtained during the acute and convalescent phases (days 6, 10, 14 and 35 after symptom onset) was investigated using in-house real-time reverse transcription polymerase chain reaction (RT-PCR) and serological assays (in-house IgM antibody capture (MAC)-enyzme-linked immunosorbent assay (ELISA) and indirect IgG ELISA) for DENV and West Nile, chikungunya and Toscana viruses (Table). For the first serum sampled on day 6, the real-time RT-PCR for DENV was positive, with a high cycle threshold (Ct) value (indicating a very low viral load), serotyping by real-time RT-PCR was negative, DENV non-structural protein 1 (NS1) detection by rapid diagnostic test (SD Bioline) was negative, but IgM and IgG antibodies against DENV antigens were detected). IgM and IgG antibodies against DENV were also detected in the next two serum samples (on days 10 and 14). For the last serum specimen, sampled on day 35, only DENV-specific IgG antibodies were detected: the specificity of these antibodies was determined by seroneutralisation against DENV serotypes 1 to 4 (DEN1–4) and West Nile virus [1]. For West Nile virus, DEN1, DEN3 and DEN4, the 90% neutralisation titre was <1/20. A 90% neutralisation titre of 1/160 against DEN-2 was highly suggestive of an infection of this patient by DEN-2.

Background

Infection with DENV – a member of the family *Flaviviridae*, genus Flavivirus – leading to dengue haemorrhagic fever and shock syndrome, is responsible for substantial morbidity and mortality in populations living in the tropics and among travellers to these

FIGURE 1

Timeline of epidemiological features and laboratory results of sera from autochthonous and imported case of dengue, Bouches-du-Rhône, France, September–November 2013



DENV: dengue virus; EID: Entente Interdépartementale pour la Démoustification du littoral Méditerranéen; ELISA: enzyme-linked immunosorbent assay; ND: not done; NLR: National Reference Laboratory for arboviruses; RHA: regional health agency ; RT-PCR: reverse transcription polymerase chain reaction.

[a] Initially reported as a suspected case.

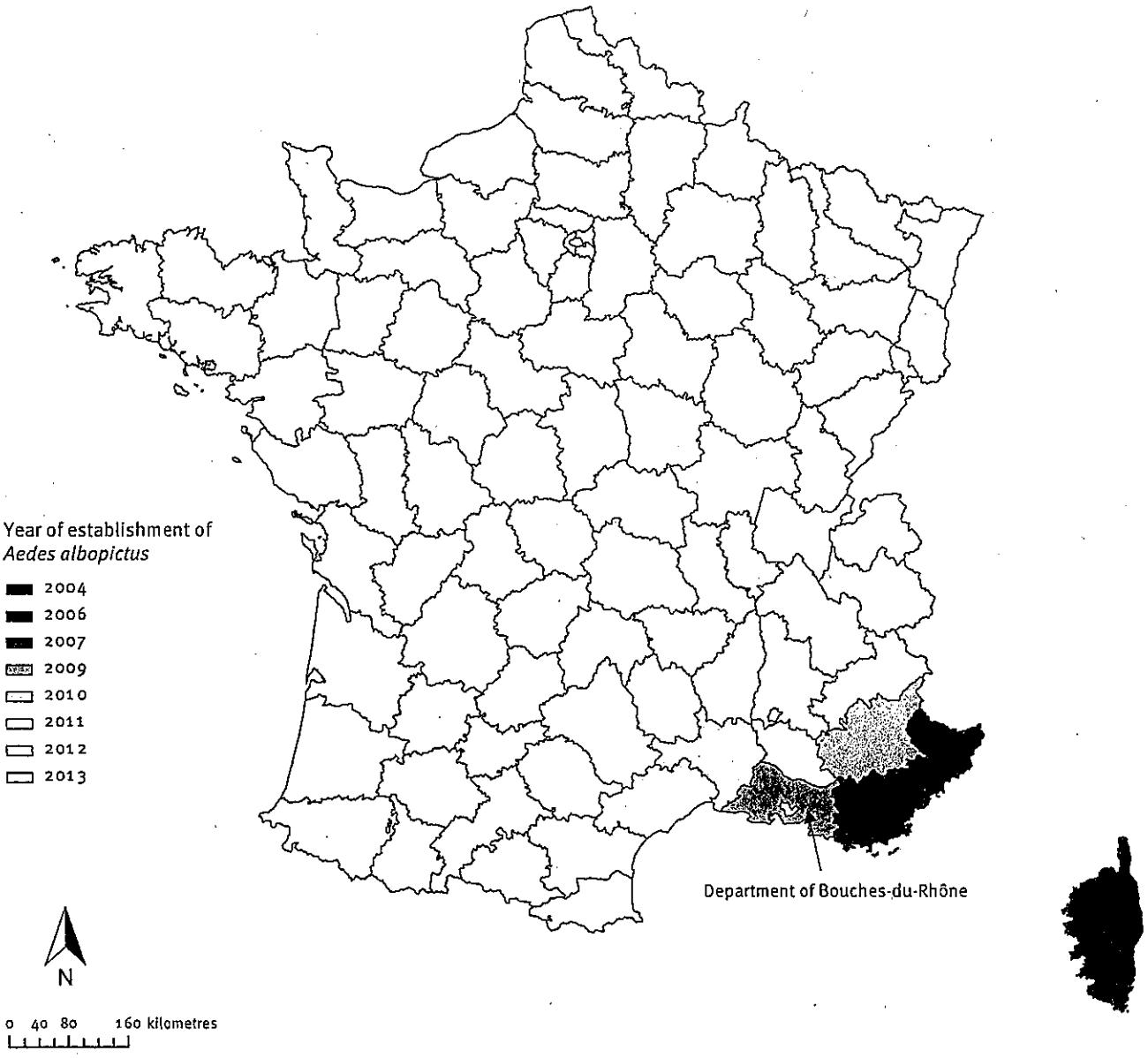
regions [2,3]. The global burden of dengue ranges from an estimated 50–100 million DENV infections worldwide every year according to the World Health Organization [3] to a recent estimate of 390 million DENV infections per year [4]. The virus is considered an emerging threat to Europe because of its recent detection in southern Europe due to the continuous spread of *Aedes (Stegomyia) albopictus (Skuse)*, an invasive mosquito species and well-known vector of chikungunya virus and DENV [5,6]. Public health concern has been heightened since limited foci of local transmission of DENV were reported in September 2010 in Nice, southern France, and Croatia and more recently in 2012 in Madeira, Portugal (where the vector was *Ae. aegypti*) [7-9].

Ae. albopictus was introduced in southern France in 2004, near the Italian border [10]. Since then, it has continuously spread eastwards and northwards and has to date colonised 17 departments (administrative districts) including Bouches-du-Rhône since 2009 (Figure 2) [10,11]. Prevention of the introduction of DENV and chikungunya virus in the departments where *Ae. albopictus* is established falls under a national preparedness and response plan created in 2006 [12]. Implemented each year during the vector activity period, from 1 May to 30 November, the plan is based on enhanced surveillance aiming at the early detection of imported dengue and chikungunya cases.

In mainland France, dengue has been a mandatorily notifiable disease since April 2006 [13]: a case should be reported immediately after laboratory confirmation [12]. In addition, in the areas and period of vector activity, physicians and laboratories are asked to immediately notify suspected imported cases to the local health authorities and to send blood samples directly to the NRL for laboratory confirmation. These actions

FIGURE 2

Departments colonised by *Aedes albopictus*, France, 2004–2013



Source: IGN-GéoFLA, 1999; French Institute for Public Health Surveillance (Institut de Veille Sanitaire, InVS), 2013.

guide entomological investigations followed by vector control measures when appropriate.

The investigation

As the case could have been exposed to blood during her laboratory work, two hypotheses on the source of infection and mode of DENV transmission were explored: occupational transmission through accidental exposure to blood from a viraemic patient or local vector-borne transmission.

Occupational transmission

Considering an incubation period of 2 to 14 days, in agreement with earlier reports of occupational DENV infection [14-16], we defined the period of likely exposure as 27 September to 8 October. The autochthonous case carried out on average 60 venous punctures a day from patients. She had no recollection of any direct blood exposure during this period. However, she reported not wearing gloves while collecting blood and presented skin excoriations on her fingers. Among patients sampled in her workplace, none had blood taken for dengue fever suspected by a physician. Nevertheless, we screened for DENV all patients sampled in the laboratory during the case's likely period of infection who presented with symptoms of or a history compatible with dengue. The criteria for screening were fever without diagnosis, reported travel in an

area where dengue was reported, leucopenia, thrombocytopenia, hepatic cytolysis, negative viral serology and negative rapid or blood smear tests for malaria. The NRL conducted DENV real-time RT-PCR and serology on the 15 blood samples still available from the 18 patients who met the selection criteria. All tested negative.

Vector-borne transmission

The patient reported no mosquito bites but remembered a sudden pricking sensation compatible with a mosquito bite on the evening of 3 October (eight days before symptom onset), when she was near her workplace in Bouches-du-Rhône. She reported no recent contact with travellers returning from an area with current epidemics or endemic for dengue.

A review of the surveillance database did not identify any case of imported dengue confirmed by the NRL in Bouches-du-Rhône since 1 August 2013. However, one suspected case had been notified in a neighbouring department in a woman who developed fever and a rash on 19 September, five days after returning from the Caribbean island of Guadeloupe, where a dengue outbreak was ongoing [17]. Sera collected on day 2 of her illness tested negative for DENV, West Nile, chikungunya and Saint Louis encephalitis viruses by our in-house real-time RT-PCR and serological assays. An entomological investigation of her residential area and places visited had been carried out on 24 September, before the negative test results were available. Among the places visited, the woman mentioned a short visit, the day before symptom onset, close to (less than 200 metres from) the workplace of the autochthonous case. Although an ovitrap placed nearby this workplace had been found colonised with 43 eggs of *Ae. albopictus* in late September 2013, no evidence of mosquito activity was found during an investigation on 23 September, hence no vector control measures were implemented at that time.

After the detection of the autochthonous case, we retested the serum sample of the suspected imported case by sero-specific real-time RT-PCR for DENV and by rapid diagnostic test for NS1 detection. An additional serum sample was collected on day 56 for serology testing. The NS1 test and the pan-DENV real-time RT-PCR were negative, the real-time RT-PCR for DEN-2 was positive with a high Ct value. In the later serum sample (day 56), only IgG antibodies against DENV were detected. These laboratory findings confirmed an infection with DEN-2 for this patient returning from Guadeloupe (considered the index case).

Control measures

Under the hypothesis of local vector-borne transmission, two places were chosen for identifying primary or secondary cases of DENV infection and for conducting immediate control measures: the autochthonous case's home, where she stayed while viraemic, and her place of work close to which eggs of *Ae. albopictus* had been detected in September.

The local health authorities and vector control operators jointly carried out the following activities in an area of 200 metres around the autochthonous case's home and workplace: door-to-door case finding; any mosquito breeding sites treated by mechanical destruction or larvicide treatment sites; and adulticide sprayings. Physicians and laboratories in the area were asked to report any patients with symptoms compatible with DENV infection since 1 August, including sudden onset of fever (>38.5 °C) and at least one pain symptom, including headache, arthralgia, myalgia, lower back pain or retro-orbital pain. Two suspected cases were identified. Neither tested positive for DENV by real-time RT-PCR or serology.

Discussion

This second report of autochthonous dengue in mainland France follows a cluster of two locally acquired cases in Alpes-Maritimes in 2010 [7]. Because our patient was a laboratory technician who daily collected blood specimens, we not only explored vector-borne local transmission of DENV but also thoroughly investigated potential occupational transmission. The latter hypothesis appears unlikely since, unlike the situation for other occupational dengue cases [14-16], our investigation pinpointed neither a viraemic or infected patient sampled nor any accidental exposure to blood at the laboratory during the likely exposure period of the case.

Several findings are in favour of vector-borne transmission in the Bouches-du-Rhône department. Firstly, our retrospective laboratory confirmation of an imported case of dengue, who had visited the immediate vicinity of the autochthonous case's workplace, while potentially viraemic (one day before symptom onset). Secondly, the 22-day delay between symptom onset of the imported and autochthonous case, which is compatible with the intrinsic (1–14 days) and extrinsic (10 days) incubation period for DENV [18]. Thirdly, the presence of *Ae. albopictus* eggs in the ovitraps in September, indicating the presence of the potential vector. Finally, laboratory confirmation of DENV infection of the same serotype, DEN-2, in both the autochthonous case and the case imported from Guadeloupe. In August to October 2013, DEN-2 was not the prevailing circulating serotype in Guadeloupe, but remained frequent [17].

The virological data presented in this paper on the two human cases of DENV infection do not follow the classical and average kinetics of viraemia and antibody response. The individual host response is known to be variable regarding the viral load in blood, the duration of viraemia and the duration of IgM detection and is also dependent on the DENV responsible for the infection [19,20]. Unfortunately, although viral RNA was detected in the acute phase sample of the autochthonous case,

we were unable to serotype and sequence the amplified product due to a very low viral load. Further comparison of the virus isolates by sequencing is therefore impossible. Two blind passages on Vero and C6/36 cell lines will be carried out to try to isolate the virus from the acute phase sera. NS1 was not detected for these two cases. However, the detection of NS1 is generally less sensitive than viral genome detection by real-time RT-PCR [20,21].

This local transmission of dengue highlights once again that mainland France is subject to overspill of dengue outbreaks, particularly from the French Antilles in the Caribbean. The implemented investigations and control measures were derived from a national plan against dengue and chikungunya that provides a framework for rapid review and exchange of information between epidemiological, entomological, laboratory and medical experts and decision-makers.

The autochthonous case was diagnosed only after a third and specialised medical consultation. We need therefore to further raise the awareness of physicians and laboratories regarding diagnosis of dengue in international travellers and the possibility of autochthonous transmission in areas where *Ae. albopictus* is established. Similarly, we should not discontinue our efforts to inform travellers to areas affected by dengue about individual protection against mosquito bites and early symptoms of dengue.

No further case could be related to this local transmission cycle of dengue in Bouches-du-Rhône. Although precautionary mosquito control was applied, this could very well have been a self-limiting viral dissemination since it occurred shortly before the end of the vector activity period in late November.

Conclusion

Although limited, this autochthonous transmission of DENV in southern France is a clear reminder that local transmission can be triggered in Europe by the introduction of the virus in areas colonised by *Ae. albopictus*, as it occurred already in 2010 in Nice. The French preparedness and response plan, in operation since 2006, proved pivotal to detect and control this threat.

Reducing the risk of local DENV dissemination to zero appears an elusive goal in the context of the continuous spread of Ae albopictus. Coordinated enhanced surveillance and response are therefore the backbone of the prevention of the occurrence of autochthonous cases and the containment of possible outbreaks. Such a plan requires, however, multidisciplinary expertise and resources and should be adapted wisely and regularly to ensure its sustainability and efficiency. In addition, innovative vector control methods and further elucidation of the dynamics of DENV transmission in non-endemic areas are needed to keep Europe safe from dengue.

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Conflict of interest

None declared.

Authors' contributions

All authors contributed to the writing of this manuscript and approved the final version. Elodie Marchand, Caroline Six, Harold Noel, Veronique Vaillant, Marie-Claire Paty drafted the manuscript and contributed to the epidemiological investigation. Caroline Six, Harold Noel designed the protocol for the rapid survey. Thibaut Bergmann, Nicolas Roux, Jeanne Rizzi contributed to the epidemiological investigation. Elisabeth Lafont and Valerie Busso conducted interviews and took part in the clinical management of the patients. Joël Deniau contributed to the epidemiological investigation and managed the national database for enhanced surveillance of dengue and chikungunya. Caroline Six coordinated the investigation at the regional level. Christine Prat, Olivier Flusin, Isabelle Leparc-Goffart were central in the laboratory investigation. Marie-Claire Paty coordinated the investigation at the national level.

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別紙様式第２－１

医薬品　研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
一般的名称	－		研究報告の公表状況	第 61 回日本ウイルス学会学術集会(2013.11.10,11,12)/兵庫県神戸市()197/()	公表国 日本	
販売名(企業名)	－					

研究報告の概要

重症熱性血小板減少症候群(severe fever With thrombocytopenia syndrome、SFTS)はブニヤウイルス科フレボウイルス属に分類される新規ウイルス(SFTS ウイルス、SFTSV)によって引き起こされるダニ媒介性感染症で、致死率 10%を超える重篤な疾患を引き起こす。数年前に中国で最初に発生が確認され、国内においても 2013 年 1 月、SFTS 患者の存在が明らかにされた。
このことから、SFTS の診断法を確立し、国内の本感染症の病態解明、疫学調査、感染リスクの評価を進めていく必要がある。本研究では、SFTSV 感染細胞および組換え核蛋白質(rNP)を用いた SFTS 血清診断法を開発し、その有用性について検討した。
SFTSV 感染細胞を抗原とした IFA および IgG－ELISA 法により、患者の回復期血清から抗 SFTSV 抗体が検出され、両者の結果はよく一致した。急性期の SFTS 患者で、血清中の SFTSV 遺伝子検出 RT－PCR で陽性を呈した 2 検体からも抗体が検出された。SFTSV rNP 発現 HeLa 細胞を用いた IFA および rNP を抗原とした IgG－ELISA 法でも、抗 SFTSV 抗体が検出可能であったが、ウイルス感染細胞を用いた場合に比べその抗体価は低く、感染性 SFTSV を抗原とした抗体検出法に比べ感度は低かった。
ウイルス感染細胞を抗原とした血清学的診断法は、血清中 SFTSV 抗体を高感度に検出することができ、SFTSV 感染のサーベイランスに有用であると考えられた。rNP を用いた血清診断法は、感染性ウイルスを用いないため安全に抗原を調整できるものの、感度を向上させるため抗原の発現方法の改良等さらなる検討が必要である。

使用上の注意記載状況・その他参考事項等

報告企業の意見	今後の対応
重症熱性血小板減少症候群(SFTS)は、SFTSV によって引き起こされるダニ媒介性感染症で、国内においても 2013 年 1 月に患者が確認された。 本情報は、国立感染症研究所における本疾患の血清学的診断法の開発に関する情報である。 現時点まで血漿分画製剤から伝播が疑われた報告はないが、今後の情報に注目していきたい。	今後とも SFTSV に関する情報等に留意していく。

O1-6-12

重症熱性血小板減少症候群（SFTS）の血清学的診断法の開発

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【目的と意義】
重症熱性血小板減少症候群(severe fever with thrombocytopenia syndrome、SFTS）はブニヤウイルス科フレボウイルス属に分類される新規ウイルス（SFTSウイルス、SFTSV）によって引き起こされるダニ媒介性感染症で、致死率10%を超える重篤な疾患を引き起こす。数年前に中国で最初に発生が確認され、国内においても2013年1月、SFTS患者の存在が明らかにされた。このことから、SFTSの診断法を確立し、国内の本感染症の病態解明、疫学調査、感染リスクの評価を進めていく必要がある。本研究では、SFTSV感染細胞および組換え核蛋白質（rNP）を用いたSFTS血清診断法を開発し、その有用性について検討した。
【材料と方法】
SFTS疑い患者の急性期および回復期に採取された血清を用いた。SFTSV感染Vero細胞を固定して用いた間接蛍光抗体法（IFA）および、SFTSV感染Huh7細胞ライセートを抗原としたIgG-ELISA法により、血清中SFTSV抗体価測定を行った。また、SFTSV NP発現HeLa細胞、バキュロウイルス発現系で発現させたrNPを抗原として、感染性SFTSVを抗原としない抗体検出法について、その診断における有用性を検討した。
【結果】
SFTSV感染細胞を抗原としたIFAおよびIgG-ELISA法により、患者の回復期血清から抗SFTSV抗体が検出され、両者の結果はよく一致した。急性期のSFTS患者で、血清中のSFTSV遺伝子検出RT-PCRで陽性を呈した2検体からも抗体が検出された。SFTSV rNP発現HeLa細胞を用いたIFAおよびrNPを抗原としたIgG-ELISA法でも、抗SFTSV抗体が検出可能であったが、ウイルス感染細胞を用いた場合に比べその抗体価は低く、感染性SFTSVを抗原とした抗体検出法に比べ感度は低かった。
【考察】
ウイルス感染細胞を抗原とした血清学的診断法は、血清中SFTSV抗体を高感度に検出することができ、SFTSV感染のサーベイランスに有用であると考えられた。rNPを用いた血清診断法は、感染性ウイルスを用いないため安全に抗原を調整できるものの、感度を向上させるため抗原の発現方法の改良等さらなる検討が必要である。

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日 2014年2月24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	Emerg Infect Dis. 2014;20:211-216	公表国 米国	
販売名（企業名）	別紙のとおり。					

研究報告の概要	問題点：米国で、暫定的にSosuga virusと命名された新規なparamyxovirus科のウイルスが確認された。 暫定的にSosuga virusと命名された新規なparamyxovirus科のウイルスが、南スーダン及びウガンダにおける調査から帰国したアメリカ人野生生物学者から検出された。彼女は調査地でげっ歯類及びコウモリを捕獲しており、発熱、倦怠感、関節痛、広範囲の発疹、および出血の症状を呈したが、入院14日目に退院した。患者検体について、いくつかの既知の病原体に係る検査が実施されたがいずれも陰性であり、大規模な遺伝子配列解析及びメタゲノム解析の結果、上記の新規なウイルスが確認された。このウイルスはオオコウモリから分離された風疹様ウイルスに最も近いウイルスであったが、患者の感染源は不明である。	使用上の注意記載状況・その他参考事項等 記載なし。

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

別紙

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤人免役グロブリン、⑥乾燥ペプシン処理人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン、⑧乾燥スルホ化人免疫グロブリン、⑨乾燥スルホ化人免疫グロブリン、⑩乾燥スルホ化人免疫グロブリン、⑪乾燥スルホ化人免疫グロブリン*、⑫乾燥濃縮人活性化プロテインC、⑬乾燥濃縮人血液凝固第Ⅷ因子、⑭乾燥濃縮人血液凝固第Ⅷ因子、⑮乾燥濃縮人血液凝固第Ⅷ因子、⑯乾燥濃縮人血液凝固第Ⅸ因子、⑰乾燥濃縮人血液凝固第Ⅸ因子、⑱乾燥濃縮人血液凝固第Ⅸ因子、⑲乾燥抗破傷風人免疫グロブリン、⑳抗HBs人免疫グロブリン、㉑抗HBs人免疫グロブリン、㉒抗HBs人免疫グロブリン、㉓トロンビン、㉔トロンビン、㉕トロンビン、㉖フィブリノゲン加第ⅩⅢ因子*、㉗フィブリノゲン加第ⅩⅢ因子、㉘乾燥濃縮人アンチトロンビンⅢ、㉙ヒスタミン加人免疫グロブリン製剤、㉚人血清アルブミン*、㉛人血清アルブミン*、㉜乾燥ペプシン処理人免役グロブリン*、㉝乾燥濃縮人アンチトロンビンⅢ
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
報告企業の意見	paramyxovirus科のウイルスは150～350nmの多形性で、核酸は一本鎖RNA、エンベロープを有するウイルスであり、ムンプスウイルスや麻疹ウイルス等が知られている。 今回の報告は、米国で、南スーダン及びウガンダにおける調査から帰国した野生生物学者が発熱や倦怠感等の症状を呈して入院し、その患者検体から新規なparamyxovirus科のウイルスが確認された、というものである。 上記製剤の製造工程には、冷エタノール分画工程及びウイルス除去膜ろ過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。今回報告した新規なparamyxovirus科の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新規なparamyxovirus科のウイルスへの感染報告例は無い。 以上の点から、上記製剤は新規なparamyxovirus科のウイルスへのウイルス感染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

Novel Paramyxovirus Associated with Severe Acute Febrile Disease, South Sudan and Uganda, 2012

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In 2012, a female wildlife biologist experienced fever, malaise, headache, generalized myalgia and arthralgia, neck stiffness, and a sore throat shortly after returning to the United States from a 6-week field expedition to South Sudan and Uganda. She was hospitalized, after which a maculopapular rash developed and became confluent. When the patient was discharged from the hospital on day 14, arthralgia and myalgia had improved, oropharynx ulcerations had healed, the rash had resolved without desquamation, and blood counts and hepatic enzyme levels were returning to reference levels. After several known suspect pathogens were ruled out as the cause of her illness, deep sequencing and metagenomics analysis revealed a novel paramyxovirus related to rubula-like viruses isolated from fruit bats.

Paramyxoviruses comprise a large family of viruses, including pathogens that cause severe disease in humans (*1*). Worldwide, >100 paramyxoviruses have been identified in bats and rodents (*2–4*). Among these, few have been shown to be pathogenic to humans, possibly because of limited host range and/or infrequent exposure. We describe a novel rubula-like virus that was associated with a severe acute febrile illness in a woman. The patient was a wildlife biologist who had participated in a 6-week field expedition to South Sudan and Uganda. During this expedition, she had been exposed to bats and rodents of >20 species while wearing different levels of personal protective equipment.

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Clinical Presentation

During the summer of 2012, a 25-year-old female wildlife biologist participated in a 6-week field expedition to South Sudan and Uganda, where she traveled to remote rural areas collecting bats and rodents for ecologic research. In the course of her duties, she manipulated animals in traps and mist nets, performed dissections, collected blood and tissues, and visited caves with large bat populations. She received no injuries from sharp objects and no bites or scratches from the animals with which she was working. She occasionally used respiratory protection when working with animals and specimens, and she wore a respirator while in caves. During her trip, she had no known contact with ill members of the field team, no contact with health care facilities, and no sexual contacts. She had been vaccinated against hepatitis A and B, yellow fever, measles, mumps, rubella, rabies, polio, tetanus/diphtheria, and typhoid fever, and she fully complied with a malaria prophylaxis regimen of atovaquone/proguanil. Her medical history included migraines and treatment of presumptive malaria with artemether/lumefantrine during a similar expedition the previous year.

Five days after returning to the United States, the woman was evaluated in the emergency department for a 2-day history of fever, malaise, headache, generalized myalgia and arthralgia, neck stiffness, a metallic taste, and sore throat. Results of rapid malaria test, performed on the day of fever onset, were negative. Other laboratory results and the patient's vital signs at the time of admission are summarized in the Table. The patient seemed to be fatigued but alert and oriented; she was anicteric, and she had no nuchal rigidity or focal neurologic deficits. Mild erythema of the soft palate without ulcerations or exudates was noted. The spleen tip was palpable despite absence of adenopathy.

Examination of heart, abdomen, and lungs (including chest radiographs) revealed no abnormalities. No rash or

Table. Vital signs and laboratory results for patient infected with a novel paramyxovirus related to rubula-like viruses isolated from fruit bats

DSO	DH	Max temp	Max pulse	SBP	WBC	Plate	Creat	AST	ALT	LDH,	TB	PT/INR ratio	TG	Ferr	qRT-PCR, C_t†	IgM ELISA†	IgG ELISA†
2	1	40.1	90	112	1.62	115	0.8	93	19	687	0.2						
3	2	40.1	79	112	1.53	93	0.7	133	20		0.1	17.2/1.45			29.5	<50	<50
4	3	40.4	77	103	1.06	77	0.6	164	28		0.1	15.8/1.29	120				
5	4				1.02	65	0.7	319	134		0.1	13.6/1.06		17,840			
6	5	38.6	77	102	0.95	62	0.6	615	261		0.1	13.7/1.07	127	11,595			
7	6				1.46	79	0.6	589	298		0.2	14.4/1.14		7,309			
8	7				1.64	84	0.5	516	299		0.1	15.5/1.26		3,371			
9	8				0.96	123	0.5	342	259		0.1	16.9/1.41			36.3	≥1,600	≥1,600
10	9				1.20	154	0.4	170	186		0.1	17.4/1.47			36.9	≥1,600	≥1,600
11	10				2.19	222	0.4	185	188		0.1	14.7/1.18			Neg	≥1,600	≥1,600
12	11				2.61	220	0.4	107	149		0.2	13.2/1.02			Neg	≥1,600	≥1,600
13	12				5.62	335	0.4								Neg		
14	13				5.79	387	0.4								Neg		
15	14	36.8	67	98	4.71	437	0.5						212		Neg	≥1,600	≥1,600
30					3.71	221		19	15		0.3						
60					5.44	348								12.4	Neg	≥400	≥1,600

*Shading indicates values outside reference range; blank cells indicate data not obtained. DSO, days from symptom onset; DH, day of hospitalization; max temp, maximum temperature, °C; max pulse; maximum pulse rate, beats/minute; SBP, systolic blood pressure, mm Hg; WBC, leukocytes ,× 1,000/μL; plate, platelets × 1,000/μL; creat, creatinine, mg/dL; AST, aspartate aminotransferase, IU/L; ALT, alanine aminotransferase, IU/L; LDH, lactate dehydrogenase, IU/L; TB, total bilirubin, mg/dL; PT/INR, prothrombin time/international normalized ratio; TG, triglycerides, g/dL; ferr, ferritin, ng/mL; qRT-PCR, quantitative reverse transcription PCR; C_t, cycle threshold; neg, negative.
†Assays were developed after the virus genome was determined.

synovitis was noted. Treatment with intravenous ceftriaxone was begun for possible typhoid fever, and artemether/lumefantrine was continued for presumptive malaria.

On hospital day 2, a maculopapular rash erupted over the patient's trunk (Figure 1, panel A), several small aphthous ulcers appeared on her soft palate, and she had mild diarrhea. As long as the fever persisted, clear pulse/temperature dissociation was present (positive Faget sign); however, hemodynamics, oxygenation, and renal function were stable. Doxycycline was added for the expanded differential diagnosis of a rickettsial illness or plague. On hospital day 3, fever, headache, and myalgia persisted, and the patient experienced bloody emesis, mild diarrhea positive for occult blood but without frank hematochezia or melena, and minimal diffuse abdominal tenderness. Her menstrual period occurred without substantial menorrhagia. The rash became confluent; a centrifugal distribution and prominent petechia appeared at sites of trauma or pressure.

The possibility of hemophagocytosis was considered, and a bone marrow biopsy sample was obtained on day 4. The sample showed a mild increase in macrocytic hemophagocytosis and pancytopenia with a hypocellular marrow with myeloid hyperplasia and erythroid hypoplasia (Figure 1, panel B).

The fever slowly but progressively decreased, and the patient improved over the next few days; the last recorded fever was on hospital day 9. Abdominal pain and diarrhea resolved. Ceftriaxone was discontinued after 8 days. When the patient was discharged on hospital day 14, arthralgia and myalgia had improved, oropharynx ulcerations had healed, the rash had resolved without desquamation, and blood counts and hepatic enzyme levels were returning to reference limits. Considerable sequelae (myalgia, arthralgia, headache, malaise, and fatigue) persisted for several months.

Diagnostic Workup

The initial suspected diagnosis was hematophagocytic syndrome (hemophagocytic lymphohistiocytosis). This clinical syndrome has been associated with a variety of viral, bacterial, fungal, and parasitic infections, as well as collagen–vascular diseases and malignancies. Initial diagnostic testing for various infectious diseases included blood screening for respiratory viruses, HIV, cytomegalovirus, and malaria parasites; all results were negative.

On hospital day 2, a diagnosis of a viral hemorrhagic fever was considered, and blood specimens were sent to the Centers for Disease Control and Prevention (CDC) for testing. Molecular testing results were negative for rickettsiae, filoviruses (Marburgviruses and Ebolaviruses), selected bunyaviruses (Rift Valley fever virus, Crimean Congo hemorrhagic fever virus), arenaviruses (Lassa, Lujo, and lymphocytic choriomeningitis viruses), and several arboviruses (yellow fever, dengue, O'nyong-nyong, chikungunya, and Zika viruses).

A pathogen-discovery deep-sequencing protocol was followed, as described (*5*,*6*). In brief, total RNA was extracted from blood and serum samples obtained 3 days after symptom onset; RNA was nonspecifically amplified with previously described primers (*7*). The cDNA library was sequenced on a 454 FLX Genome Sequencer (Roche Diagnostics, Indianapolis, IN, USA). Unassembled sequences

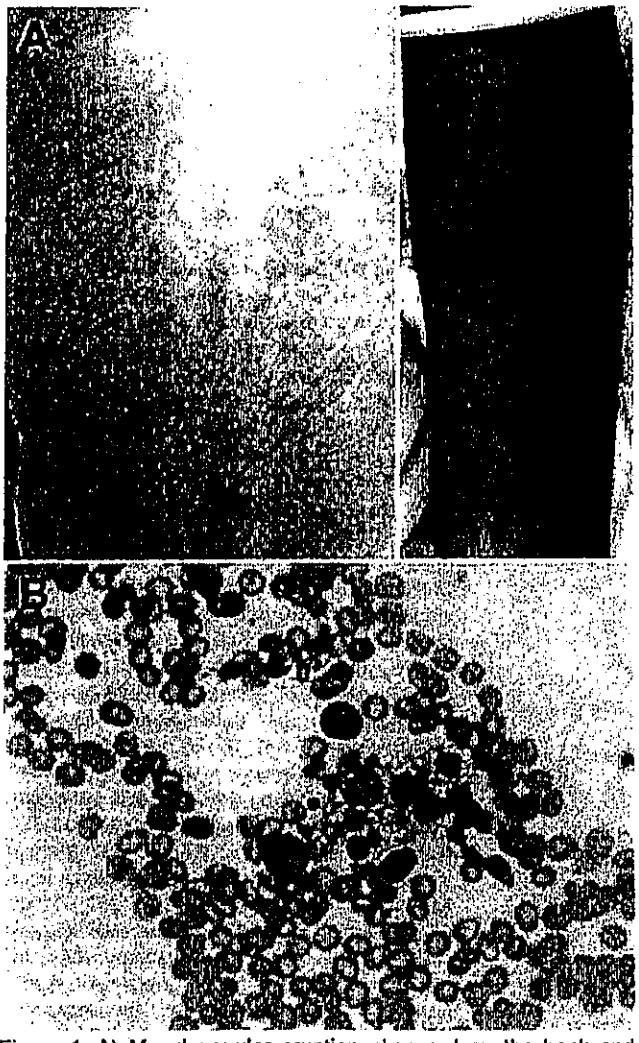

Figure 1. A) Maculopapular eruption observed on the back and arms of 25-year-old female wildlife biologist infected with a novel paramyxovirus related to rubula-like viruses isolated from fruit bats, on hospitalization day 2. B) Bone marrow biopsy sample showing macrocytic hemophagocytosis (possible granulocyte infiltration).

were translated and compared with the nonredundant protein database from the National Center for Biotechnology Information by using a BLASTx algorithm (www.ncbi.nlm.nih.gov/blast/Blast.cgi). The sequence reads linked to the BLASTx results were distributed into taxa by using MEGAN (*8*). Metagenomic analysis revealed a novel paramyxovirus in the patient's blood and serum; the virus was most closely related to Tuhoko virus 3, a rubula-like virus (family *Paramyxoviridae*) recently isolated from *Rousettus leschenaultii* fruit bats in southern China (*4*). The next-generation sequence reads with homology to Tuhoko virus 3 covered ≈25% of the expected complete virus genome. Based on the sequences obtained, a series of primers were designed to amplify overlapping fragments spanning the complete genome of this novel virus. A detailed list of primers is available upon request. Amplicons of different sizes were obtained by standard reverse transcription PCR (RT-PCR) and sequenced by the standard Sanger method (*5*,*6*).

This novel paramyxovirus is provisionally named Sosuga virus in recognition of its probable geographic origin (South Sudan, Uganda). The complete genome of Sosuga virus was 15,480 nt long and conformed to the paramyxovirus rule of 6 (*1*). The genome organization (Figure 2, panels A, B) resembled that of most paramyxoviruses, containing 6 genes, *N*, *V/P*, *M*, *F*, *HN*, and *L*, encoding the 7 viral proteins: nucleocapsid (N), V protein (V), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin–neuraminidase (HN), and polymerase (L). The sequence of the RNA editing site in the *V/P* gene is identical to that of Tuhoko virus 3 (*4*). The faithful transcription of V/P generates the V mRNA, and a GG insertion at the editing site generates the P mRNA. In addition, the terminal 5′ and 3′ sequences of the virus were experimentally determined (Figure 2, panel C) by rapid amplification of cDNA ends as described (*9*).

Pairwise comparison of the full-length sequence of Sosuga virus with the closest related viruses showed 61.6%, 53.1%, and 51.4% identities, respectively, with Tuhoko virus 3, Achimota virus 1, and Achimota virus 2 (Achimota viruses were isolated from the *Eidolon helvum* fruit bat in Ghana) (*3*). When the deduced amino acid sequences of Sosuga virus were compared with those of Tuhoko virus, 3 proteins revealed overall amino acid identities ranging from 57.4% (HN) to 84% (N). Phylogenetic analysis of Sosuga virus and other paramyxoviruses clearly showed that Sosuga virus clusters with other bat-borne rubula-like viruses, which are closely related to rubulaviruses but have not yet been classified as such (Figure 2, panel D).

Virus Isolation

Virus isolation was attempted by inoculating monolayers of Vero-E6, Vero-SLAM, and H292 cells (mucoepidermoid carcinoma cells from human lungs) with patient blood and serum collected 3 days after symptom onset, but no virus isolate was obtained. As an alternative, 10 μL of the blood sample was also inoculated intracranially and intraperitoneally into 2-day-old suckling mice, which were then observed for 28 days for signs of illness. Neurologic signs developed 9–10 days after inoculation in 2 of the 20 mice; these 2 mice were euthanized 2 days later. To confirm the presence of the virus, we extracted total RNA from liver, spleen, and brains of the euthanized animals and used it as input in a specific RT-PCR designed to amplify a 2,188-bp fragment partially spanning the virus *HN* and *L* genes. Consistent with virus replication and observed neurologic signs, viral RNA was found in the brain but not in liver or spleen samples (Figure 3, panel A).

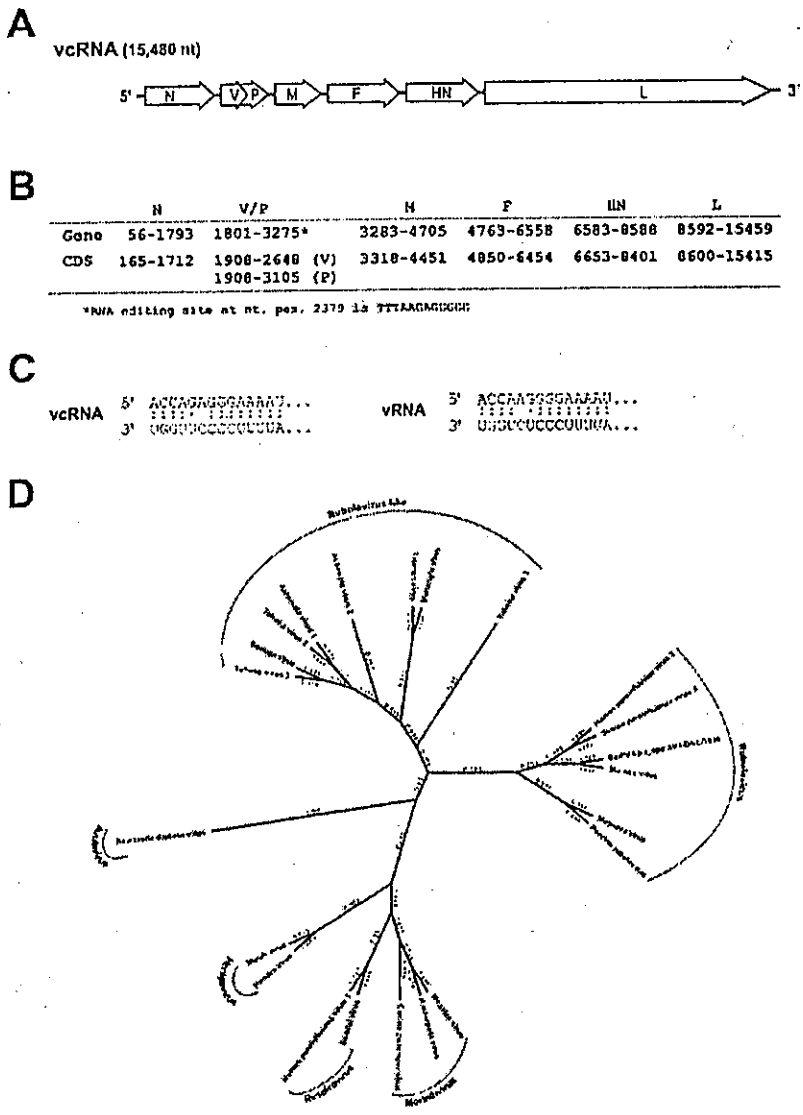

Figure 2. A) Organization of the viral genome of novel paramyxovirus related to rubula-like viruses isolated from fruit bats was determined from the full-length sequence. B) Localization of the predicted viral genes and open reading frames (ORFs). The V/P edition site is predicted from the similarity to Tuhoko virus 3. C) Terminal sequences were determined by standard rapid amplification of cDNA ends (RACE) methods. The complementarity of terminal sequences is shown in vRNA and vcRNA sense. D) Amino acid sequences of the nucleocapsid (N) protein of 22 representative paramyxovirus sequences were aligned by using the MUSCLE algorithm (CLC Genomics Workbench version 6.0.1; CLC bio, Cambridge, MA, USA). The phylogenetic analysis was conducted with a Bayesian algorithm (Mr. Bayes, Geneious version 6.1.5, www.geneious.com/). NP sequences were extracted from the complete genomic sequences in GenBank: KF774436 (Sosuga virus [SosV]), GU128082 (Tuhoko virus 3), GU128081 (Tuhoko virus 2), GU128080 (Tuhoko virus 1), AF298895 (Tioman virus), NC_007620 (Menangle virus), JX051319 (Achimota virus 1), JX051320 (Achimota virus 2), NC_003443 (human parainfluenza virus type 2), AF052755 (simian parainfluenza virus 5), HQ660095 (bat paramyxovirus Epo_spe/AR1/DRC/2009), NC_002200 (mumps virus), NC_009489 (Mapuera virus), NC_009640 (porcine rubulavirus), NC_001498 (measles virus), NC_006296 (rinderpest virus), NC_001921 (canine distemper virus), NC_001552 (Sendai virus), NC_003461 (human parainfluenza virus type 1), NC_002728 (Nipah virus), NC_001906 (Henra virus), NC_002617 (Newcastle disease virus). vcRNA, viral complementary RNA; N, nucleocapsid protein; V/P, V protein; M, matrix protein; F, fusion protein; HN, hemagglutinin-neuraminidase; L, molecular weight DNA ladder; CDS, coding sequence; nt pos. nucleotide position; vRNA, viral RNA.

Brain homogenates from the euthanized mice were inoculated into fresh monolayers of Vero-E6 cells and H292 cells; 12 days after infection, a cytopathic effect, with cell rounding but no syncytia formation, became evident. Virus antigen was detected by immunofluorescence in both cell lines by using patient's convalescent-phase serum, collected 50 days after symptom onset (Figure 3, panel B). Moreover, transmission electron microscopy used to examine virus morphology showed pleomorphic virions, consistent with those of paramyxoviruses (Figure 3, panel C).

Development of New Diagnostic Assays

Because the patient seemed to have acquired the infection during her African research expedition, where she had had extensive contact with rodents and bats, other persons who also come in contact with bats or rodents, such as field biologists, local residents, or ecotourists, might be at risk for infection. This potential public health threat prompted us to develop diagnostic assays for the rapid detection of Sosuga virus.

First, we developed a TaqMan real-time RT-PCR selective for the *N* gene and tested it on all available serum and blood samples from the patient. This test showed that the patient's viremia peaked early in the course of the infection (cycle threshold 29.5 on day 3 after symptom onset), coinciding with the period of high fever and diverse irregularities in blood parameters (Table). By day 9, the viremia had decreased (cycle threshold 36.3); viremia was undetectable 11 days after symptom onset.

Second, we developed a new ELISA specific for Sosuga virus by using the virus recombinant nucleocapsid protein produced and purified from *Escherichia coli*. This assay was tested on all available serum samples from the patient (Table). Although IgG and IgM were not detectable on day

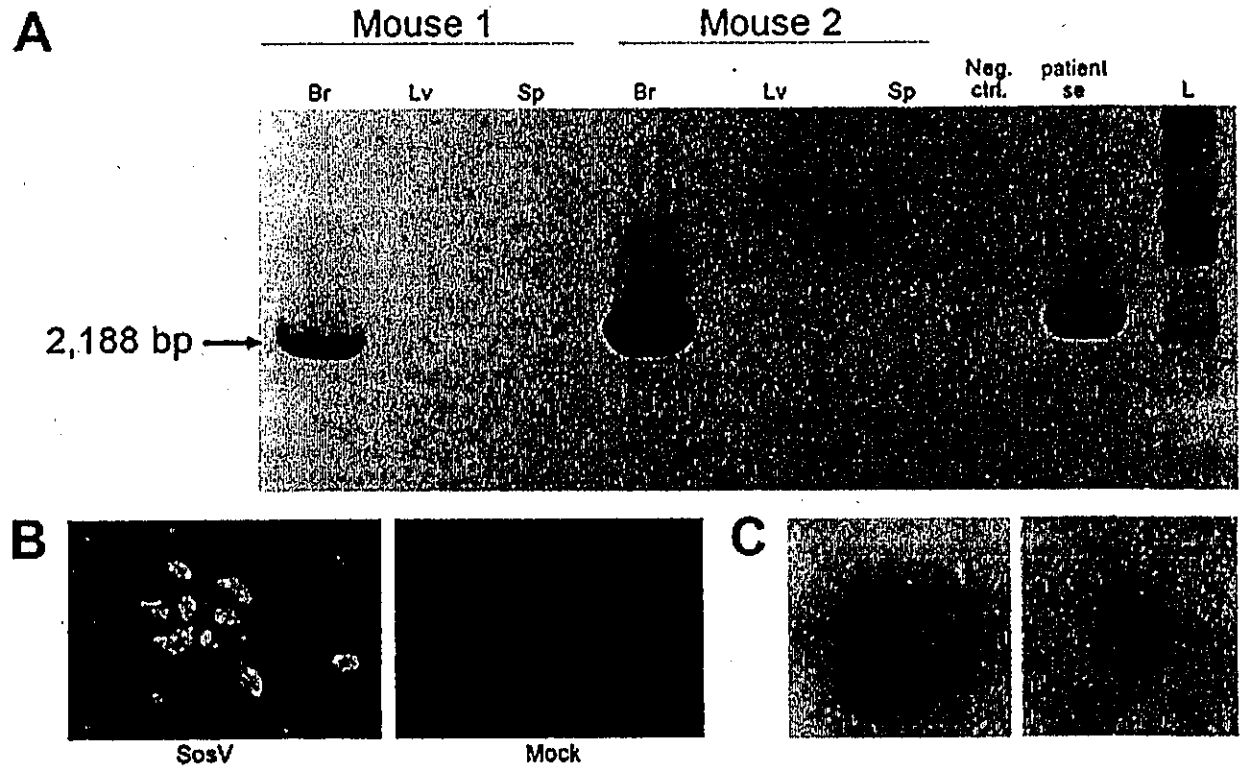

Figure 3. A) Virus isolation confirmed by reverse transcription PCR. SosV was isolated after intracranial and intraperitoneal inoculation into 2-day-old suckling mice. A specific reverse transcription PCR designed to amplify 2,188 bp of the SosV genome was performed by using RNA from brains (Br), liver (Lv), and spleen (Sp) of the euthanized animals. Viral RNA was found only in the brain, not in liver or spleen. B) Propagation of SosV in cell culture. Homogenized tissues (brain, liver, and spleen) were used to infect H292 cells. Fixed monolayers were stained with convalescent-phase serum from the patient and anti-human AlexaFluor 488 antibody (Invitrogen, Grand Island, NY, USA). C) SosV particle. Virus morphologic appearance was examined by taking supernatants from infected Vero-E6 cells, clarifying by slow-speed centrifugation, and depositing on grids for negative staining and examination by transmission electron microscopy. Pleomorphic virions can be observed. Neg.ctrl, negative control; Se, serum; SosV, Sosuga virus; L, molecular mass ladder.

3 after symptom onset (titers <50), seroconversion (IgG and IgM titers ≥1,600) occurred 11 days after symptom onset. As expected, IgM levels later decreased (titer ≥400), and IgG levels remained high 50 days after symptom onset.

In addition, the new ELISA was tested for potential cross-reactivity with some common paramyxoviruses, including mumps and measles viruses. No cross-reactivity was detected on the ELISA plates when control serum from patients with high levels of IgG against mumps and measles viruses was used, a desired feature in a new diagnostic assay because most persons have IgG to these viruses as a result of vaccination or natural infection.

Conclusions

A severe disease affected a wildlife biologist shortly after her return from rural Africa to the United States. Because of the disease characteristics (high fever and blood abnormalities) and travel history, a viral hemorrhagic fever was suspected, and clinical samples were rushed to CDC for investigation of a possible high-risk virus. After molecular and serologic diagnostic assays ruled out several well-known human pathogens (e.g., filoviruses, arenaviruses, phleboviruses, flaviviruses, and rickettsiae) as the cause of the patient's illness, a next-generation sequence approach was followed to detect a possible new infectious agent.

The combination of next-generation sequencing and metagenomic analysis identified a novel paramyxovirus; the virus genome was completely characterized by use of standard sequencing techniques. The complete virus sequence clearly indicated a relationship with other rubula-like viruses isolated from bats. Moreover, the novel virus was isolated from acute-phase serum samples by infecting suckling mice and propagating the virus in cell culture.

The specific molecular and serologic diagnostic assays that we developed will facilitate rapid identification of this novel infectious agent should new cases occur. We used these assays to retrospectively investigate all available

clinical samples from the patient, and the results revealed periods of viremia and seroconversion.

Although the exact source of the patient's infection remains unknown, the sequence similarity with bat-derived rubula-like viruses is highly suggestive. In recent years, a large number of diverse paramyxoviruses have been detected in bats (*2*,*10*), but only Nipah and Hendra viruses (genus *Henipavirus*) are known to cause severe disease in humans (*11*). An investigation to detect Sosuga virus in African bats is currently under way.

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Dr Albariño is a senior research fellow at CDC in Atlanta, Georgia. His research is focused on different aspects of RNA viruses with the goal of developing new diagnostic techniques and evaluating potential vaccines.

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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

○アンジオテンシン変換酵素II(ACE2)受容体を用いるコウモリSARS様コロナウイルス(SL-CoV)の分離と特徴
重症呼吸器症候群(SARS)CoVや中東呼吸器症候群(MERS)CoVは、コウモリが自然宿主である可能性が示されているが、コウモリからのSARS-CoV前駆ウイルスの分離は成功していない。近年、様々なSL-CoVが報告されているが、いずれも系統学的不一致及びSARS-CoV受容体分子であるヒトのACE2をスパイクタンパク質として用いることが出来ないことから、SARS-CoVの直接の前駆ウイルスとは考えられていない。
今回、中国雲南省のキクガシラコウモリから分離された2種類の新規コウモリCoV(RsSHC014、Rs3367)は、特にスパイクタンパク質の受容体結合ドメインにおいて、既知のコウモリCoVと比較しSARS-CoVとの近縁性が遙かに高いことが示された。さらに、コウモリの糞便サンプルから、典型的なCoV形態及びRs3367と99.9%の配列同一性を持ち、ヒト、ジャコウネコ、中国のキクガシラコウモリのACE2を細胞侵入のために用いることができる、SL-CoV(コウモリSL-CoV-WIV1)を分離することに初めて成功した。in vitroの予備実験の結果、WIV1は広い種指向性を持つことも示された。
これらの結果は、中国のキクガシラコウモリがSARS-CoVの保有宿主であり、一部のコウモリSL-CoVのヒト感染には中間宿主が必要ではないことを示す、これまでで最も強い証拠である。

報告企業の意見	今後の対応
中国のキクガシラコウモリの糞便から、細胞侵入のためにACE2を用いるSL-CoVを分離することに初めて成功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
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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

LETTER

doi:10.1038/nature12711

Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor

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The 2002–3 pandemic caused by severe acute respiratory syndrome coronavirus (SARS-CoV) was one of the most significant public health events in recent history[1]. An ongoing outbreak of Middle East respiratory syndrome coronavirus[2] suggests that this group of viruses remains a key threat and that their distribution is wider than previously recognized. Although bats have been suggested to be the natural reservoirs of both viruses[3–5], attempts to isolate the progenitor virus of SARS-CoV from bats have been unsuccessful. Diverse SARS-like coronaviruses (SL-CoVs) have now been reported from bats in China, Europe and Africa[5–8], but none is considered a direct progenitor of SARS-CoV because of their phylogenetic disparity from this virus and the inability of their spike proteins to use the SARS-CoV cellular receptor molecule, the human angiotensin converting enzyme II (ACE2)[9,10]. Here we report whole-genome sequences of two novel bat coronaviruses from Chinese horseshoe bats (family: Rhinolophidae) in Yunnan, China: RsSHC014 and Rs3367. These viruses are far more closely related to SARS-CoV than any previously identified bat coronaviruses, particularly in the receptor binding domain of the spike protein. Most importantly, we report the first recorded isolation of a live SL-CoV (bat SL-CoV-WIV1) from bat faecal samples in Vero E6 cells, which has typical coronavirus morphology, 99.9% sequence identity to Rs3367 and uses ACE2 from humans, civets and Chinese horseshoe bats for cell entry. Preliminary *in vitro* testing indicates that WIV1 also has a broad species tropism. Our results provide the strongest evidence to date that Chinese horseshoe bats are natural reservoirs of SARS-CoV, and that intermediate hosts may not be necessary for direct human infection by some bat SL-CoVs. They also highlight the importance of pathogen-discovery programs targeting high-risk wildlife groups in emerging disease hotspots as a strategy for pandemic preparedness.

The 2002–3 pandemic of SARS[1] and the ongoing emergence of the Middle East respiratory syndrome coronavirus (MERS-CoV)[2] demonstrate that CoVs are a significant public health threat. SARS-CoV was shown to use the human ACE2 molecule as its entry receptor, and this is considered a hallmark of its cross-species transmissibility[11]. The receptor binding domain (RBD) located in the amino-terminal region (amino acids 318–510) of the SARS-CoV spike (S) protein is directly involved in binding to ACE2 (ref. 12). However, despite phylogenetic evidence that SARS-CoV evolved from bat SL-CoVs, all previously identified SL-CoVs have major sequence differences from SARS-CoV in the RBD of their S proteins, including one or two deletions[6,9]. Replacing the RBD of one SL-CoV S protein with SARS-CoV S conferred the ability to use human ACE2 and replicate efficiently in mice[9,13]. However, to date, no SL-CoVs have been isolated from bats, and no wild-type SL-CoV of bat origin has been shown to use ACE2.

We conducted a 12-month longitudinal survey (April 2011–September 2012) of SL-CoVs in a colony of *Rhinolophus sinicus* at a single location in Kunming, Yunnan Province, China (Extended Data Table 1). A total of 117 anal swabs or faecal samples were collected from individual bats using a previously published method[5,14]. A one-step reverse transcription (RT)-nested PCR was conducted to amplify the RNA-dependent RNA polymerase (RdRP) motifs A and C, which are conserved among alphacoronaviruses and betacoronaviruses[15].

Twenty-seven of the 117 samples (23%) were classed as positive by PCR and subsequently confirmed by sequencing. The species origin of all positive samples was confirmed to be *R. sinicus* by cytochrome b sequence analysis, as described previously[16]. A higher prevalence was observed in samples collected in October (30% in 2011 and 48.7% in 2012) than those in April (7.1% in 2011) or May (7.4% in 2012) (Extended Data Table 1). Analysis of the S protein RBD sequences indicated the presence of seven different strains of SL-CoVs (Fig. 1a and Extended Data Figs 1 and 2). In addition to RBD sequences, which closely matched previously described SL-CoVs (Rs672, Rf1 and HKU3)[5,8,17,18], two novel strains (designated SL-CoV RsSHC014 and Rs3367) were discovered. Their full-length genome sequences were determined, and both were found to be 29,787 base pairs in size (excluding the poly(A) tail). The overall nucleotide sequence identity of these two genomes with human SARS-CoV (Tor2 strain) is 95%, higher than that observed previously for bat SL-CoVs in China (88–92%)[5,8,17,18] or Europe (76%)[6] (Extended Data Table 2 and Extended Data Figs 3 and 4). Higher sequence identities were observed at the protein level between these new SL-CoVs and SARS-CoVs (Extended Data Tables 3 and 4). To understand the evolutionary origin of these two novel SL-CoV strains, we conducted recombination analysis with the Recombination Detection Program 4.0 package[19] using available genome sequences of bat SL-CoV strains (Rf1, Rp3, Rs672, Rm1, HKU3 and BM48-31) and human and civet representative SARS-CoV strains (BJ01, SZ3, Tor2 and GZ02). Three breakpoints were detected with strong P values ($<10^{-20}$) and supported by similarity plot and bootscan analysis (Extended Data Fig. 5a, b). Breakpoints were located at nucleotides 20,827, 26,553 and 28,685 in the Rs3367 (and RsSHC014) genome, and generated recombination fragments covering nucleotides 20,827–26,533 (5,727 nucleotides) (including partial open reading frame (ORF) 1b, full-length S, ORF3, E and partial M gene) and nucleotides 26,534–28,685 (2,133 nucleotides) (including partial ORF M, full-length ORF6, ORF7, ORF8 and partial N gene). Phylogenetic analysis using the major and minor parental regions suggested that Rs3367, or RsSHC014, is the descendent of a recombination of lineages that ultimately lead to SARS-CoV and SL-CoV Rs672 (Fig. 1b).

The most notable sequence differences between these two new SL-CoVs and previously identified SL-CoVs is in the RBD regions of their S proteins. First, they have higher amino acid sequence identity to SARS-CoV (85% and 96% for RsSHC014 and Rs3367, respectively). Second, there are no deletions and they have perfect sequence alignment with the SARS-CoV RBD region (Extended Data Figs 1 and 2). Structural

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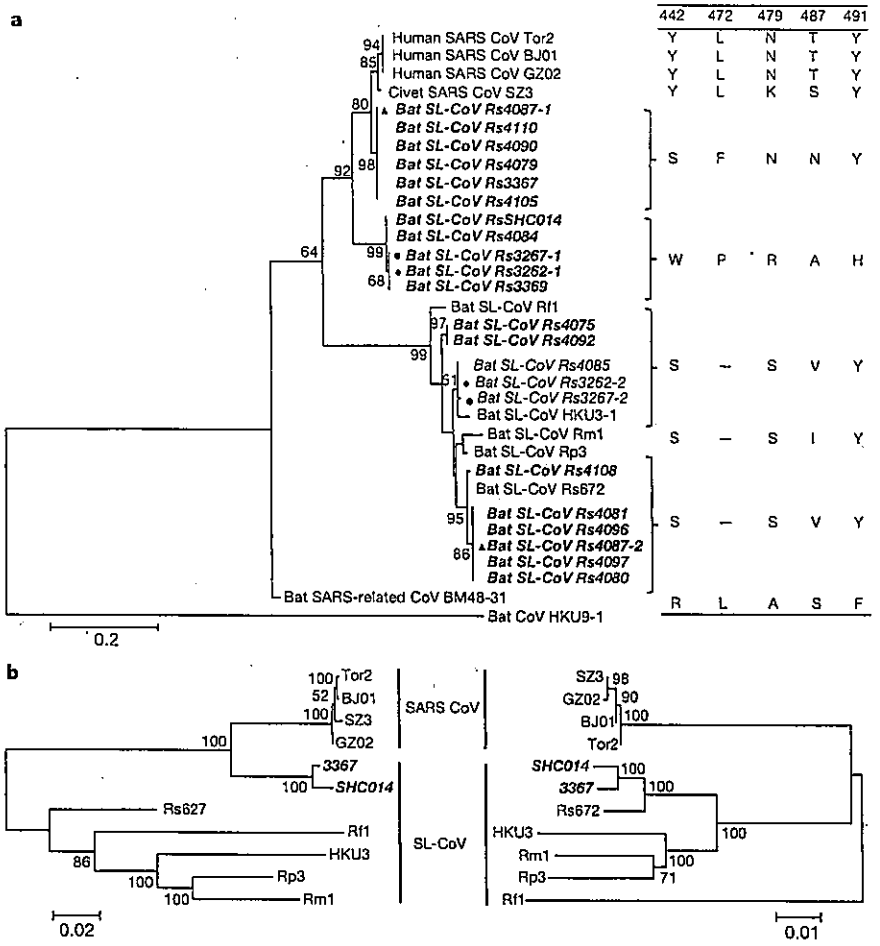

Figure 1 | Phylogenetic tree based on amino acid sequences of the S RBD region and the two parental regions of bat SL-CoV Rs3367 or RsSHC014. a, SARS-CoV S protein amino acid residues 310–520 were aligned with homologous regions of bat SL-CoVs using the ClustalW software. A maximum-likelihood phylogenetic tree was constructed using a Poisson model with bootstrap values determined by 1,000 replicates in the MEGA5 software package. The RBD sequences identified in this study are in bold and named by the sample numbers. The key amino acid residues involved in interacting with the human ACE2 molecule are indicated on the right of the tree. SARS-CoV GZ02, BJ01 and Tor2 were isolated from patients in the early, middle and late phase, respectively, of the SARS outbreak in 2003. SARS-CoV SZ3 was identified from *Paguma larvata* in 2003 collected in Guangdong, China. SL-CoV Rp3, Rs672 and HKU3-1 were identified from *R. sinicus* collected in China (respectively: Guangxi, 2004; Guizhou, 2006; Hong Kong, 2005). Rf1 and Rm1 were identified from *R. ferrumequinum* and *R. macrotis*, respectively, collected in Hubei, China, in 2004. Bat SARS-related CoV BM48-31 was identified from *R. blasii* collected in Bulgaria in 2008. Bat CoV HKU9-1 was identified from *Rousettus leschenaultii* collected in Guangdong, China in 2005/2006 and used as an outgroup. All sequences in bold and italics were identified in the current study. Filled triangles, circles and diamonds indicate samples with co-infection by two different SL-CoVs. '–' indicates the amino acid deletion. b, Phylogenetic origins of the two parental regions of Rs3367 or RsSHC014. Maximum likelihood phylogenetic trees were constructed from alignments of two fragments covering nucleotides 20,827–26,533 (5,727 nucleotides) and 26,534 –28,685 (2,133 nucleotides) of the Rs3367 genome, respectively. For display purposes, the trees were midpoint rooted. The taxa were annotated according to strain names: SARS-CoV, SARS coronavirus; SARS-like CoV, bat SARS-like coronavirus. The two novel SL-CoVs, Rs3367 and RsSHC014, are in bold and italics.

and mutagenesis studies have previously identified five key residues (amino acids 442, 472, 479, 487 and 491) in the RBD of the SARS-CoV S protein that have a pivotal role in receptor binding[20,21]. Although all five residues in the RsSHC014 S protein were found to be different from those of SARS-CoV, two of the five residues in the Rs3367 RBD were conserved (Fig. 1 and Extended Data Fig. 1).

Despite the rapid accumulation of bat CoV sequences in the last decade, there has been no report of successful virus isolation[6,22,23]. We attempted isolation from SL-CoV PCR-positive samples. Using an optimized protocol and Vero E6 cells, we obtained one isolate which caused cytopathic effect during the second blind passage. Purified virions displayed typical coronavirus morphology under electron microscopy (Fig. 2). Sequence analysis using a sequence-independent amplification method[14] to avoid PCR-introduced contamination indicated that the isolate was almost identical to Rs3367, with 99.9% nucleotide genome sequence identity and 100% amino acid sequence identity for the S1 region. The new isolate was named SL-CoV-WIV1.

To determine whether WIV1 can use ACE2 as a cellular entry receptor, we conducted virus infectivity studies using HeLa cells expressing or not expressing ACE2 from humans, civets or Chinese horseshoe bats. We found that WIV1 is able to use ACE2 of different origins as an entry receptor and replicated efficiently in the ACE2-expressing cells (Fig. 3). This is, to our knowledge, the first identification of a wild-type bat SL-CoV capable of using ACE2 as an entry receptor.

To assess its cross-species transmission potential, we conducted infectivity assays in cell lines from a range of species. Our results (Fig. 4 and Extended Data Table 5) indicate that bat SL-CoV-WIV1 can grow in human alveolar basal epithelial (A549), pig kidney 15 (PK-15) and *Rhinolophus sinicus* kidney (RSKT) cell lines, but not in human cervix (HeLa), Syrian golden hamster kidney (BHK21), *Myotis davidii* kidney (BK), *Myotis chinensis* kidney (MCKT), *Rousettus leschenaultii* kidney (RLK) or *Pteropus alecto* kidney (PaKi) cell lines. Real-time RT–PCR indicated that WIV1 replicated much less efficiently in A549, PK-15 and RSKT cells than in Vero E6 cells (Fig. 4).

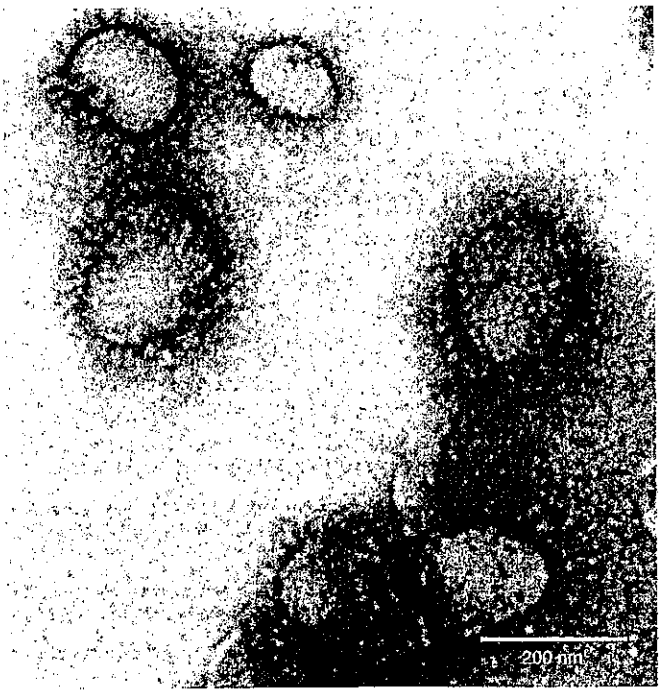

Figure 2 | Electron micrograph of purified virions. Virions from a 10-ml culture were collected, fixed and concentrated/purified by sucrose gradient centrifugation. The pelleted viral particles were suspended in 100 µl PBS, stained with 2% phosphotungstic acid (pH 7.0) and examined directly using a Tecnai transmission electron microscope (FEI) at 200 kV.

To assess the cross-neutralization activity of human SARS-CoV sera against WIV1, we conducted serum-neutralization assays using nine convalescent sera from SARS patients collected in 2003. The results showed that seven of these were able to completely neutralize 100 tissue culture infectious dose 50 ($TCID_{50}$) WIV1 at dilutions of 1:10 to 1:40, further confirming the close relationship between WIV1 and SARS-CoV.

Our findings have important implications for public health. First, they provide the clearest evidence yet that SARS-CoV originated in bats. Our previous work provided phylogenetic evidence of this[5], but the lack of an isolate or evidence that bat SL-CoVs can naturally infect human cells, until now, had cast doubt on this hypothesis. Second, the lack of capacity of SL-CoVs to use of ACE2 receptors has previously been considered as the key barrier for their direct spillover into humans, supporting the suggestion that civets were intermediate hosts for SARS-CoV adaptation to human transmission during the SARS outbreak[24]. However, the ability of SL-CoV-WIV1 to use human ACE2 argues against the necessity of this step for SL-CoV-WIV1 and suggests that direct bat-to-human infection is a plausible scenario for some bat SL-CoVs. This has implications for public health control measures in the face of potential spillover of a diverse and growing pool of recently discovered SARS-like CoVs with a wide geographic distribution.

Our findings suggest that the diversity of bat CoVs is substantially higher than that previously reported. In this study we were able to demonstrate the circulation of at least seven different strains of SL-CoVs within a single colony of *R. sinicus* during a 12-month period. The high genetic diversity of SL-CoVs within this colony was mirrored by high phenotypic diversity in the differential use of ACE2 by different strains. It would therefore not be surprising if further surveillance reveals a broad diversity of bat SL-CoVs that are able to use ACE2, some of which may have even closer homology to SARS-CoV than SL-CoV-WIV1. Our results—in addition to the recent demonstration of MERS-CoV in a Saudi Arabian bat[25], and of bat CoVs closely related to MERS-CoV in China, Africa, Europe and North America[3,26,27]—suggest that bat coronaviruses remain a substantial global threat to public health.

Finally, this study demonstrates the public health importance of pathogen discovery programs targeting wildlife that aim to identify the 'known unknowns'—previously unknown viral strains closely related to known pathogens. These programs, focused on specific high-risk wildlife groups and hotspots of disease emergence, may be a critical part of future global strategies to predict, prepare for, and prevent pandemic emergence[28].

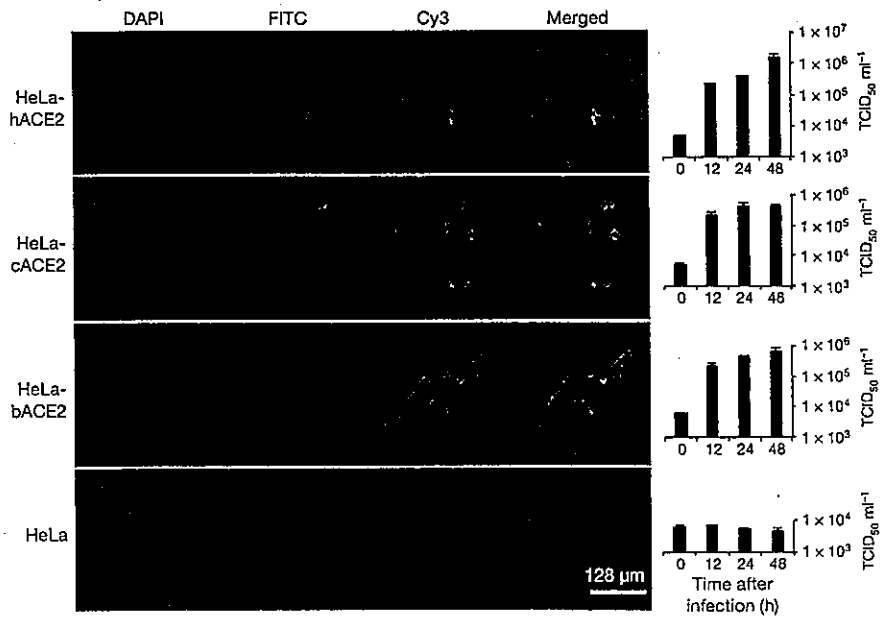

Figure 3 | Analysis of receptor usage of SL-CoV-WIV1 determined by immunofluorescence assay and real-time PCR. Determination of virus infectivity in HeLa cells with and without the expression of ACE2. b, bat; c, civet; h, human. ACE2 expression was detected with goat anti-humanACE2 antibody followed by fluorescein isothiocyanate (FITC)-conjugated donkey anti-goat IgG. Virus replication was detected with rabbit antibody against the SL-CoV Rp3 nucleocapsid protein followed by cyanine 3 (Cy3)-conjugated mouse anti-rabbit IgG. Nuclei were stained with DAPI (4′,6-diamidino-2-phenylindole). The columns (from left to right) show staining of nuclei (blue), ACE2 expression (green), virus replication (red), merged triple-stained images and real-time PCR results, respectively. ($n = 3$); error bars represent standard deviation.

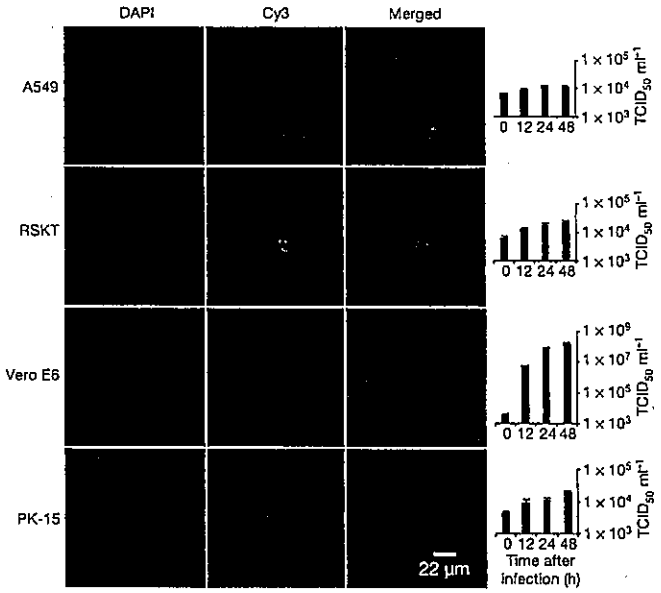

Figure 4 | Analysis of host range of SL-CoV-WIV1 determined by immunofluorescence assay and real-time PCR. Virus infection in A549, RSKT, Vero E6 and PK-15 cells. Virus replication was detected as described for Fig. 3. The columns (from left to right) show staining of nuclei (blue), virus replication (red), merged double-stained images and real-time PCR results, respectively. $n = 3$; error bars represent s.d.

METHODS SUMMARY

Throat and faecal swabs or fresh faecal samples were collected in viral transport medium as described previously[14]. All PCR was conducted with the One-Step RT-PCR kit (Invitrogen). Primers targeting the highly conserved regions of the RdRP gene were used for detection of all alphacoronaviruses and betacoronaviruses as described previously[15]. Degenerate primers were designed on the basis of all available genomic sequences of SARS-CoVs and SL-CoVs and used for amplification of the RBD sequences of S genes or full-length genomic sequences. Degenerate primers were used for amplification of the bat *ACE2* gene as described previously[29]. PCR products were gel purified and cloned into pGEM-T Easy Vector (Promega). At least four independent clones were sequenced to obtain a consensus sequence. PCR-positive faecal samples (in 200 μl buffer) were gradient centrifuged at 3,000–12,000*g* and supernatant diluted at 1:10 in DMEM before being added to Vero E6 cells. After incubation at 37 °C for 1 h, inocula were removed and replaced with fresh DMEM with 2% FCS. Cells were incubated at 37 °C and checked daily for cytopathic effect. Cell lines from different origins were grown on coverslips in 24-well plates and inoculated with the novel SL-CoV at a multiplicity of infection of 10. Virus replication was detected at 24 h after infection using rabbit antibodies against the SL-CoV Rp3 nucleocapsid protein followed by Cy3-conjugated goat anti-rabbit IgG.

Online Content Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions Z.-L.S. and P.D. designed and coordinated the study. X.-Y.G., J.-L. L. and X.-L.Y. conducted majority of experiments and contributed equally to the study. A.A.C., B.H., W.Z., C.P., Y.-J.Z., C.-M.L., B.T., N.W. and Y.Z. conducted parts of the experiments and analyses. J.H.E., J.K.M. and S.-Y.Z. coordinated the field study. X.-Y.G., J.-L.L., X.-L.Y., B.T. and G.-J.Z. collected the samples. G.C. and L.-F.W. designed and supervised part of the experiments. All authors contributed to the interpretations and conclusions presented. Z.-L.S. and X-Y.G. wrote the manuscript with significant contributions from P.D. and L-F.W. and input from all authors.

Author Information Sequences of three bat SL-CoV genomes, bat SL-CoV RBD and *R. sinicus ACE2* genes have been deposited in GenBank under accession numbers KC881005–KC881007 (genomes from SL-CoV RsSHC014, Rs3367 and WIV1, respectively), KC880984–KC881003 (bat SL-CoV RBD genes) and KC881004 (*R. sinicus ACE2*), respectively. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to P.D. (daszak@ecohealthalliance.org) or Z.-L.S. (zlshi@wh.iov.cn).

METHODS

Sampling. Bats were trapped in their natural habitat as described previously[5]. Throat and faecal swab samples were collected in viral transport medium (VTM) composed of Hank's balanced salt solution, pH 7.4, containing BSA (1%), amphotericin (15 μg ml^{-1}), penicillin G (100 U ml^{-1}) and streptomycin (50 μg ml^{-1}). To collect fresh faecal samples, clean plastic sheets measuring 2.0 by 2.0 m were placed under known bat roosting sites at about 18:00 h each evening. Relatively fresh faecal samples were collected from sheets at approximately 05:30–06:00 the next morning and placed in VTM. Samples were transported to the laboratory and stored at −80 °C until use. All animals trapped for this study were released back to their habitat after sample collection. All sampling processes were performed by veterinarians with approval from Animal Ethics Committee of the Wuhan Institute of Virology (WIVH05210201) and EcoHealth Alliance under an inter-institutional agreement with University of California, Davis (UC Davis protocol no. 16048).

RNA extraction, PCR and sequencing. RNA was extracted from 140 μl of swab or faecal samples with a Viral RNA Mini Kit (Qiagen) following the manufacturer's instructions. RNA was eluted in 60 μl RNAse-free buffer (buffer AVE, Qiagen), then aliquoted and stored at −80 °C. One-step RT–PCR (Invitrogen) was used to detect coronavirus sequences as described previously[15]. First round PCR was conducted in a 25-μl reaction mix containing 12.5 μl PCR 2× reaction mix buffer, 10 pmol of each primer, 2.5 mM MgSO4, 20 U RNase inhibitor, 1 μl SuperScript III/ Platinum Taq Enzyme Mix and 5 μl RNA. Amplification of the RdRP-gene fragment was performed as follows: 50 °C for 30 min, 94 °C for 2 min, followed by 40 cycles consisting of 94 °C for 15 s, 62 °C for 15 s, 68 °C for 40 s, and a final extension of 68 °C for 5 min. Second round PCR was conducted in a 25-μl reaction mix containing 2.5 μl PCR reaction buffer, 5 pmol of each primer, 50 mM $MgCl_2$, 0.5 mM dNTP, 0.1 μl Platinum Taq Enzyme (Invitrogen) and 1 μl first round PCR product. The amplification of RdRP-gene fragment was performed as follows: 94 °C for 5 min followed by 35 cycles consisting of 94 °C for 30 s, 52 °C for 30 s, 72 °C for 40 s, and a final extension of 72 °C for 5 min.

To amplify the RBD region, one-step RT–PCR was performed with primers designed based on available SARS-CoV or bat SL-CoVs (first round PCR primers; F, forward; R, reverse: CoVS931F-5′-VWGADGTTGTKAGRTTYCCT-3′ and CoVS1909R-5′-TAARACAVCCWGCYTGWGT-3′; second PCR primers: CoVS 951F-5′-TGTKAGRTTYCCTAAYATTAC-3′ and CoVS1805R-5′-ACATCYTG ATANARAACAGC-3′). First-round PCR was conducted in a 25-μl reaction mix as described above except primers specific for the S gene were used. The amplification of the RBD region of the S gene was performed as follows: 50 °C for 30 min, 94 °C for 2 min, followed by 35 cycles consisting of 94 °C for 15 s, 43 °C for 15 s, 68 °C for 90 s, and a final extension of 68 °C for 5 min. Second-round PCR was conducted in a 25-μl reaction mix containing 2.5 μl PCR reaction buffer, 5 pmol of each primer, 50 mM $MgCl_2$, 0.5 mM dNTP, 0.1 μl Platinum Taq Enzyme (Invitrogen) and 1 μl first round PCR product. Amplification was performed as follows: 94 °C for 5 min followed by 40 cycles consisting of 94 °C for 30 s, 41 °C for 30 s, 72 °C for 60 s, and a final extension of 72 °C for 5 min.

PCR products were gel purified and cloned into pGEM-T Easy Vector (Promega). At least four independent clones were sequenced to obtain a consensus sequence for each of the amplified regions.

Sequencing full-length genomes. Degenerate coronavirus primers were designed based on all available SARS-CoV and bat SL-CoV sequences in GenBank and specific primers were designed from genome sequences generated from previous rounds of sequencing in this study (primer sequences will be provided upon request). All PCRs were conducted using the One-Step RT–PCR kit (Invitrogen). The 5′ and 3′ genomic ends were determined using the 5′ or 3′ RACE kit (Roche), respectively. PCR products were gel purified and sequenced directly or following cloning into pGEM-T Easy Vector (Promega). At least four independent clones were sequenced to obtain a consensus sequence for each of the amplified regions and each region was sequenced at least twice.

Sequence analysis and databank accession numbers. Routine sequence management and analysis was carried out using DNAStar or Geneious. Sequence alignment and editing was conducted using ClustalW, BioEdit or GeneDoc. Maximum Likelihood phylogenetic trees based on the protein sequences were constructed using a Poisson model with bootstrap values determined by 1,000 replicates in the MEGA5 software package.

Sequences obtained in this study have been deposited in GenBank as follows (accession numbers given in parenthesis): full-length genome sequence of SL-CoV RsSHC014 and Rs3367 (KC881005, KC881006); full-length sequence of WIV1 S (KC881007); RBD (KC880984-KC881003); ACE2 (KC8810040). SARS-CoV sequences used in this study: human SARS-CoV strains Tor2 (AY274119), BJ01 (AY278488), GZ02 (AY390556) and civet SARS-CoV strain SZ3 (AY304486). Bat coronavirus sequences used in this study: Rs672 (FJ588686), Rp3 (DQ071615), Rf1 (DQ412042), Rm1 (DQ412043), HKU3-1 (DQ022305), BM48-31 (NC_014470), HKU9-1 (NC_009021), HKU4 (NC_009019), HKU5 (NC_009020), HKU8 (DQ249228), HKU2 (EF203067), BtCoV512 (NC_009657), 1A (NC_010437). Other coronavirus sequences used in this study: HCoV-229E (AF304460), HCoV-OC43 (AY391777), HCoV-NL63 (AY567487), HKU1 (NC_006577), EMC (JX869059), FIPV (NC_002306), PRCV (DQ811787), BWCoV (NC_010646), MHV (AY700211), IBV (AY851295).

Amplification, cloning and expression of the bat ACE2 gene. Construction of expression clones for human and civet ACE2 in pcDNA3.1 has been described previously[29]. Bat ACE2 was amplified from a *R. sinicus* (sample no. 3357). In brief, total RNA was extracted from bat rectal tissue using the RNeasy Mini Kit (Qiagen). First-strand complementary DNA was synthesized from total RNA by reverse transcription with random hexamers. Full-length bat *ACE2* fragments were amplified using forward primer bAF2 and reverse primer bAR2 (ref. 29). The *ACE2* gene was cloned into pCDNA3.1 with KpnI and XhoI, and verified by sequencing. Purified *ACE2* plasmids were transfected to HeLa cells. After 24 h, lysates of HeLa cells expressing human, civet, or bat ACE2 were confirmed by western blot or immunofluorescence assay.

Western blot analysis. Lysates of cells or filtered supernatants containing pseudoviruses were separated by SDS–PAGE, followed by transfer to a nitrocellulose membrane (Millipore). For detection of S protein, the membrane was incubated with rabbit anti-Rp3 S fragment (amino acids 561–666) polyantibodies (1:200), and the bound antibodies were detected by alkaline phosphatase (AP)-conjugated goat anti-rabbit IgG (1:1,000). For detection of HIV-1 p24 in supernatants, monoclonal antibody against HIV p24 (p24 MAb) was used as the primary antibody at a dilution of 1:1,000, followed by incubation with AP-conjugated goat anti-mouse IgG at the same dilution. To detect the expression of ACE2 in HeLa cells, goat antibody against the human ACE2 ectodomain (1:500) was used as the first antibody, followed by incubation with horseradish peroxidase-conjugated donkey anti-goat IgG (1:1,000).

Virus isolation. Vero E6 cell monolayers were maintained in DMEM supplemented with 10% FCS. PCR-positive samples (in 200 μl buffer) were gradient centrifuged at 3,000–12,000*g*, and supernatant were diluted 1:10 in DMEM before being added to Vero E6 cells. After incubation at 37 °C for 1 h, inocula were removed and replaced with fresh DMEM with 2% FCS. Cells were incubated at 37 °C for 3 days and checked daily for cytopathic effect. Double-dose triple antibiotics penicillin/streptomycin/amphotericin (Gibco) were included in all tissue culture media (penicillin 200 IU ml^{-1}, streptomycin 0.2 mg ml^{-1}, amphotericin 0.5 μg ml^{-1}). Three blind passages were carried out for each sample. After each passage, both the culture supernatant and cell pellet were examined for presence of virus by RT–PCR using primers targeting the RdRP or S gene. Virions in supernatant (10 ml) were collected and fixed using 0.1% formaldehyde for 4 h, then concentrated by ultracentrifugation through a 20% sucrose cushion (5 ml) at 80,000*g* for 90 min using a Ty90 rotor (Beckman). The pelleted viral particles were suspended in 100 μl PBS, stained with 2% phosphotungstic acid (pH 7.0) and examined using a Tecnai transmission electron microscope (FEI) at 200 kV.

Virus infectivity detected by immunofluorescence assay. Cell lines used for this study and their culture conditions are summarized in Extended Data Table 5. Virus titre was determined in Vero E6 cells by cytopathic effect (CPE) counts. Cell lines from different origins and HeLa cells expressing ACE2 from human, civet or Chinese horseshoe bat were grown on coverslips in 24-well plates (Corning) incubated with bat SL-CoV-WIV1 at a multiplicity of infection = 10 for 1 h. The inoculum was removed and washed twice with PBS and supplemented with medium. HeLa cells without ACE2 expression and Vero E6 cells were used as negative and positive controls, respectively. At 24 h after infection, cells were washed with PBS and fixed with 4% formaldehyde in PBS (pH 7.4) for 20 min at 4 °C. ACE2 expression was detected using goat anti-human ACE2 immunoglobulin (R&D Systems) followed by FITC-labelled donkey anti-goat immunoglobulin (PTGLab). Virus replication was detected using rabbit antibody against the SL-CoV Rp3 nucleocapsid protein followed by Cy3-conjugated mouse anti-rabbit IgG. Nuclei were stained with DAPI. Staining patterns were examined using a FV1200 confocal microscope (Olympus).

Virus infectivity detected by real-time RT–PCR. Vero E6, A549, PK15, RSKT and HeLa cells with or without expression of ACE2 of different origins were inoculated with 0.1 $TCID_{50}$ WIV-1 and incubated for 1 h at 37 °C. After removing the inoculum, the cells were cultured with medium containing 1% FBS. Supernatants were collected at 0, 12, 24 and 48 h. RNA from 140 μl of each supernatant was extracted with the Viral RNA Mini Kit (Qiagen) following manufacturer's instructions and eluted in 60 μl buffer AVE (Qiagen). RNA was quantified on the ABI StepOne system, with the TaqMan AgPath-ID One-Step RT–PCR Kit (Applied Biosystems) in a 25 μl reaction mix containing 4 μl RNA, 1 × RT–PCR enzyme mix, 1 × RT–PCR buffer, 40 pmol forward primer (5′-GTGGTGGTGACGGCA AAATG-3′), 40 pmol reverse primer (5′-AAGTGAAGCTTCTGGGCCAG-3′) and 12 pmol probe (5′-FAM-AAAGAGCTCAGCCCCAGATG-BHQ1-3′). Amplification parameters were 10 min at 50 °C, 10 min at 95 °C and 50 cycles of 15 s at 95 °C and 20 s at 60 °C. RNA dilutions from purified WIV-1 stock were used as a standard.

Serum neutralization test. SARS patient sera were inactivated at 56 °C for 30 min and then used for virus neutralization testing. Sera were diluted starting with 1:10

and then serially twofold diluted in 96-well cell plates to 1:40. Each 100 µl serum dilution was mixed with 100 µl viral supernatant containing 100 $TCID_{50}$ of WIV1 and incubated at 37 °C for 1 h. The mixture was added in triplicate wells of 96-well cell plates with plated monolayers of Vero E6 cells and further incubated at 37 °C for 2 days. Serum from a healthy blood donor was used as a negative control in each experiment. CPE was observed using an inverted microscope 2 days after inoculation. The neutralizing antibody titre was read as the highest dilution of serum which completely suppressed CPE in infected wells. The neutralization test was repeated twice.

Recombination analysis. Full-length genomic sequences of SL-CoV Rs3367 or RsSHC014 were aligned with those of selected SARS-CoVs and bat SL-CoVs using Clustal X. The aligned sequences were preliminarily scanned for recombination events using Recombination Detection Program (RDP) 4.0 (ref. 19). The potential recombination events suggested by RDP owing to their strong *P* values ($<10^{-20}$) were investigated further by similarity plot and bootscan analyses implemented in Simplot 3.5.1. Phylogenetic origin of the major and minor parental regions of Rs3367 or RsSHC014 were constructed from the concatenated sequences of the essential ORFs of the major and minor parental regions of selected SARS-CoV and SL-CoVs. Two genome regions between three estimated breakpoints (20,827–26,553 and 26,554–28,685) were aligned independently using ClustalX and generated two alignments of 5,727 base pairs and 2,133 base pairs. The two alignments were used to construct maximum likelihood trees to better infer the fragment parents. All nucleotide numberings in this study are based on Rs3367 genome position.

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
				2014. 12. 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		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)		ProMED 20131204.2092523	インド

研究報告の概要

○クリミア・コンゴ出血熱(CCHF)ーインド、グジャラート州での発生増加
2011年にインドで初めてCCHFが報告されたグジャラート州において、アウトブレイクの規模が拡大している。専門家によると、3年間でCCHFに感染した患者が30人報告され、14人が死亡した。感染の約半数はBharwadと呼ばれる牛飼いの集団において発生している。

報告企業の意見	今後の対応
インドのグジャラート州で、クリミア・コンゴ出血熱アウトブレイクが拡大しているとの報告である。	日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、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

JRC2013T-046

Published Date: 2013-12-04 21:58:46
Subject: PRO/AH/EDR> Crimean-Congo hem. fever - India (04): (GJ) increasing incidence
Archive Number: 20131204.2092523

CRIMEAN-CONGO HEMORRHAGIC FEVER - INDIA (04): (GUJARAT), INCREASING INCIDENCE
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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: 4 Dec 2013
Source: The Indian Express [edited]
http://www.indianexpress.com/news/congo-fever-outbreak-in-state-doubles-this-year/1202997/

Outbreaks of Crimean-Congo hemorrhagic fever [CCHF] seem to have doubled in Gujarat, the 1st state to have reported the prevalence of this tick-borne viral disease in the country.

In the last 3 years [2011-2013] alone, there have been 30 positive cases and 14 deaths due to CCHF, half of which were reported in a cattle-rearing community known as the Bharwads, stated experts who participated in the day-long conference "Healthy Gujarat: Agenda For Action," organised by the Gujarat Health and Family Welfare Department on Tuesday [3 Dec 2013].

"The 1st instance of CCHF was detected in Kolat village near Ahmedabad in January 2011. That year, there were 4 outbreaks of CCHF. The year 2012 was reportedly milder, with 2 outbreaks. However, in 2013, there were as many as 8 outbreaks," said Dr Kamlesh Upadhyay, a professor in the Department of Medicine at Ahmedabad-based BJ Medical College & Civil Hospital.

"In the last 3 years, there have been 30 positive cases of virus infection, 50 percent of which are from the Bharwad community," added Upadhyay, who was speaking on the topic of "Disease Burden" of Gujarat. "Today, there are 14 sites in Gujarat from where CCHF has been reported," he said.

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[Crimean-Congo haemorrhagic fever (CCHF) outbreaks have a case fatality rate of up to 40 per cent. The virus is primarily transmitted to people from ticks harboured by livestock animals. Human-to-human transmission can occur resulting from close contact with the blood, secretions, organs, or other bodily fluids of those infected. Infected domestic animals do not usually show signs of infection, which may contribute to the general lack of awareness of the disease even in endemic areas and a toleration of tick bites.

CCHF is endemic in Africa, the Balkans, the Middle East and Asia, in countries south of the 50th parallel north. There is no vaccine available for either people or animals.

A map of the states and territories of India showing the location of Gujarat in the northwest can be accessed at http://www.mapsofindia.com/maps/india/india-political-map.gif. - Mod.CP

A HealthMap/ProMED-mail map can be accessed at: http://healthmap.org/r/1pSH.]

See Also

Crimean-Congo hem. fever - India (03): (GJ) fatal 20130817.1886236
Crimean-Congo hem. fever - India (02): (GJ) human deaths 20130730.1854571
Crimean-Congo hem. fever - India: (GJ) 20130715.1826284
2012

Crimean-Congo hem. fever - India (03): (GJ) 20120722.1210673
Crimean-Congo hem. fever - India (02): (GJ) nosocomial 20120625.1180179
Crimean-Congo hem. fever - India: (GJ) nosocomial 20120623.1178585
2011

Crimean-Congo hem. fever - India (05): (GJ) 20110530.1653
Crimean-Congo hem. fever - India (04): (ND) alert 20110519.1518
Crimean-Congo hem. fever - India (03): (MP) susp. 20110221.0573
Crimean-Congo hem. fever - India (02): treatment 20110129.0353
Crimean-Congo hemorrhagic fever - India: (GJ) 20110123.0285
.....sb/cp/msp/lm

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. 12. 2	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	Emerging Infectious Disease Journal, Vol.19 No.12; Available from: http://wwwnc.cdc.gov/eid/article/19/12/13-0589_article.htm	公表国 ブラジル
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

○ブラジル・パラ州の人獣共通感染ワクシニアウイルスの再興
2010年7月、ブラジルのアマゾン地域に位置するパラ州において、乳牛44頭と酪農従事者3人が重症発疹性のワクシニアウイルスに感染した。感染したウシの痂皮及びウシとヒトの血清サンプルからウイルスを分離し系統発生解析を行ったところ、1963年にパラー州の齧歯類から分離されたBeAN58058株(BAV)及び南米で使用された天然痘ワクチンウイルス株とは異なる株であることが明らかとなり、この地域で新規のワクシニアウイルスが定着していることが示された。

報告企業の意見	今後の対応
2010年にブラジルのパラ州で乳牛と酪農労働者に重症発疹性のワクシニアウイルス感染が発生し、ウイルス分離株の分析を行ったところ、1963年に同地域で分離されたウイルス株及び天然痘ワクチン株とは異なることが明らかとなり、新規株の定着が示されたとの報告である。	日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。今後も引き続き、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
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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

Reemergence of Vaccinia Virus during Zoonotic Outbreak, Pará State, Brazil

Felipe L. de Assis, Wagner M. Vinhote, José D. Barbosa, Cairo H.S. de Oliveira, Carlos M.G. de Oliveira, Karinny F. Campos, Natália S. Silva, Giliane de Souza Trindade, Jônatas S. Abrahão, and Erna G. Kroon

In 2010, vaccinia virus caused an outbreak of bovine vaccinia that affected dairy cattle and rural workers in Pará State, Brazil. Genetic analyses identified the virus as distinct from BeAn58058 vaccinia virus (identified in 1960s) and from smallpox vaccine virus strains. These findings suggest spread of autochthonous group 1 vaccinia virus in this region.

Over the past decade, several exanthematous vaccinia virus (VACV) outbreaks that affected dairy cattle and rural workers have been reported in Brazil. During outbreaks, lesions developed on teats and udders of dairy cattle and caused a decrease in milk production (*1,2*). Infected milkers usually had lesions on their hands; the infection was apparently transmitted by unprotected contact with infected cattle (*1,2*).

Molecular studies have shown that autochthonous VACVs from Brazil (VACV-BR) can be divided into 2 groups: group 1 and group 2 (*3,4*). Group 1 includes isolates Cantagalo, Araçatuba, Passatempo, Guarani P2, Mariana, and Pelotas 2; group 2 includes isolates Guarani P1, Pelotas1, and BeAN58058 (BAV). This molecular dichotomy is also reflected in certain biologic properties of the isolates, including virulence in the BALB/c mouse model and plaque phenotype in BSC-40 cells (*1*). Although each VACV strain has unique genetic characteristics, most of them are similar to each other within the same group, especially those belonging to group 1; they most likely share a common ancestor. Although some researchers believed that VACV vaccine strains could have spread from humans to domestic animals and adapted to the rural environment (*2*), recent studies have suggested an independent origin for VACV isolates from South America, which is distinct from vaccine strains used in South America during the World Health Organization vaccination campaign. (*3,4*).

Despite emergence of VACV in the past decade, VACV was also isolated during the 1960s and 1970s during government efforts to investigate emerging viruses in forests in Brazil (*5–7*). One of those isolates, BAV, was obtained in 1963 from the blood of a rodent in Pará State in the Amazon region of Brazil that belonged to the genus *Oryzomys* (*6,7*). BAV was characterized during the 1990s, and restriction pattern and nucleotide sequence data supported its classification as a VACV (*6*). However, since its isolation, VACV circulation has not been reported in Pará State, even after VACV outbreaks in southeastern Brazil (*1*).

In this report, we describe reemergence VACV during a severe exanthematous outbreak in Pará State, 47 years after isolation of BAV. Our molecular data showed that this new VACV isolate clusters with group 1 VACV-BR isolates, which is the same VACV clade related to most viruses that caused zoonotic outbreaks in rural areas of Brazil in the past decade.

The Study

The outbreak was reported in July 2010 in Bom Jesus do Tocantins County (5°2′ 60″S, 48°36′36″W), Pará State, in the Amazon region of Brazil (Figure 1, Appendix, wwwnc.cdc.gov/EID/article/19/12/13-0589-F1.htm).

Dairy cattle and workers were affected. However, the source (index case) of this outbreak was not identified. At the study site, 44 lactating dairy cows became sick and had painful vesicular lesions on teats, udders, and inner thighs that rapidly progressed to ulcerative lesions and scabs (Figure 2, panels B and D). Two animals had extensive necrosis because of secondary infections, which led to loss of teats. Lesions were also observed on lips, muzzles, oral cavities, and tongues of calves (Figure 2, panel D). Three dairy workers became sick during the outbreak after direct contact with sick animals and had typical orthopoxvirus (OPV) lesions on their hands, forearms, and abdomen (Figure 2, panel A). Pain in the lesion region, fever, and fatigue were also reported by sick patients.

We collected 4 scabs and 44 serum samples from the 44 sick animals and 3 serum samples from the 3 dairy workers. Serum samples were tested by using 50% plaque reduction neutralization tests as described (*8*). Neutralizing antibodies were detected in 40 (90.0%) bovine and in 3 (100%) human samples, and titers ranged from 20 to 640 neutralizing units/mL. Scabs were macerated in buffer and centrifuged. Supernatants were diluted 1:100 in phosphate-buffered saline and used in a nested PCR specific for the C11R viral

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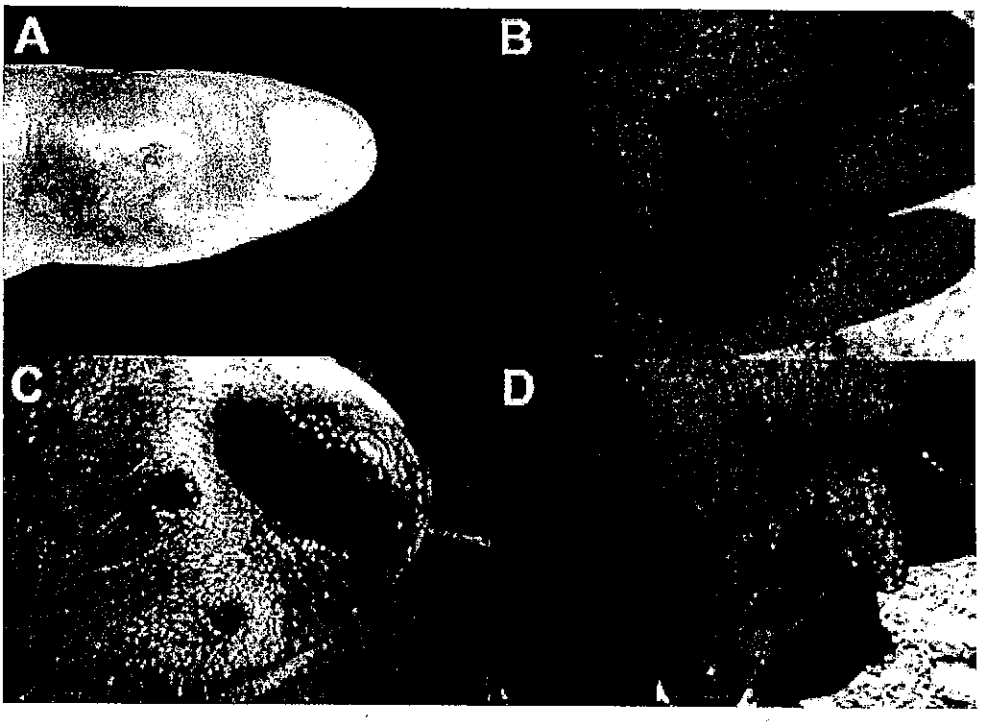

Figure 2. Exanthematic lesions caused by vaccinia virus (VACV) infection during this outbreak. A) Vesicular lesion on milker's finger that advanced to an ulcerative stage. B and D) Typical lesions on teats and udder of a dairy cow infected by VACV at different stages, ranging from ulceration to scabs. C) Lesions on a calf's muzzle probably caused by VACV infection during suckling.

growth factor gene as described (*9,10*). OPV-specific fragments from 2 scab samples were amplified. Samples were also subjected to virus isolation in Vero cells.

We isolated virus from 1 of the samples that was positive for viral growth factor by nested PCR. Negative results for VACV by PCR and virus isolation might have been caused by loss of virus titers and DNA degradation during sample transportation. After a typical poxvirus cytopathic effect was observed, virus was plaque purified and placed on Vero cell monolayers for viral amplification. This new VACV isolate was named Pará virus (PARV).

To investigate the relationship between PARV and BAV, virus gene A56R (hemagglutinin) was amplified and sequenced (*11*). The A56R gene is traditionally used for phylogenetic analysis. In addition, PARV A26L (A-type inclusion body) was also sequenced (*12*). The PCR fragments obtained were directly sequenced in both orientations and in triplicate by using a Mega-BACE 1000 Sequencer) (GE Healthcare, Little Chalfont, UK). Sequences were aligned with OPV sequences from GenBank by using ClustalW (www.ncbi.nlm.nih.gov/pmc/articles/PMC308517), and alignments were manually checked with MEGA version 4.0 software (Arizona State University, Phoenix, AZ, USA).

Optimal alignment of the A56R gene showed high identity among PARV and several group 1 VACV-BR isolates (average identity 99.8%), including VACV-TO CA (GU322359) (identity 99.9%), an amplicon obtained from blood of an Amazon monkey in Tocantins State, Brazil, in 2002 (Figure 3). PARV and BAV showed 98.3% identity with each other. PARV also showed a signature deletion of 18 nt that was also present in A56R sequences of other group 1 VACV-BR isolates

Phylogenetic trees of the A56R (Figure 3, panel A) or A26L (Figure 3, panel B) genes were constructed by using the neighbor-joining method, 1,000 bootstrap replicates, and the Tamura 3-parameter model (MEGA version 4). PARV sequences clustered with several group 1 VACV-BRs isolated during several bovine vaccinia outbreaks in Brazil. In both trees, PARV clustered in group 1 VACV-BR, whereas BAV clustered in group 2.

Conclusions

Our results confirm circulation of a new group 1 VACV-BR isolate in Pará State in the Amazon region of Brazil in the same location where BAV (group 2 VACV-BR) was isolated (*6,7*). In recent years, Bovine vaccinia outbreaks in southeastern Brazil rapidly spread to neighboring states (*1*). Epidemiologic studies suggest that movement of sick humans and the animal trade are the main causes of this circulation (*1*). The relevance of VACV circulation in the context of bovine vaccinia outbreaks has been discussed (*13*).

Several isolates belong to group 1, which is most commonly isolated from sick cattle or cow milkers; some isolates were detected in peridomestic rodents and wild monkeys (*8,13*). Therefore, although our data demonstrated that PARV does not cluster with BAV, it is not possible to phylogenetically define which group 1 isolate specifically generated PARV or caused the outbreak because of limited number of available gene sequences from VACV-BR

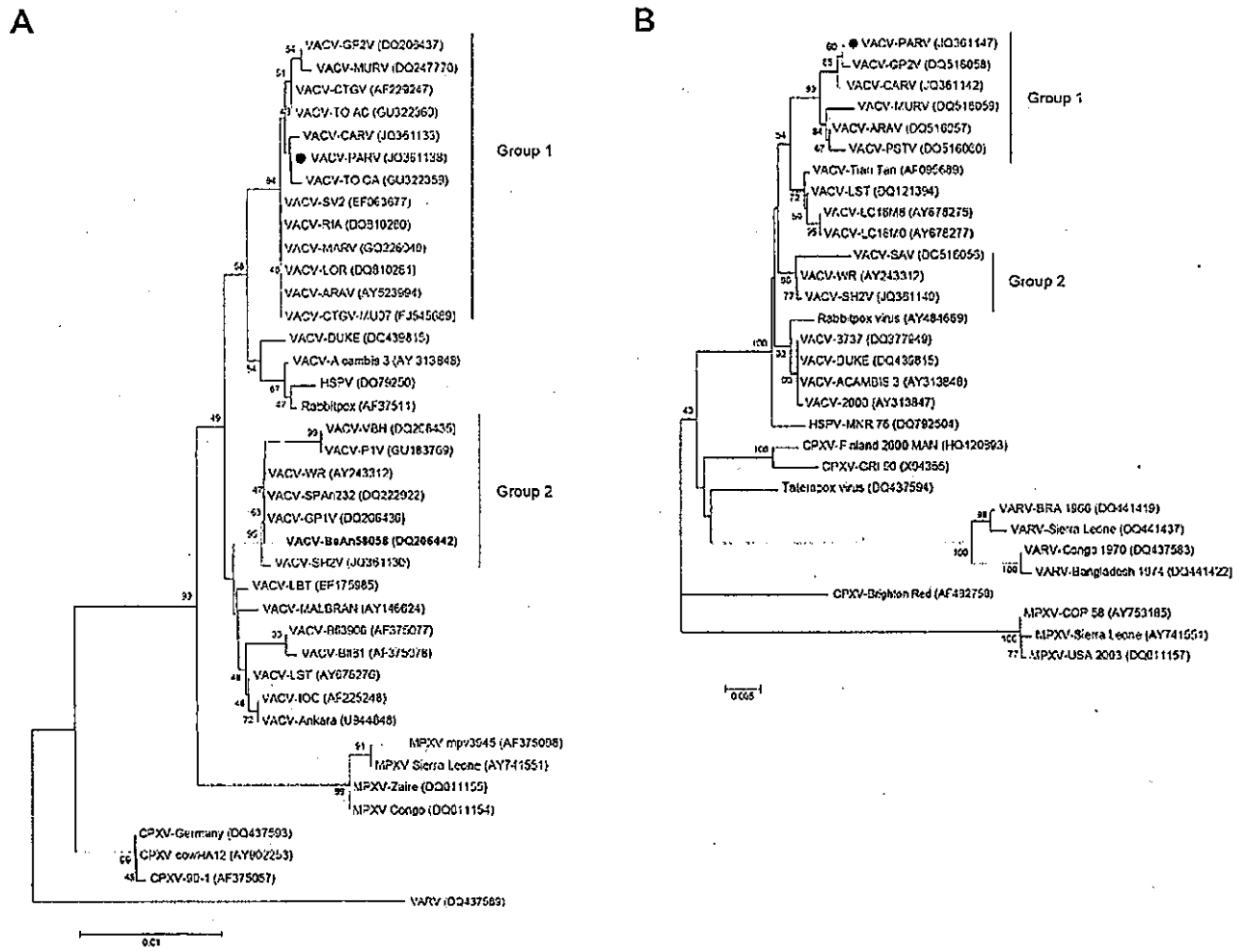

Figure 3. Phylogenetic trees based on orthopoxvirus nucleotide sequences of A56R (A) and A26L (B) genes of vaccinia virus (VACV), Pará State, Brazil. Pará virus (PARV) clusters with VACV group 1 from Brazil. Phylogenetic analysis showed that PARV (black dots) clustered in the VACV-BR-G1 clade and that BeAN58058 virus (BAV) clustered in the VACV-BR-G2 clade. A26L sequence was obtained only from PARV. Trees were constructed by using the neighbor-joining method, the Tamura-Nei model of nucleotide substitutions, and bootstrap of 1,000 replicates in MEGA version 4.0 software (Arizona State University, Phoenix, AZ, USA). In panel A, BAV is shown in boldface. GenBank accession numbers are indicated in parentheses. Values along the branches indicate bootstrap values. Scale bars indicate nucleotide substitutions per site. GP2V, Guarani P2 virus; MURV, Muriae virus; CTGV, Cantagalo virus; CARV, Carangola virus; MARV, Mariana virus; ARAV, Araçatuba virus; HSPV, horsepox virus; GP1V, Guarani P1 virus; MPXV, monkeypox virus; PSTV, Passatempo; CPXV, cowpox virus; VARV, variola virus.

isolates. Nevertheless, we believe the presence of this new isolate in Pará State likely resulted from virus spread from Tocantins, Maranhão, or Mato Grosso, 3 neighboring states of Pará State, which had Bovine vaccinia outbreaks in recent years, and not from reemergence of BAV (*1*).

Although group 2 VACV-BR isolates, including BAV, have not been detected in the Amazon region of Brazil in recent years, we believe that these viruses may be silently circulating or associated with bovine vaccinia outbreaks. As in other regions in Brazil, VACV outbreaks are underreported in the Amazon region because of its large size and the natural complexity involved in surveillance of zoonotic diseases. Our results reinforce the need for studies on VACV diversity and its transmission chain, which would be useful for the Amazon region in Brazil.

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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
				2013. 12. 26	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	Emerging Infectious Disease Journal, Vol.20 No.1; Available from: http://wwwnc.cdc.gov/eid/article/20/1/13-1071_article.htm	公表国 日本
販売名(企業名)	新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)			

研究報告の概要

○シャーガス病母子感染、日本
2012年10月、日本在住の13歳の少年が2年前からの慢性的な便秘のため防衛医科大学病院に入院した。両親及び祖父母は1992年までボリビアに住んでいた日系人である。教会で受けた*Trypanosoma cruzi*血清学的スクリーニングで患者と母親が*T.cruzi*抗体陽性であり、巨大結腸症を伴う先天性シャーガス病であると診断された。ベンズニダゾールの経口投与による治療が行われ、末梢血における原虫血症及び*T.cruzi* DNAは陰性となった。
本報告は、日本を含むシャーガス病非流行国における*T.cruzi*垂直感染を防ぐための、適切な措置の必要性を示している。

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
日本におけるシャーガス病母子感染の報告である。	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。

Mother-to-Child Transmission of Congenital Chagas Disease, Japan

Kazuo Imai, Takuya Maeda, Yusuke Sayama, Kei Mikita, Yuji Fujikura, Kazuhisa Misawa, Morichika Nagumo, Osamu Iwata, Takeshi Ono, Ichiro Kurane, Yasushi Miyahira, Akihiko Kawana, and Sachio Miura

We report a patient with congenital Chagas disease in Japan. This report reemphasizes the role of neglected and emerging tropical diseases in the era of globalization. It also indicates the need for increased vigilance for detecting Chagas disease in non–disease-endemic countries.

Chagas disease, which is caused by the pathogenic protozoa *Trypanosoma cruzi*, was previously endemic only to Central and South America but is now estimated to affect up to 10 million persons worldwide (*1*). Recent unprecedented trends in globalization have been accompanied by the migration of ≈14 million persons from disease-endemic regions to North America, Europe, Japan, and Australia. Consequently, and as predicted, sporadic reports of patients with chronic Chagas disease have emerged, and documented cases have presumably been caused by chronically infected persons who migrated from disease-endemic countries (*2*). Despite the wide geographic spread of patients with Chagas disease, cases of congenital transmission in non–disease-endemic countries have been documented (Table 1) (*3*)

It is estimated that ≈300,000 immigrants from Latin America, to which Chagas disease is endemic, are currently living in Japan and that ≈34,000 births from these immigrants have occurred in the past 10 years. However, vertical transmission of the disease in Japan has not been detected, probably because of the lack of screening programs for at-risk pregnant women and the disregard for the silent clinical manifestation of congenital Chagas disease.

The World Health Organization recommends that each country should strengthen its national and regional capacity to prevent and control congenital transmission of infectious pathogens while improving case management (*4*). We report a patient with congenital Chagas disease in Japan. We also highlight the need for increasing awareness of congenital transmission and urge establishment of an appropriate diagnostic and treatment system for Chagas disease in nonendemic countries.

The Patient

In October 2012, a 13-year-old boy in Japan was admitted to the National Defense Medical College Hospital in Saitama, Japan, for chronic constipation. His parents and grandparents were Bolivian nationals of Japanese descent who had lived in Chagas disease–endemic areas in Bolivia until 1992. In 1999, the boy was delivered full-term after an uncomplicated pregnancy in Japan but had a low birthweight. He was in excellent health and showed no signs of disease until 2 years before his admission, when he began to report chronic constipation. At that time, he had a medical examination at a Catholic church because most hospitals in Japan could not make a definitive diagnosis of Chagas disease. He underwent serodiagnostic screening for *T. cruzi* infection. The boy and his mother were seropositive for *T. cruzi*.

After admission, he reported extreme constipation and explained that he defecated only once per week. Results of laboratory tests at admission, including those for serum brain natriuretic peptide, were generally within reference ranges. However, abdominal radiography showed major distension of the colon that extended 65 mm (Figure).

The definitive diagnosis, including effectiveness of antiparasitic treatment, was confirmed by using serologic, genomic, and parasitologic methods (Table 2). An ELISA (ORTHO *T. cruzi* ELISA Test System; Ortho-Clinical Diagnostics, Raritan, NJ, USA) was performed according to the manufacturer's protocol. A nested PCR that amplifies a DNA fragment of a repetitive TCZ sequence was performed as described (*5*). The parasite was also isolated by blood culture on Novy, McNeal, and Nicolle agar (*6*) and examined by light microscopy and real-time PCR. All tests showed positive results. It was later determined that the boy's mother was also seropositive for Chagas disease. The boy was given a diagnosis of congenital Chagas disease accompanied by megacolon.

The patient was treated with oral benznidazole (5 mg/kg/d for 60 days) and showed no adverse effects. Parasitemia and DNA of *T. cruzi* in peripheral blood could not be detected by the end of treatment. To ensure successful treatment and cure, we intend to clinically follow up the patient for several decades until serologic results eventually become negative (*7*).

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Table 1. Patients with congenital Chagas disease in non–disease-endemic countries*

Country	No. patients	Mother's country of origin	Age, y, at time of diagnosis	Symptoms at birth
Sweden	1	Chile	5	Asymptomatic
Spain	7	Argentina (2), Bolivia (5)	At birth (5), 2 (1), after death (1)	Asymptomatic (5), symptomatic (2)
Switzerland	2	Bolivia (2)	At birth (2)	Asymptomatic (2)
United States	1	Bolivia	At birth	Symptomatic
Japan	1†	Bolivia	13	Asymptomatic

*Values in parentheses are no. patients.
†Patient in this study.

Conclusions

Chagas disease is usually regarded as one of the most serious health problems in rural areas of Central and South America. However, recent successful vector control programs to reduce vector-borne transmission have dramatically changed the epidemiology of this disease (*8*). Mass migration of chronically infected and asymptomatic persons has caused globalization of Chagas disease, and has made nonvectorial infection, including vertical and blood-borne transmission, more of a threat to human communities than vectorial infection (*9*).

On the basis of local and limited serologic surveys, the presumptive number of chagasic patients living in Japan is currently 4,500, compared with >100,000 in the United States and >6,000 in Spain (*2*). Sporadic imported cases have been recognized and reported in Japan in the past decade, but the exact incidence is unknown. Most cases were diagnosed only after patients had critical complications, including severe cardiac involvement (*10*). It is also conceivable that chagasic patients with less severe cardiac symptoms or gastrointestinal involvement have sought treatment at local hospitals in Japan, where the potential for missing or misdiagnosing the disease would likely be high. The difficulty in making a correct diagnosis of Chagas disease is compounded in Japan by low awareness and recognition of the disease by medical staff; scarcity of epidemiologic or statistical data; and lack of diagnostic tools, resources, and facilities available to help with the differential diagnosis.

There is currently no laboratory test–based screening system for donated blood to detect Chagas disease in Japan. Instead, a questionnaire is used to determine if donors have any connections with disease-endemic regions. As of October 2012, to avoid transmission through transfusions, Japanese Red Cross Blood Centers no longer use donated blood for transfusions or producing blood products if the donor or donor's mother has spent ≥4 weeks in Latin America. Therefore, before 2012, it is difficult to estimate how many contaminated blood donations were overlooked in Japan.

The estimated vertical transmission rate from an infected mother to her newborn is ≈5% in Bolivia (*11*). If one considers that 34,000 children were born to Latin American women during the past decade in Japan and that the seroprevalence of *T. cruzi* is estimated to be 1.8%, the number of infected newborns in the past decade is ≈30. However, there are no current screening programs for Chagas disease in Japan to detect chronically infected persons, including pregnant women and newborns.

The patient in this study had congenital chagasic infection, accompanied by advanced gastrointestinal complications. The delay in diagnosis for this patient case was caused by the absence of a screening program in Japan, a problem which also makes it impossible to determine the precise number of pregnant women and newborns with *T. cruzi* infection in this country. In Spain, the most affected country in Europe, a specific program was developed to focus on migrants from Latin American woman of childbearing age. Since its introduction, the program has contributed not only to the early diagnosis of Chagas disease but also to improvements in the quality of life and prognosis for patients (*12*).

Because the therapeutic efficacy of treatment, including benznidazole, for infection with *T. cruzi* is >90% in infants

Figure. Abdominal radiograph of a 13-year-old boy with congenital Chagas disease, Japan, showing megacolon and marked dilatation at the splenic flexure.

Table 2. Clinical course of a 13-year-old boy with congenital Chagas disease, Japan, after treatment with benznidazole

Characteristic	Before treatment	Days after starting treatment		
		30	60	180
Antibody titer*	160	160	160	160
Nested PCR result	+	+	–	–
Blood culture result	+	–	–	–

*Antibodies against *Trypanosoma cruzi*.

with congenitally transmitted Chagas disease if treated during the first year of life (*13*), it would be ideal for all pregnant women entering Japan from disease-endemic countries to be screened for the presence of serum antibody against *T. cruzi*. This report indicates the urgent need for implementing proper measures to prevent the vertical transmission of *T. cruzi* in non–Chagas disease–endemic countries, including Japan.

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感染症定期報告に関する今後の対応について

平成１６年度第５回
運営委員会確認事項
（平成１６年９月１７日）

１ 基本的な方針

運営委員会に報告する資料においては、

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

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

２ 具体的な方法

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

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

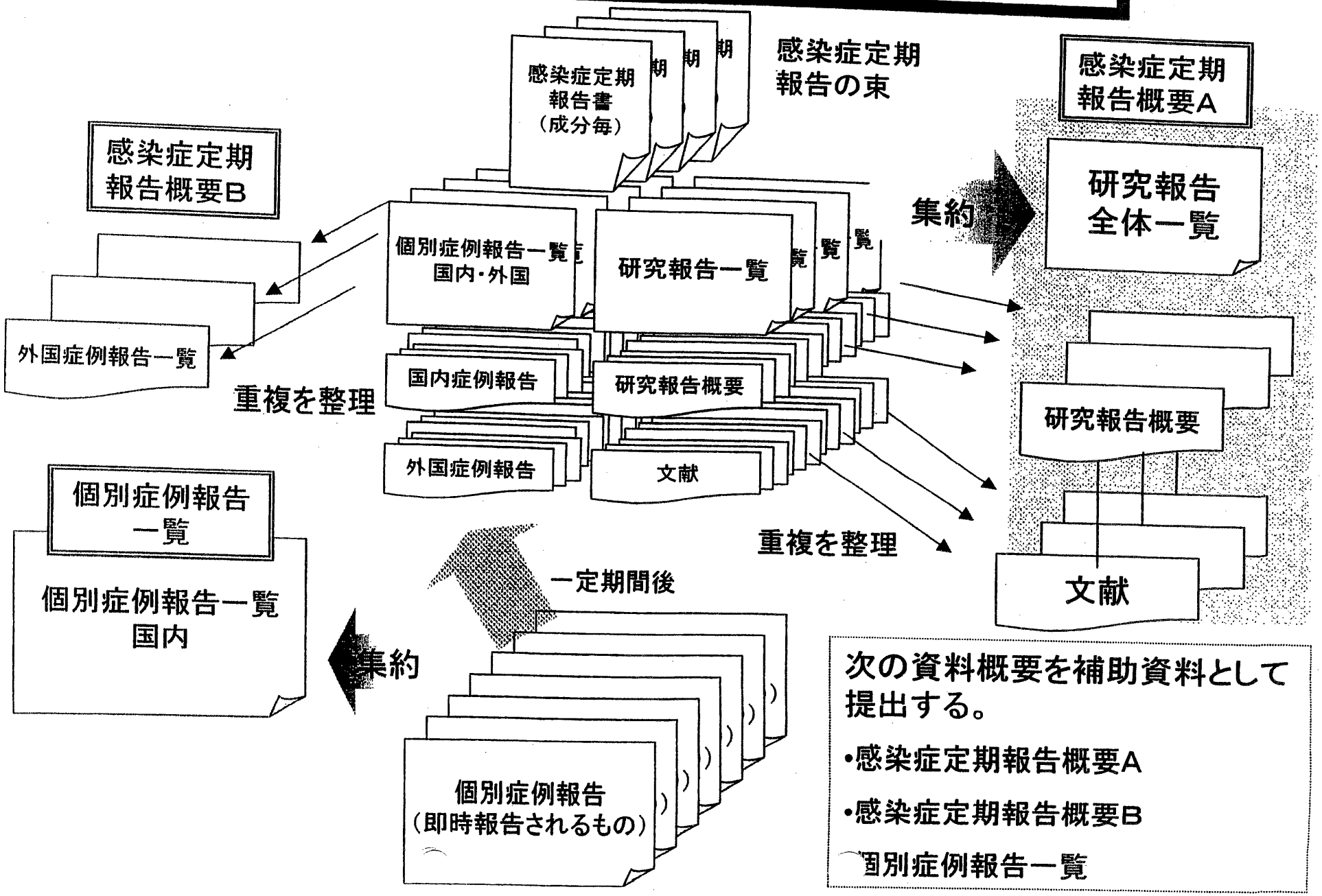
② 感染症発生症例報告のうち、発現国が「外国」の血漿分画製剤の使用による症例は、同一製品毎に報告期間を代表する感染症発生症例一覧（別紙様式第4）をまとめた**「資料概要B」**を事務局が作成し、送付する。

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

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

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

感染症定期報告・感染症個別症例報告の取り扱い



次の資料概要を補助資料として提出する。

- 感染症定期報告概要A
- 感染症定期報告概要B
- 個別症例報告一覧