資料２－２

Ａ　研究報告（詳細版）

平成２８年５月３１日
（平成２８年２月～平成２８年４月受理分）

別紙様式第2-1

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2015. 11. 4	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	Baylis SA, Crossan C, Corman VM, et al. Vox Sang. 2015 Nov ;109(4) :406-9.	公表国 ドイツ	
販売名（企業名）	新鮮凍結血漿-LR「日赤」120（日本赤十字社） 新鮮凍結血漿-LR「日赤」240（日本赤十字社） 新鮮凍結血漿-LR「日赤」480（日本赤十字社）					

研究報告の概要

○再感染と考えられる血漿供血者のE型肝炎ウイルス（HEV）に対する稀な血清学的反応。
2010年から2012年に供血され、ミニプール血漿のスクリーニング検査により検出されたHEV RNA陽性血漿供血を対象として、血清学的方法によるHEV IgM抗体およびHEV IgG抗体の検査を実施した。IgG抗体陽性となった供血について親和性測定を行った結果、2名の供血者に親和性の高いHEV IgG抗体並びに高いウイルス量が認められたが、HEV IgM抗体は認められなかった。HEV RNAとHEV IgG抗体が陽性で、IgM抗体が存在しない急性HEV感染は再感染を示唆し、この時に産生される抗体は決まって高親和性である。高親和性抗体を持つウイルス血症状態の供血者の再感染の他の可能性は、HEV慢性感染かもしれないが、慢性感染は免疫抑制状態の個人にて報告されているのみであり供血者においては報告されていない。当該データはごく一部の血漿供血者における再感染を示唆しており、過去に報告例はない。

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
E型肝炎ウイルスに再感染したことを示す血漿供血者が検出されたという報告である。	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、治療等に関する研究」の一環として、重症化が懸念されるHEV Genotype 4の輸血感染報告があった北海道赤十字血液センターで輸血用血液について試行的個別NATを実施している。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。

VoxSanguinis

The International Journal of Transfusion Medicine

JRC2015T-034

ISBT International Society of Blood Transfusion

SHORT REPORT

Vox Sanguinis (2015) 109, 406–409
© 2015 International Society of Blood Transfusion
DOI: 10.1111/vox.12294

Unusual serological response to hepatitis E virus in plasma donors consistent with re-infection

S. A. Baylis,[1] C. Crossan,[2] V. M. Corman,[3,4] J. Blümel,[1] L. Scobie[2] & H. R. Dalton[5]

[1]Paul-Ehrlich-Institut, Langen, Germany

[2]Glasgow Caledonian University, Glasgow, UK

[3]Institute of Virology, University of Bonn Medical Centre, Bonn, Germany

[4]German Centre for Infection Research (DZIF), Partner Site Bonn-Cologne, Bonn, Germany

[5]Royal Cornwall Hospital Trust and European Centre for Environment and Human Health, University of Exeter, Truro, UK

Vox Sanguinis

Received: 10 March 2015, revised 8 April 2015, accepted 8 April 2015, published online 29 May 2015

Hepatitis E virus (HEV)-positive plasma donations, identified by a plasma mini-pool screening approach, were analysed using serological methods for the presence of anti-HEV IgM and IgG. Avidity testing was performed on the IgG-reactive donations. Anti-HEV IgG with high avidity was observed in two donors together with high viral loads, but with the absence of anti-HEV IgM. These data are suggestive of re-infection in a small proportion of plasma donors, which has not previously been reported.

Key words: avidity, HEV, NAT testing, reinfection.

Introduction

Screening studies for hepatitis E virus (HEV) in blood and plasma donor populations have demonstrated that there is a high prevalence of viraemic donors in several different countries in Europe including Germany, Sweden, the Netherlands, UK and France as well as Spain [1–8]. The rate of HEV RNA-positive donations in Europe has been reported to be as high as ~1 per 1000 donors or more in parts of Germany for example [2], with the rate being dependent upon the analytical sensitivity of assays, pool sizes and local epidemiological factors. Thus far, in this region, the HEV RNA-positive donations have been identified as zoonotic genotype 3, with the majority being window periods, that is negative for anti-HEV IgM and IgG [1, 2, 4, 6, 7]. Plasma viraemia may last up to ~2 months, and where it has been possible, to follow up such HEV viraemic donors, there has been evidence of seroconversion (4; K. Matsubayashi, personal communication). In our study, we have investigated the serological response in a group of HEV RNA-positive plasma donors, and using antibody avidity assays, we have identified donors with high viral loads in the presence of high avidity anti-HEV IgG.

Correspondence: Sally A. Baylis, Viral Safety Section, Paul-Ehrlich-Institut, Paul-Ehrlich-Strasse 51-59, D-63225 Langen, Germany
E-mail: Sally.Baylis@pei.de

Materials and methods

Sequence analysis

HEV RNA was extracted using the MinElute Virus Spin kit (Qiagen GmbH, Hilden, Germany); sequencing of a partial region of the HEV RNA-dependent RNA polymerase (*RdRp*) gene was performed as previously described [3]. Phylogenetic analysis was performed using MEGA6 (www.megasoftware.net).

Viral loads

For viral load determinations, HEV RNA was extracted as described above and quantitative real-time PCR was performed as previously described [1] or else using the RealStar® HEV RT-PCR Kit 1·0 (Altona Diagnostics GmbH, Hamburg, Germany) in accordance with the manufacturer's instructions. Calibration was performed using a standard curve prepared by dilutions of the World Health Organization (WHO) international standard for HEV RNA (6329/10).

Anti-HEV antibody testing

Anti-HEV IgM and IgG assays were performed using kits from Wantai (Wantai, Beijing, China) and used in accordance with the manufacturer's instructions. Anti-HEV IgG

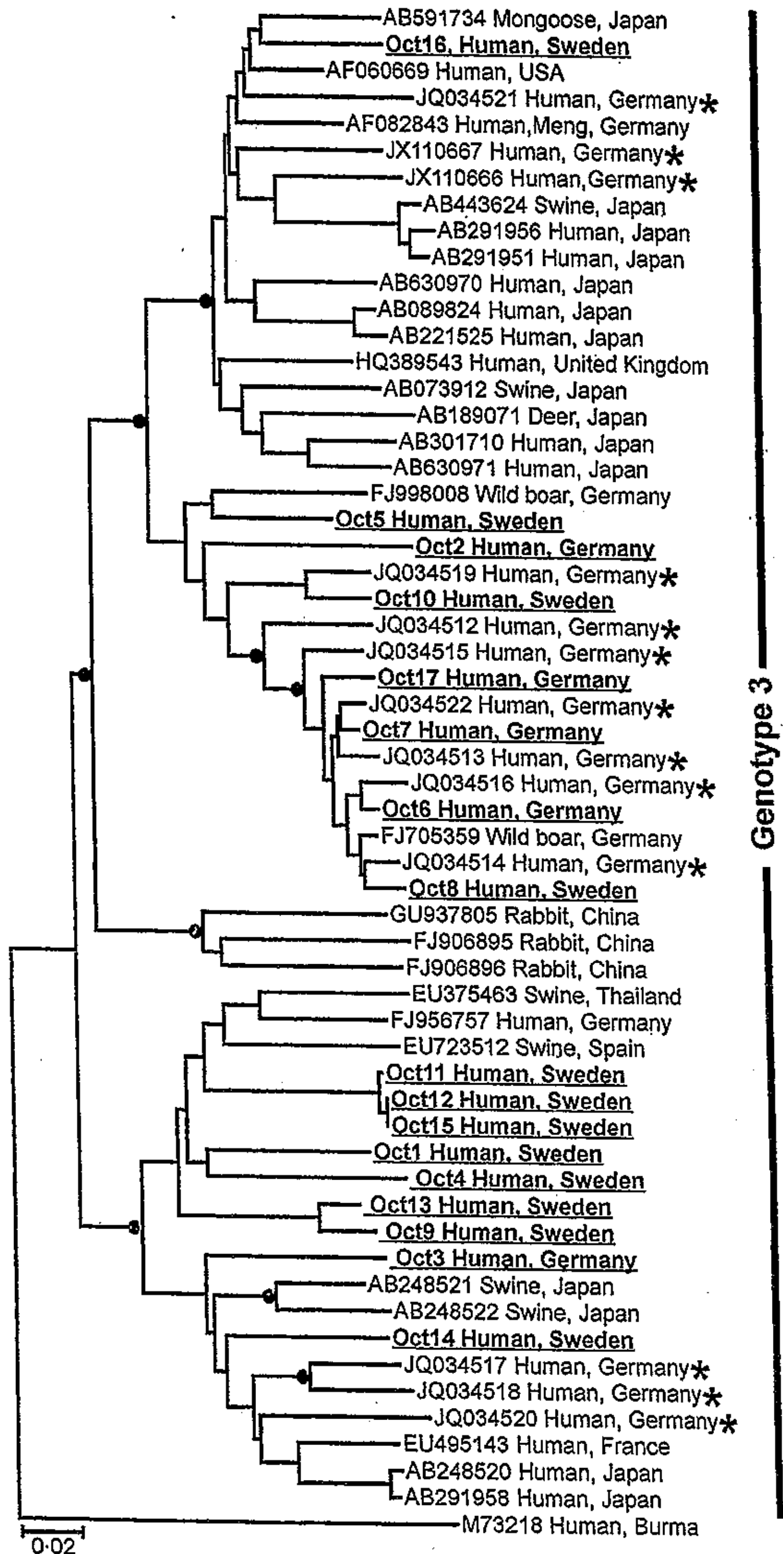


Fig. 1 Partial *RdRp* phylogeny of genotype 3 HEV strains. Phylogeny was conducted in MEGA6 (www.megasoftware.net) using the neighbour-joining algorithm. Filled circles, at deep nodes, represent bootstrap values exceeding 0·7 of 1000 repetitive analyses for confidence testing. Scale bar indicates genetic distance. The sequences that correspond to samples Oct1–17 (Oct1–12 [1] and this paper) are given in bold and underlined and are compared to other HEV strains identified in other German blood donors [3] marked with asterisks. Human genotype 1 strain 'Burma' (GenBank AccNo M73218) was used as an out-group. Accession numbers available for the sequences are as follows: Oct1–Oct12 correspond to JN995562-73 and Oct13–Oct17 correspond to KP994449-KP994453.

antibody titres were determined using the WHO international reference reagent (IRR – 95/584) for anti-HEV IgG. The Wantai anti-HEV IgG assay cut-off equates to 0·25 WHO U/ml [9], although the linear range of the assay extends from 0·2 WHO U/ml to 5 WHO U/ml (S/Co 0·721–14·079). Antibody avidity testing was performed as previously described [9].

© 2015 International Society of Blood Transfusion

Results and discussion

Seventeen HEV RNA-positive plasma samples from German and Swedish plasma donors some of which had been previously characterized, were genotyped [1]. The donations were collected between 2010 and 2012. Sequence analysis confirmed that the HEV RNA-positive donors were all infected with HEV genotype 3, of unique sequence and clustering within several different subgenotypes. Phylogenetic analyses of the HEV-positive donations are shown in Fig. 1. Analysis of a partial sequence of the RNA-dependent RNA polymerase *(RdRp)* gene and the differences observed in these sequences suggest that HEV infection, presumably via zoonotic transmission routes, originated in the majority of cases, from multiple and diverse sources.

Four of the viraemic donations were reactive for anti-HEV IgG, and two of these were also anti-HEV IgM positive (1; S. Baylis, unpublished observations); these data were confirmed by independent laboratories. Acute hepatitis E infection is associated with low avidity anti-HEV IgG; high avidity antibodies are observed during convalescence [9, 10]. Acute HEV infections presenting with HEV RNA, anti-HEV IgG and absence of anti-HEV IgM are suggestive of re-infection, and antibodies produced during re-infection are usually of high avidity [9]. Therefore, we investigated the antibody avidity of anti-HEV IgG in the HEV RNA-positive/anti-HEV IgG-positive donations (Table 1). The two HEV RNA-positive plasma samples, positive for both anti-HEV IgG and IgM, had respective antibody avidities of 14·89% and 11·34%; the low avidity is consistent with primary infection with HEV. However, the HEV RNA-positive plasma samples containing only anti-HEV IgG had respective antibody avidities of 99·16% and 70·75%. The high avidities point to re-infection of these donors. In the case of sera from convalescent hepatitis E patients (>6 months after onset), avidities exceeding 50% are normally observed with lower avidities (<25%) seen during the acute stage of infection [9]. There is ample opportunity for re-exposure to the zoonotic HEV genotype 3, for example via consumption of pork and pork-derived products or direct contact with infected animals, and donors, where immunity has waned may potentially become re-infected with HEV. An alternative possibility to re-infection in the viraemic plasma donors with high avidity anti-HEV IgG is that they may be chronically infected with HEV, although chronic infection has only been reported in immunosuppressed individuals and not blood donors. Previous reports have identified immunocompetent and immunocompromized hepatitis E patients with high viral loads, high avidity IgG and absence of IgM [9–11]. Reinfection by HEV has recently been demonstrated in immunosuppressed transplant patients in an HEV endemic area [12]. In long-term follow-up studies of the recombinant hepatitis E vaccine Hecolin®, approximately 4·5 years after vaccination, it was found that 7 breakthrough cases of hepatitis E were identified in the vaccinated group compared to 53 cases in the control group [13]; three of the seven patients received the full three-dose course of Hecolin® vaccine. High avidity anti-HEV was detected during the acute phase of infection in the vaccinated subjects that developed hepatitis E compared to low avidity anti-HEV in the control subjects who developed the disease. These data are consistent with infection by HEV, in subjects with prior immunity. Where possible, patients as well as donors presenting with similar profiles, that is high viral loads and high avidity anti-HEV IgG, merit further investigation.

Table 1 Antibody avidity of anti-HEV IgG/HEV RNA-positive donors

Code number	Viral load IU/ml HEV RNA (log_{10})	IgG (S/Co)	IgM (S/Co)	IgG (WHO units/ml)	IgG avidity (%)
2	3·26	1·1	1·37	0·37 ± 0·01	14·89 ± 5·43
5	4·95	1·62	0·09	0·48 ± 0·03	99·16 ± 3·12
12	5·68	0·73[a]	0·05	0·21 ± 0·01	70·75 ± 13·15
14	4·70	0·83[a]	1·63	0·27 ± 0·05	11·34 ± 2·31

Samples, representing four separate donors, were tested using the Wantai EIA kits (Wantai) and used in accordance with the manufacturer's instructions. Sample 2 was obtained from a German donor; the remaining samples came from Swedish donors. Donations were collected between 2010 and 2012 as part of a pilot study and code numbers are continued according to [1].

[a]These samples fell below the assay cut-off similar to the WHO IRR.

Acknowledgements

We thank Thomas Gärtner for kindly providing samples.

References

1 Baylis SA, Gärtner T, Nick S, *et al.*: Occurrence of hepatitis E virus RNA in plasma donations from Sweden, Germany and the United States. *Vox Sang* 2012; 103:89–90

2 Vollmer T, Diekmann J, Johne R, *et al.*: Novel approach for detection of hepatitis E virus infection in German blood donors. *J Clin Microbiol* 2012; 50:2708–2713

3 Corman VM, Drexler JF, Eckerle I, *et al.*: Zoonotic hepatitis E virus strains in German blood donors. *Vox Sang* 2013; 104:179–180

© 2015 International Society of Blood Transfusion

4 Slot E, Hogema BM, Riezebos-Brilman A, *et al.*: Silent hepatitis E virus infection in Dutch blood donors, 2011 to 2012. *Euro Surveill* 2013; 18: pii=20550

5 Cleland A, Smith L, Crossan C, *et al.*: Hepatitis E virus in Scottish blood donors. *Vox Sang* 2013; 105:283–289

6 Gallian P, Lhomme S, Piquet Y, *et al.*: Prevalence of silent HEV infections in blood donors, France. *Emerg Infect Dis* 2014; 20:1914–1917

7 Hewitt PE, Ijaz S, Brailsford SR, *et al.*: Hepatitis E virus in blood components: a prevalence and transmission study in southeast England. *Lancet* 2014; 384:1766–1773

8 Sauleda S, Ong E, Bes M, *et al.*: Seroprevalence of hepatitis E virus (HEV) and detection of HEV RNA with a transcription-mediated amplification assay in blood donors from Catalonia (Spain). *Transfusion* in press; doi: 10.1111/trf.12929

9 Bendall R, Ellis V, Ijaz S, *et al.*: Serological response to hepatitis E virus genotype 3 infection: IgG quantitation, avidity, and IgM response. *J Med Virol* 2008; 80:95–101

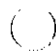
10 Bigaillon C, Tessé S, Lagathu G, *et al.*: Use of hepatitis E IgG avidity for diagnosis of hepatitis E infection. *J Virol Met* 2010; 164:127–130

11 Zhao Q, Zhang J, Wu T, *et al.*: Antigenic determinants of hepatitis E virus and vaccine-induced immunogenicity and efficacy. *J Gastroenterol* 2013; 48:159–168

12 Abravanel F, Lhomme S, Chapuy-Regaud S, *et al.*: Hepatitis E virus reinfections in solid-organ-transplant recipients can evolve into chronic infections. *J Infect Dis* 2014; 209: 1900–1906

13 Zhang J, Zhang X-F, Huang SJ, *et al.*: Long-term efficacy of a hepatitis E vaccine. *N Engl J Med* 2015; 372:914–922

© 2015 International Society of Blood Transfusion



医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
			2015. 11. 6	該当なし	
一般的名称	新鮮凍結人血漿	研究報告の公表状況	Louise Lundgren, Lene Holm HarritshøJ, et al. IDWeek 2015, Oct. 7-11, San Diego, CA. No.1201	公表国: デンマーク	
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

○移植患者における肝トランスアミナーゼの上昇（ALTフレア）の原因としてのE型肝炎ウイルス（HEV）感染。

背景：急性／慢性HEV感染が、固形臓器移植後並びに造血幹細胞移植（HSCT）後の経過を複雑にする可能性があることが示唆されている。ALTフレアの根本的原因として知られているものは僅かであった。ALTフレアが発生している期間は、HEV RNA検出の最適な機会と考え、急性／慢性HEV感染の罹患率を報告する。

方法：2009年9月から2013年7月までの期間に、移植を受けた患者1,002例（腎臓（328例）、肝臓（170例）、肺（120例）、心臓（47例）、HSCT（337例））でALTフレアが発生していた期間に採取した血漿検体を対象として、HEV RNAのスクリーニングを行った（Grifols Procleix）。

結果：1,002例の内、2回以上のALTフレアが502例に発生していた（根本的原因については不明；50%、感染；12%、薬物性肝障害；11%であり、残りはその他）。502例中215例（43%）の移植患者から、ALTフレアが発生していた期間に採取した血漿661検体を対象としてHEV RNAのスクリーニングを実施した結果、1検体が陽性となった。追加検査の結果、当該患者は移植前にHEVに急性感染していたが、2回目のALTフレアが起きていた時点では既にHEVが自然消失していた。

結論：約50%の移植患者においてALTフレアが頻回に発生するが、多くの場合は原因が不明である。HEV感染が原因となることは稀であることから、定期的なHEVに対するスクリーニングは必要ではない。また、複数回のALTフレアと超過死亡率との間には関連が認められるため、HEV以外のALTフレア誘発要因を特定するための協調行動が必要である。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見

移植患者における肝トランスアミナーゼの上昇（ALTフレア）の原因となるE型肝炎ウイルス感染は極めて稀であるという報告である。

今後の対応

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、治療等に関する研究」の一環として、重症化が懸念されるHEV Genotype 4の輸血感染報告があった北海道赤十字血液センターで輸血用血液について試行的個別NATを実施している。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。

Findings in the abstracts are embargoed until 12:01 a.m. PDT, Oct. 7th with the exception of research findings presented at the IDWeek press conferences.

1201. Hepatitis E (HEV)-Infection as Reason for Elevations in Liver Transaminase (ALT-flares) in Transplantation patients

Part of Session: 157. Transplant: Epidemiology of Infections in Transplant Patients and Other Patients with Impaired Immunity

LOUISE LUNDGREN, PREGRADUATE RESEARCH FELLOW[1], LENE HOLM HARRITSHøJ, MD[2], HENRIK ULLUM, MD PHD[3], AMANDA MOCROFT, PROFESSOR[4], CASPAR DA CUNHA-BANG, MD PHD[1], NIKOLAI KIRKBY, MSC PHD[1], SøREN SCHWARTZ SøRENSEN, MD, D.M.SC., PROFESSOR[1], JENS LUNDGREN, MD, D.M.SC, PROFESSOR[1], MARTIN IVERSEN, MD, D.M.SC.[1], ALLAN RASMUSSEN, MD[1], FINN GUSTAFSSON, MD, PHD[1] and HENRIK SENGELøV, MD, D.M.SC.[5]; [1]Rigshospitalet, University of Copenhagen, Copenhagen, Denmark, [2]Department of Clinical Immunology, Copenhagen, AL, Denmark, [3]Department of Clinical Immunology, Copenhagen, Denmark, [4]University College London, London, England, [5]University hospital, Rigshospitalet, Copenhagen, Denmark

Background: In recent years several reports suggest that acute and chronic hepatitis E virus (HEV) infection may complicate the course after both solid-organ (SOT) and hematopoietic stem cell (HSCT) transplantation. We have previously reported that ALT-flares occur frequently in transplant-recipients and multiple episodes were associated with excess risk of death (adjusted incidence rate ratio for >2 versus no ALT-flares=5.1 (3.2-8.1), p>0.0001). Underlying reason for the ALT-flares was only known for a fraction. While patients experienced on-going ALT-flares, we assumed that the chance of detecting HEV RNA was most optimal, and prevalence of acute and chronic HEV infection is reported here.

Methods: Analysis included 1002 SOT (kidney (n=328), liver (170), lung (120), and heart (47)) and HSCT (337) recipients at our hospital, all consecutively transplant between September 2009 and July 2013. All available plasma samples collected during periods of ongoing ALT-flares were screened for the detection of HEV RNA (Grifols Procleix " HEV) [95% detection probability in pools of five: 39.5 IU/ml].

Results: 502 of 1002 patients experienced >1 ALT-flare (total number of ALT-flares 1197). The underlying reason for ALT-flares were often unknown (50%), due to infection (12%), drug-induced-liver-injury (DILI) (11%) or other reasons. Among 215 of the 502 (43%) recipients a total of 661 plasma samples taken during 487 individual ALT-flares were screened for presence of HEV RNA, and one positive sample was detected. Additional examination of other available samples suggested that the person was acutely infected prior to the transplantation, but had spontaneously cleared the infection when experiencing the next post-transplant ALT-flare (Figure).

Conclusion: Whereas ALT-flares often develop during the post-transplant course in approximately 50% of transplant recipients, and develops mostly for unknown reasons, HEV infection is a rare cause of these ALT-flares. Therefore, our data suggest that primary routine screening for HEV is not indicated to investigate the cause of ALT-flares after transplantation. Also, as multiple ALT-flares are associated with excess mortality, concerted action is required to identify other ALT-flare inducing factors than HEV.

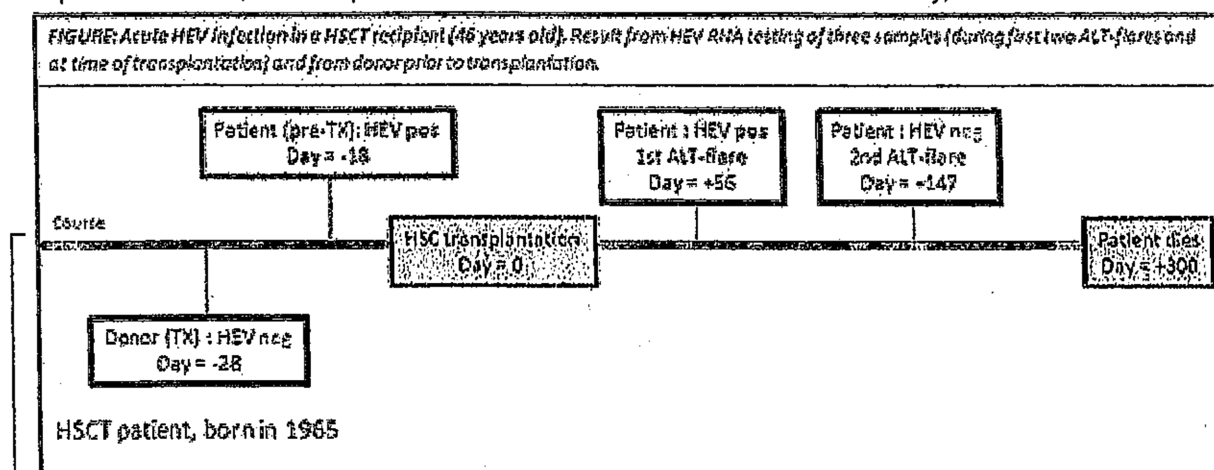


FIGURE: Acute HEV infection in a HSCT recipient (46 years old). Result from HEV RNA testing of three samples (during first two ALT-flares and at time of transplantation) and from donor prior to transplantation.

Findings in the abstracts are embargoed until 12:01 a.m. PDT, Oct. 7th with the exception of research findings presented at the IDWeek press conferences.

1202. *Epstein Barr Virus* (EBV) DNAemia and Post-Transplant Lymphoproliferative Disorders (PTLD) Among Transplant Recipients

Part of Session: 157. Transplant: Epidemiology of Infections in Transplant Patients and Other Patients with Impaired Immunity

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

<table>
<tr><td>識別番号・報告回数</td><td></td><td></td><td>報告日</td><td>第一報入手日
2016 年 01 月 18 日</td><td colspan="2">新医薬品等の区分</td><td rowspan="3">厚生労働省処理欄</td></tr>
<tr><td>一般的名称</td><td colspan="3">①②③⑨⑩pH4 処理酸性人免疫グロブリン
④⑤⑥⑦⑧ポリエチレングリコール処理人免疫グロブリン
⑪⑫人免疫グロブリン</td><td rowspan="2">研究報告の
公表状況</td><td rowspan="2">PloS one
2015; 10( ):
e0119576-</td><td rowspan="2">公表国
オーストリア</td></tr>
<tr><td>販売名
(企業名)</td><td colspan="3">①献血ポリグロビン N5%静注 0.5g/10mL（日本血液製剤機構）
②献血ポリグロビン N5%静注 2.5g/50mL（日本血液製剤機構）
③献血ポリグロビン 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献血ポリグロビン N10%静注 5g/50mL（日本血液製剤機構）
⑩献血ポリグロビン N10%静注 10g/100mL（日本血液製剤機構）
⑪グロブリン筋注 450mg/3mL「JB」（日本血液製剤機構）
⑫グロブリン筋注 1500mg/10mL「JB」（日本血液製剤機構）</td></tr>
<tr><td>研究報告の概要</td><td colspan="5">背景：近年様々な研究により、E 型肝炎（HEV）は多くの先進国で拡大する公衆衛生上の問題であることが示された。それゆえ HEV 感染は輸血による伝播リスクを包含している可能性がある。臨床的な関連性は、いまだ更なる調査を必要としている。本研究の目的は、輸血関連感染のリスク評価とオーストリア北部の献血者における急性 HEV 感染の概要を示すことにある。
方法と結果：市販の HEV RT-PCR キットを用いて合計 58,915 名分の献血者を検査した。献血者のうち 7 名（0.01%）は、PCR 陽性であったが肝炎の症状がなく臨床検査値も正常であった。リアルタイム PCR による定量で HEV-RNA 量は、2,217～293,635 IU/mL であった。献血後 2 週から 11 週の追跡検査で全ての献血者は陰性であった。加えて HEV ゲノムの ORF1 または ORF2 部位の遺伝子配列の解析を行った。HEV-RNA 陽性を示した献血者のサンプルは全てジェノタイプ 3 であった。抗 HEV IgM 抗体および抗 HEV IgG 抗体の抗体スクリーニングを ELISA を用いて実施した。血清学的試験の追跡によって抗 HEV IgM または IgG が陽転化した献血者はいなかったことが明らかとなった。さらに HEV-RNA 陰性であった献血者 1,203 名の抗 HEV IgG 抗体陽性率を調査したところ 13.55%が陽性であった。
結論：本研究において、我々は 1 年以上オーストリア北部の献血者における HEV 感染を調査した。我々は、献血者 8,416 人に 1 人が HEV-RNA 陽性であることを結論付けた。抗 HEV IgG 抗体の陽性率は、年齢と共に増加し 13.55%であった。従って、これらのデータから輸血による E 型肝炎の伝播を阻止するために HEV-PCR スクリーニングの導入を提案する。</td><td colspan="2">使用上の注意記載状況・
その他参考事項等

代表として献血ヴェノグロブリン IH5%静注 0.5g/10mL の記載を示す。
2. 重要な基本的注意
(1)本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-1 抗体陰性で、かつ ALT（GPT）値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分からポリエチレングリコール 4000 処理、DEAE セファデックス処理等により人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理、ウイルス除去膜によるろ過処理及び pH3.9～4.4 の条件下での液状インキュベー</td></tr>
</table>

別紙様式第 2-1
番号 9

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

報告企業の意見	今後の対応	
E型肝炎ウイルス（hepatitis E virus：HEV）は直径27～38nmの球状粒子で、エンベロープはなく、長さ約7,300塩基対の一本鎖RNAを内包している。万一、原料血漿にHEVが混入したとしても、各種モデルウイルスのウイルスクリアランス試験成績から、本剤の製造工程において不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。	ション処理を施しているが、投与に際しては、次の点に十分注意すること。

グロブリン

RESEARCH ARTICLE

Seroprevalence and Incidence of hepatitis E in Blood Donors in Upper Austria

Carina Fischer*, **Martina Hofmann**, **Martin Danzer**, **Katja Hofer**, **Jennifer Kaar**, **Christian Gabriel**

Red Cross Transfusion Service for Upper Austria, Linz, Austria

* carinafischer@gmx.at

OPEN ACCESS

Citation: Fischer C, Hofmann M, Danzer M, Hofer K, Kaar J, Gabriel C (2015) Seroprevalence and Incidence of hepatitis E in Blood Donors in Upper Austria. PLoS ONE 10(3): e0119576. doi:10.1371/journal.pone.0119576

Academic Editor: Xiang-Jin Meng, Virginia Polytechnic Institute and State University, UNITED STATES

Received: September 3, 2014

Accepted: January 30, 2015

Published: March 9, 2015

Copyright: © 2015 Fischer et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This research was partially funded by a grant from the Landsteiner Fonds of the Austrian Red Cross. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

Background

In recent years various studies showed, that hepatitis E virus (HEV) is a growing public health problem in many developed countries. Therefore, HEV infections might bear a transmission risk by blood transfusions. The clinical relevance still requires further investigations. The aim of this study was to provide an overview of acute HEV infections in Upper Austrian blood donors as well as a risk estimation of this transfusion-related infection.

Methods and Findings

A total of 58,915 blood donors were tested for HEV RNA using a commercial HEV RT-PCR Kit. 7 of these donors (0.01%) were PCR-positive with normal laboratory parameters in absence of clinical signs of hepatitis. Viral load determined by quantitative real-time PCR showed a HEV nucleic acid concentration of 2,217 293,635 IU/ml. At follow-up testing (2–11 weeks after donation) all blood donors had negative HEV RNA results. Additionally, genotyping was performed by amplification and sequencing of the ORF1 or ORF2 region of the HEV genome. All HEV RNA positive donor samples revealed a genotype 3 isolate. For the antibody screening, anti-HEV IgM and IgG were detected by ELISA. Follow up serological testing revealed that no donor was seropositive for HEV IgM or IgG antibodies at time of donation. Moreover, we verified the prevalence of anti-HEV IgG in 1,203 of the HEV RNA negative tested blood donors. Overall 13.55% showed positive results for anti-HEV IgG.

Conclusions

In the presented study, we investigated HEV infections in blood donations of Upper Austria over 1 year. We concluded that 1 out of 8,416 blood donations is HEV RNA positive. Seroprevalence of anti HEV IgG results in an age-related increase of 13.55%. Therefore, based on this data, we recommend HEV-PCR screening to prevent transmission of hepatitis E virus by transfusion.

Introduction

Hepatitis E Virus (HEV) is a spherical, single-stranded, positive-sense RNA virus without an envelope that belongs to the genus *hepevirus* in the hepeviridae family [1,2]. Until recently, HEV was mainly recognised as an infection in tropical countries with high endemism and as a travel-associated disease with a low mortality rate [3]. Nowadays, in developed countries, more HEV infections are autochthonous than clearly travel-associated [4]. HEV strains are divided into four major genotypes [5]. HEV genotypes 1 and 2 are restricted to humans and transmitted from person to person via the faecal-oral route e.g. contaminated water. These genotypes mainly occur in developing regions and are associated with epidemic and sporadic hepatitis E infections. HEV genotypes 3 and 4 represent a zoonotic disease and were found in several non-human primates, as well as in pigs, cows, wild boars, deers, rabbits and rodents [5–7]. Genotype 3 is common in industrialized countries, especially in Europe, in North America and in Japan, whereas genotype 4 was mainly identified in China. In Europe the most prevalent (sub) genotypes are 3c, 3e, and 3f [8–11]. In general, HEV genotype 3 and 4 infections are less pathogenic than genotype 1 and 2, they are often anicteric and asymptomatic self-limiting infections in an apparently healthy population like blood donors [12,13]. Transfusion-transmitted HEV infections are rarely reported. However, there is a broad variety of clinical courses in patients who received HEV positive blood products [14,15]. By now, some cases of transfusion-transmitted HEV infections are reported in industrialized countries, for example the first reported case in Japan 2004 [16], or cases in the UK since 2006 [14,17], as well as in Germany 2013 [18]. The usually absence of symptoms and commonly viral clearance often leads to unrecognized transfusion-transmitted HEV cases [4,5]. Caution should be taken in risk groups like pregnant women, children, immunocompromised and transplanted recipients, where HEV-infections may take a deleterious path and pose as a rare, but hazardous transfusion-associated disease, with symptoms like jaundice, abdominal pain, hepatomegaly and splenomegaly, elevated transaminases and nausea [1,4,14–17]. In some cases HEV infection can lead to fulminant liver failure and particularly solid organ transplant recipients often develop a chronic HEV infection, which can lead to liver cirrhosis and usually has to be treated with anti-viral therapy [19–21].

HEV cannot be inactivated in blood products and detection by antibody screening of IgM is not safe enough to exclude HEV in blood products [4,15,22]. Vollmer et al reported that in 4 out of 10 HEV RNA-positive samples HEV-Ag was detectable and HEV-specific IgM antibodies were only detectable in 7 out of 10 HEV RNA-positive donors [23]. Consequently, HEV antibody screening cannot reduce risks of HEV infections and therefore nucleic acid testing (NAT) becomes a gold standard to prevent active viral transmission to a growing recipient population with higher risks [23,24]. Data about IgG seroprevalence in different European countries are available and vary widely (Austria 14.3% [25], Sweden 9.3% [26], France 3.2% [27] and Southwest France 52.5% [28], United Kingdom and North Wales 10% [29], Germany 16.8% [30]). With respect to the seroprevalence, HEV is a widely spread infection among the European population and therefore represents an easily avoidable risk for transfusion-transmitted diseases, regardless whether there are frequently occurring transfusion-transmitted HEV infections or unrecognized, asymptomatic infections. To our knowledge, there are no data about the HEV RNA prevalence in Austria so far. In order to determine the current actual distribution of viremic persons by HEV RNA in the Austrian population, especially in blood donors, 58,915 donors from Upper Austria were tested by PCR and subsequently genotyped. Additionally, 1,203 blood donors as a subset of this population were randomly selected and tested for HEV IgG antibodies in order to find the prevalence of persons who have had a HEV infection in their history and moreover to determine possible differences in relation to different geographical regions. The results of this study allow an estimation of the occurrence of

autochthonous, asymptomatic HEV infections in Upper Austria in respect to consider the implementation of HEV RNA screening of blood donations.

Materials and Methods

Ethics statement

Informed consent is obtained in the donor questionnaire and signed by the donor. All questionnaires are scanned in an automatic reading system and those with a positive tick are released for further testing. The central ethics committee of Upper Austria released a statement in which it indicated that as long as samples are residual material anyway obtained by donation procedures and where these samples are not used for donor release criteria or further therapeutic use, there is no requirement for an ethics committee vote. Anyway, we still have the requirement of informed consent by the donor questionnaire.

Sample collection

Voluntary blood donations of the Upper Austrian Red Cross Transfusion Service were tested by PCR for the presence of HEV RNA. Between February 2013 and April 2014, samples were tested in pools of 96 donors, representing a total of 58,915 donations. Samples which were tested positive for HEV RNA were subsequently genotyped and tested for anti-HEV IgG and anti-HEV IgM antibodies at time of donation and at a follow-up appointment (2–11 weeks after donation). Further, HEV RNA positive blood donors were asked to complete a questionnaire to find out if they were travelling abroad in the last year and if they noticed symptoms like diarrhoea, nausea, abdominal cramping, jaundice or colour changes in urine and stool. They were also asked whether they were occupationally in contact with animals or if they have recently eaten raw meat, game or offal. Additional, women were asked whether they were pregnant and have had abortion or other problems in pregnancy in the last year. Furthermore, in the period of October 2013 to January 2014, a subset of the original samples were randomly selected and tested for anti-HEV IgG antibodies.

HEV RNA detection in blood donors

Blood donation samples were pooled by 96 samples on a Freedom EVO Clinical system (Tecan, Männedorf, Switzerland). HEV RNA was extracted from 1 ml EDTA pooled plasma using the MagNA Pure Compact system (Roche Diagnostics, Mannheim, Germany) and the Nucleic Acid Isolation Kit I—Large Volume. Reverse transcription, amplification and detection were performed on the LightCycler 480 system (Roche Diagnostics) using the RealStar HEV-RT PCR Kit (Altona Diagnostics, Hamburg, Germany). 5 µl of an Internal Control (IC) containing in the kit was added at extraction. A positive IC is required for a valid result. RNA was reverse transcribed for 10 min at 50°C. Denaturation was performed at 95°C for 10 min followed by 40 amplification cycles. Amplification constituted a denaturation step at 95°C for 15 sec, an annealing step at 55°C for 45 sec and an elongation step at 72°C for 15 sec. The sensitivity of the assay, calculated by Probit analysis, was validated on the World Health Organisation (WHO) international standard (IS) strain 6329/10 and showed a 95% detection limit of 11,6 IU/ml (CI 7.48–25.09) HEV RNA and a 99% plausibility of 21,0 IU/ml (CI 12.01–58.70) HEV RNA. Positive pools of 96 samples were tested in subpools of 12 samples and consequently those were deconstructed to confirm the individual HEV RNA positive blood donor. A positive blood sample was retested and confirmed using an additional blood sample. HEV virus load of positive samples was calculated from the Ct-values based on a calibration curve of the first WHO IS strain 6329/10.

HEV genotyping and sequence analysis of the HEV RNA ORF2 region

HEV genotyping was performed by sequencing the ORF2 region of the genome adapted from Baylis et al [31]. HEV RNA was extracted using the MagNA Pure Compact System. Reverse transcription and amplification was performed on a Veriti Thermal Cycler (Life Technologies, Darmstadt, Germany) using the OneStep RT-PCR Kit (Qiagen, Hilden, Germany) and the following primers: HEV_fw_int (5'-GTYATGYTYTGCATACATGGCT-3') and HEV_rv_int (5'-AGCCGACGAAATYAATTCTGTC-3') [31]. HEV RNA was reverse transcribed for 30 min at 50°C and a following initial polymerase activation step for 15 min at 95°C. PCR amplification is composed of a denaturation step for 30 sec at 94°C, an annealing step at 48°C for 30 sec and an elongation step at 72°C for 1 min. HEV RNA is amplified with 45 cycles. Final extension was performed for 10 min at 72°C. The amplification products were analyzed by an agarose gel electrophoresis with GelStar as a Gel Loading Solution (Biozym, Hessisch Oldendorf, Germany) and a peqGOLD Low Range HT DNA-Ladder (Peqlab, Erlangen, Germany). If genotyping of the ORF2 was unsuccessful, the ORF1 region was sequenced using the following primers according to Vollmer et al [32,33]: HEV_ORF1_F_in (5'-CTGCCCTGGCGAATGCT-3') and HEV_ORF1_R_in (5'-AGCAGTATACCAGCGCTGAACATC-3'). A specific PCR product on the gel was finally purified with ExoSAP-IT as described and sequenced using the BigDye Cycle Sequencing Kit (Life Technologies) on a Genetic Analyzer 3130xl (Life Technologies). Sequence analysis and phylogenetic analysis were assessed using the DNAStar Lasergene 8 software and alignment was done by using the BLAST service from NCBI.

Serological testing

Serum samples were tested for anti-HEV IgG and anti-HEV IgM antibodies by anti-HEV IgG and anti-HEV IgM ELISA kit (Wantai Biological Pharmacy Enterprise Co., Ltd., Beijing, China). The test and the calculation of the results were performed according to the manufacture's instruction. The absorbance was measured using Tecan Infinite 200 pro (Tecan). Positive blood donors were tested for anti-HEV IgG and anti-HEV IgM antibodies at time of donation and at a follow-up appointment after donation. To determine the occurrence of past HEV infections in Upper Austria randomly selected blood donations were tested for anti-HEV IgG only. Borderline results in this group were retested. The results were finally reported as positive or negative for presence of anti-HEV IgG or anti-HEV IgM antibodies.

Statistical analysis

Data analysis and collection was performed using MS Excel 2007 and IBM SPSS Version 21 for Windows. Categorical variables were compared by Chi-Square test or Fisher's exact test. A p-value of <0.05 was considered as statistically significant. Data were represented including descriptive statistics as means, 95% confidence intervals (CI's), standard deviation (SD) as wells as total and relative frequencies. Age-dependent risk factor for presence of anti-HEV IgG was constituted by the calculation of odds ratios and a correlation was calculated for age and the presence of anti-HEV IgG.

Results

Characteristics and clinical markers of HEV infected blood donors

In the period of February 2013 to April 2014, 58,915 blood donations in Upper Austria were screened for the presence of HEV RNA and resulted in 7 (0.01%; incidence 1:8,416) HEV RNA positive blood donations (Table 1). The age of positive donors ranged from 21 to 52 years with a mean of 35 and there was one woman among the 7 HEV RNA positive donors. All 7 positive

Table 1. Characteristics of HEV RNA positive blood donors.

Donor	Age[a]	Sex	HEV RNA [IU/ml]	Anti-HEV [at time of donation]		Anti-HEV [follow-up control][b]		Follow up appointment [weeks after donation]	HEV genotype
				IgG	IgM	IgG	IgM		
1	33	F	$2,2 \times 10^3$	-	-	+	+	6,1	3f
2	44	M	$2,9 \times 10^5$	-	-	+	+	4,1	3f
3	52	M	$3,3 \times 10^4$	-	-	+	-	11,0	3
4	23	M	$2,5 \times 10^5$	-	-	+	+	5,1	3
5	40	M	$8,0 \times 10^4$	-	-	+	+	7,1	3
6	21	M	$2,1 \times 10^4$	-	-	+	+	2,0	3
7	32	M	$9,1 \times 10^4$	-	-	+	+	5,2	3f

F: female; M: male;-: no antibody; +: positive

[a]Age at time of donation

[b]control at least 2 weeks after donation

doi:10.1371/journal.pone.0119576.t001

blood donors were confirmed shortly after the positive result to report the HEV infection to the Ministry of Health. The donors were retested for HEV RNA at a follow-up appointment to exclude a chronic HEV infection. The donors were asked to participate to the follow-up testing after 6 weeks. Unfortunately, the testing actually was performed 2–11 weeks after donation. One week after the donation all blood donors were confirmed as positive for HEV RNA. At follow-up appointment all blood donors were negative for HEV RNA. Testing for anti-HEV IgG and IgM resulted in negative results for both anti-HEV IgG and anti-HEV IgM antibodies at time of donation, which implies that these donors were in the seronegative phase of infection. At the follow-up appointment all donors were anti-HEV IgG positive and 6 of 7 had positive results for anti-HEV IgM. HEV RNA concentration was calculated by a standard curve ($r^2 = 0.9995$) and corresponds to a HEV nucleic acid concentration of 2.2×10^3 to 2.9×10^5 IU/ml (mean 11.1×10^4 IU/ml). All of the HEV RNA positive blood donors showed normal results for alanine aminotransferase (ALT) and normal C-reactive protein (CRP) at time of donation. Detailed results are supported in S1 and S2 Tables.

Analysing the questionnaires, there is no commonality between the HEV positive blood donors. 3 of 7 donors stated that they had diarrhoea or colour changes in stool in the last 6 months, which unfortunately are unspecific symptoms especially during a period of 6 months. Actually they didn't present any symptoms and didn't have health problems at time of infection, respectively. One of the 7 positive blood donors is occupationally in contact with animals. 5 of 7 blood donors had a trip abroad for holidays in the recent 6 months and visited Croatia, Italy, Spain, Portugal, Russia, Turkey and Germany. One donor had eaten some game, but no donor claimed that he/she was eating raw meat. On the basis of these results there is no reference to the source of the infection. The questions asked are only related to gastrointestinal problems, the occupational contact with animals and the eating habit. For further investigation of the possible source of the infection a more detailed questionnaire has to be prepared for the time of donation and for the follow-up testing.

Sequence alignment and pyhlogenetic analysis of the HEV RNA ORF2 region

All HEV RNA positive donor samples were genotyped and revealed genotype 3 isolates. To determine the relatedness between HEV isolates, a nucleotide alignment was performed (Fig. 1).

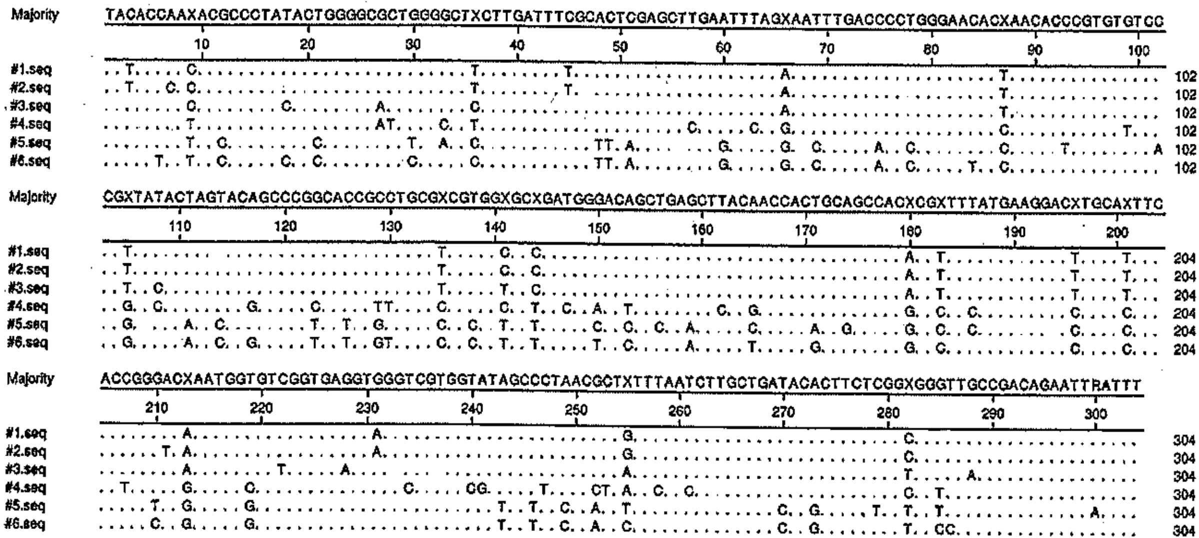


Fig 1. Sequence alignment. Comparison of a partial genome sequence in ORF2 from HEV positive samples. Dots indicate identity to the consensus sequence.

doi:10.1371/journal.pone.0119576.g001

Sequence homologies were between 80.9 and 99.3%. Sequences #1, #2 and #3 show the highest concordance with genotype 3f with nucleic acid identities ranging from 95.1–99.3% homology. A phylogenetic analysis of these sequences is illustrated in Fig. 2. Several attempts were made to obtain the sequence of ORF2 region of sample #7 with no success. Therefore, #7 was identified in the ORF1 region as genotype 3 (sequence and phylogenetic data not shown).

Seroprevalence of anti-HEV IgG in Upper Austria

In total, 163 samples of 1,203 donations were tested positive for anti-HEV IgG antibodies, which relates to a seroprevalence of 13.55% (95% CI 11.6–15.5). Blood donors were set in relation to their place of residence in Upper Austria. The distribution of anti-HEV IgG positive donors over the districts of Upper Austria is shown in Table 2 and Fig. 3. There are no results for the districts of Wels, Wels-Land and Grieskirchen, as these districts are not tested by the local blood bank.

Regarding these results, there is a higher seroprevalence in the east (Perg, Steyr-Land; mean 20.01%) than in the north-western (Schärding, Rohrbach; mean 6.80%) part of Upper Austria ($p < 0.05$). The mean seroprevalence of all districts is 12.79%, within a range from 0% to 21.01% and a standard deviation of ±5.13. Steyr shows a seroprevalence of 0.00%, whereas this result might be misleading due to the low amount of only 10 participating donors.

The mean age of the tested blood donors is 40.49 years, within a range of 19 to 69 and the median age is 42. Mean age of the donors tested positive for anti-HEV IgG is 50.14, range 22 to 69 years. Categorized age groups revealed an age-dependent increase of HEV infection ($r = 0.81$; $p < 0.01$) (Fig. 4). Only 1.91% (95% CI 0.7–4.1) of the blood donors show positive results for anti-HEV IgG at the age of 19 to 29, whereas there are 41.33% (95% CI 30.1–53.3) positive for anti-HEV IgG at the age of 60 to 69 (chi-square $p < 0.01$).

These results show that there is a 20 fold increase of the risk to undergo a HEV infection at an advanced age (> 50) than at an early age (OR = 21.62). There is no trend of different distributions between male and female donors. 13.85% male and 13.08% female blood donors were positive for anti-HEV IgG ($p > 0.1$). Detailed results are supported in S3 Table.

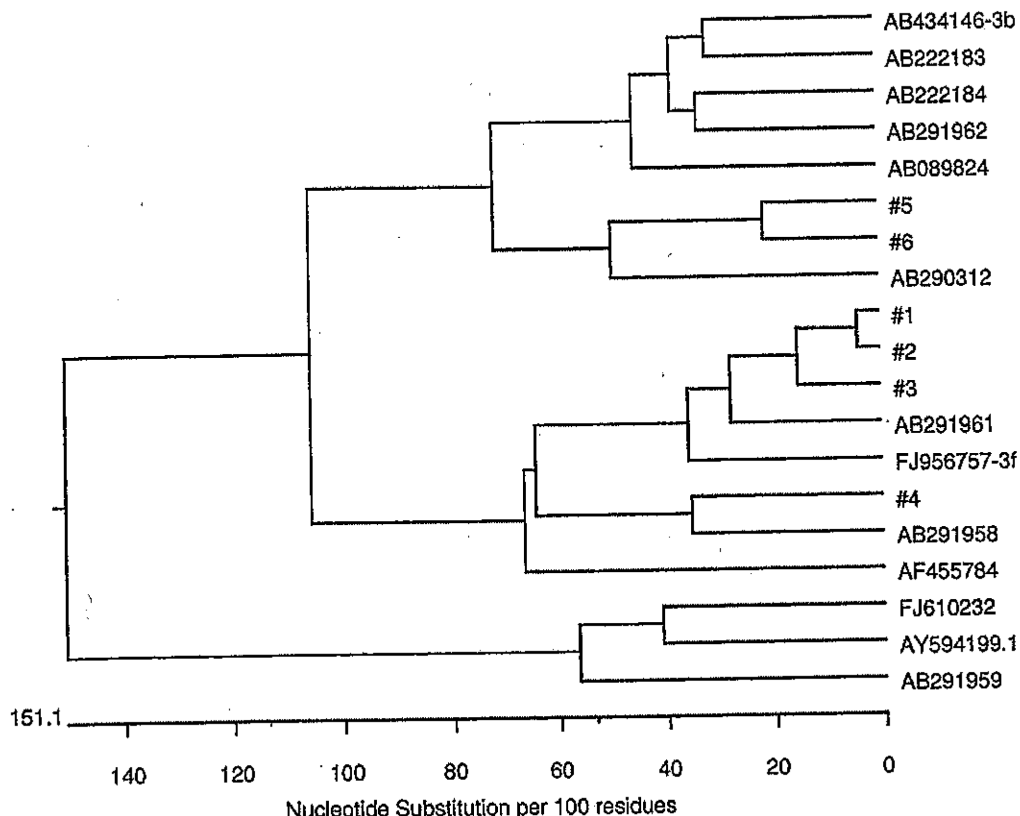


Fig 2. Phylogenetic relationship between genotype 3 HEV strains derived from Austrian blood donors (#1–6) and HEV reference strains (GenBank sequences are cited by their respective accession numbers). The tree was constructed by the Clustal W method on the basis of a 304-nt sequence fragment of ORF2 region.

doi:10.1371/journal.pone.0119576.g002

Discussion

The seroprevalence of anti-HEV IgG in the period of November 2013 to January 2014 amounts to 13.55% and our data reflects a cumulative increase with age. This confers to the wide range of seroprevalences in other European countries [25–27,29,30]. Lagler et al published the first large seroprevalence study of Austria civilians and military professionals and reported a seroprevalence of 14.3% with a very low representation of women (1.7%). Our findings are in line with this published data and we could confirm this seroprevalence rate in men (13.58%; n = 101) and women (13.08%; n = 62). However, differences in other countries may be influenced by different test systems with varying sensitivities, effects of sample selection e.g. age, exposure to contaminated food sources (uncooked meat) and contact to certain animal species e.g. pigs, wild boar and deer [25]. Possible differences in provinces in Austria published by Lagler et al could be explained by the diversity of farming, especially by the density of pig farms [25]. Nearly 40% of Austrians' pig herds are situated in Upper Austria, where the most pig husbandries are located around the centre of Upper Austria and south of the Danube [34,35]. This distribution is not exactly in concordance with the distribution map of the appearance of anti-HEV IgG antibodies in this study, where the east of Upper Austria shows the highest seroprevalence and the north-western the lowest. It is controversially discussed, that living in high pig density areas is associated with a higher risk to undergo a HEV infection, but there are many studies that confirm that there is a tendency to higher seroprevalence in these regions [36–38].

Table 2. Comparison of HEV seroprevalence in all districts of Upper Austria.

District	Number of donors	Anti-HEV IgG positive donors	% of anti-HEV IgG positive donors
Braunau am Inn	50	6	12.00%
Eferding	47	7	14.89%
Freistadt	121	15	12.40%
Gmunden	43	6	13.95%
Kirchdorf	36	5	13.89%
Linz	107	13	12.15%
Linz-Land	97	16	16.49%
Perg	138	29	21.01%
Ried im Innkreis	103	13	12.62%
Rohrbach	138	9	6.52%
Schärding	68	5	7.35%
Steyr	10	0	0.00%
Steyr-Land	65	12	18.46%
Urfahr-Umgebung	95	13	13.68%
Vöcklabruck	85	14	16.47%
Σ	**1203**	**163**	

doi:10.1371/journal.pone.0119576.t002

The prevalence of HEV RNA in 58,915 blood donors in the Upper Austrian population resulted in 7 HEV RNA positive blood donors (0.01%). A questionnaire on behavioural aspects followed by an interview and liver-specific blood tests (e.g. alanine aminotransferase) have shown no significant symptoms or clinical findings, therefore there is no surrogate marker to indicate a hepatitis E infection in blood donors. Serological testing revealed that no donor was positive for either anti-HEV IgM or anti-HEV IgG antibodies at time of donation. At a follow-up testing all donors showed negative results for HEV RNA and anti-HEV IgG were positive. With the exception of one donor, all of 7 positive HEV RNA donors were anti-HEV IgM positive at follow-up appointment. The donor who showed negative results for anti-HEV IgM was tested 11 weeks after the donation, when IgM was already negative. The combination of PCR and serology reveals that all of the HEV RNA positive donors were in the seronegative phase of infection, and therefore posed a transmission risk without testing for HEV RNA. The normal ALT and CRP levels of the HEV RNA positive donors and the absence of any symptoms and antibodies at time of donation, confirm that a HEV infection in developed countries is difficult to diagnose without molecular methods. The serological testing of all HEV RNA positive donors implies negative results, which means that the only way to detect an HEV infection at an early stage is to perform a HEV RNA PCR.

Our findings for HEV imply a relative risk to find a viremic donor as in 1:8,416 (0.01%) donors, which is in line with data from some European countries like England 1 in 7,000 [39] and Sweden with 1 in 7,986 [40] donations. Lower rates were found in Scotland with 1 in 14,520 donations [41]. A far higher rate was found in Germany with 1 in 1,240 donations positive for HEV RNA [32]. On the one hand, these results can be explained by the variations of the occurrence of HEV infections within different regions and countries. The occurrence of HEV RNA positive blood donations was lower from May to mid- September 2013, which could be explained by the lower donation frequencies in this period or by different eating habits during the summer months than during fall and winter. On the other hand increasing the sensitivity of the assay by lowering the pool size to less than 96 samples could lead to a higher rate of detected HEV infections in blood donations. The nucleic acid concentration of the samples in

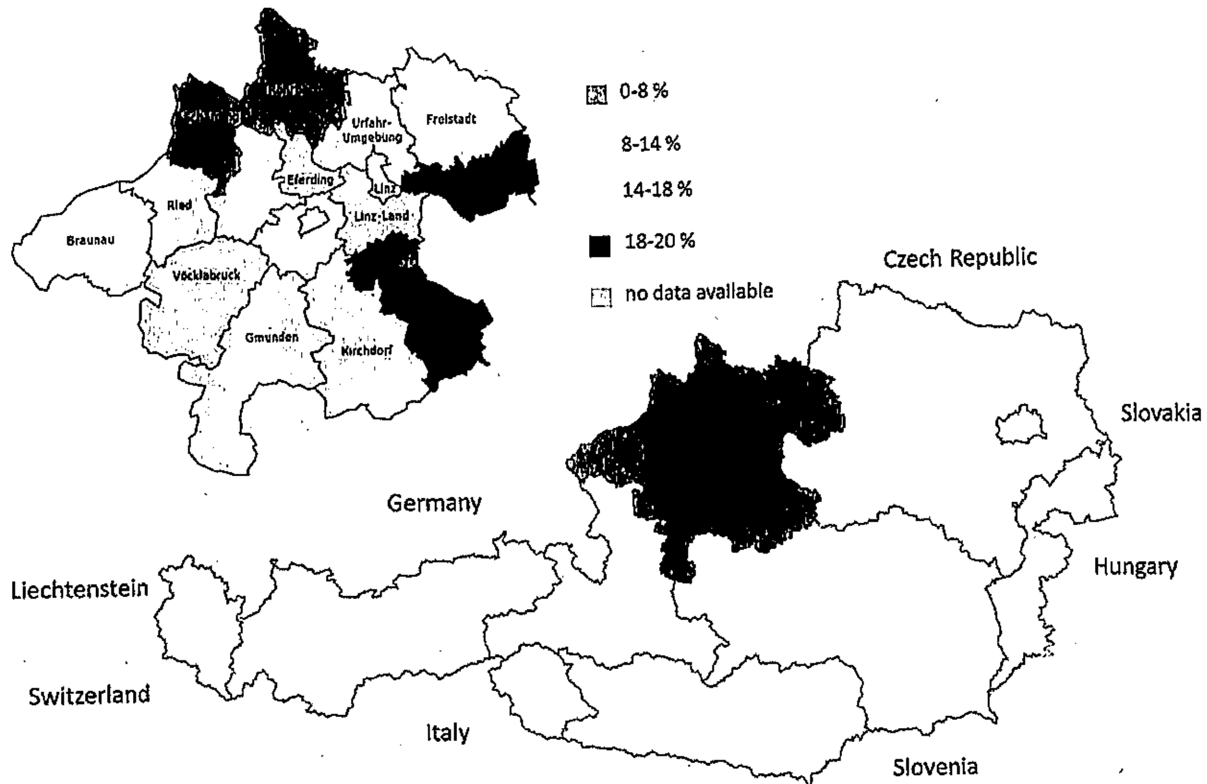


Fig 3. Geographical map of Upper Austria. A detailed analysis of seroprevalences [%] marked in green, yellow, orange and red of all districts is shown. Areas in grey are those with no available data. Copyright: The map of Austria is adapted from Statistik Austria and modified by Niklas N., http://www.statistik.at/web_de/services/interaktive_karten; The map of Upper Austria is adapted from wikipedia, author AleXXw and modified by Hofmann M., http://de.wikipedia.org/wiki/Datei:Karte_A_Ooe_ohne.svg

doi:10.1371/journal.pone.0119576.g003

this study ranged from 2.2×10^3 to 2.9×10^5 IU/ml with a mean of 1.1×10^5. This is why we regarded the 95% limit of detection (LOD) of 11.6 IU/ml (7.48–25.09), which results in a LOD for a single sample of 1,113.6 IU/ml, adequate to detect HEV viremia in pools of 96 donations.

Based on our HEV RNA screening data of 58,915 samples with HEV RNA positive attack rate of 0.01%, there is a high risk of HEV infection from a blood product which might be given to immune compromised patients, a fact that might have been neglected. To ensure that at least these patients receive HEV free blood products, mandatory HEV screening should be considered in blood centres serving many young or immune compromised patients. In two published cases HEV was transmitted by transfusion of platelets, red cells and even Intercept-treated fresh frozen plasma and verified by 100% sequence homology of the transfusion-related isolates [15,22,42]. Despite of a few confirmed and published cases of transfusion-transmitted HEV infections consequences for young or immune compromised patients or those in poor health should be considered.

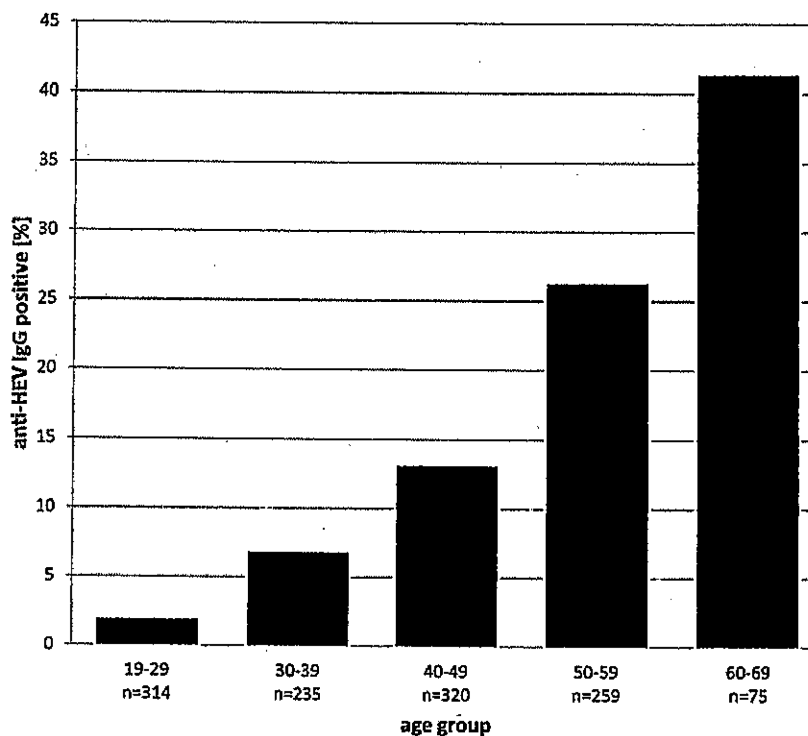


Fig 4. Prevalence of anti-HEV IgG. Estimated prevalence of anti-HEV IgG in 1,203 of Upper Austrian blood donors by age group.

doi:10.1371/journal.pone.0119576.g004

Supporting Information

S1 Table. HEV positiv blood donors
(XLSX)

S2 Table. IgG and IgM antibodies from HEV RNA positive donors.
(XLSX)

S3 Table. Anti-HEV IgG of the HEV RNA negative tested blood donors
(XLSX)

Acknowledgments

We would like to thank Norbert Niklas for graphical assistance.

Author Contributions

Conceived and designed the experiments: CF CG. Performed the experiments: MH JK. Analyzed the data: MH JK. Wrote the paper: CF MH. Critical review of the manuscript: MD KH CG.

References

1. Dalton HR, Bendall R, Ijaz S, Banks M. Hepatitis E: an emerging infection in developed countries. Lancet Infect Dis. 2008; 8: 698–709. doi: 10.1016/S1473-3099(08)70255-X PMID: 18992406

2. Reyes GR, Purdy MA, Kim JP, Luk KC, Young LM, Fry KE, et al. Isolation of a cDNA from the virus responsible for enterically transmitted non-A, non-B hepatitis. Science. 1990; 247: 1335–1339. PMID: 2107574
3. Wichmann O, Schimanski S, Koch J, Kohler M, Rothe C, Plentz A, et al. Phylogenetic and case-control study on hepatitis E virus infection in Germany. J Infect Dis. 2008; 198: 1732–1741. doi: 10.1086/593211 PMID: 18983248
4. Dreier J, Juhl D. Autochthonous hepatitis e virus infections: a new transfusion-associated risk? Transfus Med Hemother. 2014; 41: 29–39. doi: 10.1159/000357098 PMID: 24659945
5. Teshale EH, Hu DJ. Hepatitis E: Epidemiology and prevention. World J Hepatol. 2011; 3: 285–291. doi: 10.4254/wjh.v3.i12.285 PMID: 22216368
6. Echevarria JM. Light and Darkness: Prevalence of Hepatitis E Virus Infection among the General Population. Scientifica (Cairo). 2014; 2014: 481016. doi: 10.1155/2014/481016 PMID: 24672733
7. Pavio N, Meng XJ, Renou C. Zoonotic hepatitis E: animal reservoirs and emerging risks. Vet Res. 2010; 41: 46. doi: 10.1051/vetres/2010018 PMID: 20359452
8. Lu L, Li C, Hagedorn CH. Phylogenetic analysis of global hepatitis E virus sequences: genetic diversity, subtypes and zoonosis. Rev Med Virol. 2006; 16: 5–36. PMID: 16175650
9. Meng XJ, Purcell RH, Halbur PG, Lehman JR, Webb DM, Tsareva TS, et al. A novel virus in swine is closely related to the human hepatitis E virus. Proc Natl Acad Sci U S A. 1997; 94: 9860–9865. PMID: 9275216
10. Rutjes SA, Lodder WJ, Lodder-Verschoor F, van den Berg HH, Vennema H, Duizer E, et al. Sources of hepatitis E virus genotype 3 in The Netherlands. Emerg Infect Dis. 2009; 15: 381–387. doi: 10.3201/eid1503.071472 PMID: 19239749
11. Zwettler D, Fink M, Revilla-Fernandez S, Steinrigl A, Winter P, Kofer J. First detection of hepatitis E virus in Austrian pigs by RT-qPCR. Berl Munch Tierarztl Wochenschr. 2012; 125: 281–289. PMID: 22919921
12. Shata MT, Navaneethan U. The mystery of hepatitis E seroprevalence in developed countries: is there subclinical infection due to hepatitis E virus? Clin Infect Dis. 2008; 47: 1032–1034. doi: 10.1086/591971 PMID: 18781881
13. Navaneethan U, Al MM, Shata MT. Hepatitis E and pregnancy: understanding the pathogenesis. Liver Int. 2008; 28: 1190–1199. doi: 10.1111/j.1478-3231.2008.01840.x PMID: 18662274
14. Boxall E, Herborn A, Kochethu G, Pratt G, Adams D, Ijaz S, et al. Transfusion-transmitted hepatitis E in a 'nonhyperendemic' country. Transfus Med. 2006; 16: 79–83. PMID: 16623913
15. Colson P, Coze C, Gallian P, Henry M, De MP, Tamalet C. Transfusion-associated hepatitis E, France. Emerg Infect Dis. 2007; 13: 648–649. PMID: 17561564
16. Matsubayashi K, Nagaoka Y, Sakata H, Sato S, Fukai K, Kato T, et al. Transfusion-transmitted hepatitis E caused by apparently indigenous hepatitis E virus strain in Hokkaido, Japan. Transfusion. 2004; 44: 934–940. PMID: 15157263
17. Hewitt PE, Ijaz S, Brailsford SR, Brett R, Dicks S, Haywood B, et al. Hepatitis E virus in blood components: a prevalence and transmission study in southeast England. Lancet. 2014; 384: 1766–1773. doi: 10.1016/S0140-6736(14)61034-5 PMID: 25078306
18. Huzly D, Umhau M, Bettinger D, Cathomen T, Emmerich F, Hasselblatt P, et al. Transfusion-transmitted hepatitis E in Germany, 2013. Euro Surveill. 2014; 19.
19. de NA, Zaaijer HL, ten B I, Weegink CJ, Reesink HW, Beuers U. Chronic hepatitis E after solid organ transplantation. Neth J Med. 2012; 70: 261–266. PMID: 22859417
20. Riezebos-Brilman A, Verschuuren EA, van Son WJ, van Imhoff GW, Brugemann J, Blokzijl H, et al. The clinical course of hepatitis E virus infection in patients of a tertiary Dutch hospital over a 5-year period. J Clin Virol. 2013; 58: 509–514. doi: 10.1016/j.jcv.2013.08.022 PMID: 24055152
21. Peron JM, Bureau C, Poirson H, Mansuy JM, Alric L, Selves J, et al. Fulminant liver failure from acute autochthonous hepatitis E in France: description of seven patients with acute hepatitis E and encephalopathy. J Viral Hepat. 2007; 14: 298–303. PMID: 17439518
22. Hauser L, Roque-Afonso AM, Beyloune A, Simonet M, Deau FB, Burin des RN, et al. Hepatitis E transmission by transfusion of Intercept blood system-treated plasma. Blood. 2014; 123: 796–797. doi: 10.1182/blood-2013-09-524348 PMID: 24482503
23. Vollmer T, Knabbe C, Dreier J. Comparison of real-time PCR and antigen assays for detection of hepatitis E virus in blood donors. J Clin Microbiol. 2014; 52: 2150–2156. doi: 10.1128/JCM.03578-13 PMID: 24740079

24. Gupta E, Pandey P, Pandey S, Sharma MK, Sarin SK. Role of hepatitis E virus antigen in confirming active viral replication in patients with acute viral hepatitis E infection. J Clin Virol. 2013; 58: 374–377. doi: 10.1016/j.jcv.2013.07.019 PMID: 23965185

25. Lagler H, Poeppl W, Winkler H, Herkner H, Faas A, Mooseder G, et al. Hepatitis E virus seroprevalence in Austrian adults: a nationwide cross-sectional study among civilians and military professionals. PLoS One. 2014; 9: e87669. doi: 10.1371/journal.pone.0087669 PMID: 24498349

26. Olsen B, Axelsson-Olsson D, Thelin A, Weiland O. Unexpected high prevalence of IgG-antibodies to hepatitis E virus in Swedish pig farmers and controls. Scand J Infect Dis. 2006; 38: 55–58. PMID: 16338839

27. Boutrouille A, Bakkali-Kassimi L, Cruciere C, Pavio N. Prevalence of anti-hepatitis E virus antibodies in French blood donors. J Clin Microbiol. 2007; 45: 2009–2010. PMID: 17460057

28. Mansuy JM, Bendall R, Legrand-Abravanel F, Saune K, Miedouge M, Ellis V, et al. Hepatitis E virus antibodies in blood donors, France. Emerg Infect Dis. 2011; 17: 2309–2312. doi: 10.3201/eid1712.110371 PMID: 22172156

29. Beale MA, Tettmar K, Szypulska R, Tedder RS, Ijaz S. Is there evidence of recent hepatitis E virus infection in English and North Welsh blood donors? Vox Sang. 2011; 100: 340–342. doi: 10.1111/j.1423-0410.2010.01412.x PMID: 21392024

30. Faber MS, Wenzel JJ, Jilg W, Thamm M, Hohle M, Stark K. Hepatitis E virus seroprevalence among adults, Germany. Emerg Infect Dis. 2012; 18: 1654–1657. doi: 10.3201/eid1810.111756 PMID: 23018055

31. Baylis SA, Hanschmann KM, Blumel J, Nubling CM. Standardization of hepatitis E virus (HEV) nucleic acid amplification technique-based assays: an initial study to evaluate a panel of HEV strains and investigate laboratory performance. J Clin Microbiol. 2011; 49: 1234–1239. doi: 10.1128/JCM.02578-10 PMID: 21307208

32. Vollmer T, Diekmann J, Johne R, Eberhardt M, Knabbe C, Dreier J. Novel approach for detection of hepatitis E virus infection in German blood donors. J Clin Microbiol. 2012; 50: 2708–2713. doi: 10.1128/JCM.01119-12 PMID: 22675127

33. Preiss JC, Plentz A, Engelmann E, Schneider T, Jilg W, Zeitz M, et al. Autochthonous hepatitis E virus infection in Germany with sequence similarities to other European isolates. Infection. 2006; 34: 173–175. PMID: 16804663

34. STATISTIK AUSTRIA [Internet]. AS2010 Haltungsverfahren Schweine. Available: http://www.statistik.at/web_de/statistiken/land_und_forstwirtschaft/viehbestand_tierische_erzeugung/tierbestand/index.html. Last updated 31–10–2010. Accessed 21–7–2014.

35. STATISTIK AUSTRIA [Internet]. Schweinebestand nach Bundesländern von 1946 bis 2013. Available: http://www.statistik.at/web_de/statistiken/land_und_forstwirtschaft/viehbestand_tierische_erzeugung/tierbestand/index.html. Last updated 14–2–2014. Accessed 21–7–2014.

36. Krumbholz A, Joel S, Dremsek P, Neubert A, Johne R, Durrwald R, et al. Seroprevalence of hepatitis E virus (HEV) in humans living in high pig density areas of Germany. Med Microbiol Immunol. 2014; 203: 273–282. doi: 10.1007/s00430-014-0336-3 PMID: 24744181

37. Schielke A, Sachs K, Lierz M, Appel B, Jansen A, Johne R. Detection of hepatitis E virus in wild boars of rural and urban regions in Germany and whole genome characterization of an endemic strain. Virol J. 2009; 6: 58. doi: 10.1186/1743-422X-6-58 PMID: 19442307

38. Chaussade H, Rigaud E, Allix A, Carpentier A, Touze A, Delzescaux D, et al. Hepatitis E virus seroprevalence and risk factors for individuals in working contact with animals. J Clin Virol. 2013; 58: 504–508. doi: 10.1016/j.jcv.2013.08.030 PMID: 24084601

39. Ijaz S, Szypulska R, Tettmar KI, Kitchen A, Tedder RS. Detection of hepatitis E virus RNA in plasma mini-pools from blood donors in England. Vox Sang. 2012; 102: 272. doi: 10.1111/j.1423-0410.2011.01554.x PMID: 21957873

40. Baylis SA, Gartner T, Nick S, Ovemyr J, Blumel J. Occurrence of hepatitis E virus RNA in plasma donations from Sweden, Germany and the United States. Vox Sang. 2012; 103: 89–90. doi: 10.1111/j.1423-0410.2011.01583.x PMID: 22220775

41. Cleland A, Smith L, Crossan C, Blatchford O, Dalton HR, Scobie L, et al. Hepatitis E virus in Scottish blood donors. Vox Sang. 2013; 105: 283–289. doi: 10.1111/vox.12056 PMID: 23763589

42. Boxall E, Herborn A, Kochethu G, Pratt G, Adams D, Ijaz S, et al. Transfusion-transmitted hepatitis E in a 'nonhyperendemic' country. Transfus Med. 2006; 16: 79–83. PMID: 16623913

別紙様式第2-1

No. 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
				2015. 11. 4	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	Hu LP, Liu DP, Chen QY, et al. PLoS One. 2015 Oct 12;10(10):e0138552.	公表国 中国	
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

○ オカルトB型肝炎ウイルス（HBV）感染（OBI）は密接な接触を介して伝播し、顕性感染として発症する可能性がある。

輸血、臓器移植、血液透析を介するOBIの伝播の重大性は広く認識されているが、密接な接触を介するOBIの伝播に関するデータは依然として僅かである。本研究では、1名の男児およびその両親から血清検体を採取した。当該男児は出生時に標準的な計画によるワクチン接種を受けており、防御抗体が産生されていた。HBVの血清検査とHBV DNAのnested PCR法を行った。アンプリコンをクローニングし、その配列の系統発生学的解析を行った。両親の双方にOBIを認め、男児には顕性感染を認めた。父親から検出された血清型はadrq+、ayw1、aywおよびayrであり、母親から検出された血清型はayw1、adw2およびadwq+であった。男児から検出された血清型はadrq+のみであった。父親についてはGenotype B、Subgenotype C2および組換え型が同定され、母親についてはGenotype B、Subgenotype C5および3種類の組換え型が同定された。男児についてはSubgenotype C2のみが同定された。系統発生学的解析により、男児の全ての配列と父親の大半の配列は同一のクラスターに属することが判明したが、母親の配列と男児の配列については、同一のクラスターに属するものはなかった。男児と父親から得たHBs抗原遺伝子におけるアミノ酸置換のパターンは同一であった（T118K、T123NおよびG145A）。我々は、父親が男児におけるHBV感染の感染源であるという結論に達した。これによりOBIも密接な接触を介して伝播し、顕性感染として出現する可能性があることが示唆された。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見

オカルトB型肝炎ウイルス感染は密接な接触を介して伝播し、感染した患者は顕性感染として発症する可能性があるという報告である。

今後の対応

日本赤十字社では、化学発光酵素免疫測定法（CLEIA）によりHBs抗原、HBc抗体検査を実施することに加え、20プールでスクリーニングNATを行っていたが、更なる安全対策を目的に2012年8月よりHBc抗体検査の判定基準を強化し、さらに2014年8月よりNATシステムを変更し、全検体に対し個別検体によるNAT（個別NAT）スクリーニングを開始している。HBV感染に関する新たな知見等について、今後も情報の収集に努める。

別紙3 4

MedDRA/J Ver.18.1J

RESEARCH ARTICLE

Occult HBV Infection May Be Transmitted through Close Contact and Manifest as an Overt Infection

Li-Ping Hu[1,2], De-Ping Liu[3], Qin-Yan Chen[1], Tim J. Harrison[4], Xiang He[5], Xue-Yan Wang[1], Hai Li[1], Chao Tan[1], Qing-Li Yang[1], Kai-Wen Li[1,2], Zhong-Liao Fang[1]*

1 Guangxi Zhuang Autonomous Region Center for Disease Prevention and Control, Guangxi Key Laboratory for the Prevention and Control of Viral Hepatitis, Nanning, Guangxi 530028, China, 2 School of Preclinical Medicine, Guangxi Medical University, 22 ShuangYong Road, Nanning, Guangxi 530021, China, 3 Chong Zuo Center for Disease Prevention and Control, Lijiang road, Chong Zuo, Guangxi 532200, China, 4 Division of Medicine, UCL Medical School, London, United Kingdom, 5 Guangdong Provincial Institute of Public Health, Guangdong Provincial Center for Disease Control and Prevention, Guangzhou 511430, China

* zhongliaofang@hotmail.com

CrossMark
click for updates

OPEN ACCESS

Citation: Hu L-P, Liu D-P, Chen Q-Y, Harrison TJ, He X, Wang X-Y, et al. (2015) Occult HBV Infection May Be Transmitted through Close Contact and Manifest as an Overt Infection. PLoS ONE 10(10): e0138552. doi:10.1371/journal.pone.0138552

Editor: Tatsuo Kanda, Chiba University, Graduate School of Medicine, JAPAN

Received: July 14, 2015

Accepted: September 1, 2015

Published: October 12, 2015

Copyright: © 2015 Hu et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: Data are available from http://dx.doi.org/10.6084/m9.figshare.1554852, http://dx.doi.org/10.6084/m9.figshare.1554851, http://dx.doi.org/10.6084/m9.figshare.1554850, and http://dx.doi.org/10.6084/m9.figshare.1554848.

Funding: This work was supported by the National Natural Science Foundation of China (Grant No. 81260439/H2609) and the Guangxi Science Foundation (Gui Ke Gong 1140003A-37).

Competing Interests: The authors have declared that no competing interests exist.

Abstract

The importance of transmission of occult HBV infection (OBI) via transfusion, organ transplantation and hemodialysis has been widely recognized. However, data regarding the transmission of OBI through close contact remain limited. In this study, serum samples were obtained from a child and his parents. The child had received the standard vaccination regimen at birth and produced protective antibody. Sera were tested for HBV serological markers. Nested PCR assays were used to detect HBV DNA and the amplicons were cloned and their sequences subjected to phylogenetic analysis. The results showed that both parents had occult infections while the child had an overt infection. Twelve, eleven and nine clones, from the father, mother and son, respectively, were sequenced. Serotypes adrq+, ayw1, ayw and ayr were found in the father and ayw1, adw2 and adwq+ in the mother; adrq+ was the only serotype in son. Genotype B, subgenotype C2 and a recombinant were identified in the father and genotype B, subgenotype C5 and three recombinants were found in the mother. Subgenotype C2 was the only genotype identified in the child. A phylogenetic tree showed that all of the child's sequences and most of the father's sequences clustered together. However, none of mother's sequences clustered with those of the child. The surface gene from the child and his father had the same amino acid substitution pattern (T118K, T123N and G145A). We concluded that the father was the source of the son's HBV infection, suggesting that occult HBV infection may be transmitted through close contact and manifest as an overt infection.

Introduction

Persistent infection with hepatitis B virus (HBV) remains a major global public health problem. Infection with hepatitis B virus may lead to a wide spectrum of liver disease that range, in acute infection, from mild, self-limited to fulminant hepatitis and, in persistent infection, from an asymptomatic carrier state to severe chronic hepatitis, cirrhosis and hepatocellular carcinoma. More than two billion people, one third of the world's population alive today, have been infected with HBV at some time in their lives and around 240 million of them remain infected (chronic HBsAg carriers) [1]. However, these classes do not include all HBV infections; there is another form of HBV infection, occult HBV infection (OBI). This was first reported by Tabor et al. 36 years ago in a case report of HBV infection following blood transfusion with blood from donors positive for anti-HBc only [2]. OBI is defined by the absence of HBsAg despite the presence of HBV DNA in the liver, blood serum, or peripheral blood mononuclear cells, irrespective of the presence of other hepatitis B viral antibodies and antigens [3].

The prevalence of OBI varies widely across the globe and ranges from 1% to 95% worldwide, depending on the level of endemic disease, the assays used in the studies and the various populations studied [4, 5]. However, it is well known that certain groups of patients are at a much higher risk of having occult HBV infection, regardless of the geographical location, such as those with chronic HCV infection and HIV infection, hemodialysis, liver transplant and hepatocellular carcinoma (HCC) patients and injection drug users [6]. Occult infection may be reactivated, leading to acute and severe forms of classical hepatitis B. The long-term persistence of the virus in the liver may favor the progression of the chronic liver disease to cirrhosis and HCC [7].

One of the major public health problems of occult HBV infection is the potential for transmission. Blood transfusion and liver transplantation remains the major routes of transmission, although the risk of HBV transmission through blood transfusion has decreased following the introduction of sensitive and specific diagnostic assays [8]. In additional, it has also been reported that intrauterine HBV infection is possible in pregnant women who are HBsAg and HBeAg negative [9]. The possibility of horizontal transmission of HBV from individuals with occult infection to close contacts does exist [10]. When occult viruses are transmitted to other individuals, the outcomes in terms of liver disease are the same as those following transmission from overt cases [8].

It has been reported that the prevalence of OBI is higher in HBV endemic areas such as East Asia and lower in low endemic areas such as North America [5]. Guangxi is one of the provinces in China with the highest prevalence of persistent HBV infection, affecting 9.2% of the general population [11]. We reported previously that the prevalence of OBI among family members of children from Long An county, Guangxi who were positive for both HBsAg and anti-HBs after vaccination is 11.5% [12], suggesting that occult HBV infection is common in Guangxi. In this study, we provide evidence of transmission of occult HBV from a family contact to a child who was vaccinated successfully at birth but became infected overtly.

Materials and Methods

Study population and sample design

The study subjects were a three member family, a boy and his parents. Serum samples were obtained from the three individuals in April, 2015.

The father is 44 years old. He was given a full course of immunization (10 μg doses of vaccine given at 0, 1 and 6 months) in1994. He was negative for serological markers of HBV infection before vaccination and after the last dose. He was vaccinated again in 1999 according to

the same program but the doses were 30 μg, 20 μg and 10 μg, respectively. He remained negative for all of HBV serological markers after the last dose. He became weakly positive for anti-HBc in 2004 and positive for both anti-HBe and anti-HBc in 2008. He was vaccinated again in 2009 with one 60 μg dose. He was positive for anti-HBc only after vaccination.

The woman is also 44 years old and had married in 1999. She was found to be positive for anti-HBs in 1996. She was immunized with a full course of vaccination (10 μg doses of vaccine given at 0, 1 and 6 months) in 2004 when she found to be weakly positive for anti-HBs. She was positive for anti-HBs (298 IU/ml), anti-HBe and anti-HBc in 2008.

The child was born in 2001. He received in time a full course of vaccination (10 μg doses of vaccine given at 0, 1 and 6 months). He was anti-HBs positive with a titer of ≥10 IU/L after the last dose. However, he became positive for HBsAg in 2004. He was positive for HBsAg, anti-HBe and anti-HBc in 2007.

All vaccines used above are yeast-derived recombinant hepatitis B vaccine (National Vaccine and Serum Institute, Beijing, China).

Informed consent in writing was obtained from the parents and that of the child was from the parents in his behalf. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and has been approved by the Guangxi Institutional Review Board.

Serological Testing

Sera were tested for HBsAg/anti-HBs, HBeAg/anti-HBe, anti-HBc and anti-hepatitis C virus (HCV) using enzyme immunoassays (Zhong Shan Biological Technology Company, Limited, Guangzhou, China). Alanine aminotransferase (ALT) levels were determined using a Reitman kit (Sichuan Mike Scientific Technology Company, Limited, Chengdu, China).

Nested polymerase chain reaction (PCR) for HBV DNA and nucleotide sequencing

DNA was extracted from 85 μl serum by pronase digestion followed by phenol/chloroform extraction. In order to avoid false positive, two regions of the HBV genome were amplified using nested PCR, from PreS1 to the X gene and a smaller region covering the S gene only.

For PreS1 to the X gene, the first round PCR was carried out in a 50 μl reaction using primers LSOB1 (nt 2739–2762, 5'-GGCATTATTTGCATACCCTTTGG-3') and P2 (nt 1823–1806 5'-CCGGAAAGCTTGAGCTCTTCAAAAAGTTGCATGGTGCTGG-3') [13], with 5 min hot start followed by 30 cycles of 94°C for 30 sec, 50°C for 30 sec, and 72°C for 90 sec. Second round PCR was carried out on 5 μl of the first round products in a 50 μl reaction using primers LSBI1 (nt 2809–2829, 5'-TTGTGGGTCACCATATTCTT-3') and POLSEQ2 (nt1168-1188, 5'-AGCAAACACTTGGCATAGGC-3') and the same amplification protocol as first round.

For the S gene, the first round PCR was carried out in a 50 μl reaction using primers MD14 (nt 418–433, 5'-GCGCTGCAGCTATGCCTCATCTTC-3') and HCO2 (nt 761–776, 5'-GCG AAGCTTGCTGTACAGACTTGG-3'), with 5 min hot start followed by 35 cycles of 94°C for 45 sec, 45°C for 45 sec, and 72°C for 120 sec. The second round PCR was carried out on 5 μl of the first round products in a 50 μl reaction using primersME15(nt 455–470, 5'-GCGCTGCAG CAAGGTATGTTGCCCG-3') and HDO3 (nt 734–748, 5'-GCGAAGCTTCATCATCCATA TAGC-3') with 5 min hot start followed by 30 cycles of 94°C for 45 sec, 55°C for 45 sec, and 72°C for 120 sec.

Amplicons from the second round were confirmed by agarose gel electrophoresis and cloned into the vector pUCm-T (The Sangon Biotech (Shanghai, China)). Plasmid DNA was extracted using a SK1191 UNIQ-10 kit (The Sangon Biotech (Shanghai, China)) and the purified DNA was sequenced using a BigDye Terminator V3.1 Cycle Sequencing kit (Applied

Biosystems, Foster City, USA) with sequencing primer PSISEQ2F (nt 65–84, 5'-GGCTCCA ATTCCGGAACAGC-3') and POLSEQ2. Meanwhile, PreS1/S2 region of HBV from each sample was sequenced directly without cloning using sequencing primer LSBI1 in The Sangon Biotech (Shanghai, China).

Measurement of Viral loads

Serum HBV DNA concentrations were quantified by real time PCR using commercial reagents (Shanghai ZJ Bio-Tech Co., Ltd. (Shanghai, China)) in an ABI Prism 7500 sequence detection system (Applied Biosystems, Foster City, CA, California, USA), using HBV primers and a dual labeled TaqMan probe, as described previously [14].

HBV serotyping

Serotypes were determined according to a single amino acid at the following position: adrq- (122 K + 127P + 134 F + 159 V + 160R +177A +178P), adrq + (122 K + 127P + 134 F + 159A +160R + 177 V +178P), adw2 (122 K + 127P + 134 F +159A + 160 K + 177 V +178P), adwq+ (122 K + 127 L +134 F + 159A + 160 K + 177 V +178P), ayr (122R + 127P +134 F + 159A + 160R + 177 V +178P), ayw1 (122R +127P + 134 F + 159A + 160 K + 177 V +178P), ayw2 (122R + 127P + 134Y + 159G + 160 K + 177 V +178P), ayw3 (122R + 127 T + 134 F + 159G + 160 K + 177V +178P) [15–17].

HBV genotyping

HBV genotypes were determined using phylogenies reconstructed on the basis of the complete S region (678 nt) of the viruses. The sequences were alighed to 21 HBV sequences of all known genotypes retrieved from GenBank using Clustal W and visually confirmed with the sequence editor BioEdit [18]. The reference sequences were A1_M57663_Philippines, B1_D23677_Japan, B2_AY217358_China, B2_AF121249_Vietnam, B3_AB033555_Sumatra, B4_AB073835_Vietnam, B5_AB219427_Philippines, B6_DQ463801_Canada, C1_AF458664_China, C2_AY217371_China, C3_X75656_Polynesia, C4_AB048704_Australia, C5_JN827415_Thailand, D1_AF280817_China, E_AB091255_Ivory Coast, F_AY090458_Costa Rica, G_AF160501_USA, H_AY090460_USA, I1_AB231908_Vietnam, I1_FR714504_Longan_China, I2_FJ023664_Laos. Neighbor-Joining trees were reconstructed under the Kimura 2-parameter substitution model with the program MEGA [19]. The reliability of clusters was evaluated using interior branch test with 1000 replicates and the internal nodes with over 95% support were considered reliable.

Sequences that were not determined by phylogenies were genotyped using the NCBI Genotyping Tool (http://www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi).

Identification of MHR mutations and overlapping polymerase mutations

Amino acid substitutions in the major hydrophilic region (MHR; aa 99–169) were originally evaluated using the Genafor/Arevir-geno2pheno drug resistance tool (http://hbv.geno2pheno.org/index.php). Those identified as MHR substitutions by the tool were then aligned to HBV reference sequences (JQ688404, EU410081 and AB776908), which were obtained from GenBank and used to exclude subgenotypes and polymorphisms. The mutations were categorized into general mutations and escape mutations.

Table 1. Serological characteristics of the study subjects.

Samples	Ages	HBsAg	Anti-HBs	HBeAg	Anti-HBe	Anti-HBc	Viral loads	ALT (IU/ml)
Father	44	-	-	-	-	+	8×10^5 IU/ml	<40
Mother	44	-	+	-	-	+	3.53×10^3 IU/ml	<40
Son	14	+	-	+	-	+	5.42×10^5 IU/ml	170

doi:10.1371/journal.pone.0138552.t001

Results

General information

Successful PCR amplification of two regions of the viral genome was achieved for all three individuals. Both parents have OBI, the father is positive for anti-HBc only and the mother is positive for anti-HBs and anti-HBc. The ALT levels of the parents are normal but that of the child is abnormal. The viral load of the father is the highest and that of the mother is the lowest (Table 1). All three individuals are negative for anti-HCV. Complete PreS1/S2 sequences were obtained for the three individuals; 12, 11 and 9 clones were constructed from the father's, mother's and son's amplicons, respectively. The S-gene was sequenced in both directions, covering the entire MHR (GenBank accession number: KT585753-KT585784).

Serotypes in different clone sequence

Serotypes adrq+, ayw1, ayw and ayr were predicted from the father's twelve sequences. Four serotypes, including ayw1, adw2 and adwq+ were predicted from the mother's sequences. All nine sequences from the son's sample predicted serotype adrq+ (Table 2). These data suggest that transmission between father and son is possible because they have the same serotype adrq + and the transmission is from father to son because the father has more serotypes. Transmission between the father and mother also is possible because they share serotype: ayw1. However, transmission between the mother and son is not possible for because they do not share any serotype.

Table 2. Serotypes and genotypes predicted from the sequences from each study subject.

Study subject	Number of clones	Serotypes	Genotypes
Father			
	9	adrq+	C2
	1	ayw1	B
	1	ayw	B
	1	ayr	Recombinant (B/C)
Total	12		
Mother			
	7	adwq+	C5
	1	ayw1	B
	1	ayw1	Recombinant (B/C)
	1	adw2	Recombinant (B/C)
	1	adwq+	Recombinant (C/G)
Total	11		
Son			
	9	adrq+	C2
Total	9		

doi:10.1371/journal.pone.0138552.t002

Genotypes in different clone sequences

Using phylogenetic analysis and the NCBI genotyping analysis, three genotypes, subgenotype C2, genotype B and a recombinant were identified from the father's sequences. Subgenotype C5, genotype B and three recombinants were identified in the mother. Subgenotype C2 was the only genotype identified in the son (Table 2 and Fig 1). The phylogenetic tree shows that all of the son's sequences and most of the father's sequences cluster together. However, none of the mother's sequences cluster with her son's sequences (Fig 1). Clearly, the transmission was from father to son. There is no evidence of transmission between the mother and son. It is also possible for transmission to have occurred between the parents because some of their sequences cluster together, supported by a 77% bootstrap value.

Amino acid substitutions within the "a" determinant and MHR

Amino acid substitutions in the major hydrophilic region predicted from twelve clones of HBV from the father's sample include T115I, T116A, S117G, T118K, 123N, Q129L, T131N, M133L, M133S, F134L, G145A and I152V. Half of them are located within "a" determinant (aa 124–147). Five of the twelve substitutions, including T118K, T123N, T131N, M133L and N 145A, are associated with antibody escape. The T118K and N145A substitutions may result in vaccine escape. T118K and T123N may result in failure to detect of HBsAg. The 123N and 145A mutations may result from escape from immunoglobin therapy. Seven clones have the same amino acid substitution pattern as that in the son: T118K, T123N and N145A. Two clones have T118K and N145A substitutions, two other clones have T131N and the final clone has M133L (Fig 2).

Amino acid substitutions in the major hydrophilic region predicted from nine clones of HBV from the son's sample include L104S, T118K, T123N, S143L and G145A. Only two of them are located within the "a" determinant. Except for L104S, all are escape substitutions. All of the nine clones from the son have the same amino acid substitution pattern (T118K, T123N and G145A) as seen in his father. Furthermore, one of the nine sequences has an amino acid substitution at position 143, which may result in vaccine escape and failure to detect HBsAg (Fig 2).

Amino acid substitution mutations in the major hydrophilic region predicted from eleven clones of HBV from the mother's sample include D99N, T131N, F161S and V168A. Only the T131N mutation causes detection failure and this mutation could be seen in one clone only (Fig 2).

Clearly, the frequency of amino acid substitution mutations within the "a" determinant and MHR is highest in the father's sample. The next is that from the son. It is possible that there was transmission between father and son because they have the same amino acid substitution pattern. The transmission was from father to son because the father has a more complex pattern of mutations. Transmission between father and mother or mother and son is impossible because they do not share the same amino acid substitution pattern.

Mutations in the preS1/preS2 region

PreS1 contains 357 bases, encoding 119 amino acids and PreS2 contains 165 bases, encoding 55 amino acids. No deletion was found in either PreS1 or PreS2 from the three samples. No point mutation was found in the initiation codon of preS2 in the three samples.

The impact of mutations in the S gene on the overlapping polymerase region

Mutations leading to amino acid substitutions in the small S protein may produce amino acid changes in the overlapping polymerase. In this study, there are eleven amino acid substitutions

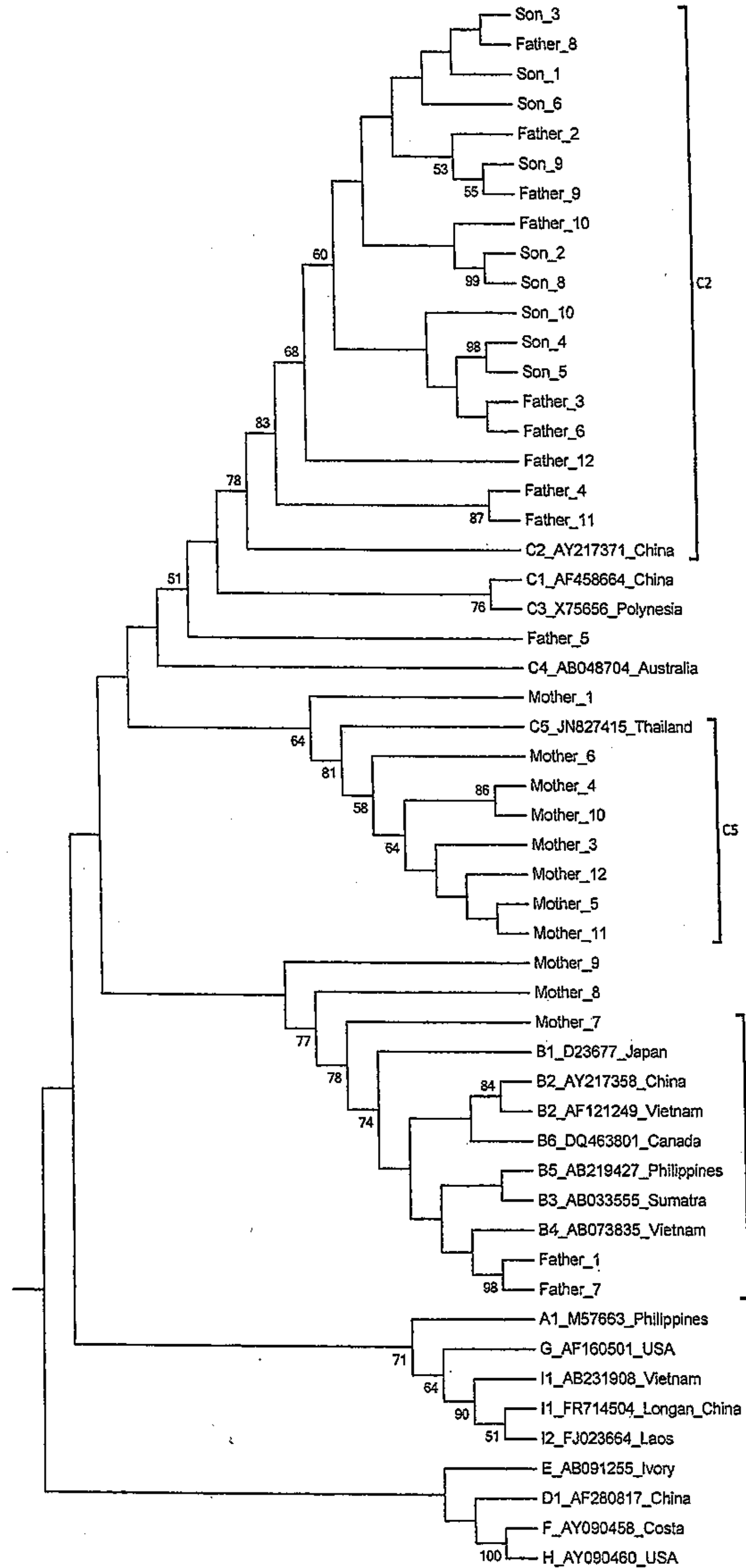

Son_3
Father_8
Son_1
Son_6
Father_2
Son_9
Father_9
Father_10
Son_2
Son_8
Son_10
Son_4
Son_5
Father_3
Father_6
Father_12
Father_4
Father_11
C2_AY217371_China
C1_AF458664_China
C3_X75656_Polynesia
Father_5
C4_AB048704_Australia
Mother_1
C5_JN827415_Thailand
Mother_6
Mother_4
Mother_10
Mother_3
Mother_12
Mother_5
Mother_11
Mother_9
Mother_8
Mother_7
B1_D23677_Japan
B2_AY217358_China
B2_AF121249_Vietnam
B6_DQ463801_Canada
B5_AB219427_Philippines
B3_AB033555_Sumatra
B4_AB073835_Vietnam
Father_1
Father_7
A1_M57663_Philippines
G_AF160501_USA
I1_AB231908_Vietnam
I1_FR714504_Longan_China
I2_FJ023664_Laos
E_AB091255_Ivory
D1_AF280817_China
F_AY090458_Costa
H_AY090460_USA
C2
C5
B

Fig 1. Neighbor-Joining trees. The trees were reconstructed on the basis of the complete S region (678 nt) of the viruses under the Kimura 2-parameter substitution model with the program MEGA [19]. The branch lengths represent the number of substitutions per site. The reliability of clusters was evaluated using the interior branch test with 1000 replicates and the internal nodes with over 95% support are considered reliable.

doi:10.1371/journal.pone.0138552.g001

in the overlapping polymerase in all clones from the son's sample, including R15L, V23I, T38A, T38K, H55Q, S57F, L72P, L77S, H126Q, V191I and H197R. There are twenty-one amino acid substitutions in the overlapping polymerase in all clones from father's sample, including F46S, R51K, H55Q, H55R, S57F, P109S, N118T, N124R, Y124H, Q125R, H126Q, 127R, N134D, C136R, N139K, Y141F, S143T, H160R, A211T, S213T and Q215H. Amino acid substitutions in mother include T16I, R41S, V44A,N53S, H55R, W58R, N76D, S81T, V103I, G107E, N121I, I122L, N123D, Q125K, H126Y, N134D, N139H, N139Q, Y158H, I163V, F178L, S185N, V207M, Q215L,Y221F, A222T, I224V, G232R. However, none of these is associated with drug resistance.

Discussion

The major finding in the study is that the son has one serotype (adrq+) only and this was seen in the father but not the mother. The son also has one genotype (subgenotype C2) only and this could also be seen in the father but not the mother. All sequences from the son clustered with that from father in the phylogenetic tree. All of the sequences from the son have the same

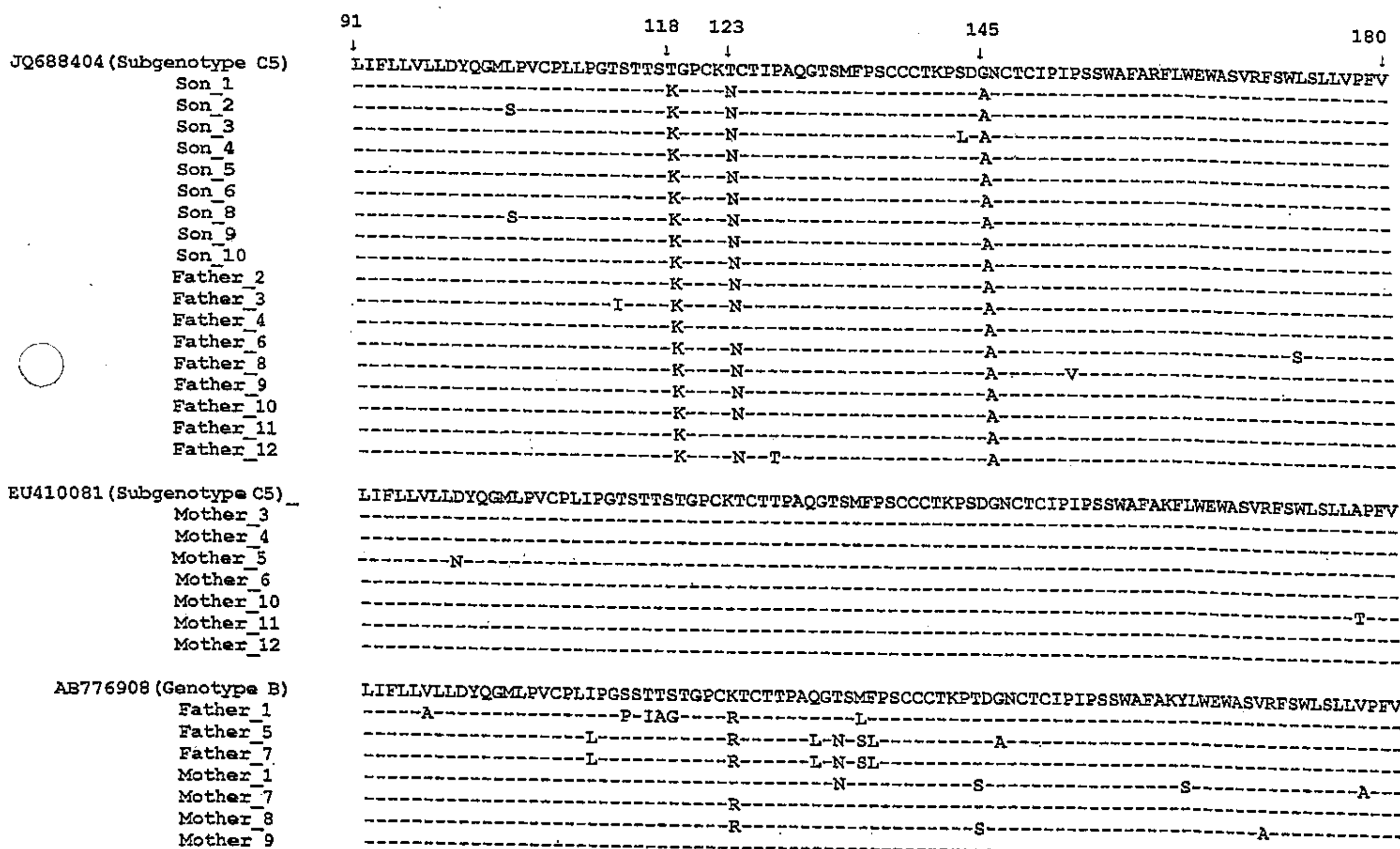


Fig 2. Frequency and distribution of amino acid substitutions in the MHR of HBsAg from each clone.

doi:10.1371/journal.pone.0138552.g002

amino acid substitution pattern in the S protein as that seen in the father. Furthermore, the son was found to be positive for HBsAg when he was tested prior to entrance to kindergarten, suggesting that household contact is the only likely pattern of transmission. These findings provide strong evidence of transmission from father to son. The father shares one serotype and genotype with the mother but not the amino acid substitution pattern in the S protein. Meanwhile, it also is suggested that the mother became infected outside the family. The strength of the study is that detailed medical records are available for the study subjects, which may provide additional evidence of transmission. The weakness of the study is that we did not test HBsAg with different commercial diagnostic kits, which may provide information about the association of amino acid substitutions with detection failure.

The recognized patterns of spread of HBV include perinatal, sexual and parenteral/percutaneous routes. Routes of parenteral transmission include injection drug use, transfusions and dialysis, acupuncture and tattooing; household contact with infected individuals and working in a health-care setting also are risk factors for horizontal transmission [20,21]. Compared to overt HBV infection, the routes of transmission of occult HBV have been studied less. Most these studies focused on blood transfusion and liver transplantation because these may transmit hepatitis B [8]. In addition, it has also been reported that intrauterine HBV infection is possible in pregnant women with OBI [9].

Occult HBV infection may be common in household contacts of individuals with chronic hepatitis B [22]. A study from India found that sequences from both occult HBV and overt HBV are similar in terms of genotype and surface variants or non-variants (wild-type) and clustered together in the phylogenetic tree. The authors considered the possibility of horizontal transmission of HBV from individuals with occult infection to their contacts [10]. However, this claim is not strong enough because it remains possible that the transmission of HBV was from individuals with overt infection but resulted in occult infection. In our study, with cloning and sequencing, we found that the father has more serotypes and genotypes than that of the son, suggesting that transmission was from father to son.

Immunization with hepatitis B vaccine is the most effective means of preventing acute infection by HBV [23]. However, the titer of vaccine-induced antibody decays exponentially over time, irrespective of the population immunized [24]. It has been claimed that neonatal HBV immunization is efficacious in inducing long-term immunity and cell-mediated immune memory for up to two decades and booster vaccinations are not required [25]. The fact that the child produced protective levels of anti-HBs after immunization but became infected by H clearly challenges these findings, suggesting that the monitoring of the level of anti-HBs among vaccinated subjects for booster vaccination is necessary. Our findings also suggested that occult HBV infection may be transmitted through close contact. Therefore, susceptible individuals should be vaccinated against hepatitis B in endemic regions. Nucleic acid testing for OBI is necessary for the staff in some occupations, such as nursery teachers, in regions where HBV is endemic.

Currently, many HBsAg immunoassays use monoclonal antibodies with epitopes directed against the MHR, in particular against the "a" determinant, and amino acid substitution in this region may result in changes to critical epitopes and account for false-negative results in immunoassays [26,27]. The T118K, T123N and N145A substitutions in the MHR have been reported to lead to failure of detection [26, 28– 30]. In this study, ten of twelve clones from the father have these mutations. The remaining two also have detection escape mutations (T131N and M133L) [26, 29]. The father tested negative for HBsAg. All of son's clones have the same mutations. However, he is positive for HBsAg. It is not clear why the same diagnostic assay produced different test results.

A study from Taiwan showed that most non-responders among anti-HBc positive subjects apparently had occult HBV infection [31]. However, this finding was not supported by a subsequent study from Iran [32]. In our study, the father was negative for all HBV serological markers before and after the first two full courses of vaccination. He became weakly positive for anti-HBc many years later. Then, he was immunized for the third time but remained negative for anti-HBs. Clearly, our data support the result from Taiwan and suggest that nucleic acid testing should be considered for non-responders to exclude OBI, especially in regions where HBV is endemic.

The predominant genotype in Guangxi is genotype C, followed by genotypes B and I (a recombinant) [33]. In the study, both parents are infected with genotype B and C and recombinants (between genotype B and C). In the future, we will determine whether the recombinant sequences are from genotype B and C in the same person or from outside sources, which may provide more information about the occurrence of recombination.

Acknowledgments

We are indebted to the family providing serum samples and staff members of Chong Zuo Center for Disease Prevention and Control for their help in handling the sera.

Author Contributions

Conceived and designed the experiments: ZLF TJH DPL. Performed the experiments: LPH QYC HL QLY. Analyzed the data: XH XYW. Contributed reagents/materials/analysis tools: DPL CT KWL. Wrote the paper: ZLF TJH.

References

1. World Health Organization. Hepatitis B Fact sheet N°204. (2014) Available: http://www.who.int/mediacentre/factsheets/fs204/en/
2. Tabor E, Hoofnagle JH, Smallwood LA, Drucker JA, Pineda-Tamondong GC, Ni LY, et al. Studies of donors who transmit posttransfusion hepatitis. Transfusion. 1979; 19: 725–731. PMID: 230620
3. Martinez MC, Kok CC, Baleriola C, Robertson P, Rawlinson WD. Investigation of occult hepatitis B virus infection in anti-hbc positive patients from a liver clinic. PLoS One. 2015; 10: e0117275. doi: 10.1371/journal.pone.0117275 PMID: 25763579
4. Gutiérrez-García ML, Fernandez-Rodriguez CM, Lledo-Navarro JL, Buhigas-Garcia I. Prevalence of occult hepatitis B virus infection. World J Gastroenterol. 2011; 17: 1538–42. doi: 10.3748/wjg.v17.i12 PMID: 21472117
5. Kwak MS, Kim YJ. Occult hepatitis B virus infection. World J Hepatol. 2014; 6: 860–9. doi: 10.4254/wjh.v6.i12.860 PMID: 25544873
6. Samal J, Kandpal M, Vivekanandan P. Molecular mechanisms underlying occult hepatitis B virus infection. Clin Microbiol Rev. 2012; 25: 142–63. doi: 10.1128/CMR.00018-11 PMID: 22232374
7. Squadrito G, Spinella R, Raimondo G. The clinical significance of occult HBV infection. Ann Gastroenterol. 2014; 27: 15–19. PMID: 24714731
8. Raimondo G, Caccamo G, Filomia R, Pollicino T. Occult HBV infection. Semin Immunopathol. 2013; 35: 39–52. doi: 10.1007/s00281-012-0327-7 PMID: 22829332
9. Gui QD, Yue YF, Li SH, Zhang F. Study on intrauterine infection of hepatitis B virus in pregnant women with hepatitis B surface antigen and hepatitis B e antigen negative. Chinese Journal of Obstetrics and Gynecology. 2005; 40: 99–102.
10. Datta S, Banerjee A, Chandra PK, Chowdhury A, Chakravarty R. Genotype, phylogenetic analysis, and transmission pattern of occult hepatitis B virus (HBV) infection in families of asymptomatic HBsAg carriers. J Med Virol. 2006; 78: 53–9. PMID: 16299727
11. Fang ZL, Harrison TJ, Yang JY, Chen QY, Wang XY, Mo JJ. Prevalence of hepatitis B virus infection in a highly endemic area of southern China after catch-up immunization. J Med Virol. 2012; 84: 878–84. doi: 10.1002/jmv.23278 PMID: 22499009

12. Fang ZL, Zhuang H, Wang XY, Ge XM, Harrison TJ. Hepatitis B virus genotypes, phylogeny and occult infection in a region with a high incidence of hepatocellular carcinoma in China. World J Gastroenterol. 2004; 10: 3264–8. PMID: 15484297

13. Gunther S, Li BC, Miska S, Krüger DH, Meisel H, Will H. A novel method for efficient amplification of whole hepatitis B virus genomes permits rapid functional analysis and reveals deletion mutants in immunosuppressed patients. Journal of Virology. 1991; 69: 5437–44.

14. Fang ZL, Sabin CA, Dong BQ, Wei SC, Chen QY, Fang KX, et al. The association of HBV core promoter double mutations (A1762T and G1764A) with viral load differs between HBeAg positive and anti-HBe positive individuals: a longitudinal analysis. J Hepatol. 2009; 50: 273–80. doi: 10.1016/j.jhep.2008.09.014 PMID: 19070921

15. Courouce-Pauty AM, Plancon A, Soulier JP. Distribution of HBsAg subtype in the world. Vox Sang. 1983; 44:197–211. PMID: 6845678

16. Okamoto H, Tsuda F, Sakugawa H, Sastrosoewignjo RI, Imai M, Miyakawa Y, et al. Typing hepatitis B virus by homology in nucleotide sequence: comparison of surface antigen subtypes. J Gen Virol. 1988; 69:2575–2583. PMID: 3171552

17. Chen X, Gao J, Ji Z, Zhang W, Zhang L, Xu R, et al. A description of the hepatitis B virus genomic background in a high-prevalence area in China. Virol J. 2014; 11:101. doi: 10.1186/1743-422X-11-101 PMID: 24884702

18. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series. 1999; 41: 95–98.

19. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution. 2013; 30: 2725–2729. doi: 10.1093/molbev/mst197 PMID: 24132122

20. Yao GB. Importance of perinatal versus horizontal transmission of hepatitis B virus infection in China. Gut. 1996; 38 Suppl 2, S39–42. PMID: 8786052

21. Gerlich WH. Medical virology of hepatitis B: how it began and where we are now. Virol J. 2013; 10:239. doi: 10.1186/1743-422X-10-239 PMID: 23870415

22. Kumar GT, Kazim SN, Kumar M, Hissar S, Chauhan R, Basir SF, et al. Hepatitis B virus genotypes and hepatitis B surface antigen mutations in family contacts of hepatitis B virus infected patients with occult hepatitis B virus infection. J Gastroenterol Hepatol. 2009; 24:588–98. doi: 10.1111/j.1440-1746.2008.05727.x PMID: 19207682

23. Zhou YH, Wu C, Zhuang H. Vaccination against hepatitis B: the Chinese experience. Chin Med J (Engl). 2009; 122: 98–102.

24. Jack AD, Hall AJ, Maine N, Mendy M, Whittle HC. What level of hepatitis B antibody is protective? J Infect Dis. 1999; 179: 489–492. PMID: 9878036

25. Saffar H, Saffar MJ, Ajami A, Khalilian AR, Shams-Esfandabad K, Mirabi AM. Long-term T-cell-mediated immunologic memory to hepatitis B vaccine in young adults following neonatal vaccination. Hepat Mon. 2014; 14: e22223. doi: 10.5812/hepatmon.22223 PMID: 25368659

26. Ireland JH, O'Donnell B, Basuni AA, Kean JD, Wallace LA, Lau GK, et al. Reactivity of 13 in vitro expressed hepatitis B surface antigen variants in 7 commercial diagnostic assays. Hepatology. 20 31: 1176–82. PMID: 10796895

27. Ly TD, Servant-Delmas A, Bagot S, Gonzalo S, Férey MP, Ebel A, et al. Sensitivities of four new commercial hepatitis B virus surface antigen (HBsAg) assays in detection of HBsAg mutant forms. J Clin Microbiol. 2006; 44: 2321–6. PMID: 16825343

28. Kfoury Baz EM, Zheng J, Mazuruk K, Van Le A, Peterson DL. Characterization of a novel hepatitis B virus mutant: demonstration of mutation-induced hepatitis B virus surface antigen group specific "a" determinant conformation change and its application in diagnostic assays. Transfus Med. 2001; 11: 355–62. PMID: 11696228

29. Cooreman MP, Leroux-Roels G, Paulij WP. Vaccine- and hepatitis B immune globulin-induced escape mutations of hepatitis B virus surface antigen. J Biomed Sci. 2001; 8: 237–47. PMID: 11385295

30. Seddigh-Tonekaboni S, Lim WL, Young B, Hou JL, Waters J, Luo KX, et al. Hepatitis B surface antigen variants in vaccinees, blood donors and an interferon-treated patient. J Viral Hepat. 2001; 8: 154–8. PMID: 11264736

31. Yen YH, Chen CH, Wang JH, Lee CM, Changchien CS, Lu SN. Study of hepatitis B (HB) vaccine non-responsiveness among health care workers from an endemic area (Taiwan). Liver Int. 2005; 25: 1162–8. PMID: 16343067

32. Aghasadeghi MR, Banifazl M, Aghakhani A, Eslamifar A, Vahabpour R, Ramezani A. No evidence for occult HBV infection in hepatitis B vaccine non-responders. Iran J Microbiol. 2014; 6: 350–3. PMID: 25848527

33. Li GJ, Hue S, Harrison TJ, Yang JY, Chen QY, Wang XY, et al. Hepatitis B virus candidate subgenotype I1 varies in distribution throughout Guangxi, China and may have originated in Long An county, Guangxi. J Med Virol. 2013; 85: 799–807. doi: 10.1002/jmv.23533 PMID: 23508905

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2016 年 02 月 17 日	新医薬品等の区分 該当なし	厚生労働省処理欄

一般的名称	①②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	http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/UCM486360.pdf/2016/02/16	公表国 アメリカ
販売名（企業名）	①抗 HBs 人免疫グロブリン筋注 200 単位/1mL「JB」（日本血液製剤機構） ②抗 HBs 人免疫グロブリン筋注 1000 単位/5mL「JB」（日本血液製剤機構） ③ヘブスブリン筋注用 200 単位（日本血液製剤機構） ④ヘブスブリン筋注用 1000 単位（日本血液製剤機構） ⑤ヘブスブリン IH 静注 1000 単位（日本血液製剤機構）			

研究報告の概要

「業界向けガイダンス：ジカウイルスの輸血による伝播リスクを低減するためのドナースクリーニング，献血延期，および製品管理に関する勧告」

米 FDA は，新規のジカウイルスのアウトブレイクに対する安全対策として，新たなガイダンス「業界向けガイダンス：ジカウイルスの輸血による伝播リスクを低減するためのドナースクリーニング，献血延期，および製品管理に関する勧告」を発行した。 ジカウイルス伝播が活発ではない地域においては，感染リスクがあるドナー（過去 4 週間に感染を示唆する症状があった人など）に対する 4 週間の献血延期が勧告されている。また，ジカウイルス伝播が活発な地域においては，輸血用全血および血液成分は米国の伝播が活発ではない地域から調達するよう勧告している。なお、本ガイダンスは Source Plasma（原料血漿）には適用されない。

報告企業の意見

ジカウイルス（Zika virus）は 1947 年にウガンダの Zika forest（ジカ森林）から発見されたウイルスで、デングウイルス、日本脳炎ウイルス、ウエストナイルウイルスと同じフラビウイルス科フラビウイルス属に属するエンベロープを有する RNA ウイルスで、蚊（ネッタイシマカ、ヒトスジシマカ）によって媒介される。万一、原料血漿にジカウイルスが混入したとしても、各種モデルウイルスのウイルスクリアランス試験成績から、本剤の製造工程において不活化・除去されると考えている。

今後の対応

本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

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

抗 HBs

5

Recommendations for Donor Screening, Deferral, and Product Management to Reduce the Risk of Transfusion-Transmission of Zika Virus

---

Guidance for Industry

This guidance is for immediate implementation.

FDA is issuing this guidance for immediate implementation in accordance with 21 CFR 10.115(g)(2) without initially seeking prior comment because the agency has determined that prior public participation is not feasible or appropriate.

FDA invites comments on this guidance. Submit one set of either electronic or written comments on this guidance at any time. Submit electronic comments to http://www.regulations.gov. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*. FDA will review any comments we receive and revise the guidance when appropriate.

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD), 10903 New Hampshire Ave., Bldg. 71, Rm. 3128, Silver Spring, MD 20993-0002, or by calling 1-800-835-4709 or 240-402-8010, or email ocod@fda.hhs.gov, or from the Internet at http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

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

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
February 2016

Table of Contents

I. INTRODUCTION 1

II. BACKGROUND 1

III. RECOMMENDATIONS 4

A. Recommendations for Areas without Active Transmission of ZIKV 4
B. Recommendations for Areas with Active Transmission of ZIKV 6
C. Post-Donation Information and Product Management 9
D. Product Disposition and Labeling 10

IV. IMPLEMENTATION 10

V. REFERENCES 12

Recommendations for Donor Screening, Deferral, and Product Management to Reduce the Risk of Transfusion-Transmission of Zika Virus

---

Guidance for Industry

This guidance represents the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

I. INTRODUCTION

We, FDA, are providing you, blood establishments that collect Whole Blood and blood components, with recommendations for donor screening, donor deferral and product management to reduce the risk of transfusion-transmitted Zika virus (ZIKV). The recommendations contained in this guidance apply to the collection of Whole Blood and blood components intended for transfusion. This guidance does not apply to the collection of Source Plasma.[1]

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

II. BACKGROUND

ZIKV is an arbovirus from the *Flaviviridae* family, genus *Flavivirus*. It is transmitted to humans primarily by the *Aedes aegypti* mosquito, but it may also be transmitted by the *Aedes albopictus* mosquito (Refs. 1, 2). In addition, intrauterine, perinatal and sexual transmission of ZIKV has been reported (Refs. 3, 4, 5). It was first isolated in 1947 from a rhesus monkey in the Zika Forest of Uganda, and isolated from a human in 1968 in Nigeria (Ref. 2). Epidemiological studies showed that the virus has circulated in humans between 1951 and 1981 in African and Asian countries (Ref. 6). ZIKV illness was first recognized outside of Africa and Asia in 2007

---

[1] This guidance does not apply to the collection of Source Plasma, which is used for further manufacture of plasma-derived products. Viral inactivation and removal methods are currently used to clear viruses in the manufacturing process for plasma-derived products.

during an outbreak on Yap Island, Micronesia (Refs. 7, 8). An outbreak of ZIKV was next reported in French Polynesia from October 2013 to February 2014, when about 11% of the population had symptomatic infection (Refs. 9, 10).

Recent outbreaks of arboviral disease in the Americas have included large dengue and chikungunya virus outbreaks. ZIKV reached the region of the Americas in early 2015 with local transmission first reported in Brazil (Refs. 11, 12) and as of February 10, 2016, there are 30 countries and territories worldwide with active local transmission of the virus (Refs. 13, 14). Outside of the Americas, local transmission of ZIKV has been reported in the Pacific Islands of Samoa, American Samoa and Tonga, and Cape Verde in Africa. As of February 10, 2016, local mosquito-borne transmission of ZIKV has not been reported in the continental United States, but cases have been reported in travelers returning to the United States from areas with local transmission (Ref. 14).

In February 2016, the World Health Organization (WHO) Director Dr. Margaret Chan declared that "the recent cluster of microcephaly cases and other neurological disorders reported in Brazil constitutes a Public Health Emergency of International Concern." In January 2016, Zika virus disease was added to the list of nationally notifiable conditions in the U.S. as a subtype of Arboviral diseases (Ref. 15).

ZIKV disease symptoms include: fever, arthralgia, maculopapular rash, and conjunctivitis. Less frequently, observed symptoms include digestive problems (abdominal pain, diarrhea and constipation), mucous membrane ulcerations (aphthae), and pruritus (Refs. 16, 17). In addition, neurological manifestations and congenital anomalies have been temporally and spatially associated with ZIKV disease outbreaks (Ref. 17). Association of ZIKV infection with Guillain-Barré syndrome cases has been reported during outbreaks in Polynesia (Ref. 18) and in Brazil (Ref. 19). In Brazil, there has also been a marked increase in the incidence of microcephaly in regions most affected by the ZIKV epidemic (Refs. 19, 20).

Sexual transmission of ZIKV has been reported (Refs. 4, 5). The two reported cases involved. transmission of the virus through sexual contact. In a separate report, ZIKV was isolated from semen at least two weeks and possibly up to 10 weeks after illness onset (Ref. 21). The duration of persistence of ZIKV in semen remains unknown. Data are currently not available regarding the presence of ZIKV in vaginal fluids. Sexual transmission of ZIKV from infected women to their sexual partners has not been reported.

Two instances of possible transfusion-transmission have been described in media announcements in Campinas, Brazil (Refs. 20, 22). In French Polynesia, 3% of samples from asymptomatic blood donors contained detectable ZIKV RNA during the outbreak in French Polynesia in 2013-14, indicating the likelihood of transmission by blood transfusion (Refs. 9, 23, 24). For these reasons, measures should be taken to prevent transfusion-transmission.

Regarding measures to help prevent ZIKV transmission through blood products, ZIKV is likely cleared by the existing viral inactivation and removal methods that are currently used to clear viruses in the manufacturing processes for plasma-derived products. For example, these viral clearance steps for various products may include pasteurization, solvent/detergent (S/D) treatment and incubation at low pH (Refs. 25, 26, 27). These methods are highly effective in

clearing lipid-enveloped viruses in plasma-derived products, but are not generally applicable for use in blood and blood components intended for transfusion. However, an S/D treated pooled plasma product has been FDA-licensed and is commercially available.

A pathogen reduction device (amotosalen + UV illumination) for plasma and platelets has recently been approved by the Center for Biologics Evaluation and Research (CBER), FDA (Refs. 28, 29) and demonstrated effective reduction of a panel of viruses, including flaviviruses, such as dengue and West Nile virus. A recent publication showed that the same pathogen reduction technology (PRT) can effectively reduce ZIKV in plasma (Ref. 24). These devices have been used to reduce the risk of ZIKV infection by plasma or apheresis platelet components that are collected in areas experiencing ZIKV outbreaks (Ref. 9).

Risk of ZIKV Transmission by Blood Transfusion

The risk of transmission of ZIKV by blood transfusion is considered likely based on the following evidence:

(a) two possible cases of transfusion-transmission in Campinas, Brazil (Refs. 20, 22);

(b) there have been documented transfusion-transmissions of other flaviviruses such as West Nile virus, dengue virus and Yellow Fever vaccine virus (Refs. 30, 31, 32), all of which have been shown to produce detectable viremia (the presence of virus in the blood) during asymptomatic and symptomatic infections;

(c) ZIKV infection produces viremia with up to 8.1×10^6 copies/ml that may last up to (and possibly beyond) 14 days, with varying reports of viremia from 2 days before to 11 days after onset of symptoms (Refs. 3, 7, 23, 33);

(d) the pre-symptomatic period for ZIKV infection varies (if symptoms develop) from 3 to 12 days, during which viremia may occur (Refs. 34, 35);

(e) an estimated 80% of ZIKV infections remain asymptomatic (Refs. 8, 9);

(f) perinatal transmission of ZIKV, most likely by transplacental transmission or during delivery, has been reported (Ref. 3); and,

(g) blood donations positive for ZIKV viral RNA by nucleic acid testing (NAT) were detected during the French Polynesia outbreak in 2013-2014 (Refs. 9, 23).

III. RECOMMENDATIONS

Consistent with existing regulations and applicable guidance, donors must be in good health at the time of donation (21 CFR 640.3(b)) as indicated by, among other things, freedom from any disease transmissible by blood transfusion, as can be determined by history and examination (21 CFR 640.3(b)(6))[2]. Standard operating procedures that are already in place should result in the deferral of individuals who have symptoms consistent with ZIKV at the time of donation. The following recommendations are intended to reduce the risk of collecting blood and blood components from at-risk donors who could be potentially infected with ZIKV and do not display clinical symptoms during the incubation period or have an asymptomatic infection.

For the purpose of this guidance, an area with "*active transmission of ZIKV*" is an area included on the CDC website listing of countries and U.S. states and territories with local vector-borne (i.e., mosquito-acquired) transmission of ZIKV: http://www.cdc.gov/zika/geo/index.html.[3]

A. Recommendations for Areas without Active Transmission of ZIKV

1. Donor Educational Material and Donor History Questionnaire

   a. Donor Educational Material

      i. We recommend that you update your donor educational material to include the risk factors for and signs and symptoms of ZIKV infection so that donors can self-defer. Relevant information on the signs and symptoms of ZIKV infection can be found on CDC's website at: http://www.cdc.gov/zika/symptoms/index.html.

      ii. The educational material should instruct donors as follows:

         a. A donor with a history of ZIKV infection should self-defer for 4 weeks after the resolution of symptoms.

         b. A donor who exhibits signs and symptoms of ZIKV infection within 2 weeks of departure from an area with active transmission of ZIKV should self-defer for 4 weeks after the resolution of symptoms.

---

[2] Under 21 CFR 630.10(a), which will replace 21 CFR 640.3 and will be effective May 23, 2016, (80 FR 29842, May 22, 2015), a donor must be in good health and free from transfusion-transmitted infections as can be determined by the processes set out in the new rule. Additionally, under 21 CFR 630.10(a) a donor is not eligible if you identify any factor(s) that may cause the donation to adversely affect the safety, purity, or potency of the blood or blood component. Such factors include travel to, or residence in, an area endemic for a transfusion-transmitted infection (21 CFR 630.10(e)(2)(iii)). Accordingly, the recommendations in this guidance will continue to apply under the new rule when it becomes effective.

[3] In general, an area is considered to have active transmission of ZIKV when locally transmitted, mosquito-borne ZIKV has been reported.

c. In addition to the above instructions, a donor should self-defer for 4 weeks after the last sexual contact with a man who has been diagnosed with ZIKV or who traveled to or resided in an area with active transmission of ZIKV in the 3 months prior to that instance of sexual contact.[4]

iii. In addition, we recommend that the educational material instruct donors with a history of recent travel to or residence in an area with active ZIKV transmission to inform the blood collection establishment promptly if they are diagnosed with ZIKV infection or if they develop symptoms suggestive of ZIKV infection within 2 weeks following donation.

b. Donor History Questionnaire

i. We recommend that you update your donor history questionnaire, including full length and abbreviated donor history questionnaires, and accompanying materials and standard operating procedures, as necessary, to incorporate the recommendations provided in this guidance.

ii. We recommend that your donor history questionnaire assess prospective donors for a history of residence in or travel to an area with active transmission of ZIKV in the past 4 weeks.

iii. We recommend that you refer to the CDC website for a listing of countries and territories with active transmission of ZIKV: http://www.cdc.gov/zika/geo/index.html.

iv. Blood collection establishments that use capture questions (e.g., "Have you traveled outside the United States within the past 3 years?") should review these questions to ensure they are adequate to identify travel to or residence in areas with active transmission of ZIKV, including U.S. territories. As necessary, capture questions may be revised or followed up with questions specific to areas with active transmission of ZIKV.

---

[4] Male donors with a history of sexual contact with another male would already be deferred for 12 months from last sexual contact consistent with the recommendations contained in the FDA Guidance, "Revised Recommendations for Reducing the Risk of Human Immunodeficiency Virus Transmission by Blood and Blood Products," dated December 2015.

2. Donor Deferral

We recommend that you defer for 4 weeks[5] donors at risk for ZIKV infection as follows:

a. Defer for 4 weeks after the resolution of symptoms a donor with a history of ZIKV infection.

b. Defer for 4 weeks after the resolution of symptoms a donor who reports symptoms suggestive of ZIKV that arose within 2 weeks of departure from an area with active transmission of ZIKV.

c. Defer for 4 weeks after the last sexual contact a donor who has had sexual contact with a man who has been diagnosed with ZIKV or who traveled to or resided in an area with active transmission of ZIKV in the 3 months prior to that instance of sexual contact.[4]

d. Defer for 4 weeks from the date of his or her departure, a donor who has been a resident of or has traveled to an area with active transmission of ZIKV.

e. A deferred donor may be considered eligible after the deferral period has lapsed provided that all donor eligibility criteria are met.

B. Recommendations for Areas with Active Transmission of ZIKV

For areas with active transmission of ZIKV, we recommend the following strategies to reduce the risk of transfusion-transmitted ZIKV:

- Obtain Whole Blood and blood components for transfusion from areas of the U.S. without active transmission of ZIKV to fulfill orders, except that you may,
    - Collect and prepare platelets and plasma locally if you implement pathogen reduction technology for platelets and plasma using an FDA-approved pathogen reduction device as specified in the Instructions for Use of the device, or
    - Collect blood components locally and test blood donations with an FDA-licensed blood donor screening test for ZIKV, when available.[6]

Note: Use of an investigational donor screening test under an investigational new drug (IND) application or investigational pathogen reduction under an investigational device

[5] The deferral for 4 weeks provides a margin of safety in excess of the known incubation period of 3 to 12 days plus 14 days of viremia post symptom onset.
[6] Note: An FDA-licensed blood donor screening test for ZIKV is not currently available.

exemption (IDE) may be permitted in situations where approved technologies are unavailable.

Specifically, we recommend the following:

1. **Whole Blood and Red Blood Cells (RBCs)**

   - Obtain Whole Blood and blood components for transfusion from areas of the U.S. without active transmission of ZIKV to fulfill orders, except that you may,

     - Collect blood components locally and test blood donations with an FDA-licensed blood donor screening test for ZIKV, when available.[6]

2. **Platelets and Plasma**

   - Obtain Whole Blood and blood components for transfusion from areas of the U.S. without active transmission of ZIKV to fulfill orders, except that you may,

     - Collect and prepare platelets and plasma locally if you implement pathogen reduction technology for platelets and plasma using an FDA-approved pathogen reduction device as specified in the Instructions for Use of the device, or

     - Collect blood components locally and test blood donations with an FDA-licensed blood donor screening test for ZIKV, when available.[6]

   NOTE: Pathogen reduction may only be applied to products as specified in the Instructions for Use of the relevant device. At this time, PRT has only been approved by FDA for the treatment of plasma and certain apheresis platelets.

3. **Donor Educational Material, Donor History Questionnaire and Donor Deferral**

If you continue to collect blood components locally because you have implemented FDA-approved pathogen reduction technology or an FDA-licensed blood donor screening test (when available),[6] we recommend the following:

a. Donor Educational Material

i. We recommend that you update your donor educational material to include the signs and symptoms of ZIKV infection. Relevant information on the signs and symptoms of ZIKV infection can be found on CDC's website at: http://www.cdc.gov/zika/symptoms/index.html.

ii. The educational materials should instruct donors as follows:

a. A donor that exhibits signs and symptoms of ZIKV infection or has a history of ZIKV infection should self-defer for 4 weeks after the resolution of symptoms.

b. In addition to the above instructions, a donor should self-defer for 4 weeks after the last sexual contact with a man who has been diagnosed with or had symptoms suggestive of ZIKV infection in the 3 months prior to that instance of sexual contact.[4]

iii. In addition, we recommend that the donor educational material instruct donors to inform the blood collection establishment promptly if they are diagnosed with ZIKV infection or develop symptoms suggestive of ZIKV within 2 weeks following donation.

b. Donor History Questionnaire

i. We recommend that you update your donor history questionnaire, including full length and abbreviated donor history questionnaires, and accompanying materials and standard operating procedures, as necessary, to incorporate the recommendations provided in this guidance.

ii. We recommend that your donor history questionnaire assess prospective donors for:

a. A history of ZIKV infection or symptoms suggestive of ZIKV in the past 4 weeks;

b. A history of sexual contact in the past 4 weeks with a man who has been diagnosed with or had symptoms suggestive of ZIKV in 3 months prior to that instance of sexual contact.[4]

c. Donor Deferral

We recommend that you defer for 4 weeks donors at risk for ZIKV infection as follows:

i. Defer for 4 weeks after the resolution of symptoms a donor who reports a history of ZIKV infection.

ii. Defer for 4 weeks after the resolution of symptoms a donor who reports symptoms suggestive of ZIKV.

iii. Defer for 4 weeks after the last sexual contact a donor who has had sexual contact with a man diagnosed with or had symptoms suggestive of ZIKV infection in the 3 months prior to that instance of sexual contact.[4]

iv. A deferred donor may be considered eligible after the deferral period has lapsed provided that all donor eligibility criteria are met.

C. Post-Donation Information and Product Management

Except for blood components that have been pathogen-reduced, we recommend that you take the following actions if you determine that blood or blood components have been collected from a donor who should have been deferred according to the recommendations in Section III.A.2 or Section III.B.3.

1. If you collected blood or blood components from a donor who should have been deferred according to the recommendations in Section III.A.2 or Section III.B.3, or who has reported post-donation symptoms or diagnosis of ZIKV infection within 2 weeks of collection, we recommend that you quarantine and destroy any undistributed in-date blood or blood components collected from that donor.

2. If you distributed blood or blood components collected from a donor who should have been deferred according to the recommendations in Section III.A.2 or Section III.B.3, or who has reported post-donation symptoms or diagnosis of ZIKV infection within 2 weeks of collection, we recommend that you advise the transfusion service to quarantine and destroy any in-date blood or blood components collected from that donor.

3. Additionally, if blood components collected from a donor with a history of ZIKV in the past 4 weeks or from a donor who reports post-donation symptoms or ZIKV infection within 2 weeks of donation have been transfused, we recommend that you advise the transfusion service to inform the transfusion recipient's physician of record regarding the potential need for monitoring the recipient for a possible ZIKV infection.

D. Product Disposition and Labeling

1. We recommend that you destroy or re-label blood and blood components that were collected from a donor who should have been deferred according to the recommendations in Section III.A.2. or Section III.B.3. of this guidance document.

2. We recommend that you destroy or re-label blood and blood components that were collected from a donor who reports post-donation symptoms or diagnosis of ZIKV within 2 weeks of donation.

3. If you re-label such blood or blood components, they may be released for research, or for manufacture into non-injectable products or in vitro diagnostic reagents for which there is no alternative source, if labeled appropriately as described below.

4. You should use the following statements to prominently re-label the blood and blood components:

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

   and

   b. "Caution: For Laboratory Research Only"

   or

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

   You should not label these products with a U.S. license number unless FDA specifically approves such action.

IV. IMPLEMENTATION

A. Recommendations for Areas without Active Transmission of ZIKV

We recommend that you implement the recommendations in this guidance as soon as feasible, but not later than 4 weeks after the guidance issue date.

Consistent with 21 CFR 601.12, licensed establishments implementing these recommendations should update their annual reports indicating the date that the establishment revised and implemented their standard operating procedures consistent with these recommendations. These changes do not require our prior approval.

B. Recommendations for Areas with Active Transmission of ZIKV

We recommend that you implement the recommendations in this guidance as follows:

- For collections intended for intrauterine transfusion, transfusion in pregnant women, or transfusion in other at-risk recipients when requested by the prescribing physician, we recommend that you implement the recommendations in the guidance immediately, and use locally collected blood components only if blood components from an area without active transmission or pathogen-reduced blood components are unavailable and the urgent need for transfusion is judged by the prescribing physician to outweigh the risk.

- For all other collections, we recommend that you implement the recommendations in the guidance as soon as feasible, but not later than 2 weeks after the guidance issue date.

- We recommend that in-date blood components (e.g., red blood cells or frozen plasma) collected in areas with active transmission and remaining in inventory after you implement the recommendations be destroyed or re-labeled consistent with the recommendations in Section III.D. above.

Consistent with 21 CFR 601.12, licensed establishments implementing these recommendations should update their annual reports indicating the date that the establishment revised and implemented their standard operating procedures consistent with these recommendations. These changes do not require our prior approval.

V. REFERENCES

1. Grard G, Caron M, Mombo IM, Nkoghe D, Mboui Ondo S, Jiolle D et al. Zika Virus in Gabon (Central Africa) – 2007: A New Threat from *Aedes albopictus*? PLoS Negl Trop Dis 2014; 8(2): e2681. doi:10.1371/journal.pntd.0002681

2. Hayes EB. Zika virus outside Africa. Emerg Infect Dis. 2009; 15(9):1347-50. http://dx.doi.org/10.3201/eid1509.090442

3. Besnard M, Lastère S, Teissier A, Cao-Lormeau VM, Musso D. Evidence of perinatal transmission of Zika virus, French Polynesia, December 2013 and February 2014. Euro Surveill. 2014;19(13):pii=20751.

4. Foy BD, Kobylinski KC, et al. Probable non-vector-borne transmission of Zika virus, Colorado, USA. Emerg Infect Dis. 2011 May; 17(5):880-2.

5. Dallas County Health and Human Services http://www.dallascounty.org/department/hhs/press/documents/PR2-2-16DCHHSReportsFirstCaseofZikaVirusThroughSexualTransmission.pdf

6. Haddow AD, Schuh AJ, Yasuda CY, Kasper MR, Heang V, Huy R, et al. (2012) Genetic Characterization of Zika Virus Strains: Geographic Expansion of the Asian Lineage. PLoS Negl Trop Dis 6(2): e1477. doi:10.1371/journal.pntd.0001477

7. Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Johnson AJ et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. Emerg Infect Dis. 2008; 14:1232–9. DOI: 10.3201/eid1408.080287

8. Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL, Lanciotti RS et al. Zika Virus Outbreak on Yap Island, Federated States of Micronesia. N Engl J Med. 2009; 360:2536-43.

9. Musso D, Nhan T, Robin E, Roche C, Bierlaire D, Zisou K et al. Potential for Zika virus transmission through blood transfusion demonstrated during an outbreak in French Polynesia, November 2013 to February 2014. Euro Surveill. 2014 Apr 10; 19(14). pii: 20761.

10. Cao-Lormeau VM, Roche C, Teissier A, Robin E, Berry AL, Mallet HP, et al. Zika virus, French Polynesia, South Pacific, 2013 [letter]. Emerg Infect Dis. 2014 Jun http://dx.doi.org/10.3201/eid2006.140138

11. Musso D, Nhan T-X. Emergence of Zika Virus. Clin Microbiol 2015, 4:5 http://dx.doi.org/10.4172/2327-5073.1000222

12. Zanluca C, de Melo VC, Mosimann AL, Dos Santos GI, Dos Santos CN, Luz K. First report of autochthonous transmission of Zika virus in Brazil. Mem Inst Oswaldo Cruz. 2015; 110:569–72. http://dx.doi.org/10.1590/0074-02760150192

13. Pan American Health Organization (PAHO). Zika Virus Infection: Geographic Distribution. Available online: http://www.paho.org/hq/index.php?option=com_topics&view=readall&cid=8096&Itemid=41484&lang=en

14. Zika virus at CDC. Areas with Zika: Countries and territories with active Zika virus transmission http://www.cdc.gov/zika/geo/index.html

15. Nationally Notifiable Conditions 2016. http://wwwn.cdc.gov/nndss/conditions/notifiable/2016/

16. European Centre for Disease Prevention and Control (ECDC). Factsheet for health professionals. Available online: http://ecdc.europa.eu/en/healthtopics/zika_virus_infection/factsheet-health-professionals/Pages/factsheet_health_professionals.aspx

17. European Centre for Disease Prevention and Control (ECDC). Rapid risk assessment: Zika virus epidemic in the Americas: potential association with microcephaly and Guillain-Barré syndrome –10 Dec 2015. Stockholm: ECDC; 2015 http://ecdc.europa.eu/en/publications/Publications/zika-virus-americas-association-with-microcephaly-rapid-risk-assessment.pdf

18. Oehler E, Watrin L, Larre P, Leparc-Goffart I, Lastere S, Valour F et al. Zika virus infection complicated by Guillain-Barre syndrome--case report, French Polynesia, December 2013. Euro Surveill. 2014 Mar 6;19(9). pii: 20720. doi:http://dx.doi.org/10.2807/1560-7917.ES2014.19.9.20720

19. Pan American Health Organization (PAHO). 1 December 2015: Neurological syndrome, congenital malformations, and Zika virus infection. Implications for public health in the Americas – Epidemiological Alert. Available online: http://www.paho.org/hq/index.php?option=com_topics&view=readall&cid=7880&Itemid=41484&lang=en

20. European Centre for Disease Prevention and Control (ECDC). Rapid risk assessment: Zika virus epidemic in the Americas: potential association with microcephaly and Guillain-Barré syndrome –21 Jan 2016. Stockholm: ECDC; 2016 http://ecdc.europa.eu/en/publications/Publications/rapid-risk-assessment-zika-virus-first-update-jan-2016.pdf

21. Musso D, et al. Potential Sexual Transmission of Zika Virus. Emerg Infect Dis. 2015 February; 21: 359-361.

22. Outbreak News Today. http://outbreaknewstoday.com/transfusion-associated-zika-virus-reported-in-brazil-76935/

23. Aubry M, Finke J, Teissier A, Roche C, Broult J, Paulous S et al. Seroprevalence of arboviruses among blood donors in French Polynesia, 2011-2013. Int J Infect Dis. 2015 Dec; 41:11-2. doi: 10.1016/j.ijid.2015.10.005.

24. Aubry M, Richard V, Green J, Broult J, Musso D. Inactivation of Zika virus in plasma with Amotosalen and ultraviolet A illumination. Transfusion. 2016 Jan; 56(1):33-40. doi: 10.1111/trf.13271.

25. Dichtelmuller, H., et al., "Robustness of solvent/detergent treatment of plasma derivatives: a data collection from Plasma Protein Therapeutics Association member companies", Transfusion, 2009; 49:1931-1943.

26. Xie, Yi-Wu, et al., Clearance of dengue virus in the plasma-derived therapeutic proteins. Transfusion. 1008; 48:1342-1347.

27. Leydold, Sandra M., et al., Chikungunya virus and the safety of plasma products. Transfusion. 2012; 52:2122-2130.

28. FDA News Release: FDA approves pathogen reduction system to treat platelets, (2014). Available at http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm427500.htm.

29. Snyder E L, et al. The Safety of the Blood Supply — Time to Raise the Bar. N Engl J Med 2015; 372:1882-1885. DOI: 10.1056/NEJMp1500154

30. Pealer et al. Transmission of West Nile virus through blood transfusion in the United States. N Engl J Med 2003; 349(13):1236-45.

31. Chuang et al. Review of dengue fever cases in Hong Kong during 1998 to 2005. Hong Kong Med J 2008; 14(3):170-7.

32. MMWR Morb Mortal Wkly Rep Yellow Fever 2010; 59(2):34-7.

33. Gourinat AC, O'Connor O, et al. Detection of Zika Virus in Urine. Emerg Infect Dis. 2015; 21:84-86 DOI: http://dx.doi.org/10.3201/eid2101.140894.

34. Ioos S, Mallet HP, et al. Current Zika virus epidemiology and recent epidemics. Med Mal Infect. 2014 Jul; 44(7):302-7.

35. European Centre for Disease Prevention and Control (ECDC). Rapid risk assessment: Zika virus infection outbreak, Brazil and the Pacific region – 25 May 2015. Stockholm: ECDC; 2015 http://ecdc.europa.eu/en/publications/Publications/rapid-risk-assessment-Zika%20virus-south-america-Brazil-2015.pdf

別紙様式第 2-1
番号 12

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2016 年 02 月 29 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	http://www.who.int/csr/resources/publications/zika/Safe-blood_supply18Feb2016.pdf?ua=1/2016/02	公表国 スイス	
販売名（企業名）	①抗 HBs 人免疫グロブリン筋注 200 単位/1mL「JB」（日本血液製剤機構） ②抗 HBs 人免疫グロブリン筋注 1000 単位/5mL「JB」（日本血液製剤機構） ③ヘブスブリン筋注用 200 単位（日本血液製剤機構） ④ヘブスブリン筋注用 1000 単位（日本血液製剤機構） ⑤ヘブスブリン IH 静注 1000 単位（日本血液製剤機構）					

研究報告の概要

ジカウイルスのアウトブレイク期間における安全で十分な血液供給の維持について－暫定的ガイダンス，2016 年 2 月，WHO：

2013 年 11 月～2014 年 2 月のフランス領ポリネシアにおけるジカウイルスのアウトブレイク期間中，健常ドナー1505 例が核酸増幅法（NAT）ベースの試験による検査を受け，そのうち 42 例（2.8%）がジカウイルスの RNA に対し陽性の結果が得られた。最近，輸血によるジカウイルス感染の可能性例 2 例が，ブラジルのカンピナスから報告されている。ジカウイルスの伝播が継続中の国における安全で十分な血液供給の維持，およびジカウイルスの伝播がない国における輸血サービスに関する対策などが示されている。ジカウイルス伝播がない国においては、流行地からの帰国後 28 日間は一時的に供血停止にする。また過去 3 ヶ月に感染した又は可能性のある男性との性パートナーの供血停止を考慮すべきとしている。

報告企業の意見

ジカウイルス（Zika virus）は 1947 年にウガンダの Zika forest（ジカ森林）から発見されたウイルスで、デングウイルス、日本脳炎ウイルス、ウエストナイルウイルスと同じフラビウイルス科フラビウイルス属に属するエンベロープを有する RNA ウイルスで、蚊（ネッタイシマカ、ヒトスジシマカ）によって媒介される。万一、原料血漿にジカウイルスが混入したとしても、各種モデルウイルスのウイルスクリアランス試験成績から、本剤の製造工程において不活化・除去されると考えている。

今後の対応

本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

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

抗 HBs

Maintaining a safe and adequate blood supply during Zika virus outbreaks

Interim guidance

February 2016
WHO/ZIKV/HS/16.1

1. Introduction

1.1 Background

These guidelines have been developed in recognition that infection with Zika virus may present a risk to blood safety, and in consideration of the declaration on 1 February 2016 by the WHO Director-General of a Public Health Emergency of International Concern with regard to clusters of microcephaly and other neurological disorders, potentially associated with Zika virus. Currently there is limited knowledge of Zika virus biology and lack of definitive evidence of a link between infection and potential complications. These guidelines will be regularly reviewed and updated as new information becomes available.

Zika virus is a mosquito-borne flavivirus, related to dengue. It is transmitted to humans through the bite of an infected mosquito from the *Aedes* genus. This mosquito also transmits dengue, chikungunya and yellow fever viruses [23].

Zika virus infection is followed by an incubation period prior to the development of clinical symptoms, which occur in only a minority of infected individuals. Asymptomatic infections are common, as described for other flaviviral infections such as dengue and West Nile fevers. It has been reported that only one in five individuals infected with Zika virus develops symptoms [4, 13]. The symptoms of Zika virus infection are similar to those of other arboviruses such as dengue, and include fever, skin rashes, conjunctivitis, muscle and joint pain, malaise, and headache. These symptoms are usually mild and typically last for 2–7 days. The incubation period is likely to be a few days to a week [4, 24], with some publications suggesting that it may be as long as twelve days [9]. Zika virus RNA has been detected in blood, urine, and saliva during the acute phase of the disease, and in seminal fluid after acute illness; infectious virus was detected in semen more than two, and possibly up to ten weeks, after recovery from clinical symptoms of Zika virus infection, and probable cases of sexual transmission have been described [7, 8, 14, 15].

A link between Zika virus infection during pregnancy and microcephaly in neonates is suspected and currently being investigated for causal association [12, 21]. An association of Zika virus with Guillain-Barré syndrome (GBS) and other autoimmune neurological complications was suspected during a 2013–2014 outbreak in French Polynesia and remains under investigation [6, 16].

During the Zika virus outbreak in French Polynesia between November 2013 and February 2014, a total of 1,505 healthy blood donors were tested by nucleic acid amplification technology (NAT) -based assays, with 42 (2.8 %) confirmed positive for Zika virus RNA. Blood donors positive for Zika virus RNA were contacted retrospectively to investigate the occurrence of 'Zika fever-like syndrome' (rash and/or conjunctivitis and/or arthralgia) after their blood donation. Of the 42 donors that tested positive, 11 declared that they had a Zika fever-like syndrome from 3–10 days after they gave blood. No transmission of Zika virus through transfusion was documented in this study [3, 13]. However, transmission of related flaviviruses (dengue and West Nile viruses) by blood transfusion has been documented [2, 18, 22]. Recently two probable cases of Zika virus transmission by blood transfusion have been reported from Campinas, Brazil [19].

1.2 Target audience

This guidance is intended for use by national health authorities and blood transfusion services, to provide a generic basis on which guidelines applicable to their own circumstances and local context may be developed.

2. Maintaining safe and adequate blood supplies in countries with ongoing Zika virus transmission

2.1 Ensuring blood supply through reinforcing blood collection in non-affected areas

Blood supply during a Zika virus outbreak should ideally be maintained by increasing blood collections in non-affected areas. In non-affected areas, consideration may be given to defer donors who have recently visited areas with ongoing transmission of Zika virus for a period of 28 days after their departure from the area (twice the assumed maximum incubation period [9]). It is crucial that public health authorities work with the blood transfusion service (BTS) to establish mechanisms to access regular, up-to-date

epidemiological information on Zika virus transmission in the country.

Effective public awareness campaigns on the need for blood donation, and education and motivation of potential blood donors are important elements in low risk areas, along with strategies to appropriately defer donors recently exposed to Zika virus in an affected area.

2.2 Measures to reduce risk to blood supply in areas with active transmission

Blood collection may need to continue in affected areas during a Zika virus outbreak in order to ensure ongoing and timely access to sufficient blood and blood components. This may be necessary when an outbreak affects most of or the entire country, or when it is logistically impossible to source blood from non-affected regions of the country.

The following measures for reducing the risk of Zika virus transmission through transfusion may be considered in areas with active Zika virus transmission.

a. Temporary donor deferral

The following donors should be deferred for a period not less than 28 days following the full resolution of symptoms:

i. donors with confirmed recent Zika virus infection; and

ii. donors with a recent clinical history consistent with Zika virus disease, for example a combination of fever or rash with conjunctivitis, or arthralgia, or headache or malaise [17].

Sexual partners of men with confirmed or suspected Zika virus infection in the last three months should be deferred for 28 days after their last sexual contact.

Blood donors must be informed and encouraged to provide post-donation information and asked to report to BTS if they subsequently become unwell with signs and symptoms suggestive of Zika virus infection, or if diagnosed with Zika virus infection within 14 days after blood donation. Implicated blood components that have not been transfused should be recalled. Tracing of patients who have already received blood or blood components from implicated donations should be performed, and evidence for transfusion-related transmission collected.

b. Testing of blood donations

Blood donations may be tested for the presence of Zika virus by appropriate tests.

Viral RNA is the first detectable marker in Zika virus infection. NAT-based tests are therefore the most appropriate for donor screening. However, there are currently no commercially available NAT assays for Zika virus RNA detection designed to screen blood donors. Sensitive NAT tests designed for diagnostic purposes may be used for small-scale screening of blood donors after respective validation. In-house developed NAT tests may also be suitable, but should be properly validated for donor screening.

Theoretically, viral antigen is another marker potentially detectable in the viraemic period of incubation and during asymptomatic infections. However, antigen tests are generally associated with lower sensitivity when compared to NAT, and commercial Zika virus antigen tests are not yet available. Zika virus antibodies become detectable at the later stage of infection and are not estimated to be indicative for active infection. A potential problem is the cross reactivity of antibodies against related flaviviruses (e.g. dengue, yellow fever) in anti-Zika virus assays.

WHO is currently working on the provision of international reference preparations for Zika virus RNA and for Zika virus antibodies to be used for comparative evaluation of both diagnostic and screening assays.

c. Pathogen reduction of blood components

Pathogen reduction technology (PRT) may be implemented. PRT is currently available for plasma and platelets, but not for whole blood or red blood cells. Different PRTs have been shown to be effective against other flaviviruses (e.g. West Nile, dengue) [10,11,20] and, in the absence of Zika-specific information, are presumed as equally effective against Zika virus.

d. Quarantine of blood components

Blood components of appropriate shelf-life (e.g. red blood cells) may be quarantined for a period of 7–14 days, and subsequently released following confirmation from the donor that they have not experienced symptoms consistent with the acute phase of Zika virus infection during the quarantine period. Despite the majority of Zika virus infections taking an asymptomatic course, this measure could prevent at least a proportion of viraemic blood components from being transfused. As platelets are characterized by a more restricted shelf-life, a quarantine period of three days may be considered.

2.3 Selecting an appropriate risk-reduction strategy

The decision to stop donation activities in affected areas or to proceed with appropriate risk-reduction strategies should be based on epidemiology and risk assessment. In addition, a number of factors should also be taken into consideration in decision-making [1].

Donor deferral to reduce the risk of transmission of Zika virus has low sensitivity and specificity. The sensitivity of donor deferral procedures is a particular problem due to the high rate of asymptomatic infection [13].

Implementation of additional testing is expensive and is likely to be difficult for some countries. Development, validation and implementation of in-house developed RNA

tests will be challenging, particularly for countries with limited BTS laboratory infrastructure or capacity.

Pathogen reduction technology involves additional steps in processing which could lead to possible delays in release of components. Its impact will be very limited when most transfusions are whole blood or red cells. The benefit of this technology should be balanced against both cost and the overall risk of Zika virus infection in the area.

Quarantine of blood components is sometimes already in place for other pathogens, such as chikungunya. This measure could thus be easily adapted to quarantine for Zika virus infection. A quarantine period of 7–14 days for red blood cells is proposed, based on the limited scientific data currently available regarding the incubation period of Zika virus infection; however, a quarantine measure is expected to be less effective for Zika virus infection due to the relatively high proportion of asymptomatic infections.

2.4. Potential high-risk blood recipient groups

According to current evidence, Zika virus infection in pregnant women may be potentially associated to severe complications for the pregnancy and fetus. Until more is known and based on precautionary principles, risk-reduction strategies should be applied to pregnant women and other groups who may be at higher risk of severe complications following Zika virus infection.

3. Measures for blood transfusion services in countries without active Zika virus transmission

In countries without active Zika virus transmission, consideration may be given to the temporary deferral of potential donors who have recently visited areas or countries with ongoing Zika virus transmission, for a period of 28 days (twice the assumed maximum incubation period) after their departure from the affected area. A temporary deferral also should be considered for sexual partners of men previously infected or potentially exposed in the previous three months [15].

In some countries the existing donor deferral policy may already involve temporary deferral of donors who have travelled to countries with mosquito-borne pathogens associated with a transfusion-transmission risk, such as dengue virus or malaria parasites. Thus donors returning from many countries currently affected by Zika virus transmission will already be deferred by a pre-existing temporary donor deferral policy. Countries with many visitors to affected countries may need to assess the impact of deferral on blood supply availability and weight the risk-benefits of implementing this measure. Selective testing of blood donors returning from affected countries may be considered as an alternative to deferral.

BTS in all countries should monitor epidemiological information and strengthen haemovigilance to identify any potential transfusion transmission of Zika virus. It is recommended that countries with a likelihood of future Zika virus transmission (e.g. countries with *Aedes* mosquitoes) consider developing a preparedness plan to ensure maintaining safe and adequate blood supplies during a period of Zika virus transmission.

4. Guidance development

4.1 Acknowledgements

This interim guidance has been jointly developed by the WHO Departments of Service Delivery and Safety (SDS) and Essential Medicines and Health Products (EMP) Geneva and the WHO AMRO Medicines and Health Technologies, Health Systems and Services (MT/HSS).

The feedback given by experts from Agence Nationale de Sécurité du Médicament et des Produits de Santé (ANS) (France), Blood Products Laboratory, National University of Cordoba (Argentina), National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention (CDC) (USA), Centro Nazionale Sangue (Italy), European Centre for Disease Prevention and Control (Sweden), Food and Drug Administration (USA), Foundation Prosangue (Brazil), Health Canada (Canada), Health Science Authority (Singapore), Ibero-American collaborative group of transfusional medicine (G-CIAMT), Paul-Ehrlich-Institut (Germany), National Heart, Lung, and Blood Institute, NIH (USA), National Blood Programs (Argentina, Brazil, Ecuador, Mexico), National Health Institute, (INS) (Colombia), New Zealand National Blood Services (New Zealand), NHSBT Blood and Transplant (UK), Northshore University Hospital (USA). Regional Hemotherapy Center, Pediatric Hospital Dr. JP Garrahan, (Argentina), Swissmedic (Switzerland), University of Campinas (Brazil), WHO AFRO Division of Health Systems and Services, and WHO EMRO Blood and Transfusion Safety programme is highly acknowledged.

4.2 Guidance development methods

Drafts of this interim guidance were developed by WHO and circulated for feedback to external experts with recognized expertise and interest in the field, including members of the Blood Regulatory Network (BRN), Ibero-American collaborative group of transfusional medicine (G-CIAMT) and Ministries of Health/National Blood Programmes in affected countries.

There is currently limited available evidence on Zika virus biology, incubation period, viraemic period of infection, and causal link with potential severe complications. The evidence on the effectiveness of measures to ensure blood safety and supply during Zika virus outbreaks is limited and recommendations are drawn from best practice during

outbreaks of other mosquito-borne virus diseases (e.g. dengue, chikungunya and West Nile).

4.3 Declaration of interests

No conflicts of interest identified from any of the contributors.

4.4 Review date

These recommendations have been produced under emergency procedures and will remain valid until August 2016, unless revised earlier. The Department of Service Delivery and Safety at WHO headquarters in Geneva will be responsible for reviewing this guideline at that time, and updating it as appropriate to reflect the evolving knowledge base and development and availability of new technologies.

5. References

1. Asia Pacific Blood Network (APBN), APBN white paper: Dengue and the blood supply. 14 March 2011. Available at: https://apbnonline.com/images/apbn%20dengue%20white%20paper.pdf
2. Chuang VW, Hong TY, Leung YH, et al. Review of dengue transmission. Emerg. Infect. Dis. 2005; 11 : 775.
3. Bierlaire D, Beau F, Lastere S, Musso D, Broult J. Virus Zika en Polynesia francaise: hemovigilance receveur. Transfusion Clinique et Biologique 2014; 21:234–242
4. Centre for Disease Control and Prevention(CDC), Atlanta. [internet]. Zika virus. Available at: http://www.cdc.gov/zika/index.html
5. Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL, Lanciotti RS, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med. 2009 Jun 11;360(24):2536-43.
6. European Centre for Disease Prevention and Control. Rapid risk assessment: Zika virus epidemic in the Americas: potential association with microcephaly and Guillain-Barré syndrome – 10 December 2015. Stockholm: ECDC. Available at: http://ecdc.europa.eu/en/publications/Publications/zika-virus-americas-association-with-microcephaly-rapid-risk-ssessment.pdf
7. Foy BD, Kobylinski KC, Chilson Foy JL, Blitvich BJ, Travassos da Rosa A, Haddow AD. Probable non-vector-borne transmission of Zika virus, Colorado, USA. Emerg Infect Dis. 2011;17:880–2.
8. Gourinat A, O'Connor O, Calvez E, Goarant C, Dupont-Rouzeyrol M. Detection of Zika Virus in Urine, Emerg Infect Dis. 2015 Jan; 21(1): 84–86
9. Ioos S, Mallet HP, et al. Current Zika virus epidemiology and recent epidemics. Médecine et Maladies Infectieuses. 2014 Jul; 44 (7): 302-7
10. Irscha J., Lin L. Pathogen Inactivation of Platelet and Plasma Blood Components for Transfusion Using the INTERCEPT Blood System™,Transfus Med Hemother. 2011 Feb; 38(1): 19–31. doi: 10.1159/000323937
11. Marschner S., Goodrich R. Pathogen Reduction Technology Treatment of Platelets, Plasma and Whole Blood Using Riboflavin and UV Light. Transfus Med Hemother 2011;38:8–18 . (DOI:10.1159/000324160)
12. Mlakar J, Korva M, Tul N, et al. Zika virus associated with microcephaly. N Engl J Med. DOI: 10.1056/NEJMoa1600651
13. Musso D, Nhan T, Robin E, Roche C, Bierlaire D, Zizou K. Potential for Zika virus transmission through blood transfusion demonstrated during an outbreak in French Polynesia, November 2013 to February 2014. Euro Surveill. 2014;19:20771.
14. Musso D,Roche C, Tu-Xuan N, Robin E, Teissier A, Cao-Lormeau VM, Detection of Zika virus in saliva, Journal of Clinical Virology 68 (2015) 53–55
15. Musso D, Roche C, Robin E, Nhan T, Teissier A, Cao-Lormeau VM. Potential sexual transmission of Zika virus. Emerg Infect Dis 2015;21:359–61.
16. Pan American Health Organization / World Health Organization. Epidemiological Update: Neurological syndrome, congenital anomalies and Zika virus infection. 17 January, Washington, D.C.: PAHO/WHO; 2016
17. Pan American Health Organization / World Health Organization. 2016 Epidemiological Update: Zika virus infection. 16 October Washington, D.C., PAHO/WHO, 2015. http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&Itemid=270&gid=32021&lang=en
18. Pealer LN, Marfin AA, Petersen LR, et al. Transmission of West Nile virus through blood transfusion in the United States. N. Egnl. J. Med. 2003; 349(139: 1234-45
19. Reuters. Brazil reports Zika infection from blood transfusions. 4 February 2016, available at http://www.reuters.com/article/us-health-zika-brazil-blood-idUSKCN0VD22N (accessed 10 February 2016).
20. Seltsam A. and Müller T.H., UVC Irradiation for Pathogen Reduction of Platelet Concentrates and Plasma.Transfus Med Hemother 2011;38:43–54 (DOI:10.1159/000323845).
21. Schuler-Faccini L, Ribeiro EM, Feitosa IM, et al. Possible association between Zika virus infection and microcephaly - Brazil, 2015. MMWR Morb Mortal Wkly Rep 2016;65:59-62
22. Tambyah PA, Koay ES, Poon ML, et al. Dengue haemorrhagic fever transmitted by blood transfusion. N. Engl. J. Med. 2008: 259: 1526-7
23. World Health Organization. Factsheet Zika virus. Available at http://www.who.int/mediacentre/factsheets/zika/en/
24. World Health Organization Regional office for Western Pacific. Fact sheet: Zika virus. Available at: http://www.wpro.who.int/mediacentre/factsheets/fs_05182015_zika/en/

© World Health Organization 2016

All rights reserved. Publications of the World Health Organization are available on the WHO website (www.who.int) or can be purchased from WHO Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (tel.: +41 22 791 3264; fax: +41 22 791 4857; e-mail: bookorders@who.int).

Requests for permission to reproduce or translate WHO publications –whether for sale or for non-commercial distribution– should be addressed to WHO Press through the WHO website (www.who.int/about/licensing/copyright_form/en/index.html).

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

All reasonable precautions have been taken by the World Health Organization to verify the information contained in this publication. However, the published material is being distributed without warranty of any kind, either expressed or implied. The responsibility for the interpretation and use of the material lies with the reader. In no event shall the World Health Organization be liable for damages arising from its use.

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日 2015. 12. 14	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	新鮮凍結人血漿		研究報告の公表状況	Rapid risk assessment: Zika virus epidemic in the Americas: potential association with microcephaly and Guillain-Barré syndrome, 10 December 2015 - 10 December 2015 European Centre for Disease Prevention and Control (ECDC)	公表国 スウェーデン（ECDC；欧州疾病予防管理センター）	
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

○南北アメリカ大陸におけるジカウイルスのアウトブレイクと小頭症およびギラン・バレー症候群（GBS）との関連性：リスク評価。
【欧州疾病予防管理センター（ECDC）によるリスク評価】
【欧州疾病予防管理センター（ECDC）によるリスク評価】欧州連合（EU）/欧州経済地域（EEA）の公衆衛生当局はリスク低減措置を検討する必要がある。また加盟国の公衆衛生当局は、ジカ
欧州連合（EU）/欧州経済地域（EEA）の公衆衛生当局は、ジカウイルス感染地域からの旅行者によるジカウイルスの輸入症例を検出するために警戒を強化し、臨床医とトラベルヘルスクリニックにお
ウイルス感染地域からの旅行者によるジカウイルスの輸入症例を検出するために警戒を強化し、臨床医とトラベルヘルスクリニックにお
いてはアウトブレイクおよび流行地域に対する認識を高める必要がある。更に、当局は感染地域への旅行者に対し、個人的に蚊の刺
咬に対する防御手段を講じるようにアドバイスを与える必要がある。
【ジカウイルス感染と小頭症および他の合併症との関連性（調査中）】
【ジカウイルス感染と小頭症および他の合併症との関連性（調査中）】ブラジルの保健省により北東部の州における小頭症症例の異常増加が報告されている。ブラジルでは2010年
2015年の10月からは、ブラジルの保健省により北東部の州における小頭症症例の異常増加が報告されている。ブラジルでは2010年
から2014年までの期間における1年当たりの小頭症児出生数は150人から200人であったが、2015年には1,248例であった。ブラジルの
保健省は2015年11月11日に当該事象が公衆衛生緊急事態であることを宣言し、以降は妊娠期間におけるジカウイルス感染と小頭症
との関連性について調査を行っている。これと同時に、ジカウイルス感染と他の神経学的症候群（特にGBS）との間に関連性があるか
調査が進められている。フランス領ポリネシアでは、ジカウイルスのアウトブレイク期間中に、感染症状を呈していた74名の患者が神経
学的症候群または自己免疫症候群を発症し、このうちの42名はGBSの診断を受けた。ブラジルでは神経学的症状とGBSの症例が121
例報告されており、全例がジカウイルス感染様症状の病歴を有していた。

以下の措置がリスク評価の本文中に記載されている。
【供血に関連したジカウイルス感染のリスク】
フランス領ポリネシアでは供血者1,505名中42名（3%）が、供血時に無症候であるにもかかわらずジカウイルス遺伝子検査が陽性を示
し、輸血による伝播のリスクが報告されている。従って、EUの血液事業規制当局は、流行国への渡航歴を持つ者に対して一時的な供
血延期（デングで適用した14日間）を検討するかもしれない。
流行地域における血液の安全性対策は、ジカウイルス感染症と診断され症状が終息後28日間の供血延期、血小板と新鮮凍結血漿製
剤への病原体不活化及び供血後にジカ熱症状を呈した供血者からの供血後情報の強化が挙げられる。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
南北アメリカ大陸および南太平洋地域においてジカウイルスのアウトブレイクが急速に拡大し、小頭症、ギラン・バレー症候群およびその他の神経学的合併症とジカウイルスとの関連性が示唆された。欧州疾病予防管理センター（ECDC）は欧州連合（EU）地域から感染地域に渡航する旅行者に注意喚起しているという報告である。	日本赤十字社では、輸血感染症対策として献血時に海外渡航歴の有無を確認し、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。

Zika outbreaks in the Americas and the potential link to microcephaly and Guillain-Barré syndrome: Risk assessment

11 Dec 2015

Following the Zika virus outbreak evolving rapidly in the Americas and the South Pacific and the potential association of the virus to microcephaly, Guillain-Barré syndrome and other neurological complications being investigated, ECDC assesses the risk of Zika virus transmission in the EU, as well as the risk to EU travellers to the affected countries.

ECDC risk assessment

With the Zika outbreak in the South Pacific and Americas evolving, the risk of imported travel-related Zika cases in the EU is increasing, while the risk of onward local transmission in the EU by the *Aedes* mosquitoes – potential vectors of the virus, remains extremely low during the winter season, states the ECDC risk assessment.

Public health authorities in EU/EEA should consider mitigation options. The authorities in the Member States should enhance their vigilance to detect imported Zika cases in travellers from affected areas, increase the awareness of clinicians and travel health clinics about the outbreak and the endemic areas as well as advise travellers to affected areas to take personal preventive measures against mosquito bites.

Travellers to affected countries are at risk of getting infected with Zika virus through mosquito bites. Since neither treatment nor vaccines are available for Zika, prevention is based on personal protection measures against mosquito bites. These should be taken during the day, as the *Aedes* mosquitoes, vectors of Zika, bite during daytime.

Zika outbreaks in the Pacific region and the Americas

In 2013 and 2014, Zika virus outbreaks were notified in several islands of the Pacific region: French Polynesia reported an outbreak with 8 750 suspected cases, the autonomous transmission further spread to other islands in the Pacific region.

In 2015, autochthonous cases of Zika virus infection have been reported in the Pacific region (Samoa and Solomon Islands, New Caledonia, Fiji, Vanuatu) and into the Americas, with autonomous transmission in Brazil, Colombia, El Salvador, Guatemala, Mexico, Panama, Paraguay, Venezuela and Cape Verde.

Possible link of Zika virus infection to microcephaly and other complications - under investigation

Since October 2015 an unusual increase in microcephaly cases was reported by the Brazilian Ministry of Health in the north-eastern states of Brazil: 1 248 suspected cases of microcephaly in 2015, compared to 150–200 children with microcephaly born per year in Brazil between 2010 and 2014. On 11 November 2015, the Brazilian Ministry of Health declared the event a public health emergency and is since investigating the possible links between Zika virus infection in pregnancy and microcephaly.

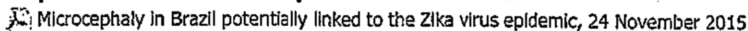
In parallel, investigations are ongoing in Brazil and French Polynesia to establish if a link exists between the Zika virus infection and other neurological syndromes, in particular Guillain–Barré syndrome. During the Zika virus outbreak in French Polynesia, 74 patients which had had Zika symptoms, later developed neurological or autoimmune syndromes - out of them, 42 were diagnosed as Guillain–Barré syndrome. In Brazil, 121 cases of neurological manifestations and Guillain–Barré syndrome (GBS) were reported, all cases with a history of Zika-like symptoms.

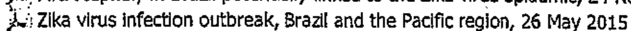
Rapid risk assessment: Zika virus epidemic in the Americas: potential association with microcephaly and Guillain-Barré syndrome, 10 December 2015 - 10 December 2015

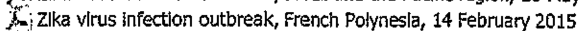
More information

Zika virus infection: Epidemiological updates
Zika: Factsheet for health professionals
Zika: Countries reporting local transmission of confirmed Zika virus infections in past nine months

Rapid Risk assessments:Rapid Risk assessments:


Microcephaly in Brazil potentially linked to the Zika virus epidemic, 24 November 2015


Zika virus infection outbreak, Brazil and the Pacific region, 26 May 2015


Zika virus infection outbreak, French Polynesia, 14 February 2015

ecdc
EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

RAPID RISK ASSESSMENT

Zika virus epidemic in the Americas: potential association with microcephaly and Guillain-Barré syndrome

10 December 2015

Main conclusions

A Zika virus outbreak in the Americas and the South Pacific is evolving rapidly, and its spread is likely to continue as the vector species *Aedes aegypti* and *Aedes albopictus* are widely distributed there. While a significant increase in the number of newborns presenting with a low head circumference seems established in the north-eastern states of Brazil, the magnitude of the increase cannot be precisely estimated. Similarly, a link with Zika virus infection cannot be confirmed until the ongoing investigations are completed.

In the light of the current disease trend – and the possible association with severe complications – public health authorities in EU/EEA Member States should consider the following mitigation options:

- Enhance vigilance towards the detection of imported cases of Zika virus infection in EU Member States, EU Overseas Countries and Territories, and EU Outermost Regions, in particular where vectors or potential vectors are present, in order to reduce the risk of autochthonous transmission.
- Strengthen laboratory capacity to confirm suspected Zika virus infections in the European region in order to differentiate Zika virus infections from other arboviral infections (e.g. dengue, chikungunya).
- Blood safety authorities should consider the deferral of donors with a relevant travel history to areas with active Zika virus transmission, in line with measures defined for dengue virus.
- Increase awareness of clinicians and travel health clinics about the evolution of the Zika virus outbreak and the endemic areas so that they can include Zika virus infection in their differential diagnosis for travellers from those areas. Fever and/or macular or papular rash not attributable to dengue or chikungunya infection among travellers returning from areas currently experiencing a Zika virus outbreak should be considered indications for further investigation of Zika virus infection.
- Advise residents and travellers visiting affected areas, particularly pregnant women, to take individual protective measures to prevent mosquito bites all day round as Zika virus disease, chikungunya and dengue are transmitted by a daytime-biting mosquito. Consequently, protective measures should be taken, especially during the day.
- Ensure that Zika virus-infected patients in areas with *Aedes* mosquitoes avoid getting bitten during the first week of illness (mosquito net, screened doors and windows as recommended by WHO/PAHO).
- Increase awareness among health professionals who provide prenatal care of the possible association of Zika virus and microcephaly and adapt prenatal monitoring in accordance with the level of exposure to the vector.

Suggested citation: European Centre for Disease Prevention and Control. Rapid risk assessment: Zika virus epidemic in the Americas: potential association with microcephaly and Guillain-Barré syndrome – 10 December 2015. Stockholm: ECDC; 2015.

© European Centre for Disease Prevention and Control, Stockholm, 2015

Source and date of request

ECDC internal decision on 3 December 2015; request from the European Commission on 4 December 2015.

Public health issue

This document assesses the risks associated with the evolving Zika virus epidemic in the Americas, and in particular the possible association between Zika virus infection and congenital microcephaly; the association between Zika virus infection and Guillain–Barré syndrome; and other severe outcomes possibly linked to the disease. We further assess the potential risks associated with Zika virus infection for travellers to affected areas.

Previous ECDC rapid risk assessments on Zika virus outbreaks:

- 'Zika virus infection outbreak, French Polynesia', 14 February 2014 [1];
- 'Zika virus infection outbreak, Brazil and the Pacific region', 25 May 2015 [2];
- 'Microcephaly in Brazil potentially linked to the Zika virus epidemic', 24 November 2015 [3].

Detailed information on the epidemiology of the Zika can be found in an ECDC factsheet for health professionals [4].

Consulted experts

ECDC internal response team in alphabetical order: Kaja Kaasik Aaslav, Sergio Brusin, Denis Coulombier, Niklas Danielsson, Dragoslav Domanovic, Romit Jain, Thomas Mollet, Joana Revez, Bertrand Sudre, Wim Van Bortel and Hervé Zeller.

Consulted external experts and acknowledgements. The following experts contributed to this risk assessment:

- WHO Regional Office for Europe, WHO Regional Office for America/Pan American Health Organization and World Health Organization Regional Office for the Western Pacific
- Fernando Bozza, MD, PhD, National Institute of Infectious Disease, Oswaldo Cruz Foundation, ministry of health, Rio de Janeiro, Brazil
- Vanessa Field, National Travel Health Network and Centre (NaTHNaC), Public Health England.

Acknowledgements: ECDC acknowledges the valuable contributions of all experts. The experts have submitted declarations of interest which were reviewed by ECDC and found not to be in conflict with the comments and suggestions made. The opinions expressed by individual experts do not necessarily represent the opinions of their institutions.

Disease background information

Zika virus disease

Zika virus disease is a mosquito-borne viral disease caused by the Zika flavivirus [5]. There are two main lineages of Zika virus, the African lineage and the Asian lineage [6-8].

The disease symptoms are usually mild and last for 2 to 7 days. Infection may go unrecognised or be misdiagnosed as dengue, chikungunya or other viral infections giving fever and rash. Asymptomatic infections are common, as described with flaviviral infections such as dengue and West Nile fever, and only one in four people infected with Zika virus are believed to develop symptoms [9,10].

An association with Guillain–Barré syndrome (GBS) and other autoimmune neurological complications was suspected during the 2013–2014 outbreak in French Polynesia and remains under investigation [11-14].

There is some evidence that mother-to-child transmission can occur, most probably transplacental or during the delivery of a viraemic mother [15]. On 2 December, the National Institute of Women's Health, Child and Adolescent Fernandes Figueira, the Oswaldo Cruz Foundation, and the Brazilian Network of Human Milk Banks, released a statement providing guidance on Zika virus and breastfeeding. The document states that there is insufficient evidence to modify current breastfeeding practices [16].

Transmission of Zika virus via transfusion of infected blood or blood products remains a possibility. Three percent (3%) of asymptomatic blood donors (42/1 505) were found positive for Zika virus by PCR during the Zika virus outbreak in French Polynesia between November 2013 and February 2014, but there are no documented cases of infections via transfusion. The presence of a viable virus was detected in semen more than two weeks after recovery from an illness consistent with Zika virus infection [9,17]. Possible cases of sexual transmission of Zika virus have been reported [17,18].

Aedes aegypti is considered the most important vector for Zika virus transmission to humans, but other *Aedes* mosquitoes can also transmit Zika virus. *Aedes albopictus* has been identified as a potential vector of Zika virus [19,20].

More information on Zika virus disease can be found in the previous risk assessment and in the ECDC factsheet for health professionals [1-4].

Laboratory diagnosis mainly consists of detection of viral RNA genome through polymerase chain reaction (PCR), virus isolation, or the detection of specific Zika virus IgM or IgG antibodies through serological tests. More detailed information on laboratory diagnosis is provided in the Rapid Risk Assessment dated 25 May 2015 [2] as well as in the PAHO epidemiological alert of 1 December 2015 [21].

Event background information

Zika epidemic evolution

In 2013 and 2014, Zika virus outbreaks were notified in several islands of the Pacific region:

- French Polynesia reported an outbreak with 8 750 suspected cases of Zika virus infection, identified by the syndromic surveillance sentinel network of French Polynesia. There were 383 confirmed cases, and Zika virus disease may have been the cause of an estimated 32 000 patients presenting to healthcare facilities between October 2013 and April 2014 [22].
- Further spread to New Caledonia, the Cook Islands and later to Easter Island (Chile) has shown the propensity of this arbovirus to spread in the Pacific region, outside its usual geographical range in Africa and south-east Asia [23]. The virus found on Easter Island was closely related to the virus identified during the French-Polynesian outbreak, and cases were reported until June 2014 [24,25].

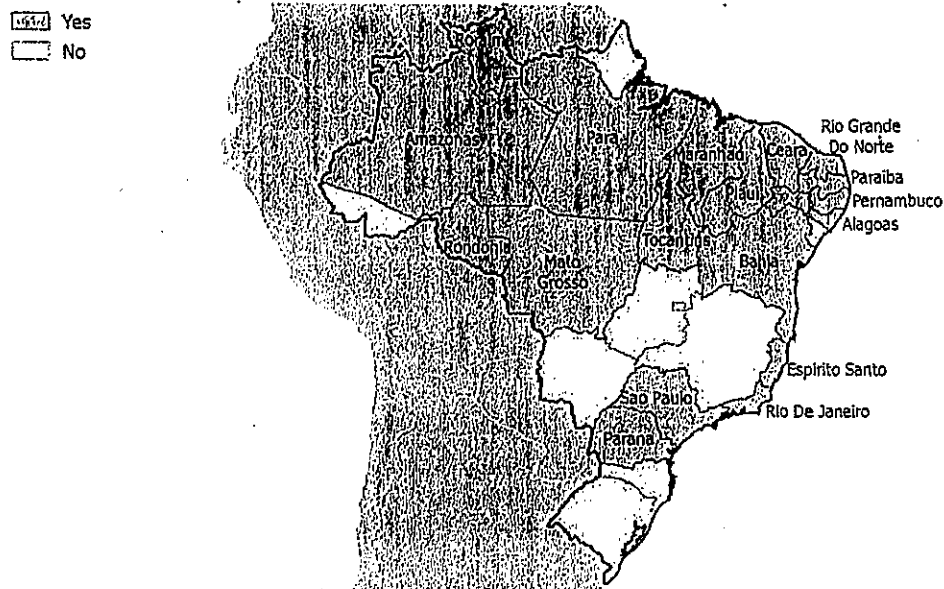
In 2015, autochthonous cases of Zika virus infection have been reported from Samoa and Solomon Islands (310 cases from February to May), New Caledonia (January to early August), Fiji (August), and at least one confirmed case in Vanuatu [26-28]. In 2015, Zika virus infections have spread to the Americas [25]:

- **Brazil**: Zika virus infections have been laboratory confirmed in 18 states in Brazil since February 2015. The following states and regions are affected: northeast (Bahia, Maranhão, Pernambuco, Rio Grande do Norte, Paraíba, Alagoas, Ceará and Piauí), north (Amazonas, Pará, Rondônia, Roraima and Tocantins), midwest (Mato Grosso), southeast (Espírito Santo, Rio de Janeiro and São Paulo) and south (Paraná), see Figure 1. The Brazilian National IHR Focal Point also reports that cases of rash illness without laboratory confirmation have been notified in the state of Sergipe. The samples were negative for dengue and chikungunya. Investigations in Sergipe by the Field Epidemiology Training Program (EpiSUS/FETP-Brazil) are ongoing. In May 2015, autochthonous transmission of Zika virus was confirmed in the states of Bahia and Rio Grande do Norte [29]. The surveillance model for Zika virus in Brazil is based on a sentinel network. Laboratory confirmation is done by RT-PCR in order to confirm autochthonous circulation as there are no standardised specific serological tests for Zika virus IgM or IgG antibodies available (cross-reaction with other flaviviruses especially with dengue fever).

  In a study conducted by the Salvador Health Authorities, twelve health districts in Salvador City – the third-largest city in Brazil – reported 14 835 cases of exanthematous illness between 15 February and 25 June 2015, with a peak incidence in May and an overall attack rate of 5.5 cases/10 000 inhabitants [30]. The authors suggest that the outbreak was caused by Zika virus because the number of confirmed dengue cases did not vary substantially during the period; only 58 cases were diagnosed as chikungunya, and confirmed Zika virus infections occurred at the same time in other cities within metropolitan Salvador [30-32]. A phylogenetic analysis of serum samples from patients hospitalised in March at Santa Helena Hospital in Camaçari, Bahia, showed that the identified Zika virus sequences belonged to the Asian lineage and were 99% identical with one partial Zika virus envelope gene region from a Zika virus isolate from French Polynesia (KJ776791) [32].

  According to preliminary estimates from the Brazilian ministry of health, between 440 000 to 1 300 000 cases of Zika virus infections may have occurred in 2015 in Brazilian states with laboratory-confirmed autochthonous cases of Zika virus [33]. As of 4 December 2015, the Brazilian ministry of health reported 9 300 suspected cases of chikungunya and approximately half a million probable cases of dengue to PAHO [34,35].

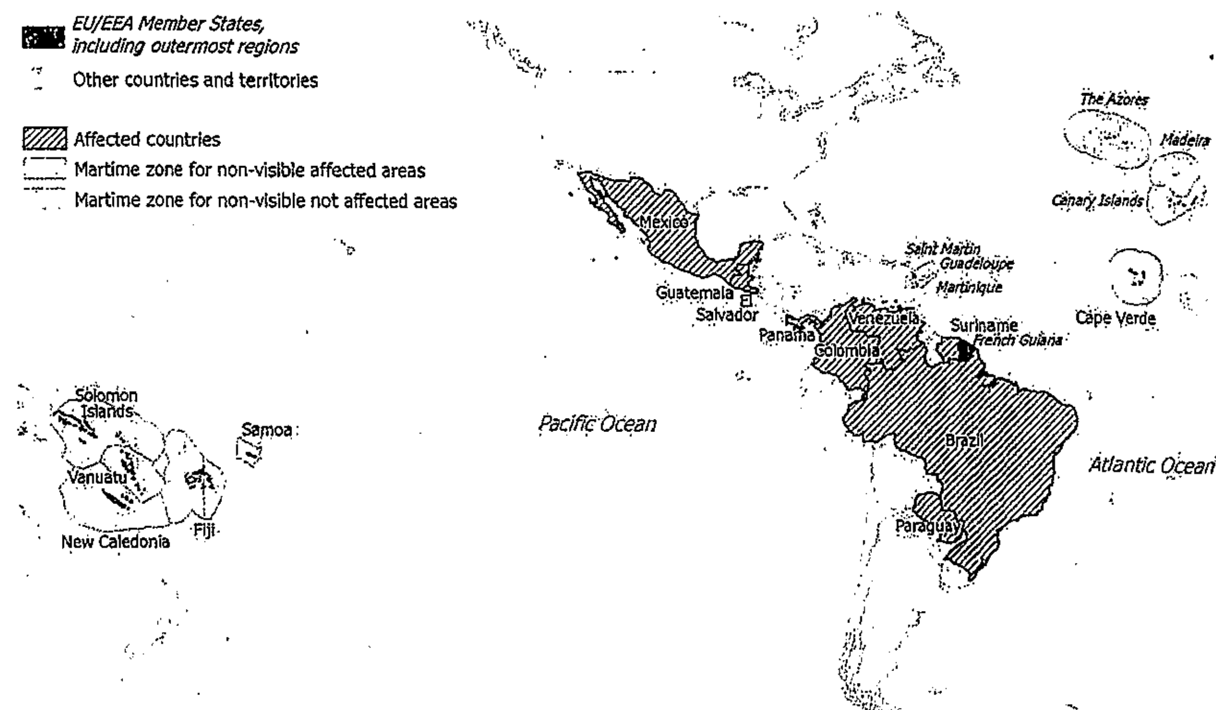
Figure 1. States with laboratory-confirmed cases Zika virus disease, Brazil, 2015, as of 23 November 2015



ECDC. Map produced on 7 Dec 2015. Administrative boundaries: ©EuroGeographics, ©UN-FAO
Data on the courtesy of MoH Brazil (VS/MS – Last Update: 23 Nov 2015)

- **Colombia:** In September, the state of Bolívar reported nine confirmed autochthonous cases of Zika virus disease. As of week 47, Colombia has reported 578 confirmed and 3 700 suspected cases, from 26 of Colombia's 36 territorial entities [36].
- **El Salvador:** On 24 November, the IHR National Focal Point of El Salvador notified three confirmed autochthonous cases of Zika virus infection. On 3 December, the media reported 240 cases across the country [37].
- **Guatemala:** On 1 December, the media, quoting authorities, reported 17 suspected cases of Zika virus infection, 14 of which were among hospital employees. Blood samples were collected and sent to the US CDC for analysis [38]. So far, one of the samples has been reported as positive.
- **Mexico:** On 26 November, the Mexican ministry of health acknowledged three Zika virus cases, including two autochthonous cases reported from Nuevo León and Chiapas. The imported case had a recent travel history in Colombia [39].
- **Panama:** On 3 December, the local health authorities reported three autochthonous cases among residents of the district of Ailigandi, in the north-eastern province of Guna Yala. [40].
- **Paraguay:** On 27 November, Paraguay reported the confirmation of six Zika virus cases in the city of Pedro Juan Caballero, which borders Brazil, after an increase in the number of notified fever cases [41].
- **Venezuela:** On 27 November, the Venezuelan IHR National Focal Point notified seven Zika virus cases (autochthonous transmission is suspected), four of which were confirmed by RT-PCR [42].
- On 3 November 2015, the **Cape Verdean** ministry of health reported that 17 out of 64 blood samples sent for confirmation to Pasteur Institute in Dakar were positive for Zika virus. According to the ministry, approximately 1 000 suspected cases with symptoms consistent with Zika virus infection were recorded as of 1 November 2015 [43].The overall distribution is reported in Figure 2 below (as of 4 December).

Figure 2. Countries with reported confirmed autochthonous cases of Zika virus infection in 2015, as of 4 December



Note: Map does not indicate the extent of the autochthonous transmission in the countries.

Update on microcephaly and central nervous system malformations increase in Brazil

In October 2015, following reports of an unusual increase of cases of microcephaly among newborns in the state of Pernambuco, an analysis of data from the Brazilian live birth information system (SINASC) identified a significant increase in the number of microcephaly cases compared with previous years [44].

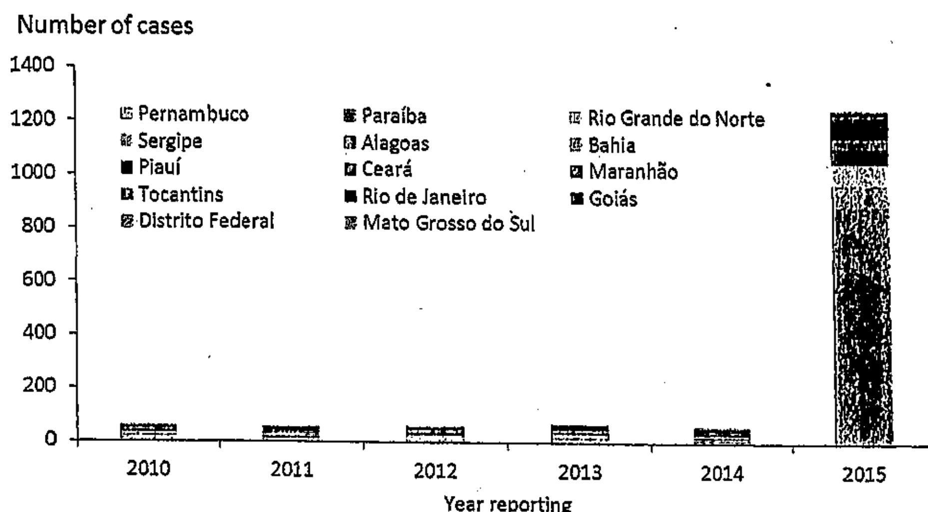
Possible links between Zika virus infection in pregnancy and microcephaly of the foetus have been under investigation since October 2015 when the Brazilian ministry of health reported an unusual increase in cases of microcephaly in the north-eastern states of Brazil [21]. On 11 November 2015, the Brazilian ministry of health declared a public health emergency in response to the dramatic increase over the expected incidence of microcephaly in Pernambuco state [45].

On average, between 150 and 200 children per year were born with microcephaly in Brazil between 2010 and 2014 (Figure 3 and Table 1). In 2015, as of 28 November, 1 248 suspected cases of microcephaly have been identified in 311 municipalities across 14 of the 26 states and one federal district of Brazil, of which 509 cases were reported between 21 and 28 November 2015 [46]. Pernambuco state has reported the highest number of cases (646), followed by the states of Paraíba (248), Rio Grande do Norte (79), Sergipe (77), Alagoas (59), Bahia (37), Piauí (36), Ceará (25), Rio de Janeiro (13), Maranhão (12), Tocantins (12), Goiás (2), Distrito Federal (1) and Mato Grosso do Sul (1) (see Figure 4) [47,48]. This is a significant increase compared to previous years (see Table 1) [46].

Brazilian health authorities have established an emergency operations centre for public health (COES, Centro de Operações de Emergências em Saúde Pública) and deployed rapid response teams to the affected states to support the investigations, provide guidance on the notification and surveillance processes, establish prenatal monitoring, and issue recommendations on prevention and control measures. The ministry of health of Brazil emphasises the importance of recommendations for pregnant women to avoid the consumption of alcohol, drugs, medications without prescription, and contact with people presenting with fever or infection. In addition, specific recommendations were issued relating to protection from mosquito bites, such as keeping doors and windows closed or screened, wearing trousers and long-sleeved shirts, and using repellents authorised during pregnancy. Clinical, laboratory and ultrasound analyses of pregnant women, mothers and newborns are being carried out.

PAHO/WHO published an epidemiological alert on the increase of microcephaly in the northeast of Brazil asking Member States to report similar events though the channels established under the International Health Regulations (IHR) [49]. On 1 December, PAHO/WHO issued an updated alert on neurological syndromes, congenital malformations, and Zika virus infection [49].

Figure 3. Notified cases of microcephaly in Brazil from 2010 to 2015, with 14 states under investigation, as of 28 November 2015



Baseline of notification of microcephaly for Brazil: 2010 (n=153), 2011 (n=139), 2012 (n=175), 2013 (n=167) and 2014 (n=147).

Adapted from [48]

Table 1. Summary of number of microcephaly cases per 1 000 live births reported annually in the fourteen Brazilian states that investigate microcephaly; 2010–2014; 2015 data as of 28 November

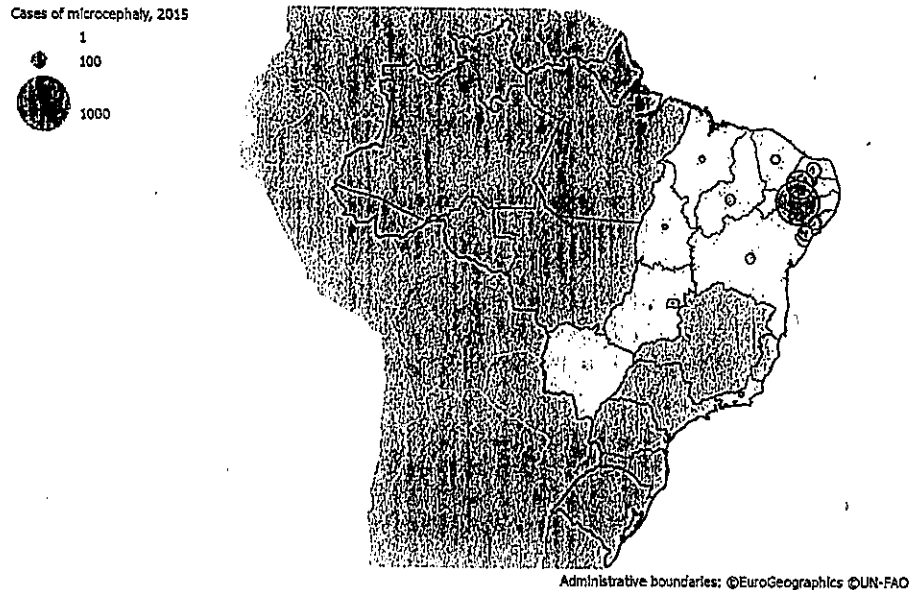
State	2009–2013	2010–2014		2015 as of 28 November		
	Yearly average number of live births [illegible]	Yearly average number of microcephaly cases [illegible]	Number of microcephaly per 1 000 LB [illegible]	Number of microcephaly cases [illegible]	Number of microcephaly cases per 1 000 LB [illegible]	Ratio between 2015/2010–2014 [illegible]
Pernambuco	140 264	8.6	0.06	646	4.61	77
Paraíba	47 998	4.2	0.09	248	5.17	57
Rio Grande do Norte	47 698	1.8	0.04	79	1.66	42
Sergipe	34 477	1.6	0.05	77	2.23	45
Alagoas	44 331	3.4	0.08	59	1.33	17
Bahia	211 660	10.6	0.05	37	0.17	3
Piauí	48 989	3	0.06	36	0.73	12
Ceará	128 112	6.6	0.05	25	0.2	4
Rio de Janeiro	219 876	12.4	0.06	13	0.06	1
Tocantins	24 586	1.2	0.05	12	0.49	10
Maranhão	119 069	3	0.03	12	0.1	3
Goiás	90 559	3	0.03	2	0.02	0.7
Distrito Federal	43 935	2.6	0.06	1	0.02	0.3
Mato Grosso do Sul	41 421	0.8	0.02	1	0.02	1
Total	**1 242 975**	**62.8**	**0.05**	**1248**	**1**	**20**

Adapted from [48]

** The denominator used for this calculation is the average number of live births per year (2009–2013)*

Data source: a) [50], b) [48]

Figure 4. Reported cases of microcephaly in Brazilian states under investigation in 2015, as of 28 November



Note: Data for other states are unavailable.

On 17 November, the Brazilian ministry of health reported the presence of Zika virus RNA in amniotic fluid samples collected from two pregnant women with foetal microcephaly from the state of Paraíba. This finding was confirmed by RT-PCR, at the Flavivirus laboratory of the Oswaldo Cruz Institute [51]. The two mothers had symptoms compatible with Zika virus disease at gestation weeks 18 and 19. Foetal Zika virus infection was confirmed through detection of Zika virus genome by RT-PCR in amniotic fluid from the two children born with microcephaly. Ultrasonography done at gestation week 20 revealed calcifications in the foetuses' brains, and a repeat scan at gestation week 28 confirmed the diagnosis of microcephaly. Urine and serum samples from the mothers were negative for Zika virus genome detection at gestation week 28 but amniocenteses were positive, with a viral load 10 000 times higher than what is normally found in blood from adults with acute infection and exanthema.

On 28 November 2015, the Brazilian ministry of health reported the presence of Zika virus genome in the blood and tissue samples of a baby from the state of Pará with microcephaly. The newborn presented with microcephaly and other congenital anomalies and died within five minutes of being born. The confirmation of the presence of the viral genome was provided by the Evandro Chagas Institute, the national reference laboratory for arboviruses in Belém, Pará [21].

On 24 November 2015, the health authorities of French Polynesia reported an unusual increase of at least 17 cases of central nervous system malformations in foetuses and infants during 2014–2015, coinciding with the Zika outbreaks in the French Polynesian islands. Investigations are still ongoing. Based on the temporal correlation of these cases with the Zika epidemic, the health authorities of French Polynesia hypothesise that Zika virus infection may be associated with these abnormalities if mothers are infected during the first or second trimester of pregnancy [3].

Update on Guillain–Barré syndrome

During the Zika virus outbreak in French Polynesia with 8 750 suspected cases, 74 patients presented with neurological syndromes or autoimmune syndromes following an illness with symptoms compatible with Zika virus infection in previous days. Of these, 42 were diagnosed as Guillain–Barré syndrome, 37 of which had presented with a previous viral syndrome [3,22]. Investigations are still ongoing in Brazil and French Polynesia to establish if Zika virus infection increases the risk of developing Guillain–Barré syndrome.

In Brazil, 121 cases of neurological manifestations and Guillain–Barré syndrome (GBS) were notified. All 121 cases had a history of rash illness and had been notified in north-eastern states between January and July 2015 [52]. Investigations are ongoing to assess a possible association between Zika virus infection and neurological manifestations and GBS [25].

According to the PAHO/WHO alert on 1 December 2015, 76 patients with neurological syndrome had been identified up to 13 July, the majority in the state of Bahia: 42 cases were classified as GBS, five were diagnosed as other neurological conditions, and 29 were either discarded or remain under investigation. Among patients with GBS, 62% (26/42) had symptoms consistent with a Zika virus infection preceding the onset of the neurological symptoms [21].

According to news reports quoting the Brazilian ministry of health on 1 December, 28 cases of GBS reported in Sergipe State were potentially linked to dengue or Zika infection [53]. According to media reports, seven cases of Guillain–Barré syndrome (GBS) reported from Pernambuco State last week were linked to Zika virus infection [54].

Fatalities potentially linked to Zika virus infection

On 30 November 2015, the COES (Centro de Operações de Emergências em Saúde Pública sobre microcefalias) reported three deaths attributed to Zika virus infection:

- A newborn with congenital anomalies (microcephaly, fetal anasarca and polyhydramnios) who died within the first five minutes of life was tested positive for Zika virus genome in Ceara State the 18 of November. Analysed samples were blood and tissue samples.
- An adult male with co-morbidities and immunosuppressive treatment. Zika virus RNA was identified in blood, brain, liver, spleen and viscera pool (kidney, lung and heart). RT-PCR for dengue genome detection was negative. Results of the other tests performed are not reported (chikungunya, West Nile fever, Saint Louis encephalitis, and yellow fever).
- A 16-year-old female from the city of Benevides (state of Pará) with onset of symptoms on 29 September 2015 (headache, nausea and petechiae) and notification on 6 October 2015. The patient was initially thought to have dengue fever, however further laboratory testing confirmed Zika virus infection by RT-PCR on a blood sample collected seven days after onset of symptoms. Further laboratory tests are ongoing (dengue fever and chikungunya infection).

Seven deaths were reported with a potential link to Zika virus infection and are now being investigated by the Brazilian ministry of health in Rio Grande do Norte (n=5), Ceará (n=1) and Piauí (n=1) [47].

ECDC threat assessment for the EU

Risk of Zika virus importation and transmission in the continental EU

Few travel-associated cases of Zika virus infections have been reported in the EU. Infections followed exposure in Asia or in French Polynesia [55-58]. With the spread of the Zika virus epidemic in the Americas, the likelihood of travel-related cases of Zika virus infection in the EU is increasing.

The *Aedes albopictus* mosquito species is established in many parts of the EU, primarily around the Mediterranean [59]. Onward transmission from imported cases within the continental EU is possible because *Aedes albopictus* is probably a competent vector for the transmission of Zika virus, even though this has not been confirmed for European mosquito populations [19,20]. The risk for transmission of Zika virus infections is extremely low in the EU during winter season as the climatic conditions are not suitable for the activity of potential vectors.

Risk of Zika virus importation and transmission for EU Overseas Countries and Territories and Outermost Regions

The probability of introduction of the virus from Zika virus-affected countries to EU Overseas Countries and Territories and EU Outermost Regions, especially in South America and in the Caribbean, has increased since the rapid risk assessment published on 25 May 2014 [2] as the epidemic is currently spreading in South America. Considering the presence of *Aedes aegypti* and *Aedes albopictus* – two competent vector species in these Overseas Countries, Territories and Outermost Regions – the establishment of local transmission is possible once the virus is introduced. This risk also includes Madeira because of its close relationship and intense trade and travel with Brazil and Cape Verde, where Zika virus is currently circulating, and the presence of competent vectors (*Aedes aegypti*).

Risk of Zika virus infection for travellers to affected regions

Travellers to countries where Zika virus is circulating are at risk of developing the disease through mosquito bites.

As neither treatment nor vaccines are available, prevention is based on personal protection measures similar to the ones against dengue and chikungunya. *Aedes* mosquitoes bite during the day as well as in the late afternoon and early evening.

Risk of Zika virus infection associated with blood donations

According to Musso et al., 42 of 1 505 (3%) blood donors in French Polynesia, although asymptomatic at the time of blood donation, were found positive for Zika virus genome by PCR, supporting a potential risk of transfusion-derived transmission [9]. Transfusion-transmitted Zika virus infection has not been reported. However, transmission is possible through blood donated by viraemic, symptomatic residents or travellers returning from affected areas [9,60,61]. Therefore, EU blood authorities may consider a temporary deferral from blood donation of persons with a travel history to affected areas (14 days as used for dengue).

Blood safety strategy in affected areas entails the deferral of donors with a diagnosis of Zika infection for 28 days from cessation of disease symptoms, pathogen inactivation of platelets and fresh frozen plasma, and enhanced post-donation reporting from donors who develop symptoms compatible with Zika fever [62].

In areas endemic for *Aedes* species, a preparedness plan to respond to future outbreaks of Zika virus infection should consider to include emergency plans to sustain blood supply.

Potential association between Zika virus infection and severe outcomes

Epidemiological data available as of November 2015 indicate, on average, a twenty-fold increase (range 0–77) in the incidence of microcephaly among newborns in the fourteen Brazilian states with Zika virus circulation that investigate microcephaly.

Initial reports of increased numbers of newborns with a low head circumference detected in the Brazilian live birth information system were a strong alert signal.

The case definition of microcephaly used so far stated a head circumference at birth of 33 centimetres or below (newborns after 37–43 weeks of gestation). However, the use of a single head circumference cut-off point for male and female newborns is not a very effective tool to assess the extent of microcephaly and establish a possible link with central nervous malformations in newborns. Investigations and follow-ups of newborns with a low head circumference will allow a more precise quantification of the malformations.

According to a protocol published on 8 December 2015, the Brazilian authorities are implementing a new case definition for microcephaly based on a head circumference for newborns of 32 centimetres or below. The new protocol will result in a decrease in the number of newborns suspected of microcephaly. Previously reported cases of microcephaly with head circumferences of up to 33 centimetres will remain under surveillance and may be reclassified [33].

Zika virus genome has been detected in the blood and tissue samples of a baby from the state of Pará. The newborn presented with microcephaly and other congenital anomalies and died. In addition, foetal Zika virus infection has been confirmed through detection of Zika virus genome by RT-PCR in the amniotic fluid of two children born with microcephaly. These observations support the conclusion of the rapid risk assessment of 24 November that a causal association between microcephaly in newborns and Zika virus infection during pregnancy is plausible [3]. However, more evidence is needed to confirm this association.

On 1 December, the PAHO/WHO issued an epidemiological alert noting that according to the preliminary analysis of the investigation conducted by the Brazil health authorities, the risk of microcephaly or congenital anomalies in newborns that is possibly associated with Zika virus infection is likely to be greatest in the first trimester of pregnancy.

In addition, investigations are still ongoing regarding a possible association between Zika virus infection and Guillain–Barré syndrome (GBS) in Brazil and French Polynesia. As of 1 December 2015, three additional fatal cases of Zika virus disease have been reported by Brazil.

In conclusion, there is limited but increasing knowledge about Zika virus infection in humans [63,64]. The disease symptoms are usually mild and last for 2 to 7 days. However, important uncertainties remain about disease complications, genetic susceptibility and levels of risk for pregnant women, newborns or patients presenting with specific co-morbidities. The spread of Zika virus infections to South, Central and North America constitutes a significant development in the epidemiology of this emerging vector-borne disease.

Conclusions and options for mitigation

The Zika virus outbreak in the Americas and the South Pacific is evolving rapidly, and its spread is likely to continue as the vector species *Aedes aegypti* and *Aedes albopictus* are widely distributed there. While a significant increase in the number of newborns presenting with a low head circumference seems established in the north-eastern states of Brazil, the magnitude of the increase cannot be precisely estimated. Similarly, a link with Zika virus infection cannot be confirmed until the ongoing investigations are completed.

In the light of the current disease trend – and the possible association with severe complications – public health authorities in EU/EEA Member States should consider the following mitigation options:

- Enhance vigilance towards the detection of imported cases of Zika virus infection in EU Member States, EU Overseas Countries and Territories, and EU Outermost Regions, in particular where vectors or potential vectors are present, in order to reduce the risk of autochthonous transmission.
- Strengthen laboratory capacity to confirm suspected Zika virus infections in the European region in order to differentiate Zika virus infections from other arboviral infections (e.g. dengue, chikungunya).
- Blood safety authorities should consider the deferral of donors with a relevant travel history to areas with active Zika virus transmission, in line with measures defined for dengue virus.
- Increase awareness of clinicians and travel health clinics about the evolution of the Zika virus outbreak and the endemic areas so that they can include Zika virus infection in their differential diagnosis for travellers from those areas. Fever and/or macular or papular rash not attributable to dengue or chikungunya infection among travellers returning from areas currently experiencing a Zika virus outbreak should be considered indications for further investigation of Zika virus infection.
- Advise residents and travellers visiting affected areas, particularly pregnant women, to take individual protective measures to prevent mosquito bites all day round as Zika virus disease, chikungunya and dengue are transmitted by a daytime-biting mosquito. Consequently, protective measures should be taken, especially during the day.
- Ensure that Zika virus-infected patients in areas with *Aedes* mosquitoes avoid getting bitten during the first week of illness (mosquito net, screened doors and windows as recommended by WHO/PAHO).
- Increase awareness among health professionals who provide prenatal care of the possible association of Zika virus and microcephaly and adapt prenatal monitoring in accordance with the level of exposure to the vector.

Information for travellers to areas with circulation of Zika virus disease

- Travellers visiting countries where Zika virus is circulating should be made aware of the ongoing outbreak of Zika virus infection.
- Travellers visiting these countries should use personal preventive measures based on protection against mosquito bites. As *Aedes* mosquitoes bite during the day, both indoors and outdoors, personal protection measures should be applied all day long, especially during the hours of highest mosquito activity (mid-morning, late afternoon to dusk).
- Personal protection measures to avoid mosquito bites should include the following:
  - Using mosquito repellents in accordance with the instructions indicated on the product label. DEET*-based repellent use is not recommended in children under three months of age but can be used in concentrations up to 50% in pregnant women.
  - Wearing long-sleeved shirts and long pants, especially during the hours of highest mosquito activity.
  - Using insecticide-treated mosquito nets is essential if accommodations are not adequately screened or air conditioned.
- Travellers that are pregnant, have immune disorders or severe chronic illnesses, or are accompanied by young children should consult their doctor or seek advice from a travel clinic before travelling in order to receive recommendations on the use of repellents and other preventive measures.
- Travellers showing symptoms compatible with dengue, chikungunya or Zika virus disease within three weeks after returning from an affected area should contact their healthcare provider.
- Pregnant women who have travelled to areas with Zika virus transmission should mention their travel during antenatal visits in order to be assessed and monitored appropriately.

---

* DEET: N,N-Diethyl-meta-toluamide or diethyltoluamide, a common active ingredient in insect repellents.

References

1. European Centre for Disease Prevention and Control. Rapid risk assessment: Zika virus infection outbreak, French Polynesia [Internet]. ECDC; 2014 [updated 2014 Feb 14; cited 2014 Feb 14]. Available from: http://ecdc.europa.eu/en/publications/_layouts/forms/Publication_DispForm.aspx?List=4f55ad51-4aed-4d32-b960-af70113dbb90&ID=1025.
2. European Centre for Disease Prevention and Control. Zika virus infection outbreak, Brazil and the Pacific region [Internet]. ECDC; 2014 [updated 2015 May 26; cited 2015 Nov 20]. Available from: http://ecdc.europa.eu/en/publications/Publications/rapid-risk-assessment-Zika%20virus-south-america-Brazil-2015.pdf.
3. European Centre for Disease Prevention and Control. Microcephaly in Brazil potentially linked to the Zika virus epidemic [Internet]. Stockholm: ECDC; 2015 [cited 2015 Nov 25]. Available from: http://ecdc.europa.eu/en/publications/_layouts/forms/Publication_DispForm.aspx?List=4f55ad51-4aed-4d32-b960-af70113dbb90&ID=1407.
4. European Centre for Disease Prevention and Control. Zika virus infection (factsheet for health professionals) [Internet]. Stockholm: ECDC; 2015 [cited 2015 18 May 2015]. Available from: http://ecdc.europa.eu/en/healthtopics/zika_virus_infection/factsheet-health-professionals/Pages/factsheet_health_professionals.aspx.
5. Dick GW, Kitchen SF, Haddow AJ. Zika virus. I. Isolations and serological specificity. Trans R Soc Trop Med Hyg. 1952 Sep;46(5):509-20.
6. Kuno G, Chang GJ, Tsuchiya KR, Karabatsos N, Cropp CB. Phylogeny of the genus Flavivirus. J Virol. 1998 Jan;72(1):73-83.
7. Faye O, Freire CC, Iamarino A, Faye O, de Oliveira JV, Diallo M, et al. Molecular Evolution of Zika Virus during Its Emergence in the 20(th) Century. PLoS Negl Trop Dis. 2014;8(1):e2636.
8. Haddow AD, Schuh AJ, Yasuda CY, Kasper MR, Heang V, Huy R, et al. Genetic characterization of Zika virus strains: geographic expansion of the Asian lineage. PLoS Negl Trop Dis. 2012;6(2):e1477.
9. Musso D, Nhan T, Robin E, Roche C, Bierlaire D, Zisou K, et al. Potential for Zika virus transmission through blood transfusion demonstrated during an outbreak in French Polynesia, November 2013 to February 2014. Euro Surveill. 2014;19(14).
10. Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL, Lanciotti RS, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med. 2009 Jun 11;360(24):2536-43.
11. Millon P. Epidémiologie des syndromes de Guillain–Barré en Nouvelle-Calédonie entre 2011 et 2014: influence des arboviroses. Faculte de Medecine de Grenoble: Universite Joseph Fourier; 2015.
12. Ioos S, Mallet HP, Leparc Goffart I, Gauthier V, Cardoso T, Herida M. Current Zika virus epidemiology and recent epidemics. Med Mal Infect. 2014 Jul;44(7):302-7.
13. Oehler E, Watrin L, Larre P, Leparc-Goffart I, Lastere S, Valour F, et al. Zika virus infection complicated by Guillain-Barre syndrome--case report, French Polynesia, December 2013. Euro Surveill. 2014;19(9).
14. Mons S, Ghawche F, Oehler E, Lastere S, Larre P, Mallet HP. Epidémie de syndromes de Guillain-Barre durant l'épidémie de Zika en Polynésie française. Bulletin de Veille Sanitaire Antilles. 2015;8-9:14-5.
15. Besnard M, Lastere S, Teissier A, Cao-Lormeau V, Musso D. Evidence of perinatal transmission of Zika virus, French Polynesia, December 2013 and February 2014. Euro Surveill. 2014;19(13).
16. Instituto Fernandes Figueira da Fiocruz and Centro de Referência da Rede de Banco de Leite Humano. Comunicação e informação (7 Dec 2015) [Internet]. Rio de Janeiro Rede Nacional de Bancos de Leite Humano; 2015 [cited 2015 Dec 3]. Available from: http://www.redeblh.fiocruz.br/cgi/cgilua.exe/sys/start.htm?infoid=1847&sid=368.
17. Musso D, Roche C, Robin E, Nhan T, Teissier A, Cao-Lormeau VM. Potential sexual transmission of Zika virus. Emerg Infect Dis. 2015 Feb;21(2):359-61.
18. Foy BD, Kobylinski KC, Chilson Foy JL, Blitvich BJ, Travassos da Rosa A, Haddow AD, et al. Probable non-vector-borne transmission of Zika virus, Colorado, USA. Emerg Infect Dis. 2011 May;17(5):880-2.
19. Wong PS, Li MZ, Chong CS, Ng LC, Tan CH. *Aedes* (Stegomyia) *albopictus* (Skuse): a potential vector of Zika virus in Singapore. PLoS Negl Trop Dis. 2013 Aug;7(8):e2348.
20. Grard G, Caron M, Mombo I, Nkoghe D, Mboui Ondo S, Jiolle D, et al. Zika Virus in Gabon (Central Africa) – 2007: A New Threat from *Aedes albopictus*? PLoS Negl Trop Dis. 2014.

21. Pan American Health Organization, World Health Organization. Regional Office for the Americas. Epidemiological Alert: Neurological syndrome, congenital malformations, and Zika virus infection. Implications for public health in the Americas [Internet]. Washington: World Health Organization; 2015 [updated 2015 Dec 1; cited 2015 Dec 1]. Available from: http://www.paho.org/hq/index.php?option=com_docman&task=doc_download&Itemid=&gid=32405&lang=en.
22. Mallet HP, Vial AL, Musso D. BISES: Bulletin d'information sanitaires, épidémiologiques et statistiques [Internet]. Papeete: Bureau de veille sanitaire (BVS) Polynésie française,; 2015 [cited 13 Mai]. Available from: http://www.hygiene-publique.gov.pf/IMG/pdf/no13_-_mai_2015_-_zika.pdf.
23. Roth A, Mercier A, Lepers C, Hoy D, Duituturaga S, Benyon E, et al. Concurrent outbreaks of dengue, chikungunya and Zika virus infections - an unprecedented epidemic wave of mosquito-borne viruses in the Pacific 2012-2014. Euro Surveill. 2014;19(41).
24. Tognarelli J, Ulloa S, Villagra E, Lagos J, Aguayo C, Fasce R, et al. A report on the outbreak of Zika virus on Easter Island, South Pacific, 2014. Arch Virol. 2015 Nov 26.
25. Pan American Health Organization -World Health Organization - Regional Office for the Americas. Epidemiological Alert: Zika virus infection: 7 May 2015 [Internet]. Washington: PAHO; 2015. Available from: http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&Itemid=270&gid=30075&lang=en.
26. WHO WPR, World Health Organization. Regional Office for the Western Pacific Region. Pacific syndromic surveillance report (Week 33, ending 16 August, 2015) [Internet]. Manila, 2015 [cited 2015 Aug 16]. Available from: http://www.wpro.who.int/southpacific/programmes/communicable_diseases/disease_surveillance_response/PSS-16-August-2015/en/.
27. WHO WPR, World Health Organization. Regional Office for the Western Pacific Region. Pacific syndromic surveillance report (Week 17, ending 26 April, 2015) [Internet]. Manila, 2015 [cited 2015 Apr 26]. Available from: http://www.wpro.who.int/southpacific/programmes/communicable_diseases/disease_surveillance_response/PSS-26-April-2015/en/.
28. Auckland Regional Public Health Service. Dengue Fever, Zika and Chikungunya (Situation Update: October 2015) [Internet]. Auckland2015 [updated 2015 Nov 27; cited 2015 Nov 27]. Available from: http://www.arphs.govt.nz/health-information/communicable-disease/dengue-fever-zika-chikungunya#.VlHRNMuFPDc
29. Ministério da Saúde (Brazil). Confirmação do Zika Vírus no Brasil, [Internet]. Brasília: Ministério da Saúde (Brazil); 2015 [updated 2015 May 14; cited 2015 May 14]. Available from: http://portalsaude.saude.gov.br/index.php/cidadao/principal/agencia-saude/17701-confirmacao-do-zika-virus-no-brasil.
30. Cristiane WC, Igor ADP, Mariana K, Moreno SR, Monaise MOS, Gubio SC, et al. Outbreak of Exanthematous Illness Associated with Zika, Chikungunya, and Dengue Viruses, Salvador, Brazil. Emerging Infectious Disease journal. 2015;21(12):2274.
31. Zammarchi L, Tappe D, Fortuna C, Remoli ME, Gunther S, Venturi G, et al. Zika virus infection in a traveller returning to Europe from Brazil, March 2015. Euro Surveill. 2015;20(23).
32. Gubio SC, Antonio CB, Silvia IS. Zika Virus Outbreak, Bahia, Brazil. Emerging Infectious Disease journal. 2015;21(10):1885.
33. Blog da Saúde (Ministério da Saúde ; Brazil). Ministério da Saúde publica Protocolo de Vigilância sobre microcefalia e vírus Zika [Internet]. Brasilia2015 [cited 2015 Dec 8]. Available from: http://www.blog.saude.gov.br/agenda-ms/50437-ministerio-da-saude-publica-protocolo-de-vigilancia-sobre-microcefalia-e-virus-zika.
34. Pan American Health Organization, World Health Organization. Regional Office for the Americas. Number of Reported Cases of Chikungunya Fever in the Americas by Country or Territory, 2015 [Internet]. 2015 [cited 2015 Dec 7]. Available from: http://www.paho.org/hq/index.php?option=com_docman&task=doc_download&Itemid=&gid=32483&lang=en.
35. Pan American Health Organization, World Health Organization. Regional Office for the Americas. Number of Reported Cases of Dengue and Severe Dengue (SD) in the Americas by Country or Territory, 2015 [Internet]. 2015 [cited 2015 Dec 7]. Available from: http://www.paho.org/hq/index.php?option=com_docman&task=doc_download&Itemid=&gid=32449&lang=en.
36. Instituto Nacional de Salud. Boletín Epidemiológico Semanal. Semena 47. [Internet]. 2015 [cited 2015 Dec 7]. Available from: http://www.ins.gov.co/boletin-epidemiologico/Paginas/default.aspx.
37. World Health Organization. Disease Outbreak News: Zika virus infection – El Salvador [Internet]. WHO; 2105 [cited 2015 Nov 27]. Available from: http://www.who.int/csr/don/27-november-2015-zika-el-salvador/en/.

38. Gómez V. Autoridades sospechan de otros posibles casos del virus del zika. Prensa Libre [Internet]. 2015. Available from: http://www.prensalibre.com/guatemala/zacapa/sospechan-de.
39. World Health Organization. Disease Outbreak News: Zika virus infection – Mexico [Internet]. 2015 [cited 2015 Dec 3]. Available from: http://www.who.int/csr/don/03-december-2015-zika-mexico/en/.
40. Ministry of Health of the Republic of Panama. Ministry of Health informed the Panamanian population on the Zika virus [Internet]. Ancon2015 [cited 2015 Dec]. 3]. Available from: http://www.minsa.gob.pa/noticia/ministerio-de-salud-comunica-la-poblacion-panamena-sobre-el-virus-zika.
41. Ministry of Public Health and Social Welfare Paraguay. Introducción de virus Zika (ZIKAV) en el Paraguay [Internet]. 2015 [cited 2015 Nov 30]. Available from: http://www.mspbs.gov.py/v3/wp-content/uploads/2015/12/Alerta-Introduccion-de-virus-Zika.pdf.pdf.
42. World Health Organization. Disease Outbreak News: Zika virus infection – Venezuela [Internet]. 2015 [cited 2015 Dec 3]. Available from: http://www.who.int/csr/don/03-december-2015-zika-venezuela/en/.
43. Ministério da Saúde (Capo Verde). Ministério da Saúde confirma infecção por Vírus Zika no concelho da Praia [Internet]. Santiago Island2015 [updated 2015 Nov 2; cited 2015 Nov 2]. Available from: http://www.minsaude.gov.cv/index.php/rss-noticias/912-ministerio-da-saude-confirma-infeccao-por-virus-zika-no-concelho-da-praia.
44. Secretaria de Vigilância em Saúde - Departamento de Vigilância Epidemiológica. Nota informativa Nº 01/2015 – COES Microcèfalias. Brasília: Ministério da Saúde , Brazil, 2015 2015 Nov 17. Report No.
45. Ministério da Saúde (Brazil). Ministério da Saúde investiga aumento de casos de microcefalia em Pernambuco [Internet]. 2015 [updated 2015 Nov 11; cited 2015 Nov 11]. Available from: http://portalsaude.saude.gov.br/index.php/cidadao/principal/agencia-saude/20629-ministerio-da-saude-investiga-aumento-de-casos-de-microcefalia-em-pernambuco.
46. Ministério da Saúde (Brazil). Ministério divulga boletim epidemiológico sobre microcefalia [Internet]. Brasilia2015 [cited 2015 Nov 24]. Available from: http://portalsaude.saude.gov.br/index.php/o-ministerio/principal/secretarias/svs/noticias-svs/20929-ministerio-divulga-boletim-epidemiologico-sobre-microcefalia.
47. Centro de operações de emergências em saúde pública sobre microcefalias. Monitoramento dos casos de microcefalias no brasil informe epidemiológico Nº 02/2015 – semana epidemiológica 47 (22 a 28/11/2015) [Internet]. 2015 [cited 2015 Nov 30]. Available from: http://portalsaude.saude.gov.br/images/pdf/2015/novembro/30/coes-microcefalias---informe-epidemiol--gico---se-47.pdf.
48. Ministério da Saúde (Brazil). Ministério da Saúde divulga novos dados de microcefalia [Internet]. 2015 [cited 2015 Nov 30]. Available from: http://portalsaude.saude.gov.br/index.php/o-ministerio/principal/secretarias/svs/noticias-svs/21020-ministerio-da-saude-divulga-novos-dados-de-microcefalia.
49. Pan American Health Organization, World Health Organization. Regional Office for the Americas. Epidemiological Alert: Increase of microcephaly in the northeast of Brazil - 17 November 2015 [Internet]. 2015 [cited 2015 Nov 11]. Available from: http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&Itemid=270&gid=32285&lang=en.
50. Departemento de Informatica do SUS. Informações de Saúde (TABNET): Demográficas e Socioeconômicas [Internet]. 2015 [cited 2015 Nov 25]. Available from: http://www2.datasus.gov.br/DATASUS/index.php?area=02.
51. Ministério da Saúde (Brazil). Microcefalia - Ministério da Saúde divulga boletim epidemiológico [Internet]. Brasília: Ministério da Saúde; 2015 [updated 2015 Nov 17; cited 2015 Nov 17]. Available from: http://portalsaude.saude.gov.br/index.php/cidadao/principal/agencia-saude/20805-ministerio-da-saude-divulga-boletim-epidemiologico.
52. Ministério da Saúde (Brazil). Evento de saúde pública relacionado aos casos de Febre do Zika [Internet]. 2015 [updated 2015 Aug 13; cited 2015 Aug 13]. Available from: http://portalsaude.saude.gov.br/index.php/o-ministerio/principal/secretarias/svs/noticias-svs/19139-evento-de-saude-publica-relacionado-aos-casos-de-febre-do-zika.
53. NN. Sergipe registra 28 casos da Síndrome de Guillian-Barré. Globocom. 2015 2015 Dec 1.
54. Coutinho K. Pesquisa estuda relação do zika com síndrome que afeta sistema nervoso. Globocom. 2015 2015 Nov 25.
55. Zammarchi L, Stella G, Mantella A, Bartolozzi D, Tappe D, Gunther S, et al. Zika virus infections imported to Italy: clinical, immunological and virological findings, and public health implications. J Clin Virol. 2015 Feb;63:32-5.
56. Tappe D RJ, Gabriel M, Emmerich P, Günther S, Held G, Smola S, Schmidt-Chanasit J. First case of laboratory-confirmed Zika virus infection imported into Europe, November 2013. Eurosurveillance. 2014 30 January 2014;19(4)::pii=20685.

57. Tappe D, Nachtigall S, Kapaun A, Schnitzler P, Gunther S, Schmidt-Chanasit J. Acute zika virus infection after travel to malaysian borneo, september 2014. Emerg Infect Dis. 2015 May;21(5):911-3.

58. Waehre T, Maagard A, Tappe D, Cadar D, Schmidt-Chanasit J. Zika virus infection after travel to Tahiti, December 2013. Emerg Infect Dis. 2014 Aug;20(8):1412-4.

59. European Centre for Disease Prevention and Control. Mosquito maps. [Internet]. Stockholm: European Centre for Disease Prevention and Control; 2015 [cited 2015 Nov 20]. Available from: http://ecdc.europa.eu/en/healthtopics/vectors/vector-maps/Pages/VBORNET_maps.aspx.

60. Aubry M, Finke J, Teissier A, Roche C, Broult J, Paulous S, et al. Seroprevalence of arboviruses among blood donors in French Polynesia, 2011-2013. Int J Infect Dis. 2015 Oct 23;41:11-2.

61. Aubry M, Richard V, Green J, Broult J, Musso D. Inactivation of Zika virus in plasma with amotosalen and ultraviolet A illumination. Transfusion (Paris). 2015 Aug 18.

62. Pan American Health Organization, World Health Organization. Regional Office for the Americas. Eligibility for Blood Donation: Recommendations for Education and Selection of Prospective Blood Donors [Internet]. Washington2009 [cited 2015 Dec 8]. Available from: http://www1.paho.org/hq/dmdocuments/2009/EligiBlood09EN.pdf?ua=1.

63. Pan American Health Organization - World Health Organization - Regional Office for the Americas. Epidemiological Update: Zika virus infection [Internet]. 2015 [cited 2015 Nov 20]. Available from: http://www.paho.org/hq/index.php?option=com_topics&view=article&id=427&Itemid=41484&lang=fr.

64. World Health Organization Regional Office for the Western Pacific - World Health Organization. Zika virus [Internet]. 2015 [cited 2015 Nov 20]. Available from: http://www.wpro.who.int/mediacentre/factsheets/fs_05182015_zika/en/#.

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 2016年2月12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	MMWR/February 19, 2016/Vol.65/No.6		公表国 ブラジル	
販売名（企業名）	別紙のとおり。					

研究報告の概要	使用上の注意記載状況・その他参考事項等
問題点：ブラジルで、先天性感染の新生児2例および死亡した胎児2例からの脳および胎盤組織におけるジカウイルス感染のエビデンスが報告された。 ジカウイルス疾患の疑い例が多くみられているブラジルにおいて，2015年中旬～2016年1月，小頭症の疑い例4700例以上が報告された。これを受けて，ブラジルのMinistry of Healthは，同ウイルスと新生児における脳の異常が関連する可能性について追加の調査を実施するためにタスクフォースを設立した。 小頭症の乳児からの脳組織および早期流産例からの胎盤組織におけるウイルスRNAと抗原の検出を通して，ジカウイルスと胎児死亡および小頭症との関連がエビデンスとして報告された。	記載なし。

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

MedDRA/J ver.18.1

別紙

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤人免役グロブリン、⑥乾燥ペプシン処理人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン、⑧乾燥スルホ化人免疫グロブリン、⑨乾燥スルホ化人免疫グロブリン、⑩乾燥スルホ化人免疫グロブリン、⑪乾燥スルホ化人免疫グロブリン*、⑫乾燥濃縮人活性化プロテインC、⑬乾燥濃縮人血液凝固第Ⅷ因子、⑭乾燥濃縮人血液凝固第Ⅷ因子、⑮乾燥濃縮人血液凝固第Ⅷ因子、⑯乾燥濃縮人血液凝固第Ⅸ因子、⑰乾燥濃縮人血液凝固第Ⅸ因子、⑱乾燥濃縮人血液凝固第Ⅸ因子、⑲乾燥抗破傷風人免疫グロブリン、⑳抗 HBs 人免疫グロブリン、㉑抗 HBs 人免疫グロブリン、㉒抗 HBs 人免疫グロブリン、㉓トロンビン、㉔トロンビン、㉕トロンビン、㉖フィブリノゲン加第XⅢ因子*、㉗フィブリノゲン加第XⅢ因子、㉘乾燥濃縮人アンチトロンビンⅢ、㉙ヒスタミン加人免疫グロブリン製剤、㉚人血清アルブミン*、㉛人血清アルブミン*、㉜乾燥ペプシン処理人免役グロブリン*、㉝乾燥濃縮人アンチトロンビンⅢ、㉞乾燥濃縮人血液凝固第X因子加活性化第Ⅶ因子
販売名（企業名）	①献血アルブミン 20 "化血研"、②献血アルブミン 25 "化血研"、③人血清アルブミン "化血研" *、④ガンマーグロブリン筋注 450mg/3mL「化血研」、⑤ガンマーグロブリン筋注 1500mg/10mL「化血研」、⑥献血グロブリン注射用 2500mg「化血研」、⑦献血ベニロン－I 静注用 500mg、⑧献血ベニロン－I 静注用 1000mg、⑨献血ベニロン－I 静注用 2500mg、⑩献血ベニロン－I 静注用 5000mg、⑪ベニロン*、⑫注射用アナクトC2,500 単位、⑬コンファクトF注射用 250、⑭コンファクトF注射用 500、⑮コンファクトF注射用 1000、⑯ノバクトM静注用 400 単位、⑰ノバクトM静注用 800 単位、⑱ノバクトM静注用 1600 単位、⑲テタノセーラ筋注用 250 単位、⑳ヘパトセーラ筋注 200 単位/mL、㉑ヘパトセーラ筋注 200 単位/1mL、㉒ヘパトセーラ筋注 1000 単位/5mL、㉓トロンビン "化血研"、㉔献血トロンビン経口・外用5千「化血研」、㉕献血トロンビン経口・外用1万「化血研」、㉖ボルヒール*、㉗ボルヒール組織接着用、㉘アンスロビンP500 注射用、㉙ヒスタグロビン皮下注用、㉚アルブミン 20%化血研*、㉛アルブミン 5%化血研*、㉜静注グロブリン*、㉝アンスロビンP1500 注射用、㉞バイクロット配合静注用、㉟ノバクトM静注用 500 単位、㊱ノバクトM静注用 1000 単位、㊲ノバクトM静注用 2000 単位
報告企業の意見	ジカウイルスは、日本脳炎ウイルス、デングウイルス、ウエストナイルウイルスなどと同属のフラビウイルス属の蚊媒介性感染症ウイルスとして知られており、一本鎖 RNA 及びエンベロープを有するウイルスである。今回の報告は、ジカウイルス感染と胎児死亡および小頭症との関連を示すもので、母子感染を引き起こす可能性を示唆する。 上記製剤の製造工程には、冷エタノール分画工程、ウイルス除去膜ろ過工程、加熱工程といった原理の異なるウイルスクリアランス工程が複数導入されており、各工程のウイルスクリアランス効果は「血漿分画製剤のウイルスに対する安全性確保に関するガイドライン（医薬発第1047号、平成11年 8 月 30 日）」に基づく、モデルウイルスを用いたウイルスプロセスバリデーションにより確認されている。今回報告したジカ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。なお、念のため、上流で添加されるヘパリンナトリウムが BVDV クリアランス効果に影響がないか評価中である。また、これまでに上記製剤によるジカウイルスへの感染報告例は無い。 以上の点から、上記製剤はジカウイルスに対する安全性を確保していると考える。

*：現在製造を行っていない

INF2015－007

Notes from the Field

Evidence of Zika Virus Infection in Brain and Placental Tissues from Two Congenitally Infected Newborns and Two Fetal Losses — Brazil, 2015

Roosecelis Brasil Martines, MD, PhD[1]; Julu Bhatnagar, PhD[1]; M. Kelly Keating, DVM[1]; Luciana Silva-Flannery, PhD[1]; Atis Muehlenbachs, MD, PhD[1]; Joy Gary, DVM, PhD[1]; Cynthia Goldsmith, MS[1]; Gillian Hale, MD[1]; Jana Ritter, DVM[1]; Dominique Rollin, MD[1]; Wun-Ju Shieh, MD, PhD[1]; Kleber G. Luz, MD, PhD[2]; Ana Maria de Oliveira Ramos, MD, PhD[3]; Helaine Pompeia Freire Davi, MD, PhD[4]; Wanderson Kleber de Oliveria, MD[5]; Robert Lanciotti, PhD[6]; Amy Lambert, PhD[6]; Sherif Zaki, MD, PhD[1]

On February 10, 2016, this report was posted as an MMWR Early Release on the MMWR website (http://www.cdc.gov/mmwr).

Zika virus is a mosquito-borne flavivirus that is related to dengue virus and transmitted primarily by *Aedes aegypti* mosquitoes, with humans acting as the principal amplifying host during outbreaks. Zika virus was first reported in Brazil in May 2015 (*1*). By February 9, 2016, local transmission of infection had been reported in 26 countries or territories in the Americas.* Infection is usually asymptomatic, and, when symptoms are present, typically results in mild and self-limited illness with symptoms including fever, rash, arthralgia, and conjunctivitis. However, a surge in the number of children born with microcephaly was noted in regions of Brazil with a high prevalence of suspected Zika virus disease cases. More than 4,700 suspected cases of microcephaly were reported from mid-2015 through January 2016, although additional investigations might eventually result in a revised lower number (*2*). In response, the Brazil Ministry of Health established a task force to further investigate possible connections between the virus and brain anomalies in infants (*3*).

Since November 2015, CDC has been developing assays for Zika virus testing in formalin-fixed, paraffin-embedded (FFPE) tissue samples. In December 2015, FFPE tissues samples from two newborns (born at 36 and 38 weeks gestation) with microcephaly who died within 20 hours of birth and two miscarriages (fetal losses at 11 and 13 weeks) were submitted to CDC, from the state of Rio Grande do Norte in Brazil, for histopathologic evaluation and laboratory testing for suspected Zika virus infection. All four mothers had clinical signs of Zika virus infection, including fever and rash, during the first trimester of pregnancy, but did not have clinical signs of active infection at the time of delivery or miscarriage. The mothers were not tested for antibodies to Zika virus. Samples included brain and other autopsy tissues from the two newborns, a placenta from one of the newborns, and products of conception from the two miscarriages.

FFPE tissues were tested by Zika virus reverse transcription-polymerase chain reaction (RT-PCR) targeting the nonstructural protein 5 and envelope genes using general methods for RT-PCR (*4*), and by immunohistochemistry using a mouse polyclonal anti-Zika virus antibody, using methods previously described (*5*). Specific specimens from all four cases were positive by RT-PCR, and sequence analysis provided further evidence of Zika virus infection, revealing highest identities with Zika virus strains isolated from Brazil during 2015. In the newborns, only brain tissue was positive by RT-PCR assays. Specimens from two of the four cases were positive by immunohistochemistry: viral antigen was noted in mononuclear cells (presumed to be glial cells and neurons within the brain) of one newborn, and within the chorionic villi from one of the miscarriages. Testing for dengue virus was negative by RT-PCR in specimens from all cases.

For both newborns, significant histopathologic changes were limited to the brain, and included parenchymal calcification, microglial nodules, gliosis, and cell degeneration and necrosis. Other autopsy tissues and placenta had no significant findings. Tests for toxoplasmosis, rubella, cytomegalovirus, herpes simplex, and HIV were negative in the two mothers who experienced miscarriages. Placental tissue from one miscarriage showed heterogeneous chorionic villi with calcification, fibrosis, perivillous fibrin deposition, and patchy intervillositis and focal villitis, while tissue from the other miscarriage had sparsely sampled normal-appearing chorionic villi.

This report describes evidence of a link between Zika virus infection and microcephaly and fetal demise through detection of viral RNA and antigens in brain tissues from infants with microcephaly and placental tissues from early miscarriages. Histopathologic findings indicate the presence of Zika virus in fetal tissues. These findings also suggest brain and early gestational placental tissue might be the preferred tissues for postmortem viral diagnosis. Nonfrozen, formalin-fixed specimens or FFPE blocks are the preferred sample type for histopathologic evaluation and immunohistochemistry, and RT-PCR can be performed on either fresh frozen or formalin-fixed specimens. To better understand the pathogenesis of Zika virus infection and associated congenital anomalies and fetal death, it is necessary to evaluate autopsy and placental tissues from additional cases, and to determine the effect of gestational age during maternal illness on fetal outcomes.

* Updated information about local transmission of Zika virus is available online (http://www.cdc.gov/zika/geo/index.html).

[1]Infectious Diseases Pathology Branch, Division of High-Consequence Pathogens and Pathology, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), CDC; [2]Department of Infectious Diseases, Federal University of Rio Grande do Norte, Natal, RN, Brazil; [3]Department of Pathology, Federal University of Rio Grande do Norte, Natal, Brazil; [4]Service for Ascertaining Death in the State of Rio Grande do Norte (SVO/RN), Natal/RN, Brazil; [5]Ministry of Health, Brazil; [6]Arboviral Diseases Branch, Division of Vector-Borne Diseases, NCEZID, CDC.

Corresponding author: Roosecelis Brasil Martines, RBrasilMartines@cdc.gov, 404-639-3886.

References

1. Zanluca C, de Melo VC, Mosimann ALP, Dos Santos GI, Dos Santos CN, Luz K. First report of autochthonous transmission of Zika virus in Brazil. Mem Inst Oswaldo Cruz 2015;110:569–72. http://dx.doi.org/10.1590/0074-02760150192.
2. Victoria CG, Schuler-Faccini L, Matijasevich A, Ribeiro E, Pessoa A, Barros FC. Microcephaly in Brazil: how to interpret reported numbers? Lancet 2016. Epub February 5, 2016. http://dx.doi.org/10.1016/S0140-6736(16)00273-7.
3. Schuler-Faccini L, Ribeiro EM, Feitosa IML, et al.; Brazilian Medical Genetics Society–Zika Embryopathy Task Force. Possible association between Zika virus infection and microcephaly—Brazil, 2015. MMWR Morb Mortal Wkly Rep 2016;65:59–62. http://dx.doi.org/10.15585/mmwr.mm6503e2.
4. Bhatnagar J, Blau DM, Shieh WJ, et al. Molecular detection and typing of dengue viruses from archived tissues of fatal cases by rt-PCR and sequencing: diagnostic and epidemiologic implications. Am J Trop Med Hyg 2012;86:335–40. http://dx.doi.org/10.4269/ajtmh.2012.11-0346.
5. Shieh WJ, Blau DM, Denison AM, et al. 2009 pandemic influenza A (H1N1): pathology and pathogenesis of 100 fatal cases in the United States. Am J Pathol 2010;177:166–75. http://dx.doi.org/10.2353/ajpath.2010.100115.

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
				2016. 1. 29	該当なし	
一般的名称	人血小板濃厚液		研究報告の公表状況	Hennessey M, Fischer M, Staples JE. MMWR Morb Mortal Wkly Rep. 2016 Jan 29;65(3):55-8. doi: 10.15585/mmwr.mm6503e1.	公表国 米国	
販売名(企業名)	濃厚血小板-LR「日赤」(日本赤十字社) 照射濃厚血小板-LR「日赤」(日本赤十字社) 濃厚血小板HLA-LR「日赤」(日本赤十字社) 照射濃厚血小板HLA-LR「日赤」(日本赤十字社)					

研究報告の概要

○新たな地域へのジカウイルスの拡散 — アメリカ大陸地域、2015年5月～2016年1月の調査結果。
ジカウイルスは蚊により媒介されるフラビウイルスで、1947年にウガンダで最初に発見され、アフリカ及びアジアで孤発的に人への感染が報告されていたが、2007年にミクロネシア連邦ヤップ州にて3歳以上の人口の73%が感染するアウトブレイクが報告され、その後に東南アジアと西太平洋におけるアウトブレイクと続いた。2015年5月に、WHOはアメリカ大陸で初めてとなるブラジルにおけるジカウイルスの局地的な伝播を報告した。2016年1月中旬までに、アメリカ大陸の19地域とプエルトリコにてジカウイルスの伝播が全米保健機構に報告され、他の地域へのさらなる拡散が注視されている。アメリカ合衆国本土ではジカウイルスの局地的な伝播は確認されておらず、感染は帰国した旅行者あるいは合衆国の訪問者において報告されてきた。しかし、これらの輸入感染症例が増えてくると、媒介する蚊が存在するアメリカ合衆国本土の限られた地域では、ヒト-蚊-ヒトの拡散をもたらす結果となるかもしれない。

使用上の注意記載状況・その他参考事項等

濃厚血小板-LR「日赤」
照射濃厚血小板-LR「日赤」
濃厚血小板HLA-LR「日赤」
照射濃厚血小板HLA-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
アメリカ大陸の19地域とプエルトリコにてジカウイルスの伝播拡散が全米保健機構に報告された。	日本赤十字社では、輸血感染症対策として献血時に海外渡航歴の有無を確認し、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。

Zika Virus Spreads to New Areas — Region of the Americas, May 2015–January 2016

Morgan Hennessey, DVM[1]; Marc Fischer, MD[1]; J. Erin Staples, MD, PhD[1]

On January 22, this report was posted as an MMWR Early Release on the MMWR website (http://www.cdc.gov/mmwr).

Zika virus is a mosquito-borne flavivirus that was first identified in Uganda in 1947 (*1*). Before 2007, only sporadic human disease cases were reported from countries in Africa and Asia. In 2007, the first documented outbreak of Zika virus disease was reported in Yap State, Federated States of Micronesia; 73% of the population aged ≥3 years is estimated to have been infected (*2*). Subsequent outbreaks occurred in Southeast Asia and the Western Pacific (*3*). In May 2015, the World Health Organization reported the first local transmission of Zika virus in the Region of the Americas (Americas), with autochthonous cases identified in Brazil (*4*). In December, the Ministry of Health estimated that 440,000–1,300,000 suspected cases of Zika virus disease had occurred in Brazil in 2015 (*5*). By January 20, 2016, locally-transmitted cases had been reported to the Pan American Health Organization from Puerto Rico and 19 other countries or territories in the Americas* (Figure) (*6*). Further spread to other countries in the region is being monitored closely.

Although local transmission of Zika virus has not been documented in the continental United States, Zika virus infections have been reported in returning travelers (*7*). In light of the recent outbreaks in the Americas, the number of Zika virus disease cases among travelers visiting or returning to the United States is likely to increase. These imported cases might result in local human-to-mosquito-to-human spread of the virus in limited areas of the continental United States that have the appropriate mosquito vectors.

Zika virus is transmitted primarily by *Aedes aegypti* mosquitoes (*1,7*). *Aedes albopictus* mosquitoes also might transmit the virus. *Aedes aegypti* and *Ae. albopictus* mosquitoes are found throughout much of the Americas, including parts of the United States, and also transmit dengue and chikungunya viruses. In addition to mosquito-to-human transmission, Zika virus infections have been documented through intrauterine transmission resulting in congenital infection, intrapartum transmission from a viremic mother to her newborn, sexual transmission, blood transfusion, and laboratory exposure (*5*). There is a theoretical concern that transmission could occur through organ or tissue transplantation, and although Zika virus RNA has been detected in breast milk, transmission through breastfeeding has not been documented (*5*).

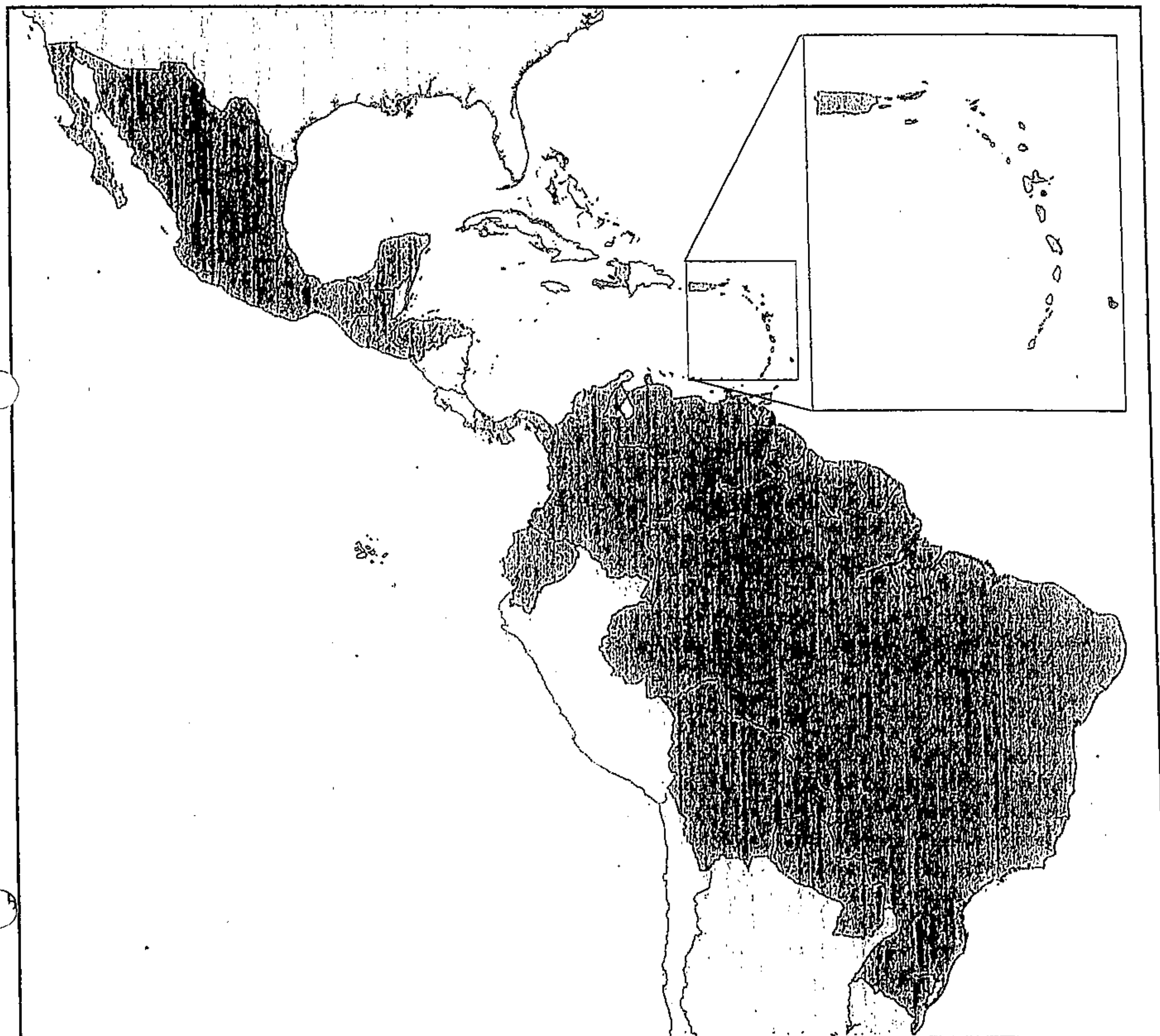
During outbreaks, humans are the primary amplifying host for Zika virus. An estimated 80% of persons who are infected with Zika virus are asymptomatic (*2*). Symptomatic disease generally is mild and characterized by acute onset of fever, maculopapular rash, arthralgia, or nonpurulent conjunctivitis. Symptoms usually last from several days to 1 week. Based on information from previous outbreaks, severe disease requiring hospitalization is uncommon, and fatalities are rare. During the current outbreak in Brazil, Zika virus RNA has been identified in tissues from several infants with microcephaly and from fetal losses in women who were infected during pregnancy (*5,7,8*). The Brazil Ministry of Health has reported a marked increase in the number of infants born with microcephaly in 2015, although it is not known how many of these cases are associated with Zika virus infection (*8*). Guillain-Barré syndrome also has been reported in patients following suspected Zika virus infection (*5*). Studies are under way to evaluate the risks for Zika virus transmission during pregnancy, the spectrum of outcomes associated with congenital infection, and the possible association between Zika virus infection and Guillain-Barré syndrome.

Zika virus infection should be considered in patients with acute onset of fever, maculopapular rash, arthralgia, or conjunctivitis, who traveled to areas with ongoing transmission in the 2 weeks preceding illness onset. Because dengue and chikungunya virus infections share a similar geographic distribution with Zika virus and symptoms of infection are similar, patients with suspected Zika virus infections also should be evaluated and managed for possible dengue or chikungunya virus infection (*9,10*). Other considerations in the differential diagnosis include malaria, rubella, measles, parvovirus, adenovirus, enterovirus, leptospirosis, rickettsia, and group A streptococcal infections.

There is no commercially available test for Zika virus. Zika virus testing is performed in the United States at CDC and four state health department laboratories, and CDC is working to expand laboratory diagnostic testing to additional states. Health care providers should contact their state or local health department to facilitate testing. To evaluate for evidence of Zika virus infection, reverse transcription–polymerase chain reaction (RT-PCR) testing should be performed

* Barbados, Bolivia, Brazil, Colombia, Ecuador, El Salvador, French Guiana, Guadeloupe, Guatemala, Guyana, Haiti, Honduras, Martinique, Mexico, Panama, Paraguay, Puerto Rico, Saint Martin, Suriname, and Venezuela.

FIGURE. Countries and territories with documented local transmission of Zika virus infection reported to the Pan American Health Organization — Region of the Americas, 2015–2016



on serum specimens collected within the first week of illness (*11*). Immunoglobulin M and neutralizing antibody testing should be performed on specimens collected ≥4 days after onset of illness; however, these serologic assays can be positive because of cross-reacting antibodies against related flaviviruses (e.g., dengue and yellow fever viruses). Virus-specific cross-neutralization testing can be used to discriminate between cross-reacting antibodies in primary flavivirus infections, although neutralizing antibodies might still yield cross-reactive results in persons who were previously infected or vaccinated against a related flavivirus (i.e., secondary flavivirus infection).

No specific antiviral treatment is available for Zika virus disease. Treatment is generally supportive and can include rest, fluids, and use of analgesics and antipyretics. Aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) should be avoided until dengue can be ruled out to reduce the risk of hemorrhage. Febrile pregnant women should be treated with acetaminophen. Persons infected with Zika, dengue, or

chikungunya virus should be protected from further mosquito exposure during the first few days of illness to reduce the risk for local transmission.

No vaccine to prevent Zika virus infection is available. The best way to prevent Zika virus infection is to avoid mosquito bites by using air conditioning or window and door screens when indoors, wearing long sleeves and pants, using permethrin-treated clothing and gear, and using insect repellents when outdoors. Most Environmental Protection Agency (EPA)–registered repellents, including N,N-diethyl-m-toluamide (DEET), can be used on children aged >2 months (*12*). When used according to the product label, EPA-registered insect repellents also are safe for pregnant and lactating women. All travelers should take steps to avoid mosquito bites to prevent Zika virus infection and other mosquito-borne diseases.

Until more is known, and out of an abundance of caution, pregnant women should consider postponing travel to any area where Zika virus transmission is ongoing.[†] Pregnant women who do travel to one of these areas should talk to their health care provider before traveling and strictly follow steps to avoid mosquito bites during travel. Pregnant women who develop a clinically compatible illness during or within 2 weeks of returning from an area with Zika virus transmission should be tested for Zika virus infection (*13*). Fetuses and infants of women infected with Zika virus during pregnancy should be evaluated for possible congenital infection.

Health care providers are encouraged to report suspected Zika virus disease cases[§] to their state or local health departments to facilitate diagnosis and mitigate the risk for local transmission in areas where *Aedes* species mosquitoes are currently active. State health departments are requested to report laboratory-confirmed cases to CDC. CDC is working with the Council of State and Territorial Epidemiologists and other partners to develop a surveillance case definition, to provide further guidance and mechanisms for evaluating and reporting cases, and to track the outcomes of pregnant women infected with Zika virus and their babies.

[†] CDC. Traveler's health notices. http://wwwnc.cdc.gov/travel/notices/.

[§] The interim case definition for suspected Zika virus disease is an illness characterized by acute onset of two or more of the following: fever, maculopapular rash, arthralgia, or nonpurulent conjunctivitis not explained by other medical conditions, in a person who resides in or has visited an area with ongoing Zika virus transmission within 2 weeks before the onset of symptoms.

[1] Division of Vector-Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, CDC.

Corresponding author: Marc Fischer, mfischer@cdc.gov, 970-221-6400.

Summary

What is already known on this topic?

Zika virus is a mosquito-borne flavivirus transmitted primarily by *Aedes aegypti* mosquitoes. Most infections are asymptomatic, and symptomatic disease generally is mild. In May 2015, the first local transmission of Zika virus in the Region of the Americas was reported in Brazil. Following the spread of Zika virus in Brazil, there has been a marked reported increase in the number of infants born with microcephaly; it is not known how many of these cases are associated with Zika virus infection.

What is added by this report?

By mid-January 2016, local Zika virus transmission had been reported to the Pan American Health Organization from 20 countries or territories in the Region of the Americas; spread to other countries in the region is likely. Although local transmission of Zika virus has not been documented in the continental United States, infections have been reported among travelers visiting or returning to the United States, and these likely will increase. Imported cases might result in local transmission in limited areas of the continental United States.

What are the implications for public health practice?

The best way to prevent Zika virus infection is to avoid mosquito bites by avoiding exposure and eliminating mosquito breeding areas. Until more is known, pregnant women should consider postponing travel to any area with ongoing Zika virus transmission. Health care providers should contact their state or local health department about testing patients with symptoms of Zika virus infection and a compatible travel history.

References

1. Hayes EB. Zika virus outside Africa. Emerg Infect Dis 2009;15:1347–50. http://dx.doi.org/10.3201/eid1509.090442.
2. Duffy MR, Chen TH, Hancock WT, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med 2009;360:2536–43. http://dx.doi.org/10.1056/NEJMoa0805715.
3. Musso D, Nilles EJ, Cao-Lormeau VM. Rapid spread of emerging Zika virus in the Pacific area. Clin Microbiol Infect 2014;20:O595–6. http://dx.doi.org/10.1111/1469-0691.12707.
4. Zanluca C, de Melo VC, Mosimann AL, Dos Santos GI, Dos Santos CN, Luz K. First report of autochthonous transmission of Zika virus in Brazil. Mem Inst Oswaldo Cruz 2015;110:569–72.
5. European Centre for Disease Prevention and Control. Zika virus epidemic in the Americas: potential association with microcephaly and Guillain-Barré syndrome. Stockholm, Sweden: European Centre for Disease Prevention and Control; 2015. http://ecdc.europa.eu/en/publications/Publications/zika-virus-americas-association-with-microcephaly-rapid-risk-assessment.pdf.
6. Pan American Health Organization. Zika virus infection. Washington, DC: World Health Organization, Pan American Health Organization; 2016. http://www.paho.org/hq/index.php?option=com_topics&view=article&id=427&Itemid=41484&lang=en.
7. CDC. Zika virus. Atlanta, GA: US Department of Health and Human Services, CDC; 2016. http://www.cdc.gov/zika/index.html.

8. Pan American Health Organization. Epidemiological alert: neurological syndrome, congenital malformations, and Zika virus infection. Implications for public health in the Americas. Washington, DC: World Health Organization, Pan American Health Organization; 2015. http://www.paho.org/hq/index.php?option=com_docman&task=doc_download&Itemid=&gid=32405&lang=en.
9. CDC. Chikungunya virus: clinical evaluation & disease. Atlanta, GA: US Department of Health and Human Services, CDC; 2015. http://www.cdc.gov/chikungunya/hc/clinicalevaluation.html.
10. World Health Organization. Dengue: guidelines for diagnosis, treatment, prevention and control. Geneva, Switzerland: World Health Organization; 2009. http://apps.who.int/iris/bitstream/10665/44188/1/9789241547871_eng.pdf.
11. Lanciotti RS, Kosoy OL, Laven JJ, et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. Emerg Infect Dis 2008;14:1232–9. http://dx.doi.org/10.3201/eid1408.080287.
12. Nasci RS, Wirtz RA, Brogdon WG. Protection against mosquitoes, ticks, and other arthropods. In: CDC health information for international travel, 2016. New York, NY: Oxford University Press; 2015. http://wwwnc.cdc.gov/travel/yellowbook/2016/the-pre-travel-consultation/protection-against-mosquitoes-ticks-other-arthropods.
13. Petersen EE, Staples JE, Meaney-Delman D, et al. Interim guidelines for pregnant women during a Zika virus outbreak—United States, 2016. MMWR Morb Mortal Wkly Rep 2016;65:30–3 http://dx.doi.org/10.15585/mmwr.mm6502e1.

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2016 年 02 月 08 日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①②抗 HBs 人免疫グロブリン ③④乾燥抗 HBs 人免疫グロブリン ⑤ポリエチレングリコール処理抗 HBs 人免疫グロブリン		研究報告の公表状況	www.cdc.gov/mmwr/volumes/65/wr/mm6505e1er.htm?s_cid=mm6505e1.htm_w/2016/02/05	公表国 アメリカ	
販売名（企業名）	①抗 HBs 人免疫グロブリン筋注 200 単位/1mL「JB」（日本血液製剤機構） ②抗 HBs 人免疫グロブリン筋注 1000 単位/5mL「JB」（日本血液製剤機構） ③ヘブスブリン筋注用 200 単位（日本血液製剤機構） ④ヘブスブリン筋注用 1000 単位（日本血液製剤機構） ⑤ヘブスブリン IH 静注 1000 単位（日本血液製剤機構）					

研究報告の概要

ジカウイルスの性感染予防に関する暫定的ガイドライン―米国，2016 年

ジカウイルスは性行為による伝播が可能であり，これは特に妊娠中に懸念される。ジカウイルスの性感染の可能性に関する最新の情報は，3 事例の報告に基づいている。最初の 1 例は，男性から女性へのジカウイルスの性感染の可能性が高い事例である。2 例目は性感染で調査中であり，3 例目は発症から少なくとも 2 週間から最大 10 週まで精子中からウイルスが分離された男性（性交渉はなし）の症例である。
ジカウイルス伝播地域に在住または渡航した男性とそのパートナー（妊婦または妊娠していない）は性交渉に際して避妊する様あるいは避妊を考慮に入れる様勧告している。

使用上の注意記載状況・その他参考事項等

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

報告企業の意見

ジカウイルス（Zika virus）は 1947 年にウガンダの Zika forest（ジカ森林）から発見されたウイルスで、デングウイルス、日本脳炎ウイルス、ウエストナイルウイルスと同じフラビウイルス科フラビウイルス属に属するエンベロープを有する RNA ウイルスで、蚊（ネッタイシマカ、ヒトスジシマカ）によって媒介される。万一、原料血漿にジカウイルスが混入したとしても、各種モデルウイルスのウイルスクリアランス試験成績から、本剤の製造工程において不活化・除去されると考えている。

今後の対応

本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

抗 HBs

Interim Guidelines for Prevention of Sexual Transmission of Zika Virus — United States, 2016

Weekly / February 12, 2016 / 65(5);120–121

On February 5, 2016, this report was posted as an MMWR *Early Release on the* MMWR *website (http://www.cdc.gov/mmwr*(http://www.cdc.gov/mmwr)*).*

Alexandra M. Oster, MD[1]; John T. Brooks, MD[1]; Jo Ellen Stryker, PhD[1]; Rachel E. Kachur, MPH[2]; Paul Mead, MD[3]; Nicki T. Pesik, MD[4]; Lyle R. Petersen, MD[3] (View author affiliations)

View suggested citation

Zika virus is a mosquito-borne flavivirus primarily transmitted by *Aedes aegypti* mosquitoes (*1,2*). Infection with Zika virus is asymptomatic in an estimated 80% of cases (*2,3*), and when Zika virus does cause illness, symptoms are generally mild and self-limited. Recent evidence suggests a possible association between maternal Zika virus infection and adverse fetal outcomes, such as congenital microcephaly (*4,5*), as well as a possible association with Guillain-Barré syndrome. Currently, no vaccine or medication exists to prevent or treat Zika virus infection. Persons residing in or traveling to areas of active Zika virus transmission should take steps to prevent Zika virus infection through prevention of mosquito bites (http://www.cdc.gov/zika/prevention/ (http://www.cdc.gov/zika/prevention/)).

Sexual transmission of Zika virus is possible, and is of particular concern during pregnancy. Current information about possible sexual transmission of Zika is based on reports of three cases. The first was probable sexual transmission of Zika virus from a man to a woman (*6*), in which sexual contact occurred a few days before the man's symptom onset. The second is a case of sexual transmission currently under investigation (unpublished data, 2016, Dallas County Health and Human Services). The third is a single report of replication-competent Zika virus isolated from semen at least 2 weeks and possibly up to 10 weeks after illness onset; reverse transcriptase-polymerase chain reaction testing of blood plasma specimens collected at the same time as the semen specimens did not detect Zika virus (*7*). The man had no sexual contacts. Because no further testing was conducted, the duration of persistence of Zika virus in semen remains unknown.

In all three cases, the men developed symptomatic illness. Whether infected men who never develop symptoms can transmit Zika virus to their sex partners is unknown. Sexual transmission of Zika virus from infected women to their sex partners has not been reported. Sexual transmission of many infections, including those caused by other viruses, is reduced by consistent and correct use of latex condoms.

The following recommendations, which apply to men who reside in or have traveled to areas with active Zika virus transmission (http://wwwnc.cdc.gov/travel/notices/ (http://wwwnc.cdc.gov/travel/notices/)) and their sex partners, will be revised as more information becomes available.

Recommendations for men and their pregnant partners

^ Top

Men who reside in or have traveled to an area of active Zika virus transmission who have a pregnant partner should abstain from sexual activity or consistently and correctly use condoms during sex (i.e., vaginal intercourse, anal intercourse, or fellatio) for the duration of the pregnancy. Pregnant women should discuss their male partner's potential exposures to mosquitoes and history of Zika-like illness (http://www.cdc.gov/zika/symptoms (http://www.cdc.gov/zika/symptoms)) with their health care provider; providers can consult CDC's guidelines for evaluation and testing of pregnant women (*8*).

Recommendations for men and their nonpregnant sex partners

^ Top

Men who reside in or have traveled to an area of active Zika virus transmission who are concerned about sexual transmission of Zika virus might consider abstaining from sexual activity or using condoms consistently and correctly during sex. Couples considering this personal decision should take several factors into account. Most infections are asymptomatic, and when illness does occur, it is usually mild with symptoms lasting from several days to a week; severe disease requiring hospitalization is uncommon. The risk for acquiring vector-borne Zika virus in areas of active transmission depends on the duration and extent of exposure to infected mosquitoes and the steps taken to prevent mosquito bites (http://www.cdc.gov/zika/prevention (http://www.cdc.gov/zika/prevention)). After infection, Zika virus might persist in semen when it is no longer detectable in blood.

Zika virus testing has been recommended to establish a diagnosis of infection in some groups, such as pregnant women (*8*). At present, Zika virus testing for the assessment of risk for sexual transmission is of uncertain value, because current understanding of the incidence and duration of shedding in the male genitourinary tract is limited to one case report in which Zika virus persisted longer than in blood (*7*). At this time, testing of men for the purpose of assessing risk for sexual transmission is not recommended. As we learn more about the incidence and duration of seminal shedding from infected men and the utility and availability of testing in this context, recommendations to prevent sexual transmission of Zika virus will be updated.

Acknowledgments

^ Top

Brian Foy, Colorado State University, Ft. Collins, Colorado; Joel Gallant, Southwest CARE Center, Santa Fe, New Mexico; King Holmes, University of Washington, Seattle, Washington; Tom Quinn, Johns Hopkins University, Baltimore, Maryland; Wendy Chung, Dallas County Health and Human Services, Dallas Texas.

---

^ Top

Corresponding author: John T. Brooks, zud4@cdc.gov (mailto:zud4@cdc.gov), 404-639-3894.

[1]Division of HIV/AIDS Prevention, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, CDC; [2]Division of STD Prevention, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, CDC; [3]Division of Vector-Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, CDC; [4]Office of the Director, National Center for Emerging and Zoonotic Infectious Diseases, CDC.

^ Top

^ Top

References

1. Hayes EB. Zika virus outside Africa. Emerg Infect Dis 2009;15:1347–50. CrossRef (http://dx.doi.org/10.3201/eid1509.090442) PubMed (http://www.ncbi.nlm.nih.gov/pubmed/19788800)
2. CDC. Zika virus. Atlanta, GA: US Department of Health and Human Services, CDC; 2016. http://www.cdc.gov/zika/index.html (http://www.cdc.gov/zika/index.html).
3. Duffy MR, Chen TH, Hancock WT, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med 2009;360:2536–43. CrossRef (http://dx.doi.org/10.1056/NEJMoa0805715) PubMed (http://www.ncbi.nlm.nih.gov/pubmed/19516034)
4. European Centre for Disease Prevention and Control. Rapid risk assessment: Zika virus epidemic in the Americas: potential association with microcephaly and Guillain-Barré syndrome. Stockholm, Sweden: European Centre for Disease Prevention and Control; 2015. http://ecdc.europa.eu/en/publications/Publications/zika-virus-americas-association-with-microcephaly-rapid-risk-assessment.pdf (http://ecdc.europa.eu/en/publications/Publications/zika-virus-americas-association-with-microcephaly-rapid-risk-assessment.pdf).
5. Oliveira Melo AS, Malinger G, Ximenes R, Szejnfeld PO, Alves Sampaio S, Bispo de Filippis AM. Zika virus intrauterine infection causes fetal brain abnormality and microcephaly: tip of the iceberg? Ultrasound Obstet Gynecol 2016;47:6–7. CrossRef (http://dx.doi.org/10.1002/uog.15831) PubMed (http://www.ncbi.nlm.nih.gov/pubmed/26731034)
6. Foy BD, Kobylinski KC, Chilson Foy JL, et al. Probable non-vector-borne transmission of Zika virus, Colorado, USA. Emerg Infect Dis 2011;17:880–2. CrossRef

(http://dx.doi.org/10.3201/eid1705.101939) PubMed (http://www.ncbi.nlm.nih.gov/pubmed/21529401)

7. Musso D, Roche C, Robin E, Nhan T, Teissier A, Cao-Lormeau VM. Potential sexual transmission of Zika virus. Emerg Infect Dis 2015;21:359–61. CrossRef (http://dx.doi.org/10.3201/eid2102.141363) PubMed (http://www.ncbi.nlm.nih.gov/pubmed/25625872)
8. Oduyebo T, Petersen EE, Rasmussen SA, et al. Update: interim guidelines for health care providers caring for pregnant women and women of reproductive age with possible Zika virus exposure—United States, 2016. MMWR Morb Mortal Wkly Rep 2016;65.

^ Top

Suggested citation for this article: Oster AM, Brooks JT, Stryker JE, et al. Interim Guidelines for Prevention of Sexual Transmission of Zika Virus — United States, 2016. MMWR Morb Mortal Wkly Rep 2016;65:120–121. DOI: http://dx.doi.org/10.15585/mmwr.mm6505e1 (http://dx.doi.org/10.15585/mmwr.mm6505e1).

Use of trade names and commercial sources is for identification only and does not imply endorsement by the U.S. Department of Health and Human Services.
References to non-CDC sites on the Internet are provided as a service to *MMWR* readers and do not constitute or imply endorsement of these organizations or their programs by CDC or the U.S. Department of Health and Human Services. CDC is not responsible for the content of pages found at these sites. URL addresses listed in *MMWR* were current as of the date of publication.

All HTML versions of *MMWR* articles are generated from final proofs through an automated process. This conversion might result in character translation or format errors in the HTML version. Users are referred to the electronic PDF version (http://www.cdc.gov/mmwr (http://www.cdc.gov/mmwr)) and/or the original *MMWR* paper copy for printable versions of official text, figures, and tables. An original paper copy of this issue can be obtained from the Superintendent of Documents, U.S. Government Printing Office (GPO), Washington, DC 20402-9371; telephone: (202) 512-1800. Contact GPO for current prices.

Questions or messages regarding errors in formatting should be addressed to mmwrq@cdc.gov (javascript:sendIt ();).

File Formats Help:
How do I view different file formats (PDF, DOC, PPT, MPEG) on this site? (http://www.cdc.gov/Other/plugins/)

(http://www.cdc.gov/Other/plugins/#pdf)

Page last reviewed: February 11, 2016

Page last updated: February 11, 2016

Content source: Centers for Disease Control and Prevention (http://www.cdc.gov/)

別紙様式第2－1

4

医薬品　研究報告　調査報告書

識別番号・報告回数		報告日	第一報入手日 2016年3月14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	Disease Outbreak News 7 March 2016	公表国 フランス領Polynesia	
販売名（企業名）	別紙のとおり。				

研究報告の概要

問題点：ジカウイルス感染とギラン・バレー症候群発症との関連を示す強力なエビデンスとなり得る調査結果が報告された。

2013年10月～2014年4月，フランス領Polynesiaでは，これまで同国で記録された中で最大のジカウイルスのアウトブレイクが発生した。疑い例8750例が報告され，このうち383例がRT-PCR法により検査確定された。
このジカウイルスのアウトブレイク中，42例の患者がギラン・バレー症候群（GBS）で入院した。これは，フランス領Polynesiaにおける GBS の発生率としては過去4年間と比較して20倍の増加である。これらの症例の大多数（88%）が，神経学的症状を発症する前の数日（中央値：6日）間に症候性のジカウイルス感染症を報告した。

GBSとの関連が知られている，または関連する可能性がある感染の潜在的役割を明らかとするために，更なる調査が行われた結果，GBS症例42例のうち41例がジカウイルスに対するIgM抗体またはIgG抗体を有していたことが示された。さらに，全例（100%）がジカウイルスに対して血清中和反応陽性であった。非熱性疾患対照群の結果には有意差が認められた：対照患者98例のうち35例においてIgM抗体またはIgG抗体の増加が検出された。また，対照患者54例（56%）においてジカウイルスに対する中和抗体が検出された。デングウイルスの血清学的分析では，大半の症例（95%）がデングウイルスに対する既存の免疫があったものの，最近のデングウイルス感染は裏付けられなかった。

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。

MedDRA/J ver.19.0

別紙

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤人免役グロブリン、⑥乾燥ペプシン処理人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン、⑧乾燥スルホ化人免疫グロブリン、⑨乾燥スルホ化人免疫グロブリン、⑩乾燥スルホ化人免疫グロブリン、⑪乾燥スルホ化人免疫グロブリン*、⑫乾燥濃縮人活性化プロテインC、⑬乾燥濃縮人血液凝固第Ⅷ因子、⑭乾燥濃縮人血液凝固第Ⅷ因子、⑮乾燥濃縮人血液凝固第Ⅷ因子、⑯乾燥濃縮人血液凝固第Ⅸ因子、⑰乾燥濃縮人血液凝固第Ⅸ因子、⑱乾燥濃縮人血液凝固第Ⅸ因子、⑲乾燥抗破傷風人免疫グロブリン、⑳抗 HBs 人免疫グロブリン、㉑抗 HBs 人免疫グロブリン、㉒抗 HBs 人免疫グロブリン、㉓トロンビン、㉔トロンビン、㉕トロンビン、㉖フィブリノゲン加第XⅢ因子*、㉗フィブリノゲン加第XⅢ因子、㉘乾燥濃縮人アンチトロンビンⅢ、㉙ヒスタミン加人免疫グロブリン製剤、㉚人血清アルブミン*、㉛人血清アルブミン*、㉜乾燥ペプシン処理人免役グロブリン*、㉝乾燥濃縮人アンチトロンビンⅢ、㉞乾燥濃縮人血液凝固第X因子加活性化第Ⅶ因子
販売名（企業名）	①献血アルブミン 20 "化血研"、②献血アルブミン 25 "化血研"、③人血清アルブミン "化血研" *、④ガンマーグロブリン筋注 450mg/3mL「化血研」、⑤ガンマーグロブリン筋注 1500mg/10mL「化血研」、⑥献血グロブリン注射用 2500mg「化血研」、⑦献血ベニロン－Ⅰ静注用 500mg、⑧献血ベニロン－Ⅰ静注用 1000mg、⑨献血ベニロン－Ⅰ静注用 2500mg、⑩献血ベニロン－Ⅰ静注用 5000mg、⑪ベニロン*、⑫注射用アナクトC2,500 単位、⑬コンファクトF注射用 250、⑭コンファクトF注射用 500、⑮コンファクトF注射用 1000、⑯ノバクトM静注用 400 単位、⑰ノバクトM静注用 800 単位、⑱ノバクトM静注用 1600 単位、⑲テタノセーラ筋注用 250 単位、⑳ヘパトセーラ筋注 200 単位/mL、㉑ヘパトセーラ筋注 200 単位/1mL、㉒ヘパトセーラ筋注 1000 単位/5mL、㉓トロンビン "化血研"、㉔献血トロンビン経口・外用５千「化血研」、㉕献血トロンビン経口・外用１万「化血研」、㉖ボルヒール*、㉗ボルヒール組織接着用、㉘アンスロビンP500 注射用、㉙ヒスタグロビン皮下注用、㉚アルブミン 20%化血研*、㉛アルブミン 5%化血研*、㉜静注グロブリン*、㉝アンスロビンP1500 注射用、㉞バイクロット配合静注用、㉟ノバクトM静注用 500 単位、㊱ノバクトM静注用 1000 単位、㊲ノバクトM静注用 2000 単位
報告企業の意見	ジカウイルスは、日本脳炎ウイルス、デングウイルス、ウエストナイルウイルスなどと同属のフラビウイルス属の蚊媒介性感染症ウイルスとして知られており、一本鎖 RNA 及びエンベロープを有するウイルスである。今回の報告では、ジカウイルス感染によるギランバレー症候群の発生率の増加リスクが示された。 上記製剤の製造工程には、冷エタノール分画工程、ウイルス除去膜ろ過工程、加熱工程といった原理の異なるウイルスクリアランス工程が複数導入されており、各工程のウイルスクリアランス効果は「血漿分画製剤のウイルスに対する安全性確保に関するガイドライン（医薬発第 1047 号、平成 11 年 8 月 30 日）」に基づく、モデルウイルスを用いたウイルスプロセスバリデーションにより確認されている。今回報告したジカ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ジカウイルスへの感染報告例は無い。 以上の点から、上記製剤はジカウイルスに対する安全性を確保していると考える。

*：現在製造を行っていない

WORLD IMMUNIZATION WEEK 2016

Emergencies preparedness, response

Guillain-Barré syndrome – France - French Polynesia

Disease Outbreak News
7 March 2016

On 24 November 2015, health authorities in French Polynesia reported unknown and unspecified causes of morbidity and mortality in the context of concomitant outbreaks of Zika and dengue (serotypes 1 and 3) viruses. This update provides additional information on the clinical findings as well as the epidemiological and laboratory investigations of these cases.

Between October 2013 and April 2014, French Polynesia experienced the largest Zika virus outbreak ever recorded in the country. During this period of time, 32,000 patients (11.5% of the population) were assessed for the infection and 8,750 suspected cases were reported by the national surveillance system. Of the suspected cases, 383 were later laboratory-confirmed by reverse transcription polymerase chain reaction (RT-PCR).

During the Zika virus outbreak, 42 patients were admitted to hospital with Guillain-Barré syndrome (GBS). This represents a 20-fold increase in incidence of GBS in French Polynesia compared with the previous four years. Of the 42 patients, 16 (38%) required admission to an intensive care unit (ICU) and 12 (29%) received mechanical ventilation. The duration of hospital stay for patients that were not admitted to ICU ranged between 7 to 20 days (median=11). The duration of hospital stay for patients that were admitted to intensive care went from 16 to 70 days (median=51). No deaths were reported. The majority of these cases (88%) reported symptomatic Zika virus infection in the days (median=6) that preceded the onset of neurological symptoms.

Further investigations were carried out to identify the potential role of previous infections known to be associated, or potentially associated, with GBS. The investigations carried out by the Bureau de Veille Sanitaire-Direction de la Santé de Polynésie Française showed that 41 of the 42 cases of GBS (98%) had IgM or IgG antibodies against Zika virus; furthermore, all GBS cases (100%) had positive seroneutralisation against Zika virus. Results for the matched non-febrile illness control group were significantly different: elevated IgM or IgG antibodies were detected in 35 of the 98 control patients (36%); additionally, neutralising antibodies against Zika virus were detected in 54 control patients (56%).

Analysis of dengue serology (immunofluorescent assay, microsphere immunoassay, and seroneutralisation) did not support recent dengue infection, even though most cases (95%) had pre-existing dengue

immunity. Other known causes of GBS were investigated and excluded, including Campylobacter jejuni, Cytomegalovirus, HIV, Epstein–Barr and Herpes simplex viruses.

WHO risk assessment

This is the first report of a large number of patients who developed GBS after contracting Zika virus. The study provides strong evidence of a possible causal relationship between Zika virus infection and GBS. Since all 42 cases had serological tests suggesting successive dengue and Zika virus infections, this association might be a predisposing factor for developing GBS. Further investigations are needed to understand the implications of pre-existing dengue infections, together with recent Zika infections, in the pathogenesis of GBS.

Similarly to French Polynesia, it is likely that countries that are currently reporting autochthonous Zika virus transmission will face a rise in the number of GBS cases in the coming months. A number of countries in Latin America have already started to report an increase in the incidence of GBS while experiencing a rise in the cases of Zika virus infection. Nevertheless, it is critical to ensure that in all these countries, the reported increases in the incidence of GBS are the result of a real change rather than enhanced surveillance. WHO continues to monitor the epidemiological situation and conduct risk assessment based on the latest available information.

WHO advice

WHO recommends Member States affected or susceptible to Zika virus outbreaks to:

- monitor the incidence and trends of neurological disorders, especially GBS, to identify variations against their expected baseline values;
- develop and implement sufficient patient management protocols to manage the additional burden on health care facilities generated by a sudden increase in patients with Guillain-Barre Syndrome;
- raise awareness among health care workers and establish and/or strengthen links between public health services and clinicians in the public and private sectors.

The proximity of mosquito vector breeding sites to human habitation is a significant risk factor for Zika virus infection. Prevention and control relies on reducing the breeding of mosquitoes through source reduction (removal and modification of breeding sites) and reducing contact between mosquitoes and people. This can be achieved by reducing the number of natural and artificial water-filled habitats that support mosquito larvae, reducing the adult mosquito populations around at-risk communities and by using barriers such as insect screens, closed doors and windows, long clothing and repellents. Since the Aedes mosquitoes (the primary vector for transmission) are day-biting mosquitoes, it is recommended that those who sleep during the daytime, particularly young children, the sick or elderly, should rest under mosquito nets (bed nets), treated with or without insecticide to provide protection.

During outbreaks, space spraying of insecticides may be carried out following the technical orientation provided by WHO to kill flying mosquitoes. Suitable insecticides (recommended by the WHO Pesticide

Evaluation Scheme) may also be used as larvicides to treat relatively large water containers, when this is technically indicated.

Basic precautions for protection from mosquito bites should be taken by people traveling to high risk areas, especially pregnant women. These include use of repellents, wearing light colored, long sleeved shirts and pants and ensuring rooms are fitted with screens to prevent mosquitoes from entering.

WHO does not recommend any travel or trade restriction to France and its overseas departments based on the current information available.

Related links

More GBS outbreak news

Zika situation report – 4 March 2016

More on Zika virus

PAHO/WHO Algorithm for detecting Zika virus

WHO Pesticide Evaluation Scheme

France country profile

別紙様式第2-1

No. 11

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2015. 11. 27	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	Gérardin P, Couderc T, Bintner M, et al. Neurology. 2016 Jan 5;86(1):94-102.	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

○チクングニヤウイルス関連脳炎:レユニオン島におけるコホート研究(2005年～2009年)。
目的:レユニオン島のアウトブレイク期間におけるチクングニヤウイルス(CHIKV)関連中枢神経系(CNS)疾患の累積罹患率(CIR)を予測し、並びに疾病の状況と3年後の患者転帰を評価する。
方法:RT-PCR法でCHIKV陽性、若しくは脳脊髄液中のCHIKV IgM抗体が陽性であり、なおかつ脳炎または脳症に関するInternational Encephalitis Consortiumの基準を満たしている患者のコホートについて、CHIKV関連CNS疾患の評価を後ろ向きに行った。神経学的後遺症の評価は3年後に行った。
結果:2005年9月から2006年6月までの期間に、CHIKV関連脳炎患者24例を含む57例の患者がCHIKV関連CNS疾患の診断を受けており、CIRは10万人あたり8.6人であった。脳炎患者は年齢範囲の両端で確認された。10万人あたりのCIRは1歳未満の患者で187人、65歳超の患者で37人であり、共に米国の同年齢区分における、あらゆる原因による脳炎のCIRをはるかに上回っていた。CHIKV関連脳炎の致死率は16.6%であり、後遺症を有する状態で退院した小児の割合は30%から45%の間であると推定された。新生児期以降の臨床症状と転帰については、幼児における重症度は成人と比較してより低くなった。
結論:大規模なアウトブレイクではCHIKV感染がCNS疾患の重大な要因となる。他の原因による症例と同様に、CHIKV関連脳炎症例の年齢別の分布は、U字型の放物線を描く。

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

2005年以降にレユニオン島でアウトブレイクしたチクングニヤウイルス(CHIKV)罹患患者における神経学的後遺症の評価を3年後に行った。中枢神経系疾患の累積罹患率は米国のあらゆる原因による脳炎に比べ高かった。CHIKV関連脳炎の致死率は16.6%であり、後遺症を有する状態で退院した小児の割合は30%から45%と推定されたという報告である。

今後の対応

日本赤十字社では、輸血感染症対策として献血時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。国内でチクングニヤ熱が確認されたため、渡航歴確認の徹底を図っている。また、チクングニヤ熱の既往歴がある場合、治癒後6ヵ月間は献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

MedDRA/J Ver.18.1J

12

Chikungunya virus–associated encephalitis

A cohort study on La Réunion Island, 2005–2009

Patrick Gérardin, MD, PhD
Thérèse Couderc, PhD
Marc Bintner, MD
Patrice Tournebize, MD
Michel Renouil, MD
Jérome Lémant, MD
Véronique Boisson, MD
Gianandrea Borgherini, MD
Frédérik Staikowsky, MD, PhD
Frédéric Schramm, MD, PhD
Marc Lecuit, MD, PhD
Alain Michault, PharmD†
On behalf of the Encephalchik Study Group

Correspondence to Dr. Gérardin:
patrick.gerardin@chu-reunion.fr

ABSTRACT

Objective: To estimate the cumulative incidence rate (CIR) of Chikungunya virus (CHIKV)–associated CNS disease during the La Réunion outbreak, and assess the disease burden and patient outcome after 3 years.

Methods: CHIKV-associated CNS disease was characterized retrospectively in a cohort of patients with positive CHIKV reverse transcriptase PCR or anti-CHIKV immunoglobulin M antibodies in the CSF and fulfilling International Encephalitis Consortium criteria for encephalitis or encephalopathy. Neurologic sequelae were assessed after 3 years.

Results: Between September 2005 and June 2006, 57 patients were diagnosed with CHIKV-associated CNS disease, including 24 with CHIKV-associated encephalitis, the latter corresponding to a CIR of 8.6 per 100,000 persons. Patients with encephalitis were observed at both extremes of age categories. CIR per 100,000 persons were 187 and 37 in patients below 1 year and over 65 years, respectively, both far superior to those of cumulated causes of encephalitis in the United States in these age categories. The case-fatality rate of CHIKV-associated encephalitis was 16.6% and the proportion of children discharged with persistent disabilities estimated between 30% and 45%. Beyond the neonatal period, the clinical presentation and outcomes were less severe in infants than in adults.

Conclusions: In the context of a large outbreak, CHIKV is a significant cause of CNS disease. As with other etiologies, CHIKV-associated encephalitis case distribution by age follows a U-shaped parabolic curve. *Neurology*® 2016;86:94–102

GLOSSARY

ADEM = acute disseminated encephalomyelitis; **CHIKV** = Chikungunya virus; **CFR** = case-fatality rate; **CIR** = cumulative incidence rate; **DQ** = development quotient; **DWI** = diffusion-weighted imaging; **ECSA** = East Central South African; **IEC** = International Encephalitis Consortium; **IgM** = immunoglobulin M; **LP** = lumbar puncture; **NECACD** = nonencephalitic Chikungunya virus-associated CNS disease; **WNV** = West Nile virus.

Chikungunya virus (CHIKV) is a re-emerging alphavirus.[1] Alphaviruses are divided into arthritogenic viruses (old world) and encephalitogenic viruses (new world) including equine encephalitis viruses.[2]

Until its reemergence in the Indian Ocean in 2004 and the worldwide spread that followed, beyond the burden of arthritis, known for lasting weeks to years,[3] Chikungunya was considered as a nonfatal disease with spontaneous resolution, not causing lifelong disabilities, even though rare cases of CNS disease had been reported.[4,5]

The major outbreaks that have occurred since 2005 in the Indian Ocean islands were attributable to a new Indian Ocean lineage that evolved from the East Central South African (ECSA) lineage and selected the mutation E1-A226V, which favors transmission by *Aedes albopictus*.[6,7]

Supplemental data at Neurology.org

†Deceased.

From the Centre Hospitalier Universitaire (CHU) (P.G., M.B., P.T., M.R., J.L., V.B., G.B., A.M.) and Centre d'Investigation Clinique–Épidémiologie Clinique (CIC1410) de La Réunion (Inserm, CHU, Université de La Réunion, Union Régionale des Médecins Libéraux de la Réunion) (P.G.), Saint Pierre, La Réunion; UMR PIMIT (Inserm U 1187, CNRS 9192, IRD 249, Université de La Réunion) (P.G., A.M.), CYROI, Saint-Denis, La Réunion; Biology of Infection Unit (T.C., M.L.), Institut Pasteur, Paris; Inserm U1117 (T.C., M.L.), Paris; CHU Henri Mondor (F. Staikowsky), Créteil; Université de Strasbourg (F. Schramm), EA 7290, Faculté de Médecine, Strasbourg; and Université Paris Descartes (M.L.), Sorbonne Paris Cité, Division of Infectious Diseases and Tropical Medicine, Necker Enfants Malades University Hospital, Institut Imagine (M.L.), Paris, France.

Coinvestigators of the Encephalchik Study Group are listed on the *Neurology*® Web site at Neurology.org.

Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

© 2015 American Academy of Neurology
© 2015 American Academy of Neurology. Unauthorized reproduction of this article is prohibited.

Recently, Asian lineage CHIKV emerged in the Caribbean and expanded to the Americas, and recent clinical and experimental data indicate differences in the pathogenicity between Asian and American lineages.[8–10]

The 2005–2006 epidemic on La Réunion Island affected 300,000 persons and enabled the observation of severe forms of the disease.[11] These included rare severe or fatal cases with CNS involvement, in both adults and neonates.[12–16]

We report the results of an ambispective cohort study aimed at characterizing clinical and biological features of CHIKV-associated CNS disease, disease burden, and 3-year neurologic outcome of patients with this condition.

METHODS We conducted this study in the Groupe Hospitalier Sud Réunion, the largest hospital on the island, which covers a population of 277,602 inhabitants.[17]

Retrospective cohort study. We considered all patients hospitalized between September 1, 2005, and June 30, 2006, with CHIKV infection and neurologic symptoms that led to lumbar puncture (LP) eligible for the study. Patients with positive CSF for CHIKV RNA or anti-CHIKV immunoglobulin M (IgM) antibodies were studied further.

Anti-CHIKV IgM assay in the CSF was performed by ELISA using the ETIMAX 3000 (Diasorin, Italy). A one-step TaqMan real-time quantitative PCR was performed from CSF samples using the LightCycler 2.0 system (Roche Diagnostics; Basel, Switzerland).

Standard protocol approvals, registrations, and patient consents. Each patient provided oral consent for the use of clinical, biological, and imaging data, in accordance with the recommendations of the local Committee for Clinical Research.[13]

Case definition. We used positive CSF findings (CHIKV RNA or IgM) to provide the more specific case definition. Additionally, we used International Encephalitis Consortium (IEC) criteria to classify our patients according to an up-to-date definition of encephalitis.[18] These combine the major criterion altered mental status (defined as decreased or altered level of consciousness, lethargy, or personality change lasting ≥24 hours with no alternative cause identified) with a set of minor criteria: fever (≥38°C) within the 72 hours before or after presentation; general or partial seizures not fully attributable to epilepsy; new onset of focal neurologic signs; CSF leukocyte count ≥5/mm³; brain parenchyma on neuroimaging suggestive of encephalitis either new from prior studies or appearing acute in onset; EEG consistent with encephalitis and not attributable to another cause.

Exclusion criteria were the main causes of encephalopathy and of noninfectious encephalitis: positive HIV status, pyogenic meningitis, thrombophlebitis, brain abscess, empyema, cerebral malaria, acute disseminated encephalomyelitis, voltage-gated potassium channels, NMDA receptor antibodies, systemic vasculitis, multiple sclerosis, paraneoplastic-related encephalitis, prion disease, encephalopathy of primary tumor, or hematologic, toxic, or metabolic origin.[19]

Thus, we defined probable CHIKV-associated encephalitis in the presence of the major criterion and at least 3 minor criteria, possible CHIKV-associated encephalitis in the presence of the major criterion and 2 minor criteria,[18] and nonencephalitic CHIKV-associated CNS disease (NECACD) in the presence of major criterion alone or with one minor criterion, or in the presence of 2 minor criteria other than fever.

Prospective follow-up study. We followed up each patient with CHIKV CNS disease to search for neurologic sequelae over a 3-year period using the framework of the extended Glasgow Outcome Scale (adult and pediatric versions).[20] For children, trained psychometrists assured neuropsychological evaluation using the revised neurodevelopmental scale of Brunet-Lézine, a standardized psychometric test routinely used in francophone countries. For adults, neurologists performed clinical and EEG examinations. CT or MRI scans were performed on clinical indication.

Statistical analysis. We compared characteristics of CHIKV-associated encephalitis and NECACD globally and between adults and children using χ^2 or Fisher exact test for proportions. We compared distributions using Mann-Whitney tests. We tested correlations between CSF and serum viral loads in children, or between CSF and serum IgM levels in adults, using Spearman correlation coefficients.

We provided cumulative incidence rates (CIR) for CHIKV-associated encephalitis (probable, possible, or both), overall and by age groups, applying weights for subpopulation structure using data from the 2006 census.[17] We then compared these estimates to US standards.[21]

We assessed the range of neurologic sequelae in applying the same actual rate or null for the missing observations, this assumption being likely conservative, given the low probability of loss to follow-up due to death or sequelae in our insular population.

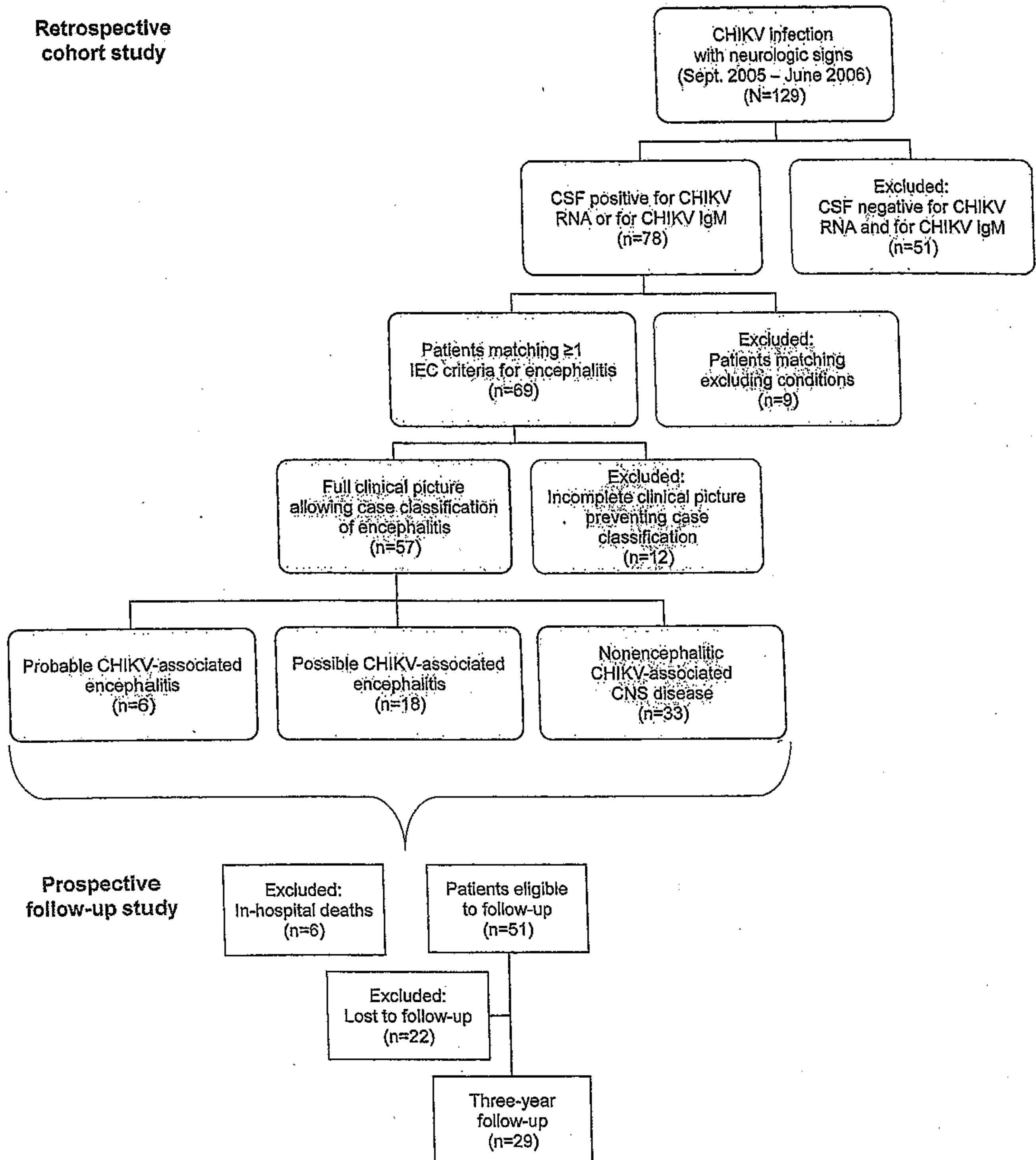
We used Stata (v10.0, StataCorp., College Station, TX) for comparisons. Statistical significance was set at $p = 0.05$.

RESULTS Retrospective cohort study. ***CHIKV acute patients with neurologic symptoms.*** Among the 129 CHIKV-infected patients with CNS disease, biological analysis of the CSF was positive for 55 CHIKV RNA or 30 anti-CHIKV IgM in 78 patients and negative in 51 patients (figure 1).

We excluded 9 patients because they exhibited additional conditions, which invalidated CHIKV as a unique cause for the neurologic symptoms. Briefly, these consisted of encephalopathy of primary metabolic origin (n = 3), alcohol-related encephalopathy (n = 3), posterior reversible encephalopathy syndrome in systemic lupus erythematosus (n = 1), *Streptococcus pneumoniae* meningitis (n = 1), and neurocysticercosis (n = 1). The 69 remaining patients showed at least one IEC encephalitis criterion but 12 of them were excluded because incomplete charts did not allow definite classification. Thus, a total of 57 patients diagnosed with CHIKV-associated CNS disease were enrolled in the study. Among them, 24 (42.1%) patients with altered mental status matched IEC encephalitis definition, whereas 33 (57.9%) others did not and were designated as NECACD in further analysis.

© 2015 American Academy of Neurology. Unauthorized reproduction of this article is prohibited.

Figure 1 Study population of CHIKV-associated CNS disease, Réunion Island (2005-2006)



The flow chart classifies the Chikungunya virus (CHIKV)-associated CNS disease cases at inclusion and at follow-up. IEC = International Encephalitis Consortium; IgM = immunoglobulin M.

We identified 6 confirmed cases of CHIKV-associated encephalitis (i.e., altered mental status plus at least 3 minor criteria), while 18 patients were classified as possible cases (i.e., 2 minor criteria). Both groups shared the same clinical and biological profiles, which confirmed the appropriateness of the IEC classification that allow these groups to be pooled for case registration (data not shown).

CHIKV encephalitic cases were more likely to exhibit severe CNS disease than were cases of NE-CACD, which consisted almost exclusively of mild to moderate behavioral changes (table 1). Two

© 2015 American Academy of Neurology. Unauthorized reproduction of this article is prohibited.

Table 1	Clinical features of CHIKV-associated CNS disease in La Réunion Island (2005–2006)		
Variables	Probable or possible encephalitis (n = 24), n (%)	NECACD[a] (n = 33), n (%)	p Value
Age group, y			0.003[b]
≤1	9 (37.5)	27 (81.8)	
1–19	0 (0)	0 (0)	
20–44	2 (8.3)	1 (3.0)	
45–64	4 (16.7)	1 (3.0)	
≥65	9 (37.5)	4 (12.1)	
Female sex	11 (45.8)	16 (48.5)	0.843
History of fever (≥ 38°C)	24 (100)	33 (100)	1
Onset of fever ≤7 d	12 (60.0)	18 (85.7)	0.063
Altered mental status[c]	24 (100)	33 (100)	1
Decreased consciousness[d]	8 (33.3)	2 (6.1)	0.012
Coma[e]	4 (16.7)	1 (3.0)	0.151
General or partial seizures	3 (12.5)	1 (3.0)	0.300
Focal neurologic signs	5 (20.8)	0 (0)	0.010
Other behavioral changes[f]	15 (62.5)	32 (97.0)	0.001
Skin rash	8 (33.3)	17 (51.5)	0.190
Intensive care support	10 (41.7)	3 (9.1)	0.009
Length of stay >4 d	14 (58.3)	13 (39.4)	0.187
Deaths	4 (16.7)	3 (9.1)	0.439

Abbreviations: CHIKV = Chikungunya virus; IgM = immunoglobulin M; NECACD = nonencephalitic Chikungunya virus–associated CNS disease.
[a] Unless stated, the data were available for 24 encephalitic cases and 33 cases of NECACD. The onset of fever was available for 20 encephalitic cases and 21 cases of NECACD.
[b] Fisher exact overall p value testing the 5 age categories.
[c] Decreased or altered level of consciousness, lethargy, or personality change (disorientation, agitation).
[d] Glasgow Coma Scale score <15.
[e] Glasgow Coma Scale score ≤9.
[f] Attention disorders, memory troubles, excessive pain feeling (irritability).

indicators of CSF inflammation, leukocyte count and protein level, were higher in patients with encephalitis than encephalopathy, but viral loads or IgM titers in serum or in CSF were not significantly different between these groups of patients (table 2). Importantly, encephalitic cases required more intensive care support than did NECACD cases (table 3).

The cohort contained 21 adults (mean age, 63.9 years; SD, 15.6 years; range, 33–88 years) and 36 infants (mean age, 1.6 months; SD, 1.15 months; range, 4 days–5.4 months).

Five infants were in the early neonatal period (<7 days) and 4 in the late neonatal period (7–28 days), corresponding to cases of mother-to-child and postnatal mosquito-borne transmission, respectively.

Infants were more likely to experience a recent onset of fever prior to hospitalization, behavioral changes, skin rash, or survival and adults were more likely to experience decreased consciousness, coma, focal neurologic signs, seizures, or a fatal issue (table e-1 on the *Neurology*® Web site at Neurology.org). Protein, glucose, and chloride CSF levels were higher in adults than infants (table e-2). CHIKV loads in the CSF or serum were higher in infants than adults, whereas it was the opposite for IgM. These results are in line with the fact that adults were observed later than infants in the course of the CHIKV-associated CNS disease. CHIKV loads in the CSF and serum for infants and between IgM levels in the CSF and serum for adults were positively correlated (data not shown). In infants, CHIKV loads in serum negatively correlated with age, while in adults IgM levels in serum positively correlated with age (data not shown).

Except for one neonate exhibiting cerebral edema MRI features, no early (<7 days) diffusion-weighted imaging (DWI) MRI scan was available for CHIKV-associated encephalitic cases, although DWI is increasingly recognized as the most sensitive technique for timely diagnosis of acute brain parenchyma inflammation.[22] Subsequently, no radiologic image evocative of acute stage of CHIKV-associated encephalitis was observed among the 22 other patients submitted to brain CT scans, late MRI scans, or both.

Cumulative incidence rates of CHIKV-associated encephalitis. The overall CIR estimate of CHIKV-associated encephalitis was 8.6 per 100,000 persons (95% confidence interval 6.9–10.4). Importantly, the age distribution pattern of CHIKV-associated CNS disease (figure 2A) or CHIKV-associated encephalitis incidence (figure 2B) exhibited a U-shaped parabolic pattern with a clear trend to the highest incidence towards the youngest age than the oldest.

Prospective cohort study. Six adult patients died (mean age 67.5 years; SD 15.7 years; range 41–83 years) during hospitalization (case-fatality rate [CFR] 10.5%). Detailed cause-specific mortality were cardiac failure (n = 2), septic shock (n = 2), respiratory failure (n = 1), and sudden death (n = 1). Death certificates mentioned Chikungunya as the primary cause for degradation in each case. As a consequence, 51 patients were eligible for the follow-up study.

Eight adults were discharged with neurologic sequelae (table e-3). One died 3 months after discharge (case 6). He was a 72-year-old man free from medical history presenting with altered mental status, classified as NECACD. He deteriorated gradually towards dementia and died in a clinical picture of metabolic encephalopathy due to dehydration and hypernatremia. EEG revealed a global slowdown without spike. Subacute stage CT scans showed extensive demyelination and cerebral subcortical atrophy. Four adult survivors were lost to follow-up and the 10 others were assessed clinically at 3 years. Of these, we diagnosed 3 patients with neurologic

© 2015 American Academy of Neurology. Unauthorized reproduction of this article is prohibited.

Table 2 Biological parameters of CHIKV-associated CNS disease in La Réunion Island (2005–2006)

Variables	Probable or possible encephalitis (n = 24)	NECACD (n = 33)	p Value
CSF leukocytes ≥5/mm³, n (%)	1 (70.8)	4 (12.1)	<0.001
CSF proteins ≥40 mg/dL, n (%)	21 (91.3)	16 (50.0)	0.001
CSF leukocytes/mm³, mean (SD)	12.8 (21.9)	1.7 (2.7)	<0.001
CSF proteins, mg/dL, mean (SD)	75.3 (39.4)	47.2 (23.7)	<0.001
CSF glucose, mmol/L, mean (SD)	4.2 (1.3)	3.7 (1.0)	0.086
CSF chloride, mmol/L, mean (SD)	123.3 (4.3)	121.1 (5.8)	0.039
CSF CHIKV load, cp/mL, mean (SD)	578,815 (1,787,072)	221,914 (796,673)	0.126
Serum CHIKV load, cp/mL, mean (SD)	1.2×10^8 (2.2×10^8)	9.0×10^7 (1.7×10^8)	0.330
CSF anti-CHIKV IgM, UI/L, mean (SD)	101.0 (83.0)	143.7 (168.7)	0.885
Serum anti-CHIKV IgM, UI/L, mean (SD)	139.3 (115.6)	57.3 (92.9)	0.219
CSF/serum CHIKV loads ratio, mean (SD)[a]	0.63 (0.14)	0.55 (0.20)	0.055
CSF/serum IgM levels ratio, mean (SD)[b]	0.41 (0.33)	0.96 (0.57)	0.069

Abbreviations: CHIKV = Chikungunya virus; IgM = immunoglobulin M; NECACD = nonencephalitic Chikungunya virus associated CNS disease.
CSF proteins were available for 23 encephalitic cases and 32 cases of NECACD. CHIKV loads were measured in the CSF available for 52 patients (36 infants and 16 adults). CSF of all infants, of whom 9 are encephalitic, and CSF of 4 adults with encephalitis are positive. Among the 12 CSF-negative adults, 9 were encephalitic. CHIKV loads were measured in the serum available for 37 patients (32 infants and 5 adults). Among infants, 31 were positive and 9 of them were encephalitic, while the 5 adults were negative. CHIKV IgM were searched in the CSF for 52 patients and in the serum for 37 patients. CSF was positive for 21 of 52 patients (2 weakly positive infants with NECACD and 19 highly positive adults, of whom 10 had encephalitis) and serum was positive for 32 of 37 patients (13 weakly positive infants of whom 6 had encephalitis; 19 highly positive adults, of whom 10 had encephalitis).
[a] Data are complete for 9 infants with encephalitis, 22 infants with NECACD.
[b] Data are complete for 12 adults with encephalitis, 4 adults with NECACD.

sequelae (epilepsy, postinfectious dementia, cognitive disorder, respectively) and 4 with an absence of detectable sequelae.

Nineteen infants were lost to follow-up, and 17 were evaluated at an average of 38 months of age. One developed severe cerebral palsy and blindness. He was a full-term normal for gestational age boy free from obstetrical history presenting with hemorrhagic fever on day 4 of life (case 2, table e-3). Subacute and late-stage MRI findings evidenced progressive decrease of cerebral and cerebellar hemorrhages and replacement of brain edema features by subsequent demyelination of the white matter, whose evolution contrasted with monophasic or multiphasic patterns of acute disseminated encephalomyelitis (ADEM). Four infants exhibited poor neurodevelopmental performance (Brunet-Lézine development quotients [DQ] ≤85), irrespective of prenatal alcohol exposure; the other 8 had age-appropriate skills (mean DQ 98, SD 9, range 86–120). Of these 5 children, 2 were infected vertically and 3 in the postneonatal period (day 17, day 35, and day 73, respectively) (table e-3). The medical history of the lost-to-follow-up infants was uneventful, except for one who developed Langherhans histiocytosis.

Given the high attrition in the follow-up and the risk for information bias, the burden of neurologic sequelae resulting from CHIKV-associated CNS disease could not be calculated precisely and was estimated to be in the range of 17.6% (9/51) to 43.1% (22/51). Nevertheless, lost-to-follow-up children corresponded to the milder forms of CNS disease, so that our estimates are likely conservative owing to the fact that the incidence of sequelae often correlates with the intensity of the acute stage of infection in a previously healthy population. For CHIKV-associated encephalitis, the CFR was 16.6% (4/24) and the 3-year burden of neurologic sequelae in the range of 30% (6/20) to 45% (9/20). Importantly, we observed an age difference in 3-year outcome of CHIKV-associated encephalitis, poor prognosis (i.e., death or sequelae) being predominant in adults (52.6% vs 18.2%, $p = 0.020$).

DISCUSSION This study reports findings on CHIKV-associated CNS disease (encephalitis and encephalopathy) using both CSF examination findings and IEC criteria for encephalitis. Our data reveal that during the 2005–2006 CHIKV outbreak in La Réunion Island, the incidence of CHIKV-associated encephalitis contributed to a twofold increase of the regional overall incidence (14.6 vs 6.0 cases per 100,000 persons per year at baseline) of all encephalitis. Remarkably, this burden far exceeds the annual rate of encephalitis calculated

© 2015 American Academy of Neurology. Unauthorized reproduction of this article is prohibited.

Table 3	Outcomes of CHIKV-associated CNS disease in La Réunion Island (2005–2009)		
Variables	Probable or possible encephalitis (n = 24), n (%)	NECACD[a] (n = 33), n (%)	p Value
Intensive care support	10 (41.7)	3 (9.1)	0.009
Length of stay >4 d	14 (58.3)	13 (39.4)	0.187
Extended Glasgow Outcome Scale			0.946[b]
Dead	3 (18.7)	4 (21.0)	
Vegetative state	0 (0)	0 (0)	
Lower severe disability	1 (6.3)	2 (10.5)	
Upper severe disability	0 (0)	2 (10.5)	
Lower moderate disability	1 (6.3)	1 (5.3)	
Upper moderate disability	3 (18.7)	2 (10.5)	
Lower good recovery	1 (6.3)	1 (5.3)	
Upper good recovery	7 (43.8)	7 (36.8)	
Not assessed	8	14	

Abbreviations: CHIKV = Chikungunya virus; GOSE = Extended Glasgow Outcome Scale; IgM = immunoglobulin M; NECACD = nonencephalitic Chikungunya virus-associated CNS disease.

[a] Unless stated, the data were available for 24 encephalitic cases and 33 NECACD cases. The GOSE was assessed at discharge for nonsurvivors and at 3 years postinfection for the survivors. We used both adult and pediatric versions of the GOSE. Percentages are calculated on a total of 35 patients.

[b] Fisher exact overall p value testing 7 of the 8 outcome categories.

for mainland France in 2000–2002 concerning encephalitis of infectious or specified etiology,[23] as well as the rate reported in the United States between 1998 and 2010 for all encephalitis.[21] Of note, the CIR of CHIKV-associated encephalitis in La Réunion Island was also superior to those observed with West Nile virus (WNV) and other neuroinvasive arboviral infections in the United States between 1999 and 2007,[24] or to the global incidence observed with Japanese encephalitis.[25]

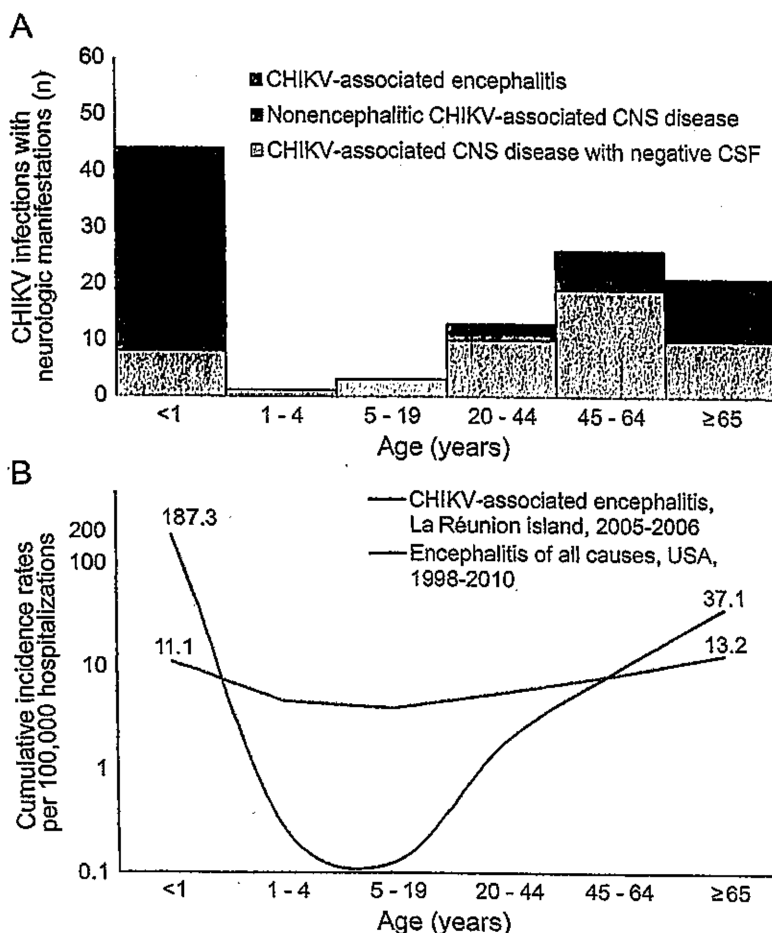
Though no similar study has been reported previously to our knowledge, our findings are consistent with earlier report of CNS conditions complicating CHIKV infection, ranging from mild neurocognitive or behavioral disorders to severe neurologic syndromes including acute stage encephalopathy/encephalitis, postinfective ADEM (encephalomyeloradiculitis), and postinfective Guillain-Barré syndrome (polyradiculoneuritis).[14–17] They are also in agreement with earlier observational studies, even though the criteria used to define encephalitis differ from those we used.[5,26–28] CHIKV-associated CNS disease prognosis seems similar to that of other viral etiologies. It was associated in our setting with more pejorative figures than previously reported in India,[26,27] or even recently in Thailand.[28] This substantial toll is compatible with that of other virus-associated encephalitis.[29–31]

The age distribution of encephalitis incidence was U-shaped, with 2 peaks observed in young infants and older adults, consistent with the distribution of encephalitis in general populations,[21,29,30] or with overall neuroinvasive disease of viral origin reported in the United States.[21] The CIR of CHIKV-associated encephalitis in these extreme age groups is 25-fold higher for children under 1 year and 6-fold higher for people over 65 years than those found for encephalitis of specified etiology in the United States.[21] This also contrasts the bimodal age distribution of herpes simplex virus encephalitis, which peaks between 60 and 64 years[32]; that of WNV, which has a greater effect on the elderly[24,33]; or that of La Crosse virus, which targets young children.[24,34] Higher susceptibility of children to CHIKV-associated encephalitis is also supported by the cerebral edema features observed in a neonate infected by mother-to-child transmission. Accordingly, young mice are more susceptible to CHIKV than adult mice.[35] CHIKV targets the choroid plexuses and meningeal and ependymal envelopes, but does not invade brain parenchyma of adult mice deficient for type 1 interferon and adult monkeys,[35,36] despite the presence of viral RNA or infectious virus in the CSF of animals during the acute phase of infection, whereas CHIKV infects neurons of neonatal/suckling mice.[37] A defective host response may contribute to the higher susceptibility of neonates to CHIKV, as suggested by studies showing that the neonatal immune response is quantitatively and qualitatively distinct from that of adults.[38] Thus, in contrast to New World alphaviruses that cause encephalitis in humans and in animal models as a consequence of viral invasion of the brain parenchyma,[39] CHIKV is not a neurotropic virus in experimentally infected adult animals, although it disseminates and replicates in the meningeal and ependymal envelopes.[35]

In contrast to what is observed in adults, CSF and serum of infants contained CHIKV RNA, which is likely explained by their earlier presentation to the hospital in our cohort. Moreover, the higher the CHIKV load was in the serum, the higher it was in the CSF, and we made the same observation in adult CHIKV-specific IgM (CSF/serum ratios smaller than 1). Therefore a passive diffusion of viral RNA or IgM from the serum to the CSF, either by traumatic LP or as a result of a leakage in the blood–brain barrier, rather than CHIKV replication or IgM production in the CNS, cannot be excluded.

Importantly, although infants appear more susceptible to CHIKV-associated encephalitis, the clinical presentation and 3-year outcome of CHIKV-associated encephalitis were more severe in adults than in infants, except for one neonate. As also observed in the CHIMERE cohort study,[40] they consisted exclusively of behavioral changes and neurocognitive impairment in infants, while they affected cortical functioning and led to disabling sequelae in

© 2015 American Academy of Neurology. Unauthorized reproduction of this article is prohibited.



(A) Total case reports (probable + possible) of Chikungunya virus (CHIKV)-associated encephalitis, nonencephalitic CHIKV CNS disease, and CHIKV-associated CNS disease with negative CSF (i.e., CHIKV infections with neurologic manifestations compatible with aforementioned diagnoses but negative CSF for immunoglobulin M and PCR) by age categories. (B) Age-stratified cumulative incidence rates of CHIKV-associated encephalitis (probable + possible) in La Réunion or the age-stratified cumulative incidence rates of all encephalitis in the United States.

adults. These data are in line with French national data and WNV encephalitis in the United States showing milder presentation and better outcomes in children.[20,31,33]

Our study has some limitations. First, we have not searched for CHIKV RNA and IgM systematically in patients presenting neurologic manifestations. Second, LP was not repeated in the absence of clinical deterioration. We may therefore have missed pathologic changes in CSF protein level or leukocyte count, notably in neonates who are prone to prohemorrhagic conditions.[14] Third, we have not performed neuroradiologic examination routinely, so that patients with mild neurologic forms were probably underestimated, while in turn, cases of major CNS disease could be unstable to undergo timely MRI scans. Thus, as our study was not population-based, we may have underestimated the real burden and slightly overestimated the CFR and incidence of neurologic sequelae. Fourth, the data collection was partially retrospective and we may have missed some minor symptoms, such as tremors or other movement disorders indicative of thalamic or basal ganglia involvement.[22] We focused on the symptoms whose presence was constantly noted, so that our description of CHIKV-associated CNS disease is likely conservative and limits information bias. Fifth, our study was restricted to a fairly localized area, so we cannot rule out that the extent of CHIKV-associated CNS disease in recent years may reflect a stronger neurovirulence of the ECSA sublineage. Encephalitis has not yet been described everywhere the ECSA genotype has circulated. The occurrence of encephalitis may depend of the magnitude of the outbreak, by targeting susceptible hosts to CHIKV-associated CNS disease. The study of host and CHIKV genetic factors underlying CHIKV-associated CNS disease may help better understand the pathogenesis of CHIKV-associated CNS disease. In this regard, we have much to learn from current outbreaks throughout the world due to African and Asian lineage viruses.

CHIKV-associated CNS disease, including encephalitis as defined by the IEC, may complicate CHIKV infection. Altogether these data contribute to improve the knowledge of CHIKV-associated neuropathology and illustrate the clinical neurotropism of CHIKV and its deleterious consequences, especially in neonates.

AUTHOR CONTRIBUTIONS

Patrick Gérardin: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, accepts responsibility for conduct of research and final approval, acquisition of data, statistical analysis. Thérèse Couderc: drafting/revising the manuscript, analysis or interpretation of data, accepts responsibility for conduct of research and final approval. Marc Bintner: drafting/revising the manuscript, analysis or interpretation of data, accepts responsibility for conduct of research and final approval, acquisition of data. Patrice Tournebize: drafting/revising the manuscript, analysis or interpretation of data, accepts responsibility for conduct of research and final approval, acquisition of data. Michel Renouil: drafting/revising the manuscript, analysis or interpretation of data, accepts responsibility for conduct of research and final approval, acquisition of data. Jérome Lémant: drafting/revising the manuscript, accepts responsibility for conduct of research and final approval, acquisition of data. Véronique Boisson: analysis or interpretation of data, accepts responsibility for conduct of research and final approval, acquisition of data. Gianandrea Borgherini: analysis or interpretation of data, accepts responsibility for conduct of research and final approval. Frédérik Staikowsky: drafting/revising the manuscript, accepts responsibility for conduct of research and final approval, acquisition of data. Frédéric Schramm: drafting/revising the manuscript, analysis or interpretation of data, accepts responsibility for conduct of research and final approval, acquisition of data. Marc Lecuit: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, accepts responsibility for conduct of research and final approval, study supervision, obtaining funding. Alain Michault: drafting/revising the manuscript, accepts responsibility for conduct of research and final approval.

ACKNOWLEDGMENT

The authors thank the coinvestigators and contributors and the staff of the Microbiology Laboratory and the Center for Clinical Investigation. Alain Michault (1950–2015) dedicated his career to epidemiology of

© 2015 American Academy of Neurology. Unauthorized reproduction of this article is prohibited.

communicable diseases in Réunion Island and South West Indian Ocean. Alain facilitated most of the major partnerships and publications made on the Chikungunya virus by our institution. He died on August 5, 2015, of cancer. The authors convey their sympathy to his wife, his 2 sons, and his family.

STUDY FUNDING

Supported by Institut Pasteur, Inserm, ICRES FP7, and LabEx IBEID, Ville de Paris, Fondation BNP Paribas.

DISCLOSURE

The authors report no disclosures relevant to the manuscript. Dr. Michault is deceased; disclosures are not included for this author. Go to Neurology.org for full disclosures.

Received February 18, 2015. Accepted in final form August 31, 2015.

REFERENCES

1. Staples JE, Breiman RF, Powers AM. Chikungunya fever: an epidemiological review of re-emerging infectious disease. Clin Infect Dis 2009;49:942–948.
2. Weaver SC, Winegar R, Manger ID, Forrester NL. Alphaviruses: population genetics and determinants of emergence. Antivir Res 2012;94:242–257.
3. Brighton SW, Prozesky OW, de La Harpe AL. Chikungunya virus infection: a retrospective study of 107 cases. S Afr Med J 1983;63:313–315.
4. Chastel C. Human infections in Cambodia by the Chikungunya virus or a closely related agent: serology. Bull Soc Path Exot 1963;56:892–915.
5. Carey DE, Myers RM, DeRanitz CM, Jadhav M, Reuben R. The 1964 Chikungunya epidemic at Vellore, South India, including observations on concurrent dengue. Trans R Soc Trop Med Hyg 1969;63:434–445.
6. Schuffenecker I, Iteman I, Michault A, et al. Genome microevolution of Chikungunya viruses causing the Indian Ocean outbreak. PLoS Med 2006;3:e263.
7. Tsetsarkin KA; Vanlandingham DL, Mc Gee CE, Higgs S. A single mutation in Chikungunya virus affects vector specificity and epidemic potential. PLoS Pathog 2007;3:e201.
8. Leparc-Goffart I, Nougairede A, Cassadou S, Prat C, de Lamballerie X. Chikungunya in the Americas. Lancet 2014;383:514.
9. Miner JJ, Aw-Yeang YH, Fox JM, et al. Chikungunya viral arthritis in the United States: a mimic of seronegative rheumatoid arthritis. Arthritis Rheumatol 2015;67: 1214–1220.
10. Teo TH, Her Z, Tan JJ, et al. Carribbean and La Réunion Chikungunya virus isolates differ in the capacity to induce pro-inflammatory Th1 and NK cell responses and acute joint pathology. J Virol 2015;89: 7955–7969.
11. Gérardin P, Guernier V, Perrau J, et al. Estimating Chikungunya prevalence in La Réunion Island outbreak by serosurveys: two methods for two critical times of the epidemic. BMC Infect Dis 2008;8:99.
12. Lemant J, Boisson V, Winer A, et al. Serious acute Chikungunya virus infection requiring intensive care during the Réunion Island outbreak in 2005-2006. Crit Care Med 2008;36:2536–2541.
13. Gérardin P, Barau G, Michault A, et al. Multidisciplinary prospective study of mother-to-child Chikungunya virus infections on the island of La Réunion. PLoS Med 2008;5:e60.
14. Tournebize P, Charlin C, Lagrange M. Neurological manifestations in Chikungunya: about 23 cases collected in Réunion Island [in French]. Rev Neurol 2009;165:48–51.
15. Ganesan K, Diwan A, Shankar SK, et al. Chikungunya encephalomyeloradiculitis: report of 2 cases with neuroimaging and 1case with autopsy findings. AJNR Am J Neuroradiol 2008;29:1636–1637.
16. Lebrun G, Khadda K, Reboux AH, Martinet O, Gaüzère BA. Guillain-Barré syndrome after Chikungunya infection. Emerg Infect Dis 2009;15:495–496.
17. Gérardin P, Fianu A, Malvy D, et al. Perceived morbidity and community burden after a Chikungunya outbreak: the TELECHIK survey, a population-based-cohort study. BMC Med 2011;9:5.
18. Venkatesan A, Tunkel AR, Bloch KC, et al. Case definitions, diagnostic algorithms, and priorities in encephalitis: consensus statement of the International Encephalitis Consortium. Clin Infect Dis 2013;57:1114–1128.
19. Mailles A, Stahl JP. Infectious encephalitis in France in 2007: a national prospective study. Clin Infect Dis 2009; 49:1838–1847.
20. Beers SR, Wisniewski SR, Garcia-Fillion P, et al. Validity of a pediatric version of the Glasgow Outcome Scale–Extended. J Neurotrauma 2012;29:1126–1139.
21. Vora NM, Holman RC, Mehal JM, Steiner CA, Blanton J, Sejvar J. Burden of encephalitis-associated hospitalizations in the United States, 1998–2010. Neurology 2014;82:443–451.
22. Solomon T, Michael BD, Smith PE, et al. Management of suspected viral encephalitis in adults: Association of British Neurologists and British Infection Association National guidelines. J Infect 2012;64:347–363.
23. Mailles A, Vaillant V, Stahl JP. Encéphalites infectieuses: données et limites du PMSI pour l'étude épidémiologique, France métropolitaine 2000–2002. Med Mal Infect 2007; 37:95–102.
24. Reimann CA, Hayes EB, DiGuiseppi C, et al. Epidemiology of neuroinvasive arboviral disease in the United States, 1999–2007. Am J Trop Med Hyg 2008;76: 974–979.
25. Campbell GL, Hills SL, Fischer M, et al. Estimated global incidence of Japanese encephalitis: a systematic review. Bull World Health Organ 2011;89:766–774.
26. Rampal, Sharda M, Meena H. Neurological complications in Chikungunya fever. J Assoc Physicians India 2007;55: 765–769.
27. Lewthwaite P, Vasanthapuram R, Osborne JC, et al. Chikungunya virus and central nervous system infections in children, India. Emerg Infect Dis 2009;15:329–331.
28. Chusri S, Siripaitoon P, Hirunpat S, Silpapojakul K. Short report: cases reports of neurochikungunya in southern Thailand. Am J Trop Med Hyg 2011;85:386–389.
29. Glaser CA, Honarmand S, Anderson LJ, et al. Beyond viruses: clinical profiles and etiologies associated with encephalitis. Clin Infect Dis 2006;43:1565–1577.
30. Granerod J, Ambrose HE, Davies NWS, Clewley JP, Walsh AL, Morgan D. Causes of encephalitis and differences in their clinical presentations in England: a multicentre, population-based prospective study. Lancet Infect Dis 2010;10:835–844.
31. Mailles A, De Broucker T, Costanzo P, et al. Long-term outcome of patients presenting with acute infectious encephalitis of various causes in France. Clin Infect Dis 2012;54:1455–1464.

© 2015 American Academy of Neurology. Unauthorized reproduction of this article is prohibited.

32. Steiner I, Kennedy PGE, Pachner AR. The neurotropic herpes viruses: herpes simplex and varicella-zoster. Lancet Neurol 2007;6:1015–1028.
33. Sejvar J. The long-term outcomes of human West Nile virus infection. Clin Infect Dis 2007;44:1617–1624.
34. Gaensbauer JT, Lindsey NP, Messacar K, Staples JE, Fischer M. Neuroinvasive arboviral disease in the United States: 2003 to 2012. Pediatrics 2014;134:e642–e650.
35. Couderc T, Chrétien F, Schilte C, et al. A mouse model for Chikungunya: young age and inefficient type-I interferon signaling are risk factors for severe disease. PLoS Pathog 2008;4:e29.
36. Labadie K, Larcher T, Joubert C, et al. Chikungunya disease in nonhuman primates involves long-term viral persistence in macrophages. J Clin Invest 2010;3: 894–906.
37. Fraisier C, Koraka P, Belghazi M, et al. Kinetic analysis of mouse brain proteome alterations following Chikungunya virus infection before and after appearance of clinical symptoms. PLoS One 2014;9:e91397.
38. Adkins B, Marshall-Clarke S, Leclerc C. Neonatal adaptive immunity comes of age. Nat Rev Immunol 2004;4:553–564.
39. Zacks MA, Paessler S. Encephalitic alphaviruses. Vet Microbiol 2010;140:281–286.
40. Gérardin P, Sampériz S, Ramful D, et al. Neurocognitive outcome of children exposed to perinatal mother-to-child Chikungunya virus infection: the CHIMERE cohort study on Réunion Island. PLoS Negl Trop Dis 2014;8:e2996.

20 Minutes Pack a Punch

***Neurology*® Podcasts**

- Interviews with top experts on new clinical research in neurology
- Editorial comments on selected articles
- Convenient—listen during your commute, at your desk, or even at the gym
- On demand—it's there when you want it
- Fun and engaging
- New topic each week
- FREE

Listen now at ***www.aan.com/podcast***

Introducing EBM Online—FREE to AAN Members!

The American Academy of Neurology again demonstrates its commitment to high-quality neurology education by converting and refining its popular classroom evidence-based medicine training into a convenient on-demand, self-paced online program. Only EBM Online from the AAN provides the trusted expertise of the source of the world's most respected neurology guidelines.

EBM Online:

- Features five hours of convenient, interactive courses in 10 modules
- Uses common, real-life neurologic clinical examples .
- Measures outcomes with pre-test, post-test, and module evaluations
- Provides helpful feedback on individual knowledge and more

This program is available now to residents and fellows; practicing neurologists are encouraged to register when CME is available in 2016.

Learn more at *AAN.com/view/EBMOnline.*

© 2015 American Academy of Neurology. Unauthorized reproduction of this article is prohibited.

Neurology®

Chikungunya virus–associated encephalitis: A cohort study on La Réunion Island, 2005–2009

Patrick Gérardin, Thérèse Couderc, Marc Bintner, et al.

Neurology 2016;86;94-102 Published Online before print November 25, 2015
DOI 10.1212/WNL.0000000000002234

This information is current as of November 25, 2015

Updated Information & Services	including high resolution figures, can be found at: http://www.neurology.org/content/86/1/94.full.html
Supplementary Material	Supplementary material can be found at: http://www.neurology.org/content/suppl/2015/12/18/WNL.0000000000002234.DC1.html http://www.neurology.org/content/suppl/2015/12/18/WNL.0000000000002234.DC2.html
References	This article cites 40 articles, 11 of which you can access for free at: http://www.neurology.org/content/86/1/94.full.html##ref-list-1
Subspecialty Collections	This article, along with others on similar topics, appears in the following collection(s): **Cohort studies** http://www.neurology.org//cgi/collection/cohort_studies **Encephalitis** http://www.neurology.org//cgi/collection/encephalitis **Prognosis** http://www.neurology.org//cgi/collection/prognosis **Viral infections** http://www.neurology.org//cgi/collection/viral_infections
Permissions & Licensing	Information about reproducing this article in parts (figures,tables) or in its entirety can be found online at: http://www.neurology.org/misc/about.xhtml#permissions
Reprints	Information about ordering reprints can be found online: http://www.neurology.org/misc/addir.xhtml#reprintsus

Neurology ® is the official journal of the American Academy of Neurology. Published continuously since 1951, it is now a weekly with 48 issues per year. Copyright © 2015 American Academy of Neurology. All rights reserved. Print ISSN: 0028-3878. Online ISSN: 1526-632X.

別紙様式第2-1

No. 12

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日 2016. 1. 7	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	新鮮凍結人血漿		研究報告の公表状況	Oehler E, Fournier E, Leparc-Goffart I, et al. Euro Surveill. 2015 Dec 3;20(48). doi: 10.2807/1560-7917.ES.2015.20.48.30079.	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

○フランス領ポリネシアで発生したチクングニヤ熱のアウトブレイクにおけるギラン・バレー症候群（GBS）症例の増加（2014年～2015年）。
フランス領ポリネシアでは近年チクングニヤ熱のアウトブレイクが発生しており、発生期間（2014年10月～2015年3月）における神経障害患者数が異常に多いことを確認した。臨床症状及び補完的検査により、9名の患者でGBSとチクングニヤ熱の関連性が疑われた。9名全員が少し前にデング熱様症状を有しており、血清学的検査により8名にはチクングニヤウイルス（CHIKV）に対するIgM及びIgG抗体が検出され、1名はCHIKVに対するRT-PCR法で陽性となった。全員がデングウイルスIgG抗体、8名がジカウイルスIgG抗体は陽性であったが、双方のウイルスに対するIgM抗体については全員が陰性であった。当該期間にGBSの発症率が例年の4～9倍に増加したため、CHIKV感染との関連が示唆された。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見

フランス領ポリネシアではチクングニヤ熱のアウトブレイクが発生した期間に、ギラン・バレー症候群の症例数が例年の4～9倍に増加し、チクングニヤウイルス感染との関連が示唆されたという報告である。

今後の対応

日本赤十字社では、輸血感染症対策として献血時に海外渡航歴の有無を確認し、帰国（入国）後4週間は献血不適としている。国内でチクングニヤ熱が確認されたため、渡航歴確認の徹底を図っている。また、チクングニヤ熱の既往歴がある場合、治癒後6ヵ月間は献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

RAPID COMMUNICATIONS

Increase in cases of Guillain-Barré syndrome during a Chikungunya outbreak, French Polynesia, 2014 to 2015

E Oehler [1], E Fournier [2], I Leparc-Goffart [3], P Larre [4], S Cubizolle [4], C Sookhareea [4], S Lastère [5], F Ghawche [4]
1. Internal Medicine Department, French Polynesia Tertiary Hospital, Tahiti, French Polynesia
2. Clinical Neurophysiology Department, Pitié Salpétrière, APHP, Paris, France
3. French National Reference Centre for Arboviruses, IRBA, Marseille, France
4. Neurology Department, French Polynesia Tertiary Hospital, Tahiti, French Polynesia
5. Clinical Virology Laboratory, French Polynesia Tertiary Hospital, Tahiti, French Polynesia

Correspondence: Erwan Oehler (erwan.oehler@cht.pf)

Citation style for this article:
Oehler E, Fournier E, Leparc-Goffart I, Larre P, Cubizolle S, Sookhareea C, Lastère S, Ghawche F. Increase in cases of Guillain-Barré syndrome during a Chikungunya outbreak, French Polynesia, 2014 to 2015. Euro Surveill. 2015;20(48):pii=30079. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2015.20.48.30079

Article submitted on 24 November 2015 / accepted on 03 December 2015 / published on 03 December 2015

During the recent chikungunya fever outbreak in French Polynesia in October 2014 to March 2015, we observed an abnormally high number of patients with neurological deficit. Clinical presentation and complementary exams were suggestive of Guillain–Barré syndrome (GBS) for nine patients. All nine had a recent dengue-like syndrome and tested positive for chikungunya virus (CHIKV) in serology or RT-PCR. GBS incidence was increased four- to nine-fold during this period, suggesting a link to CHIKV infection.

Between October 2014 and March 2015, an estimated 66,000 cases of chikungunya virus infections were reported in French Polynesia, with an overall attack rate of 25% [1]. At the same time, we observed an epidemiological cluster of cases Guillain–Barré syndrome (GBS), an acute and probably autoimmune demyelinating polyradiculoneuropathy that appears after a triggering (viral or bacterial) infection in almost two thirds of cases. GBS had already been associated with several arboviral diseases including chikungunya virus (CHIKV) infections [2,3], but a cluster as we reported here had never been described.

Cluster description

Among the reported chikungunya fever cases, ca 50 people developed complications (myocarditis, meningoencephalitis, GBS) and 18 died; most of them had comorbidities. Nine patients were admitted for GBS based on clinical assessment, in the French Polynesia tertiary hospital: three in November, three in December, two in January and one in February. Six patients were male (male/female ratio: 2), with a median age of 48 years (range: 37–77 years). Eight patients were Polynesian and one was Caucasian from Metropolitan France. Two patients had hypertension and one had hypertension and diabetes mellitus; the six others did not have any underlying conditions.

All nine were referred and then admitted to the department of neurology with a median length of stay of 11 days (range: 6–21 days). The patients were hospitalised within a median of eight days (range: 3–40 days) after the onset of symptoms of infection. Upon admission none had any symptoms of this acute phase which had presented as a dengue-like syndrome: all patients were febrile, eight had arthromyalgia, and three had a rash. GBS, presenting as a sensorimotor deficit beginning in the lower limbs and evolving to the upper limbs, was reported for eight patients; the last patient only had facial diplegia with sensory disorders of the face. Seven patients presented with signs of cranial nerve involvement, facial paralysis, dysphonia, dysarthria or dysphagia, associated with severity of GBS [4]. Among them, four were hospitalised in the intensive care unit for a median length of stay of six days (range: 2–15 days).

Diagnostic findings in Guillain–Barré syndrome patients

Eight patients had IgM and IgG antibodies against CHIKV and one was positive for CHIKV in RT-PCR positive. Serological evidence of past dengue or Zika virus infection (IgG) was found in nine and eight patients, respectively, IgM for both infections was negative in all cases; no PCR was done for Zika virus. No PCR was performed for dengue virus either but when performed, it was negative.

Brain and spinal magnetic resonance imaging (MRI) was performed in seven patients within a median of nine days (range: 5–15 days) after the onset of neurological symptoms. Among the seven patients presenting with cranial nerves involvement, MRI showed signs of neuritis of the facial nerves in two patients and neuritis of the facial and left trigeminal nerves in one patient; no abnormalities were seen in the others. MRIs which disclosed signs of neuritis were performed at least eight

days after the beginning of GBS; those whose MRI was normal had it at day 5 and day 6; two patients only had a computerised tomography brain scan without contrast enhancement.

Electromyography was performed in all nine patients within a median of seven days (range: 3–14 days) after the start of GBS. It disclosed significant prolongation of the motor distal latencies with reduction of distal motor amplitudes, which attested a severe and predominant impairment of motor conduction in distal part of the nerves; motor conduction of more proximal nerve segments was less affected and little sensory impairment was seen.

All patients underwent a lumbar puncture, which was performed within a median of six days after the onset of neurological deficit (range: 2–15 days). Cerebrospinal fluid (CSF) was characterised by an albuminocytologic dissociation (elevated total protein concentration without CSF cell count abnormality) in all cases; median spinal protein concentration was 1.26 g/L (range: 0.82–4.97; norm <0.5), whereas the median cell count was 2/mm³ (range: 1–6; norm <5). Glucose levels were normal, with a median CSF/plasma ratio of 0.6 (range: 0.43–0.70; norm <0.75); CSF lactate levels were slightly elevated at a median of 167 mg/L (range: 117–251; norm <190). Plasma level of antiganglioside antibodies was measured in eight patients (anti-GM1, GM2, GD1a, GD1b, anti GQ1b IgM and IgG antibodies); a low positivity of anti-GM2 was noted in one case and of anti-GD1a in another case.

All patients were treated with intravenous immunoglobulin. Six patients were discharged to a functional rehabilitation centre for a median stay of 28 days (range: 19–60 days). In all patients, electrophysiological parameters quickly returned to almost normal levels within less than three months.

Discussion

Chikungunya fever is an arboviral disease that usually manifests as a self-liming dengue-like syndrome with high fever, severe arthralgias and myalgias, and a maculopapular rash. Rare but severe complications may occur, such as myocarditis, hepatitis and neurological manifestations [5].

Neurological tropism of CHIKV seems to be lower than of other arboviruses such as dengue, West Nile or yellow fever viruses, but several studies have described, especially during epidemics, neurological manifestations such as meningoencephalitis, seizures or GBS [6]. In some cases, IgM antibodies against CHIKV have been found in CSF of patients with meningitis, supporting the theory of neuroinvasion [7].

While the annual incidence of GBS in French Polynesia is between 1 and 2 per 100,000 inhabitants, nine cases of GBS were admitted to our hospital during this six-month outbreak of chikungunya, a four- to nine-fold increased incidence, leading us to suspect a causal relationship between CHIKV infection and GBS. To our knowledge, this is the first series of GBS temporally associated with a chikungunya fever outbreak. Characteristics of the neurological presentation of our patients are not different from GBS related with, or following other aetiologies. We thus recommend keeping in mind that GBS is not an uncommon possibility in neurological disorders associated with CHIKV virus infection, especially in an epidemic context. Finally, it is interesting to note that this is the second arbovirus-triggered outbreak of GBS in French Polynesia within two years: the first one, during the Zika fever outbreak from 2013 to 2014, resulted in 42 cases and a 20-fold increase in the annual incidence of GBS [8].

Our report adds to the mounting body of evidence about the possibility of severe neurological disease following CHIKV infections.

Conflict of interest

None declared.

Authors' contributions

EO, SL, EF, ILG wrote the manuscript. EO, PL, SC, CS, FG took part in the clinical management of patients during the outbreak. SL collaborated in molecular biology techniques. ILG collaborated on the virological investigation. EF collaborated on the neurophysiological investigation. All authors read and approved the final manuscript.

References

1. Nhan TX, Musso D. The burden of chikungunya in the Pacific. Clin Microbiol Infect. 2015;21(6):e47-8. DOI: 10.1016/j.cmi.2015.02.018 PMID: 25749562
2. Lebrun G, Chadda K, Reboux AH, Martinet O, Gaüzère BA. Guillain-Barré syndrome after chikungunya infection.Emerg Infect Dis. 2009;15(3):495-6. DOI: 10.3201/eid1503.071482 PMID: 19239775
3. Villamil-Gómez W, Silvera LA, Páez-Castellanos J, Rodriguez-Morales AJ. Guillain-Barré syndrome after Chikungunya infection: A case in Colombia.Enferm Infecc Microbiol Clin. 2015. DOI: 10.1016/j.eimc.2015.05.012 PMID: 26164263
4. Bhargava A, Banakar BF, Pujar GS, Khichar S. A study of Guillain-Barré syndrome with reference to cranial neuropathy and its prognostic implication. J Neurosci Rural Pract. 2014;5(Suppl 1):43-7.
5. Lemant J, Boisson V, Winer A, Thibault L, André H, Tixier F, et al. Serious acute chikungunya virus infection requiring intensive care during the Reunion Island outbreak in 2005-2006. Crit Care Med. 2008;36(9):2536-41. DOI: 10.1097/CCM.0b013e318183f2d2 PMID: 18679124
6. Lewthwaite P, Vasanthapuram R, Osborne JC, Begum A, Plank JL, Shankar MV, et al. Chikungunya virus and central nervous system infections in children, India. Emerg Infect Dis. 2009;15(2):329-31. DOI: 10.3201/eid1502.080902 PMID: 19193287
7. Chusri S, Siripaitoon P, Hirunpat S, Silpapojakul K. Case reports of neuro-Chikungunya in southern Thailand.Am J Trop Med Hyg. 2011;85(2):386-9. DOI: 10.4269/ajtmh.2011.10-0725 PMID: 21813863
8. Oehler E, Watrin L, Larre P, Leparc-Goffart I, Lastère S, Valour F, et al. Zika virus infection complicated by Guillain-Barre syndrome--case report, French Polynesia, December 2013. Euro Surveill. 2014;19(9):20720. DOI: 10.2807/1560-7917.ES2014.19.9.20720 PMID: 24626205

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2015. 11. 4	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	Duong V, Lambrechts L, Paul RE, et al. Proc Natl Acad Sci U S A. 2015 Nov 24;112(47):14688-93.	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

○デング：無症候者が蚊にデングウイルス(DENV)を伝播。
毎年3億9千万症例が発生すると推定されているDENV感染症例の4分の3は臨床的に不顕性である。DENV不顕性感染者は、ウイルス血症が蚊を感染させる上で十分な高いレベルには達しないため、一般的に感染の終末宿主であると考えられている。しかしながら、無症候者はウイルス血症の平均レベルが低い状態であっても蚊に対して感染力を有している可能性があることが分かった。更に、ウイルス血症が一定のレベルに達している場合、不顕性感染または発症前の状態にあるDENV感染者の蚊に対する感染力は、発症した感染者と比較して有意に高くなる。臨床症状が認められないDENV血症患者は、日常生活において発症した患者よりも更に多くの蚊に曝露される可能性がある。また無症候のDENV血症患者はDENV感染症例の大半を占める可能性がある。したがって我々のデータは、蚊へのウイルスの伝播において、無症候のDENV血症患者が寄与する可能性のレベルは、従来認識されていたものよりも著しく高いことを示している。

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

臨床症状が認められないデングウイルス血症患者は、発症している患者よりも蚊に対してより感染力があるという報告である。

今後の対応

日本赤十字社では、輸血感染症対策として献血時に海外渡航歴の有無を確認し、帰国（入国）後4週間は献血不適としている。また、国内においてデング熱感染が確認された場合は、感染が確認された地区への訪問歴を確認し、最後の訪問から4週間献血不適とするとともに、献血前後の発熱等に関する情報収集を強化することとしている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

JRC2015T-038 CrossMark click for updates

Asymptomatic humans transmit dengue virus to mosquitoes

Veasna Duong[a,1], Louis Lambrechts[b,c,1], Richard E. Paul[c,d], Sowath Ly[e], Rath Srey Lay[a], Kanya C. Long[f], Rekol Huy[g], Arnaud Tarantola[e], Thomas W. Scott[f,h], Anavaj Sakuntabhai[c,d], and Philippe Buchy[a,i,2]

[a]Virology Unit, Institut Pasteur in Cambodia, International Network of Pasteur Institutes, 12201 Phnom Penh, Cambodia; [b]Insect–Virus Interactions Group, Department of Genomes and Genetics, Institut Pasteur, 75015 Paris, France; [c]Centre National de la Recherche Scientifique, Unité de Recherche Associée 3012, 75015 Paris, France; [d]Functional Genetics of Infectious Diseases Unit, Department of Genomes and Genetics, Institut Pasteur, 75015 Paris, France; [e]Epidemiology and Public Health Unit, Institut Pasteur in Cambodia, International Network of Pasteur Institutes, 12201 Phnom Penh, Cambodia; [f]Department of Entomology and Nematology, University of California, Davis, CA 95616; [g]National Center for Malariology, Ministry of Health, 12302 Phnom Penh, Cambodia; [h]Fogarty International Center, National Institutes of Health, Bethesda, MD 20892; and [i]GlaxoSmithKline, Vaccines Value Health Sciences, Singapore 189720

Edited by Luciano A. Moreira, Centro de Pesquisas René Rachou FIOCRUZ-MG, Belo Horizonte, Brazil, and accepted by the Editorial Board October 7, 2015 (received for review April 25, 2015)

Three-quarters of the estimated 390 million dengue virus (DENV) infections each year are clinically inapparent. People with inapparent dengue virus infections are generally considered dead-end hosts for transmission because they do not reach sufficiently high viremia levels to infect mosquitoes. Here, we show that, despite their lower average level of viremia, asymptomatic people can be infectious to mosquitoes. Moreover, at a given level of viremia, DENV-infected people with no detectable symptoms or before the onset of symptoms are significantly more infectious to mosquitoes than people with symptomatic infections. Because DENV viremic people without clinical symptoms may be exposed to more mosquitoes through their undisrupted daily routines than sick people and represent the bulk of DENV infections, our data indicate that they have the potential to contribute significantly more to virus transmission to mosquitoes than previously recognized.

mosquito experimental infection | Cambodia | *Aedes aegypti* | human-to-mosquito transmission | dengue

With 3.97 billion people living in 128 countries currently at risk for infection, dengue viruses (DENV-1 to -4) cause more human morbidity and mortality worldwide than any other arthropod-borne virus (1, 2). *Aedes aegypti* mosquitoes are the primary vectors of DENV throughout the tropics (3). Dengue prevention relies on the control of *Ae. aegypti* populations, which is failing in most parts of the world due to lack of resources, lack of political will, and/or ineffective implementation (4).

Virus transmission from infected humans to mosquitoes is a critical step in dengue epidemiology, but due to logistical constraints it has been directly examined only in a handful of studies to date (5). In initial experimental infections of human volunteers during the 1920s (6, 7), the onset of clinical symptoms occurred 4–9 d after virus inoculation by mosquito bite (8). DENV-infected humans were infectious to mosquitoes from 2 d before to 2 d after the onset of symptoms, and *Ae. aegypti* fed on viremic people were able to transmit virus to another person after at least 11 d of extrinsic incubation (8). Results from later studies indicated that, for naturally infected people with clinically apparent dengue, the duration of detectable viremia was on average 4–5 d after the onset of symptoms, but could range from 2 to 12 d (9, 10). Investigators in Vietnam fed *Ae. aegypti* directly on 208 symptomatic, hospitalized dengue patients and reported that the probability of successful human-to-mosquito DENV transmission was coincident with the kinetics of viremia (11). Dengue patients were infectious up to 5 d after the onset of symptoms, which generally corresponded with "defervescence" (11).

All previous studies on human-to-mosquito DENV transmission were limited to people with overt illness and did not consider subclinical infections. An estimated 300 million of the total 390 million DENV infections per year are clinically inapparent or mildly symptomatic, i.e., no illness that disrupted a person's daily routine (1). Following Grange et al. (12), we use "inapparent" or "subclinical" interchangeably to denote infections confirmed by virus detection or seroconversion, but with insufficient symptoms to be detected by existing surveillance systems and health care providers. "Asymptomatic" refers to a confirmed DENV infection in the complete absence of reported or detected symptoms. Inapparent human DENV infections are a potentially important component of the overall burden of dengue because they can serve as a previously unrecognized source of mosquito infection (12). Epidemic transmission of DENV associated with low viremia levels and mild illness has been reported (13). It has long been assumed, but not empirically verified, that people with inapparent infections fail to infect mosquitoes because they do not reach sufficiently high viremia levels (5). This assumption is based on the observation that disease severity is positively correlated with the magnitude of DENV viremia (10, 11, 14). To our knowledge, the only study that quantified viral RNA levels in a limited number of asymptomatic DENV infections in humans did not detect a significantly lower viremia (15), but infectiousness to mosquitoes was not evaluated. The aim of the present study was to document variation in DENV infectiousness of naturally infected humans across the spectrum of disease manifestations, including fully asymptomatic infections, and to verify the assumption that people with inapparent infections are not infectious to mosquitoes.

Significance

Our work provides evidence that people who are infected with dengue virus without developing detectable clinical symptoms or prior to the onset of symptoms are infectious to mosquitoes. At a given level of viremia, symptom-free people were markedly more infectious to mosquitoes than clinically symptomatic patients. Our results fundamentally change the current paradigm for dengue epidemiology and control, based on detection of dengue virus-infected cases with apparent illness.

Author contributions: V.D., L.L., R.E.P., S.L., K.C.L., R.H., A.T., T.W.S., A.S., and P.B. designed research; V.D., S.L., R.S.L., R.H., A.T., and P.B. performed research; V.D., L.L., R.E.P., R.S.L., T.W.S., A.S., and P.B. analyzed data; and V.D., L.L., R.E.P., R.S.L., T.W.S., A.S., and P.B. wrote the paper.

Conflict of interest statement: P.B. is currently an employee of GlaxoSmithKline Vaccines, but the research presented does not have any relation with his current position.

This article is a PNAS Direct Submission. L.A.M. is a guest editor invited by the Editorial Board.

Freely available online through the PNAS open access option.

[1]V.D. and L.L. contributed equally to this work.

[2]To whom correspondence should be addressed. Email: buchyphilippe@hotmail.com.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1508114112/-/DCSupplemental.

Results

Before the development of studies based on geographic cluster sampling around index cases (16, 17), inapparent DENV infections could only be detected in very small numbers or retrospectively in cohort studies after the viremic period had ended (18, 19). We overcame this obstacle by capturing DENV-infected people across the continuum of disease manifestations while they were viremic using a comprehensive catchment system combining passive, hospital-based surveillance and cluster investigations in and around the households of hospitalized index cases (*Materials and Methods*). Following informed consent, wild-type but laboratory-reared *Ae. aegypti* mosquitoes were allowed to feed on the blood of study participants through direct (biting the person) and indirect (artificial feeder) methods (*Materials and Methods*). Legs and wings of blood-engorged mosquitoes that survived a 2-wk extrinsic incubation period (EIP) were tested by serotype-specific, quantitative reverse transcription–PCR (qRT-PCR) to detect and quantify DENV RNA. Mosquitoes with DENV-positive wings and legs indicated that virus had disseminated from their midguts, which is a recognized proxy for their mosquito-to-human transmission potential (20).

Our analyses included a total of 181 study participants who were either viremic at the time of mosquito feeding ($n = 176$) or were viremic at the time of inclusion, but had already reached an undetectable level of viremia at the time of experimental mosquito exposure and were nevertheless subsequently found to be infectious to mosquitoes ($n = 5$). Most study participants (89%) were children under 16 y of age [mean, 9.32; median, 8; interquartile range (IQR), 5–12 y] with a slight male-biased sex ratio (1.2). All four DENV serotypes were represented with a marked predominance of DENV-1 (51.4%), followed by DENV-2 and DENV-4 (24.9% and 22.7%, respectively), and two DENV-3–infected participants (1.1%). There were more secondary (46.3%) than primary DENV infections (33.1%); immune status was undetermined for 20.6% of the infected people. Overall, 126 (69.6%) participants had symptomatic dengue when experimental mosquito feeding took place, 42 (23.2%) developed symptoms after mosquitoes had fed, and 13 (7.2%) did not report and/or exhibit any detectable symptom at recruitment and throughout the 10-d follow-up period. Relative frequencies of DENV serotypes did not differ between asymptomatic, presymptomatic, and symptomatic disease categories ($P = 0.639$). Although DENV transmission to mosquitoes from naturally infected people was recently studied in a symptomatic cohort in Vietnam (11), by extending the present study design to include people with presymptomatic and asymptomatic infections we addressed an unexplored knowledge gap in dengue epidemiology.

Most participants ($n = 156$; 86.2%) underwent both direct and indirect mosquito feedings; a small subset participated in only direct ($n = 8$; 4.4%) or only indirect ($n = 17$; 9.4%) mosquito feedings. Direct mosquito feedings were only performed with children above 4 y of age. A total of 3,163 individual *Ae. aegypti* was assayed, of which 1,645 were fed directly on the infected person (mean per participant, 10.0; median, 9; IQR, 5–14) and 1,518 indirectly on viremic blood (mean per participant, 8.8; median, 7; IQR, 5–11). The proportion of positive mosquitoes was positively and significantly correlated between direct and indirect feedings on the same participant, although indirect feedings generally resulted in a significantly lower proportion of infected mosquitoes (weighted linear regression of infection percentage: indirect = 1.05 + 0.768*direct; $r^2 = 0.69$; *SI Appendix*, Fig. S1). This result indicates that indirect feeding is a less sensitive method for assessing human-to-mosquito transmission than direct feeding, but may nevertheless represent a reasonable alternative when direct feeding is not possible.

First, we examined the relationship between illness and infectiousness to mosquitoes in people who developed symptoms during the course of their DENV infection. Our study design allowed us to identify viremic people before the onset of fever and associated symptoms. Across the presymptomatic and symptomatic cohorts, participants were exposed to mosquitoes from 3 d before to 8 d after the onset of symptoms. Collectively, viremia peaked around 0–2 d after the onset of symptoms and then gradually decreased, but viral RNA remained detectable in some participants on their eighth day of illness (Fig. 1*A*). Infectiousness to mosquitoes closely matched the kinetics of viremia (Fig. 1 *B* and *C*). There was no successful DENV transmission to *Ae. aegypti* beyond the sixth day of illness. One participant's blood did successfully infect mosquitoes by indirect feeding on the seventh day of illness (Fig. 1*C*), but this was not confirmed by direct feeding (Fig. 1*B*). Some participants were infectious as early as 2 d ($n = 3$) or 1 d ($n = 8$) before the onset of symptoms (Fig. 1*B*), confirming results previously reported by Siler et al. (6). Although duration of infectiousness is best measured with longitudinal profiles of the same individuals, our composite time course at the population level suggests that for the viruses we studied humans naturally infected with DENV can be infectious to mosquitoes from 2 d before to 6 d after the onset of illness.

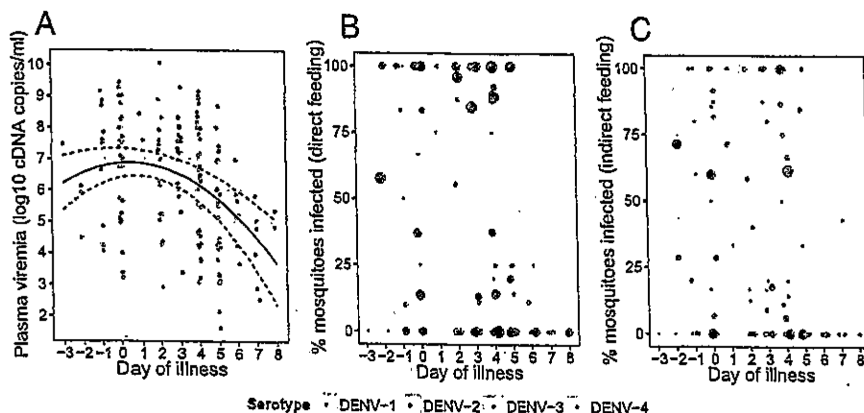


Fig. 1. Profiles of plasma viremia and infectiousness by day of illness. Plasma viremia expressed as DENV cDNA copy number per milliliter (*A*) and percentage of infected mosquitoes following direct (*B*) and indirect feeding (*C*) are shown as a function of day of illness for presymptomatic/symptomatic participants. Day 0 is the onset of clinical symptoms. Each dot corresponds to an individual participant and is color-coded by DENV serotype. *A*, *B*, and *C* include 163, 142, and 144 participants who developed symptoms, respectively. In *A*, solid lines indicate the quadratic regression and dotted lines represent the SE of regression parameter estimates. In *B* and *C*, the size of the dots is proportional to the number of mosquitoes tested per participant.

Next, we examined the human, virological, and entomological factors that were associated with infectiousness to mosquitoes. In multivariate regression analyses, both viremia level and disease category were consistently and significantly associated with the likelihood of mosquito infection (Table 1). Conversely, infection immune status (primary, secondary, or indeterminate status), age, and EIP were not significant predictors of successful mosquito infection. A significant effect of serotype and gender was only detected for one mosquito feeding method, but not both, which may be due to the fact that the cohorts of individuals involved in each mosquito feeding method were not exactly the same.

Our data confirmed that the level of viremia is one of the most important determinants of human infectiousness to mosquitoes (11). The amount of detectable DENV RNA in participants' plasma (expressed in cDNA copies per milliliter) ranged from 1.61 to 10.1 $\log_{10}$ cDNA copies per mL of plasma across all study participants (mean ± SE: 6.17 ± 0.14). The observed magnitude of viremia was not associated with age, gender, DENV serotype, or immune status. It was, however, significantly different between disease categories (*SI Appendix*, Table S1). Viremia level measured in people with asymptomatic infections (mean ± SE: 4.75 ± 0.39 $\log_{10}$ cDNA copies per mL) was lower on average than in people with presymptomatic (mean ± SE: 6.74 ± 0.25 $\log_{10}$ cDNA copies per mL) or symptomatic (mean ± SE: 6.12 ± 0.17 $\log_{10}$ cDNA copies per mL) infections. Because viremia level depends on when it was measured during the course of infection (Fig. 1*A*), we also compared the average viremia level of asymptomatic infections with that of people who developed symptoms, stratified by day of illness. The average viremia level of asymptomatic infections did not differ from viremia levels observed 2–3 d before or 5–8 d after the onset of symptoms. It was, however, significantly lower than between −1 and 4 d of illness. Thus, the average DENV viremia level of asymptomatic infections corresponded to the magnitude or viremia observed during the early and late viremic period of people who develop symptoms. To confirm that the dynamic nature of the viremic phase (Fig. 1*A*) did not confound our time-independent analysis, we repeated the multivariate regression analysis with temporal stratification of disease categories. Although the effect of viremia level decreased, overall results did not change when symptomatic people were split into groups of 0–2, 3–4, and 5–8 d of illness (*SI Appendix*, Table S5). Asymptomatic DENV-infected people were always significantly more infectious than symptomatic people, with the exception of group 0–2 d of illness, which was marginally insignificant for indirect feeding. Presymptomatic DENV infections did not differ significantly from asymptomatic infections (*SI Appendix*, Table S5).

Viremia levels measured by qRT-PCR and expressed, like in this study, as cDNA copy numbers are generally well correlated with infectious titers, but should be interpreted with caution. The number of infectious virus particles measured by the mosquito inoculation technique can be 2–5 logs lower, depending on the virus strain, cell type used, level of viremia, host immune response, and several other factors such as handling and storage conditions of viremic blood sample (21). RT-PCR detects RNA from infectious viruses, but also from immature virions and defective particles. On the other hand, five participants with undetectable DENV viremia by qRT-PCR were infectious to mosquitoes. This further highlights the limited correlation between infectious titer and concentration of viral RNA in plasma. It also suggests that the amount of virus might differ between venous blood (used to measure viremia) and capillary or venule blood (imbibed by mosquitoes) and/or between plasma and whole blood. As previously reported, it is also possible that the high sensitivity of the mosquito infection model may help to detect low levels of viremia that are below the limit of detection by qRT-PCR, but are actually sufficient to infect mosquitoes (21). In our multivariate analysis (Table 1), factors other than the amount of viral RNA in plasma (e.g., serotype and disease category) contributed to differences in participant infectiousness.

Strikingly, asymptomatic and presymptomatic infections were associated with increased overall probability of mosquito infection, independently of viremia level measured as cDNA copy numbers. Asymptomatic and presymptomatic DENV infections did not differ significantly from each other (direct: $P = 0.430$; indirect: $P = 0.496$). Dose–response scatterplots revealed that asymptomatic and presymptomatic infections were more infectious to mosquitoes than symptomatic infections at any given viremia level (Fig. 2). By direct feeding, the 50% mosquito infectious doses [95% confidence interval (CI)] were 5.31 (4.81–5.80), 5.68 (5.30–6.00), and 7.21 (7.05–7.36) $\log_{10}$ viral cDNA copies per mL of plasma in the asymptomatic, presymptomatic, and symptomatic categories, respectively. By indirect feeding, the corresponding estimates were 5.68 (5.14–6.55), 5.97 (5.55–6.33), and 7.78 (7.59–7.99), respectively.

We analyzed viral loads in the wings and legs of infected mosquitoes because they are positively correlated with DENV in *Ae. aegypti* saliva and thus mosquito transmission potential (20). The strongest predictor of viral loads in positive mosquitoes was human viremia, followed by disease category and DENV serotype.

Table 1. Multivariate regression analysis of successful human-to-mosquito DENV transmission

	Direct feeding		Indirect feeding	
Factor	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Serotype	N.S.			
DENV-1			3.66 (1.76–7.63)	<0.001
DENV-2			1.74 (0.72–4.19)	0.213
DENV-4			Ref.	
Gender			N.S.	
Male	2.08 (1.07–4.04)	0.032		
Female	Ref.			
Viremia, +1 $\log_{10}$ copies/mL	2.05 (1.64–2.56)	<0.001	1.81 (1.52–2.16)	<0.001
Disease category				
Asymptomatic	10.05 (1.76–57.51)	0.010	6.72 (1.90–23.9)	0.003
Presymptomatic	4.84 (2.02–11.58)	<0.001	4.19 (1.94–9.05)	<0.001
Symptomatic	Ref.		Ref.	

Minimal adequate model based on marginal logistic regression of mosquito infection status. DENV-3 was excluded due to small sample size. Direct and indirect feedings are analyzed separately as indicated in the table heading. Test statistics for the full model are in *SI Appendix*, Table S2. CI, confidence interval; N.S., not significant; OR, odds ratio; Ref., reference level.

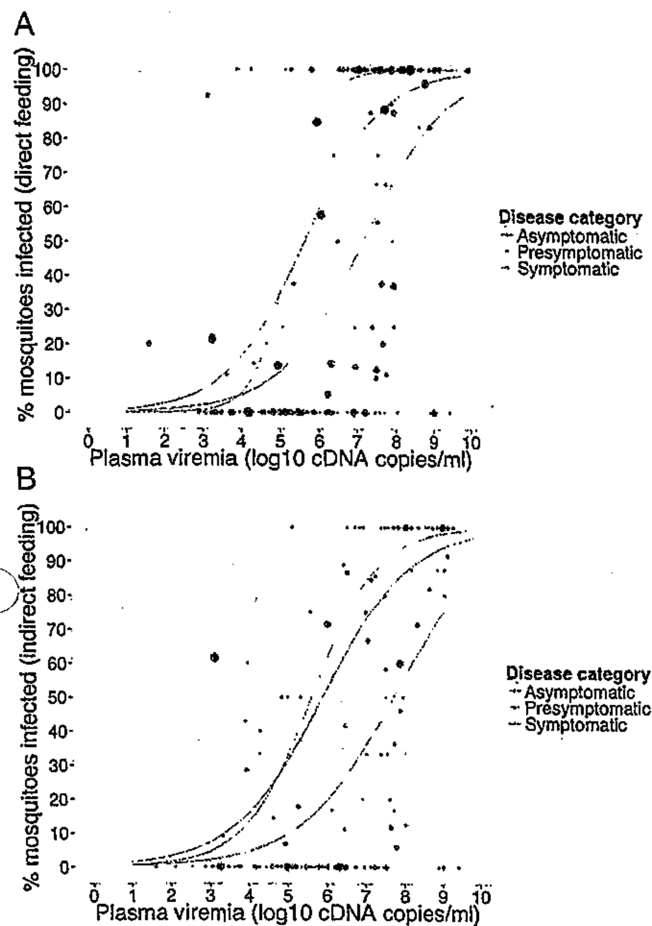


Fig. 2. Dose-response profiles by disease category. The percentage of infected mosquitoes is shown as a function of viremia (measured as cDNA copies per milliliter of plasma) for each study participant involved in direct (*A*) and indirect (*B*) mosquito feedings. Solid symbols represent positive viremia levels. Open symbols correspond to undetectable viremia levels in five participants that resulted in mosquito infection. The size of the dot is proportional to the number of mosquitoes tested per participant (direct feeding: mean, 10.0; median, 9; IQR, 5–14; indirect feeding: mean, 8.8; median, 7; IQR, 5–11). *A* includes 8 asymptomatic, 37 presymptomatic, and 117 symptomatic participants. *B* includes 10 asymptomatic, 41 presymptomatic, and 120 symptomatic participants. Curves are logistic regressions of the data (excluding participants with undetectable viremia). The minimum adequate model for direct feedings explained 34.2% of the variation in the proportion of mosquitoes infected, and disease category alone accounted for 8% of the overall variation. For the indirect feedings, the percentage of variation explained by the model was 37.4% and 7.8% for disease category alone.

(*SI Appendix*, Tables S3 and S4). For both direct and indirect feedings, asymptomatic and presymptomatic infections resulted in a significantly higher viral load in infected mosquitoes than symptomatic infections ($P < 0.001$). This conclusion was confirmed with temporal stratification of symptomatic infections as a function of day of illness (*SI Appendix*, Table S6). Following direct feeding, the mean viral loads (±SE) of infected mosquitoes expressed in $\log_{10}$ cDNA copies per milliliter were higher for mosquitoes that fed on asymptomatic people (6.52 ± 0.26) and presymptomatic people (5.29 ± 0.16) than on symptomatic patients in the 0–2 d (4.91 ± 0.18), 3–4 d (5.20 ± 0.13), and 5–8 d of illness (5.14 ± 0.16). Following indirect feeding, the mean viral loads (±SE) of infected mosquitoes were also higher after feeding on the blood drawn from asymptomatic people (6.06 ± 0.32) and presymptomatic people (5.06 ± 0.22) than on blood from symptomatic patients in the 0–2 d (4.33 ± 0.25), 3–4 d (4.86 ± 0.18), and 5–8 d of illness (3.43 ± 0.21). Thus, not only a larger proportion of mosquitoes became infected when they fed on the blood of participants without symptoms, but mosquitoes that became infected had significantly more viral genome copies in their bodies than those infected by feeding on the blood of symptomatic participants.

Discussion

Our results show that people with natural DENV infections and no clinical symptoms can contribute to virus transmission dynamics by efficiently infecting mosquito vectors. Moreover, people with asymptomatic and presymptomatic infections had an ~100-fold lower 50% mosquito infectious dose and resulted in larger viral loads in infected mosquitoes, which we interpret as increased transmission potential (20). The dynamic nature of changes in human infectiousness to mosquitoes could reflect a shift in the ratio of noninfectious to infectious viral particles (undetected by qRT-PCR alone) through the course of infection and/or the influence of differential cofactors in asymptomatic compared with symptomatic infections. The strong immune response and high cytokine levels developed during illness may play a role in reducing human infectiousness to mosquitoes (22). Consistent with this hypothesis, reduced risk of human-to-mosquito transmission has been associated with increasing day of illness and rising IgG and IgM titers (11). Understanding and characterizing the mechanistic basis of variation in infectiousness among DENV-infected people should be a priority for future research.

Regardless of the underlying mechanism(s), our results are consistent with the hypothesis that DENV transmission from humans to mosquitoes can be "silent" because it occurs before the onset of symptoms or in the absence of apparent illness. Quantitative estimates of the relative contribution to DENV transmission among disease categories remain to be determined by combining assessments of magnitude and duration of infectiousness across the infectious period of different people with measures of various people's exposure to biting mosquitoes during the course of their infection (23). Larger sample sizes than in the present study and sequential mosquito feedings on the same person will help to further clarify the role of presymptomatic and asymptomatic infections in DENV transmission. Asymptomatic infections had a lower average level of viremia, which is thought to be associated with a shorter time window of infectiousness (11). On the other hand, people without symptoms may be more likely to visit multiple locations during their daily routines where they are cumulatively bitten by more mosquitoes than sick people who are hospitalized or who stay at home and are exposed to only their resident mosquitoes (24). Additional sources of heterogeneity to consider include duration of the mosquito's EIP (25), genetic variation among DENV strains, and intrinsic differences among human and mosquito populations (26, 27).

The observation that clinically inapparent human DENV infections can contribute to the population of infected mosquitoes supports the hypothesis that they are not dead-end hosts for transmission (5). Inapparent infections could contribute to DENV persistent circulation during interepidemic periods and may provide new insights and approaches into outbreak detection and response. For example, inapparent cholera infections play a central role in driving the shape of epidemic curves; people with severe disease are relatively minor contributors (28). Inapparent infections force a more rapid rise and fall of cholera epidemics, shifting the peak of the epidemic curve to an earlier time than previously recognized, and accelerating the process of pathogen epidemic transmission and geographic spread. This has important public health implications for dengue because it reemphasizes the need to rapidly detect and effectively respond to an outbreak, which will depend on the amount of transmission occurring before detection of an increase in disease (29). It also encourages measurement of infection, not just disease, in dengue vaccine and vector control trials. Evaluating the risk of transmission in the absence of symptoms could be important for

other arboviruses with a high proportion of inapparent infections such as yellow fever virus (30).

Global efforts to reverse the increasing burden of dengue disease will require more than overcoming the lack of resources, lack of political will, or ineffective intervention implementation. Equally important will be an improved understanding and application of influential features of virus transmission dynamics. It is our hope that changing views about how people with inapparent and mild DENV infections contribute to transmission will improve the theory of DENV transmission dynamics, lead to innovation in outbreak detection and response, and refine assessment of new prevention strategies.

Materials and Methods

Ethics Statement. This study was approved by the Cambodian National Ethics Committee for Health Research (Protocol 063NECHR) and by the Institut Pasteur Ethics Board for European Projects. A participant's enrollment was subject to obtaining written consent signed by the participant or by a legal representative for participants under 16 y of age.

Study Participants. Patients presenting with acute dengue-like illness between June and October of 2012 and 2013 were enrolled at Kampong Cham City Provincial Hospital and at two district hospitals of the Kampong Cham province (*SI Appendix*, Fig. S2). This province is ~120 km northeast from Phnom Penh, the capital of Cambodia, where the Institut Pasteur in Cambodia (IPC) is located. Participant inclusion criteria were as follows: (*i*) age ≥2 y (age ≥4 y for direct mosquito feeding); (*ii*) axillary temperature >38.0 °C; (*iii*) two or more of the following symptoms: headache, retroorbital pain, muscle pain, joint pain, rash, and any bleeding; and (*iv*) written informed consent from the participant or a legal representative for participants under 16 y of age.

DENV infection of hospitalized patients was confirmed by NS1 antigen detection using a commercial rapid diagnostic test (for details, see *Dengue Diagnosis and Classification* below) followed by qRT-PCR on the plasma sample obtained during the acute febrile phase of disease (*SI Appendix*, Fig. S3) (31). Patients with a confirmed DENV infection by NS1 antigen detection and/or qRT-PCR were considered dengue index cases (DICs) that initiated geographic cluster investigations. Cluster participants were enrolled from family members at the DIC's household as well as from people living in houses within a 200-m radius of the DIC's home or in the 20 closest houses when the population density of the area exceeded the logistical capacity of the field team. Cluster participants were aged from 2 to 40 y and lived in villages located within 30 km from the provincial hospital (*SI Appendix*, Fig. S2). Cluster participants or representative for the participants under 16 y of age provided a written informed consent before enrollment. Exclusion criteria were as follows: (*i*) pregnancy or breastfeeding; (*ii*) symptoms inconsistent with dengue and obvious nondengue acute infection (e.g., otitis media, pneumonia, meningitis); or (*iii*) known chronic illness.

Clinical Data and Blood Collection. DICs and cluster participants were examined during sequential visits as shown in *SI Appendix*, Figs. S3 and S4. At each visit, data were collected using a standardized questionnaire designed for either hospitalized patients or cluster participants.

For hospitalized patients, three blood samples were collected: shortly after hospital admission during the febrile acute phase (visit 1), at the time of defervescence (visit 2), and during the convalescent phase at hospital discharge (visit 3), which in general corresponded to days 3–5 of hospitalization. The severity of the disease was assessed according to the 1997 World Health Organization (WHO) criteria using clinical, biological, and paraclinical examination data recorded at admission and throughout the entire hospitalization period (32).

To screen cluster participants for DENV infection, home visits were conducted within 24 h after DIC identification [home visit 0 (HV0)] and then again at day 2 (HV2) and day 7 (HV7) during 2012. In 2013, only HV0 and then a visit 4 d later (HV4) were conducted (*SI Appendix*, Fig. S4).

Blood samples drawn during visits were sent with ice packs to IPC within 5–10 h for laboratory diagnosis. Blood samples were collected in tubes with EDTA anticoagulant, and volume drawn was adapted according to participant weight: 3.0 mL for children below 20 kg of weight and 5.0 mL for individuals over 20 kg.

Cluster Investigation Participant Follow-Up. Once confirmed positive for DENV infection, cluster investigation participants were visited at different time points depending on their clinical presentation. Cluster participants with any dengue-like clinical symptoms described above, at any point in their observation period, were considered as symptomatic cases and those who did not report or present any symptom during examination in the follow-up period were classified as asymptomatic individuals. The participant was classified as symptomatic if any symptoms appeared or were reported by the participant or their caregiver during the follow-up period or if the participant or their caregiver reported antipyretic drug intake.

Symptomatic cluster participants were visited immediately after confirmation of DENV infection at three time points: day 0 (D0), D2, and D7. A longer and closer monitoring was conducted in the asymptomatic group at D0, D1, D2, D3, D4, D5, D6, D7, and D10 (*SI Appendix*, Fig. S4). During each daily visit, a blood sample was collected and a standard questionnaire was administered regarding current symptoms or history of symptoms during at least 2 consecutive days for the last 7 d including dengue-like symptoms (headache, retroorbital pain, muscle pain, joint pain, rash, and any bleeding), body temperature, and intake of an antipyretic drug. D0 of the cluster investigation follow-up usually corresponded to the second day after the participant was enrolled (at HV0) due to the time required for blood sample transportation to IPC and subsequent laboratory confirmation.

Dengue Diagnosis and Classification. Blood samples collected at admission (visit 1) of hospitalized patients were tested for DENV NS1 antigen by SD BIOLINE Dengue Duo rapid diagnostic kit (Standard Diagnostic) according to the manufacturer's recommendations. This test was performed by well-trained nurses at the patient's bedside and the result was confirmed at IPC by DENV RNA detection in the same blood sample using a serotype-specific multiplex qRT-PCR method (31). The qRT-PCR had a limit of detection of 0.5–3 plaque-forming units (PFU) and 5–10 cDNA copies per 20-µL reaction, depending on the serotype (31). To screen for additional DENV infections among household members and neighbors, blood samples that were collected at HV0, HV2, HV7 (2012), or HV0 and HV4 (2013) were tested by qRT-PCR at IPC. Results were communicated to the field team on a daily basis to initiate cluster investigations, perform mosquito feedings when required, and ensure the follow-up of cluster participants. DENV viremia was expressed as cDNA copy number per milliliter of plasma.

Serological tests were performed on sera collected during the acute and convalescent phase of the infection in both symptomatic and asymptomatic groups for detection of antibodies against DENV. Because of potential cross-reactivity among flaviviruses, all specimens were tested for both anti-DENV and anti-Japanese encephalitis virus (JEV) using an in-house IgM capture ELISA (MAC-ELISA) and hemagglutination inhibition (HI) assay as previously described (33, 34). Primary or secondary immune status of DENV infections was determined by HI test according to WHO criteria (32).

***Aedes aegypti* for Human-to-Mosquito Transmission Experiments.** Wild immature stages of *Ae. aegypti* were repeatedly collected at ~2-mo intervals at different locations in Kampong Cham province from areas where the study participants resided. All *Ae. aegypti* females that were used in human-to-mosquito DENV transmission experiments were from laboratory-reared F_2 or F_3 generations derived from F_1 parental females that tested negative for DENV, JEV, and chikungunya virus (CHIKV) by virus-specific RT-PCR and for other potential flaviviruses by pan-flavivirus RT-PCR (31, 35–37).

Briefly, field-caught *Ae. aegypti* larvae (F_0) were pooled, reared in distilled water, and fed on commercial dry fish food. Following emergence, adult mosquitoes were fed twice weekly directly on immobilized mice and maintained on cotton soaked with 10% (wt/vol) sugar solution in an environmental chamber with 12:12 light/dark hours, at 27 °C, and 70% relative humidity. F_1 eggs were collected on paper towels lining oviposition cups placed in the cages. The F_1 generation was reared as described above, and F_1 adults were kept in cages containing males and females. F_1 females were provided blood meals for multiple gonotrophic cycles over a period of 30 d. At the end of this period, surviving F_1 adults were removed and freeze-killed. F_1 adults that died before 30 d were also collected for testing. All F_1 mosquitoes were sorted by sex in a Petri dish placed on ice, pooled into vials of 10 mosquitoes (male and female) per vial, and homogenized. Extracted RNA was tested for DENV, JEV, CHIKV, and other flaviviruses (31, 35–37).

Eggs from cages that included only F_1 females and that tested negative for all tested viruses were hatched and reared to produce F_2 and F_3 adults for experimental feeding as described above. Nulliparous female *Ae. aegypti* of the F_2 and F_3 generations were used in human-to-mosquito transmission experiments.

Experimental Exposure of *Ae. aegypti* to DENV Viremic Blood. Following confirmation of DENV infection, each study participant was asked, or through his/her legal guardian, to participate in human-to-mosquito transmission experiments by direct and/or artificial membrane (indirect) feeding methods (*SI Appendix*, Fig. S5). Written informed consent was provided for all enrolled participants. Both hospitalized patients (DICs) and cluster participants were involved in mosquito feedings (*SI Appendix*, Fig. S6).

For direct feeding, two mesh-covered 500-mL paper cups, each containing 25 female 5- to 9-d-old *Ae. aegypti* that had been starved for 24 h, were placed on

the participant's legs for 5 min. A nurse monitored participants for 30 min for any side effects and was prepared to advise the participant to seek treatment if fever or any other symptoms occurred in the following 2 wk. Antihistamine cream was applied to the sites of mosquito bites to relieve itchiness.

For indirect feeding, venous blood was drawn from the participant into a tube with EDTA anticoagulant within 30 min after direct mosquito feeding. Briefly, 3.0 mL of whole blood was thoroughly mixed with 30 µL of 10 mM ATP used as a phagostimulant, and the remaining blood was kept to quantify DENV RNA by qRT-PCR. Approximately 1.5 mL of mixture was loaded into two glass feeders maintained at 37 °C by a connected heated water source. Two mesh-covered 500-mL paper cups, each containing 25 female 5- to 9-d-old *Ae. aegypti* that had been starved for 24 h, were placed under the feeders for 30 min to allow blood feeding through a piece of desalted porcine intestine used as a membrane.

After direct or indirect feeding, mosquitoes were cold anesthetized at 4 °C for 10–15 min and sorted on a Petri dish placed on ice. Unfed or partially fed females were discarded. Only fully engorged females were transferred to a new 500-mL paper cup and maintained in an environmental chamber maintained in a biosafety level 2+ facility with 12:12 light/dark hours, at 27 °C, and 70% relative humidity for ~2 wk. EIP ranged from 10 to 19 d, but for most participants (92%) surviving females were harvested between 13 and 16 d after blood feeding. Variation in EIP was taken into account in the statistical analyses.

DENV Detection in Mosquitoes. When harvested for processing, mosquitoes were freeze-killed, and their legs and wings were separated from the rest of the body with sterile forceps and, for each mosquito, placed individually in a vial containing 300 µL of sterile PBS with 10% (vol/vol) FCS and 15–20 ceramic beads. The mixture was homogenized using a MagNA Lyser instrument (Roche Life Science Thailand, catalog no. 03358976001) at 6,500 rpm for 50 s. Viral load in individual mosquito body parts was measured by qRT-PCR and the results expressed as cDNA copy number per milliliter of legs and wings suspension (31).

Statistical Analyses. All analyses were based on the final cohort of 181 infected human participants including 13 asymptomatic, 42 presymptomatic, and 126 symptomatic people. Analyses of mosquito infection data were performed separately for direct and indirect feeding methods. Detectable plasma viremia levels were $\log_{10}$-transformed and analyzed with an analysis of variance. The proportion of infected mosquitoes was analyzed as a function of the covariates using marginal logistic regression models. Dose–response curves and 50% mosquito infectious dose estimates were derived from the logistic regression coefficients. Because of the small size of the asymptomatic cohort, dose–response curves could not be further stratified within each disease category. Viral load in infected mosquitoes was $\log_{10}$-transformed and analyzed with a generalized linear mixed model that included the random effect of the participant and fixed effects of other covariates. Full models were reduced to minimal adequate models by backward elimination of nonsignificant terms in a stepwise fashion. Dispersion was estimated from the data, and P values were obtained with F statistics. All statistical analyses were performed in GenStat (38).

ACKNOWLEDGMENTS. We express our gratitude to patients who were eagerly willing to participate in the study, particularly in the mosquito transmission experiments. We also acknowledge the staff of Virology and Epidemiology Units at Institut Pasteur in Cambodia for their technical support. We thank doctors and nurses of the three hospitals in Kampong Cham Province for their contribution in patients' enrollment, sample collection, and patience. We extend our thanks to our entomology assistants, Mr. Neung Choueth and Mr. Chhin Deth, for their valuable assistance, and to all the team from the Dengue National Control Program. We thank A. Fontaine and S. Lequime for assistance with the figures, and three anonymous reviewers for constructive comments that significantly improved the manuscript. The research leading to these results received funding from the European Union Seventh Framework Programme (FP7/2007/2011) under Grant Agreement 282 378. L.L. received funding from the French Government's Investissement d'Avenir Program, Laboratoire d'Excellence Integrative Biology of Emerging Infectious Diseases (Grant ANR-10-LABX-62-IBEID), and from the Emergence(s) Program of the City of Paris. T.W.S. was supported by the Research and Policy for Infectious Disease Dynamics Program of the Science and Technology Directory, Department of Homeland Security, and Fogarty International Center, National Institutes of Health (NIH). L.L., K.C.L., and T.W.S. received support from NIH Grant 1P01AI098670-01A1.

MICROBIOLOGY

1. Bhatt S, et al. (2013) The global distribution and burden of dengue. *Nature* 496(7446): 504–507.
2. Brady OJ, et al. (2012) Refining the global spatial limits of dengue virus transmission by evidence-based consensus. *PLoS Negl Trop Dis* 6(8):e1760.
3. Lambrechts L, Scott TW, Gubler DJ (2010) Consequences of the expanding global distribution of *Aedes albopictus* for dengue virus transmission. *PLoS Negl Trop Dis* 4(5):e646.
4. Morrison AC, Zielinski-Gutierrez E, Scott TW, Rosenberg R (2008) Defining challenges and proposing solutions for control of the virus vector *Aedes aegypti*. *PLoS Med* 5(3):e68.
5. Carrington LB, Simmons CP (2014) Human to mosquito transmission of dengue viruses. *Front Immunol* 5:290.
6. Siler JF, Hall MW, Hitchens AP (1926) Dengue: Its history, epidemiology, mechanism of transmission, etiology, clinical manifestations, immunity, and prevention. *Philipp J Sci* 29:1–302.
7. Simmons JS, St. John JH, Reynolds FHK (1931) Experimental studies of dengue. *Philipp J Sci* 44:1–247.
8. Nishiura H, Halstead SB (2007) Natural history of dengue virus (DENV)-1 and DENV-4 infections: Reanalysis of classic studies. *J Infect Dis* 195(7):1007–1013.
9. Gubler DJ, Suharyono W, Tan R, Abidin M, Sie A (1981) Viraemia in patients with naturally acquired dengue infection. *Bull World Health Organ* 59(4):623–630.
10. Murgue B, Roche C, Chungue E, Deparis X (2000) Prospective study of the duration and magnitude of viraemia in children hospitalised during the 1996–1997 dengue-2 outbreak in French Polynesia. *J Med Virol* 60(4):432–438.
11. Nguyet MN, et al. (2013) Host and viral features of human dengue cases shape the population of infected and infectious *Aedes aegypti* mosquitoes. *Proc Natl Acad Sci USA* 110(22):9072–9077.
12. Grange L, et al. (2014) Epidemiological risk factors associated with high global frequency of inapparent dengue virus infections. *Front Immunol* 5:280.
13. Gubler DJ, Suharyono W, Lubis I, Eram S, Gunarso S (1981) Epidemic dengue 3 in central Java, associated with low viremia in man. *Am J Trop Med Hyg* 30(5):1094–1099.
14. Vaughn DW, et al. (2000) Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. *J Infect Dis* 181(1):2–9.
15. Duong V, et al. (2011) Clinical and virological factors influencing the performance of a NS1 antigen-capture assay and potential use as a marker of dengue disease severity. *PLoS Negl Trop Dis* 5(7):e1244.
16. Yoon IK, et al. (2012) Underrecognized mildly symptomatic viremic dengue virus infections in rural Thai schools and villages. *J Infect Dis* 206(3):389–398.
17. Mammen MP, et al. (2008) Spatial and temporal clustering of dengue virus transmission in Thai villages. *PLoS Med* 5(11):e205.
18. Endy TP, et al. (2011) Determinants of inapparent and symptomatic dengue infection in a prospective study of primary school children in Kamphaeng Phet, Thailand. *PLoS Negl Trop Dis* 5(3):e975.
19. Gordon A, et al. (2013) The Nicaraguan pediatric dengue cohort study: Incidence of inapparent and symptomatic dengue virus infections, 2004–2010. *PLoS Negl Trop Dis* 7(9):e2462.
20. Lambrechts L, et al. (2012) Dengue-1 virus clade replacement in Thailand associated with enhanced mosquito transmission. *J Virol* 86(3):1853–1861.
21. Choy MM, Ellis BR, Ellis EM, Gubler DJ (2013) Comparison of the mosquito inoculation technique and quantitative real time polymerase chain reaction to measure dengue virus concentration. *Am J Trop Med Hyg* 89(5):1001–1005.
22. Rathakrishnan A, et al. (2012) Cytokine expression profile of dengue patients at different phases of illness. *PLoS One* 7(12):e52215.
23. Liebman KA, et al. (2014) Determinants of heterogeneous blood feeding patterns by *Aedes aegypti* in Iquitos, Peru. *PLoS Negl Trop Dis* 8(2):e2702.
24. Stoddard ST, et al. (2013) House-to-house human movement drives dengue virus transmission. *Proc Natl Acad Sci USA* 110(3):994–999.
25. Christofferson RC, Mores CN (2011) Estimating the magnitude and direction of altered arbovirus transmission due to viral phenotype. *PLoS One* 6(1):e16298.
26. Pongsiri A, et al. (2014) Differential susceptibility of two field *Aedes aegypti* populations to a low infectious dose of dengue virus. *PLoS One* 9(3):e92971.
27. Coffey LL, et al. (2009) Human genetic determinants of dengue virus susceptibility. *Microbes Infect* 11(2):143–156.
28. King AA, Ionides EL, Pascual M, Bouma MJ (2008) Inapparent infections and cholera dynamics. *Nature* 454(7206):877–880.
29. Fraser C, Riley S, Anderson RM, Ferguson NM (2004) Factors that make an infectious disease outbreak controllable. *Proc Natl Acad Sci USA* 101(16):6146–6151.
30. Chastel C (2011) [Asymptomatic infections in man: A Trojan horse for the introduction and spread of mosquito-borne arboviruses in non-endemic areas?] *Bull Soc Pathol Exot* 104(3):213–219. French.
31. Hue KD, et al. (2011) Validation of an internally controlled one-step real-time multiplex RT-PCR assay for the detection and quantitation of dengue virus RNA in plasma. *J Virol Methods* 177(2):168–173.
32. WHO/TDR (1997) *Dengue Hemorrhage Fever: Diagnosis, Treatment, Prevention, and Control* (World Health Organization, Geneva), 2nd Ed.
33. Vong S, et al. (2010) Dengue incidence in urban and rural Cambodia: Results from population-based active fever surveillance, 2006-2008. *PLoS Negl Trop Dis* 4(11):e903.
34. Clarke DH, Casals J (1958) Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. *Am J Trop Med Hyg* 7(5):561–573.
35. Shirato K, Miyoshi H, Kariwa H, Takashima I (2005) Detection of West Nile virus and Japanese encephalitis virus using real-time PCR with a probe common to both viruses. *J Virol Methods* 126(1-2):119–125.
36. Pastorino B, et al. (2005) Development of a TaqMan RT-PCR assay without RNA extraction step for the detection and quantification of African Chikungunya viruses. *J Virol Methods* 124(1-2):65–71.
37. Scaramozzino N, et al. (2001) Comparison of flavivirus universal primer pairs and development of a rapid, highly sensitive heminested reverse transcription-PCR assay for detection of flaviviruses targeted to a conserved region of the NS5 gene sequences. *J Clin Microbiol* 39(5):1922–1927.
38. VSN International (2011) GenStat for Windows (VSN International, Hemel Hempstead, UK), 15th Ed.

別紙様式第 2-1
番号 10

医薬品
医薬部外品　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2016 年 02 月 15 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	http://www.who.int/csr/don/8-february-2016-gbs-brazil/en/#/2016/02/08	公表国 ブラジル	
販売名（企業名）	①抗 HBs 人免疫グロブリン筋注 200 単位/1mL「JB」（日本血液製剤機構） ②抗 HBs 人免疫グロブリン筋注 1000 単位/5mL「JB」（日本血液製剤機構） ③ヘブスブリン筋注用 200 単位（日本血液製剤機構） ④ヘブスブリン筋注用 1000 単位（日本血液製剤機構） ⑤ヘブスブリン IH 静注 1000 単位（日本血液製剤機構）					

研究報告の概要

2016 年 1 月 22 日，ブラジルのナショナル IHR フォーカルポイントは，全国レベルでのギラン・バレー症候群の増加について PAHO/WHO に通知した。病院のサーベイランスシステムのデータにより，2015 年 1 月～11 月，国内全域で GBS 症例 1708 例が登録されたことが明らかとされた。多数の州（特にアラゴアス（516.7%），バイア（196.1%），リオ・グランデ・ド・ノルテ（108.7%），ピアウイ（108.3%），エスピリトサント（78.6%），リオリオデジャネイロ（60.9%））において著しい増加が報告された一方で，他の州では 2014 年と比較してギラン・バレー症候群の症例数は安定している，または減少していることが報告された。ブラジルの大部分の州において，ジカウイルス，チクングニアウイルス，デングウイルスが伝播しているとした上で，GBS 及び先天異常とジカウイルスとの因果関係は今後のケースコントロール研究の結果明らかになる可能性があるとしている。

使用上の注意記載状況・その他参考事項等

代表として静注用ヘブスブリン－IH の記載を示す。

2. 重要な基本的注意

(1)本剤の原材料となる血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体陰性で、かつ ALT（GPT）値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した高力価の抗 HBs 抗体を含有する血漿を原料として、Cohn の低温エタノール分画で得た画分からポリエチレングリコール 4000 処理、DEAE セファデックス処理等により抗 HBs 人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及びろ過膜処理（ナノフィルトレーション）を施しているが、投与に際しては、次の点に十分注意すること。

代表としてヘブスブリン IH 静注 1000 単位の記載を示す。

報告企業の意見

Zika ウイルスについて：ジカウイルス（Zika virus）は 1947 年にウガンダの Zika forest（ジカ森林）から発見されたウイルスで、デングウイルス、日本脳炎ウイルス、ウエストナイルウイルスと同じフラビウイルス科フラビウイルス属に属するエンベロープを有する RNA ウイルスで、蚊（ネッタイシマカ、ヒトスジシマカ）によって媒介される。万一、原料血漿にジカウイルスが混入したとしても、各種モデルウイルスのウイルスクリアランス試験成績から、本剤の製造工程において不活化・除去されると考えている。

チクングニアウイルスについて：チクングニアウイルス（Chikungunya virus：CHIKV）は、トガウイルス科（Togaviridae）アルファウイルス属（Alphavirus）に分類される直径70nmのエンベロープを有する球状の1本鎖RNAウイルスで、蚊（ヤブカ属のネッタイシマカやヒトスジシマカ）によって媒介される。万一、原料血漿にCHIKVが混入したとしても、各種モデルウイルスのウイルスクリアランス試験成績から、本剤の製造工程において不活化・除去されると考える。

デングウイルスについて：デングウイルス（dengue virus）は、フラビウイルス科フラビウイルス属に属する直径40～50nmのエンベロープを有する球形のRNAウイルスで、血清型の違いからD1、D2、D3、D4の4種類があり、主としてネッタイシマカによって媒介される。万一、原料血漿にデングウイルス混入したとしても、各種モデルウイルスのウイルスクリアランス試験成績から、本剤の製造工程において不活化・除去されると考える。

今後の対応

本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

抗 HBs

15

Emergencies preparedness, response

Guillain-Barré syndrome – Brazil

Disease Outbreak News
8 February 2016

On 22 January 2016, the National IHR Focal Point of Brazil notified PAHO/WHO of an increase of Guillain-Barre Syndrome (GBS) recorded at the national level.

Data from the hospital-based surveillance system reveal that, between January and November 2015, 1,708 cases of GBS were registered nationwide. While a number of states reported significant increases in reported cases – especially, Alagoas (516.7%), Bahia (196.1%), Rio Grande do Norte (108.7%), Piauí (108.3%), Espirito Santo (78.6%), and Rio de Janeiro (60.9%) – other states reported stable or even diminishing number of GBS cases as compared to 2014. Most of the states in Brazil are experiencing the circulation of Zika, chikungunya, and dengue virus.

WHO risk assessment

At present, available information is insufficient to interpret the observed differences in GBS incidence globally and among Brazilian states. The potential cause of the reported increase of GBS incidence in certain Brazilian states remains unknown. Case-control studies are ongoing to determine the cause of the increase. These studies may provide evidence that corroborates or disproves a causal relationship between Zika virus, GBS and other congenital malformations. WHO continues to monitor the epidemiological situation and conduct risk assessment based on the latest available information.

WHO recommends Member States affected or susceptible to Zika virus outbreaks to:

- monitor the incidence and trends of neurological disorders, especially GBS, to identify variations against their expected baseline values;
- develop and implement sufficient patient management protocols to manage the additional burden on health care facilities generated by a sudden increase in patients with Guillain-Barre Syndrome;
- raise awareness among health care workers and establish and/or strengthen links between public health services and clinicians in the public and private sectors.

WHO advice

The proximity of mosquito vector breeding sites to human habitation is a significant risk factor for Zika virus infection. Prevention and control relies on reducing the breeding of mosquitoes through source reduction (removal and modification of breeding sites) and reducing contact

between mosquitoes and people. This can be achieved by reducing the number of natural and artificial water-filled habitats that support mosquito larvae, reducing the adult mosquito populations around at-risk communities and by using barriers such as insect screens, closed doors and windows, long clothing and repellents. Since the Aedes mosquitoes (the primary vector for transmission) are day-biting mosquitoes, it is recommended that those who sleep during the daytime, particularly young children, the sick or elderly, should rest under mosquito nets (bed nets), treated with or without insecticide to provide protection.

During outbreaks, space spraying of insecticides may be carried out following the technical orientation provided by WHO to kill flying mosquitoes. Suitable insecticides (recommended by the WHO Pesticide Evaluation Scheme) may also be used as larvicides to treat relatively large water containers, when this is technically indicated.

Basic precautions for protection from mosquito bites should be taken by people traveling to high risk areas, especially pregnant women. These include use of repellents, wearing light colored, long sleeved shirts and pants and ensuring rooms are fitted with screens to prevent mosquitoes from entering.

WHO does not recommend any travel or trade restriction to Brazil based on the current information available.

別紙様式第 2-1
番号 7

医薬品
医薬部外品　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2015 年 12 月 03 日	新医薬品等の区分		厚生労働省処理欄
一般的名称	①②③⑨⑩pH4 処理酸性人免疫グロブリン ④⑤⑥⑦⑧ポリエチレングリコール処理人免疫グロブリン ⑪⑫人免疫グロブリン			研究報告の公表状況	www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/UCM475072.pdf/2015/12/02	公表国 アメリカ	
販売名（企業名）	①献血ポリグロビン N5%静注 0.5g/10mL（日本血液製剤機構） ②献血ポリグロビン N5%静注 2.5g/50mL（日本血液製剤機構） ③献血ポリグロビン 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献血ポリグロビン N10%静注 5g/50mL（日本血液製剤機構） ⑩献血ポリグロビン N10%静注 10g/100mL（日本血液製剤機構） ⑪グロブリン筋注 450mg/3mL「JB」（日本血液製剤機構） ⑫グロブリン筋注 1500mg/10mL「JB」（日本血液製剤機構）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
業界向けガイダンス（案）：エボラウイルスへの対応における血液ドナーの適合性，血液ドナー延期，および血液製剤管理の評価に対する勧告 Ⅰ．緒言 この手引書、広範囲にわたる伝播のよるエボラ・ウイルス疾患（EVD）のアウトブレイクが少なくとも 1 ヶ国で宣言された場合、ドナーの適合性、ドナー延期および血液製剤の管理を評価するための我々（FDA）の勧告に、輸血や原料血漿を含む、更なる製造のための血液および血液成分を収集するあなた（血液事業者）に提供する。この手引書は、主にエボラウイルス（ザイールエボラウイルス種）に適用されるが、勧告はスーダンウイルス、Bundibugyo ウイルス、および TAI 森林ウイルスなどのエボラウイルス属の他のウイルスに適用することが期待される。手引書の第 III 章の勧告は、輸血や原料血漿を含む更なる製造のための血液および血液成分の日常採取に適用される。EVD 生存者からの回復期血漿の採取は、手引書の第 V 章に対処している。	代表として献血ヴェノグロブリン IH5%静注 0.5g/10mL の記載を示す。 2. 重要な基本的注意 (1)本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-1 抗体陰性で、かつ ALT（GPT）値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分からポリエチレングリコール 4000 処理、DEAE セファデックス処理等により人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理、ウイルス除去膜によるろ過

グロブリン

16

医薬品
医薬部外品　研究報告　調査報告書
化粧品

報告企業の意見	今後の対応	
エボラウイルス（ebola virus）は、フィロウイルス科（filoviridae）エボラウイルス属（ebola virus）に属し、ザイール型、スーダン型、コートジボアール型、レストン型の4種の存在が確認されている。ウイルスの大きさは短径が80～100nm、長径が700～1,500nmのエンベロープを有するRNAウイルスで、U字状、ひも状、ぜんまい状など多形成を示す。万一、原料血漿にエボラウイルスが混入したとしても、各種モデルウイルスのウイルスクリアランス試験成績から、本剤の製造工程において不活化・除去されると考える。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。	処理及びpH3.9～4.4の条件下での液状インキュベーション処理を施しているが、投与に際しては、次の点に十分注意すること。

グロブリン

Recommendations for Assessment of Blood Donor Suitability, Donor Deferral and Blood Product Management in Response to Ebola Virus

---

Draft Guidance for Industry

This guidance document is for comment purposes only.

Submit one set of either electronic or written comments on this draft guidance by the date provided in the *Federal Register* notice announcing the availability of the draft guidance. Submit electronic comments to http://www.regulations.gov. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD), 10903 New Hampshire Ave., Bldg. 71, Rm. 3128, Silver Spring, MD 20993-0002, or by calling 1-800-835-4709 or 240-402-8010, or email ocod@fda.hhs.gov, or from the Internet at http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

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

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
December 2015

Table of Contents

I. INTRODUCTION 1
II. BACKGROUND 1
III. RECOMMENDATIONS 3
- A. Donor Educational Material and Donor History Questionnaire 3
- B. Donor Deferral 4
- C. Product Retrieval and Quarantine and Notification 6

IV. REPORTING A BIOLOGICAL PRODUCT DEVIATION (BPD) 7
V. CONVALESCENT PLASMA 7
VI. IMPLEMENTATION 8
VII. REFERENCES 9

Recommendations for Assessment of Blood Donor Suitability, Donor Deferral and Blood Product Management in Response to Ebola Virus

Draft Guidance for Industry

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

I. INTRODUCTION

This guidance document provides, you, blood establishments that collect blood and blood components for transfusion or further manufacture, including Source Plasma, with our (FDA) recommendations for assessing donor suitability, donor deferral and blood product management in the event that an outbreak of Ebola virus disease (EVD) with widespread transmission is declared in at least one country. This guidance document applies primarily to Ebola virus (species *Zaire ebolavirus*), but recommendations are expected to apply to other viruses of the Ebolavirus genus such as Sudan virus, Bundibugyo virus, and Taï Forest virus. The recommendations in section III. of the guidance document would apply to the routine collection of blood and blood components for transfusion or further manufacture, including Source Plasma. The collection of convalescent plasma from EVD survivors is addressed in section V. of the guidance document.

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

II. BACKGROUND

Ebola virus is a member of the family *Filoviridae* that can cause severe hemorrhagic fever in humans and non-human primates (NHPs) with historically high morbidity and mortality rates of up to 90% (Refs. 1 and 2). However, in the 2014 outbreak in West Africa, the mortality rate has been lower, with 28,617 suspected, probable and confirmed cases and 11,314 deaths reported as

of early November 2015.[1] Ebola virus is a lipid-enveloped zoonotic pathogen that, when studied in the laboratory, requires the highest level of biosafety containment (BSL-4). The Centers for Disease Control and Prevention (CDC) has classified it as a "Category A" bioterrorism agent/disease.[2] Ebola virus is reported to be inactivated by heating at 60°C for 60 minutes, and also following incubation at pH 2.5 (Ref. 3). Solvent detergent treatment and pathogen inactivation technologies are also known to inactivate lipid-enveloped viruses (Refs. 4 through 8).

In humans, EVD is typically characterized at onset by fever, severe headache, muscle pain and weakness, followed by diarrhea, vomiting, abdominal pain and sometimes diffuse hemorrhage (bleeding or bruising). In previous outbreaks of EVD, symptoms generally appeared within 21 days and most often within 4-10 days following infection (Refs. 9 and 10). Based on mathematical models, symptom onset later than 21 days is estimated as possible in 0.1 to 12% of cases (Refs. 10 and 11). In a retrospective study in which 500 patients diagnosed in 2014 recalled their likely source of infection, 5% reported symptom onset > 21 days (up to a maximum of 43 days) post-exposure (Ref. 10).

Viremia and virus shedding escalate rapidly after onset of symptoms and infectivity appears to correlate with severity and stage of disease. Although viremia in survivors typically resolves within 21 days of disease onset, infectious virus and viral RNA has been detected in other body components or fluids (e.g., aqueous humor, semen and vaginal fluids) for longer periods. For instance, viable Ebola virus was detected in aqueous humor obtained from the eye 14 weeks after the onset of the initial symptoms of EVD and 9 weeks after the clearance of viremia (Ref. 12). Infectious virus and viral RNA have been detected in semen up to 82 and 272 days post EVD onset, respectively (Refs. 13 through 17).[3] Further, a case of sexual transmission of Ebola virus was reported in which the patient was exposed to Ebola virus through sexual contact with a survivor 179 days after likely disease onset (Refs. 18 and 19). These findings raise the theoretical possibility, which has not been documented in humans or animal models, of an intermittent low level viremia after recovery from illness. In addition, there have been isolated reports of apparently asymptomatic Ebola virus infection in individuals who had contact with Ebola patients (Ref. 18), and of antibody to Ebola virus in rural African populations reportedly unassociated with acute illness (Ref. 19). These reports raise the possibility that there may be an asymptomatic infection or mild disease in some individuals; if this condition exists, the infectivity of these individuals is uncertain but likely to be less than that of severely ill persons.

Ebola virus is transmitted from human to human by direct contact with body fluids (such as blood, urine, stool, saliva, semen, vaginal fluids or vomit) of symptomatic infected individuals. Therefore, blood and blood products from symptomatic individuals, if they were to donate, would have the potential of transmitting Ebola virus to recipients. The theoretical possibility of pre-symptomatic viremia has not been extensively investigated. If this condition exists the

---

[1] See CDC website: http://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/case-counts.html.
[2] See CDC website: http://www.bt.cdc.gov/agent/agentlist-category.asp.
[3] See also the CDC Review of Human-to-Human Transmission of Ebola Virus, http://www.cdc.gov/vhf/ebola/transmission/human-transmission.html.

infectivity is uncertain, but likely to be less than of symptomatic persons. Healthcare providers caring for symptomatic Ebola patients, and family and friends in close contact with symptomatic Ebola patients, are at the highest risk of becoming infected because they may come in direct contact with infected blood or other body fluids of sick patients.

III. RECOMMENDATIONS

A donor must be in good health with a normal temperature at the time of donation (21 CFR 640.3(b) and 21 CFR 640.63(b)(3)). Standard procedures that are already in place to assure that the donor feels healthy at the time of donation serve as an effective safeguard against collecting blood or blood components from a donor who seeks to donate after the onset of clinical symptoms. The following recommendations are intended to reduce the risks of collecting blood and blood components from potentially Ebola virus-infected persons during the asymptomatic incubation period before the onset of clinical symptoms, as well as from individuals with a history of Ebola virus infection or disease.

The guidance contains recommendation for updating your donor educational materials in section III.A.1. The remaining recommendations should be implemented when the CDC has classified one or more countries as having widespread transmission of Ebola virus. When there are no countries classified by CDC as having widespread transmission of Ebola virus it is appropriate to discontinue asking donors questions related to risk of Ebola virus infection or disease. (See http://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/distribution-map.html.)

A. Donor Educational Material and Donor History Questionnaire

1. Donor Educational Material

We expect very few individuals with a history of Ebola virus infection or disease to present as blood donors. When there are no countries classified as having widespread transmission of Ebola virus, self-deferral of donors with a history of Ebola virus infection or disease should provide sufficient protection. You may update your donor educational materials to instruct donors with a history of Ebola virus infection or disease to not donate blood or blood components.

2. Donor History Questionnaire

In the event that one or more countries is designated as having widespread transmission of Ebola virus, we recommend that you update your donor history questionnaire (DHQ), including your full-length and abbreviated DHQ, and accompanying materials to incorporate the recommendations provided in this guidance.

We recommend that the updated DHQ include the following elements to assess prospective donors for risk of Ebola virus infection or disease.

a. A history of Ebola virus infection or disease.

b. A history of residence in or travel in the past 8 weeks to a country with widespread transmission of Ebola virus disease or cases in urban areas with uncertain control measures. (See http://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/distribution-map.html).

c. A history of close contact in the past 8 weeks with a person confirmed to have Ebola virus infection or disease or any person under investigation (PUI) for Ebola virus infection or disease in whom diagnosis is pending. For the purposes of this guidance, close contact is defined as contact that could have resulted in direct exposure to body fluids. Individuals falling into this close contact category include healthcare workers and other persons who care for, have lived with, or have otherwise been in contact with a PUI or a person confirmed to have Ebola virus infection or disease.[4]

- Additionally, this close contact category includes individuals with a history of sexual contact in the past 8 weeks with a person known to have recovered from EVD prior to that instance of sexual contact.

d. A history of notification by a public health authority that he or she may have been exposed in the past 8 weeks to a person with Ebola virus disease.

We note that educational material may assist donors in assessing their risk factors for Ebola virus infection or disease as described above. Relevant information on risk factors can be found on CDC's website at http://www.cdc.gov/vhf/ebola.

B. Donor Deferral

1. We recommend that you defer indefinitely[5] a donor with a history of Ebola virus infection or disease.

Note: This recommendation excludes the collection of convalescent plasma for treatment of EVD as described in section V. of this guidance.

---

[4] For additional information on epidemiologic risk factors to consider when evaluating a person for exposure to Ebola virus see: http://www.cdc.gov/vhf/ebola/exposure/risk-factors-when-evaluating-person-for-exposure.html. We expect individuals in the "high risk," "some risk," and "low risk" categories would fall into this close contact category.

[5] Until more data regarding the persistence of Ebola virus in survivors becomes available, we recommend you defer such donors indefinitely.

2. We recommend that you defer for 8 weeks[6] from the date of his or her departure a donor who has been a resident of or has travelled to a country with widespread transmission of Ebola virus disease or with cases in urban areas with uncertain control measures. (See http://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/distribution-map.html.)

   Note: Longer deferral periods may apply based on recommendations for deferral due to risk of malaria exposure. See FDA document entitled, "Guidance for Industry: Recommendations for Donor Questioning, Deferral, Reentry and Product Management to Reduce the Risk of Transfusion-Transmitted Malaria," dated August 2014. http://www.fda.gov/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/guidances/blood/ucm365191.htm.

3. We recommend that you defer for 8 weeks[6] after the last contact a donor who has had close contact with any PUI or confirmed to have Ebola virus infection or disease, in whom the diagnosis is pending. Individuals falling into this category include healthcare workers and other persons who care for, or have lived with, a PUI or person confirmed to have Ebola virus infection or disease.[4]

   - We recommend that you defer for 8 weeks[6] after the last sexual contact a donor who has had sexual contact with a person known to have recovered from Ebola virus disease.[7]

4. We recommend that you defer for a period of 8 weeks after exposure[6] a donor who has been notified by a federal, state, or local public health authority that he or she may have been exposed to a person with Ebola virus disease.

---

[6] Although symptoms generally appear within 21 days of infection, we recommend an extended deferral period of 8 weeks to prevent blood and blood component collection from an individual who could be infected and have an extended incubation period. In addition, 8 weeks is consistent with the inter-donation interval for Whole Blood donations.

[7] Until additional data regarding the length of time semen could be infectious post Ebola virus disease becomes available, we recommend that you defer for 8 weeks after the last sexual contact a donor who has had sexual contact with a person known to have recovered from Ebola virus disease.

C. Product Retrieval and Quarantine and Notification

1. Blood and Blood Components Collected from Donors at Risk for Ebola Virus Infection or Disease Because of Risk Factors Related to Residency, Travel or Close Contact.

   a. If you collected blood or blood components intended for transfusion or further manufacturing from a donor who should have been deferred for risk factors for EVD related to residency, travel, or close contact, according to the recommendations in section III.B. of this document, we recommend that you quarantine and destroy all undistributed in-date blood and blood components from that donor.

   b. If you distributed blood or blood components intended for transfusion or further manufacture from a donor who should have been deferred for risk factors for EVD related to residency, travel or close contact according to the recommendations in section III.B. of this document, we recommend that you notify consignees to retrieve, quarantine and destroy the in-date blood and blood components collected from that donor.

   c. We do not recommend retrieval or quarantine of plasma pooled for further manufacturing into products that are manufactured under processes that include multiple validated viral clearance steps, which have been shown to be robust in the clearance of lipid-enveloped viruses.

2. Blood and Blood Components Collected from Donors Later Determined to Have Ebola Virus Infection or Disease.

   We recommend you contact FDA[8] as soon as possible upon learning that you collected blood or blood components from a donor later determined to have Ebola virus infection or disease. In addition, blood establishments should consider the need to notify state and local public health authorities.

   a. If you collected blood or blood components within a recommended deferral period as specified in section III.B. of this document from a donor later determined to have Ebola virus infection or disease, you should promptly retrieve and quarantine the blood and blood components collected in the 8 weeks prior to disease onset and after disease onset.

      - If such blood components were transfused, we recommend that consignees notify the transfusion recipient's physician of record

[8] Contact CBER's Office of Communication, Outreach and Development (OCOD) by calling 1-800-835-4709 or 240-402-8010. After regular business hours and on weekends, call the FDA emergency number: 1-866-300-4374 or 301-796-8240.

regarding the need for notification and monitoring of the recipient for possible Ebola virus infection or disease.

b. Manufacturers should contact FDA to discuss their conduct of an adequate risk analysis if plasma collected from a donor later determined to have Ebola virus infection or disease has been pooled for further manufacturing or manufactured into a finished product. Finished products manufactured from such plasma pools should not be released prior to completion of an adequate risk analysis demonstrating that the product will not place patients at risk of Ebola virus disease. Finished products manufactured from such plasma pools that have been released should also undergo a risk analysis.

IV. REPORTING A BIOLOGICAL PRODUCT DEVIATION (BPD)

If you have distributed blood or blood components for transfusion or further manufacture collected from a donor at risk for or known to have Ebola virus infection or disease according to section III.B. of this document, you should report a BPD as soon as possible but you must report at a date not to exceed 45 calendar days from the date you acquire the information reasonably suggesting that a reportable event has occurred (21 CFR 606.171).

If you have distributed finished products manufactured from blood or blood components collected from a donor later determined to have Ebola virus infection or disease according to section III.B. of this guidance, you should report a BPD as soon as possible but you must report at a date not to exceed 45 calendar days from the date you acquire the information reasonably suggesting that a reportable event has occurred (21 CFR 600.14).

V. CONVALESCENT PLASMA

As of the date of issuance of this guidance, there are no FDA-approved therapeutics or licensed vaccines for Ebola virus disease. Standard treatment for Ebola virus disease is limited to supportive care, which includes intravenous fluids and electrolytes, treatment of secondary infections, and pain control.

Serum and plasma therapies have been used to treat many infectious diseases, including Junin Virus, a virus that also causes hemorrhagic fever (Ref. 22). There is similar interest in whether convalescent serum or plasma collected from Ebola virus disease survivors may be an effective therapy in Ebola virus outbreaks. Neutralizing antibodies are generated during filovirus infection in humans (Ref. 23). Ebola virus-infected individuals develop humoral immune responses (Ref. 24) that include neutralizing antibodies in some survivors. In previous studies conducted using non-human primates, passive transfer of certain neutralizing monoclonal antibodies (Refs. 25 and 26) and convalescent immunoglobulin concentrate prepared from non-

human primates that were vaccinated and virus challenged (Ref. 27) have resulted in protection against lethal challenge with Ebola virus. However, whole blood from Ebola virus vaccinated and challenged monkeys did not protect against Ebola virus challenge in non-human primates (Ref. 28).

Treatment of Ebola virus disease patients with convalescent human sera has been used in uncontrolled studies (Refs. 29 through 31). Based on the available scientific evidence, the World Health Organization (WHO) has developed interim guidance for national health authorities and blood transfusion services, entitled, "Use of Convalescent Whole Blood or Plasma Collected from Patients Recovered from Ebola Virus Disease for Transfusion, as an Empirical Treatment during Outbreaks," dated September 2014, http://www.who.int/csr/resources/publications/ebola/convalescent-treatment/en/.

As noted, although this investigational treatment has not yet been proven effective, its effectiveness is biologically plausible and has been prioritized by WHO for investigation. Convalescent plasma or serum collected from donors who have recovered from Ebola virus disease is an investigational product, and controlled studies with an adequate number of patients are needed to assess safety and effectiveness. Blood establishments wishing to collect or distribute convalescent plasma intended for transfusion in the United States must submit an investigational new drug application in accordance with 21 CFR Part 312, and sponsors seeking to develop devices for this use are subject to the investigational device regulations in 21 CFR Part 812 (see 21 CFR 601.21). We encourage such sponsors to contact FDA.[9]

VI. IMPLEMENTATION

This guidance is being issued for comment purposes only. If you elect to implement the recommendations contained in this draft guidance, you may do so without prior approval of FDA.

We will provide recommendations in the final guidance to licensed establishments on reporting implementation of the recommendations contained in the guidance, including revised donor history questionnaires and accompanying materials, to FDA under 21 CFR 601.12.

[9] Please contact the Office of Blood Research and Review, CBER in accordance with CBER SOPP 8101.1: Scheduling and Conduct of Regulatory Review Meetings with Sponsors and Applicants. See http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/ProceduresSOPPs/ucm079448.htm.

VII. REFERENCES

1. Feldmann, H., Jones, S., Klenk, H.D., et al., "Ebola Virus: from Discovery to Vaccine. Nature Review," *Immunology*, 2003; 3:677-685.

2. Towner, J.S., Sealy, T.K., Khristova, M.L., et al., "Newly Discovered Ebola Virus Associated With Hemorrhagic Fever Outbreak in Uganda," *PLoS pathogens*, 2008; 4:e1000212.

3. Mitchell, S.W., McCormick, J.B., "Physicochemical inactivation of Lassa, Ebola, and Marburg Viruses and Effect on Clinical Laboratory Analyses," *J Clin Microbiol*, 1984; 20:486-489.

4. Horowitz, B., Bonomo, R., Prince, A.M., et al., "Solvent/Detergent-Treated Plasma: a Virus-Inactivated Substitute for Fresh Frozen Plasma," *Blood*, 1992; 79:826-831.

5. Dichtelmuller, H., et al., "Robustness of Solvent/Detergent Treatment of Plasma Derivatives: A Data Collection from Plasma Protein Therapeutics Association Member Companies," *Transfusion*, 2009; 49:1931-1943.

6. El-Ekiaby, M., et al., "Solvent-Detergent Filtered (S/D-F) Fresh Frozen Plasma and Cryoprecipitate Minipools Prepared in a Newly Designed Integral Disposable Processing Bag System," *Transfusion Medicine*, 2010; 20:48-61.

7. Singh, Y., et al., "Photochemical Treatment of Plasma with Amotosalen and Long Wavelength Ultraviolet Light Inactivates Pathogens While Retaining Coagulation Function," *Transfusion*, 2006; 46:1168-1177.

8. Lin, L., et al., "Inactivation of Viruses in Platelet Concentrates by Photochemical Treatment with Amotosalen and Long-Wavelength Ultraviolet Light," *Transfusion*, 2005; 45:580-590.

9. Eichner, M., Dowell, S.F., Firese, N., "Incubation Period of Ebola Hemorrhagic Virus Subtype Zaire," *Osong Public Health Res. Perspect*, 2011; 2:3-7.

10. World Health Organization Ebola Response Team. "Ebola Virus Disease in West Africa--the First 9 Months of the Epidemic and Forward Projections," *N Engl J Med*, 2014; 371:1481-1495.

11. Haas, C.N., "On the Quarantine Period for Ebola Virus," *PLOS Currents Outbreaks*. 2014 Oct 14, 1st ed.

12. Varkey, J.B., et al., "Persistence of Ebola Virus in Ocular Fluid during Convalescence," *N Engl J Med*, 2015; 372: 2423-2437.

13. Bausch, D.G., Towner, J.S., Dowell, S.F., et al., "Assessment of the Risk of Ebola Virus Transmission from Bodily Fluids and Fomites," *The Journal of Infectious Diseases*, Nov 15, 2007; 196 Suppl 2:S142-147.

14. Rodriguez, L.L., De Roo, A., Guimard, Y, et al., "Persistence and Genetic Stability of Ebola Virus During the Outbreak in Kikwit, Democratic Republic of the Congo, 1995," *The Journal of Infectious Diseases*, Feb 1999; 179 Suppl 1:S170-176.4.

15. Rowe, A.K., Bertolli, J., Khan, A.S., et al., "Clinical, Virologic, and Immunologic Follow-Up of Convalescent Ebola Hemorrhagic Fever Patients and Their Household Contacts, Kikwit, Democratic Republic of the Congo. Commission de Lutte contre les Epidemies a Kikwit," *The Journal of Infectious Diseases*, Feb 1999; 179 Suppl 1:S28-35.

16. Richards, G.A., Murphy, S., Jobson, R., et al., "Unexpected Ebola virus in a Tertiary Setting: Clinical and Epidemiologic Aspects," *Critical Care Medicine*, Jan 2000; 28(1):240-244.

17. Deen, G.F. et al. "Ebola RNA Persistence in Semen of Ebola Virus Disease Survivors – Preliminary Report," *N Engl J Med,* October 2015; DIO: 10.1056/NEJMoas1511410.

18. Centers for Disease Control and Prevention, "Possible Sexual Transmission of Ebola Virus – Liberia, 2015," Morbidity and Mortality Weekly May 2015; 64(17): 479-481.

19. Mate, S.E. et al. "Molecular Evidence of Sexual Transmission of Ebola Virus," *N Engl J Med,* October 2015; DIO:10.1056/NEJMoa1509773.

20. Leroy, E.M. et al., "Human Asymptomatic Ebola Infection and Strong Inflammatory Response," *The Lancet,* 2000; 255: 2210-2215.

21. Nkoghe, D., Padilla, C., Becquart, P., et al., "Risk Factors for Zaire Ebolavirus--Specific IgG in Rural Gabonese Populations," *J Infect Dis*, 2011; 204 Suppl 3:S768-775.

22. Maiztegui, J.I., Fernandez, N.J., de Damilano, A.J., "Efficacy of Immune Plasma in Treatment of Argentine Haemorrhagic Fever and Association between Treatment and A Late Neurological Syndrome," *The Lancet,* 1979; 2:1216-1217.

23. Maruyama, T., Rodriguez, L.L., Jahrling, P.B., et al., "Ebola Virus Can Be Effectively Neutralized by Antibody Produced in Natural Human Infection," *J Virol*, 1999; 73:6024-6030.

24. Bradfute, S.B., Bavari, S., "Correlates of Immunity to Filovirus Infection," *Viruses*, 2011; 3:982-1000.

25. Qiu, X., Audet, J., Wong, G., et al., "Successful Treatment of Ebola Virus-Infected Cynomolgus Macaques with Monoclonal Antibodies," *Sci Transl Med*, 2012; 4:138ra181.

26. Olinger, G.G., Pettitt, Jr., J., Kim, D., et al., "Delayed Treatment of Ebola Virus Infection with Plant-Derived Monoclonal Antibodies Provides Protection in Rhesus Macaques," *Proc Natl Acad Sci USA*, 2012.

27. Dye, J.M., Herbert, A.S., Kuehne, A.I., et al., "Postexposure Antibody Prophylaxis Protects Nonhuman Primates from Filovirus Disease," *Proc Natl Acad Sci USA*, 2012; 109:5034-5039.

28. Jahrling, P.B., Geisbert, J.B., Swearengen, J.R., et al., "Ebola Hemorrhagic Fever: Evaluation of Passive Immunotherapy in Nonhuman Primates," *J Infect Dis*, 2007; 196 Suppl 2:S400-403.

29. International Commission, "Ebola Haemorrhagic Fever in Zaire, 1976," Bull World Health Organ 1978; 56:271-293.

30. Emond, R.T., Evans, B., Bowen, E.T., et al., "A Case of Ebola Virus Infection," *Br Med J*, 1977; 2:541-544.

31. Mupapa, K., Massamba, M., Kibadi, K., et al., "Treatment of Ebola Hemorrhagic Fever with Blood Transfusions from Convalescent Patients. International Scientific and Technical Committee," *J Infect Dis*, 1999; 179 Suppl 1:S18-23.

別紙様式第2-1

No. 8

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2016. 1. 7	該当なし	
一般的名称	新鮮凍結人血漿		研究報告の公表状況	Mate SE, Kugelman JR, Nyenswah TG, et al. N Engl J Med. 2015 Dec 17;373(25):2448-54. doi: 10.1056/NEJMoa1509773. Epub 2015 Oct 14.	公表国 米国	
販売名（企業名）	新鮮凍結血漿-LR「日赤」120（日本赤十字社） 新鮮凍結血漿-LR「日赤」240（日本赤十字社） 新鮮凍結血漿-LR「日赤」480（日本赤十字社）					

研究報告の概要

○性交渉によるエボラウイルス伝播の分子生物学的エビデンス。
リベリアにおいてエボラウイルス病（EVD）の生存者である男性から女性パートナー（報告対象患者）への性感染が疑われた。
2015年3月に発生した当該事例では、患者の血液検体及び男性生存者の精液検体からエボラウイルス（EBOV）のゲノムが抽出され、直接感染を支持する解析結果が得られた。両ゲノムに共通した3箇所の塩基置換は、西アフリカで確認された他の全てのEBOVのゲノムには認められず、今回の感染がそれ以前にリベリアで記録された一連の感染とも関連していないことを示すものであった。疫学データを結びつけた本ゲノム解析は、EBOVの性感染のエビデンス並びにEVD発症後179日以上、感染性EBOVが精液中に存在することを示すエビデンスを提供している。

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

エボラウイルス病（EVD）の生存者である男性との性交渉により相手女性がエボラウイルス（EBOV）に感染した。EVD発症後179日目の精液中には、感染性EBOVが存在するという報告である。

今後の対応

日本赤十字社は、輸血感染症対策として献血時に海外渡航歴の有無を確認し、帰国（入国）後4週間は献血不適とし、更にリベリア滞在者にはマラリア流行地域の為1年間の採血延期の措置をとっている。また、発熱などの体調不良者を献血不適としている。今後も引き続き情報の収集に努める。

MedDRA/J Ver.18.1J

BRIEF REPORT

Molecular Evidence of Sexual Transmission of Ebola Virus

S.E. Mate, J.R. Kugelman, T.G. Nyenswah, J.T. Ladner, M.R. Wiley, T. Cordier-Lassalle, A. Christie, G.P. Schroth, S.M. Gross, G.J. Davies-Wayne, S.A. Shinde, R. Murugan, S.B. Sieh, M. Badio, L. Fakoli, F. Taweh, E. de Wit, N. van Doremalen, V.J. Munster, J. Pettitt, K. Prieto, B.W. Humrighouse, U. Ströher, J.W. DiClaro, L.E. Hensley, R.J. Schoepp, D. Safronetz, J. Fair, J.H. Kuhn, D.J. Blackley, A.S. Laney, D.E. Williams, T. Lo, A. Gasasira, S.T. Nichol, P. Formenty, F.N. Kateh, K.M. De Cock, F. Bolay, M. Sanchez-Lockhart, and G. Palacios

SUMMARY

A suspected case of sexual transmission from a male survivor of Ebola virus disease (EVD) to his female partner (the patient in this report) occurred in Liberia in March 2015. Ebola virus (EBOV) genomes assembled from blood samples from the patient and a semen sample from the survivor were consistent with direct transmission. The genomes shared three substitutions that were absent from all other Western African EBOV sequences and that were distinct from the last documented transmission chain in Liberia before this case. Combined with epidemiologic data, the genomic analysis provides evidence of sexual transmission of EBOV and evidence of the persistence of infective EBOV in semen for 179 days or more after the onset of EVD. (Funded by the Defense Threat Reduction Agency and others.)

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Address reprint requests to Dr. Palacios at the U.S. Army Medical Research Institute for Infectious Diseases, 1425 Porter St., Rm. 622, Fort Detrick, Frederick, MD 21702-5011, or at gustavo.f.palacios.ctr@us.army.mil.

Dr. Mate, Dr. Kugelman, Mr. Nyenswah, Dr. Ladner, and Dr. Wiley contributed equally to this article.

This article was published on October 14, 2015, at NEJM.org.

N Engl J Med 2015;373:2448-54.
DOI: 10.1056/NEJMoa1509773
Copyright © 2015 Massachusetts Medical Society.

In December 2013, EBOV emerged in Guinea and quickly spread to several neighboring countries, resulting in the largest recorded outbreak of EVD in history.[1] On September 3, 2015, Liberia was declared to be free from EVD for the second time, and although new cases were still being reported in Guinea and Sierra Leone as of September 9, 2015, weekly numbers were just a fraction of those reported during the peak of the outbreak.[1] As the EVD outbreak in western Africa wanes, the affected countries must transition from controlling an EVD epidemic to addressing the needs of an unprecedented number of survivors of EVD who often have substantial medical sequelae.[2]

EBOV is detectable in the bloodstream only during acute illness, but the virus may persist for longer periods of time within immune-privileged sites. For instance, among convalescent patients, EBOV RNA has been detected in breast milk up to 15 days after the onset of the disease, in vaginal secretions up to 33 days after onset, in ocular aqueous humor up to 98 days after onset, and in semen up to 101 days after onset.[3-5] In addition, EBOV has been cultured from semen samples that were collected 40, 61, and 82 days after disease onset when EBOV was cleared from the blood.[3,4,6]

This long-term persistence may provide an opportunity for the transmission of EBOV from survivors even after the official end of an outbreak, which is currently defined by the World Health Organization (WHO) as 42 days after the last direct contact with a patient or burial (i.e., two incubation periods after blood

The New England Journal of Medicine
Downloaded from nejm.org at J R C CENTRAL BLOOD INSTITUTE on February 15, 2016. For personal use only. No other uses without permission.
Copyright © 2015 Massachusetts Medical Society. All rights reserved.

samples from the last patient with confirmed disease have tested negative twice for the virus). Because Marburg virus, a distant relative of EBOV, has been sexually transmitted at least once,[7] sexual transmission of EBOV is thought to be plausible. Consequently, the WHO and the Centers for Disease Control and Prevention advised survivors of EVD to abstain from sexual intercourse or to use condoms during sexual relations for at least 3 months after the onset of EVD. (Note that the epidemiologic investigation of this case[8] has resulted in a change to this recommendation[9].) However, evidence of the sexual transmission of EBOV has thus far been limited to scarce and inconclusive data.[10]

CASE REPORT

HISTORY AND EPIDEMIOLOGY

On March 20, 2015, a 44-year-old woman from Montserrado County, Liberia, was confirmed to have EVD. Blood samples from the patient were confirmed to be positive for EBOV RNA by quantitative reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay at the Eternal Love Winning Africa (ELWA) Laboratory in Paynesville, Liberia. The patient died on March 27, 2015.

The case investigation did not reveal an immediate source of infection, such as contact with patients with acute EVD. However, the patient reported that on March 7, 2015, she had had unprotected vaginal intercourse with a male Liberian survivor of EVD.[8] Subsequent to the patient's EVD diagnosis, 192 contacts were identified,[11] all of whom were free from clinical signs.

The survivor also lived in Montserrado County. Several members of his family had had EVD, beginning in late August 2014. The survivor's older brother, who presented with clinical signs of EVD on August 22, died during the night on September 5–6, 2014, and was confirmed to be positive for EBOV RNA by means of a postmortem quantitative RT-PCR assay. The survivor is thought to have had symptoms of EVD beginning on September 9, 2014, which is the estimated triage date,[8] and he was admitted to the nearby Island Clinic Ebola Virus Disease Treatment Unit on September 23.

Quantitative RT-PCR testing for EBOV RNA in the survivor's first blood sample on September 28 yielded ambiguous results. Repeated testing of this sample on September 29 yielded a negative result for EBOV. A subsequent test performed on October 3 (presumably from a second blood sample, although this information could not be confirmed owing to the absence of a sample record) was also negative. The survivor was discharged from the Ebola treatment unit on October 7 and reported no subsequent illness.

On September 20, clinical signs of EVD developed in the survivor's former wife, who was estranged from the survivor. She was admitted to an ELWA Ebola treatment unit on September 24 and died the following day.

As a result of the case investigation into the patient's illness, the survivor voluntarily provided a blood sample on March 23, 2015, and a semen sample on March 27, 2015 (199 days after the estimated onset of EVD and 175 days after the survivor's blood tested negative for EBOV). The blood sample tested negative for EBOV RNA on quantitative RT-PCR assay, but the sample tested positive for EBOV glycoprotein-specific and nucleoprotein-specific IgG antibodies.[8] The semen sample tested positive for EBOV RNA on quantitative RT-PCR assay, but attempts to culture virus were unsuccessful.

On April 28 (32 days later), the survivor provided a second semen sample for diagnostic testing at the Liberian National Public Health Reference Laboratory in Margibi County. No EBOV RNA was detected by the quantitative RT-PCR assay. A third semen sample, collected 3 days later, on May 1, also tested negative for EBOV on quantitative RT-PCR assay, which suggests that there was possible EBOV clearance from semen 231 days after the estimated onset of EVD and 207 days after the survivor's blood tested negative. A timeline of the events is shown in Fig. 1.

Specific consent was obtained from the survivor. For all other samples collected for testing during the EVD outbreak, informed consent was not obtained because this work was conducted at the Liberian Institute for Biomedical Research (LIBR) as part of the EVD response and EBOV surveillance. With the consent of the National Incident Management System of the Ebola Virus Disease Outbreak and the Liberian Ministry of Health and Social Welfare, the work was supervised by the LIBR institutional review board. All the information obtained from the participants was anonymized for this report. The WHO Liberia

The New England Journal of Medicine
Downloaded from nejm.org at J R C CENTRAL BLOOD INSTITUTE on February 15, 2016. For personal use only. No other uses without permission.
Copyright © 2015 Massachusetts Medical Society. All rights reserved.

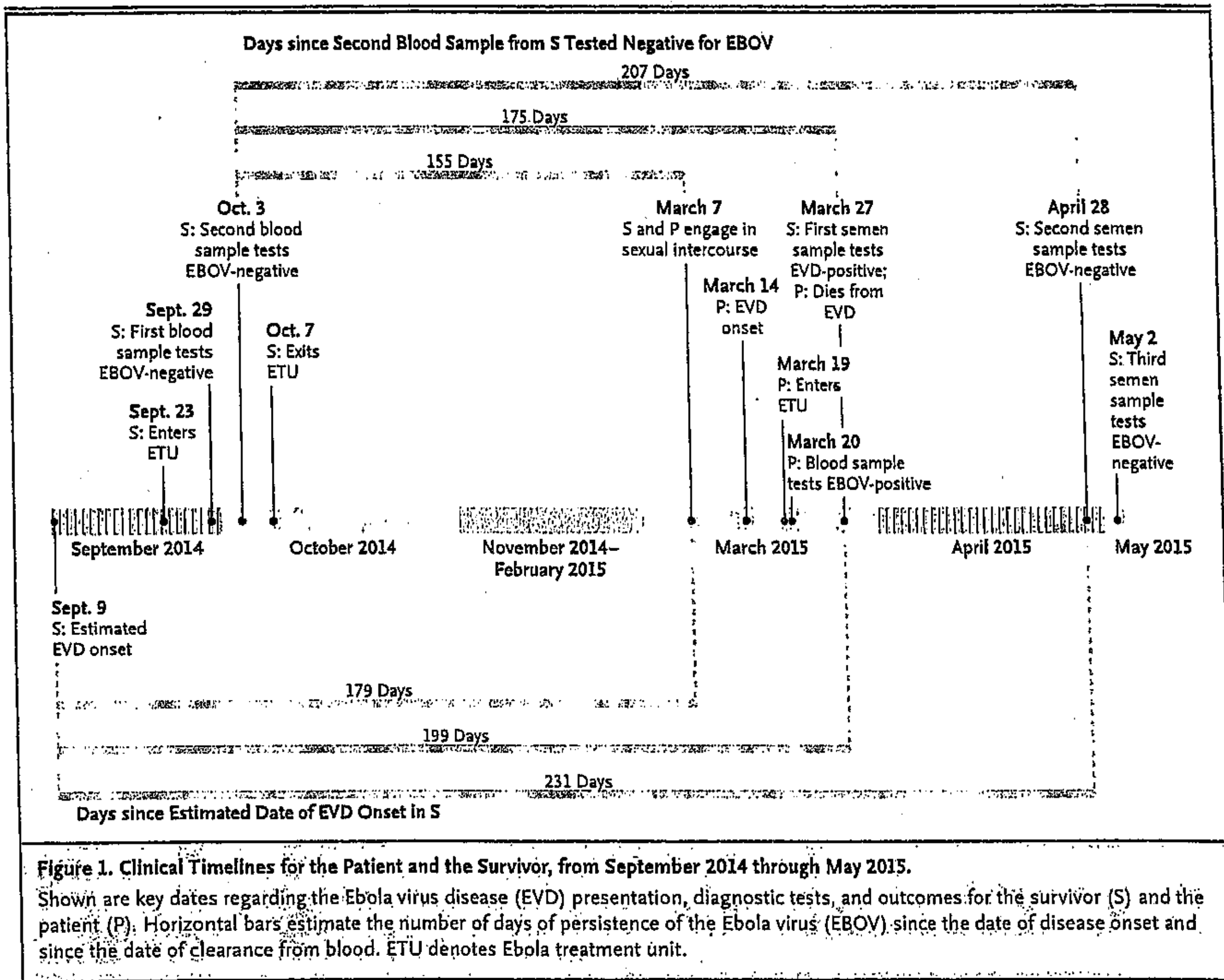


Figure 1. Clinical Timelines for the Patient and the Survivor, from September 2014 through May 2015.

Shown are key dates regarding the Ebola virus disease (EVD) presentation, diagnostic tests, and outcomes for the survivor (S) and the patient (P). Horizontal bars estimate the number of days of persistence of the Ebola virus (EBOV) since the date of disease onset and since the date of clearance from blood. ETU denotes Ebola treatment unit.

Country Office team coordinated field epidemiologic investigations and support to the survivor and the patient.

MOLECULAR INVESTIGATION

As part of the investigation into the source of the patient's EBOV infection, the following samples were examined: whole blood from the patient was tested on March 20 and 21, 2015 (two samples); whole blood from the survivor's older brother was tested on September 9, 2014; whole blood from the survivor's former wife was tested on September 24, 2014; and semen from the survivor was tested on March 27, 2015. Viral RNA that was potentially present in all five samples was initially sequenced on an Illumina MiSeq at the LIBR with the use of methods that have been described previously.[12]

Nearly complete EBOV genome sequences (97.4 to 99.7% coverage) were assembled from the samples obtained from the patient, the survivor's older brother, and the survivor's former wife. No EBOV sequences were obtained from the survivor's semen sample with the use of this sequencing method. Therefore, we enriched the semen sample for EBOV genomic RNA using the TruSeq RNA Access kit (Illumina) with custom capture probes designed against EBOV, along with other modifications (see the Supplementary Appendix, available with the full text of this article at NEJM.org). The semen sample was processed and sequenced separately to avoid contamination. With the combined data from four independent enrichment libraries, 85.1% genome coverage was achieved. A minimum of 3× sequencing depth was required to determine a genome position. However, the enrichment process resulted in a large number of duplicate reads.

The New England Journal of Medicine
Downloaded from nejm.org at J R C CENTRAL BLOOD INSTITUTE on February 15, 2016. For personal use only. No other uses without permission.
Copyright © 2015 Massachusetts Medical Society. All rights reserved.

Table 1. Distinct Ebola Virus Genome Substitutions in the Patient, the Survivor, and the Survivor's Older Brother.*

Position†	Reference	Alternative	Samples with Alternative	Survivor-Corrected Depth‡	Nature of Substitution§
4,107	G	A	P, S	1	VP35, V327I
8,592	A	T	P, S	1	VP30, synonymous
16,636	G	A	P, S	5	L, G1686S
4,384	A	C	P, S, SB	3	Noncoding
12,996	C	A	P, S, SB	1	L, synonymous
18,399	AAAAAA	AAAAAAA	P, S, SB	2	Noncoding
11,263	C	T	S	1	Noncoding

* The GenBank accession numbers for the tested genomes are as follows: for the patient (P), the number is KT587343, for the survivor (S), the number is KT587344, and for the survivor's older brother (SB), the number is KT587346. L denotes RNA-dependent RNA polymerase, and VP viral protein.

† Positions were relative to the reference genome Ebola virus/H.sapiens-wt/GIN/2014/Makona-C15 (GenBank accession number, KJ660346.2).

‡ The number indicates the depth at each position from the survivor after correction for duplicates resulting from polymerase-chain-reaction amplification.

§ The gene abbreviation is provided for substitutions within coding regions, followed by a description of the amino acid change for substitutions that are nonsynonymous.

Therefore, the duplicate-adjusted sequencing depth for the semen sample was less than 3× in some positions (Table 1). The assembled genomes are available at GenBank under accession numbers KT587343, KT587344, KT587346, and KT587345.

The four assembled EBOV genomes were compared with all publicly available sequences (796 genomes, including 56 from cases in Liberia[12-19]) from the outbreak in Western Africa (Makona variant).[20] The results were consistent with sexual transmission of EBOV from the survivor to the patient. First, the EBOV genome from the patient grouped phylogenetically with other genomes obtained from Liberian patients and was distinct from sequences from patients in Guinea, Sierra Leone, and Mali (Fig. 2). Thus, it is unlikely that the patient was infected owing to an undocumented reintroduction of EBOV to Liberia from a neighboring country with ongoing transmission.

Second, before the confirmation of EVD in the patient, the last known cluster of EVD cases in Liberia (December 29, 2014, to February 19, 2015) was linked to a single index case from a village near Saint Paul River Bridge, and the three sequenced EBOV genomes from this cluster (LIBR0993, LIBR1195, and LIBR1413) grouped together in an evolutionary lineage (SPB in Fig. 2) that was unrelated to the EBOV genome from the patient.[21] Therefore, the infection in this patient is unlikely to have originated from this cluster of EVD cases.

Finally, the EBOV genomes from the patient and the survivor differed in only one position (11,263) across 15,808 nucleotides, a finding that is consistent with direct EBOV transmission (Fig. 2 and Table 1). Notably, EBOV genomes from the patient and the survivor shared eight substitutions relative to the ancestral haplotype (SL2)[15] that is thought to have been originally introduced into Liberia,[12] and three of these substitutions have thus far been seen only in the viruses that infected the patient and the survivor (Fig. 2 and Table 1). Although only 1× sequencing depth was obtained for several of these positions (after correction for duplicate reads), the detection in the survivor's semen sample of every substitution that distinguished the patient's EBOV sequence from the ancestral SL2 haplotype is indicative of a close epidemiologic link. The EBOV genome obtained from the survivor's older brother shared five of eight substitutions with the EBOV genomes of the patient and the survivor, which suggests involvement of the survivor's older brother in the same transmission chain (Fig. 2 and Table 1). The EBOV genome from the survivor's former wife, however, was distinct (Fig. 2).

The EBOV genomes from the patient and the survivor differed at a single position (11,263),

The New England Journal of Medicine
Downloaded from nejm.org at J R C CENTRAL BLOOD INSTITUTE on February 15, 2016. For personal use only. No other uses without permission.
Copyright © 2015 Massachusetts Medical Society. All rights reserved.

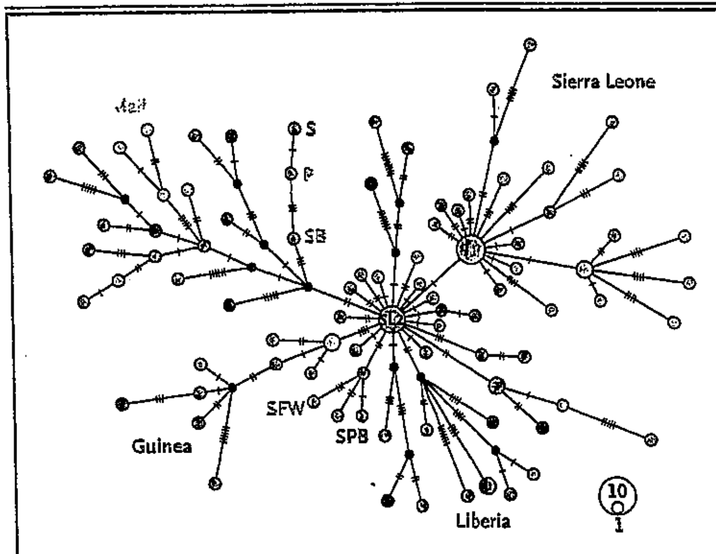


Figure 2. Median-Joining Haplotype Network.

This network was constructed from a full genome alignment of 100 clinical sequences of the Ebola virus Makona variant, including those assembled from blood samples obtained from the patient (P), the survivor's older brother (SB), and the survivor's former wife (SFW), from a semen sample from the survivor (S), and from 96 additional genomes chosen from the 796 genomes that were analyzed to be representative of samples collected in Guinea, Liberia, Mali, and Sierra Leone. For visual clarity, the network was limited to 100 genomes. The GenBank accession numbers for the tested genomes are as follows: for P, the number is KT587343; for S, the number is KT587344; for SB, the number is KT587346, and for SFW, the number is KT587345. Each colored vertex represents a sampled viral haplotype. The vertex size is proportional to the number of sampled sequences. Genomes sequenced in this study are shown in pink. Purple vertexes (SPB) indicate samples from the last known cluster of EVD cases in Liberia before the infection of the patient discussed in this report. Other colors indicate the respective countries of origin. Edges are not drawn to scale; hatch marks indicate the number of substitutions along each edge. The vertex SL2 represents the ancestral haplotype that is thought to have been introduced into Liberia in the spring of 2014.[12,15]

thus representing an additional substitution in the survivor's EBOV genome relative to all other genomes assembled. After duplicates from PCR amplification were controlled for, this position had only 1× coverage depth. Given the low level of sequencing depth, this apparent substitution may simply represent a low-frequency allele in the survivor's EBOV population or even a sequencing artifact. Alternatively, it could represent a shift in allele frequencies of EBOV subpopulations within the survivor during the 20 days that passed between the date of sexual intercourse and potential transmission (March 7, 2015) and the date of semen collection (March 27, 2015). Nevertheless, the nearly identical EBOV genomes place the survivor and the patient in the same transmission chain, and case tracing confirms contact by vaginal intercourse.

DISCUSSION

Studies involving survivors from previous EVD outbreaks have indicated the possibility of sexual transmission owing to the presence of EBOV RNA in semen and vaginal secretions. However, our understanding of EBOV persistence in these bodily fluids is restricted to the examination of 13 samples (12 semen samples and 1 vaginal-secretion sample), each with a limited temporal span.[3,4,22] Using a combination of genomic and epidemiologic data, we found that at least one case of EVD in the ongoing Liberian outbreak probably resulted from sexual transmission through unprotected vaginal intercourse. Although we cannot exclude the possibility of EBOV transmission from sources that were not sampled, contact tracing failed to uncover any other connections of the patient to possible or confirmed EVD cases. Furthermore, the EBOV genomes assembled from the survivor and the patient shared three substitutions that were not present in 796 EBOV genomes from western Africa. Together, these data provide evidence of human-to-human EBOV transmission through sexual contact.

The analysis of the semen sample obtained from the survivor presented additional challenges beyond those encountered with whole-blood and oral-swab samples that were obtained from other patients with EVD. The high cycle-threshold values on the quantitative RT-PCR assay that were observed in the semen sample suggest a low viral load,[8] which makes it difficult to obtain enough sequencing coverage with unbiased amplification of RNA. Therefore, we implemented a new target-enrichment strategy to obtain sufficient coverage. This approach resulted in nearly complete coverage of the EBOV genome, which was necessary given the low number of substitutions that discriminate distinct transmission chains within the current (2013–2015) EVD outbreak.[15,19]

The frequency of EBOV persistence among survivors is unknown, and available information suggests that sexual transmission is a relatively rare event. Nonetheless, persistent infections, in combination with unprotected sexual intercourse, could lead to flare-ups of EVD at close-to-ran-

The New England Journal of Medicine
Downloaded from nejm.org at J R C CENTRAL BLOOD INSTITUTE on February 15, 2016. For personal use only. No other uses without permission.
Copyright © 2015 Massachusetts Medical Society. All rights reserved.

dom locations. We found that viral nucleic acids in the semen from a survivor of EVD persisted for at least 199 days after the estimated onset of EVD (175 days after the clearance from blood), which is more than four times as long as the WHO-defined waiting period for declaring a country to be free from EVD. Although the semen sample contained no detectable infectious EBOV, the assembly of a nearly complete genome suggested the possible presence of infectious particles. In addition, from the evidence of the sexual transmission between the patient and the survivor, we can infer that infectious EBOV was present in the survivor at least 179 days after the onset of disease (155 days after the clearance from blood). Larger and more systematic surveys of survivors are needed in order to determine the prevalence and risk of EBOV persistence in semen and other immunologically privileged sites.

The views expressed in this article are those of the authors and do not necessarily reflect the views or policies of the U.S. Department of Defense, the U.S. Department of Health and Human Services, the U.S. Department of the Army, or the institutions and companies affiliated with the authors.

Supported by the Defense Threat Reduction Agency, the Global Biosurveillance Technology Initiative, Global Emerging Infections Surveillance, and Illumina, by a contract (HHSN272200700016I) between Battelle Memorial Institute and the U.S. National Institute of Allergy and Infectious Diseases (NIAID), and by the Intramural Research Program, NIAID, National Institutes of Health.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank the survivor and his partner for willingly providing samples and information for the investigation; the personnel that provided infrastructure and personnel for general Ebola virus disease control in Liberia: Mark M. Bailey, Alissa L. Byrne, Gary W. Carter, Melissa A. Dugan, Joanna Fishback, Alex Hail, and Michelle A. Jefferson; Anthony R. Jones, Brian J. Kearney, Dave A. Norwood, Susana Padilla, Matthew A. Voorhees, and Neal Woollen (U.S. Army Medical Research Institute of Infectious Diseases [USAMRIID]); Trenton Bushmaker, Darryl Falzarano, Heinz Feldmann, Friederike Feldmann, Robert Fischer, Allison Groseth, Thomas Hoenen, Seth Judson, Andrea Marzi, Kristin McNally, Joseph Prescott, Kyle Rosenke, and Brandi Williamson (Rocky Mountain Laboratories, NIAID, NIH); Richard Bennett, Laura Bollinger, Michael R. Holbrook, Peter Jahrling, Krisztina Janosko, Joshua Johnson, Jason Kindrachuk, and Jonathan Marchand (Integrated Research Facility [IRF], NIAID, NIH); Augustine Fallah, Josiah S. George, Charlesetta Kanneh, Aaron T. Momolu, and Vera Yatta Walker (Liberian National Reference Laboratory); Eric Patten (Liberian Ministry of Health); U.S. Ambassador Deborah R. Malac for support of the USAMRIID, the Centers for Disease Control and Prevention, and the NIH–NIAID–IRF–Frederick Ebola Response Team; Tabitha Austill for arranging the shipment of sequencing and other equipment and supplies to the Liberian Institute for Biomedical Research; and Sean Lovett and Brad Pfeffer for the deposition of sequence data in public repositories.

APPENDIX

The authors' full names and academic degrees are as follows: Suzanne E. Mate, Ph.D., Jeffrey R. Kugelman, Ph.D., Tolbert G. Nyenswah, L.L.B., M.P.H., Jason T. Ladner, Ph.D., Michael R. Wiley, Ph.D., Thierry Cordier-Lassalle, M.B.A., D.B.S.S., Athalia Christie, M.I.A., Gary P. Schroth, Ph.D., Stephen M. Gross, Ph.D., Gloria J. Davies-Wayne, R.N., M.P.H., Shivam A. Shinde, M.B., B.S., Ratnesh Murugan, M.B., B.S., Sohpon B. Sieh, B.A., Moses Badio, M.Sc., Lawrence Fakoli, M.S., Fahn Taweh, B.S., Emmie de Wit, Ph.D., Neeltje van Doremalen, Ph.D., Vincent J. Munster, Ph.D., James Pettitt, M.S., Karla Prieto, M.S., Ben W. Humrighouse, M.S., M.P.H., Ute Ströher, Ph.D., Joseph W. DiClaro, Ph.D., Lisa E. Hensley, Ph.D., Randal J. Schoepp, Ph.D., David Safronetz, Ph.D., Joseph Fair, Ph.D., Jens H. Kuhn, M.D., Ph.D., David J. Blackley, Dr.P.H., A. Scott Laney, Ph.D., Desmond E. Williams, M.D., Ph.D., Terrence Lo, Dr.P.H., Alex Gasasira, M.B., Ch.B., M.P.H., Stuart T. Nichol, Ph.D., Pierre Formenty, D.V.M., M.P.H., Francis N. Kateh, M.D., Kevin M. De Cock, M.D., Fatorma Bolay, Ph.D., Mariano Sanchez-Lockhart, Ph.D., and Gustavo Palacios, Ph.D.

The authors' affiliations are as follows: the Center for Genome Sciences (S.E.M., J.R.K., J.T.L., M.R.W., K.P., M.S.-L., G.P.) and Diagnostic Systems Division (R.J.S.), U.S. Army Medical Research Institute of Infectious Diseases, and the Division of Clinical Research, Integrated Research Facility at Fort Detrick, National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH) (J.P., L.E.H., J.H.K.) — all in Frederick, MD; the Ministry of Health and Social Welfare (T.G.N., S.B.S., M.B., F.N.K.) and the World Health Organization (WHO) (G.J.D.-W., R.M.), Monrovia, and the Liberian Institute for Biomedical Research, Charlesville (L.F., F.T., F.B.) — all in Liberia; WHO, Geneva (T.C.-L., A.G., P.F.); the Centers for Disease Control and Prevention, Atlanta (A.C., B.W.H., U.S., D.J.B., A.S.L., D.E.W., T.L., S.T.N., K.M.D.C.); Illumina, San Diego, CA (G.P.S., S.M.G.); WHO, New Delhi, India (S.A.S); Rocky Mountain Laboratories, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, NIH, Hamilton, MT (E.W., N.D., V.J.M., D.S.); Naval Medical Research Unit 3, Cairo (J.W.D.); and the Foundation Mérieux, Washington, DC (J.F.).

REFERENCES

1. Ebola situation reports. Geneva: World Health Organization, 2015 (http://apps.who.int/ebola/ebola-situation-reports).
2. Clark DV, Kibuuka H, Millard M, et al. Long-term sequelae after Ebola virus disease in Bundibugyo, Uganda: a retrospective cohort study. Lancet Infect Dis 2015; 15:905-12.
3. Bausch DG, Towner JS, Dowell SF, et al. Assessment of the risk of Ebola virus transmission from bodily fluids and fomites. J Infect Dis 2007;196:Suppl 2:S142-S147.
4. Rodriguez LL, De Roo A, Guimard Y, et al. Persistence and genetic stability of Ebola virus during the outbreak in Kikwit, Democratic Republic of the Congo, 1995. J Infect Dis 1999;179:Suppl 1:S170-S176.
5. Varkey JB, Shantha JG, Crozier I, et al. Persistence of Ebola virus in ocular fluid during convalescence. N Engl J Med 2015; 372:2423-7.
6. Emond RT, Evans B, Bowen ET, Lloyd G. A case of Ebola virus infection. Br Med J 1977;2:541-4.
7. Martini GA, Schmidt HA. Spermatogenic transmission of the "Marburg virus": causes of "Marburg simian disease." Klin Wochenschr 1968;46:398-400. (In German.)
8. Possible sexual transmission of Ebola virus — Liberia, 2015. MMWR Morb Mortal Wkly Rep 2015;64:479-81.
9. World Health Organization. Ebola virus disease: factsheet N 103, August 2015 (http://www.who.int/mediacentre/factsheets/fs103/en/).

The New England Journal of Medicine
Downloaded from nejm.org at J R C CENTRAL BLOOD INSTITUTE on February 15, 2016. For personal use only. No other uses without permission.
Copyright © 2015 Massachusetts Medical Society. All rights reserved.

10. Rowe AK, Bertolli J, Khan AS, et al. Clinical, virologic, and immunologic follow-up of convalescent Ebola hemorrhagic fever patients and their household contacts, Kikwit, Democratic Republic of the Congo. J Infect Dis 1999;179:Suppl 1:S28-S35.
11. Cordier-Lassalle T. Summary of sector response model: a quality management system that led to Liberia's success in fighting Ebola. Geneva: World Health Organization, 2015 (http://www.who.int/csr/disease/ebola/sector-approach-liberia.pdf).
12. Kugelman JR, Wiley MR, Mate S, et al. Monitoring of Ebola virus Makona evolution through establishment of advanced genomic capability in Liberia. Emerg Infect Dis 2015;21:1135-43.
13. Baize S, Pannetier D, Oestereich L, et al. Emergence of Zaire Ebola virus disease in Guinea. N Engl J Med 2014;371:1418-25.
14. Carroll MW, Matthews DA, Hiscox JA, et al. Temporal and spatial analysis of the 2014-2015 Ebola virus outbreak in West Africa. Nature 2015;524:97-101.
15. Gire SK, Goba A, Andersen KG, et al. Genomic surveillance elucidates Ebola virus origin and transmission during the 2014 outbreak. Science 2014;345:1369-72.
16. Hoenen TS, Safronetz D, Groseth A, et al. Virology: mutation rate and genotype variation of Ebola virus from Mali case sequences. Science 2015;348:117-9.
17. Park DJ, Dudas G, Wohl S, et al. Ebola virus epidemiology, transmission, and evolution during seven months in Sierra Leone. Cell 2015;161:1516-26.
18. Simon-Loriere E, Faye O, Faye O, et al. Distinct lineages of Ebola virus in Guinea during the 2014 West African epidemic. Nature 2015;524:102-4.
19. Tong YG, Shi WF, Liu D, et al. Genetic diversity and evolutionary dynamics of Ebola virus in Sierra Leone. Nature 2015;524:93-6.
20. Kuhn JH, Andersen KG, Baize S, et al. Nomenclature- and database-compatible names for the two Ebola virus variants that emerged in Guinea and the Democratic Republic of the Congo in 2014. Viruses 2014;6:4760-99.
21. Nyenswah T, Fallah M, Sieh S, et al. Controlling the last known cluster of Ebola virus disease — Liberia, January–February 2015. MMWR Morb Mortal Wkly Rep 2015;64:500-4.
22. Richards GA, Murphy S, Jobson R, et al. Unexpected Ebola virus in a tertiary setting: clinical and epidemiologic aspects. Crit Care Med 2000;28:240-4.

Copyright © 2015 Massachusetts Medical Society.

The New England Journal of Medicine
Downloaded from nejm.org at J R C CENTRAL BLOOD INSTITUTE on February 15, 2016. For personal use only. No other uses without permission.
Copyright © 2015 Massachusetts Medical Society. All rights reserved.

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2015. 11. 18	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	Jayne Peters, Jonathon Elliott, Adrian Bloor, et al. 57th ASH Annual Meeting & Exposition, December 5-8, 2015, Orlando, FL. No.2346	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)抗体陽性血液製剤の輸血により造血幹細胞移植(HSCT)患者におけるCMV再活性化リスクが上昇することはない。
背景:英国ではSaBTO(Safety of Blood, Tissues and Organs)のガイダンスに従って、2013年4月に同種HSCT(AlloHSCT)患者への輸血はCMV抗体陰性血液製剤の非選択的使用とする方針に変更した。本解析は当該変更がCMV再活性化発生率の上昇をもたらしたか否かという点について評価を行うことを目的として実施した。
方法:1998年1月から2014年11月までの期間にHSCTを受けた患者868例(男性:561例、女性:307例)を本解析の対象とした。移植の種類の内訳は、AutoHSCTが384例、AlloHSCT(兄弟)が217例、AlloHSCT(HLA一致非血縁ドナー)が267例であった。診断の内訳は急性白血病が313例、慢性白血病が36例、骨髄腫が216例、リンパ腫または他の悪性疾患が42例であった。
結果:26,345件のCMV PCR検査の結果を評価した。AutoHSCT患者は3,100件中、109件(3.6%)が陽性となりCMV抗体陰性血液製剤の非選択的使用の前後ではCMV PCR陽性率に差異は認められなかった。追加解析の対象はAlloHSCT患者に限定し、患者を2群(A群:1998年から2013年、B群:2013年から2014年)に分けた。23,278件のうち、9.1%が陽性となった(log中央値:2.7、範囲:0.3~7.3)。CMV再活性化の発生率をA群とB群で比較した結果、差異は認められなかった(47.7% 対48.1%、p=0.93)。RIC(骨髄非破壊的前処置)を用いた移植を受けた患者群(53.2%対42.3%、p=0.02)およびドナー・レシピエント間CMVミスマッチ群(NP:24.5%、PN:80%、PP:90.8%、p<0.0001)では発生率がより高くなった。
結論:本解析により、CMV再活性化リスクはドナー・レシピエント間のCMVミスマッチおよび移植前処置の強度に関連することが示唆されている。CMV抗体陰性血液製剤の非選択的使用によりCMV再活性化リスクが上昇することはなく、CMV抗体の有無に基づく慎重なドナー選定がAlloHSCT後のCMV再活性化リスク低減における重要要素であると言える。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見

サイトメガロウイルス(CMV)抗体陽性血液製剤の輸血により造血幹細胞移植患者はCMV再活性化を受けないという報告である。

今後の対応

日本赤十字社では、CMVの感染防止に有効とされる、保存前白血球除去した血液製剤のみを供給している。さらに、必要に応じてCMV抗体が陰性であることを確認した血液製剤を供給している。今後もCMV感染に関する新たな知見等について情報の収集に努める。

ASH 57th Annual Meeting & Exposition
Orlando, FL • December 5-8, 2015

-Author name in bold denotes the presenting author
-Asterisk * with author name denotes a Non-ASH member
denotes an abstract that is clinically relevant.

denotes that this is a recommended PHD Trainee Session.

denotes that this is a ticketed session.

2346 Use of CMV Unselected Blood Products Does Not Increase the Risk of CMV Reactivation in Patients Undergoing Hematopoetic Stem Cell Transplant (HSCT)

Basic Science and Clinical Practice in Blood Transfusion
Program: Oral and Poster Abstracts
Session: 401. Basic Science and Clinical Practice in Blood Transfusion: Poster II

Sunday, December 6, 2015, 6:00 PM-8:00 PM
Hall A, Level 2 (Orange County Convention Center)

Jayne Peters, MRCP*, Jonathon Elliott, MRCP*, Adrian Bloor, FRCPath*, Michael Dennis, FRCPath*, John Murray, MSc*, Jim Cavet, FRCPath*, Tim Somervaille, Sven Armin Sommerfeld, MRCP*, Ken Mutton, FRCPath*, Kaz Mamat, MRCP*, Malcolm Gulver, PhD* and Samar Kulkarni, FRCPath*

The Christie NHS Foundation Trust, Manchester, United Kingdom

Introduction: Conventionally patients receiving allogeneic HSCT (AlloHSCT) have received CMV negative blood products to obviate the risk of transfusion related CMV transmission. In the era of leucodepletion with more than log 4 reduction in blood product WBC content, the utility of this practice has been questioned and, in line with SABTO (Safety of Blood, Tissues and Organs) guidance, most UK transplant centers have adopted the policy of using unselected blood products for this patient cohort. At this center, our policy was changed to conform to recommended national practice in April 2013. This analysis was carried out to evaluate if this change has resulted in an increased incidence of CMV reactivation.

Methods: 868 (M: 561; median age: F: 307; median age:) patients who received HSCT from January 1998 to November 2014 were included in analysis. Transplant types included AutoHSCT (n=384), AlloHSCT-Sibling (n=217) and AlloHSCT-MUD (n=267). Diagnosis was Ac Leukaemia (n=313), Chr Leukaemia (n=36), Myeloma (n=216), Lymphoma (n=261) or other malignancies (n=42). Commonest indication for AutoHSCT was myeloma or lymphoma. TBI based condition was used in 225 AlloHSCT and 50 AutoHSCT cases. RIC was used in 268 cases and full intensity in 215 cases (unknown in 1). Alemtuzumab or ATG was used in conditioning for 274 cases. Source of stem cell was PBSC (AutoHSCT: 363, AlloHSCT: 382), BM (AutoHSCT: 7, AlloHSCT: 97), both (AutoHSCT: 2, AlloHSCT: 9) and 8 AlloHSCT were UCB grafts.

Results: 26, 345 blood PCR results were evaluated. 3100 tests were requested in AutoHSCT patients and 109 (3.6%) were positive. There were no differences in the incidence of positive CMV PCR results before and after use of CMV unselected blood products. Further analysis was limited to AlloHSCT patients. AlloHSCT patients were divided in two groups, GrpA: 1998 to 2013 and GrpB: 2013 to 2014 to evaluate the effect of using CMV unselected blood products. In AlloHSCT group, 9.1% of 23278 samples tested for CMV PCR were positive (Median log: 2.7, range: 0.3 -7.3). Incidence of CMV reactivation was not different in GrpB as compared to GrpA (47.7% vs. 48.1%, $p=0.93$). There was no difference with gender (M: 45.8% vs. F: 53.1%, $p=0.13$), type of donor (Sibling: 48.6% vs. MUD: 47.9%, $p=0.98$), use of Alemtuzumab/ATG (50.6% vs. 45.4%, $p=0.51$), source of stem cells (BM: 36.4% vs. PBSC: 51.3%, $p=0.07$), use of TBI (43.8% vs. 52.3%, $p=0.06$). Higher incidence was observed with use of RIC transplant (53.2% vs. 42.3%, $p=0.02$) and donor-recipient CMV mismatch (NP: 24.5%, PN: 80%, PP: 90.8%, $p<0.0001$).

Conclusion: This analysis suggests that the risk of CMV reactivation is related to the donor-recipient CMV mis-match and the transplant intensity. Use of CMV unselected blood products does not increase the risk of CMV reactivation and careful selection of donors using CMV sero-status is the key factor to reduce the risk of CMV reactivation post AlloHSCT.

Disclosures: Cavet: *Janssen:* Consultancy , Research Funding , Speakers Bureau ; *Celgene:* Consultancy , Research Funding , Speakers Bureau . **Somervaille:** *Novartis Pharmaceuticals Corporation:* Consultancy , Membership on an entity's Board of Directors or advisory committees .

See more of: 401. Basic Science and Clinical Practice in Blood Transfusion: Poster II
See more of: Basic Science and Clinical Practice in Blood Transfusion
See more of: Oral and Poster Abstracts

<< Previous Abstract | Next Abstract >>

*signifies non-member of ASH

Copyright © 2015 by American Society of Hematology

別紙様式第2-1

No. 9

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日 2015. 11. 4	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	新鮮凍結人血漿		研究報告の公表状況	Shigemi D, Yamaguchi S, Otsuka T, et al. Am J Infect Control. 2015 Nov 1;43(11):1218-21.	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	使用上の注意記載状況・その他参考事項等
○日本の妊婦における血清サイトメガロウイルス(CMV)IgG抗体陽性率(2009年～2014年)。 背景:CMVは先天性感染症の主要原因である。日本女性(母親)における血清CMV IgG抗体の陽性率は低下してきている。本研究において、我々はCMV感染率並びにCMV感染のリスク因子を評価した。 方法:当院で分娩を行った女性7,074例の医療記録を後ろ向きに検討した。血清陰性であった患者には予防のための教材が提供され、妊娠後期に再度CMV IgG抗体価が測定された。セロコンバージョンが認められた母親から出生した新生児における先天性感染を尿検査によって判定した。 結果:全体のCMV IgG抗体陽性率は69.1%であった。CMV IgG抗体保有率は年齢、経産回数と共に上昇した。多変量ロジスティック回帰分析では、経産回数がCMV IgG抗体陽性の独立決定因子であった。妊娠期間におけるセロコンバージョン率は0.37%であった。セロコンバージョンが認められた女性の37.5%において新生児先天性CMV感染が発生した。 結論:我々は経産回数が血清CMV IgG抗体陽性の独立決定因子であることを確認した。これにより育児が母親におけるCMV感染の高リスク因子である可能性が示唆された。衛生情報の提供が、有効かつ安価なCMV感染の予防方法となる可能性がある。	新鮮凍結血漿-LR「日赤」120 新鮮凍結血漿-LR「日赤」240 新鮮凍結血漿-LR「日赤」480 血液を介するウイルス、細菌、原虫等の感染 vCJD等の伝播のリスク

報告企業の意見	今後の対応
日本の妊婦における血清サイトメガロウイルス(CMV)のIgG抗体の陽性率は1996年の82.5%から2014年には69.1%まで低下してきている。妊娠期間中のセロコンバージョン率は0.37%であり、その37.5%において新生児先天性CMV感染が発生したという報告である。	日本赤十字社では、CMVの感染防止に有効とされる、保存前白血球除去した血液製剤のみを供給している。さらに、必要に応じてCMV抗体が陰性であることを確認した血液製剤を供給している。今後もCMV感染に関する新たな知見等について情報の収集に努める。

American Journal of Infection Control 43 (2015) 1218-21

Contents lists available at ScienceDirect

American Journal of Infection Control

journal homepage: www.ajicjournal.org

Major article

Seroprevalence of cytomegalovirus IgG antibodies among pregnant women in Japan from 2009-2014

Daisuke Shigemi MD[a,b,*], Satoru Yamaguchi MD, PhD[b,c], Toshiaki Otsuka MD, PhD[d], Seiryu Kamoi MD, PhD[a,b], Toshiyuki Takeshita MD, PhD[a]

[a] *Department of Obstetrics and Gynecology, Nippon Medical School, Tokyo, Japan*
[b] *Department of Obstetrics and Gynecology, Nippon Medical School, Chiba Hokusoh Hospital, Chiba, Japan*
[c] *Yamaguchi Women's Hospital, Chiba, Japan*
[d] *Department of Hygiene and Public Health, Nippon Medical School, Tokyo, Japan*

Key Words:
Congenital cytomegalovirus infection
IgG antibody
Pregnancy

Background: Human cytomegalovirus (CMV) is a major cause of congenital infection. The seroprevalence of maternal CMV IgG antibodies among Japanese women is decreasing. In this study, we assessed the rate of and risk factors for CMV infection. This article includes a description of a method for the prevention of CMV infection.

Methods: Medical records of 7,074 women who delivered a baby at our hospital were retrospectively reviewed. For seronegative patients, preventive educational materials were provided, and CMV IgG antibody levels were reassessed during late pregnancy. Congenital infection in neonates from seroconverted mothers was determined by urine analysis.

Results: The overall CMV IgG seropositivity rate was 69.1%. The prevalence of CMV IgG increased with age and parity. In a multivariate logistic regression analysis, parity remained an independent determinant of CMV IgG seropositivity. The seroconversion rate for CMV IgG antibody during pregnancy was 0.37%. Neonatal congenital CMV infection occurred in 37.5% of seroconverted women. The risk of primary CMV infection in mothers during their first pregnancy was 7.0%, with an average follow-up period of 2.1 years.

Conclusion: We found that parity was an independent determinant of CMV IgG seropositivity, suggesting that child-rearing may be a high risk factor for maternal CMV infection. The provision of information on hygiene may be an effective and inexpensive method for preventing CMV infection.

Copyright © 2015 by the Association for Professionals in Infection Control and Epidemiology, Inc. Published by Elsevier Inc. All rights reserved.

Pregnancy usually occurs with an absence of a maternal immune response against the fetus and placenta. This immunologic tolerance allows maternal IgG antibodies to pass through the placenta to the fetus to protect it against infections; however, it can also increase the risk of a number of infections of mothers.[1] Human cytomegalovirus (CMV), a member of the human herpesviruses, is the major cause of congenital infections. Clinical manifestations include asymptomatic forms, severe fetal damage, and in rare cases, death because of spontaneous abortion. A relevant meta-analysis reported that approximately 0.64% of live-born neonates are infected with CMV and that this infection can cause symptoms ranging from sensorineural hearing defects to multiple organ failure.[2]

Once infected, CMV persists in the host for life, but it often induces few symptoms with no long-term sequelae. Moreover, recurrence of disease rarely occurs, except in patients with compromised immunity. In contrast, CMV infection is a major concern for seronegative pregnant women because of maternal immunosuppression and the risk of infection of the fetus, which has an immature immune system.

In pregnancy, recurrent CMV infection can be caused by reactivation of a latent CMV infection or reinfection with a different CMV strain.[3] The incidence of primary infection among pregnant women who are seronegative for CMV IgG ranges from 1%-4%,[4] whereas the rate of maternal transmission to the fetus in such cases is 30%-40%.[2] Recent studies have reported a decrease in the prevalence of anti-CMV IgG antibodies among pregnant women in

* Address correspondence to Daisuke Shigemi, MD, Nippon Medical School, 1-1-5, Sendagi, Bunkyo-ku, Tokyo, 113-8602, Japan.
E-mail address: dshigemi@nms.ac.jp (D. Shigemi).

Funding/Support: This study was supported in part by Health and Labor Sciences Research Grants of Japan (grant no. H25-Jisedai-Shitei-003).
Conflicts of interest: None to report.

0196-6553/$36.00 - Copyright © 2015 by the Association for Professionals in Infection Control and Epidemiology, Inc. Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ajic.2015.06.026

Table 1
Characteristics of study participants and neonates (N = 7,074)

Characteristics	Value
Maternal age (y)	31.80 ± 4.72
Parity (no.)	0.61 ± 0.72
Infant birth weight (g)	3,089 ± 380
pH of umbilical artery	7.31 ± 0.07
Apgar score, 1 min	8.93 ± 0.35
Apgar score, 5 min	9.94 ± 1.24
Maternal CMV-IgG seroprevalence (%)	69.1

NOTE. Values are mean ± SD or as otherwise indicated.
CMV, cytomegalovirus.

Table 2
Age group and parity

Age group (Y)	Total (n)	Parity			
		1st	2nd	3rd	≥4th
<24	472	363	102	6	1
25-29	1,728	1,164	461	87	16
30-34	2,792	1,368	1,109	274	41
35-39	1,780	666	825	245	44
≥40	302	101	143	44	14
Total	7,074	3,662	2,640	656	116

Japan,[5,6] indicating an increased risk of more severe congenital CMV infections in this patient group. Ikuta et al reported on the relevance of the CMV glycoprotein H subtypes of mothers and congenital CMV infection.[7]

The aim of this study was to assess the rate of CMV infection, identify risk factors for infection, and describe methods to prevent infection by screening pregnant women in Japan for CMV IgG antibodies over a 5-year period, from 2009-2014.

METHODS

The medical records of 7,074 women who delivered from May 2009-January 2014 at Yamaguchi Women's Hospital (Chiba, Japan) and underwent CMV IgG antibody screening were retrospectively reviewed. A blood sample was collected from each consenting participant to assess CMV IgG antibody levels during the early stage of pregnancy. For seronegative patients, preventive education was provided and CMV IgG antibody levels were reassessed during the late stage of pregnancy. The infants of women positive for CMV IgG antibodies during pregnancy (identified by a positive test results in the early or late stage of pregnancy) were evaluated for neonatal CMV infection by urine analysis using polymerase chain reaction. Our hospital is not a perinatal center; therefore, cases at high risk for preterm labor or small for gestational age are transferred to the perinatal center. For this reason, there were no severe preterm or small for gestational age babies who required intensive treatment or neonatal intensive care unit admission at our hospital.

Educational materials designed to prevent CMV infection in pregnant women were distributed at our hospital. These materials advised frequent handwashing after contact with the saliva or urine of a child, avoiding direct contact with a child's saliva, and avoiding kissing a child on the mouth.

Serum CMV IgG antibody titers were measured using an enzyme immunoassay (DENKA SEIKEN, Tokyo, Japan). The cutoff value used to determine IgG positivity was 2.0 IU/mL. Therefore, samples with a concentration >2.0 IU/mL were considered positive for CMV IgG, whereas those with a concentration <2.0 IU/mL were considered negative.

Table 3
Maternal age group and cytomegalovirus IgG seropositivity

Age group (Y)	Maternal cytomegalovirus IgG seropositivity (%)
<24	66.1
25-29	67.7
30-34	69.2
35-39	70.2
≥40	74.5

Table 4
Maternal parity and cytomegalovirus IgG seropositivity

Parity	Maternal cytomegalovirus IgG seropositivity (%)
1st	67.1
2nd	70.0
3rd	75.2
≥4th	77.8

Table 5
Multivariate logistic regression analysis for the association between parity, age, and cytomegalovirus seropositivity

Maternal factor	OR	95% CI	P value
Parity (per delivery)	1.18	1.09-1.27	<.001
Age (per 1-y increase)	1.01	1.00-1.02	.18

CI, confidence interval; *OR*, odds ratio.

SPSS version 21 for Windows (IBM Japan, Tokyo, Japan) was used for all statistical analyses. Clinical characteristics are presented as the means ± SDs. Categorical data are presented as percentages. The χ^2 test for trends was used to evaluate whether the prevalence of CMV IgG seropositivity increased with age or parity. Multivariate logistic regression analysis was performed to determine the odds ratios and 95% confidence interval for the associations between CMV IgG seropositivity and age and parity. All statistical tests were 2 sided, and a probability (P) value <.05 was considered significant.

RESULTS

The mean clinical characteristics of all study participants (N = 7,074) are shown in Table 1. Women of 30-34, 35-39, and 25-29 years of age accounted for most of the study cohort (n = 2,792; [39.5%]; n = 1,780 [25.2%], and n = 1,728 [24.4%], respectively), and Table 2 shows that most women were para 0 or 1 (n = 3,662 [48.2%] and n = 2,640 [37.1%], respectively).

As shown in Table 3, CMV IgG seropositivity increased with age (66.1% for <24 years of age, 67.7% for 25-29 years of age, 69.2% for 30-34 years of age, 70.2% for 35-39 years of age, and 74.5% for ≥40 years of age; for the trend, χ^2 $P < .001$). Seropositivity also significantly increased with the number of previous deliveries (67.1% for para 1, 70.0% for para 2, 75.2% for para 3, and 77.8% for para ≥4; for the trend, χ^2 $P < .001$) (Table 4).

Although the prevalence of CMV IgG seropositivity appeared to increase with both age and parity, the analysis of variance revealed significant differences according to age among multiparous women ($P < .001$, data not shown).

In a multivariate logistic regression analysis, the number of previous deliveries remained an independent determinant of CMV IgG seropositivity (odds ratio = 1.175; 95% confidence interval, 1.091-1.265) for each previous delivery (Table 5). Age was not an independent determinant of CMV IgG seropositivity.

The seroconversion rate for CMV IgG antibody during pregnancy was 0.37% (8/2,186 cases). The neonatal congenital CMV infection

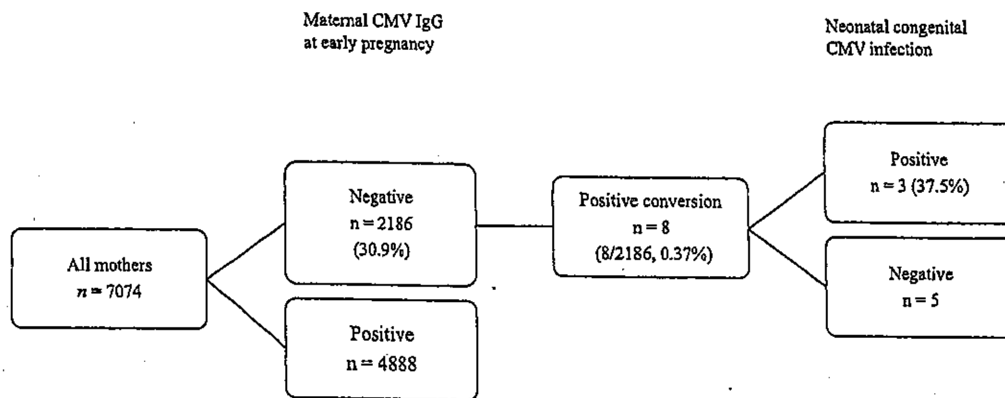


Fig 1. Neonatal congenital CMV infection rate. *CMV*, cytomegalovirus.

rate in the seroconverted maternal cases was confirmed by urine analysis to be 37.5% (Fig 1). The 3 children with congenital CMV infection were completely asymptomatic. The prevalence of new CMV infections among mothers during their child-rearing years was 7.0% (12/172 cases that were seronegative during their first pregnancy became seropositive during their second pregnancy), with an average follow-up period of 2.1 years.

DISCUSSION

The results of this study revealed a CMV IgG seropositivity rate of 69.1% among >7,000 pregnant women in Japan. This result is in accordance with a recent Japanese study[5] that revealed a trend of decreasing CMV IgG seropositivity over time, from 82.5% in 1996 to 69.1% in 2014. A major factor contributing to this decline may be the decreased risk of CMV infection during infancy because of improvements in living standards. The CMV seropositivity rate in Japan is not necessarily lower than that in other countries. For example, the reported rates of seropositivity among childbearing-aged women are 50%-88%[2] and 58.3%[8] in the United States, 68.3% in Italy,[9] 56.8% in Australia,[10] and 98.1% in Korea.[11] However, the increase in the prevalence of pregnant women without CMV antibodies generates a great need for the development of improved perinatal therapies. Congenital CMV infection involves a wide range of fetal, neonatal, and pediatric manifestations, ranging from spontaneous abortion to hearing defects. Although the efficacy of some medications for the prevention or treatment of congenital CMV infection has been investigated in recent years, such as ganciclovir,[12,13] valganciclovir,[14] and hyperimmune globulin,[15] no standard treatment has been established. Similarly, there is no vaccine for CMV.

We found that the prevalence of new CMV infections among mothers during their child-rearing years was 3.3% (7.0% per 2.1 years), which is considered high compared with the seroconversion rate of women who do not bear children. Colugnati et al reported a risk of primary CMV infection among non-Hispanic whites during a full-term pregnancy of 1.38%,[4] and this risk has not been reported among Japanese pregnant women. Based on the findings previously presented, nonpharmacologic methods for the prevention of CMV during the perinatal period should be considered. In the present study, the positive seroconversion rate for CMV IgG among pregnant women who received preventive education in the early stages of pregnancy was 0.37% (8/2,186 cases), which was lower than rates reported in the United States (1.6% among 12- to 49-year-old women, 1.38% among pregnant non-Hispanic whites, 3.40% among pregnant nonHispanic blacks, and 3.85% among pregnant Mexican Americans)[4] and France (0.6%-1.4%).[16] These results indicate that preventive education for CMV IgG-negative mothers may reduce the incidence of new CMV infections during pregnancy. Another study[17] reported that prevention programs focusing on hygiene may be beneficial for reducing primary CMV infection during pregnancy, with an infection rate of 0.19% (5/2,583 cases) in a group receiving hygiene counseling.

In addition, we determined that parity was an independent determinant of CMV IgG seropositivity, with an odds ratio of 1.175 for each previous delivery. This suggests that child-rearing may generate a high risk of maternal CMV infection.

Currently, there is no global consensus on the implementation of CMV serologic screening programs. However, the results of this study showed a decrease in the rate of neonatal congenital CMV infection among Japanese women who received educational material describing hygiene practices during pregnancy. Providing educational materials to women with insufficient CMV antibodies is inexpensive and effectively induced changes in daily behaviors among pregnant women in Japan to reduce the risk of new CMV infection of the fetus during pregnancy.

Acknowledgments

We appreciate all facilities cooperating on this nationwide survey supporting this project. We thank the Yamaguchi Women's Hospital staff for their cooperation in obtaining informed consents and gathering of blood samples. We are also grateful to the women who participated in this study.

References

1. Tanaka A, Hirota K, Takahashi K, Numazaki Y. Suppression of cell mediated immunity to cytomegalovirus and tuberculin in pregnancy employing the leukocyte migration inhibition test. Microbiol Immunol 1983;27:937-43.
2. Kenneson A, Cannon MJ. Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. Rev Med Virol 2007;17:253-76.
3. Stagno S. Cytomegalovirus. In: Remnington JS, Klein JO, editors. Infectious diseases of the fetus and newborn infant. 5th ed. Philadelphia (PA): WB Saunders Co; 2001. p. 389-424.
4. Colugnati FA, Staras SA, Dollard SC, Cannon MJ. Incidence of cytomegalovirus infection among the general population and pregnant women in the United States. BMC Infect Dis 2007;7:71.
5. Azuma H, Takanashi M, Kohsaki M, Sato H, Ishimaru F, Yamada H. Cytomegalovirus seropositivity in pregnant women in Japan during 1996-2009 [Japanese, with English abstract]. J Jpn Soc Perin Neon Med 2010;46:1273-9.
6. Taniguchi K, Watanabe N, Sato A, Jwa SC, Suzuki T, Yamanobe Y, et al. Changes in cytomegalovirus seroprevalence in pregnant Japanese women-a 10 year single center study. J Clin Virol 2014;59:192-4.
7. Ikuta K, Minematsu T, Inoue N, Kubo T, Asano K, Ishibashi K, et al. CMV glycoprotein H-based serological analysis in Japanese healthy pregnant women, and in neonates with congenital CMV infection and their mothers. J Clin Virol 2013;58:474-8.

8. Staras SA, Dollard SC, Radford KW, Flanders WD, Pass RF, Cannon MJ. Seroprevalence of cytomegalovirus infection in the United States, 1988-1994. Clin Infect Dis 2006;43:1143-51.
9. De Paschale M, Agrappi C, Manco MT, Paganini A, Clerici P. Incidence and risk of cytomegalovirus infection during pregnancy in an urban area of Northern Italy. Infect Dis Obstet Gynecol 2009;2009:206505.
10. Munro SC, Hall B, Whybin LR, Leader L, Robertson P, Maine GT, et al. Diagnosis of and screening for cytomegalovirus infection in pregnant women. J Clin Microbiol 2005;43:4713-8.
11. Seo S, Cho Y, Park J. Serologic screening of pregnant Korean women for primary human cytomegalovirus infection using IgG avidity test. Korean J Lab Med 2009;29:557-62.
12. Tanaka-Kitajima N, Sugaya N, Futatani T, Kanegane H, Suzuki C, Oshiro M, et al. Ganciclovir therapy for congenital cytomegalovirus infection in six infants. Pediatr Infect Dis J 2005;24:782-5.
13. Kimberlin DW, Lin CY, Sánchez PJ, Demmler GJ, Dankner W, Shelton M, et al. Effect of ganciclovir therapy on hearing in symptomatic congenital cytomegalovirus disease involving the central nervous system. J Pediatr 2003;143:16-25.
14. Kimberlin DW, Acosta EP, Sánchez PJ, Sood S, Agrawal V, Homans J, et al. Pharmacokinetic and pharmacodynamic assessment of oral valganciclovir in the treatment of symptomatic congenital cytomegalovirus diseases. J Infect Dis 2008;197:836-45.
15. Nigro G, Adler SP, La Torre R, Best AM, Congenital Cytomegalovirus Collaborating Group. Passive immunization during pregnancy for congenital cytomegalovirus infection. N Engl J Med 2005;353:1350-62.
16. Gratacap-Cavallier B, Bosson JL, Morand P, Dutertre N, Chanzy B, Jouk PS, et al. Cytomegalovirus seroprevalence in French pregnant women: parity and place of birth as major predictive factors. Eur J Epidemiol 1998;14:147-52.
17. Vauloup-Fellous C, Picone O, Cordier AG, Parent-du-Châtelet I, Senat MV, Frydman R, et al. Does hygiene counseling have an impact on the rate of CMV primary infection during pregnancy? Result of a 3-year prospective study in a French hospital. J Clin Virol 2009;46(Suppl 4):S49-53.

医薬品
医薬部外品　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2015 年 12 月 11 日	新医薬品等の区分		厚生労働省処理欄
一般的名称	①②③⑨⑩pH4 処理酸性人免疫グロブリン ④⑤⑥⑦⑧ポリエチレングリコール処理人免疫グロブリン ⑪⑫人免疫グロブリン			研究報告の公表状況	www.cdc.gov/mmwr/preview/mmwrhtml/mm6448a5.htm?s_cid=mm6448a5_w/2015/12/11	公表国 アメリカ	
販売名（企業名）	①献血ポリグロビン N5%静注 0.5g/10mL（日本血液製剤機構） ②献血ポリグロビン N5%静注 2.5g/50mL（日本血液製剤機構） ③献血ポリグロビン 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献血ポリグロビン N10%静注 5g/50mL（日本血液製剤機構） ⑩献血ポリグロビン N10%静注 10g/100mL（日本血液製剤機構） ⑪グロブリン筋注 450mg/3mL「JB」（日本血液製剤機構） ⑫グロブリン筋注 1500mg/10mL「JB」（日本血液製剤機構）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
Notes from the Field：セントルイス脳炎ウイルスおよびウエストナイルウイルス疾患の同時アウトブレイク－アリゾナ州、2015 年 米国における、初めてのセントルイス脳炎ウイルス（SLEV）およびウエストナイルウイルス（WNV）疾患の同時アウトブレイクに関する報告。2010 年～2014 年、537 の WNV 疾患症例および僅か 1 例の SLEV 疾患症例がアリゾナ州の保健サービス部門に報告された。2015 年 7 月末までに、アリゾナ在住のヒト 7 症例において SLEV 感染が確認された。2015 年 11 月 24 日時点で、75 の WNV 疾患症例、19 の SLEV 疾患症例、および 23 の未特定のフラビウイルス疾患症例を含む、合計 117 症例のフラビウイルス疾患がアリゾナ州保健サービス部門にに報告されている。15 ヵ所の郡のうち 8 ヵ所から症例が報告された；Maricopa 郡からは 45 の WNV 疾患症例と 18 の SLEV 疾患症例。全体的に、77 症例（66%）の患者は 50 歳以上（中央年齢=54 歳、範囲：21～89 歳）で、61 症例（52%）は男性であった。79 症例（68%）の患者は神経侵襲性疾患（髄膜炎，脳炎，急性弛緩性麻痺など）を発症した。全 117 症例の間で、86 症例（74%）が入院し、5 症例（4%）が死亡した。	代表として献血ヴェノグロブリン IH5%静注 0.5g/10mL の記載を示す。 2. 重要な基本的注意 (1)本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-1 抗体陰性で、かつ ALT（GPT）値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分からポリエチレングリコール 4000 処理、DEAE セファデックス処理等により人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理、ウイルス除去膜によるろ過処理及び pH3.9～4.4 の条件下

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

報告企業の意見	今後の対応	
セントルイス脳炎ウイルス（St. Louis encephalitis virus：SLEV）とウエストナイルウイルス（West Nile virus：WNV）は、フラビウイルス科フラビウイルス属に属し、大きさは40～60nmのエンベロープを有するRNAウイルスである。血清学的には日本脳炎ウイルス群に含まれ、蚊によって媒介される。FDAは、2005年6月の業界向けガイダンス改訂版において、「FDAは全ての血漿分画製剤について現在行われているウイルス低減工程を再調査した。現在行われている方法は、WNVとSLEVとが分類上関連しているフラビウイルスを不活化することがバリデートされている。」と評価し、EMAもまたポジションステートメントにおいて、血漿分画製剤の製造工程でWNVとSLEVは不活化・除去されると評価している。万一、原料血漿にWNVとSLEVが混入したとしても、各種モデルウイルスのウイルスクリアランス試験成績から、本剤の製造工程において不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。	での液状インキュベーション処理を施しているが、投与に際しては、次の点に十分注意すること。

グロブリン

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives. Protecting People.™
Morbidity and Mortality Weekly Report (*MMWR*)

Notes from the Field: Concurrent Outbreaks of St. Louis Encephalitis Virus and West Nile Virus Disease — Arizona, 2015

Weekly

December 11, 2015 / 64(48);1349-50

Heather Venkat, DVM1,2,3,*; Elisabeth Krow-Lucal, PhD1,4,*; Morgan Hennessey, DVM1,4; Jefferson Jones, MD1,2,3; Laura Adams, DVM3,6; Marc Fischer, MD4; Tammy Sylvester, MSN2; Craig Levy, MS2; Kirk Smith, PhD5; Lydia Plante, MSPH3; Kenneth Komatsu, MPH3; J. Erin Staples, MD4; Susan Hills, MBBS4

St. Louis encephalitis virus (SLEV) and West Nile virus (WNV) are closely related mosquito-borne flaviviruses that can cause outbreaks of acute febrile illness and neurologic disease. Both viruses are endemic throughout much of the United States and have the same *Culex* species mosquito vectors and avian hosts (*1*); however, since WNV was first identified in the United States in 1999, SLEV disease incidence has been substantially lower than WNV disease incidence, and no outbreaks involving the two viruses circulating in the same location at the same time have been identified. Currently, there is a commercially available laboratory test for diagnosis of acute WNV infection, but there is no commercially available SLEV test, and all SLEV testing must be performed at public health laboratories. In addition, because antibodies against SLEV and WNV can cross-react on standard diagnostic tests, confirmatory neutralizing antibody testing at public health laboratories is usually required to determine the flavivirus species (*2*). This report describes the first known concurrent outbreaks of SLEV and WNV disease in the United States.

During 2010–2014, 537 WNV disease cases and only one SLEV disease case were reported to the Arizona Department of Health Services. However, during 2015, by the end of July, SLEV infection had been confirmed in seven ill Arizona residents. In addition, the Maricopa County Vector Control Division identified 60 pools of *Culex tarsalis* or *Culex quinquefasciatus* mosquitoes that tested positive for SLEV RNA by reverse transcription polymerase chain reaction, and 97 pools that tested positive for WNV RNA. An investigation was initiated to ascertain the magnitude and describe the epidemiology of the outbreaks. Cases were defined according to national surveillance case definitions (*3*). If the patient had immunoglobulin M antibody against both WNV and SLEV, and insufficient sample or inconclusive results on neutralizing antibody testing, the case was classified as an unspecified flavivirus infection.

As of November 24, 2015, a total of 117 cases of flavivirus disease had been reported to the Arizona Department of Health Services, including 75 WNV, 19 SLEV, and 23 unspecified flavivirus disease cases. Laboratory testing is ongoing, and some cases will likely be reclassified. Among all cases, 103 (88%) occurred from July through September. Eight (53%) of 15 counties reported cases; 45 (60%) WNV and 18 (95%) SLEV disease cases were reported from Maricopa County. Overall, 77 (66%) patients were aged ≥50 years (median = 54 years, range = 21–89 years), and 61 (52%) were male. Seventy-nine (68%) patients had neuroinvasive disease (e.g., meningitis, encephalitis, or acute flaccid paralysis), including 47 (63%) with WNV infection, 17 (89%) with SLEV infection, and 15

(65%) with unspecified flavivirus infection. Among all 117 cases, 86 (74%) patients were hospitalized and five (4%) died.

This is the first known outbreak of concurrent WNV and SLEV disease. Enhanced clinical and laboratory surveillance activities in Arizona will continue through the end of the arboviral transmission season in late November to characterize the outbreak. WNV and SLEV disease cases will be compared to better understand differences in the epidemiology and outcomes of these diseases. Because of the similarity in clinical presentation for WNV and SLEV disease cases, cross reactivity between WNV and SLEV antibodies, and the lack of availability of a commercial SLEV test, SLEV disease cases could be incorrectly diagnosed as WNV disease cases or remain undetected if clinicians only request WNV testing and no confirmatory testing is conducted. Health care providers should consider both WNV and SLEV infections in the differential diagnosis of cases of aseptic meningitis and encephalitis and obtain appropriate cerebrospinal fluid, serum specimens, or both for laboratory testing (*4*). Confirmatory testing at state health departments or CDC will be required to distinguish these flavivirus infections. When feasible, vector control programs should test mosquitoes for SLEV in addition to WNV. Clinical management for both diseases involves supportive care. Because human vaccines against domestic arboviruses are not available, prevention of arboviral infection depends on local vector control, community, and household efforts to reduce vector populations (e.g., removal of standing water), and individual efforts to decrease exposure to mosquitoes (e.g., applying mosquito repellant and eliminating mosquito breeding sites).

Acknowledgments

Tammy Kafenbaum, Andrew Strumpf, Carrie Walker, Rebecca Sunenshine, Maricopa County Department of Public Health, Phoenix, Arizona; Jennifer Chevinsky, Kathryn Fitzpatrick, Jennifer Pistole, Arizona Department of Health Services; John Townsend, Maricopa County Environmental Services, Mesa, Arizona; Robert Lanciotti, Jennifer Lehman, Nicole Lindsey, Ingrid Rabe, Arboviral Diseases Branch, Division of Vector-Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, CDC.

1Epidemic Intelligence Service, CDC; 2Maricopa County Department of Public Health, Phoenix, Arizona; 3Arizona Department of Health Services; 4Division of Vector-Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, CDC; 5Maricopa County Environmental Services Vector Control Division; 6Career Epidemiology Field Officer Program, CDC.

Corresponding authors: Heather Venkat, HeatherVenkat@mail.maricopa.gov, 602-531-4422; Elisabeth Krow-Lucal, ekrowlucal@cdc.gov, 970-266-3565.

References

1. Reimann CA, Hayes EB, DiGuiseppi C, et al. Epidemiology of neuroinvasive arboviral disease in the United States, 1999–2007. Am J Trop Med Hyg 2008;79:974–9.
2. Martin DA, Noga A, Kosoy O, Johnson AJ, Petersen LR, Lanciotti RS. Evaluation of a diagnostic algorithm using immunoglobulin M enzyme-linked immunosorbent assay to differentiate human West Nile virus and St. Louis Encephalitis virus infections during the 2002 West Nile virus epidemic in the United States. Clin Diagn Lab Immunol 2004;11:1130–3.
3. CDC. Arboviral diseases, neuroinvasive and non-neuroinvasive: 2015 case definition. Atlanta, GA: US Department of Health and Human Services, CDC; 2015. Available at http://wwwn.cdc.gov/nndss/conditions/arboviral-diseases-neuroinvasive-and-non-neuroinvasive/case-definition/2015/.
4. CDC. West Nile virus—for healthcare providers: diagnostic testing. Atlanta, GA: US Department of Health and Human Services, CDC; 2015. Available at

http://www.cdc.gov/westnile/healthCareProviders/healthCareProviders-Diagnostic.html.

* These authors contributed equally to this report.

Use of trade names and commercial sources is for identification only and does not imply endorsement by the U.S. Department of Health and Human Services.

References to non-CDC sites on the Internet are provided as a service to *MMWR* readers and do not constitute or imply endorsement of these organizations or their programs by CDC or the U.S. Department of Health and Human Services. CDC is not responsible for the content of pages found at these sites. URL addresses listed in *MMWR* were current as of the date of publication.

All *MMWR* HTML versions of articles are electronic conversions from typeset documents. This conversion might result in character translation or format errors in the HTML version. Users are referred to the electronic PDF version (http://www.cdc.gov/mmwr) and/or the original *MMWR* paper copy for printable versions of official text, figures, and tables. An original paper copy of this issue can be obtained from the Superintendent of Documents, U.S. Government Printing Office (GPO), Washington, DC 20402-9371; telephone: (202) 512-1800. Contact GPO for current prices.

**Questions or messages regarding errors in formatting should be addressed to mmwrq@cdc.gov.

Page last reviewed: December 11, 2015
Page last updated: December 11, 2015
Content source: Centers for Disease Control and Prevention

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

別紙様式第2-1

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
			2015. 12. 3	該当なし	
一般的名称	新鮮凍結人血漿	研究報告の公表状況	Gong Z, Gu S, Zhang Y, et al. Clin Microbiol Infect. 2015 Dec;21(12):1115-20.	公表国 中国	
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

○中国南東部における重症熱性血小板減少症候群ウイルス(SFTSV)のエアロゾル伝播の可能性。
中国では2010年の時点においてSFTSVの集団感染が複数報告されていた。しかしながらSFTSVのエアロゾル伝播に関する疫学的エビデンスは今日に至るまで報告されていない。2014年の5月に発端患者と、感染確認症例11例並びに臨床診断症例1例を含む計13例のSFTSV感染者集団が確認された後、疫学的調査が実施された。発端患者は4月23日にSFTSV感染を発症し、5月1日に死亡した。発端患者に続いて発症した患者集団はSFTSV感染を5月10日から16日の期間に発症しており、最も発症者数が多かった日は5月13日であった。また、そのうちの8例は発端患者の家族であり、他の4例は発端患者の隣人であった。疫学的調査により、1、3、4、5、6、7、9、および12番目の患者は発端患者の血液に接触することにより感染していたことが判明した。特筆すべき点は、8番目と10番目の患者には発端患者の血液への接触歴がなく、両名が葬儀会場に長時間滞在していたことである。SFTSVは直接接触および/またはエアロゾルにより、ヒトからヒトへ伝播する可能性がある。エアロゾルによる伝播を可能性のある伝播経路の一つとして考慮することは重要である。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見

重症熱性血小板減少症候群ウイルス(SFTSV)の集団感染は、感染者の遺体との接触および/または遺体から発生したエアロゾルによりSFTSVが伝播した可能性があるとする報告である。

今後の対応

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

ORIGINAL ARTICLE VIROLOGY

Probable aerosol transmission of severe fever with thrombocytopenia syndrome virus in southeastern China

Z. Gong[1], S. Gu[2], Y. Zhang[1], J. Sun[1], X. Wu[2], F. Ling[1], W. Shi[1], P. Zhang[3], D. Li[4], H. Mao[1], L. Zhang[1], D. Wen[3], B. Zhou[2], H. Zhang[2], Y. Huang[2], R. Zhang[1], J. Jiang[1], J. Lin[1], S. Xia[1], E. Chen[1] and Z. Chen[1]

1) *Zhejiang Provincial Centre for Disease Control and Prevention, Hangzhou,* 2) *Anji Centre for Disease Control and Prevention, Anji,* 3) *Huzhou Municipal Centre for Disease Control and Prevention, Huzhou and* 4) *National Institute for Viral Disease Control and Prevention, Beijing, China*

Abstract

Some clusters of severe fever with thrombocytopenia syndrome virus (SFTSV) infection were reported in China as of 2010. However, to date, there has been no epidemiologic evidence of aerosol transmission of SFTSV. Epidemiologic investigations were conducted after a cluster of 13 cases of SFTSV in May 2014. A total of 13 cases, including 11 confirmed cases and one clinically diagnosed case, were identified besides the case of the index patient. The index patient experienced onset of SFTSV on 23 April and died on 1 May. The patients with secondary cases had onset from 10 to 16 May, peaking on 13 May. Moreover, eight secondary cases occurred in family members of the index patient, and the other five cases occurred in neighbors of the index patient. According to epidemiologic investigations, patients 1, 3, 4, 5, 6, 7, 9 and 12 contracted the disease through contact with blood of the index patient. Notably, patients 8 and 10 did not have a history of contact with the blood of the index patient, but they stayed in the mourning hall for hours. SFTSV could be transmitted from person to person by direct contact and/or aerosol transmission, and it is important to consider aerosol transmission as a possible transmission route.

Clinical Microbiology and Infection © 2015 The Authors. Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases.

Keywords: Aerosol transmission, cluster, emerging infectious disease, person to person, severe fever with thrombocytopenia syndrome virus
Original Submission: 19 January 2015; **Revised Submission:** 27 July 2015; **Accepted:** 28 July 2015
Editor: I. Gyssens
Article published online: 6 August 2015

Corresponding authors: Z. Chen, Zhejiang Provincial Centre for Disease Control and Prevention, No. 3399, Binsheng Road, Hangzhou, China
E. Chen, Zhejiang Provincial Centre for Disease Control and Prevention, No. 3399, Binsheng Road, Hangzhou, China
S. Xia, Zhejiang Provincial Centre for Disease Control and Prevention, No. 3399, Binsheng Road, Hangzhou, China
E-mails: shchxia@cdc.zj.cn (S. Xia), enfchen@cdc.zj.cn (E. Chen), zjcdcczp@126.com (Z. Chen)
The first six authors contributed equally to this article, and all should be considered first author

Introduction

Severe fever with thrombocytopenia syndrome (SFTS), with an average case fatality rate of 12%, is an emerging infectious disease caused by a newly discovered virus, severe fever with thrombocytopenia syndrome virus (SFTSV). SFTSV is classified in the family *Bunyaviridae*, genus *Phlebovirus*, and contains three segments of negative or ambisense polarity RNA, designated L, M and S segments. The major clinical symptoms and laboratory abnormalities of SFTS are fever, thrombocytopenia, leukopenia and elevated serum hepatic enzymes, and SFTS patients usually die of multiple organ failure [1]. The clinical symptoms, however, are less specific and need to be differentiated from various other infectious diseases, in particular hemorrhagic fever with renal syndrome caused by hantavirus and human anaplasmosis [2,3].

Knowledge of the transmission mode of SFTSV is fundamental to an understanding and control of the disease. SFTSV is believed to be transmitted by ticks because the virus has been detected in *Haemaphysalis longicornis* ticks. Some studies reported that SFTSV could also be transmitted from person to

Clin Microbiol Infect 2015; **21:** 1115–1120
Clinical Microbiology and Infection © 2015 The Authors. Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)
http://dx.doi.org/10.1016/j.cmi.2015.07.024

person. Bao and colleagues reported that SFTSV was transmitted from a 59-year-old man to his son and son-in-law in 2010, and another cluster showed that SFTSV was transmitted from an 80-year-old woman to six secondary patients including her daughters, nephews and sons-in-law in 2007 in Jiangsu Province [4,5]. Gai *et al.* [6] identified the person-to-person transmission of SFTSV with a cluster of six SFTS patients including intensive care unit physician, intensive care unit consultation physician, mortuary beautician and family members that occurred in 2010 in Shandong Province. Three other studies reported that person-to-person transmission of SFTSV occurred in Anhui Province, Henan Province and Hubei Province, respectively [7–9]. However, all secondary patients of these clusters contracted SFTSV infection through contact with the blood or bloody secretions of patients in the end stage of the disease. There was no epidemiologic evidence of aerosol transmission of SFTSV. In this study, we identified a cluster of person-to-person transmission of SFTSV and investigated the potential transmission routes, including aerosol transmission.

Methods

Case definition

According to "the diagnosis and treatment programs of severe fever with thrombocytopenia syndrome," issued by the Chinese Ministry of Health (http://www.moh.gov.cn/mohwsyjbgs/s8348/201010/49272.shtml), a suspected case of SFTS is defined as acute onset of fever (≥38.0°C) with other symptoms (e.g. gastrointestinal symptoms, bleeding), epidemiologic risk factors (being a farmer or being exposed to ticks 2 weeks before onset of illness) and laboratory data consisting of thrombocytopenia and leukocytopenia. Confirmed SFTS cases were defined as meeting the criteria for suspected SFTS and also met one or more of the following criteria: (a) detection of SFTSV RNA by a molecular method from patient serum, (b) seroconversion or a fourfold or more increase of antibody titers between acute and convalescent sera collected at least 2 weeks apart or (c) isolation of SFTSV in cell culture.

The ethics committee of Zhejiang Provincial Centre for Disease Control and Prevention approved this research project. Human research was carried out in compliance with the Helsinki Declaration. All participants provided written informed consent to participate in this study.

Laboratory test assays

The sera of the suspected patients were tested for SFTSV RNA by real-time reverse-transcription PCR performed as described elsewhere [10] in the Zhejiang Provincial Centre for Disease Control and Prevention.

Epidemiologic investigation

All persons who had a history of contact with the body of the index patient from 1 to 3 May were interviewed. The aims of our study were explained to all patients, and their consent was obtained before inclusion onto this study. A standardized questionnaire was used to collect information about demographic features, such as age, gender, occupation, and residential address, exposure history, clinical signs and symptoms, date of onset and date of confirmation. Exposure history included taking care of the index patient in hospital, moving the corpse into a car and from the car to home, washing and wiping the corpse, dressing the corpse, moving the corpse to a coffin, keeping vigil beside the coffin and staying in the mourning hall with no contact with the corpse.

Results

A total of 13 SFTS cases, including 11 confirmed cases and one clinically diagnosed case, were identified besides the index patient during this outbreak. Of these patients, six were men and seven were women, and the median age of the patients was 60.5 years, ranging from 41 to 74 years (Fig. 1). The index patient experienced disease onset on 23 April and died on 1 May. The secondary cases occurred from 10 to 16 May, with a peak on 13 May (Fig. 2). Eight secondary cases occurred in family members of the index patient, and the other five cases occurred in neighbors of the index patient (Fig. 1).

The index patient and all secondary patients were hospitalized, and all patients had fever, fatigue and chills. The majority of patients had headache, anorexia, myalgia and conjunctival congestion. Four patients had nausea, three diarrhea, two vomiting and two gingival hemorrhage (Table 1). Symptoms were similar for the index patient and the secondary patients.

The index patient was 66-year-old female farmer with a history of hypertension for more than 15 years. She lived in a wooded, hilly upland area with shrubs and grasses (Fig. 3). She used to pick tea leaves before onset of illness and developed a fever on 23 April. She visited a clinic on 25 April, and her temperature was 40°C. She was treated with ribavirin and azlocillin, but symptoms were not alleviated. She thus continued seeking medical consultations at several hospitals. She was hospitalized on 29 April (Fig. 4). As her condition deteriorated, her family requested that the hospital discharge her. Her son, daughter and brother brought her home on 1 May. She died

Clinical Microbiology and Infection © 2015 The Authors. Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases, *CMI*, 21, 1115–1120
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

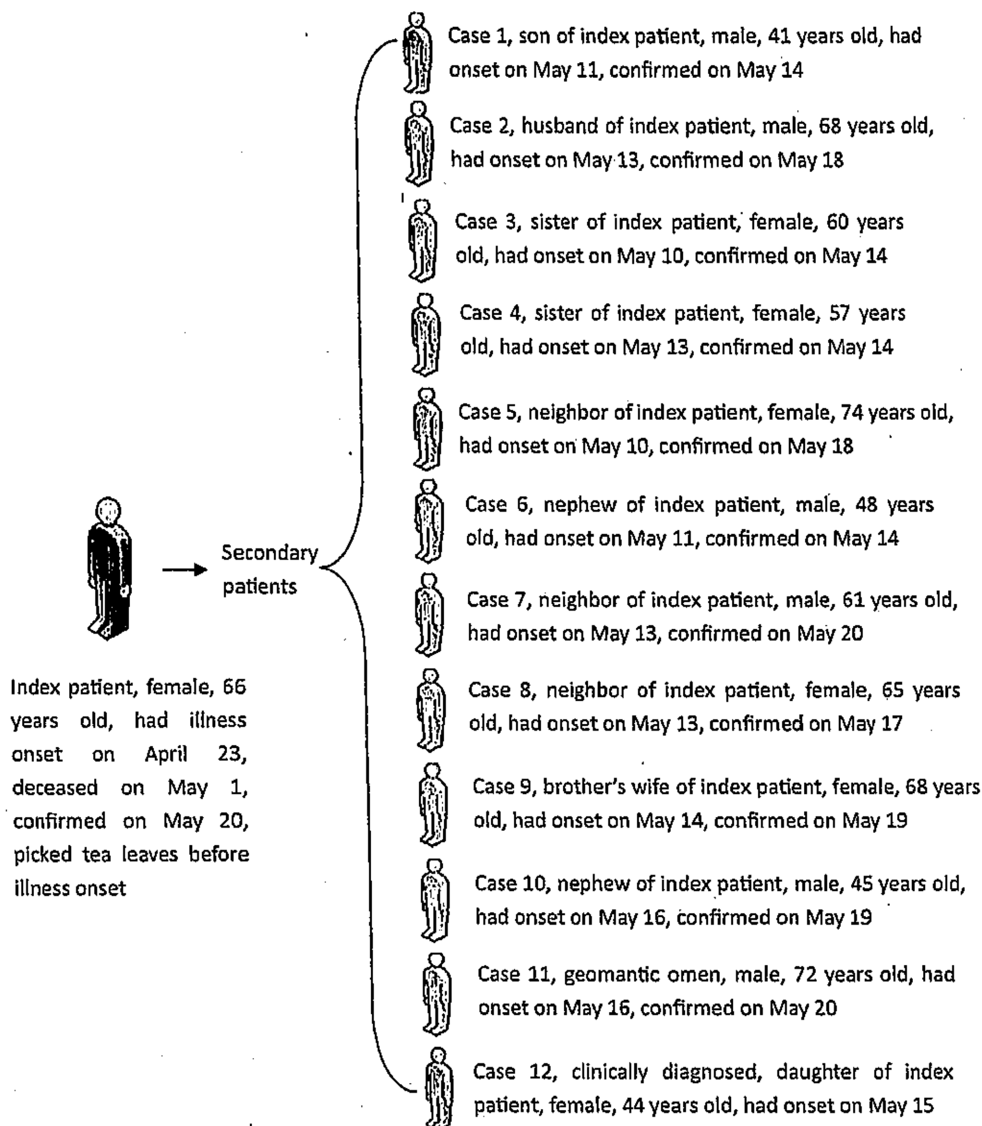


FIG. 1. Demographic features and date of illness onset of patients with severe fever with thrombocytopenia syndrome.

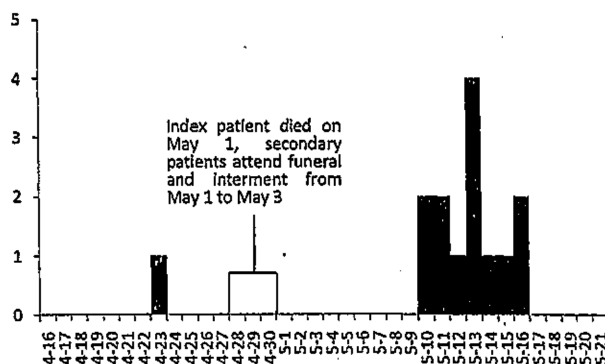


FIG. 2. Timelines of index patient and key event of severe fever with thrombocytopenia syndrome outbreak in southeastern China.

during the trip home. Because the corpse had already been buried when the outbreak occurred, blood samples were not collected from the index patient. Fortunately, we collected seven blood spot samples on the walls of her home on 18 May, and two of the samples were positive for SFTSV. The minimum platelet count of the patient was 20×10^9/L on 29 April.

Patient 1, the son of the index patient, developed fever on 11 May. He took care of the index patient in hospital, participated in moving the corpse into a car and from the car to home, moved the corpse to a coffin and kept vigil beside the coffin (Table 2).

Patient 2, the husband of the index patient, experienced onset of illness on 13 May. He did not have contact with the corpse, but he stayed in mourning hall for hours. Similarly,

Clinical Microbiology and Infection © 2015 The Authors. Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases, CMI, 21, 1115–1120
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

TABLE 1. Clinical characteristics of index patient and secondary patients

Characteristic	Index	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12
Fever	√	√	√	√	√	√	√	√	√	√	√	√	√
Fatigue	√	√	√	√	√	√	√	√	√	√	√	√	√
Chills	√	√	√	√	√	√	√	√	√	√	√	√	√
Headache	√	√		√	√	√	√		√				
Anorexia	√	√	√	√	√	√	√	√	√				
Nausea								√	√	√			√
Myalgia	√	√		√	√		√	√	√			√	√
Diarrhea	√						√		√				
Vomiting									√	√			
Gingival hemorrhage	√								√				
Conjunctival congestion	√	√		√	√					√	√	√	√

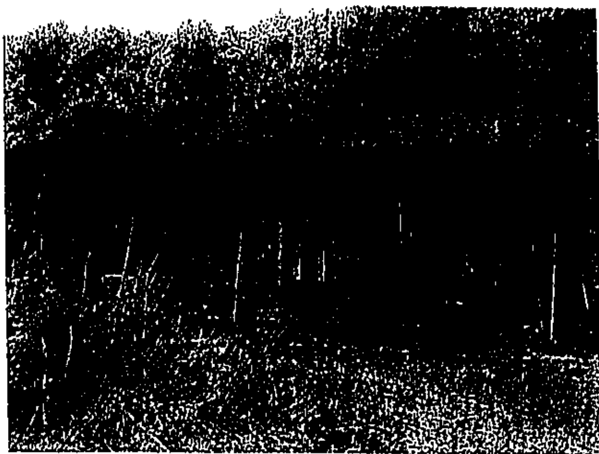


FIG. 3. House where index patient lived.

patients 8 and 10 also did not have history of contact with the corpse, although they too stayed in the mourning hall for hours.

Patients 3 and 4 were sisters of the index patient. Both of them participated in washing and wiping the corpse, and they dressed the corpse. Patients 5 and 9 dressed the corpse but had no other contact. Patient 12 was the daughter of the index patient; she had taken care of the index patient in hospital, moved the corpse into the car, washed and wiped the corpse, dressed the corpse and kept vigil beside the coffin. Three persons, patients 3, 4 and 12, participated in washing and wiping the corpse, and five people, patients 3, 4, 5, 9 and 12, participated in dressing the index patient. All these persons were infected with SFTSV.

Patients 6 and 7 participated in moving the corpse from the car to home and moving the corpse to a coffin, along with patient 1. Of note, patients 1, 6 and 7 had contact with the index patient's blood when they carried the corpse from the car into the house.

Of interest, patient 11 was a geomantic omen, and he did not have history of contact with the corpse; nor did he stay in the mourning hall for hours. However, he went to hilly areas to select the burial site for the index patient. We collected ticks in hilly areas near the cemetery, indicating that patient 11 had a history of exposure to ticks.

Discussion

In this study, we identified a cluster of person-to-person transmission of SFTSV including the index patient and 12 secondary patients. Notably, two secondary patients probably contracted the disease through aerosol transmission and/or direct contact.

A fatal outcome of SFTS was associated with high virus load in blood at admission and sustained high virus load during different illness stages [11–13]. These findings indicated that

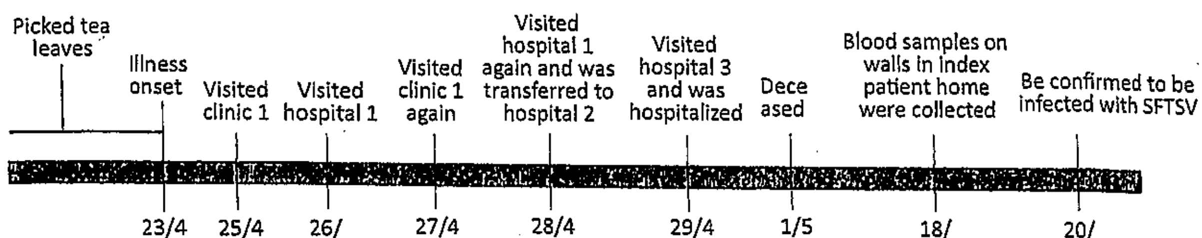


FIG. 4. Timelines of potential exposure and medical consultations of index patient.

This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

TABLE 2. Exposure modes of secondary patients with severe fever with thrombocytopenia syndrome virus infection

Patient no.	Taking care of index patient in hospital	Carrying corpse into car	Carrying corpse from car to home	Washing and wiping corpse	Dressing corpse	Carrying corpse to coffin	Keeping vigil beside coffin	Staying in mourning hall but no contact with corpse
1	√	√	√			√	√	
2							√	√
3				√	√			
4				√	√			
5					√			
6			√			√		
7			√			√		
8								√
9					√			
10								√
12	√	√		√	√		√	

the virus load of dead patients was very high in blood, and people might be infected with SFTSV through contact with the blood of dead patients. According to the intervals of the dates of the onset of illness of secondary patients from the funeral of the index patient, we conclude that the infection of secondary patients was related to the index patient. However, secondary patients might have been infected with SFTSV via different transmission modes. We believe that patients 1, 3, 4, 5, 6, 7, 9 and 12 were infected with SFTSV through direct contact with the blood of the index patient. Although we cannot rule out the notion that they were infected through tick bites, we think that such a probability is very low as a result of the timing of their illness. Firstly, the nine secondary patients had an obvious exposure history of contact with blood of the index patient. Secondly, blood samples on the walls in house of the index patient tested positive for SFTSV. Thirdly, all of the patients did not have an obvious history of tick bites. Finally, all nine patients developed the disease 10 to 15 days after exposure.

The index patient died of massive hemorrhage in a car while being transported home, and her clothes were soaked with blood on 1 May. Of note, all three people who attended washing and wiping the corpse, and all five people who dressed the index patient were infected with SFTSV, and another three secondary patients had direct contact with the blood of the index patient when they moved the corpse from the car into her house. All these exposure occurred on 1 May, indicating that SFTSV-infected blood may remain infectious even after death.

Patient 2 did not have a history of direct contact with the blood of the index patient. However, he lived with the index patient, had a history of contact with the clothes of the index patient, which were soaked with the blood of the index patient, and he stayed for hours in the mourning hall. Therefore, he might have been infected with SFTSV through contact with contaminated clothes, aerosol transmission or tick bites. The probability of infection through contact with the clothes of the index patient was the highest.

Patients 8 and 10 did not have history of contact with the blood or contaminated clothes of the index patient. They may have acquired the infection through one of three possible transmission routes, including aerosol transmission, contact with utensils which were contaminated with blood or secretion of the index patient and tick bites. We thought the probability of aerosol transmission was the highest. Firstly, both of them had a history of staying in the mourning hall for hours. Secondly, they did not have a history of direct contact with the blood of the index patient. Thirdly, the corpse of the index patient was placed in a small space, thus facilitating aerosol transmission. Fourthly, the timing of their illness was compatible with exposure to the corpse of the index patient. Fifthly, they lived in different towns and they were unlikely to have been bitten by ticks all at the same time. Moreover, aerosol transmission of other viruses in the *Bunyaviridae* family has been reported. For example, *Andes* virus, a hantavirus causing hantavirus pulmonary syndrome in South America, had documented aerosol transmission under certain circumstances [14,15]. Aerosol transmission of hantavirus was also confirmed in the laboratory, and rodents could be infected by virus aerosol generated by infected *Apodemisagrarius* and artificial virus aerosol [16–19]. SFTSV was detectable in blood, throat, urine and faecal specimens of SFTS patients, and these excreta as well as the blood of patients might generate virus aerosol [11]. As a result, family members or physicians might be infected with SFTSV through aerosol transmission.

Patient 11 was a geomantic omen and had no history of contact with the index patient. He was most probably infected with SFTSV through a tick bite when he selected the grave site for the index patient. We found ticks around the cemetery the index patient was buried in, which supports our postulate.

There were several limitations to our study. Firstly, we could not isolate virus or obtain a viral sequence from the index patient because the corpse had been buried when the outbreak was identified. Therefore, we could not conduct sequencing for virus isolate analysis between the index patient and the

This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

secondary patients. Secondly, we did not know the prevalence of SFTSV in ticks around the home of the index patient. We cannot completely rule out the notion that the majority of secondary patients, especially patients 8 and 10, were infected through tick bites, although we believe this to be unlikely.

To the our knowledge, this is the first report of probable aerosol transmission of SFTSV. Our findings suggest that SFTSV could be transmitted from person to person by direct contact and/or aerosol transmission, and it is of importance to consider aerosol transmission as a possible transmission route. Aerosol precautions should be considered and standard for doctors, nurses and family members when they take care of patients with SFTSV. Furthermore, laboratory experiments should be performed to study aerosol transmission in SFTSV.

Transparency Declaration

This work was supported by a grant from Zhejiang Province major science and techology major science and technology program (2012C13016-2), the Project of the State Scientific and Technological Development of the 12th Five Year Plan grant (2012ZX10004219), the medical research program of Zhejiang Province (2014RCA002, 2014ZDA003) and National Natural Science Foundation of China (NSFC) grant 81171609. All authors report no conflicts of interest relevant to this article.

Acknowledgements

We thank the physicians and staff at the Huzhou Centre for Disease Control and Prevention and the Anji Centre for Disease Control and Prevention.

References

[1] Yu XJ, Liang MF, Zhang SY, et al. Fever with thrombocytopenia associated with a novel *Bunyavirus* in China. N Engl J Med 2011;364:1523–32.

[2] Zhang YZ, Zou Y, Fu ZF, et al. Hantavirus infections in humans and animals, China. Emerg Infect Dis 2010;16:1195–203.

[3] Zhang LJ, Liu Y, Ni DX, et al. Nosocomial transmission of human granulocytic anaplasmosis in China. JAMA 2008;300:2263–70.

[4] Bao CJ, Qi X, Wang H. A novel *Bunyavirus* in China. N Engl J Med 2011;365:862–3.

[5] Bao CJ, Guo XL, Qi X, et al. A family cluster of infections by a newly recognized *Bunyavirus* in eastern China, 2007: further evidence of person-to-person transmission. Clin Infect Dis 2011;53:1208–14.

[6] Gai ZT, Liang MF, Zhang Y, et al. Person to person transmission of severe fever with thrombocytopenia syndrome *Bunyavirus* through blood contact. Clin Infect Dis 2012;54:249–52.

[7] Liu Y, Li Q, Hu WF, et al. Person-to-person transmission of severe fever with thrombocytopenia syndrome virus. Vector Borne Zoonot Dis 2012;12:156–60.

[8] Tang XY, Wu WL, Wang HF, et al. Human-to-human transmission of severe fever with thrombocytopenia syndrome *Bunyavirus* through contact with infectious blood. J Infect Dis 2013;207:736–9.

[9] Chen HB, Hu K, Zou JJ, et al. A cluster of cases of human-to-human transmission caused by severe fever with thrombocytopenia syndrome *Bunyavirus*. Int J Infect Dis 2013;17:e206–8.

[10] Zhang L, Wang XY, Zhang YJ, et al. Severe fever with thrombocytopenia syndrome *Bunyavirus* (SFTSV) infections in Zhejiang Province, China. Int J Infect Dis 2012;17:e137–8.

[11] Zhang YZ, He YW, Dai YA, et al. Hemorrhagic fever caused by a novel *Bunyavirus* in China: pathogenesis and correlates of fatal outcome. Clin Infect Dis 2012;54:527–33.

[12] Gai ZT, Zhang Y, Liang MF, et al. Clinical progress and risk factors for death in severe fever with thrombocytopenia syndrome patients. J Infect Dis 2012;206:1095–102.

[13] Liu W, Lu QB, Cui N, et al. Case-fatality ratio and effectiveness of ribavirin therapy among hospitalized patients in China who had severe fever with thrombocytopenia syndrome. Clin Infect Dis 2013;57:1292–9.

[14] Martinez VP, Bellomo C, San Juan J, et al. Person-to-person transmission of Andes virus. Emerg Infect Dis 2005;11:1848–53.

[15] Padula PJ, Edelstein A, Miguel SD, et al. Hantavirus pulmonary syndrome outbreak in Argentina: molecular evidence for person-to-person transmission of Andes virus. Virology 1998;241:323–30.

[16] Li JS, Lu JC, Che FX, et al. Studies on hantavirus aerosol transmission in laboratory and the field sampling. Virol Sin 2001;16:124–7.

[17] Li JS, Lu JC, Che FX, et al. Characteristic and antibody response of experimental animal infected by hantavirus aerosol. Chin J Zoonoses 1998;14:3–5.

[18] Tao KH, Zhang Y, Zhao XZ, et al. Study on EHFV spreading among experimental animals by aerosol. Chin J Public Health 1993;12:314–5.

[19] Zhang Y, Tao KH, Zhao XZ, et al. Experimental study on transmission of hemorrhagic fever with renal syndrome virus by aerosols. Chin J Exp Clin Virol 1995;9:122–5.

Clinical Microbiology and Infection © 2015 The Authors. Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases, *CMI*, 21, 1115–1120
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

別紙様式第2-1
番号6

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2015年12月02日	新医薬品等の区分		厚生労働省処理欄
一般的名称	①②③⑨⑩pH4処理酸性人免疫グロブリン ④⑤⑥⑦⑧ポリエチレングリコール処理人免疫グロブリン ⑪⑫人免疫グロブリン			研究報告の公表状況	Journal of General Virology 2015; 96(5):975-981	公表国 中国	
販売名（企業名）	①献血ポリグロビンN5%静注0.5g/10mL（日本血液製剤機構） ②献血ポリグロビンN5%静注2.5g/50mL（日本血液製剤機構） ③献血ポリグロビン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献血ポリグロビンN10%静注5g/50mL（日本血液製剤機構） ⑩献血ポリグロビンN10%静注10g/100mL（日本血液製剤機構） ⑪グロブリン筋注450mg/3mL「JB」（日本血液製剤機構） ⑫グロブリン筋注1500mg/10mL「JB」（日本血液製剤機構）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
8つの鳥インフルエンザA（H5N6）ウイルスは、ヒトH5N6感染が発生した郡に近いものも含め、2014年に中国の四川省と江西省の生きた家禽市場（LPM）から分離された。遺伝学的および系統学的分析は、これらのH5N6ウイルスはH5N1クレード2.3.4およびH6N6ウイルス間の新たな再集合体であった、そして2つの異なる系統（四川と江西）に進化したことを明らかにした。その上、ヒトH5N6ウイルスは四川系統の鳥類のソースウイルスに密接に関連していた。特に、H5N6ウイルスは、哺乳動物においてヒト様レセプターおよび病原性のためのウイルス親和性を高めるのを助けることができる、赤血球凝集素タンパク質中にT160A置換を、そしてノイラミニダーゼ茎中に11のaa欠損を含んでいた。H5N1ウイルスが直接接触を介してヒトに感染するように、新型H5N6ウイルスの感染は、H5亜型がパンデミックのための有力候補であったという重大な懸念を引き起こした。従って、LPMにおける鳥インフルエンザウイルスの大規模かつ長期の監視が不可欠である。	代表として献血ヴェノグロブリンIH5%静注0.5g/10mLの記載を示す。 2. 重要な基本的注意 (1)本剤の原材料となる献血者の血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体、抗HTLV-1抗体陰性で、かつALT（GPT）値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohnの低温エタノール分画で得た画分からポリエチレングリコール4000処理、DEAEセファデックス処理等により人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理、ウイルス除去膜によるろ過処理及

グロブリン

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

報告企業の意見	今後の対応	
インフルエンザウイルス（influenza virus）は、オルトミクソウイルス科（Orthomyxoviridae）に属するA型インフルエンザウイルス（influenzavirus A）、B型インフルエンザウイルス（influenzavirus B）、C型インフルエンザウイルス（influenzavirus C）の3属を指す。A型とB型のウイルス粒子表面にはヘマグルチニン（HA）とノイラミニダーゼ（NA）の糖蛋白があり、これらが感染防御免疫の標的抗原となっている。特にA型では、16種類のHAと9種類のNAの組み合わせにより様々なウイルスが、ヒト以外にもブタやトリなどその他の宿主に広く分布している。ウイルスの大きさは直径80～120nmの球形粒子で、エンベロープを有する1本鎖RNAウイルスで、万一原料血漿にインフルエンザウイルスが混入したとしても、各種モデルウイルスのウイルスクリアランス試験成績から、本剤の製造工程において不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。	び pH3.9～4.4 の条件下での液状インキュベーション処理を施しているが、投与に際しては、次の点に十分注意すること。

グロブリン

Journal of General Virology (2015), 96, 975–981 DOI 10.1099/vir.0.000056

Short Communication

Two novel reassortants of avian influenza A (H5N6) virus in China

Yuhai Bi,[1,2]† Kun Mei,[3,4]† Weifeng Shi,[5]† Di Liu,[1,2] Xiaolan Yu,[4] Zhimin Gao,[3] Lihua Zhao,[3] George F Gao,[1,2,3,6,7] Jianjun Chen[2,3] and Quanjiao Chen[2,3]

Correspondence
Jianjun Chen
chenjj126@126.com
Quanjiao Chen
zuochqj@126.com

[1]CAS Key Laboratory of Pathogenic Microbiology and Immunology (CASPMI), Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, PR China

[2]Center for Influenza Research and Early-warning (CASCIRE), Chinese Academy of Sciences, Beijing 100101, PR China

[3]CAS Key Laboratory of Special Pathogens and Biosafety, Wuhan Institute of Virology, Chinese Academy of Sciences, Hubei 430071, PR China

[4]Hubei University, Hubei 430062, PR China

[5]Institute of Pathogen Biology, Taishan Medical College, Shandong 271016, PR China

[6]Office of Director-General, Chinese Center for Disease Control and Prevention (China CDC), Beijing 102206, PR China

[7]Collaborative Innovation Center for Diagnosis and Treatment of Infectious Disease, Zhejiang University, Hanzhou 310003, PR China

Eight avian influenza A (H5N6) viruses were isolated from live poultry markets (LPMs) in Sichuan and Jiangxi Provinces in China in 2014, including those close to the county where the human H5N6 infection occurred. Genetic and phylogenetic analyses revealed that these H5N6 viruses were novel reassortants between H5N1 clade 2.3.4 and H6N6 viruses, and had evolved into two distinct lineages (Sichuan and Jiangxi). Moreover, the human H5N6 virus was closely related to the avian-source viruses of Sichuan lineage. Notably, H5N6 viruses contained a T160A substitution in the haemagglutinin protein and an 11 aa deletion in the neuraminidase stalk, which may aid in enhancing viral affinity for human-like receptors and virulence in mammals. As the H5N1 virus infects humans through direct contact, infection with the novel H5N6 virus raised significant concerns that the H5 subtype was a likely candidate for a pandemic. Therefore, extensive and long-term surveillance of avian influenza viruses in LPMs is essential.

Received 11 November 2014
Accepted 13 January 2015

The highly pathogenic avian influenza (HPAI) H5N1 virus was initially isolated in 1996 in China (Xu *et al.*, 1999) and caused much concern in 1997, with 18 recorded human infections and six deaths in Hong Kong (Chan, 2002). Re-emergence of the H5N1 virus was reported in 2003. Since then, transmission of H5N1 viruses from avian hosts to humans has been reported in 17 countries and areas worldwide, leading to 676 laboratory-confirmed infections and 398 deaths as of 4 December 2014 (WHO, 2014a). Importantly, the H5N1 virus can infect migratory birds, which act as vectors that contribute to the spread of the virus to new areas via long-distance migration and transmission to domestic poultry through direct or indirect contact (Liu *et al.*, 2005; Sakoda *et al.*, 2012; Wang *et al.*, 2008). Since its emergence, the HPAI H5N1 virus has been proposed as a likely candidate for a potential influenza pandemic, although effective human-to-human transmission is yet to be demonstrated. To date, the H5N1 virus has become endemic in domestic birds in China, Southeast Asia and Africa, and evolved into numerous phylogenetic lineages (WHO/OIE/FAO, 2012). Amongst the 32 distinguishable clades of H5N1, clades 2.3.2.1, 2.3.4.2 and 7.2 are predominant in China (WHO/OIE/FAO, 2012). Additionally, evolutionary clades, such as 2.3.4.5 and 2.3.4.6, have been reported (Gu *et al.*, 2013). In addition to haemagglutinin (HA) evolution, internal genes of the

†These authors contributed equally to this work.

The GenBank/EMBL/DDBJ accession numbers for the genome sequences of the H5N6 and H5N1 viruses are KP090436–KP090451, KM251463–KM251471, KM251473–KM251484, KM251486–KM251491, KM251493–KM251501, KM251503–KM251511, KM251513–KM251521, KM251523–KM251531 and KM251533–KM251541.

Two supplementary tables and two supplementary figures are available with the online Supplementary Material.

000056 © 2015 The Authors Printed in Great Britain

H5N1 virus have frequently reassorted with other subtypes of avian influenza viruses circulating in poultry (Neumann *et al.*, 2010; Zhao *et al.*, 2008). In particular, H5N5, H5N8 and H5N2 subtypes bearing the genetic backbone of clade 2.3.4 H5N1 viruses have been identified in poultry populations (Gu *et al.*, 2011; Zhao *et al.*, 2012; Wu *et al.*, 2014).

Apart from the H5N1 subtype, various reassortants of the H5 subtype with other neuraminidase (NA) subtypes have been isolated, although none of them has been documented to cause human infection. However, on 6 May 2014, a fatal H5N6 human infection was reported in Sichuan Province, China (China CDC, 2014; WHO, 2014b). The patient was a poultry dealer working directly in live poultry markets (LPMs), recently shown to play a key role in human infection with influenza A (H7N9 and H10N8) viruses (Cowling *et al.*, 2013; Gao, 2014; Zhang *et al.*, 2014). However, the origin of the novel H5N6 virus remains unknown. To determine the evolutionary history of H5N6 in China, we performed comprehensive genetic analysis of the full-length genome sequences of eight H5N6 and three H5N1 viruses isolated from LPMs.

Surveillance in five LPMs was conducted on 26 and 27 April 2014 in Nanbu County, Sichuan Province, where human infection with the H5N6 virus was reported (Fig. S1, available in the online Supplementary Material). A total of 77 oropharyngeal and cloacal mixed swab samples (one oropharyngeal and cloacal mixed swab for one bird) were collected from chickens (n=10), ducks (n=31) and pigeons (n=36), along with 19 faeces and nine poultry drinking-water samples. The surveillance additionally covered the Dunzitang LPM of Nanchang city (Jiangxi Province) where human infection with the H10N8 virus was recorded on 30 November 2013 (Chen *et al.*, 2014). Overall, 18 oropharyngeal and 18 cloacal swab samples (one oropharynx and one cloacal swab for one bird) were collected from chickens. Samples were kept in viral medium at 4 °C until transported to the laboratory and then either stored at −80 °C or directly inoculated in 10-day-old specific pathogen-free embryonated chicken eggs for 72 h at 37 °C. Complete gene segments of the HA titre-positive samples were amplified using the improved primers (Table S1) designed based on previous reports (Hoffmann *et al.*, 2001; Li *et al.*, 2007) and sequenced using an ABI 3730XL automatic DNA analyser. In total, 16 samples were H5-positive; nine H5 viruses were isolated from the oropharyngeal and cloacal mixed swabs in Sichuan Province, including six influenza A (H5N6) viruses (SC-H5N6) and three influenza A (H5N1) viruses (SC-H5N1); two influenza A (H5N6) viruses were isolated from the oropharyngeal swabs in Jiangxi Province (JX-H5N6). The other five H5-positive samples were detected to be mixed with other subtypes, such as H6, H7 and H9 viruses, in Sichuan Province and Jiangxi Province. We determined the full-length genome sequences of these strains of the 11 avian influenza viruses isolated from both Sichuan Province and Jiangxi Province (H5N6, n=8; H5N1, n=3; Table 1). Genome sequences of the other five recently identified avian and human influenza A (H5N6) viruses from China were retrieved from the Influenza Virus Resources at GenBank and the Global Initiative on Sharing Avian Influenza Database (GISAID) (Table 1). The 16 full-length avian influenza virus genomes were divided into eight datasets corresponding to the eight independent RNA segments of type A influenza virus. For the NA dataset, the three NA sequences of the H5N1 subtype (SC-H5N1) were removed. The eight datasets were subsequently used as queries to perform a BLAST search against GenBank to obtain reference sequences. BLAST hits and their corresponding query datasets were combined and aligned using Clustal Omega.

Sequence comparison disclosed high nucleotide identity amongst HA genes of the SC-H5N6 isolates (>99.3 %) and between the two JX-H5N6 isolates (99.8 %). HA genes of the SC-H5N6 isolates shared 95.1–95.6 % sequence similarity with JX-H5N6 isolates. In addition, the HA gene of the reference strain, A/environment/Zhenjiang/C13/2013 (H5N6) (EN/ZJ/C13/13), displayed higher identity with SC-H5N6 (99.3–99.6 %) than JX-H5N6 (95.4 %). HA genes of A/duck/Guangdong/GD01/2014 (H5N6) (DK/GD/GD01/14), A/environment/Shenzhen/25-24/2013 (H5N6) (EN/SZ/25-24/13) and A/duck/Jixangxi/95/2014 (H5N6) (DK/JX/95/14) shared high nucleotide identity (99.5–99.7 %), and displayed higher nucleotide identity (99.2–99.5 %) with JX-H5N6 than SC-H5N6 (95.3–95.8 %). HA genes of the three SC-H5N1 isolates possessed 94.7–95.4 % identity with those of SC-H5N6 isolates and EN/ZJ/C13/13 (H5N6), and 97.8–98.2 % identity with those of JX-H5N6, DK/GD/GD01/14 (H5N6), EN/SZ/25-24/13 (H5N6) and DK/JX/95/14 (H5N6) (Table S2).

High nucleotide identity was observed amongst NA genes of the SC-H5N6 isolates (99.6–99.9 %) and between the two JX-H5N6 isolates (99.7 %). However, NA genes of the SC-H5N6 and JX-H5N6 isolates shared only 89.3–89.6 % sequence identity. Interestingly, NA genes of SC-H5N6 isolates showed higher identity with EN/ZJ/C13/13 (H5N6) (99.0–99.2 %) than DK/GD/GD01/14 (H5N6), EN/SZ/25-24/13 (H5N6) and DK/JX/95/14 (H5N6) (89.7–90.0 %) (Table S2).

A BLAST search showed that the internal genes of H5N6 viruses from China were similar to the H5N1 viruses circulating in China and Southeast Asia. Specifically, genes of the SC-H5N6 isolates possessed 99.6–100 % nucleotide identity, whilst nucleotide identities of the JX-H5N6 isolates ranged from 99.2 to 99.9 %. In addition, internal genes of the SC-H5N6 isolates displayed 96.7–98.7, 97.9–98.8, 96.0–99.7 and 96.4–98.7 % identity with JX-H5N6, SC-H5N1, EN/ZJ/C13/13 (H5N6) and the other three H5N6 [DK/GD/GD01/14 (H5N6), EN/SZ/25-24/13 (H5N6) and DK/JX/95/14 (H5N6)] viruses, respectively, whilst JX-H5N6 isolates showed 97.8–99.2, 93.8–98.4 and 96.6–99.7 % identity with SC-H5N1, EN/ZJ/C13/13 (H5N6) and the other three H5N6 viruses (Table S2). Notably, all the

Table 1. Molecular characterization of H5N6 viruses in terms of HA, PB2, NA, M2 and PB1-F2

Virus*	Collection date	HA (H3 numbering)								PB2			NA		M2	PB1-F2
		Connecting peptide	110	158	160	224	226	228	318	591	627	701	274†	Stalk deletion	31	58–90 truncated
CK/SCH/NCJPL1/14 (H5N6)	27 April 2014	REKRRKR/GL	H	N	A	N	Q	G	T	Q	E	D	H	No	S	Yes
DK/SCH/NCJPL7/14 (H5N6)	27 April 2014	REKRRKR/GL	H	N	A	N	Q	G	T	Q	E	D	H	No	S	Yes
DK/SCH/NCXJ15/14 (H5N6)	27 April 2014	REKRRKR/GL	H	N	A	N	Q	G	T	Q	E	D	H	No	S	Yes
DK/SCH/NCXJ16/14 (H5N6)	27 April 2014	REKRRKR/GL	H	N	A	N	Q	G	T	Q	E	D	H	No	S	Yes
DK/SCH/NCXJ24/14 (H5N6)	27 April 2014	REKRRKR/GL	H	N	A	N	Q	G	T	Q	E	D	H	No	S	Yes
WR/SCH/NCLL1/14 (H5N6)	27 April 2014	REKRRKR/GL	H	N	A	N	Q	G	T	Q	E	D	H	No	S	Yes
DK/SCH/NCXN10/14 (H5N1)	27 April 2014	RERRRKR/GL	H	N	T	N	Q	G	T	Q	E	D	H	49–68	S	Yes
DK/SCH/NCXN11/14 (H5N1)	27 April 2014	RERRRKR/GL	H	N	T	N	Q	G	T	Q	E	D	H	49–68	S	Yes
PN/SCH/NCXN29/14 (H5N1)	27 April 2014	RERRRKR/GL	H	N	T	N	Q	G	T	Q	E	D	H	49–68	S	Yes
CK/JX/NCDZT1123/14 (H5N6)	29 June 2014	RERRRKR/GL	H	N	A	N	Q	G	T	Q	E	D	H	58–68	S	Yes
CK/JX/NCDZT1126/14 (H5N6)	29 June 2014	RERRRKR/GL	H	N	A	N	Q	G	T	Q	E	D	H	58–68	S	Yes
EN/SZ/25-24/13 (H5N6)‡	2 December 2013	RERRRKR/GL	H	N	A	N	Q	G	T	Q	E	D	H	58–68	S	Yes
EN/ZJ/C13/13 (H5N6)‡	12 December 2013	REKRRKR/GL	H	N	A	N	Q	G	T	Q	E	D	H	No	S	Yes
DK/JX/95/14 (H5N6)‡	1 October 2014	RERRRKR/GL	H	N	A	N	Q	G	T	Q	E	D	H	58-68	S	Yes
DK/GD/GD01/14 (H5N6)‡	March 2014	RERRRKR/GL	H	N	A	N	Q	G	T	Q	E	D	H	58-68	S	Yes
A/SCH/26221/2014 (H5N6)‡	21 April 2014	REKRRKR/GL	H	N	A	N	Q	G	T	Q	E	N	H	No	S	Yes

*CK, Chicken; DK, duck; EN, environment; PN, pigeon; WR, water; GD, Guangdong; JX, Jiangxi; SCH, Sichuan; SZ, Shenzhen; ZJ, Zhenjiang.

†N2 numbering.

‡Sequences downloaded from GenBank and GISAID databases.

eight gene segments of the human-source virus, A/Sichuan/26221/2014 (H5N6) (SCH26221), possessed the highest genetic identities (99.5–100 %) with those of SC-H5N6 (Table S2).

Phylogenetic analysis was performed using the maximum-likelihood method in RAxML. The GTRGAMMA model was applied and 1000 bootstrap replicates performed. It should be noted that for the HA dataset, the most related reference sequences were selected and combined with a dataset comprising sequences of representative HA clades. Phylogenetic trees were visualized using Dendroscope software.

From the HA phylogeny (Fig. 1a), genes of the recently identified Chinese H5N6 avian influenza viruses and the human-source virus fell within clade 2.3.4, suggesting that they were originally derived from the HPAI H5N1 viruses circulating in China. Phylogenetic trees reconstructed using the six internal gene sequences consistently showed that all identified H5N6 and H5N1 avian influenza viruses stemmed from previously circulating H5N1 lineages in China and Southeast Asia (Fig. S2). In contrast, the NA phylogenetic tree revealed that the NA genes originated from H6N6 avian influenza virus circulating in the Fujian and Guangdong Provinces of China (Fig. 1b).

Notably, in all eight gene phylogenetic trees, Chinese H5N6 avian influenza viruses formed two independent lineages (Figs 1 and S2). The human H5N6 virus (SCH26221) and avian H5N6 viruses isolated from Sichuan formed one lineage (Sichuan), with A/environment/Zhenjiang/C13/2013 (H5N6). Together with the epidemiological data analysis, it could be deduced that the patient was probably infected by direct contact with the H5N6-infected poultry (Figs 1 and S2). The two Jiangxi H5N6 isolates formed the other lineage (Jiangxi), which also included H5N6 avian influenza viruses from Guangdong and Shenzhen. Additionally, a number of H5N2 avian influenza viruses from Jiangxi fell within this lineage. Thus, based on genetic identities and phylogenies, two H5N6 lineages have been co-circulating in China, designated Sichuan and Jiangxi.

Interestingly, the three H5N1 avian influenza viruses isolated from Sichuan Province did not cluster together with the two H5N6 lineages, but formed a separate lineage with Vietnamese H5N1 isolates from 2014 (Figs 1a and S2a, c–h). Therefore, based on current surveillance data, we speculate that the H5N6 and H5N1 lineages from Sichuan Province were introduced from two independent external sources. Furthermore, in the PA (polymerase acidic) phylogenetic tree (Fig. S2e), one Sichuan H5N1 avian influenza virus, A/duck/Sichuan/NCXN10/2014, did not cluster together with the two other Sichuan H5N1 strains, suggestive of a novel H5N1 avian influenza virus reassortant. However, we do not know whether this reassortment event occurred before or after introduction of the virus into Sichuan.

The HA phylogeny also revealed multiple potentially novel HA subclades within clade 2.3.4. To date, six subclades have been reported within clade 2.3.4 (2.3.4.1–2.3.4.6). However, we identified four separate lineages, two H5N6 (Jiangxi and Sichuan), one H5N8 and one H5N1, which did not cluster together with the previously described subclades of clade 2.3.4. Next, we calculated between-group sequence divergence between the four lineages and 2.3.4.6, using the recommendations proposed by the WHO/OIE/FAO H5N1 Evolution Working Group. Sequence divergence values between H5N1, Jiangxi, H5N8 and Sichuan and clade 2.3.4.6 were calculated as 3.1, 3.0, 3.2 and 3.0 (>1.5 %), respectively. Furthermore, within-group divergence values of H5N1, Jiangxi, H5N8 and Sichuan were 0.4, 0.5, 0.2 and 0.3, respectively. Therefore, HPAI H5N1 viruses from China are experiencing rapid evolution and these lineages could be potentially assigned as novel subclades within clade 2.3.4.

HA proteins of all H5N6 and H5N1 viruses possessed multiple basic amino acids at the cleavage site (Table 1), indicating high pathogenicity to poultry. Residues at the cleavage site of SC-H5N1 isolates were identified as RERRRKR/GL, whilst the H5N6 isolates contained two motifs, REKRRKR/GL and RERRRKR/GL. The HA protein of these isolates displayed Q226 and G228 (H3 numbering) at the receptor binding site, suggesting that they favour avian-like receptors. The HA proteins of the SC-H5N1 isolates contained T160 (Table 1), whereas a T160A substitution was observed in HA of all H5N6 viruses, which is proposed to enhance binding capacity to human-like receptors (Gao *et al.*, 2009; Zhang *et al.*, 2013).

The NA stalk of SC-H5N1 viruses had a 20 aa deletion (positions 49–68), which might enhance virus adaptation to domestic fowl and increase virulence in mammals (Cauldwell *et al.*, 2014; Matsuoka *et al.*, 2009; Zhou *et al.*, 2009). This 20 aa deletion was not found in the NA stalk of SC-H5N6 and the human-H5N6 strain. Interestingly, however, JX-H5N6 isolates displayed an 11 aa deletion at positions 58–68. These findings suggested that the SC-H5N6 and JX-H5N6 isolates might have different adaptation and virulence characteristics in poultry and mammals.

Amino acids Q591, E627 and D701 were identified in the PB2 proteins of the H5N6 and H5N1 avian influenza viruses, indicating low adaptation to mammalian hosts. However, the human H5N6 virus contained a D701N mutation, which might enhance virulence for humans. Moreover, these H5N6 and H5N1 viruses contained a truncated PB1-F2 protein of 57 aa in length, which might influence their virulence in mammals (Zamarin *et al.*, 2006; McAuley *et al.*, 2010). No drug resistance-associated mutations (H274Y in NA and S31N in M2 protein) were observed, signifying that these H5 isolates are sensitive to NA and M2 inhibitors.

To further identify the antigenicity of the H5N6 and H5N1 isolates, the HA inhibition (HI) assay with the reference H5 subtype antigens and antisera was performed according to standard protocols (WHO, 2011). The reference antisera produced by the H5N1 vaccine strains, Re4 [A/chicken/

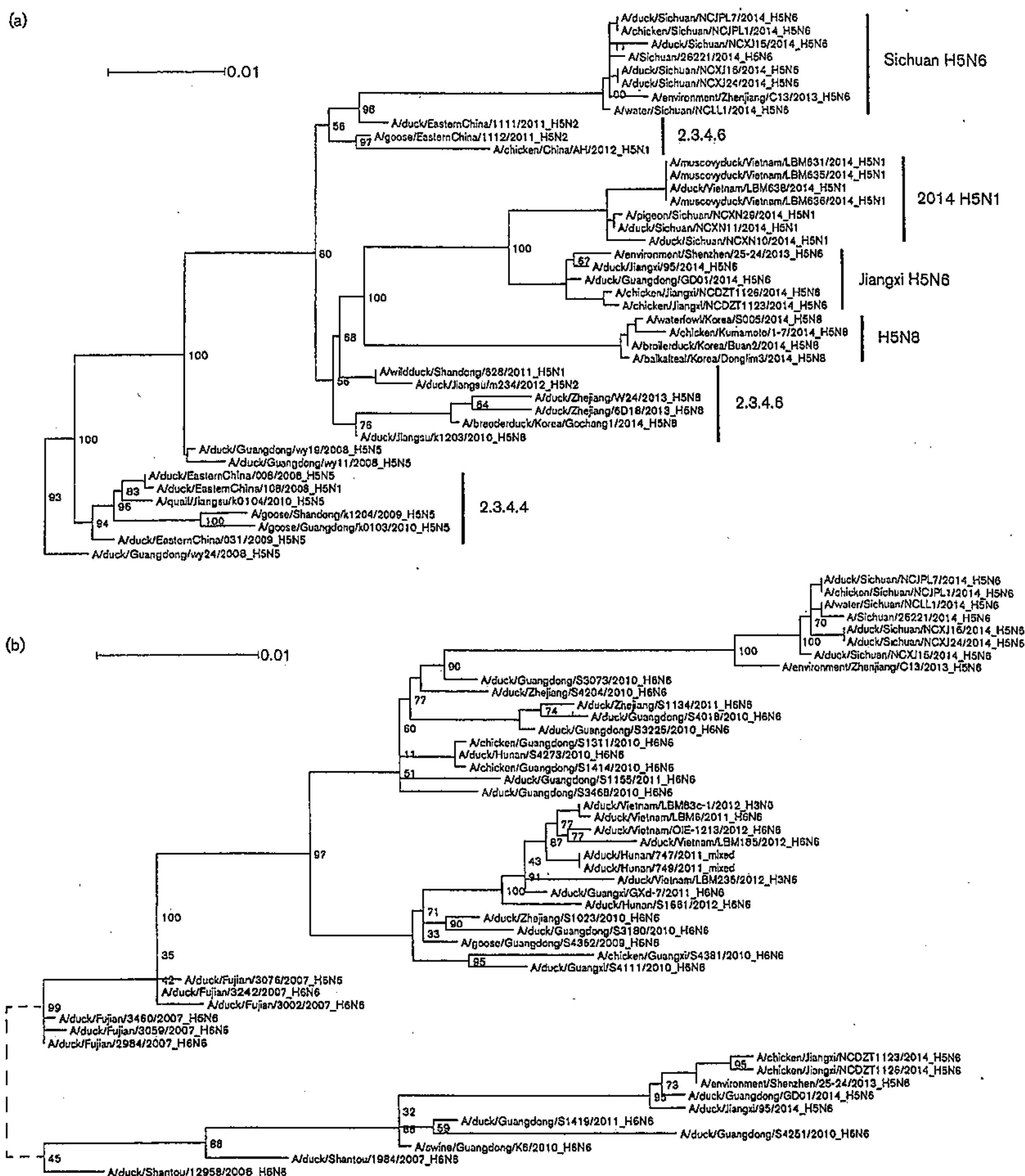


Fig. 1. Phylogenetic trees for (a) HA and (b) NA genes of H5N6 influenza A viruses isolated in the present study. Phylogenetic analysis was based on the whole ORF of H5 HA (29–1732, 1704 bp) and N6 NA (19–1431, 1413 bp). Maximum-likelihood phylogenetic trees were inferred with RAxML software under the GTRGAMMA model with 1000 bootstraps. Segments of SC-H5N6, JX-H5N6, human H5N6 and H5N1 isolates are coloured in red, blue, green and pink, respectively.

Table 2. Antigen characterization of H5N6 and H5N1 subtype avian influenza viruses isolated in China

Virus*	Clade	HI titres of vaccine strain hyperimmune chicken sera against the H5 isolates†		
		CK/SX/2/06 (H5N1) (Re4)	DK/AH/1/06 (H5N1) (Re5)	DK/GD/S1322/10 (H5N1) (Re6)
CK/SX/2/06 (H5N1) (Re4)	7	640	10	20
DK/AH/1/06 (H5N1) (Re5)	2.3.4	20	320	40
DK/GD/S1322/10 (H5N1) (Re6)	2.3.2	20	20	640
CK/SCH/NCJPL1/14 (H5N6)	2.3.4	<10	20	<10
DK/SCH/NCJPL7/14 (H5N6)	2.3.4	<10	20	<10
DK/SCH/NCXJ15/14 (H5N6)	2.3.4	<10	40	<10
DK/SCH/NCXJ16/14 (H5N6)	2.3.4	<10	20	<10
DK/SCH/NCXJ24/14 (H5N6)	2.3.4	<10	20	<10
WR/SCH/NCLL1/14 (H5N6)	2.3.4	<10	20	<10
DK/SCH/NCXN10/14 (H5N1)	2.3.4	<10	40	<10
DK/SCH/NCXN11/14 (H5N1)	2.3.4	<10	80	<10
PN/SCH/NCXN29/14 (H5N1)	2.3.4	<10	40	<10
CK/JX/NCDZT1123/14 (H5N6)	2.3.4	<10	40	<10
CK/JX/NCDZT1126/14 (H5N6)	2.3.4	<10	40	<10

*CK, Chicken; DK, duck; PN, Pigeon; WR, water; AH, Anhui; GD, Guangdong; JX, Jiangxi; SCH, Sichuan; SX, Shanxi.
†Hyperimmune chicken sera and homologous vaccine antigens were obtained from Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences.

Shanxi/2/2006, (H5N1), clade 7], Re5 [A/duck/Anhui/1/2006 (H5N1), clade 2.3.4] and Re6 [A/duck/Guangdong/S1322/2010 (H5N1), clade 2.3.2], were obtained from the Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences. High HI titres were detected when antisera reacted with the homologous vaccine strains. Notably, SC-H5N6, JX-H5N6 and SC-H5N1 viruses reacted specifically with the Re5 antiserum, but with significantly lower HI titres, compared with the Re5 homologous virus (Table 2). These findings indicated that all 11 H5 isolates described in this study could be novel clade 2.3.4 variants and have probably evolved to form a new subcluster, in accordance with the phylogenetic analysis (Figs 1a and S2a).

In 2014, H5N6 virus outbreaks in Laos (starting 13 March 2014), Vietnam (starting 13 April 2014) and China (starting 23 April 2014) resulted in the death and culling of >97 000 fowl (OIE, 2014; Wong *et al.*, 2015). In the present study, the two Jiangxi H5N6 viruses were isolated from dead chickens and the others were from apparently healthy birds. These findings suggested that the available H5N1 vaccines could not thoroughly eliminate the virus and may not completely protect poultry against H5N6 virus infection. Therefore, the infected poultry look apparently healthy; however, they can expel viruses to the surroundings. Fortunately, the H5N6 epidemic in Harbin (China) has been controlled and new vaccines will soon be deployed.

In summary, genetic and phylogenetic analyses indicate that two lineages of H5N6 viruses are currently circulating in five regions of China, designated Jiangxi and Sichuan. Both lineages may have originated from reassortment events between the prevalent H5N1 and H6N6 viruses circulating in Southeast China. Phylogenetic and antigenic analyses suggest these H5N6 viruses belong to clade 2.3.4, and might have evolved into new subclades with altered antigenicity, as there were low reactions between the H5N6 isolates and the antisera of the vaccine strains. Notably, the novel H5N6 viruses were isolated from LPMs close to the site where the human H5N6 infection occurred, and there was a very close genetic relationship between the human and avian H5N6 viruses, supporting that human infection is caused through direct contact with poultry. Furthermore, these H5N6 viruses have distinct evolutionary characteristics, such as the A160 mutation in the HA protein and an 11 aa deletion in the NA stalk, which may enhance their adaptation and infectivity in mammals, including humans. Our collective findings highlight the necessity to maintain and expand avian influenza virus surveillance in LPMs for the prevention and control of human infection.

Acknowledgements

This study was supported by grants from the National Basic Research Program (2010CB530300), National Natural Science Foundation of China (31311120063, 31070141 and 31100132), Special Project of Ministry of Science and Technology (2013FY113500), National Mega Project on Major Infectious Disease Prevention (2012ZX10004-207) and State Forestry Administration Special Fund (00472130211) and the intramural special grant for influenza virus research from the Chinese Academy of Sciences (KJZD-EW-L09). G.F.G. is a leading principal investigator of the NSFC Innovative Research Group (81321063). The funding sources had no role in the study design,

data collection and analysis, decision to publish or preparation of the manuscript. We also thank the data submitters for their H5N6 sequences in the GISAID and GenBank databases.

References

Cauldwell, A. V., Long, J. S., Moncorgé, O. & Barclay, W. S. (2014). Viral determinants of influenza A virus host range. *J Gen Virol* 95, 1193–1210.

Chan, P. K. (2002). Outbreak of avian influenza A(H5N1) virus infection in Hong Kong in 1997. *Clin Infect Dis* 34 (Suppl 2), S58–S64.

Chen, H., Yuan, H., Gao, R., Zhang, J., Wang, D., Xiong, Y., Fan, G., Yang, F., Li, X. & other authors (2014). Clinical and epidemiological characteristics of a fatal case of avian influenza A H10N8 virus infection: a descriptive study. *Lancet* 383, 714–721.

China CDC (2014). Avian influenza A(H5N6) virus was detected in a respiratory tract sample from a patient who died of severe pneumonia in Sichuan. http://www.chinacdc.cn/mtdx/crbxx/201405/t20140507_96416.htm (in Chinese).

Cowling, B. J., Jin, L., Lau, E. H., Liao, Q., Wu, P., Jiang, H., Tsang, T. K., Zheng, J., Fang, V. J. & other authors (2013). Comparative epidemiology of human infections with avian influenza A H7N9 and H5N1 viruses in China: a population-based study of laboratory-confirmed cases. *Lancet* 382, 129–137.

Gao, G. F. (2014). Influenza and the live poultry trade. *Science* 344, 235.

Gao, Y., Zhang, Y., Shinya, K., Deng, G., Jiang, Y., Li, Z., Guan, Y., Tian, G., Li, Y. & other authors (2009). Identification of amino acids in HA and PB2 critical for the transmission of H5N1 avian influenza viruses in a mammalian host. *PLoS Pathog* 5, e1000709.

Gu, M., Liu, W., Cao, Y., Peng, D., Wang, X., Wan, H., Zhao, G., Xu, Q., Zhang, W. & other authors (2011). Novel reassortant highly pathogenic avian influenza (H5N5) viruses in domestic ducks, China. *Emerg Infect Dis* 17, 1060–1063.

Gu, M., Zhao, G., Zhao, K., Zhong, L., Huang, J., Wan, H., Wang, X., Liu, W., Liu, H. & other authors (2013). Novel variants of clade 2.3.4 highly pathogenic avian influenza A(H5N1) viruses, China. *Emerg Infect Dis* 19, 2021–2024.

Hoffmann, E., Stech, J., Guan, Y., Webster, R. G. & Perez, D. R. (2001). Universal primer set for the full-length amplification of all influenza A viruses. *Arch Virol* 146, 2275–2289.

Li, O. T., Barr, I., Leung, C. Y., Chen, H., Guan, Y., Peiris, J. S. & Poon, L. L. (2007). Reliable universal RT-PCR assays for studying influenza polymerase subunit gene sequences from all 16 haemagglutinin subtypes. *J Virol Methods* 142, 218–222.

Liu, J., Xiao, H., Lei, F., Zhu, Q., Qin, K., Zhang, X. W., Zhang, X. L., Zhao, D., Wang, G. & other authors (2005). Highly pathogenic H5N1 influenza virus infection in migratory birds. *Science* 309, 1206.

Matsuoka, Y., Swayne, D. E., Thomas, C., Rameix-Welti, M.-A., Naffakh, N., Warnes, C., Altholtz, M., Donis, R. & Subbarao, K. (2009). Neuraminidase stalk length and additional glycosylation of the hemagglutinin influence the virulence of influenza H5N1 viruses for mice. *J Virol* 83, 4704–4708.

McAuley, J. L., Chipuk, J. E., Boyd, K. L., Van De Velde, N., Green, D. R. & McCullers, J. A. (2010). PB1-F2 proteins from H5N1 and 20 century pandemic influenza viruses cause immunopathology. *PLoS Pathog* 6, e1001014.

Neumann, G., Green, M. A. & Macken, C. A. (2010). Evolution of highly pathogenic avian H5N1 influenza viruses and the emergence of dominant variants. *J Gen Virol* 91, 1984–1995.

OIE (2014). Update on highly pathogenic avian influenza in animals (type H5 and H7). http://www.oie.int/en/animal-health-in-the-world/update-on-avian-influenza/2014/

Sakoda, Y., Ito, H., Uchida, Y., Okamatsu, M., Yamamoto, N., Soda, K., Nomura, N., Kuribayashi, S., Shichinohe, S. & other authors (2012). Reintroduction of H5N1 highly pathogenic avian influenza virus by migratory water birds, causing poultry outbreaks in the 2010–2011 winter season in Japan. *J Gen Virol* 93, 541–550.

Wang, G., Zhan, D., Li, L., Lei, F., Liu, B., Liu, D., Xiao, H., Feng, Y., Li, J. & other authors (2008). H5N1 avian influenza re-emergence of Lake Qinghai: phylogenetic and antigenic analyses of the newly isolated viruses and roles of migratory birds in virus circulation. *J Gen Virol* 89, 697–702.

WHO (2011). Manual for the laboratory diagnosis and virological surveillance of influenza. http://whqlibdoc.who.int/publications/2011/9789241548090_eng.pdf

WHO (2014a). Cumulative number of confirmed human cases of avian influenza A (H5N1) reported to WHO, 2003–2014. http://www.who.int/influenza/human_animal_interface/H5N1_cumulative_table_archives/en/

WHO (2014b). Influenza at the human–animal interface. http://www.who.int/influenza/human_animal_interface/about/en/

WHO/OIE/FAO (2012). Continued evolution of highly pathogenic avian influenza A (H5N1): updated nomenclature. *Influenza Other Respi Viruses* 6, 1–5.

Wong, F. Y. K., Phommachanh, P., Kalpravidh, W., Chanthavisouk, C., Gilbert, J., Bingham, J., Davies, K. R., Cooke, J., Eagles, D. & other authors (2015). Reassortant highly pathogenic influenza A(H5N6) virus in Laos. *Emerg Infect Dis* doi:10.3201/eid2103.141488 [Epub ahead of print].

Wu, H., Peng, X., Xu, L., Jin, C., Cheng, L., Lu, X., Xie, T., Yao, H. & Wu, N. (2014). Novel reassortant influenza A(H5N8) viruses in domestic ducks, eastern China. *Emerg Infect Dis* 20, 1315–1318.

Xu, X., Subbarao, K., Cox, N. J. & Guo, Y. (1999). Genetic characterization of the pathogenic influenza A/Goose/Guangdong/1/96 (H5N1) virus: similarity of its hemagglutinin gene to those of H5N1 viruses from the 1997 outbreaks in Hong Kong. *Virology* 261, 15–19.

Zamarin, D., Ortigoza, M. B. & Palese, P. (2006). Influenza A virus PB1-F2 protein contributes to viral pathogenesis in mice. *J Virol* 80, 7976–7983.

Zhang, W., Shi, Y., Lu, X., Shu, Y., Qi, J. & Gao, G. F. (2013). An airborne transmissible avian influenza H5 hemagglutinin seen at the atomic level. *Science* 340, 1463–1467.

Zhang, T., Bi, Y., Tian, H., Li, X., Liu, D., Wu, Y., Jin, T., Wang, Y., Chen, Q. & other authors (2014). Human infection with influenza virus A(H10N8) from live poultry markets, China, 2014. *Emerg Infect Dis* 20, 2076–2079.

Zhao, Z.-M., Shortridge, K. F., Garcia, M., Guan, Y. & Wan, X.-F. (2008). Genotypic diversity of H5N1 highly pathogenic avian influenza viruses. *J Gen Virol* 89, 2182–2193.

Zhao, G., Gu, X., Lu, X., Pan, J., Duan, Z., Zhao, K., Gu, M., Liu, Q., He, L. & other authors (2012). Novel reassortant highly pathogenic H5N2 avian influenza viruses in poultry in China. *PLoS One* 7, e46183.

Zhou, H., Yu, Z., Hu, Y., Tu, J., Zou, W., Peng, Y., Zhu, J., Li, Y., Zhang, A. & other authors (2009). The special neuraminidase stalk-motif responsible for increased virulence and pathogenesis of H5N1 influenza A virus. *PLoS One* 4, e6277.

別紙様式第2-1

No. 7

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 2016. 1. 7	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	新鮮凍結人血漿		研究報告の公表状況	Nielsen TS, Nielsen AY, Banner J, et al. J Clin Virol. 2016 Jan;74:78-81. doi: 10.1016/j.jcv.2015.11.028. Epub 2015 Nov 27.	公表国 ロシア	
販売名(企業名)	新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)					

研究報告の概要	使用上の注意記載状況・その他参考事項等
○ヒト心筋炎に関連するSaffoldウイルス感染。 背景:Saffoldウイルスは、2007年にカルジオウイルス属に属する最初のヒトウイルスの一つとして報告された。カルジオウイルスは動物において重篤な心筋感染を引き起こす可能性があり、複数の研究によりSaffoldウイルスとヒト疾患との関連が示されている。Saffoldウイルスは心筋を含む様々な解剖学的部位から分離されているが、現在に至るまで、付随する炎症の病理組織学的兆候は実証できていない。 目的:本研究の目的は、Saffoldウイルスがヒトの心筋に侵襲的感染を起こす能力を有する可能性について検討することであった。 研究デザイン:RT-PCR法を用いて、剖検時に心筋炎と診断された死亡患者150名のホルマリン固定パラフィン包埋心臓組織検体を検討し、結果と組織学的所見を比較した。 結果および結論:Saffoldウイルスは、小児1名の心筋組織、肺組織及び血液から検出され、同時にウイルス感染に伴う心臓及び肺の病理組織学的炎症所見が認められた。当該所見はカルジオウイルスとヒト心筋炎との関連の可能性を示唆している。	新鮮凍結血漿-LR「日赤」120 新鮮凍結血漿-LR「日赤」240 新鮮凍結血漿-LR「日赤」480 血液を介するウイルス、細菌、原虫等の感染 vCJD等の伝播のリスク

報告企業の意見	今後の対応
Saffoldウイルスが、剖検時に心筋炎と診断された患者の心筋組織、肺組織及び血液から検出され、心筋炎との関連性が示唆されたという報告である。	今後も情報の収集に努める。

MedDRA/J Ver.18.1J

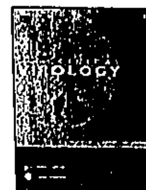
23

Journal of Clinical Virology 74 (2016) 78–81

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv



Short communication

Saffold virus infection associated with human myocarditis

Trine Skov Nielsen[a,*], Alex Yde Nielsen[b], Jytte Banner[c,d], Jakob Hansen[c], Ulrik Baandrup[a], Lars Peter Nielsen[b]

[a] Centre for Clinical Research, Vendsyssel Hospital/Department of Clinical Medicine, Bispensgade 37, 9800 Hjørring, Aalborg University, Denmark
[b] Department of Virology/Epidemiology Research, Statens Serum Institut, Artillerivej 5, 2300 Copenhagen S, Denmark
[c] Department of Forensic Medicine, Aarhus University, Brendstrupgaardsvej 100, 8200 Aarhus N, Denmark
[d] Department of Forensic Medicine, Copenhagen University, Frederik V's Vej 11, 2100 Copenhagen Ø, Denmark

ARTICLE INFO

Article history:
Received 3 July 2015
Received in revised form 9 November 2015
Accepted 25 November 2015

Keywords:
Human myocarditis
Virology
Saffold virus
PCR
Forensic medicine

ABSTRACT

Background: Saffold virus was described in 2007 as one of the first human viruses within the genus cardioviruses. Cardioviruses may cause severe infections of the myocardium in animals, and several studies have associated saffold virus with human disease. As a result, saffold virus has been isolated from different anatomical compartments, including the myocardium, but, until now, it has not been possible to demonstrate the accompanying histopathological signs of inflammation.
Objectives: The aim of the study was to examine if saffold virus is capable of causing invasive infection in the human myocardium.
Study design: Using real-time PCR, we retrospectively examined formalin-fixed paraffin embedded cardiac tissue specimens from 150 deceased individuals diagnosed with myocarditis at autopsy. The results were compared with histological findings.
Results and conclusions: Saffold virus was detected in the myocardium, lung tissue and blood of one child and was accompanied by histopathological inflammation in the heart and lungs, which was supportive of a viral infection. These findings suggest that cardioviruses may be associated with myocarditis in humans.
© 2015 Elsevier B.V. All rights reserved.

1. Background

Cardiovirus is a genus within the picornavirus family that has been known for several decades. It consists of two main species: Cardiovirus A-B. Cardiovirus A contains two serotypes of Encephalomyocarditis virus (EMCV), which are well known causes of myocarditis in animals. Human infections with EMCV were recently diagnosed using isolation and serology, but no human case of myocarditis due to EMCV has been identified [1]. The Cardiovirus B species includes Theiler's murine encephalomyelitis virus, which is known to cause encephalitis and myocarditis [2], Thera virus, Vilyuisk human encephalomyelitis virus and the recent described human virus; Saffold virus (SAFV).

SAFV was discovered in 2007 when it was isolated from a stool sample from a child with fever of unknown origin [3]. The virus is distributed worldwide. SAFV has been isolated from respiratory specimens and stool samples from children with respiratory and gastrointestinal symptoms [4,5] and, in a few studies, the virus has been detected in the cerebrospinal fluid and the myocardium [6,7]. However, the detection of SAFV has not been associated with microscopic evidence of inflammation in the affected organs. No larger studies on the occurrence of SAFV in myocardial tissue specimens from humans with myocarditis have been reported.

2. Objectives

Using real-time PCR, we examined a cohort of 150 deceased individuals diagnosed with myocarditis to investigate if SAFV is capable of causing invasive infection in the human myocardium.

3. Study design

We retrospectively examined formalin-fixed paraffin embedded (FFPE) cardiac tissue specimens from 150 deceased individuals diagnosed with myocarditis at autopsy (106 men and 44 women; median age 34.7 years, age range 3 weeks to 77 years; 13 cases below the age of 5 years). The cases were selected from a database at The Institute of Forensic Medicine, Aarhus University, Denmark and

* Corresponding author. Fax: +45 86125995.
E-mail addresses: tsn@retsmedicin.au.dk (T.S. Nielsen), alex.christian.yde.nielsen@regionh.dk (A.Y. Nielsen), jytte.banner@sund.ku (J. Banner), jah@forens.au.dk (J. Hansen), utb@rn.dk (U. Baandrup), larspn@gmail.com (L.P. Nielsen).

http://dx.doi.org/10.1016/j.jcv.2015.11.028
1386-6532/© 2015 Elsevier B.V. All rights reserved.

represented all autopsy cases diagnosed with myocarditis in the period 1992–2010. Four myocardial tissue samples from each heart were examined: one from the anterior wall of the left ventricle; one from the posterior wall of the left ventricle, one from the interventricular septum and one from the right ventricle. Three consecutive sections fro each location were stained for Hematoxylin & Eosin, CD3 and CD68.

Total nucleic acids were extracted from 20 μm tissue sections using a previously described, customized protocol that utilizes the automated Maxwell 16 system (Promega) in combination with proteinase K digestion and incubation in a lysis buffer [8]. The presence of RNA after extraction was confirmed in 91% of the myocarditis cases by reverse-transcriptase PCR amplification of a 121 basepair fragment of the mRNA transcript from the human *LONP1* gene as described previously [9]. All samples were tested for SAFV using a reverse-transcriptase real-time PCR assay and a genotyping was performed as previously described [7].

The laboratory workflow was performed according to usual guidelines where all procedures for the extraction and PCR were handled in different laboratories. Furthermore, all samples were processed separately from each other and from all other materials in order to reduce the risk of contamination. All assays included positive and negative control reactions.

4. Results

SAFV was detected at low concentrations upon repeated measurements (ct value = 39) in one of the 150 deceased individuals. This patient was a 2-year, 7-month-old previously healthy boy who died suddenly and unexpected after one day of slight fever. Using direct VP2 genotyping analysis, we determined the identified SAFV to be SAFV genotype 2. SAFV was also detectable in frozen blood (ct-value 30) and respiratory secretion (ct-value 22) while the cerebrospinal fluid was negative. As the present case was a forensic autopsy case routine examinations for other pathogens were performed prior to this study in order to establish the cause of death. *Staphylococcus aureus*, *Haemophilus influenzae* and non-hemolytic streptococci were isolated from lung tissue while enterovirus was detected in respiratory secretion. The results are shown in Table 1.

Histological examination revealed acute inflammation in the myocardium (Figs. 1 and 2) and in the lung tissue (Fig. 3) while the other examined organs revealed no signs of inflammation or other diseases. Due to the inflammatory alterations and the detection of enterovirus in the respiratory secretion, analysis for SAFV and enteroviruses was also performed on FFPE lung tissue, which despite repeated trials was negative for both viruses.

5. Discussion

Since the discovery of SAFV many efforts have been made to clarify its possible pathogenicity in humans. In addition to the positive findings in respiratory specimens and stool samples, SAFV has been detected in the cerebrospinal fluid and the myocardium, indicating an invasive potential [6,7]. In the first study, SAFV was found in the cerebrospinal fluid of two children ≤4 years of age, one of which died without preexisting symptoms. In this child, SAFV was also detected in a myocardial biopsy. In the second study, 10 myocardial biopsies were tested, and one was positive in a nested PCR assay, but not in real-time PCR. However, no histopathological alteration could be demonstrated in the myocardium of either of the two cases. In our study, we found SAFV in the respiratory secretion, blood and myocardial tissue of a child and histological evidence of inflammation in two related organs (heart and lungs). This combination is novel and may point to a role of SAFV in organ-related diseases.

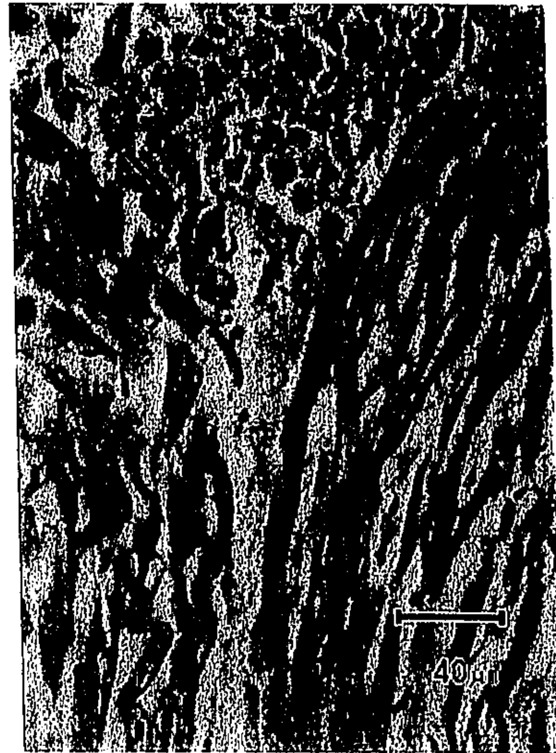


Fig. 1. Acute lymphocytic myocarditis. Histological section of the myocardium illustrating a small lymphocytic focus with associated degeneration of the myocytes (arrow). Hematoxylin and Eosin × 200.

The histological alterations in the myocardium and lung tissue primarily consisted of lymphocytes, which is suggestive of an infection of viral origin. The inflammatory changes in the myocardium were less pronounced than the changes in the lungs. Myocarditis is known to be a condition that causes both focal and diffuse inflammation, and even a small lesion may have a significant clinical impact [10]. The presence of viremia and the detection of SAFV in respiratory secretion and myocardium combined with the absence of other detectable pathogens in the myocardium, made SAFV plausible as a causative agent of the inflammatory changes. The low concentration of SAFV in the myocardium compared to blood and respiratory secretion may have been caused by the use of FFPE myocardial tissue as there is a known decreased sensitivity of virus detection in FFPE tissue. This may also be a likely explanation for the negative result of SAFV analysis on FFPE lung tissue. However, a higher concentration of viral RNA in the myocardium compared to lung tissue is also a possibility. The possibility of detecting RNA targets in postmortem tissue samples depends on several parameters including postmortem interval, fixation time, pH in fixation solution, and RNA extraction method [11]. In this study, we could detect a control RNA target in more than 90% of the study samples suggesting a reasonable RNA detection sensitivity and integrity in our FFPE material. Other studies have established that FFPE tissue material is usable for reverse-transcriptase PCR analysis of virus and messenger RNA, although the analytical sensitivity is reduced when compared to the use of matched frozen tissue material [12].

Three different types of bacteria were isolated from the lung tissue. Although *S. aureus* and *H. influenzae* may cause pneumonia, the histological picture is not in agreement with a bacterial etiology. Rather, the findings represent post-mortal growth of bacteria in the airways, and the presence of non-hemolytic streptococci supports this view.

Table 1
The results of the routine examinations and PCR analysis for saffold virus—overview.

	Routine examinations[a]	Examination of saffold virus
Histological examination		
Myocardium	Acute myocarditis	
Lung tissue	Acute interstitial pneumonia	
Other[b]	Normal tissue	
Bacteriological examination		
Lung tissue	*Staphylococcus aureusNon-hemolytic streptococciHaemophilus influenzae*	
Blood	Negative	
Cerebrospinal fluid	Negative	
Virological analysis[c]		
Myocardium	Negative	Positive
Blood	Negative	Positive
Respiratory secretion	Enterovirus	Positive
Cerebrospinal fluid	Negative	Negative
Lung tissue (FFPE)	Negative[d]	Negative

[a] Examinations performed in relation to autopsy.
[b] Liver, brain, kidney, prostate, bone marrow, pancreas, testicles, thyroid gland, thymus, spleen, epiglottis and peripheral muscle.
[c] Routine virological analysis: adenovirus, coronavirus, enterovirus, parechovirus, influenzavirus, parainfluenza virus, parvovirus B19, human herpes simplex virus 1+2, varicella zoster virus and rhinovirus.
[d] Only examined for enterovirus and saffold virus.

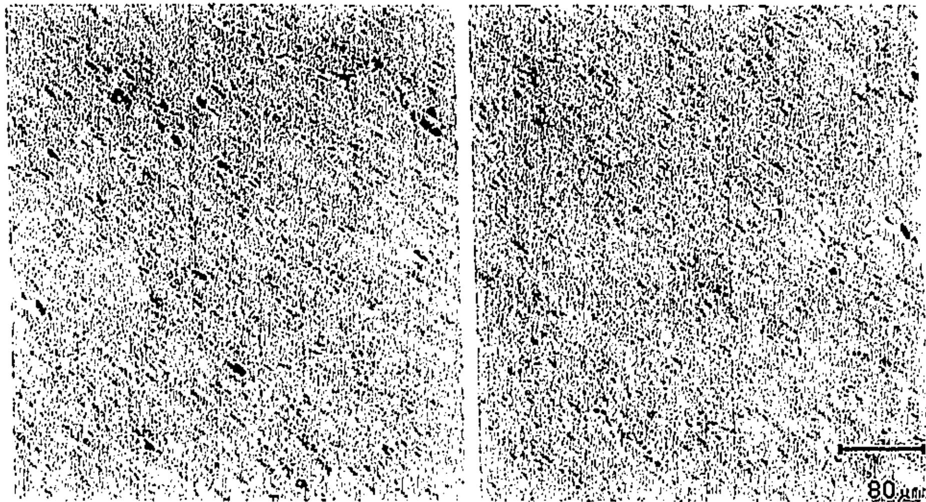


Fig. 2. Acute lymphocytic myocarditis. Histological sections of the myocardium illustrating an area with an increased amount of CD45-positive lymphocytes (left picture) compared to normal myocardium (right picture). CD45 × 100.

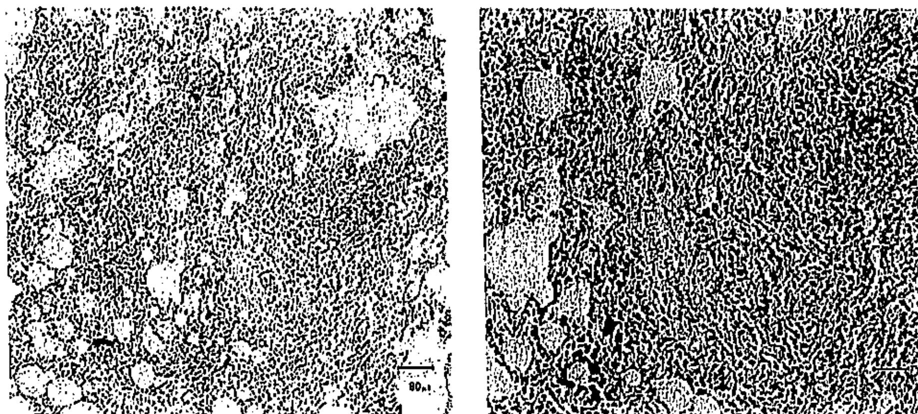


Fig. 3. Interstitial pneumonia. Histological section of the lung illustrating lymphocytic infiltration into the interstitial tissue. Hematoxylin and Eosin × 100 (left) and ×200 (right).

A limitation of our study is that a verification of intracellular RNA e.g., by in situ hybridization (ISH) was not performed. A positive PCR result in the myocardium might represent the presence of blood in the tissue. However, the sensitivity of ISH is markedly lower than RT-PCR and a negative result of ISH in our case cannot rule out the presence of the virus. Future studies preferably using unfixed material should establish the presence of the virus inside the infected myocytes. Another limitation is the age of the study cohort varying from 3 weeks to 77 years, with the mean being 34.7 years. Thirteen cases were below the age of 5 years. In prior studies as well as in this study, SAFV has mainly been detected in different compartments of young children, indicating that infection with SAFV occurs early in life [10,13,14]. Determining the exact significance of SAFV for myocarditis would require a study including a large cohort of young children.

We found one SAFV-positive case of 150 deceased individuals. Despite a low prevalence that does not allow us to make a definite conclusion on the association of SAFV with myocarditis, we find the result remarkable because cardioviruses are well-known causes of myocarditis in animals [2]. In conjunction with prior studies it seems evident that SAFV plays a role in invasive human infections and our results suggest that SAFV may also be associated with myocarditis in humans.

Conflict of interests

The authors state that they have no conflict of interest.

Funding

This work was supported by the Beckett Foundation, Kong Christian den Tiendes Foundation, Kirsten Anthonius Foundation, Aarhus University and Aalborg University.

Ethical approval

The study was approved by the national ethics committee, Denmark, protocol no. 1209317.

Acknowledgements

The authors are grateful to Mette Skov Mikkelsen, Bente Wormstrup and Anette Funder for their excellent technical assistance.

References

[1] M.S. Oberste, E. Gotuzzo, P. Blair, W.A. Nix, T.G. Ksiazek, J.A. Comer, et al., Human febrile illness caused by encephalomyocarditis virus infection, Peru, Emerg. Infect. Dis. 15 (April (4)) (2009) 640–646.

[2] R.M. Gomez, J.E. Rinehart, R. Wollmann, R.P. Roos, Theiler's murine encephalomyelitis virus-induced cardiac and skeletal muscle disease, J. Virol. 70 (December (12)) (1996) 8926–8933.

[3] M.S. Jones, V.V. Lukashov, R.D. Ganac, D.P. Schnurr, Discovery of a novel human picornavirus in a stool sample from a pediatric patient presenting with fever of unknown origin, J. Clin. Microbiol. 45 (July (7)) (2007) 2144–2150.

[4] Y. Abed, G. Boivin, New saffold cardioviruses in 3 children, Canada, Emerg. Infect. Dis. 14 (May (5)) (2008) 834–836.

[5] O. Blinkova, A. Kapoor, J. Victoria, M. Jones, N. Wolfe, A. Naeem, et al., Cardioviruses are genetically diverse and cause common enteric infections in South Asian children, J. Virol. 83 (May (9)) (2009) 4631–4641.

[6] J.F. Drexler, S. Baumgarte, M. Eschbach-Bludau, A. Simon, C. Kemen, U. Bode, et al., Human cardioviruses, meningitis, and sudden infant death syndrome in children, Emerg. Infect. Dis. 17 (December (12)) (2011) 2313–2315.

[7] A.C. Nielsen, B. Bottiger, J. Banner, T. Hoffmann, L.P. Nielsen, Serious invasive Saffold virus infections in children, 2009, Emerg. Infect. Dis. 18 (January (1)) (2012) 7–12.

[8] R. Loewe, RNA-Isolation aus FFPE-Gewebe: Säulchen- und Bead-basierte Methoden, Laborwelt 12 (05) (2011) 38–39.

[9] T.S. Nielsen, J. Hansen, L.P. Nielsen, U.T. Baandrup, J. Banner, The presence of enterovirus, adenovirus, and parvovirus B19 in myocardial tissue samples from autopsies: an evaluation of their frequencies in deceased individuals with myocarditis and in non-inflamed control hearts, Forensic Sci. Med. Pathol. 10 (September (3)) (2014) 344–350.

[10] T.S. Nielsen, J.R. Nyengaard, J. Moller, J. Banner, L.P. Nielsen, U.T. Baandrup, Quantitative diagnosis of lymphocytic myocarditis in forensic medicine, Forensic Sci. Int. 238 (May) (2014) 9–15.

[11] B.P. Bass, K.B. Engel, S.R. Greytak, H.M. Moore, A review of preanalytical factors affecting molecular, protein, and morphological analysis of formalin-fixed, paraffin-embedded (FFPE) tissue: how well do you know your FFPE specimen? Arch. Pathol. Lab. Med. 138 (November (11)) (2014) 1520–1530.

[12] A. van der Linden, B.M. Blokker, M. Kap, A.C. Weustink, J.L. Robertus, P.H. Riegman, et al., Post-mortem tissue biopsies obtained at minimally invasive autopsy: an RNA-quality analysis, PLoS One 9 (12) (2014) e115675.

[13] C.Y. Chiu, A.L. Greninger, E.C. Chen, T.D. Haggerty, J. Parsonnet, E. Delwart, et al., Cultivation and serological characterization of a human Theiler's-like cardiovirus associated with diarrheal disease, J. Virol. 84 (May (9)) (2010) 4407–4414.

[14] J. Zoll, H.S. Erkens, K. Lanke, L.F. Verduyn, W.J. Melchers, E. Schoondermark van de Ven, et al., Saffold virus, a human Theiler's-like cardiovirus, is ubiquitous and causes infection early in life, PLoS Pathog. 5 (May (5)) (2009) e1000416.

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2015. 12. 3	該当なし	
一般的名称	新鮮凍結人血漿		研究報告の公表状況	Kruger DH, Tkachenko EA, Morozov VG, et al. Emerg Infect Dis. 2015 Dec;21(12):2204-8.	公表国 ロシア	
販売名(企業名)	新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)					

研究報告の概要

○ロシアにおける致死的ソチウイルス感染。
ロシア南部のソチ市を含む行政区クラスノダールで発見された新種のハンタウイルスであるソチウイルスは、Black Sea Field Mouse(*Apodemus ponticus*)から採取した細胞培養物並びに劇症型ハンタウイルス疾患患者(ショック並びに腎不全と呼吸不全の合併症により死亡)から分離された。
2000年から2013年にソチウイルスに感染した患者62例における臨床疾患の重症度を、ロシアの標準的な基準に従い軽症、中等症、重症のいずれかに分類した。
致死率は14.5%(62例中9例)であった。患者の60%近くが重症(死亡例を含む)であったが、残る40%は中等症であった。
男性患者の割合は有意に高く($p=1.05 \times 10^{-9}$)、その大半(66.7%)は重症であったが、女性患者における重症者の割合は35.7%であった($p=0.037$)。死亡例9例のうち女性患者はわずかに2例であった。
ソチウイルス感染が確認された調査対象患者の大半が、重篤な臨床経過をたどった。ソチウイルス感染における生存率は、診断、治療及び予防における地元の医師並びに公衆衛生当局の認識の増大により改善すると思われる。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
新種のハンタウイルスであるソチウイルス感染患者の大半が重篤な臨床経過をたどり、致死率は14.5%であった。女性患者に比べ、男性患者の重症度は高かったという報告である。	今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

Life-Threatening Sochi Virus Infections, Russia

Medscape EDUCATION **ACTIVITY**

Medscape, LLC is pleased to provide online continuing medical education (CME) for this journal article, allowing clinicians the opportunity to earn CME credit.

This activity has been planned and implemented in accordance with the Essential Areas and policies of the Accreditation Council for Continuing Medical Education through the joint providership of Medscape, LLC and Emerging Infectious Diseases. Medscape, LLC is accredited by the ACCME to provide continuing medical education for physicians.

Medscape, LLC designates this Journal-based CME activity for a maximum of 1.0 *AMA PRA Category 1 Credit(s)™*. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

All other clinicians completing this activity will be issued a certificate of participation. To participate in this journal CME activity: (1) review the learning objectives and author disclosures; (2) study the education content; (3) take the post-test with a 75% minimum passing score and complete the evaluation at http://www.medscape.org/journal/eid; (4) view/print certificate.

Release date: November 12, 2015; Expiration date: November 12, 2016

Learning Objectives

Upon completion of this activity, participants will be able to:

- Analyze the demographics of patients infected with the Sochi virus in the current study
- Assess laboratory data available from patients infected with Sochi virus in the current study
- Distinguish the anatomic site of the highest concentration of Sochi virus among infected individuals
- Evaluate the prognosis of infection with Sochi virus.

CME Editor

Karen L. Foster, Technical Writer/Editor, *Emerging Infectious Diseases. Disclosure: Karen L. Foster has disclosed no relevant financial relationships.*

CME Author

Charles P. Vega, MD, Clinical Professor of Family Medicine, University of California, Irvine. *Disclosure: Charles P. Vega, MD, has disclosed the following financial relationships: served as an advisor or consultant for Lundbeck, Inc.; McNeil Pharmaceuticals; Takeda Pharmaceuticals North America, Inc.*

Authors

Disclosures: Detlev H. Kruger, MD, PhD; Evgeniy A. Tkachenko, MD, PhD; Vyacheslav G. Morozov, MD; Yulia V. Yunicheva, MD, PhD; Olga M. Pilikova, MD, PhD; Gennadiy Malkin, BS; Aydar A. Ishmukhametov, MD, PhD; Patrick Heinemann, PhD; Peter T. Witkowski, MD; Boris Klempa, PhD; and Tamara K. Dzagurova, MD, PhD, have disclosed no relevant financial relationships.

Detlev H. Kruger, Evgeniy A. Tkachenko, Vyacheslav G. Morozov, Yulia V. Yunicheva, Olga M. Pilikova, Gennadiy Malkin, Aydar A. Ishmukhametov, Patrick Heinemann, Peter T. Witkowski, Boris Klempa, Tamara K. Dzagurova

Sochi virus was recently identified as a new hantavirus genotype carried by the Black Sea field mouse, *Apodemus ponticus*. We evaluated 62 patients in Russia with Sochi virus infection. Most clinical cases were severe, and the case-fatality rate was as high as 14.5%.

Hantaviruses are zoonotic pathogens transmitted from small animals to humans. Hantavirus disease in the Americas is called hantavirus pulmonary syndrome and in Asia and Europe is called hemorrhagic fever with renal syndrome (HFRS). Both syndromes can lead to cardiopulmonary and renal failure (*1*). Recently we described a new hantavirus, Sochi virus, from the administrative region Krasnodar (including the city of Sochi), southern European Russia, which was isolated in cell culture from a Black Sea field mouse (*Apodemus ponticus*) and a

Author affiliations: Charité School of Medicine, Berlin, Germany (D.H. Kruger, P. Heinemann, P.T. Witkowski, B. Klempa); Chumakov Institute of Poliomyelitis and Viral Encephalitides, Moscow, Russia (E.A. Tkachenko, G. Malkin, A.A. Ishmukhametov, T.K. Dzagurova); Medical State University, Samara, Russia (V.G. Morozov); Anti-Plague Stations, Sochi, Russia (Y.V. Yunicheva); Anti-Plague Stations, Novorossiysk, Russia(O.M. Pilikova); Slovak Academy of Sciences, Bratislava, Slovakia (B. Klempa)

DOI: http://dx.doi.org/10.3201/eid2112.150891

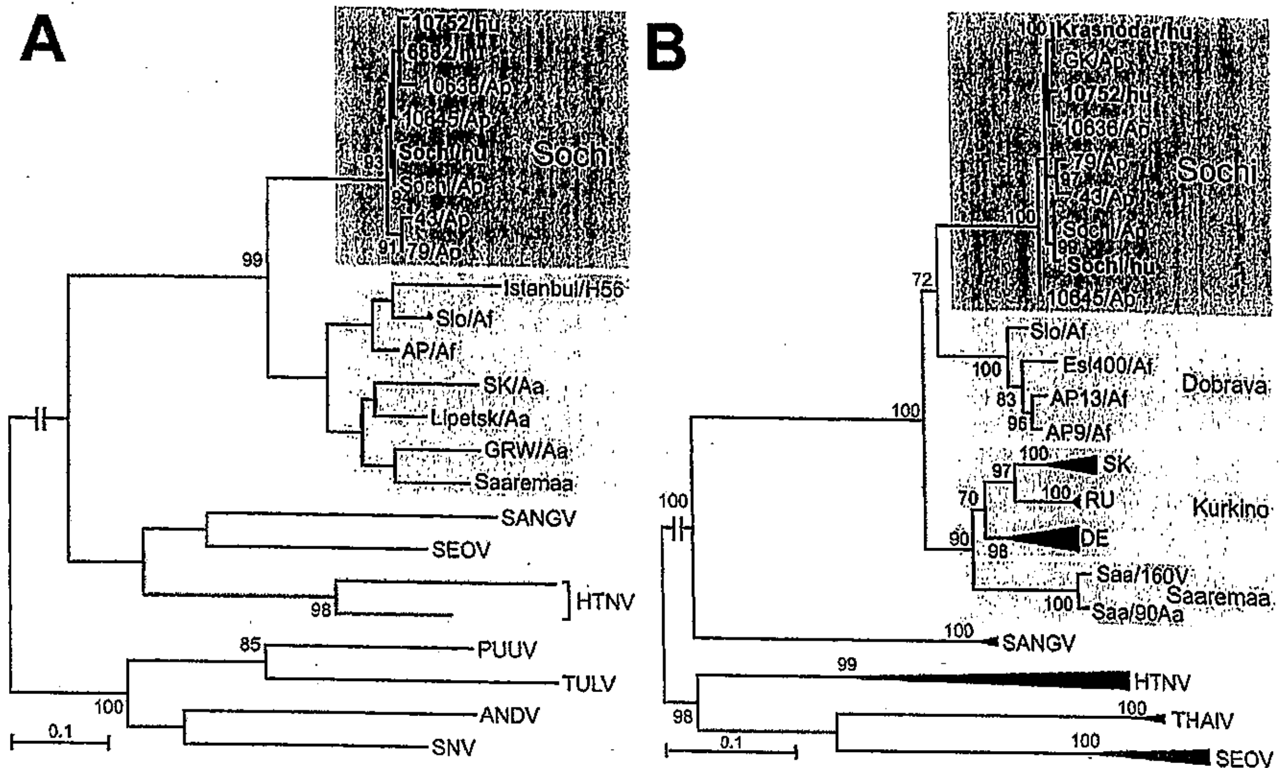


Figure 1. Phylogenetic analysis segment sequences of Sochi virus, Russia: A) 347-bp large (L) segment sequence; B) 1,197-bp small (S) segment sequence. Virus sequences derived from patients (shown in bold type) and *Apodemus ponticus* mice cluster within the Sochi genotype of DOBV. Evolutionary analysis was conducted in MEGA6 (*6*). The evolutionary history was inferred by using the maximum-likelihood method based on the Tamura 3-parameter model with a discrete gamma distribution and 5 rate categories (analysis in panel A) and on the general time reversible model with gamma rates and heterogeneous patterns (analysis in panel B), respectively, which were estimated to be the best-fit substitution model according to the Bayesian information criterion. Scale bars indicate an evolutionary distance of 0.1 substitutions per position in the sequence. Bootstrap values ≥70%, calculated from 500 replicates, are shown at the tree branches. GenBank accession numbers of all sequences used in the analysis are listed in online Technical Appendix Table 1 (http://wwwnc.cdc.gov/EID/article/21/12/15-0891-Techapp1.pdf). Dark gray shading indicates cluster of DOBV-Sochi strains; light gray shading indicates different clusters of strains from other DOBV genotypes. ANDV, Andes virus; DOBV, Dobrava-Belgrade virus; HTNV, Hantaan virus; PUUV, Puumala virus; SANGV, Sangassou virus; SEOV, Seoul virus; SNV, Sin Nombre virus; THAIV, Thailand virus; TULV, Tula virus.

patient with fulminant hantavirus disease who died of shock and combined kidney and lung failure (*2*–*4*). Molecular taxonomical analyses identified Sochi virus as a new genotype within the Dobrava-Belgrade virus (DOBV) species (*5*). Here we show that HFRS caused by Sochi virus infection occurs in the geographic region where *A. ponticus* mice are prevalent. For 62 patients infected by this virus during 2000–2013, we evaluated clinical and epidemiologic data.

The Study

Serum of patients with suspected acute hantavirus disease from the Krasnodar region were screened for hantavirus antibodies by indirect immunofluorescence assays and ELISA. Sixty-two patients showed clear DOBV IgG seropositivity. During the acute phase of illness, all patients tested positive for DOBV IgM (data not shown). For 26 patients, sufficient volumes of follow-up serum were available for additional focus reduction neutralization assays to specify neutralizing antibodies. All serum samples exhibited substantially higher neutralizing titers toward DOBV than toward Puumala virus, Hantaan virus, and Seoul virus. When the neutralizing effect of DOBV-positive patients' serum were compared against the different human pathogenic genotypes of DOBV (Dobrava, Kurkino, and Sochi), all serum predominantly reacted with the Sochi genotype (online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/21/12/15-0891-Techapp1.pdf).

We successfully obtained virus genomic large (L) segment sequences from 2 patients (no. 51, specimen no. 6882; no. 59, specimen no. 10752). In the neighborhood

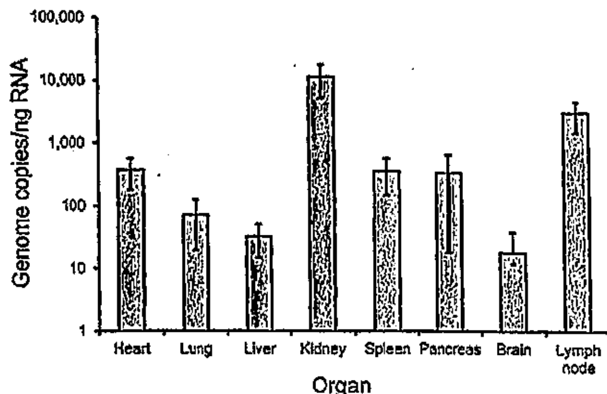


Figure 2. Quantification of hantavirus RNA in tissue biopsies from a 50-year-old Sochi virus–infected man (patient no. 59), Russia. Two independent approaches were performed to extract RNA from each organ. Quantitative reverse transcription PCR previously developed for DOBV (*7*) was used to measure virus load in the analyzed biopsy samples. Three quantitative reverse transcription PCR estimations were conducted for every RNA extraction, followed by calculation of mean values and SDs. Viral RNA levels are shown as genome copies per nanogram of total RNA isolated from the samples. Error bars indicate SD.

of the residence of patient no. 59, mice were trapped, and hantaviral L and small (S) segment regions from 2 *A. ponticus* animals (specimen nos. 10636, 10645) were amplified. The sequences obtained were deposited in GenBank under accession nos. KM192207–09 and KP878308–10 (L segment) and KP878311–13 (S segment) (online Technical Appendix Table 2). Samples from virus-positive mice were phylogenetically characterized by analysis of a 242-bp region of their *cytB* gene; all of them clustered with the previously identified *A. ponticus* animals (*3*) (data not shown). In addition, the *A. ponticus*–derived isolate Sochi/Ap (*4*), the patient-derived isolate Sochi/hu (*5*), an S segment sequence from a mouse (GK/Ap) trapped near the home of the previously described Krasnodar patient (*4*), and sequences originating from 2 *A. ponticus* mice sampled near the Black Sea coast, 43/Ap and 79/Ap, were included in the molecular analyses of the virus.

The patient-derived sequences 6882/hu, 10752/hu, and Sochi/hu clearly cluster with *A. ponticus*–derived sequences 43/Ap, 79/Ap, 10636/Ap, 10645/Ap, and Sochi/Ap (Figure 1, panel A). In the analysis of the S segment, we obtained a very similar result; the patient-derived sequences 10752/hu, Krasnodar/hu, and Sochi/hu cluster with *A. ponticus*–associated sequences 43/Ap, 79/Ap, 10636/Ap, 10645/Ap, GK/Ap, and Sochi/Ap (Figure 1, panel B). In analysis of both L and S segments, the Sochi virus strains form a unique group, clearly distinguishable from all other DOBV genotypes.

Specimens from different organs of deceased patient no. 59 were analyzed for virus load. The highest concentration was detected in kidney (11,446 copies/ng RNA) and lymph node (3,086 copies/ng RNA), whereas the least virus RNA (10–100 copies/ng RNA) was detected in lung, brain, and liver (Figure 2).

The clinical disease severity of the 62 Sochi virus–infected patients investigated (Table 1) was subdivided into mild, moderate, or severe following the standard Russian criteria (i.e., length of febrile phase, minimal blood pressure in the hypotonic phase, extent of hemorrhagic symptoms, minimal urine production, serum creatinine level, and extent of proteinuria) (online Technical Appendix Table 3). The case-fatality rate (CFR) was as high as 14.5% (9/62 patients). Including fatalities, severe disease developed in nearly 60% of patients, whereas the remaining 40% of cases were moderate. The average age of all patients was 33 years. A significantly higher proportion of patients were males ($p = 1.05 \times 10^{-9}$). Moreover, severe disease developed in most affected male patients (66.7%) but in only 35.7% of affected female patients ($p = 0.037$). The fact that only 2 of 9 fatal cases occurred in female patients (Table 1) underscores this finding.

All 9 patients with fatal infections died of multiorgan failure and shock (Table 2). Postmortem examination showed multiple hemorrhages and edema in internal organs, including kidneys and lungs. The patients died within 8.2 days (range 3–16 days) after disease onset. An extraordinary fulminant course was observed for patient no. 47, who died 3 days after onset and before he could be hospitalized. This 19-year-old man was the son-in-law of patient no. 48, who also died after Sochi virus infection. Both men lived at the same rural address, and rodent contact during work in haystacks was reported.

Table 1. Comparisons in clinical outcome, age, and sex of 62 patients with Sochi virus infection, Russia*

	Total		Sex, no. (%)		Age, y, n/N (%)	
Characteristic	No. (%)	Median age, y (range)	M, n = 48	F, n = 14	7–15	>15
No. patients	62 (100)	33.3 (7–57)	**48 (77.4)**	**14 (22.6)**	**6/62 (9.7%)**	**56/62 (90.3)**
Outcome						
Died	9 (14.5)	38.6 (19–53)	7 (14.6)	2 (14.3)	0/6	9/56 (16.1)
Survived	53 (85.5)	32.4 (7–57)	41 (85.4)	12 (85.7)	6/6 (100)	47/56 (83.9)
Illness course						
Severe, including fatal	37 (59.7)	33.1 (10–57)	**32 (66.7)**	**5 (35.7)**	3/6 (50)	34/56 (60.7)
Moderate, mild	25 (40.3)	33.6 (7–57)	**16 (33.3)**	**9 (64.3)**	3/6 (50)	22/56 (39.3)

*Bold type indicates statistically significant differences between sex or age groups. Comparison of binomial population proportions analysis as implemented in Statlets (NWP Associates, Inc., http://www.mrs.umn.edu/~sungurea/statlets/statlets.htm) indicates rejection of the null hypothesis (claiming that the 2 proportions are equal) at significance level of $p<0.05$.

Table 2. Characteristics of 9 deceased patients with Sochi virus infection, Russia*

Patient no.	Age, y/sex	Hospitalized, no. d after onset	GI symptoms	Max serum creatinine, μmol/L†	Min platelet count, × 10⁹/L‡	Died, no. d after onset	Clinical and postmortem findings
23	33/M	5	No	148	70	8	Pneumonia; renal, cardiovascular, multiorgan failure; multiple internal hemorrhages, edema
29	29/M	Same day	Yes	282	115	6	Renal, cardiovascular, multiorgan failure; multiple internal hemorrhages, edema
30	47/F	5	Yes	391	38	12	Renal, lung failure; shock; coagulation disturbance; hemorrhagic gastroenteritis; multiple internal hemorrhages, edema
34	53/M	3	Yes	250	110	10	Multiorgan failure; coagulation disturbances; multiple internal hemorrhages
42	30/M	14	Yes	186	67	16	Uremic coma; multiorgan failure; multiple internal hemorrhages
47§	41/M	Died before hospitalization	Yes	NR	NR	3	Renal failure; multiple internal hemorrhages, edema
48§	19/M	4	Yes	192	54	6	Renal, cardiovascular failure; RDS, DIC syndrome; bleedings in pituitary, adrenal gland, intestinum, etc.
56	35/F	4	Yes	410	49	6	Cardiovascular, renal, lung, liver failure; renal tubular necrosis; lung, brain edema
59	50/M	5	Yes	310	3	7	Renal,cardiovascular failure; RDS; multiple internal hemorrhages; pleurorrhea; lung, brain edema

*DIC, disseminated intravascular coagulation; GI, gastrointestinal; max, maximum; min, minimum; RDS, respiratory distress syndrome; NR, not reported.
†Reference range <96 μmol/L for female patients, <110 μmol/L for male patients.
‡Reference range 150–400 × 10⁹/L
§Patient no. 47 was the father in-law of patient no. 48; both lived in the same rural residence.

Conclusions

We have demonstrated the occurrence of human infections by Sochi virus and studied the clinical outcome for 62 patients. This virus is carried by the Black Sea field mouse (*A. ponticus*), which occurs naturally in the Transcaucasian region between the Black and Caspian Seas, including a part of southern European Russia. In anecdotal field studies in the coast region near Sochi, *A. ponticus* was the most abundant mouse species (71% of all trapped mice were identified as *A. ponticus*); moreover, 14% of trapped *A. ponticus* mice were serologically proven to be DOBV infected (*8*). This finding indicates that DOBV is the hantavirus indigenous in this geographic area and that *A. ponticus* mice are highly relevant as a hantavirus reservoir. All evidence from the natural virus reservoir, as well as serologic and molecular diagnostics of patients' serum, shows that the virus responsible for the infections is the DOBV genotype Sochi.

Most investigated patients found to be infected by Sochi virus exhibited a severe clinical course. With a calculated CFR of 14.5%, Sochi virus might be the most deadly hantavirus outside the Americas, where 35%–50% of hantavirus infections are fatal (*1,9*). Even Asian Hantaan virus is estimated to be less deadly; recent studies show CFRs of 1%–3% in China and South Korea, where Hantaan virus infections play an important role in HFRS morbidity (*10,11*). On the other hand, increased awareness in diagnostics, treatment, and prevention by local physicians and public health authorities is expected to improve survival rates for Sochi virus infections.

Among the related viruses of the DOBV species, Sochi virus seems to have the highest level of virulence, similar to Dobrava virus (carried by *A. flavicollis* mice), which has a CFR of up to 10%–12% (*12,13*). As shown in larger studies, disease caused by infection with the related Kurkino genotype (carried by the western lineage of *A. agrarius* mice) is associated with a CFR of only 0.3%–0.9% (*3,14*). These phylogenetically related viruses exert a quite different pathogenicity in humans.

Acknowledgments

We thank Brita Auste for careful execution of the molecular diagnostics.

This work was supported by Russian Science Foundation (grant no. 14-15-00619), Robert Koch Institute on behalf of the German Ministry of Public Health (grant no. 1369-382), and Deutsche Forschungsgemeinschaft (Priority Program 1596 "Ecology and species barriers in emerging viral diseases," grant no. KR1293/13-1).

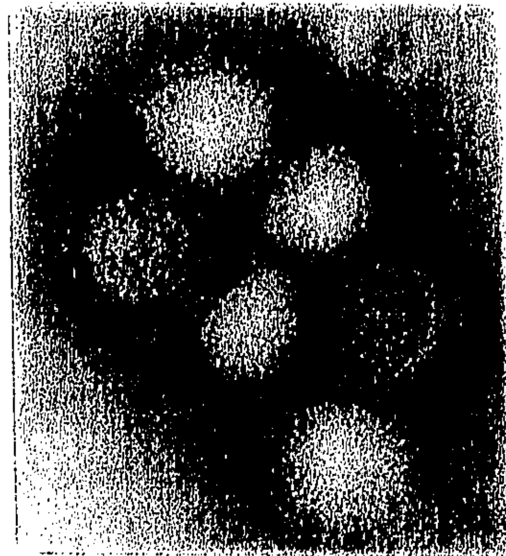
Dr. Kruger is the head of the Institute of Medical Virology, Charité–University Medicine Berlin. His research focuses on the molecular epidemiology and clinical relevance of emerging virus infections.

References

1. Kruger DH, Figueiredo LTM, Song JW, Klempa B. Hantaviruses—globally emerging pathogens. J Clin Virol. 2015;64:128–36. http://dx.doi.org/10.1016/j.jcv.2014.08.033
2. Tkachenko EA, Okulova NM, Yunicheva YV, Morzunov SP, Khaibulina SF, Riabova TE, et al. The epizootological and v irological characteristics of a natural hantavirus infection focus in the subtropic zone of the Krasnodarsk Territory [in Russian]. Vopr Virusol. 2005;50:14–9.
3. Klempa B, Tkachenko EA, Dzagurova TK, Yunicheva YV, Morozov VG, Okulova NM, et al. Hemorrhagic fever with renal syndrome caused by 2 lineages of Dobrava hantavirus, Russia. Emerg Infect Dis. 2008;14:617–25. http://dx.doi.org/10.3201/eid1404.071310
4. Dzagurova TK, Witkowski PT, Tkachenko EA, Klempa B, Morozov VG, Auste B, et al. Isolation of Sochi virus from a fatal case of hantavirus disease with fulminant clinical course. Clin Infect Dis. 2012;54:e1–4. http://dx.doi.org/10.1093/cid/cir746
5. Klempa B, Avsic-Zupanc T, Clement J, Dzagurova TK, Henttonen H, Heyman P, et al. Complex evolution and epidemiology of Dobrava-Belgrade hantavirus: definition of genotypes and their characteristics. Arch Virol. 2013;158:521–9. http://dx.doi.org/10.1007/s00705-012-1514-5
6. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol. 2013;30:2725–9. http://dx.doi.org/10.1093/molbev/mst197
7. Kramski M, Meisel H, Klempa B, Krüger DH, Pauli G, Nitsche A. Detection and typing of human pathogenic hantaviruses by real-time reverse transcription–PCR and pyrosequencing. Clin Chem. 2007;53:1899–905. http://dx.doi.org/10.1373/clinchem.2007.093245
8. Okulova NM, Khliap LA, Varshavskii AA, Dzagurova TK, Iunicheva IV, Riabova TE, et al. Spatial structure of natural foci of hantavirus on the territory of northwestern Caucasus [in Russian]. Zh Mikrobiol Epidemiol Immunobiol. 2013; Sep–Oct:47–53.
9. Mertz GJ, Hjelle B, Crowley M, Iwamoto G, Tomicic V, Vial PA. Diagnosis and treatment of new world hantavirus infections. Curr Opin Infect Dis. 2006;19:437–42. http://dx.doi.org/10.1097/01.qco.0000244048.38758.1f
10. Zhang YZ, Zou Y, Fu ZF, Plyusnin A. Hantavirus infections in humans and animals, China. Emerg Infect Dis. 2010;16:1195–203. http://dx.doi.org/10.3201/eid1608.090470
11. Noh JY, Cheong HJ, Song JY, Kim WJ, Song KJ, Klein TA, et al. Clinical and molecular epidemiological features of hemorrhagic fever with renal syndrome in Korea over a 10-year period. J Clin Virol. 2013;58:11–7. http://dx.doi.org/10.1016/j.jcv.2013.06.027
12. Avsic-Zupanc T, Petrovec M, Furlan P, Kaps R, Elgh F, Lundkvist A. Hemorrhagic fever with renal syndrome in the Dolenjska region of Slovenia—a 10-year survey. Clin Infect Dis. 1999;28:860–5. http://dx.doi.org/10.1086/515185
13. Papa A, Antoniadis A. Hantavirus infections in Greece—an update. Eur J Epidemiol. 2001;17:189–94. http://dx.doi.org/10.1023/A:1017987104363
14. Dzagurova TK, Klempa B, Tkachenko EA, Slyusareva GP, Morozov VG, Auste B, et al. Molecular diagnostics of hemorrhagic fever with renal syndrome during a Dobrava virus infection outbreak in the European part of Russia. J Clin Microbiol. 2009;47:4029–36. http://dx.doi.org/10.1128/JCM.01225-09

Address for correspondence: Detlev H. Kruger, Institute of Medical Virology, Helmut-Ruska-Haus, Charité–University Medicine Berlin, Charitéplatz 1, D-10117 Berlin, Germany; email: detlev.kruger@charite.de

Outbreak of a New Strain of Flu at a Fair



Dr. Karen Wong, an EIS officer with the Centers for Disease Control and Prevention, discusses her study about flu outbreaks at agricultural fairs.

http://www2c.cdc.gov/podcasts/player.asp?f=8627464

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 2016年1月18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	http://www.fda.gov/BiologicsBloodVaccines/GuidanceComlpianceRegulatoryInformation/Guidances/default.htm.		公表国 米国	
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
「業界向けガイダンス：改訂クロイツフェルト・ヤコブ病及び変異型クロイツフェルト・ヤコブ病の血液及び血液製剤を介した伝播のリスクを低減するための予防対策について」が発出された。 改訂のポイントは、アルブミン、血漿由来アルブミンを含む製剤を含む血漿由来製品の表示の2012年改訂案の推奨と、FDAへの表示変更報告に関する、2012年改訂案の推奨。 その他、2010年ガイダンスの修正 ・新しい表示推奨に関連した情報・グローバルでのvCJDとBSE感染状況の情報の更新 ・CJDの家族歴を持つドナーからの再登録の基準の明確化、・生物由来製品の逸脱報告に関する必要要件の明確化	重要な基本的注意 現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

報告企業の意見	今後の対応
ＦＤＡより発出された、改訂版のガイダンス：クロイツフェルト・ヤコブ病及び変異型クロイツフェルト・ヤコブ病の血液及び血液製剤を介した伝播リスクを低減するための予防対策について　の報告である。	今後ともvCJD伝播に関する情報等に留意していく。

Revised Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease and Variant Creutzfeldt-Jakob Disease by Blood and Blood Products

Guidance for Industry

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD), 10903 New Hampshire Ave., Bldg. 71, Rm. 3128, Silver Spring, MD 20993-0002, or by calling 1-800-835-4709 or 240-402-8010, or email ocod@fda.hhs.gov, or from the Internet at http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

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

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
May 2010
Updated January 2016

Table of Contents

I. INTRODUCTION....1

II. BACKGROUND....3

- A. CJD and vCJD....3
- B. Evolution of the Global BSE epidemic....6
- C. TSE Agents and Blood....7
- D. FDA Regulatory History....10
- E. Rationale for Geographic Donor Deferrals....15

III. EXPLANATION OF CURRENT VCJD RECOMMENDATIONS....17

- A. Exposure to British Beef in the U.K....17
- B. Exposure to British Beef Products Distributed Outside of the U.K....17
- C. Indigenous BSE Exposure Outside the U.K....18
- D. Potential Infection with vCJD Agent Acquired by Transfusion....21
- E. Exposure to Bovine Insulin....21
- F. Reports of Biological Product Deviations....21
- G. Definitions....22

IV. RECOMMENDATIONS FOR DONOR DEFERRAL....24

- A. Donor Deferral Criteria....24
- B. Questions to Identify Donors at an Increased Risk for CJD....25
- C. Donor Reentry after Donor Deferral for Risk of Familial CJD....26
- D. Questions for Identifying Donors at Risk for Exposure to BSE....26

V. POST-DONATION INFORMATION: RECOMMENDATIONS FOR PRODUCT RETRIEVAL AND QUARANTINE, CONSIGNEE NOTIFICATION, AND BIOLOGICAL PRODUCT DEVIATION REPORTING....29

- A. Whole Blood and Blood Components Intended for Transfusion, Cellular Blood Components Intended for Further Manufacture into Injectable Products, and Source Plasma From Donors with CJD or CJD Risk Factors29
- B. Whole Blood and Blood Components Intended for Transfusion, Source Leukocytes and Other Cellular Blood Components Intended for Further Manufacture into Injectable Products, from Donors with Geographic Risk Deferrals and/or Exposure to Bovine Insulin Made in the U.K. since 1980..30
- C. Source Plasma and Recovered Plasma from Donors with Geographic Risk Deferrals and/or Exposure to Bovine Insulin Made in the U.K. Since 1980.31
- D. Whole Blood and Blood Components Intended for Transfusion, Recovered Plasma, Source Leukocytes, Other Cellular Blood Components Intended for Manufacturing into Injectable Products, and Source Plasma from Donors with vCJD, suspected vCJD, or CJD and Age Less Than 55 Years....31
- E. Plasma Derivatives....32
- F. Disposal of Retrieved and Quarantined Products....33

VI. RECOMMENDATIONS FOR RECIPIENT TRACING AND NOTIFICATION...34

VII. LABELING RECOMMENDATIONS....34

VIII. IMPLEMENTATION OF RECOMMENDATIONS....36

IX. THE IMPACT OF GEOGRAPHIC DONOR DEFERRALS THAT ARE MORE STRINGENT THAN THOSE RECOMMENDED BY THIS GUIDANCE....37

X. SOURCES OF ADDITIONAL INFORMATION....38

XI. REFERENCES....39

APPENDIX TABLE 1: DONOR DEFERRAL, PRODUCT DISPOSITION, RECIPIENT NOTIFICATION FOR WHOLE BLOOD, BLOOD COMPONENTS INTENDED FOR TRANSFUSION, SOURCE LEUKOCYTES, AND OTHER CELLULAR BLOOD COMPONENTS INTENDED FOR FURTHER MANUFACTURE....45

APPENDIX TABLE 2: DONOR DEFERRAL, PRODUCT DISPOSTION, AND RECIPIENT NOTIFICATION FOR SOURCE PLASMA (SP), RECOVER PLASMA (RP) AND PLASMA DERIVATIVES (PD)....47

Revised Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease and Variant Creutzfeldt-Jakob Disease by Blood and Blood Products

---

Guidance for Industry

This guidance represents the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

I. INTRODUCTION

This guidance is the latest in a series of guidances addressing the risk of Creutzfeldt-Jakob Disease (CJD) and Variant Creutzfeldt-Jakob Disease (vCJD)[1] transmission by blood and blood products.

- In 1999, we, FDA, issued a document entitled "Guidance for Industry: Revised Precautionary Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease (CJD) and New Variant Creutzfeldt-Jakob Disease (nvCJD) by Blood and Blood Products" dated November 1999 (1999 guidance).[2]
- In 2002, we issued a document entitled "Guidance for Industry: Revised Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease (CJD) and Variant Creutzfeldt-Jakob Disease (vCJD) by Blood and Blood Products" dated January 2002 (2002 guidance).[3]
- In 2006, we issued a draft document entitled "Draft Guidance for Industry: Amendment (Donor Deferral for Transfusion in France Since 1980) to 'Guidance for Industry: Revised Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease (CJD) and Variant Creutzfeldt-Jakob Disease (vCJD) by Blood and Blood Products'" dated August 2006 (2006 draft guidance).

---

[1] We have retained the same nomenclature used in previous guidance documents for the new variant of CJD (originally abbreviated "nvCJD," but later as "vCJD"). We refer to all other forms of CJD (sporadic, familial and iatrogenic) as "CJD."

[2] The 1999 guidance addressed the theoretical possibility that a new variant of CJD that had been plausibly attributed to human infection with the agent of bovine spongiform encephalopathy might be transmissible from human to human through blood and blood products.

[3] The 2002 guidance superseded the 1999 guidance and recommended new deferrals for certain donors at risk of exposure to BSE.

- In 2010, we issued a document entitled "Guidance for Industry: Revised Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease (CJD) and Variant Creutzfeldt-Jakob Disease (vCJD) by Blood and Blood Products" dated May 2010 (2010 guidance).[4]
- Finally, in 2012, we issued a draft guidance entitled "Draft Guidance for Industry: Amendment to 'Guidance for Industry: Revised Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease and Variant Creutzfeldt-Jakob Disease by Blood and Blood Products,'" dated June 2012 (2012 draft guidance), which recommended revised labeling of plasma-derived products to reflect current understanding of vCJD transmission through blood and blood products.

This guidance amends the 2010 guidance and finalizes the 2012 draft guidance. This guidance incorporates the recommendations from the 2012 draft guidance for revised labeling for plasma-derived products, including albumin and products containing plasma-derived albumin. This guidance also provides manufacturers of plasma-derived products with recommendations on how to report the labeling changes to FDA under 21 CFR 601.12. All other recommendations in the 2010 guidance are unchanged.[5]

In addition, this guidance amends the 2010 guidance by: a) including information relevant to the new labeling recommendations; b) providing updated information on the global vCJD and Bovine Spongiform Encephalopathy (BSE) epidemics in Section II; c) clarifying the reentry criteria for a donor with a family history of CJD in Section IV.C.; d) clarifying the requirements related to biological product deviation reporting in Section V. and in Tables 1 and 2 of the Appendix; and e) updating, adding, and removing certain footnotes and references.

Tests are being developed to detect CJD and vCJD infections in blood and plasma donors. However, until suitable donor screening tests become available, FDA continues to recommend interim preventive measures based on the available scientific data and the evolving state of knowledge regarding these diseases.

We expect that additional epidemiological information will become available as the epidemics of vCJD and BSE continue to evolve. We may update this guidance in the future, in light of developments in testing technology, epidemiological information, and the impact of these recommendations on the supply of blood and blood-derived products.

This guidance applies to Whole Blood and blood components intended for transfusion, and blood components intended for use in further manufacturing into injectable and non-injectable products,

---

[4] The 2010 guidance finalized the donor deferral recommendation from the 2006 draft guidance (for donors who have received a transfusion of blood or blood components in France since 1980); provided updated scientific information; and revised labeling recommendations for Whole Blood and blood components intended for transfusion.

[5] FDA discussed potential changes to the geographic exposure based deferrals for risk of vCJD with its Transmissible Spongiform Encephalopathies Advisory Committee (TSEAC) in June 2015. Available at http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/TransmissibleSpongiformEncephalopathiesAdvisoryCommittee/ucm444810.htm. FDA intends to address revised recommendations for geographic donor deferrals in future guidance documents.

including recovered plasma, Source Leukocytes and Source Plasma, and plasma derivatives. Within this document, "donors" refers to donors of Whole Blood and blood components and "you" refers to blood collecting establishments or manufacturers of plasma derivatives.

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

II. BACKGROUND

A. CJD and vCJD

CJD is a rare but invariably fatal degenerative disease of the central nervous system, one of a group of transmissible diseases called transmissible spongiform encephalopathies (TSEs) or prion diseases. TSEs are associated with a poorly understood transmissible agent (Refs. 1-6), now designated TSE agents or prions (Ref. 7). Cases of sporadic CJD—the most common human TSE—occur at low frequency by an unknown mechanism. CJD may be acquired by an identified exogenous (usually iatrogenic) exposure to infectious material; or it may be familial, associated with one of a number of mutations in the prion-protein-encoding (*PRNP*) gene. Clinical latency for iatrogenic CJD, following point exposures to contaminated materials, has sometimes exceeded 30 years (Ref. 8); incubation periods of kuru—another human TSE—appear to have sometimes exceeded 50 years (Ref. 9).

In 1996, a previously unrecognized variant of CJD, now designated vCJD, was reported in the United Kingdom (U.K.) (Ref. 10). vCJD is distinguished from CJD by differences in clinical presentation, cerebral imaging and neuropathologic changes, summarized in Table 1 (Refs. 10-14).

Table 1. vCJD compared with CJD[6]

Differences in clinical presentation	vCJD	CJD
Age of onset	Earlier	Later
Median age at death	28 years	68 years
Psychiatric and sensory symptoms	Frequent in early course of illness	Appear later in course of illness
EEG changes	No diagnostic EEG changes	Diagnostic EEG changes commonly seen
Median duration of illness (Ref. 15)	13 months	4 months
MRI abnormalities (Refs. 16-17)	Hyperintensity in pulvinar; little atrophy in cerebral cortical gray matter	Hyperintensity in putamen and caudate nucleus; atrophy of cerebral cortical gray matter
Neuropathologic features	Florid prion protein plaques, surrounded by spongiform changes	Florid prion plaques uncommon
Immunohistochemistry (Ref. 18)	Abnormal accumulations of prion protein detectable in lymphoid tissues	Abnormal accumulations of prion protein not detected in lymphoid tissues

The unique accumulation of abnormal prion protein seen in vCJD lymphoid tissues led to concerns that transmission of vCJD by blood might be a greater risk than for CJD (Ref. 19). Presumptive transmissions of vCJD by transfusions and possible transmission of vCJD by plasma-derived Factor VIII were subsequently reported in the U.K. (see Section II.C. below). Neuropathologic examination of brain tissue is required to confirm a diagnosis of vCJD.

A confirmed (or definite) case of vCJD is currently defined by the following neuropathologic findings:

1. Numerous widespread kuru-type amyloid plaques, surrounded by vacuoles, in both the cerebellum and cerebrum ("florid" plaques);
2. Spongiform change most evident in the basal ganglia and thalamus, with sparse distribution in the cerebral cortex; and

[6] See Centers for Disease Control and Prevention (CDC) fact sheet at http://www.cdc.gov/prions/vcjd/index.html for more information.

3. High-density accumulations of abnormal prion protein, particularly in the cerebrum and cerebellum as shown by immunohistochemistry and other techniques (Ref. 20).

However, a clinical diagnosis of "suspected" vCJD can be made based upon certain clinical features, if adequate neuropathological specimens are unavailable. Although recommended diagnostic evaluations and criteria for vCJD are evolving, the Centers for Disease Control and Prevention (CDC) classifies cases in the United States (U.S.) with all of the following features as suspected vCJD:

1. Current age (if alive) or age at death less than 55 years;
2. Persistent painful sensory symptoms and/or psychiatric symptoms at clinical presentation;
3. Dementia, and delayed development (≥four months after illness onset) of ataxia, plus at least one of the following three neurologic signs: myoclonus, chorea, or dystonia;
4. A normal or abnormal electroencephalogram (EEG) but not the diagnostic EEG changes often seen in classic CJD;
5. Duration of illness of at least six months;
6. Routine investigations do not suggest an alternative non-CJD diagnosis;
7. A history of possible exposure to BSE (e.g., residence or travel in a BSE-affected country from 1980 to the present);
8. No history of iatrogenic exposure to CJD, such as receipt of a dura mater allograft or injection of human cadaveric pituitary-derived hormones; and
9. Absence of a mutation in the *PRNP* gene, or, if this has not been determined, no history of CJD in a first-degree relative.

As of May 2015, 228 patients, including 177 in the U.K., 27 in France and 25 in ten other countries (including four in the U.S. and two in Canada), have been diagnosed with clinical vCJD (definite and probable cases).[7] The size of the vCJD epidemic has not yet been determined with certainty. (Refs. 21-24). Deaths from vCJD in the U.K. appeared to have peaked in 2000 and have subsequently decreased.[8] However, additional "waves" of cases in the U.K. and elsewhere have been predicted by some experts and the possibility of an increased incidence of cases in the future cannot be dismissed (Refs. 22-25).[9] Of the four cases of vCJD identified in the U.S., two were in former residents of the U.K., one in a former resident of Saudi Arabia and one in a former resident of Kuwait

---

[7] The European and Allied Countries Collaborative Study Group of CJD (EUROCJD) plus the Extended European Collaborative Study Group of CJD (NEUROCJD) at http://www.eurocjd.ed.ac.uk/surveillance%20data%201.html.
[8] NCJDSU at http://www.cjd.ed.ac.uk.
[9] See also, McKie, R. "Warning over second wave of CJD cases. Scientists say that threat of brain illness returning will persist for decades," Observer, Aug. 3, 2008 at 11; Collinge, J. et al. (2006) "Kuru in the 21st century—an acquired human prion disease with very long incubation periods." Lancet 376: 2068-74.

and the former Soviet Union.[10] Cases of vCJD have also been reported from the Republic of Ireland (4), Japan (1), Italy (2), the Netherlands (3), Portugal (2), Saudi Arabia (1), Taiwan (1) and Spain (5). Most of these cases occurred in persons who had never resided in the U.K. Laboratory and epidemiologic studies have linked vCJD to human infection with the agent of BSE, probably acquired from contaminated beef products (Refs. 25-26).

B. Evolution of the Global BSE Epidemic

The vCJD and BSE epidemics have continued to evolve. BSE cases have been reported in over 20 countries of Europe, including Austria, Belgium, the Czech Republic, Denmark, Finland, France, Germany, Greece, the Republic of Ireland, Italy, Liechtenstein, Luxembourg, the Netherlands, Poland, Portugal, Slovakia, Slovenia, Spain, Sweden, Switzerland, and the U.K. BSE has also been identified in Japan (36 cases) and Israel (1 case).[11]

1. BSE in Europe

In the U.K., BSE infections probably first occurred in cattle in about 1980, although the disease was not recognized there until 1985. Cases of BSE in the U.K. peaked in 1992. That year, over 37,000 confirmed cases were reported to the World Organization for Animal Health (OIE), with reports falling to low levels by 1996 as a result of control measures. U.K. authorities reported 114 confirmed cases to the OIE in 2006.[12] While the current prevalence of BSE is much lower a few cases continue to be reported yearly in Europe.[13]

2. BSE in Asia and the Middle East

Following the first recognized case of BSE in Japan in 2001, a total of 36 cattle with the disease have been reported to OIE.[14] Israel reported a single case of BSE in 2002 but no additional cases have been reported.[15]

3. BSE in North America

BSE was first confirmed in Canada in 1993 in a cow imported from the U.K. The first reported case of BSE in a native-born Canadian cow occurred ten years later. As of February 2015, 21 cases of BSE in Canada have been detected, 20 of which

[10] See CDC fact sheet at http://www.cdc.gov/prions/vcjd/vcjd-reported.html and http://www.cdc.gov/prions/vcjd/news.html; also see Maheshwari A, et.al. Recent US case of variant Creutzfeldt-Jakob disease—global implications. Emerging Infectious Diseases 2015;21:750-9.
[11] World Organization for Animal Health (OIE) at http://www.oie.int/en/anial-health-in-the-world/bse-specific-data/.
[12] OIE at http://www.oie.int/en/animal-health-in-the-world/bse-specific-data/.
[13] OIE at http://www.oie.int/en/animal-health-in-the-world/bse-specific-data/.
[14] OIE at http://www.oie.int/en/animal-health-in-the-world/bse-specific-data/.
[15] OIE at http://www.oie.int/en/animal-health-in-the-world/bse-specific-data/.

are in native-born Canadian cattle.[16] The first case of BSE in the U.S. was confirmed in 2003 in a Canadian-born cow. Three cases were later detected in U.S.-born cows.[17] The overall prevalence of BSE in U.S. cattle was estimated by the United States Department of Agriculture (USDA), based on the results of a temporarily enhanced active surveillance program, to be very low—less than one case per million cattle at the 95 percent confidence level, based on an adult cattle population of 42 million animals.[18]

C. TSE Agents and Blood

1. Potential Risk of Transmitting CJD by Transfusion

In 1978, blood of guinea pigs experimentally infected with the CJD agent was found to transmit infection to normal guinea pigs (Ref. 27). Subsequently, blood of mice with experimentally induced TSE was also found to contain the transmissible agent (Ref. 28). Transmission of BSE has been repeatedly achieved by blood transfusions from experimentally infected sheep to normal sheep (Refs. 29-30), and infection has also been transmitted by transfusions of blood from scrapie-infected sheep (Refs. 30-31). In blood of hamsters infected with scrapie—the most thoroughly studied model of TSE—infectivity, although detectable in all components, appeared to be mainly associated with both nucleated cells and plasma (Ref. 32).

Based on repeated demonstrations that the blood of animals infected with a variety of TSE agents sometimes contained infectivity (Ref. 33) and the recognition that iatrogenic CJD had been transmitted by human cadaveric pituitary growth hormones (Ref. 34), FDA recommended in 1987[19] that persons identified by history to be at increased risk for CJD because they had received human cadaveric pituitary growth hormone injections be deferred from donating blood. These recommendations were later broadened in August 1995 and slightly revised in December 1996[20] to include deferral of donors who had been treated with human dura mater allografts, also implicated in iatrogenic transmission of CJD (Ref. 35), and donors who had a family history of CJD, because of its association with a transmissible agent similar to those found in sporadic and

---

[16] OIE at http://www.oie.int/en/animal-health-in-the-world/bse-specific-data and the Canadian Food Inspection Agency at http://www.inspection.gc.ca/animals/terrestrial-animals/diseases/reportable/bse/fact-sheet/eng/1363892691907/1363893176627.

[17] OIE at http://www.oie.int/eng/info/en_esbmonde.htm and USDA at http://www.ars.usda.gov/research/publications/publications.htm?seq_no_115=197033.

[18] USDA at http://www.usda.gov/wps/portal/usda/usdahome?contentid=BSE_Ongoing_Surveillance_Information_Center.html&contentidonly=true.

[19] See FDA memo at http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/OtherRecommendationsforManufacturers/MemorandumtoBloodEstablishments/UCM063012.pdf.

[20] June 2, 1999 TSEAC meeting transcript: http://www.fda.gov/ohrms/dockets/ac/99/transcpt/3518t1.rtf.

iatrogenic CJD (Refs. 2 and 36). Subsequently, a number of published epidemiological studies failed to suggest that CJD (sporadic, familial, and iatrogenic forms) had been transmitted by blood and blood products. This evidence included five case-control studies of over 600 CJD cases, two lookback studies tracing recipients of components from blood of donors later found to have CJD, and two autopsy studies of patients with hemophilia (Refs. 37-43). None of these studies linked CJD to receipt of blood or blood products. Nonetheless, FDA continues to recommend (1) deferrals for donors at increased risk for CJD; and (2) market withdrawal and retrieval of labile blood components from donors when post-donation information reveals an increased risk of CJD.

In 1998, FDA recommended that—with the exceptions discussed below—plasma derivatives no longer be withdrawn when post-donation information reveals that a plasma donor had been diagnosed with CJD or was at increased risk for CJD.[21] That change in policy was based mainly on the following information: (1) the CDC reviewed 3,642 reported CJD deaths over a period of 16 years (later increased to 4,468 reports) and concluded that no reported CJD case had any other diagnosis of a condition associated with frequent receipt of blood or blood products (hemophilia, thalassemia, or sickle cell disease (Ref. 44)); and (2) experimental studies with animal models suggested that procedures used in manufacture consistently and substantially lowered the amounts of infectious material present in most plasma derivatives (Ref. 45).

Also in 1998, the U.S. Surgeon General[22], in collaboration with NIH, CDC and FDA, concluded that previous withdrawals of plasma derivatives from donors who were later determined to have CJD or have been at increased risk for CJD did not improve the safety of plasma derivatives. In addition, the U.S. Surgeon General concluded that the withdrawal of plasma derivatives from such donors contributed to serious shortages of immunoglobulin products. Further withdrawals of "CJD-implicated" plasma derivatives would be indicated only if a plasma donor was later found to have vCJD (or CJD with onset before age 55 where vCJD could not be excluded on a case-by-case basis). Since then, accumulating evidence has repeatedly confirmed that several manufacturing processes commonly used to manufacture plasma derivatives are effective in removing from plasma both abnormal forms of the prion protein and infectivity spiked into blood (Refs. 46-52).[23] However, as detailed below in Section II.C.2, there has been one case of transmission of vCJD in the U.K. that may be due to

[21] December 18, 1998 TSEAC meeting transcript: http://www.fda.gov/ohrms/dockets/ac/98/transcpt/3484t1.rtf.
[22] FDA website at http://www.fda.gov/NewsEvents/Testimony/ucm115104.htm.
[23] February 20, 2003 TSEAC meeting transcript: http://www.fda.gov/ohrms/dockets/ac/03/transcripts/3923t1.htm.

treatment of a patient with a plasma derivative product.[24] Recipients of plasma derivatives are the subject of a continuing lookback study in the U.K. as part of the Transfusion Medicine Epidemiology Review.[25]

2. Evidence that vCJD Has Been Transmitted by Blood Products

Soon after the first description in the U.K. of vCJD affecting 10 young patients in 1996 (Ref. 10), vCJD was recognized to be an emerging infectious disease with several unique clinical and pathological characteristics differing from those of previously known forms of CJD. It was uncertain whether human blood might transmit the vCJD agent. FDA therefore recommended in the 1999 guidance a donor deferral policy more stringent for donors at increased risk of vCJD than for those at increased risk of the "classical" forms of the disease (see Section IV below), including a recommendation to withdraw plasma derivatives should a plasma donor later be diagnosed with vCJD (a situation never recognized in the U.S. to date) and a case-by-case review when a plasma donor is suspected of having vCJD (including all donors with onset of CJD before the age of 55 years) instead of a more common form of CJD.

In December 2003, U.K. authorities reported a case of vCJD in a recipient of non-leukoreduced red blood cell concentrate obtained from a clinically healthy donor who later developed typical vCJD (Ref. 53). In July 2004, a second recipient of non-leukoreduced red blood cell concentrate from another such donor in the U.K. was reported to have died of other causes without clinical or neuropathological evidence of vCJD, but at autopsy the recipient had abnormal accumulations of prion protein in lymphoid tissues (Ref. 54). This finding is typical of vCJD, although the recipient had a *PRNP* genotype (heterozygous for the sequences encoding methionine and valine at *PRNP* codon 129 [129 MV]) not previously found in cases of vCJD (all of which have been 129 MM homozygous). Two additional recipients of non-leukoreduced red blood cell concentrates from a donor incubating vCJD were subsequently reported by U.K. authorities in February 2006 (Refs. 55-56) and January 2007[26] to have died with confirmed vCJD. These four cases provided convincing epidemiological evidence that vCJD infections have been transmitted by non-leukoreduced red blood cell concentrates. Although no other blood components have been associated with transfusion-transmitted vCJD, experience is still too limited to allow a conclusion that other blood components cannot transmit the infection.

[24] U.K. Health Protection Agency (HPA), "vCJD abnormal prion protein found in a patient with haemophilia at post mortem," dated February 17, 2009, and "Variant CJD and plasma products," dated July 27, 2009 at http://www.hpa.org.uk.
[25] Transfusion Medicine Epidemiology Review: http://www.cjd.ed.ac.uk/TMER/TMER.htm.
[26] Transfusion Medicine Epidemiology Review: http://www.cjd.ed.ac.uk/TMER/TMER.htm.

In February 2009, the United Kingdom Health Protection Agency announced evidence of vCJD infection in a patient with type-A hemophilia at postmortem.[27] The patient had been treated with human plasma-derived Factor VIII clotting factor manufactured using plasma from U.K. donors, including one batch that was manufactured using plasma from a donor who later developed typical vCJD. This is the first report that vCJD abnormal protein has been found in a patient with hemophilia or any patient treated with plasma products. The patient, who was over 70 years old, died of other causes and may have been exposed to other risk factors for vCJD. A risk assessment performed by U.K. health authorities concluded that, assuming that the abnormal prion protein finding was a marker for asymptomatic vCJD infection, the most likely source of such an infection was plasma-derived Factor VIII, rather than dietary exposure, endoscopy procedures, or red blood cell transfusions.

At this time, plasma derivatives have not been implicated in vCJD transmission in any country other than the U.K. To date, no U.S.-licensed plasma-derived products have been manufactured from a donor known to have developed vCJD and no cases of vCJD have been reported from use of a U.S.-licensed plasma derivative. In addition, published studies and information submitted to FDA show that certain plasma derivative manufacturing steps can remove TSE infectivity, although such experiments have inherent limitations (Refs. 51, 57). Based on animal studies as well as on FDA risk assessments, the possibility of vCJD transmission by a U.S.-licensed plasma derivative is extremely small.

D. FDA Regulatory History

On December 11, 1996, we issued a memorandum to all registered blood and plasma establishments and all establishments engaged in manufacturing plasma derivatives entitled "Revised Precautionary Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease (CJD) by Blood and Blood Products." We recommended as a preventive measure that manufacturers should quarantine and destroy in-date Source Plasma and plasma derivatives and in-date transfusion products prepared from donors who were at increased risk for developing CJD or who were subsequently diagnosed with CJD. We also recommended permanent deferral of donors with CJD or CJD risks, unless, in cases of a family member with CJD, the donor underwent genetic testing that demonstrated absence of a familial-CJD-associated abnormality (mutation) of the prion protein gene—generally requiring complete nucleotide sequencing of both *PRNP* genes. We made no specific recommendations regarding vCJD in that document. Changes to

[27] U.K. Health Protection Agency (HPA), "vCJD abnormal prion protein found in a patient with haemophilia at postmortem," dated February 17, 2009, and "Variant CJD and plasma products," dated July 27, 2009, at http://www.hpa.org.uk.

those recommendations were announced on September 8, 1998, and were incorporated into an August 1999 guidance that was revised and updated in November 1999. Those changes were as follows:

- that you no longer withdraw plasma derivatives containing plasma from donors with CJD or CJD risk factors;
- that you withdraw all material collected from donors diagnosed with vCJD or suspected vCJD; and
- that you defer donors based on their potential exposure to BSE in the U.K., or injection of insulin made from bovine sources in the U.K.

Because the potential for transmission was unknown, in August 1999, we recommended that, as a preventive measure, you withdraw blood components and derivatives collected from donors diagnosed with vCJD. As a further preventive measure, we also recommended that you defer donors who have resided in the U.K. for a total of six months or more, between the beginning of 1980 and the end of 1996. We estimated that this policy would result in deferral of donors accounting for approximately 87% of total days of potential dietary exposure to the BSE agent in the U.K. ("donor exposure days").

The period from 1980 through 1996 reflects the peak years of the U.K. BSE epidemic. In 1998, FDA, advised by the Transmissible Spongiform Encephalopathies Advisory Committee (TSEAC), concluded that measures implemented in the U.K. since 1996 have been adequate to keep the BSE agent out of the human food chain there.[28] As other countries institute similar food chain protections against BSE and the prevalence of BSE in their national cattle herds declines, we expect to reconsider this and other geographic donor deferral policies for other countries.

At its meeting, on June 1, 2000, the TSEAC discussed the possible deferral of donors from other countries known or suspected to be affected by BSE.[29] The TSEAC voted not to recommend new donor deferrals for potential exposures in European countries outside the U.K. at that time. This decision was based on conclusions that: (1) the extent of the BSE epidemic in Europe was undetermined; and (2) U.S. donor deferrals for U.K. residence had only recently been fully implemented so that the potential for adverse impact on the availability of blood and blood products had not yet been fully appreciated. The TSEAC also recommended against changing the U.K. donor deferral period to one shorter than six months.

At its meeting on January 18, 2001,[30] the TSEAC reviewed more recent epidemiological information on exposure to BSE in European countries, and again discussed possible changes to donor deferrals for vCJD risk. The TSEAC again voted that epidemiological and other currently available scientific information did not support changing the current deferral for donors who had resided or traveled in the U.K. The TSEAC did recommend

---

[28] December 18, 1998 TSEAC meeting transcript: http://www.fda.gov/ohrms/dockets/ac/98/transcpt/3484t1.rtf.
[29] June 1, 2000 TSEAC meeting transcript: http://www.fda.gov/ohrms/dockets/ac/00/transcripts/3617t1.rtf.
[30] January 18, 2001 TSEAC meeting transcript: http://www.fda.gov/ohrms/dockets/ac/01/transcripts/3681t1.rtf.

that deferrals be considered for donors potentially exposed to beef products exported from the U.K. to U.S. military bases in Europe, and for donors potentially exposed to BSE since 1980 in France, Portugal, and the Republic of Ireland. In response to advice from the TSEAC that FDA should consider recommending deferral of donors for residence or travel in Portugal and the Republic of Ireland (i.e., countries where BSE exposure was not related to human consumption of British beef *per se*), we decided to re-examine the issue publicly with the TSEAC on June 28-29, 2001.[31] At this meeting, the TSEAC considered the estimated potential human exposures to the BSE agent in the U.K. and other parts of Europe, as well as estimates of risk reduction and donor loss expected to result from tightened geographic donor deferrals. Specifically, the TSEAC considered three deferral options (including the option proposed by the TSEAC at its January 2001 meeting) and voted (10 for and 7 against) to endorse instead a revised set of recommendations proposed by FDA.

The main features of the recommendation were: (1) deferral of donors for any cumulative travel or residence for a period of five years or more in any European country except the U.K. from 1980 through the present; (2) deferral of donors who spent three months or more in the U.K. from 1980 through the end of 1996; (3) deferral of donors who spent more than six months in Europe on a base of the U.S. Department of Defense (DoD) from 1980 through the end of 1996 (or 1980 through 1990 if all exposure after 1990 was on bases in Northern Europe); and (4) deferral of any recipient of a blood transfusion in the U.K. from 1980 to the present. Deferrals were to be recommended for implementation in two stages within six months of publication by FDA of a final guidance. FDA estimated that the new policy might lead to a loss of 4.6% to 5.3% of blood donors with a 72% reduction in existing vCJD risk, for a total reduction of 90% relative to the risk that had existed prior to implementation of the 1999 recommendations. The TSEAC also evaluated information suggesting that measures taken in the U.K. to prevent human exposure to food-borne BSE agents were adequate to reduce the risk there markedly after the end of 1996. The proposed deferral policy was endorsed by a majority of TSEAC members and used by FDA as the basis for the 2002 guidance.

At its meeting, held jointly with the Blood Products Advisory Committee on January 17, 2002, the TSEAC reviewed the FDA guidance of January 2002 and agreed again - by unanimous vote - that the combination of measures implemented in the U.K. by the end of 1996 to protect the human food chain from BSE contamination were sufficient to obviate the need for donor deferrals based on subsequent travel or residence in the U.K.[32] However, TSEAC members stressed that U.K. authorities must assure vigorous, sustained, and consistent application of aggressive food-protective measures with active BSE surveillance and monitoring of BSE-safety-related efforts.

In December 2003, as noted in Section II.C.2 above, the first case of presumptive transfusion-transmitted vCJD was reported from the U.K. and the first U.S. case of BSE

[31] June 28-29, 2001 TSEAC meeting transcript: http://www.fda.gov/ohrms/dockets/ac/01/transcripts/3762t1.rtf and http://www.fda.gov/ohrms/dockets/ac/01/transcripts/3762t2.rtf.

[32] January 17, 2002 TSEAC meeting transcript: http://www.fda.gov/ohrms/dockets/ac/02/transcripts/3834t2.rtf.

was diagnosed postmortem in a Canadian-born cow slaughtered in Washington State (seven months after the first native-born cow was diagnosed with BSE in the Canadian Province of Alberta). At its meeting on February 12-13, 2004, the TSEAC discussed those two events and their possible implications for U.S. blood safety.[33] The TSEAC expressed confidence that the deferral policies already in place were likely to be effective and were concerned that additional restrictions on blood donor eligibility, while probably adding little to safety, might seriously reduce supply. The TSEAC discussed the possible benefit of leukoreduction, which had been introduced in several BSE countries in the hope of reducing the risk of transfusion-transmitted vCJD (Ref. 58).[34] Experimental studies using blood of rodents infected with scrapie agent as a model for human TSE (Ref. 59) subsequently confirmed previous findings, suggesting that a substantial portion of blood-borne infectivity was in plasma and not removed by leukoreduction filtration (Ref. 32). The TSEAC concluded that, whatever its other benefits, leukoreduction remains of unproven value in reducing the risk of transfusion-transmitted vCJD and should not be relied upon to replace a donor deferral policy. At its meeting, on October 14, 2004, the TSEAC discussed: (1) whether the policies recommended by FDA in the guidance of January 2002 were still justified; and (2) whether additional preventive measures were indicated to enhance blood safety.[35] The TSEAC voted unanimously that the measures FDA had recommended in the 2002 guidance were still justified. The TSEAC voted (13 for and 1 against) that FDA should continue to recommend those deferral policies without enhancements and also should follow the situation closely and consider adding risk-reducing measures if indicated. One TSEAC member expressed the opinion that FDA should seriously consider recommending deferral of donors transfused in some BSE countries besides the U.K.

At its meeting, on February 8, 2005, the TSEAC discussed available information and recommendations for deferral of U.S. donors transfused in France and in other European countries since 1980.[36] The TSEAC voted (12 in favor, 3 against, with one abstention) to recommend deferral of blood donors with a history of transfusion in France since 1980. However, the TSEAC voted unanimously against advising deferral of both blood donors and Source Plasma donors transfused in other European countries besides France and the U.K., reasoning that many more cases of vCJD had occurred in France than in any other country except the U.K. In a closely divided vote, the TSEAC advised FDA not to recommend deferral of Source Plasma donors with a history of transfusion in France (five members favored deferral of Source Plasma donors while seven members opposed it and one abstained), based on information presented at the October 14, 2004 TSEAC meeting showing that the processes used to manufacture plasma derivatives had the capacity to

[33] February 12-13, 2004 TSEAC meeting transcript:
http://www.fda.gov/ohrms/dockets/ac/04/transcripts/4019t1.htm and
http://www.fda.gov/ohrms/dockets/ac/04/transcripts/4019t2.htm.
[34] http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1621089/pdf/pmed.0030342.htm.
[35] October 14, 2004 TSEAC meeting transcript: http://www.fda.gov/ohrms/dockets/ac/04/transcripts/2004-4075T1.htm.
[36] February 8, 2005 TSEAC meeting transcript: http://www.fda.gov/ohrms/dockets/ac/05/transcripts/2005-4088t1_01.pdf.

remove substantial amounts of TSE infectivity (Refs. 47-49 and 51-52).[37] Subsequent presentations on the capacity of processes used to manufacture plasma derivatives to remove TSE infectivity were made to the TSEAC on September 18, 2006,[38] and December 15, 2006.[39]

In the 2006 draft guidance, FDA summarized interim events, including advice from the TSEAC, and proposed to amend the 2002 guidance to include a recommendation that blood establishments indefinitely defer blood donors who have received transfusions of blood or blood components in France since 1980. In the 2006 draft guidance, FDA, while again relying on laboratory studies showing that steps used in certain processes used to manufacture fractionated plasma products reduce TSE infectivity, cautioned that "... not all products have been thoroughly studied [and] ... it remains uncertain whether the models accurately reflect the form of infectivity in blood." Therefore, we also recommended in the 2006 draft guidance that Source Plasma donors who have received a transfusion of blood or blood components in France since 1980 be indefinitely deferred, and stated that we will continue to monitor the BSE epidemic and re-evaluate the necessity of deferring donors transfused in other European countries.

After the 2006 draft guidance was issued for comment, FDA received additional information concerning the risk of transmitting vCJD by plasma derivatives (uncertain but small in most although not all scenarios analyzed by probabilistic computer models[40]) and remains concerned about the increasing number of vCJD cases reported from France. The 2010 guidance recognized new information and incorporated advice we received from the TSEAC since the 2002 guidance was issued, and included revisions made in response to comments received on the 2006 draft guidance.

In October 2010, we sought the advice of the TSEAC on our proposed labeling recommendations to reflect potential risk of vCJD in plasma-derived products. We proposed recommendations for labeling for plasma-derivatives that included mention of vCJD for the first time, and the potential risk for its transmission.

Similarly, we proposed revisions to the labeling for plasma-derived albumin and products containing plasma-derived albumin. In addition to its indications for direct infusion into patients, albumin may be used in the manufacture of other biological products. For example, it is used in the culture media of certain licensed vaccines or as a stabilizer in certain recombinant clotting factor products. Licensed albumin and albumin contained in other licensed products have never been known to transmit viruses, CJD or vCJD, and laboratory experimental evidence suggests albumin is less likely to contain CJD-like agents when compared with other fractionated products (Refs. 45, 60-61). There is no

[37] Presentation slides at: http://www.fda.gov/ohrms/dockets/ac/04/slides/2004-4075S1_05_files/frame.htm.
[38] Presentation slides at: http://www.fda.gov/ohrms/dockets/ac/06/slides/2006-4240S1-index.htm.
[39] Presentation slides at: http://www.fda.gov/ohrms/dockets/ac/06/slides/2006-4271S1_00-index.htm.
[40] September 18, 2006 TSEAC meeting transcript: http://www.fda.gov/ohrms/dockets/ac/06/slides/2006-4240S1-index.htm and December 15, 2006 TSEAC meeting transcript: http://www.fda.gov/ohrms/dockets/ac/06/slides/2006-4271S1_00-index.htm.

epidemiological evidence for transmission of CJD or vCJD in the U.S., U.K., or elsewhere by products containing plasma-derived albumin. Therefore, our recommendations for revised warning statements for vCJD risk for plasma-derived albumin and products containing plasma-derived albumin contained additional language to reflect the extremely low likelihood of vCJD and CJD transmission through these products.

TSEAC agreed unanimously that labeling for the potential risk of vCJD is warranted for plasma derivatives, including albumin and products containing albumin.[41] The revised recommendations for labeling plasma-derived products, including albumin and products containing plasma-derived albumin in this guidance are based upon current knowledge and the advice from TSEAC.

We are not recommending changes to the elements of the warning label for CJD. The transmission of CJD is currently described as a theoretical risk, given that there is no evidence that CJD is transmitted by blood (Refs. 56, 62-64).

E. Rationale for Geographic Donor Deferrals

This guidance document contains recommendations for donor deferral, product retrieval, and quarantine and disposition based upon consideration of risk in the donor and product, and the effect that withdrawals and deferrals might have on the supply of life- and health-sustaining blood, blood components, and plasma derivatives. In particular, we distinguish donors with vCJD from those with CJD or with CJD risk factors because of differences in the demonstrated risk of transfusion transmission. While no case of classical CJD has been attributed to transfusion, vCJD has several times been transmitted by blood transfusion (Ref. 65).[42]

These recommendations reflect a continuing effort to minimize the possible risk of transmitting vCJD by blood and blood products while maintaining their availability. We have previously estimated that vCJD-related donor deferrals might result in a 90% reduction in total person-days of risk-weighted (relative to U.K. risk 1980-1996) donor exposure to the agent of vCJD. We calculated risk as the sum of relative risk-weighted person-days exposure in the U.K. (weight =1.0), France (weight = 0.05), other European countries (weight = 0.015), and members of the U.S. military and their dependents (weight = 0.35).[43] We later estimated that deferring donors transfused in France after 1980 might result in the loss of fewer than 2 in 10,000 otherwise suitable blood donors.[44] Donor loss, under the policy recommendations in the 2002 guidance, was projected to be

[41] October 28, 2010 TSEAC meeting transcript: http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/TransmissibleSpongiformEncephalopathiesAdvisoryCommittee/ucm244061.htm.
[42] October 14, 2004 TSEAC meeting transcript: http://www.fda.gov/ohrms/dockets/ac/04/transcripts/2004-4075t1_01.pdf.
[43] January 18, 2001 TSEAC meeting transcript: http://www.fda.gov/ohrms/dockets/ac/01/slides/3681s1.htm.
[44] October 14, 2004 TSEAC meeting transcript: http://www.fda.gov/ohrms/dockets/ac/04/transcripts/2004-4075T1.htm.

approximately 5%, based upon analysis of data from a 1999 multi-center blood donor travel survey,[45] which was conducted using methodology described for Retrovirus Epidemiology Donor Studies (Ref. 66). We recognized that these deferrals might adversely affect the available supply of blood and plasma derivatives and warned that supplies needed to be monitored closely. The impact was expected to vary locally and regionally depending upon the dynamics of supply and demand and other characteristics such as demographics of the donor populations. More specifically, we were concerned that donors with a history of travel to the U.K. and other parts of Europe might be as much as 50% higher in urban coastal cities than in central and rural areas of the U.S.[46] As noted above BSE has been found in 36 Japanese cattle, one cow in Israel, 19 cattle in Canada and three in the U.S.[47] Residence in those countries, and residence in the U.K. after the end of 1996, has not been considered by FDA as cause to recommend donor deferral. The news media reported that other countries also received U.K. meat-and-bone meal,[48] implying that those countries might also have introduced the BSE infection into their cattle herds but have no recognized cases. We considered additional deferrals based upon possible donor exposure to BSE in Asian and other countries after the recommended deferrals were fully implemented in the fall of 2002, their impact assessed, and additional information about the potential level of BSE exposure and food chain controls in various countries sought. Following the recognition of BSE in North American cattle in 2003, the entire worldwide situation was considered by FDA and implications discussed publicly at meetings of TSEAC. We reasoned that additional deferrals would probably yield only a negligible benefit in reducing risk while compromising, to some uncertain but potentially significant degree, the continued supply of Whole Blood and blood components. The question whether additional geographically based donor deferrals should be considered for exposure in the Kingdom of Saudi Arabia was discussed with TSEAC in April 2011. Geographic deferrals were more broadly discussed with TSEAC in June 2015 in consideration of the results of a new FDA-developed quantitative assessment model for vCJD global geographic risk and the estimated risk reduction achieved by voluntary implementation of leukocyte reduction for red blood cells. We will reconsider our recommendations as appropriate based on the impact of expanded or reduced donor deferrals on the safety and availability of blood products.

[45] June 28, 2001 TSEAC meeting transcript: http://www.fda.gov/ohrms/dockets/ac/01/transcripts/3762t1.rtf.
[46] January 18-19, 2001 TSEAC meeting transcript: http://www.fda.gov/ohrms/dockets/ac/01/transcripts/3681t1.rtf and http://www.fda.gov/ohrms/dockets/ac/01/transcripts/3681t2.rtf.
[47] OIE at http://www.oie.int/eng/info/en_esb.htm.
[48] "Japan's Beef Scandal." Nature 413 (6854): 333.

III. EXPLANATION OF CURRENT vCJD RECOMMENDATIONS

A. Exposure to British Beef in the U.K.

The vCJD epidemic in the U.K., while markedly reduced since deaths peaked in 2000[49] (Ref. 21), continues. Furthermore, it has not been excluded that additional "waves" of cases may occur and that some uncertain but potentially substantial number of persons in the U.K. may have pre-clinical or sub-clinical infections (Refs. 67-70).[50]

To increase protection of the U.S. blood supply, we continue to recommend that you defer blood and plasma donors who have traveled or resided in the U.K. for a cumulative period of three or more months from the beginning of 1980 through the end of 1996.

B. Exposure to British Beef Products Distributed Outside of the U.K.

In January 2001, the TSEAC recognized two types of risk outside the U.K.: (1) exposure to BSE from infected cows in the country of residence ("indigenous" BSE exposure); and (2) exposure to BSE from bovine products exported from the U.K. during the BSE epidemic prior to full implementation of food control measures in 1996 ("imported" BSE exposure).

Available data suggest that France imported a substantial amount of beef from the U.K. during the peak years of the BSE epidemic;[51] at least 5% of beef consumed in France is estimated to have come from the U.K. during the late 1980s. The number of French vCJD cases (23) is currently about 13% of those in the U.K.[52] It has been speculated that many French vCJD cases might have been infected by consumption of British beef in France, since only one of the 23 individuals had lived in the U.K. for six or more months, and the indigenous French BSE epidemic has been much smaller and more recent than that in the U.K. Substantial amounts of British beef also were exported to the Netherlands, but it appears that much of this meat was apparently then exported from the Netherlands to a variety of other countries.[53]

On January 18, 2001, the TSEAC voted to defer potential donors who resided in France for 10 years or more, from 1980 until the present.[54] The suggested 10-year (120-month) deferral period for France reflected an estimated 5% risk of exposure to BSE, compared

---

[49] CJD Statistics from the British Department of Health at www.doh.gov.uk.

[50] See also, McKie, R. "Warning over second wave of CJD cases. Scientists say that threat of brain illness returning will persist for decades," Observer, Aug. 3, 2008 at 11; Collinge, J. et al. (2006) "Kuru in the 21st century—an acquired human prion disease with very long incubation periods." Lancet 376: 2068-74.

[51] June 1-2, 2000 TSEAC meeting transcript: http://www.fda.gov/ohrms/dockets/ac/00/transcripts/3617t1.rtf and http://www.fda.gov/ohrms/dockets/ac/00/transcripts/3617t2.rtf.

[52] Chart at: www.invs.sante.fr/publications/mcj/donnees_mcj.html.

[53] June 1-2, 2000 TSEAC meeting transcript: http://www.fda.gov/ohrms/dockets/ac/00/transcripts/3617t1.rtf and http://www.fda.gov/ohrms/dockets/ac/00/transcripts/3617t2.rtf.

[54] January 18-19, 2001 TSEAC meeting transcript: http://www.fda.gov/ohrms/dockets/ac/01/transcripts/3681t1.rtf and http://www.fda.gov/ohrms/dockets/ac/01/transcripts/3681t2.rtf.

to exposure of donors who resided in the U.K. for at least six months. However, in our 2002 guidance, FDA recommended a more stringent deferral for exposure of five or more years in Europe (see Section III.C. below) consistent with a revised recommendation of deferral for three months exposure in the U.K. Although more recent data suggest that the relative risk of BSE exposure in France compared with the U.K. may have exceeded 5%, we continue to recommend deferral of blood and plasma donors with a history of five or more years of cumulative residence or travel in France since 1980.

Some U.S. military personnel, civilian military personnel, and their dependents in Europe were also potentially exposed to British beef procured for consumption or sale on U.S. military bases between 1980 and 1996. British beef was distributed to U.S. military bases in Northern Europe (Germany, U.K., Belgium, and the Netherlands) between 1980 and 1990, and to U.S. military bases elsewhere in Europe (Greece, Turkey, Spain, Portugal, and Italy), between 1980 and 1996. While exposure varied widely, it is estimated that in some areas, up to 35% of beef consumed on U.S. military bases in Europe came from the U.K.[55] In January 2001, the TSEAC recommended deferring such donors but advised that more information was needed to assess the impact of deferral for various time periods in Europe on the supply of blood products.

Due to a history of potential consumption of U.K. beef by persons on U.S. military bases in Europe, we continue to recommend that current and former U.S. military personnel, civilian military personnel, and their dependents stationed at European bases for six months or more during the timeframes outlined in the preceding paragraph be deferred indefinitely. Based upon information provided by the DoD, we estimated that approximately 1.8% of U.S. blood donors might be deferred by this recommendation. Since as of 1996, DoD no longer procures U.K. beef for any U.S. military bases, such deferred donors now constitute a smaller percentage of otherwise suitable donors.

C. Indigenous BSE Exposure Outside the U.K.

BSE in Europe is likely to have originated from infected cattle and cattle feed that were exported from the U.K. to other parts of Europe. The risk of human exposure to the BSE agent in any country is based upon several factors, including the prevalence of BSE and the implementation of control measures to prevent the BSE agent from entering the human food chain. Control measures have included some of the following:

- prohibition of air injection stunning methods for cattle;
- active surveillance through testing of slaughtered cattle more than 30 months old for BSE;
- prohibitions on the use of carcasses from disabled cattle (so-called "downer" cattle not inspected and passed for human consumption);

[55] January 18-19, 2001 TSEAC meeting transcript: http://www.fda.gov/ohrms/dockets/ac/01/transcripts/3681t1.rtf and http://www.fda.gov/ohrms/dockets/ac/01/transcripts/3681t2.rtf.

- holding of all carcasses from cattle tested for cause until non-positive results have been received;
- exclusion of high-risk material (e.g., brain, other neural tissues, lymphoid tissues, and many parts of the intestines) from human food;
- a ban on human consumption of slaughtered cattle more than 30 months old;
- prohibition of mechanically recovered meat;
- a ban on mammalian-derived feed for ruminants;
- use of certain rendering processes; and
- additional herd control and surveillance.[56]

BSE has been detected in many European countries.[57] Food chain control measures (and their enforcement) have varied in Europe and cannot be assured for all time periods in question. Because of these uncertainties and the evolving BSE epidemic, donor deferrals on a country-by-country basis have not been practical. Therefore, FDA developed a uniform recommendation for donor deferral based on exposure in Europe outside of the U.K. The highest prevalence of BSE that has been observed in a European country with a strong surveillance program (Switzerland) is approximately 1.5% of the BSE prevalence that was observed for the U.K. between 1980 and 1996. Also, as noted in Section III.B above, residents in France may have consumed at least 5% of their total beef as imported British beef during the epidemic period, while other Europeans almost certainly consumed less. Therefore, the estimated maximum risk of BSE exposure in Europe was taken to be approximately 1.5-5% of that in the U.K. Assuming a "worst-case" relative risk of 5% per day of exposure, a European donor deferral of five years (60 months) was equivalent to a three-month deferral for cumulative travel or residence in the U.K. This remains the basis for our current recommendation to defer donors of Whole Blood and blood components intended for transfusion and Source Leukocytes who have a history of five or more years of residence or travel in Europe outside of the U.K.

As discussed in Section II.C.2., there has been one case of transmission of vCJD in the U.K. that may be due to the use of human plasma. In 2006, the TSEAC discussed risk assessments for potential exposure to vCJD risk from certain plasma-derived products.[58] The risk of transmitting vCJD by plasma derivatives was estimated based upon the probable infectivity of plasma from pre-symptomatic or asymptomatic donors with vCJD infections, the prevalence of vCJD in the donor population (mainly dependent on the

---

[56] European Commission Scientific Steering Committee opinions on the Geographical Risk of BSE: http://ec.europa.eu/food/fs/bse/scientific_advice01_en.html.

[57] January 18-19, 2001 TSEAC meeting transcript: http://www.fda.gov/ohrms/dockets/ac/01/transcripts/3681t1.rtf and http://www.fda.gov/ohrms/dockets/ac/01/transcripts/3681t2.rtf, and June 1-2, 2000 TSEAC Meeting Transcript: http://www.fda.gov/ohrms/dockets/ac/00/transcripts/3617t1.rtf and http://www.fda.gov/ohrms/dockets/ac/00/transcripts/3617t2.rtf.

[58] Risk assessments for plasma-derived factors VIII and XI presented to the TSEAC on December 15, 2006: http://www.fda.gov/ohrms/dockets/ac/cber06.html#TransmissibleSpongiform and draft risk assessments presented to the TSEAC on October 15, 2006: http://www.fda.gov/ohrms/dockets/ac/06/briefing/2006-4271b1-index.htm.

number infected in the U.K., not all of whom are deferred by recommended policies), the size of the plasma pool used for fractionation, and the removal of vCJD infectivity during the manufacturing process. In experimental studies, model TSE agents were removed from plasma products by a number of manufacturing steps, including precipitation, depth filtration, and column chromatography (Refs. 48-49, 57-60). Other unpublished data provided to FDA also suggested that the vCJD agent was similarly removed from most plasma derivatives by the same manufacturing steps.

The relative risks and benefits of excluding plasma donors who have lived or traveled in Europe for five years or more have not been established. In particular, the effect of such a donor deferral upon the supply of life and health-sustaining plasma derivatives has not been determined, but could be significant.[59] However, the implementation in October 2002, of the previous enhanced vCJD deferral policies for donors of Source Plasma was not followed by reported shortages of plasma-derived products in the U.S. Furthermore, in contrast to blood, plasma derivatives are highly processed materials. Considering the estimated low prevalence of vCJD infections in most countries of Europe compared to the U.K. and France, the likelihood that plasma fractionation processes reduce TSE infectivity, and the uncertain effect of additional deferrals upon the supply of plasma derivatives, we have not recommended that you defer Source Plasma donors who lived or traveled in other countries of Europe, although we are recommending that donors who lived in France for five or more years from 1980 to the present should be deferred from donating Source Plasma. Moreover, we are recommending, in consideration of the relatively greater risk of vCJD in persons with exposure to beef products from the U.K. that you should not collect Source Plasma from donors with a history of travel or residence in the U.K., U.S. military bases in Europe, and in France, as described in Sections III.A. and B. of this document.

Blood donors who are deferred for history of European travel or residence (except as stated for the U.K., France, and U.S. military bases in Europe) remain eligible to donate Source Plasma in a Center for Biologics Evaluation and Research (CBER) approved program. We will continue to evaluate this recommendation in light of evolving experimental and epidemiological information.

Given these considerations, we recommend that you defer donors of Whole Blood and blood components intended for transfusion, Source Leukocytes, and recovered plasma, but not donors of Source Plasma, who have resided in the countries of Europe listed in the Appendix to this document for a cumulative period of five years or more, between the beginning of 1980 and the present. We recommend that donors of Source Plasma who resided in the U.K., France, and U.S. military bases in Europe, be deferred as noted in the previous sections of this guidance.[60]

---

[59] June 28, 2001 TSEAC meeting transcript: http://www.fda.gov/ohrms/dockets/ac/01/transcripts/3762t1.rtf.
[60] We continue to refer to donor deferrals both for risk of exposure to BSE due to residence in BSE countries, consumption of British beef products, injection of U.K. bovine insulin, and history of transfusion in the U.K. or in France after 1980 as "geographic risk deferrals."

D. Potential Infection with vCJD Agent Acquired by Transfusion

As discussed in Section II.C., there have been four reports of presumptive transmissions of vCJD to humans by blood transfusions, three resulting in clinical cases of vCJD and one in an infection with typical abnormal accumulations of prion protein in lymphoid tissues. FDA has little doubt that vCJD has been efficiently transmitted by non-leukoreduced Red Blood Cells from clinically healthy donors who later became ill with vCJD. Other components, while not implicated in transfusion transmissions of vCJD to date, cannot be considered safe. In addition, there has been one reported case of vCJD transmission in the U.K. that may be due to use of plasma-derived Factor VIII. Therefore, as a preventive measure, donors who have received transfusions of blood or blood components in the U.K. and in France since 1980 should be indefinitely deferred.

E. Exposure to Bovine Insulin

No cases of transmission of vCJD have been reported in recipients of bovine insulin or other injectable products manufactured in BSE-affected countries. However, as a safeguard, most material from cattle in BSE countries should not be used in the manufacture of FDA-regulated products.[61] We are aware that some diabetic patients have imported bovine insulin for personal use.[62] Additionally, some insulin products legally distributed in the U.S. since 1980 were manufactured from cattle in the U.K. Therefore, as a preventive measure, you should indefinitely defer blood donors who have injected bovine insulin since the beginning of 1980, unless you can confirm that the product was not manufactured after 1980 from cattle in the U.K. We are not aware that bovine insulin has been imported into the U.S. from France or any other European BSE country.

F. Reports of Biological Product Deviations

The biological product deviation regulation[63] requires blood establishments to submit a biological product deviation report (BPDR) when the event meets the standard set out in 21 CFR 606.171. The regulation requires an establishment to report to FDA events that:

- occurred while the product was in the establishment's control; and
- EITHER represents a deviation from current good manufacturing practice, applicable regulations, applicable standards, or established specifications; OR represents an unexpected or unforeseeable event; and
- may affect the safety, purity or potency of a distributed product.

Some establishments have asked questions about submitting a BPDR in the context of these donor deferral recommendations.

[61] 59 FR 44591, Aug. 29, 1994.
[62] For examples, see: http://www.fda.gov/OHRMS/DOCKETS/dailys/02/Dec02/122302/80042e34.txt and http://www.gopetition.co.uk/petitions/restore-beef-insulins-to-the-united-states.html.
[63] 65 FR 6635, Nov. 7, 2000, as amended at 70 FR 14984, March 24, 2005.

Example #1: On the first day after implementing new donor criteria, a repeat donor provided information of living for seven years in France between 1981 and 1988. The donor was deferred at this donation. Must the establishment submit a BPDR with respect to units previously collected from that donor, if those units were distributed?

The regulation does not require the establishment to submit a BPDR. At the time of prior donations, collection from that donor did not represent a deviation from current good manufacturing practice, applicable regulations, applicable standards, or established specifications, and the donor would not have been deferred. Nor was the collection an unexpected or unforeseeable event.

Example #2: One year after implementing new donor criteria, the establishment discovers that one of its repeat donors provided information of living in France between 1981 and 1988. The donor donated Source Plasma eight weeks earlier and Whole Blood five months earlier. Despite the donor's unsuitability under the new donor criteria, the establishment accepted those donations. Must the establishment submit a BPDR with respect to those units, if those units were distributed?

The establishment must submit a BPDR (21 CFR 606.171). At the time of the donations, collection from that donor represented a deviation from current good manufacturing practice, applicable regulations, applicable standards, or established specifications.

Example #3: The establishment discovers that one of its repeat donors has developed CJD or vCJD. The donor donated Whole Blood three months earlier, and has a long history of donating. Must the establishment submit a BPDR with respect to units previously collected from that donor, if those units were distributed?

The establishment must submit a BPDR (21 CFR 606.171). Collection from that donor represented an unexpected or unforeseeable event that may affect the safety, purity, or potency of the product. Neither the blood establishment nor the agency expected or foresaw that the establishment would collect donations from individuals with CJD or vCJD.

Example #4: Six months after implementing new donor criteria, a repeat donor provided information of receiving a blood transfusion to treat a bleeding ulcer during a vacation in France 20 years ago. The donor donated Whole Blood three months earlier, at which time the donor provided the same information. Must the establishment submit a BPDR with respect to units previously collected from that donor, if those units were distributed?

The establishment must submit a BPDR (21 CFR 606.171). At the time of the donation, collection from that donor represented a deviation from current good manufacturing practice, applicable regulations, applicable standards, or established specifications.

G. Definitions

Audio CASI: computer assisted interactive donor questioning program that is accompanied by an audio component. The donor reads the questions on a computer display screen and hears the questions through a speaker or headphones.

Blood components intended for transfusion: Red Blood Cells, Platelets, Plasma, Cryoprecipitate, or Granulocytes derived from human blood collected by either manual Whole Blood collection or automated apheresis techniques and intended to be transfused to human recipients.

Military employee or dependent: An individual who is or was a member of one of the U.S. military services (Army, Air Force, Navy, Marines, Coast Guard), a civilian employee of one of the U.S. military services or a dependent (e.g., a spouse, child, parent, other) of a member of one of the U.S. military services or a civilian employee of one of the U.S. military services.

Recovered Plasma: the fluid portion of human blood obtained from Whole Blood or as a byproduct of apheresis procedures (e.g., plateletperesis) in conjunction with the preparation of blood components for transfusion and Source Leukocytes. Recovered plasma, an unlicensed product, is intended for further manufacturing into injectable and non-injectable products.

Source Leukocytes: a blood component derived from human blood collected by either manual or automated apheresis techniques and intended for further manufacturing into injectable products, like interferon. Source Leukocyte donors may donate once every eight weeks or more frequently and must meet Whole Blood or Source Plasma donor suitability criteria depending on the type and frequency of donation.[64]

Source Plasma: the fluid portion of human blood collected by plasmapheresis and intended for use as a source material for further manufacturing. Source Plasma may be manufactured into products intended for either injectable or non-injectable uses (21 CFR 640.60).

Source Plasma Donors:

- **Frequent Source Plasma Donor:** a donor who donates more frequently than once every four weeks. These donors are subject to the requirements in 21 CFR 630.15 and 21 CFR 640.65(b)(1)).[65]
- **Infrequent Source Plasma Donor:** a donor who has 1) not donated plasma by plasmapheresis or a co-collection of plasma with another blood component in the preceding 4 weeks and 2) not donated more than 12.0 liters of plasma (14.4 liters of plasma for donors weighing more than 175 pounds) in the past year. (See 21 CFR 630.3(e) and 21 CFR 630.25).[65]

[64] See 21 CFR 630.10 and 630.15. See Requirements for Blood and Blood Components Intended for Transfusion or for Further Manufacturing Use; Final Rule (80 FR 29842, May 22, 2015). The rule is effective May 23, 2016. Current requirements are in 21 CFR 640.3 and 640.63.

[65] See Requirements for Blood and Blood Components Intended for Transfusion or for Further Manufacturing Use; Final Rule (80 FR 29842, May 22, 2015). The rule is effective May 23, 2016.

IV. RECOMMENDATIONS FOR DONOR DEFERRAL

A. Donor Deferral Criteria

Donor deferral criteria 1-7 apply to all donors. Donor deferral criterion 8 (residence in Europe for 5 years or more between 1980 and the present) applies to all donors *with the exception of* donors of Source Plasma.

1. You should permanently defer donors who have been diagnosed with vCJD or any other form of CJD.[66]

2. You should permanently defer donors at increased risk for CJD (as identified by questions 2 and 3 in Section IV.B. Donors are considered to have an increased risk for CJD if they have received a dura mater transplant or an injection of human cadaveric pituitary-derived growth hormone. Donors with one or more blood relatives diagnosed with CJD (as identified in Section IV.B., Question 1 below) are also considered to be at increased risk of CJD, and should be indefinitely deferred (see Section IV.C. for donor reentry recommendations).

3. You should indefinitely defer donors who have spent three months or more cumulatively in the U.K. from the beginning of 1980 through the end of 1996.

4. You should indefinitely defer donors who have spent five years or more cumulatively in France from the beginning of 1980 to the present.

5. You should indefinitely defer former or current U.S. military personnel, civilian military personnel, and their dependents as follows:

   a. Individuals who resided at U.S. military bases in Northern Europe (Germany, United Kingdom, Belgium, and the Netherlands) for six months or more from 1980 through 1990, or

   b. Individuals who resided at U.S. military bases elsewhere in Europe (Greece, Turkey, Spain, Portugal, and Italy) for six months or more from 1980 through 1996.

6. You should indefinitely defer donors who have received a transfusion of blood or blood components in the U.K. or in France between the beginning of 1980 and the present.

[66] For the purposes of this document, FDA considers the less common TSEs, Gerstmann-Sträussler-Scheinker syndrome and fatal insomnia syndromes, to be equivalent in risk to familial and sporadic CJD. The blood establishment need not name these rare syndromes in the questionnaire but might consider them as equivalent in risk to CJD if, in response to a question about CJD, the donor offers information that a family member has been diagnosed with one of them.

7. You should indefinitely defer donors who have injected bovine insulin since 1980, unless you can confirm that the product was not manufactured after 1980 from U.K. cattle.

8. You should indefinitely defer donors of Whole Blood, blood components for transfusion, and Source Leukocytes, who have lived cumulatively for five years or more in Europe from the beginning of 1980 until the present. (Note this criterion includes time spent in the U.K. from 1980 through 1996 and time spent in France from 1980 to the present.) Unless otherwise unsuitable (for example, because they lived in the U.K. or France or on U.S. military bases for the periods of time noted previously), these donors remain eligible for Source Plasma donation.

NOTE: Donors who are otherwise deferred based upon the above criteria 2-8 may continue to donate if they are participating in a CBER-approved program that allows collection of Source Plasma solely for use in manufacturing of non-injectable products. We recommend special labeling for products obtained from such donors (see Section VII.A).

B. Questions to Identify Donors at an Increased Risk for CJD

You should question frequent Source Plasma donors at the first donation following implementation of the recommendations in this guidance, and annually thereafter. You should question donors of Whole Blood and blood components, infrequent Source Plasma donors and Source Leukocyte donors at each donation. If the donor is not familiar with the term "Creutzfeldt-Jakob Disease," you may take that as a negative response. These questions are similar to those in the 1999 and 2002 guidances. We consider donors who answer "Yes" to any of the questions below to have an increased risk for developing CJD.

Question 1: Have any of your blood relatives ever had Creutzfeldt-Jakob Disease?[67]

Question 2: Have you ever received growth hormone made from human pituitary glands?

NOTE: If the donor is uncertain about his or her treatment, the following question describing human pituitary-derived growth hormone injections may be asked: "Was the hormone treatment given repeatedly by injection?" This question needs to be asked only once, since human cadaveric pituitary growth hormone is no longer available.

[67] See footnote 66.

Question 3: Have you ever received a dura mater (brain covering) graft?

NOTE: This question may be preceded by the more general question "Have you ever had brain surgery?" Ask the specific question only if the donor responds "yes" to the general question.

C. Donor Reentry after Donor Deferral for Risk of Familial CJD

If you defer a donor because of family history of CJD, you may reenter that donor if:

1) The diagnosis of CJD in the family member(s) is confidently excluded, or CJD in the family member(s) is iatrogenic, or the family member(s) is (are) not a blood relative(s); or
2) Laboratory testing (gene sequencing) shows that the donor does not have a mutation associated with familial CJD. Note that gene sequencing of the donor is not necessary to demonstrate that the donor is not at risk for familial CJD. Sequencing of the family member with CJD or the appropriate parent of the donor, if the CJD-affected family member was a second-degree relative, may be sufficient to demonstrate that the donor does not have a mutation associated with familial CJD.

D. Questions for Identifying Donors at Risk for Exposure to BSE

1. Method of Donor Questioning

Due to the added complexity of screening donors for cumulative periods of potential exposure to BSE, a trained staff member should administer the revised geographic donor deferral criteria by face-to-face interview to each new donor (as defined in your blood establishment's standard operating procedures (SOP)). Instead of face-to-face interviews, you may use a computerized interactive donor interview program that includes an audio component (audio-CASI) as described in the FDA guidance entitled "Guidance for Industry: Streamlining the Donor Interview Process: Recommendations for Self-Administered Questionnaires," dated July 2003.[68] You should submit changes to your donor interview procedure according to 21 CFR 601.12. For repeat donors, you may use alternative methods for introducing and emphasizing the new questions. Your alternative method should provide the repeat donor with a detailed description of the changes to the donor questionnaire, to highlight any new questions and modifications.

[68] Available at http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/ucm075086.htm.

2. Donor Questions

You should indefinitely defer donors who answer "Yes" to the following questions:

To identify donors with geographic risk of BSE exposure.

Since the beginning of 1980, have you ever lived in or traveled to Europe?

a. If the donor answers "No," you need not take any further action.
b. If the donor answers "Yes," then ask the following questions:

    1) Between 1980 through 1996 did you spend time that adds up to three months or more in the U.K. (England, Northern Ireland, Scotland, Wales, the Isle of Man, the Channel Islands, Gibraltar, or the Falkland Islands)?
    2) Since 1980 have you received a transfusion of blood, platelets, plasma, cryoprecipitate, or granulocytes in the U.K. (England, Northern Ireland, Scotland, Wales, the Isle of Man, the Channel Islands, Gibraltar, or the Falkland Islands) or in France?[69]
    3) Between 1980 through 1996, were you a member of the U.S. military, a civilian military employee, or a dependent of a member of the U.S. military?

    If the donor answers "No," you need not take any further action.
    If the donor answers "Yes," ask the following question:
    Did you spend a total time of six months or more associated with a military base in any of the following countries:

    - From 1980 through 1990 in Belgium, the Netherlands, or Germany, or
    - From 1980 through 1996 in Spain, Portugal, Turkey, Italy, or Greece?

NOTE: For Questions 1 and 3, you need to question donors only once, because these questions encompass a discrete time frame. You should administer Question 2 to frequent Source Plasma donors at intervals of no greater than four months, and to all other donors, at each donation.

[69] For purposes of this guidance, the United Kingdom should be taken to include all of the following: England, Northern Ireland, Scotland, Wales, the Isle of Man, the Channel Islands, Gibraltar, and the Falkland Islands; France should be taken to include its overseas departments (e.g., Martinique and others).

To identify donors of Source Plasma who have additional geographic risk of BSE exposure, you should ask the following questions:

4) Since 1980, have you spent time that adds up to five years or more in France?

For donors of Whole Blood, components intended for transfusion, and Source Leukocytes, you should **substitute** the following for question 4):

Question 4 (alternative): Since 1980, have you spent time that adds up to five years or more in Europe (including time spent in the U.K. from 1980 through 1996)?

Donors deferred from donating Whole Blood based on this question remain eligible to donate Source Plasma in a CBER-approved program, unless they are otherwise unsuitable.

For Donors of Source Plasma, however, you should **continue to ask** the **original** version of **Question 4,** as described above, rather than the alternative. European countries with BSE risk that FDA has identified as a basis for donor deferral are listed in the Appendix to this document. We will periodically issue new guidance to update the list of countries with BSE risk, to be used as a basis for donor deferral. FDA does not currently consider those European and non-European countries that are not listed in the Appendix to this document to pose a BSE-exposure risk warranting deferral of donors who have spent any period of time there, even if these countries have reported cases of BSE to the OIE.[70]

To identify donors who have been injected with bovine insulin since 1980, you should ask donors with diabetes the following question:

5) Since 1980, have you ever injected bovine (beef) insulin?

Since the above question applies to a subset of potential donors, you may ask it as a secondary question to a general medication question if a donor responds that they have taken insulin. If the donor answers "Yes" or "I don't know" in response to the question, you should indefinitely defer that donor, unless it can be documented that the product was not manufactured from cattle in the U.K. after 1980.

NOTE: Donors of Source Plasma who otherwise should be indefinitely deferred based on their responses to the questions specified in Sections IV.D.2.(b)(3) and IV.D.2.(b)(4), may continue to donate if they are participating in a CBER-

[70] OIE at http://www.oie.int/en/animal-health-in-the-world/bse-specific-data.

approved program that allows collection of Source Plasma solely for use in manufacturing of non-injectable products. We recommend special labeling for products obtained from such donors. (See Section VII.A.)

V. POST-DONATION INFORMATION: RECOMMENDATIONS FOR PRODUCT RETRIEVAL AND QUARANTINE, CONSIGNEE NOTIFICATION, AND BIOLOGICAL PRODUCT DEVIATION REPORTING

A. Whole Blood and Blood Components Intended for Transfusion, Cellular Blood Components Intended for Further Manufacture into Injectable Products, and Source Plasma from Donors with CJD or CJD Risk Factors

1. Product Disposition

If you receive post-donation information about a donor with CJD or CJD risk factors, you should immediately retrieve and quarantine for subsequent destruction all in-date blood components (including Whole Blood, blood components intended for transfusion, Source Leukocytes, and Source Plasma), all in-date cellular blood components intended for manufacturing into injectable products, and all recovered plasma that are under your control. We also recommend that you follow your SOPs or update your SOPs regarding notifying consignees to immediately retrieve, quarantine, and subsequently destroy (or arrange for the destruction of) the implicated components. Such notification should occur within one week of receiving the post-donation information.

NOTE: If you have sent Source Plasma or recovered plasma to a consignee and receive post-donation information about a donor with CJD or CJD risk factors, at a time when you know the plasma units have been pooled, you should not conduct product retrieval or consignee notification for those units.

2. Biological Product Deviation Reports

If you received post-donation information about a donor with CJD, you must submit a BPDR (21 CFR 606.171) for any distributed components. The regulation requires you to submit a BPDR as soon as possible but not to exceed 45 calendar days after you discover the event (21 CFR 606.171(c)). If you received post-donation information about a donor with CJD risk factors, you must submit a BPDR (21 CFR 606.171) for any distributed components collected after the implementation of donor deferral. A BPDR is not required if components were collected prior to the implementation of donor deferral.

B. Whole Blood and Blood Components Intended for Transfusion, Source Leukocytes and Other Cellular Blood Components Intended for Further Manufacture into Injectable Products, from Donors with Geographic Risk Deferrals and/or Exposure to Bovine Insulin Made in the U.K. since 1980

<u>Donors with Geographic Risk Deferrals</u>

1. Product Disposition

If you receive post-donation information about a donor with geographic risk factors, you should immediately retrieve and quarantine for subsequent destruction all in-date blood components (including Whole Blood, blood components intended for transfusion, and Source Leukocytes), and all in-date cellular blood components intended for manufacturing into injectable products, that are under your control. We also recommend that you follow your SOPs or update your SOPs regarding notifying consignees to immediately retrieve, quarantine, and subsequently destroy (or arrange for the destruction of) the implicated components. Such notification should occur within one week of receiving the post-donation information.

2. Biological Product Deviation Reports

If you received post-donation information about a donor with geographic risk factors, you must submit a BPDR (21 CFR 606.171) for any distributed components collected after the implementation of donor deferral. A BPDR is not required if components were collected prior to the implementation of donor deferral.

<u>Donors with Exposure to Bovine Insulin Made in the U.K. since 1980</u>

1. Product Disposition

If you receive post-donation information about a donor exposure to bovine insulin made in the U.K. since 1980, you should immediately retrieve and quarantine for subsequent destruction all in-date blood components (including Whole Blood, blood components intended for transfusion, and Source Leukocytes), and all in-date cellular blood components intended for manufacturing into injectable products, that are under your control. We also recommend that you follow your SOPs or update your SOPs regarding notifying consignees to immediately retrieve, quarantine, and subsequently destroy (or arrange for the destruction of) the implicated components. Such notification should occur within one week of receiving the post-donation information.

2. Biological Product Deviation Reports

If you received post-donation information about a donor exposure to bovine insulin made in the U.K. since 1980, you must submit a BPDR (21 CFR 606.171) for any distributed components collected after the implementation of donor deferral. A BPDR is not required if components were collected prior to the implementation of donor deferral.

C. Source Plasma and Recovered Plasma from Donors with Geographic Risk Deferrals and/or Exposure to Bovine Insulin Made in the U.K. Since 1980

1. Product Disposition

If you receive post-donation information about a donor with geographic risk factors, or exposure to bovine insulin made in the U.K. since 1980, you should immediately retrieve and quarantine for subsequent destruction all in-date Source Plasma and all recovered plasma under your control. We also recommend that you follow your SOPs or update your SOPs regarding notifying consignees to immediately retrieve, quarantine, and subsequently destroy (or arrange for the destruction of) the Source Plasma and recovered plasma. Such notification should occur within one week of receiving the post-donation information.
NOTE: If you have sent Source Plasma or recovered plasma to a consignee and receive post-donation information about a donor with geographic risk factors, or exposure to bovine insulin from the U.K. at a time when you know the plasma units have been pooled, you should not conduct product retrieval or consignee notification for those units.

2. Biological Product Deviation Reports

If you received post-donation information about a donor with geographic risk factors or exposure to bovine insulin made in the U.K. since 1980, you must submit a BPDR (21 CFR 606.171) for any distributed components collected after the implementation of donor deferral. A BPDR is not required if components were collected prior to the implementation of donor deferral.

D. Whole Blood and Blood Components Intended for Transfusion, Recovered Plasma, Source Leukocytes, Other Cellular Blood Components Intended for Manufacturing into Injectable Products, and Source Plasma from Donors with vCJD, suspected vCJD, or CJD and Age Less Than 55 Years

1. Product Disposition

We recommend you contact the Office of Blood Research and Review (OBRR), CBER at 240-402-8360 as soon as possible upon receiving post-donation information about a donor with vCJD, suspected vCJD, or CJD and age less than 55 years. You should immediately retrieve and quarantine for subsequent

destruction all in-date blood components (including Whole Blood, blood components intended for transfusion, Source Leukocytes, and Source Plasma), all recovered plasma, and all in-date cellular blood components intended for manufacturing into injectable products that are under your control. We also recommend that you follow your SOPs or update your SOPs regarding notifying consignees to immediately retrieve, quarantine, and subsequently destroy (or arrange for the destruction of) the implicated components. Such notification should occur within one week of receiving the post-donation information.

You may save the collected material for use in research on vCJD by qualified laboratories (see Section VII.A for labeling recommendations).

2. Biological Product Deviation Reports

If you received post-donation information about a donor with vCJD, suspected vCJD, or CJD and age less than 55 years, you must submit a BPDR (21 CFR 606.171) for any distributed components. The regulations require you to submit a BPDR as soon as possible but not to exceed 45 calendar days after you discover the event (21 CFR 606.171(c)).

E. Plasma Derivatives

1. Plasma derivatives manufactured using plasma from donors with CJD or CJD risk factors, or geographic risk deferrals, as defined in Section IV.D. We are not recommending that you withdraw pooled plasma, intermediates, and plasma derivatives manufactured from these donors.

2. Plasma derivatives manufactured using plasma from donors diagnosed with vCJD or suspected vCJD

a. Product Disposition

We recommend you contact OBRR, CBER at 240-402-8360 as soon as possible upon receiving post-donation information about a donor with vCJD or suspected vCJD. You should immediately retrieve and quarantine for subsequent destruction any pooled plasma, intermediates, derivatives, and any other material containing plasma from such a donor. Alternatively, you may save the material for use in research on vCJD by qualified laboratories (see Section VII.A. for labeling recommendations). You should not use such material for non-injectable products.

We also recommend that you follow your SOPs or update your SOPs regarding notifying consignees to immediately retrieve, quarantine, and subsequently destroy (or arrange for the destruction of) the pooled plasma,

intermediates, and derivatives, and any other materials containing plasma from the vCJD donor. Such notification should occur within one week of receiving the post-donation information.

b. Biological Product Deviation Reports

You must submit a BPDR (21 CFR 600.14) if a plasma derivative product is manufactured using plasma collected from a donor who was diagnosed with vCJD or suspected vCJD and the product was distributed. The regulations require you to submit a BPDR as soon as possible but not to exceed 45 calendar days after you discover the event (21 CFR 606.171(c)).

3. Plasma derivatives manufactured using plasma from donors with a physician's clinical or pathological diagnosis of CJD and age less than 55 years.

a. Product Disposition

We recommend you contact OBRR, CBER at 240-402-8360 as soon as possible upon receiving information about a donor's diagnosis of CJD when less than 55 years old. We will make recommendations to quarantine and withdraw plasma derivatives from such donors on a case-by-case basis, depending upon results of the investigation. We may recommend quarantine and withdrawal of products if available information is ambiguous and does not clearly eliminate the possibility of vCJD. You should treat quarantined and withdrawn material from such donors in the same manner as for vCJD (see Section V.D.).

b. Biological Product Deviation Reports

You must submit a BPDR (21 CFR 600.14) if a plasma derivative product is manufactured using plasma collected from a donor with a physician's clinical or pathological diagnosis of CJD and age less than 55 years, and the product was distributed.

The regulations require you to submit a BPDR as soon as possible but not to exceed 45 calendar days after you discover the event (21 CFR 600.14(c)).

F. Disposal of Retrieved and Quarantined Products

TSE agents are quite resistant to most disinfecting regimens. There is no current consensus on specific details of decontamination requirements for blood products. However, methods of destruction of TSE-implicated material include steam autoclaving at 132°C for 1-4 hours, incineration, or treatment with 1 N or 2 N NaOH or concentrated

sodium hypochlorite for at least 1 hour. These treatments are known to diminish (but may not completely eliminate) infectivity (Refs. 71-72).[71] You may save blood components and plasma derivatives from donors with vCJD, or which have been withdrawn because the donor might have vCJD, to use in research on vCJD by qualified laboratories (see Section VII.A. for labeling recommendations).

VI. RECOMMENDATIONS FOR RECIPIENT TRACING AND NOTIFICATION

It may be appropriate to identify blood components for transfusion prepared from prior collections from any donor found to have CJD, vCJD, suspected vCJD, risk factors for CJD, or if withdrawal is recommended in cases under investigation for vCJD (CJD diagnosis and age less than 55). In those situations, consignee notification could enable the consignee to inform the physician, or other qualified personnel responsible for the care of the recipients, so that recipient tracing and medically appropriate notification and counseling may be performed at the discretion of health care providers.

For transfusible components from a donor with one family member diagnosed with CJD, or with risk factors for vCJD (due to geographic risk deferral, transfusion in the U.K. or in France between 1980 and the present, or due to injection of bovine insulin), we believe it is not appropriate to conduct tracing and notification of recipients of prior donations.

It may be appropriate to identify plasma derivatives prepared from prior collections from any donor found to have vCJD, suspected vCJD, or if withdrawal is recommended in cases under investigation for vCJD (CJD diagnosis and age less than 55 years). In those situations, consignee notification could enable the consignee to inform the physician, or other qualified personnel responsible for the care of the recipients, so that recipient tracing and medically appropriate notification and counseling may be performed at the discretion of health care providers.

VII. LABELING RECOMMENDATIONS

A. Labeling of Blood and Blood Components from Deferred Donors for Research, or Intended for Further Manufacture into Non-Injectable Products

You should label blood and blood components from donors with CJD, who are at increased risk for CJD, or who have potential exposure to the agent of vCJD with the following statements, as appropriate:

- "Biohazard";

[71] World Health Organization (WHO) Infection Control Guidelines for Transmissible Spongiform Encephalopathies at http://www.who.int/csr/resources/publications/bse/WHO_CDS_CSR_APH_2000_3/en/.

- "Collected from a donor determined to be at risk for CJD"; or "Collected from a donor diagnosed with CJD"; or "Collected from a donor with potential risk of exposure to variant CJD"; and
- "Caution: For laboratory research use only"; or "Caution: For use in manufacturing non-injectable products only."[72]

You should not use blood or blood components from donors diagnosed with vCJD for further manufacture into non-injectable products. However blood components and plasma derivatives from donors with vCJD, suspected vCJD, or which have been withdrawn on a case-by-case basis for suspicion of vCJD, may be used in laboratory research on vCJD by qualified laboratories. You should label these products with the following statements:

- "Biohazard";
- "Collected from a donor with variant CJD"; and
- "Caution: Only for laboratory research on variant CJD."

B. Labeling of Non-Implicated Products

As a prudent notice, we recommend that all blood, blood components, and plasma-derived products include labeling to address the possible risk of transmission of vCJD and CJD. Because albumin has never been known to transmit viral diseases, and because laboratory experiments suggest that albumin is less likely to contain CJD-like agents than other plasma fractions, the package insert for albumin, and products containing albumin, may contain a more specific statement:

1. For Whole Blood and blood components intended for transfusion, the instruction circular should include the following warning statement:

   "Because Whole Blood and blood components are made from human blood, they may carry a risk of transmitting infectious agents (e.g., viruses, bacteria, parasites, the variant Creutzfeldt-Jakob disease (vCJD) agent, and, theoretically, the Creutzfeldt-Jakob disease (CJD) agent."[73]

---

[72] Donors who are otherwise deferred based upon donor deferral criteria 2 through 8 of this guidance, may continue to donate if they are participating in a CBER approved program that allows collection of Source Plasma solely for use in manufacturing of non-injectable products (see Section IV.A.).

[73] This language is included in the AABB "Circular of Information for the Use of Human Blood and Blood Components," dated November 2013, which FDA has recognized as an acceptable mechanism that is consistent with FDA requirements and recommendations for the labeling of Whole Blood and blood components intended for transfusion. If you do not utilize the AABB Circular of Information, you may attach the recommended labeling statement to your current circular until it is revised.

2. For plasma-derived products other than albumin, you should revise the statement in the Warnings and Precautions section of your labeling to include the following statement:

   "Because this product is made from human blood, it may carry a risk of transmitting infectious agents, e.g., viruses, the variant Creutzfeldt-Jakob disease (vCJD) agent and, theoretically, the Creutzfeldt-Jakob disease (CJD) agent."

3. For plasma-derived albumin, you should revise the statement in the Warning and Precautions section of your labeling to include the following statement:

   "Albumin is a derivative of human blood. Based on effective donor screening and product manufacturing processes, it carries an extremely remote risk for transmission of viral diseases and variant Creutzfeldt-Jakob disease (vCJD). There is a theoretical risk for transmission of Creutzfeldt-Jakob disease (CJD), but if that risk actually exists, the risk of transmission would also be considered extremely remote. No cases of transmission of viral diseases, CJD or vCJD have ever been identified for licensed albumin."

4. For products containing plasma-derived albumin, you should revise the statement in the Warnings and Precautions section of your labeling to include the following statement:

   "This product contains albumin, a derivative of human blood. Based on effective donor screening and product manufacturing processes, it carries an extremely remote risk for transmission of viral diseases and variant Creutzfeldt-Jakob disease (vCJD). There is a theoretical risk for transmission of Creutzfeldt-Jakob disease (CJD), but if that risk actually exists, the risk of transmission would also be considered extremely remote. No cases of transmission of viral diseases, CJD or vCJD have ever been identified for licensed albumin or albumin contained in other licensed products."

VIII. IMPLEMENTATION OF RECOMMENDATIONS

We recommend that you implement the new recommendations contained in this guidance, (i.e., those recommendations related to labeling of plasma-derived products, including albumin and products containing plasma-derived albumin), within six months of publication of this guidance.[74] Manufacturers must submit the labeling change to FDA in accordance with 21 CFR 601.12(f)(2).

[74] As stated in the 2010 guidance, all recommendations contained therein should have been implemented no later than November 2010.

IX. THE IMPACT OF GEOGRAPHIC DONOR DEFERRALS THAT ARE MORE STRINGENT THAN THOSE RECOMMENDED BY THIS GUIDANCE

A more stringent geographic donor deferral policy (deferral for a cumulative period of six months or more in Europe since 1980 or a cumulative period of three months or more in the U.K. since 1980) was proposed as an initiative in early 2001 by a member of the blood industry. Based upon the BSE geographic relative risk model proposed by the FDA and CDC and reviewed by the TSEAC in 2001, both the industry-proposed and FDA-proposed deferrals resulted in an estimated one-log reduction of theoretical risk. Importantly, the donor loss for the industry proposal, if implemented on a national basis, was estimated by FDA to be at least 8-9% (3-4% higher than the FDA-recommended policy announced in January 2002). Some countries have recommended deferring donors who received transfusions in countries other than the U.K. and France (Ref. 58). Some authorities have noted that potential exposure of some U.S. military personnel residing in certain bases in Europe to the BSE agent between 1980-1996 might have exceeded that in France and suggested that persons transfused with their blood also be deferred as blood donors.

FDA's recommendations for donor deferral related to risk of CJD and vCJD are based on our current consideration of the relative benefits of risk reduction compared with the potential adverse effects of a decrease in availability of the blood supply, and may be updated in the future as better scientific information becomes available. Nevertheless, we recognize that some blood establishments may wish to implement geographic donor deferrals that are more stringent than the FDA-recommended policy. We are concerned that blood availability may be more severely affected by periods of deferral more stringent than those outlined by this guidance. If you wish to implement donor deferrals other than those recommended in this guidance, consider strategies for offsetting projected donor losses and maintaining an adequate blood supply to meet hospital demands for blood products.

X. SOURCES OF ADDITIONAL INFORMATION

Subject	Contact
FDA policies on CJD, vCJD and BSE exposure	Division of Emerging and Transfusion-Transmitted Diseases, OBRR, CBER at 240-402-8360
This guidance and FDA policies for implementing acceptable DHQ documents	Division of Blood Components and Devices, OBRR, CBER at 301-402-8360
Receipt of post-donation information about a donor with vCJD, suspected vCJD or CJD and under age 55.	Division of Blood Components and Devices, OBRR, CBER at 240-402-8360
The vDHQ-1.3 or other AABB DHQ documents	AABB at 301-907-6977, attention of the AABB Donor History Task Force
DHQ documents that FDA has recognized as acceptable	http://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/LicensedProductsBLAs/BloodDonorScreening/ucm164185.htm.
Biological product deviation reporting	Division of Inspections and Surveillance, OCBQ, CBER, at 240-402-9160 or by email at http://www.accessdata.fda.gov/scripts/email/cber/bpdrcontact.cfm.

XI. REFERENCES

1. DeArmond, S. J. and S. B. Prusiner (1995). "Etiology and pathogenesis of prion diseases." Am J Pathol **146**(4): 785-811.

2. Masters, C. L., J. O. Harris, et al. (1979). "Creutzfeldt-Jakob disease: patterns of worldwide occurrence and the significance of familial and sporadic clustering." Ann Neurol **5**(2): 177-88.

3. Chesebro, B. (1998). "BSE and Prions: Uncertainties About the Agent." Science **279**(5347): 42-3.

4. Erdtmann, R. and L. Sivitz, Eds. (2001). Advancing Prion Science. Guidance for the National Prion Research Program (Institute of Medicine, U.S. Committee on Transmissible Spongiform Encephalopathies. Assessment of Relevant Science). Washington, The National Academies Press.

5. Chesebro, B. (2003). "Introduction to the transmissible spongiform encephalopathies or prion diseases." Br Med Bull **66**: 1-20.

6. Manuelidis, L., Z. X. Yu, et al. (2007). "Cells infected with scrapie and Creutzfeldt-Jakob disease agents produce intracellular 25-nm virus-like particles." Proc Natl Acad Sci U S A **104**(6): 1965-70.

7. Prusiner, S. B. (1982). "Novel proteinaceous infectious particles cause scrapie." Science **216**: 136-144.

8. Croes, E. A., G. Roks, et al. (2002). "Creutzfeldt-Jakob disease 38 years after diagnostic use of human growth hormone." J Neurol Neurosurg Psychiatry **72**(6): 792-3.

9. Collinge, J., J. Whitfield, et al. (2006). "Kuru in the 21st century--an acquired human prion disease with very long incubation periods." Lancet **367**(9528): 2068-74.

10. Will, R. G., J. W. Ironside, et al. (1996). "A new variant of Creutzfeldt-Jakob disease in the UK." Lancet **347**(9006): 921-5.

11. Cousens, S. N., E. Vynnycky, et al. (1997). "Predicting the CJD epidemic in humans." Nature **385**(6613): 197-8.

12. Zeidler, M., E. C. Johnstone, et al. (1997). "New variant Creutzfeldt-Jakob disease: psychiatric features." Lancet (London) **350**(9082): 908-10.

13. Zeidler, M., G. E. Stewart, et al. (1997). "New variant Creutzfeldt-Jakob disease: neurological features and diagnostic tests." Lancet **350**(9082): 903-7.

14. Ironside, J. W. (1998). "Neuropathological findings in new variant CJD and experimental transmission of BSE." FEMS Immunol Med Microbiol **21**(2): 91-5.

15. Cousens, S. N., M. Zeidler, et al. (1997). "Sporadic Creutzfeldt-Jakob disease in the United Kingdom: analysis of epidemiological surveillance data for 1970-96." BMJ **315**(7105): 389-95.

16. Zeidler, M., R. J. Sellar, et al. (2000). "The pulvinar sign on magnetic resonance imaging in variant Creutzfeldt-Jakob disease." Lancet **355**(9213): 1412-8.

17. Collie, D. A., R. J. Sellar, et al. (2001). "MRI of Creutzfeldt-Jakob Disease: imaging features and recommended MRI protocol." Clin Radiol **56**(9): 726-39.

18. Hill, A. F., R. J. Butterworth, et al. (1999). "Investigation of variant Creutzfeldt-Jakob disease and other human prion diseases with tonsil biopsy samples." Lancet **353**(9148): 183-9.

19. Will, R. G. and R. H. Kimberlin (1998). "Creutzfeldt-Jakob disease and the risk from blood or blood products." Vox Sanguinis (Basel) **75(3)**: 178-80.

20. Zeidler, M., C. Gibbs, Jr, et al., Eds. (1998). WHO Manual for Strengthening Diagnosis and Surveillance of Creutzfeldt-Jakob Disease. Geneva, World Health Organization.

21. Andrews, N. J., C. P. Farrington, et al. (2000). "Incidence of variant Creutzfeldt-Jakob disease in the UK." Lancet **356**(9228): 481-2.

22. Ghani, A. C., N. M. Ferguson, et al. (2000). "Predicted vCJD mortality in Great Britain." Nature **406**(6796): 583-4.

23. Collinge, J. (1999). "Variant Creutzfeldt-Jakob disease." Lancet **354**(9175): 317-23.

24. Mead, S., S. Joiner, et al. (2007). "Creutzfeldt-Jakob disease, prion protein gene codon 129VV, and a novel PrPSc type in a young British woman." Arch Neurol **64**(12): 1780-4.

25. Collinge, J., K. C. Sidle, et al. (1996). "Molecular analysis of prion strain variation and the aetiology of 'new variant' CJD." Nature **383**(6602): 685-90.

26. Bruce, M. E., R. G. Will, et al. (1997). "Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent." Nature **389**(6650): 498-501.

27. Manuelidis, E. E., E. J. Gorgacs, et al. (1978). "Viremia in experimental Creutzfeldt-Jakob disease." Science **200**(4345): 1069-71.

28. Kuroda, Y., C. J. Gibbs, Jr., Amyx, H. L., et al. (1983). "Creutzfeldt-Jakob disease in mice: persistent viremia and preferential replication of virus in low-density lymphocytes." Infection and Immunity **41**: 154-161.

29. Houston, F., J. D. Foster, et al. (2000). "Transmission of BSE by blood transfusion in sheep." Lancet **356**(9234): 999-1000.

30. Hunter, N., J. Foster, et al. (2002). "Transmission of prion diseases by blood transfusion." J Gen Virol **83**(Pt 11): 2897-905.

31. Hunter, N. and F. Houston (2002). "Can prion diseases be transmitted between individuals via blood transfusion: evidence from sheep experiments." Dev Biol (Basel) **108**: 93-8.

32. Gregori, L., N. McCombie, et al. (2004). "Effectiveness of leucoreduction for removal of infectivity of transmissible spongiform encephalopathies from blood." Lancet **364**(9433): 529-31.

33. Brown, P. (2007). "Creutzfeldt-Jakob disease: reflections on the risk from blood product therapy." Haemophilia **13 Suppl 5**: 33-40.

34. Gibbs, C. J., Jr., A. Joy, et al. (1985). "Clinical and pathological features and laboratory confirmation of Creutzfeldt-Jakob disease in a recipient of pituitary-derived human growth hormone." N Engl J Med **313**(12): 734-8.

35. Thadani, V., P. L. Penar, et al. (1988). "Creutzfeldt-Jakob disease probably acquired from a cadaveric dura mater graft. Case report." J. Neurosurg. (Baltimore MD) **69**: 766-69.

36. Asher, D. M., C. L. Masters, et al. (1983). ''Familial Spongiform Encephalopathies.'' Res Publ Assoc Res Nerv Ment Dis **60**: 273-91.

37. Esmonde, T. F., R. G. Will, et al. (1993). "Creutzfeldt-Jakob disease and blood transfusion." Lancet **341**(8839): 205-7.

38. Heye, N., S. Hensen, et al. (1994). "Creutzfeldt-Jakob disease and blood transfusion." Lancet **343**(8892): 298-9.

39. Wientjens, D. P., Z. Davanipour, et al. (1996). "Risk factors for Creutzfeldt-Jakob disease: a reanalysis of case-control studies." American Academy of Neurology **46**(5): 1287-1291.

40. Sullivan, M., L. Schonberger, et al. (1997). "Creutzfeldt-Jakob disease (CJD) Investigational lookback study." Transfusion **37 suppl**: 2s.

41. Evatt, B., H. Austin, et al. (1998). "Surveillance for Creutzfeldt-Jakob disease among persons with hemophilia." Transfusion **38**(9): 817-20.

42. Lee, C., J. Ironside, et al. (1998). "Retrospective neuropathological review of prion disease in UK haemophilic patients." Thromb Haemost **80**: 909-11.

43. van Duijn, C. M., N. Delasnerie-Laupretre, et al. (1998). "Case-control study of risk factors of Creutzfeldt-Jakob disease in Europe during 1993-95. European Union (EU) Collaborative Study." Lancet (London) **351**: 1081-85.

44. Holman, R. C., A. S. Khan, et al. (1996). "Creutzfeldt-Jakob Disease in the United States, 1979-1994: Using national mortality data to assess the possible occurrence of variant cases." Emerg Infect Dis **2**(4): 333-7.

45. Brown, P., R. G. Rohwer, et al. (1998). "The distribution of infectivity in blood components and plasma derivatives in experimental models of transmissible spongiform encephalopathy." Transfusion **38**(9): 810-16.

46. Foster, P. R. (1999). "Assessment of the potential of plasma fractionation processes to remove causative agents of transmissible spongiform encephalopathy." Transfus Med **9**(1): 3-14.

47. Foster, P. R. (2000). "Prions and blood products." Ann Med **32**(7): 501-13.

48. Foster, P. R., C. McLean, et al. (2000). "Removal of abnormal prion protein by plasma fractionation." Transfus Sci **22**(1-2): 53-6.

49. Foster, P. R., A. G. Welch, et al. (2000). "Studies on the removal of abnormal prion protein by processes used in the manufacture of human plasma products." Vox Sang **78**(2): 86-95.

50. Reichl, H. E., P. R. Foster, et al. (2002). "Studies on the removal of a bovine spongiform encephalopathy-derived agent by processes used in the manufacture of human immunoglobulin." Vox Sang **83**(2): 137-45.

51. Foster, P. R. (2004). "Removal of TSE agents from blood products." Vox Sang **87 Suppl 2**: 7-10.

52. Foster, P. R., B. D. Griffin, et al. (2004). "Distribution of a bovine spongiform encephalopathy-derived agent over ion-exchange chromatography used in the preparation of concentrates of fibrinogen and factor VIII." Vox Sang **86**(2): 92-9.

53. Llewelyn, C. A., P. E. Hewitt, et al. (2004). "Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion." Lancet **363**(9407): 417-21.

54. Peden, A. H., M. W. Head, et al. (2004). "Preclinical vCJD after blood transfusion in a *PRNP* codon 129 heterozygous patient." Lancet **364**(9433): 527-9.

55. U.K. Health Protection Agency (2006). "New case of transfusion-associated vCJD." Commun Dis Resp CDR Wkly **16**: serial online.

56. Hewitt, P. E., C. A. Llewelyn, et al. (2006). "Creutzfeldt-Jakob disease and blood transfusion: results of the UK Transfusion Medicine Epidemiological Review study." Vox Sang **91**(3): 221-30.

57. Lee, D.C., Streland, J.C., et al. (2001). "A direct relationship between the partitioning of the pathogenic prion protein and transmissible spongiform encephalopathy infectivity during the purification of plasma proteins." Transfusion. **41**: 449-55.

58. Seitz, R., F. von Auer, et al. (2007). "Impact of vCJD on blood supply." Biologicals **35**(2): 79-97.

59. Gregori, L. and R. G. Rohwer (2007). "Characterization of scrapie-infected and normal hamster blood as an experimental model for TSE-infected human blood." Dev Biol (Basel) **127**: 123-33.

60. Brown, P., L. Cervenakova, et al. (1999). "Further studies of blood infectivity in an experimental model of transmissible spongiform encephalopathy, with an explanation of why blood components do not transmit Creutzfeldt-Jakob disease in humans." Transfusion **39**(11-12): 1169-78.

61. Brown, P. Rowher, RG, et al. (1998). "The distribution of infectivity in blood components and plasma derivatives in experimental models of transmissible spongiform encephalopathy." Transfusion. **38**(9):810-16.

62. Dorsey, K., Zou, S., et al. (2009). "Lack of evidence of transfusion transmission of Creutzfeldt-Jakob disease in a US surveillance study." Transfusion. **49**: 977-84.

63. Puopolo, M., Ladogana, A., et al. (2011). "Transmission of sporadic Creutzfeldt-Jakob disease by blood transfusion: risk factor or possible biases." Transfusion. **51:** 1556-66. 3004.

64. Molesworth, A., Mackenzle, J., et al. (2011). "Sporadic Creutzfeldt-Jakob disease and risk of blood transfusion in the United Kingdom." Transfusion. **51**: 1872-73.

65. Zou, S., C. T. Fang, et al. (2008). "Transfusion Transmission of Human Prion Diseases." Transfus Med Rev **22**(1): 58-69.

66. Williams, A. E., R. A. Thomson, et al. (1997). "Estimates of Infectious Disease Risk Factors in US Blood Donors. Retrovirus Epidemiology Donor Study." JAMA **277**(12): 967-72.

67. Hill, A. F. and J. Collinge (2003). "Subclinical prion infection." Trends Microbiol **11**(12): 578-84.

68. Hill, A. F. and J. Collinge (2003). "Subclinical prion infection in humans and animals." Br Med Bull **66**: 161-70.

69. Hilton, D. A., A. C. Ghani, et al. (2004). "Prevalence of lymphoreticular prion protein accumulation in UK tissue samples." J Pathol **203**(3): 733-9.

70. Ironside, J. W., M. T. Bishop, et al. (2006). "Variant Creutzfeldt-Jakob disease: prion protein genotype analysis of positive appendix tissue samples from a retrospective prevalence study." BMJ **332**(7551): 1186-8.

71. Taylor, D. M., H. Fraser, et al. (1994). "Decontamination studies with the agents of bovine spongiform encephalopathy and scrapie." Arch Virol **139**(3-4): 313-26.

72. World Health Organization (1999). "WHO Infection Control Guidelines for Transmissible Spongiform Encephalopathies." Report of a WHO consultation, Geneva, Switzerland, 23-26 March 1999 at http://whqlibdoc.who.int/hq/2000/WHO_CDS_CSR_APH_2000.3.pdf.

APPENDIX: List of European Countries with BSE or at Risk of BSE Applicable to Donor Deferral

European Countries List to be Used for Deferral of Donors Based on Geographic Risk of BSE[75]

Albania, Austria, Belgium, Bosnia-Herzegovina, Bulgaria, Croatia, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Republic of Ireland, Italy, Liechtenstein, Luxembourg, Macedonia, Netherlands, Norway, Poland, Portugal, Romania, Slovak Republic, Slovenia, Spain, Sweden, Switzerland, United Kingdom, and Federal Republic of Yugoslavia.

[75] For purposes of this guidance, the United Kingdom should be taken to include all of the following: England, Northern Ireland, Scotland, Wales, the Isle of Man, the Channel Islands, Gibraltar, and the Falkland Islands; France should be taken to include its overseas departments (e.g., Martinique and others); Spain should be taken to include the Canary Islands and Spanish North African territories; Portugal should be taken to include the Azores.

APPENDIX TABLE 1: DONOR DEFERRAL, PRODUCT DISPOSITION, RECIPIENT NOTIFICATION FOR WHOLE BLOOD, BLOOD COMPONENTS INTENDED FOR TRANSFUSION, SOURCE LEUKOCYTES, AND OTHER CELLULAR BLOOD COMPONENTS INTENDED FOR FURTHER MANUFACTURE

Risk	Deferral	Disposition of Product And Consignee Notification	BPDR (21 CFR 606.171) for previously distributed product	Recipient Tracing/ Notification
Diagnosed with vCJD or CJD, or suspected vCJD	Permanent	Immediately retrieve, quarantine, and follow /update SOPs regarding notifying consignees for all in-date products and cellular blood components intended for manufacturing into injectable products.	Yes	Consignee notified, consignee informs responsible caretaker for discretionary recipient notification, counseling
Risk factors for CJD: Receipt of pituitary-derived growth hormone, or dura mater transplant Family history of CJD in >1 family member	Permanent Indefinite; reentry if genetic testing does not reveal CJD-associated prion protein allele**	Immediately retrieve, quarantine, and follow/update SOPs regarding notifying consignees for all in-date products and cellular blood components intended for manufacturing into injectable products.	Yes*	Consignee notified, consignee informs responsible caretaker for discretionary recipient notification, counseling
CJD in only 1 family member	Indefinite; reentry if genetic testing does not reveal CJD-associated prion protein allele	Immediately retrieve, quarantine, and follow/update SOPs regarding notifying consignees for all in-date products and cellular blood components intended for manufacturing into injectable products.	Yes*	No

* As stated in Section V. of this guidance, a BPDR is not required if components were collected prior to the implementation of donor deferral.
** Note that gene sequencing of the donor is not necessary to demonstrate that the donor is not at risk for familial CJD. Sequencing of the family member with CJD or the appropriate parent of the donor, if the CJD-affected family member was a second-degree relative, may be sufficient to demonstrate that the donor does not have a mutation associated with familial CJD.

Risk	Deferral	Disposition of Product And Consignee Notification	BPDR (21 CFR 606.171) for previously distributed product	Recipient Tracing/ Notification
Geographic donor deferrals (U.K. ≥3 months 1980-1996; France ≥5 years 1980-present; military in Europe as specified)	Indefinite	Immediately retrieve, quarantine, and follow/update SOPs regarding notifying consignees for all in-date products and cellular blood components intended for manufacturing into injectable products.	Yes*	No
Geographic donor deferrals (Europe other than U.K. ≥5 years 1980-present)	Indefinite	Immediately retrieve, quarantine, and follow/update SOPs regarding notifying consignees for all in-date products and cellular blood components intended for manufacturing into injectable products.	Yes*	No
Bovine insulin injection	Indefinite, donor may be re-entered after proof of non-U.K. insulin source	Immediately retrieve, quarantine, and follow/update SOPs regarding notifying consignees for all in-date products and cellular blood components intended for manufacturing into injectable products.	Yes*	No
Transfusion in U.K. or in France from Jan 1, 1980 to the present	Indefinite	Immediately retrieve, quarantine, and follow/update SOPs regarding notifying consignees for all in-date products and cellular blood components intended for manufacturing into injectable products.	Yes*	No

APPENDIX TABLE 2: DONOR DEFERRAL, PRODUCT DISPOSTION, AND RECIPIENT NOTIFICATION FOR SOURCE PLASMA (SP), RECOVER PLASMA (RP) AND PLASMA DERIVATIVES (PD)

Risk	Deferral	Disposition of Product And Consignee Notification	BPDR (21 CFR 606.171 or 600.14) for previously distributed product	Recipient Tracing/ Notification
Diagnosed with vCJD, suspected vCJD	Permanent	SP and RP: Immediately retrieve, quarantine, and follow/update SOPs regarding notifying consignees for in-date SP and all RP PD: Immediately retrieve, quarantine, and follow/update SOPs regarding notifying consignees	SP and RP: Yes PD: Yes	Consignee notified, consignee informs responsible caretaker for discretionary recipient notification, counseling
Diagnosed with CJD (and age <55)	Permanent	SP and RP: Immediately retrieve, quarantine, and follow/update SOPs regarding notifying consignees for in-date SP and all RP PD: Disposition decided case-by-case depending upon investigation results	SP and RP: Yes PD: Decided upon case-by-case	Case-by-case recommendation, depending upon investigation results
Diagnosed CJD (and age ≥55)	Permanent	SP and RP: Immediately retrieve, quarantine, and follow/update SOPs regarding notifying consignees for in-date SP and all RP unless plasma known to be previously pooled PD: No retrieval, quarantine, consignee notification	SP and RP: Yes PD: No	SP and RP: N/A PD: No

Risk	Deferral	Disposition of Product And Consignee Notification	BPDR (21 CFR 606.171, 600.14) for previously distributed product	Recipient Tracing/ Notification
Risk factors for CJD: Receipt of pituitary-derived growth hormone, or dura mater transplant Family history of CJD in >1 family member	Permanent Indefinite	SP and RP: Immediately retrieve, quarantine, and follow/update SOPs regarding notifying consignees for in-date SP and all RP unless plasma known to be previously pooled PD: No retrieval, quarantine, consignee notification	SP and RP: Yes* PD: No	SP and RP: N/A PD: No
CJD in only 1 family member	Indefinite; reentry if genetic testing does not reveal CJD-associated prion protein allele**	SP and RP: Immediately retrieve, quarantine, and follow/update SOPs regarding notifying consignees for in-date SP and all RP unless plasma known to be previously pooled PD: No retrieval, quarantine, consignee notification	SP and RP: Yes* PD: No	SP and RP: N/A PD: No
Geographic donor deferrals (U.K. ≥3 months 1980-1996; France ≥5 years 1980-present; military in Europe as specified, transfusion in U.K. or France since 1980)	Indefinite	SP and RP: Immediately retrieve, quarantine, and follow/update SOPs regarding notifying consignees for in-date SP and all RP unless plasma known to be previously pooled PD: No retrieval, quarantine, consignee notification	SP and RP: Yes* PD: No	SP and RP: N/A PD: No

Risk	Deferral	Disposition of Product	BPDR (21 CFR 606.171, 600.14) for previously distributed product	Consignee Notification
Geographic donor deferrals (Europe other than U.K.. ≥5 years 1980-present)	RP: Indefinite SP: No deferral	RP: Immediately retrieve, quarantine, and update/follow SOPs regarding notifying consignees unless plasma known to be previously pooled SP: N/A PD: No retrieval, quarantine, notification of consignee	RP: Yes* SP: N/A PD: No	RP: N/A SP: N/A PD: No
Bovine insulin injection	Indefinite	SP and RP: Immediately retrieve, quarantine, and update/follow SOPs regarding notifying consignees for all RP and for in-date SP unless plasma known to be previously pooled PD: No retrieval, quarantine, notification of consignee	SP and RP: Yes* PD: No	SP and RP:N/A PD: No

* As stated in Section V. of this guidance, a BPDR is not required if components were collected prior to the implementation of donor deferral.
** Note that gene sequencing of the donor is not necessary to demonstrate that the donor is not at risk for familial CJD. Sequencing of the family member with CJD or the appropriate parent of the donor, if the CJD-affected family member was a second-degree relative, may be sufficient to demonstrate that the donor does not have a mutation associated with familial CJD.

医薬品 研究報告 調査報告書

識別番号・報告回数		回	報告日 年 月 日	第一報入手日 2015年10月5日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	[1] Weekly Disease Information 24 September 2015 18/09/2015: Bovine spongiform encephalopathy, Slovenia. （Immediate notification） 2015;28(39) [2] Weekly Disease Information 1 October 2015 24/09/2015: Bovine spongiform encephalopathy, Slovenia. （Follow-up report No. 1） 2015;28(40)	公表国 スロベニア	
販売名（企業名）						

研究報告の概要

問題点：スロベニアのウシ1頭（12歳齢）において非定型のウシ海綿状脳症（H-BSE）が確認された。

【概要】スロベニアにおける非定型BSE（H-BSE）に関する情報。

[1] OIE Web Site（2015年9月24日付）、[2] OIE Web Site（2015年10月1日付）

2015年9月29日付でスロベニア当局（Ministry of Agriculture, forestry and food）は、国際獣疫事務局（OIE）に対して、ノヴォ・メストのAdlesiciにある農場で死亡したウシ1頭（12歳齢）において、非定型BSE（H-BSE）が確認されたと報告した。

当該ウシは農場で死亡した12歳齢の乳牛であり、2015年9月16日にスロベニアの国立獣医学研究所（National Veterinary Institute）で実施された検査結果（迅速検査陽性、ウェスタンブロット法陽性）にてBSEが確定された。その後、当該ウシの検体は型別判定検査のため、英国ウェーブリッジにあるEUリファレンスラボラトリー（European Union Reference Laboratory）である動植物衛生局（Animal and Plant Health Agency）に送付され、最終検査にて非定型BSE（H-BSE）陽性であることが確認された。
当該ウシと同じ農場で出生した4頭のうち3頭は2005年と2012年にと畜解体処理が行われていたが、残りの1頭は本BSE発生時に他の農場で飼養されており、2015年9月29日に殺処分が行われた。本疾患の発生源または感染源は未確定であるとされている。

使用上の注意記載状況・その他参考事項等

BYL-2016-0433

[1] Weekly Disease Information 24 September 2015
18/09/2015: Bovine spongiform encephalopathy, Slovenia.
（Immediate notification）
2015;28(39)

[2] Weekly Disease Information 1 October 2015
24/09/2015: Bovine spongiform encephalopathy, Slovenia.
（Follow-up report No. 1）
2015;28(40)

報告企業の意見	今後の対応
本件は、スロベニアのウシ1頭（12歳齢）において非定型のウシ海綿状脳症（H-BSE）が確認されたことに関して国際獣疫事務局が発表したものである。本疾患の発生源または感染源は未確定であるとされている。 コージネイトFSの製造工程においてアフィニティークロマトグラフィーを用いており、このリガンドであるマウスIgGモノクロナール抗体産生細胞の培養液にウシインスリンが添加されている。このウシインスリンの一連の製造・精製工程は細菌やウイルスを高率に除去できることが確認されている。なお、2007年4月以降、コージネイトFSの販売は行っていない。	現時点で新たな安全対策上の措置を講じる必要はないと考える。 今後も、新規人畜共通感染症や新たなウイルス感染症に関する情報収集に努める。

MedDRAバージョン19.0

BYL-2016-0433

Bovine spongiform encephalopathy, Slovenia

Print
Close

Information received on 18/09/2015 from Mrs Simona Salamon, Head of Audit, Administration Directorate, Ministry of Agriculture, forestry and food, Administration of the Republic of Slovenia for food safety, veterinary sector and plant protection, LJUBLJANA, Slovenia

Summary

Report type	Immediate notification
Date of start of the event	16/09/2015
Date of confirmation of the event	16/09/2015
Report date	18/09/2015
Date submitted to OIE	18/09/2015
Date event resolved	29/09/2015
Reason for notification	Reoccurrence of a listed disease
Date of previous occurrence	2007
Manifestation of disease	Clinical disease
Causal agent	Prion
Nature of diagnosis	Laboratory (advanced)
This event pertains to	the whole country
Related reports	Immediate notification (18/09/2015) Follow-up report No. 1 (29/09/2015)

New outbreaks (1)

Outbreak 1 (1/2015)	Adlesici, NOVO MESTO
Date of start of the outbreak	16/09/2015
Outbreak status	Resolved (29/09/2015)
Epidemiological unit	Backyard

Affected animals

Species	Susceptible	Cases	Deaths	Destroyed	Slaughtered
Cattle	2	1	1	1	0

Affected population: Bovine spongiform encephalopathy was confirmed in a 12-year-old cow, which died on the farm. The cohort included four animals, three of them were slaughtered in 2005 and 2012 and one animal was still alive and kept in another holding. This animal was killed on 29 September 2015.

Summary of outbreaks: Total outbreaks: 1

Total animals affected

Species	Susceptible	Cases	Deaths	Destroyed	Slaughtered
Cattle	2	1	1	1	0

Outbreak statistics

Species	Apparent morbidity rate	Apparent mortality rate	Apparent case fatality rate	Proportion susceptible animals lost*
Cattle	50.00%	50.00%	100.00%	100.00%

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

Epidemiology

Source of the outbreak(s) or origin of infection	Unknown or inconclusive
Epidemiological comments	The sample will be sent to the European Union Reference Laboratory, Animal and Plant Health Agency (APHA), Weybridge (United Kingdom) for BSE typing.

Control measures

Measures applied	Traceability No vaccination No treatment of affected animals
Measures to be applied	Stamping out

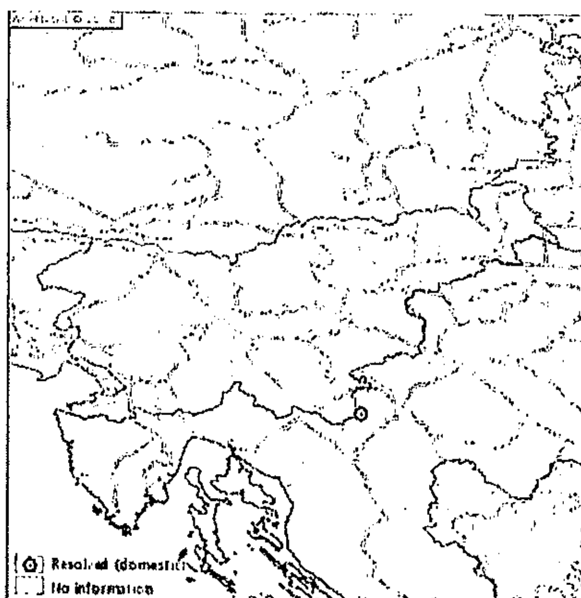
Diagnostic test results

Laboratory name and type	Species	Test	Test date	Result
National Veterinary Institute (National laboratory)	Cattle	rapid tests	16/09/2015	Positive
National Veterinary Institute (National laboratory)	Cattle	western blot	16/09/2015	Positive

Future Reporting

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

Map of outbreak locations



Bovine spongiform encephalopathy,
Slovenia

Print
Close

Information received on 29/09/2015 from Mrs Simona Salamon, Head of Audit, Administration Directorate, Ministry of Agriculture, forestry and food, Administration of the Republic of Slovenia for food safety, veterinary sector and plant protection, LJUBLJANA, Slovenia

Summary

Report type	Follow-up report No. 1 (Final report)
Date of start of the event	16/09/2015
Date of confirmation of the event	16/09/2015
Report date	29/09/2015
Date submitted to OIE	29/09/2015
Date event resolved	29/09/2015
Reason for notification	Reoccurrence of a listed disease
Date of previous occurrence	2007
Manifestation of disease	Clinical disease
Causal agent	Prion
Nature of diagnosis	Laboratory (advanced)
This event pertains to	the whole country
Related reports	Immediate notification (18/09/2015) Follow-up report No. 1 (29/09/2015)

Outbreaks There are no new outbreaks in this report

Epidemiology

Source of the outbreak(s) or origin of infection	Unknown or inconclusive
Epidemiological comments	European Union Reference Laboratory, Animal and Plant Health Agency (APHA), Weybridge (United Kingdom) informed about final results - positive for atypical H-type BSE.

Control measures

Measures applied	Traceability Stamping out No vaccination No treatment of affected animals
Measures to be applied	No other measures

Future Reporting

The event is resolved. No more reports will be submitted.

別紙様式第２－１

2

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2016年2月9日	該当なし。	
一般的名称	別紙のとおり。		研究報告の公表状況	Published online February5, 2016 http://dx.doi.org/10.1016/s1473-3099(15)00464-8	公表国 米国	
販売名（企業名）	別紙のとおり。					

研究報告の概要	使用上の注意記載状況・その他参考事項等
問題点：ヒトにライム病を引き起こす新たな原因菌種が発見された。 米CDCは，Mayo Clinicおよび米Minnesota，Wisconsin，North Dakotaの保健当局者と共同で，ヒトにライム病を引き起こす新種の菌（Borrelia mayonii）の発見について報告した。これまで北米でライム病を引き起こすと考えられている種はBorrelia burgdorferiのみであった。 Minnesota，RochesterのMayo Clinicの科学者は，ライム病が疑われるヒト6例でこれまでのボレリア菌種では見られなかった症状等（スピロヘータ血症、遊走性紅斑→バラ疹、39℃≦、嘔気・嘔吐）が見られた後，新たな菌の可能性を疑った。Mayo ClinicとCDCにおける追加の遺伝子検査により，暫定的にBorrelia mayoniiと名付けられた菌がB. burgdorferiの近縁種であることが判明した。	記載なし。

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

MedDRA/J ver.18.1

27

別紙

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤人免役グロブリン、⑥乾燥ペプシン処理人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン、⑧乾燥スルホ化人免疫グロブリン、⑨乾燥スルホ化人免疫グロブリン、⑩乾燥スルホ化人免疫グロブリン、⑪乾燥スルホ化人免疫グロブリン*、⑫乾燥濃縮人活性化プロテインC、⑬乾燥濃縮人血液凝固第Ⅷ因子、⑭乾燥濃縮人血液凝固第Ⅷ因子、⑮乾燥濃縮人血液凝固第Ⅷ因子、⑯乾燥濃縮人血液凝固第Ⅸ因子、⑰乾燥濃縮人血液凝固第Ⅸ因子、⑱乾燥濃縮人血液凝固第Ⅸ因子、⑲乾燥抗破傷風人免疫グロブリン、⑳抗 HBs 人免疫グロブリン、㉑抗 HBs 人免疫グロブリン、㉒抗 HBs 人免疫グロブリン、㉓トロンビン、㉔トロンビン、㉕トロンビン、㉖フィブリノゲン加第XⅢ因子*、㉗フィブリノゲン加第XⅢ因子、㉘乾燥濃縮人アンチトロンビンⅢ、㉙ヒスタミン加人免疫グロブリン製剤、㉚人血清アルブミン*、㉛人血清アルブミン*、㉜乾燥ペプシン処理人免役グロブリン*、㉝乾燥濃縮人アンチトロンビンⅢ、㉞乾燥濃縮人血液凝固第X因子加活性化第Ⅶ因子
販売名(企業名)	①献血アルブミン 20 "化血研"、②献血アルブミン 25 "化血研"、③人血清アルブミン "化血研" *、④ガンマーグロブリン筋注 450mg/3mL「化血研」、⑤ガンマーグロブリン筋注 1500mg/10mL「化血研」、⑥献血グロブリン注射用 2500mg「化血研」、⑦献血ベニロン－Ⅰ静注用 500mg、⑧献血ベニロン－Ⅰ静注用 1000mg、⑨献血ベニロン－Ⅰ静注用 2500mg、⑩献血ベニロン－Ⅰ静注用 5000mg、⑪ベニロン*、⑫注射用アナクトC2,500 単位、⑬コンファクトF注射用 250、⑭コンファクトF注射用 500、⑮コンファクトF注射用 1000、⑯ノバクトM静注用 400 単位、⑰ノバクトM静注用 800 単位、⑱ノバクトM静注用 1600 単位、⑲テタノセーラ筋注用 250 単位、⑳ヘパトセーラ筋注 200 単位/mL、㉑ヘパトセーラ筋注 200 単位/1mL、㉒ヘパトセーラ筋注 1000 単位/5mL、㉓トロンビン "化血研"、㉔献血トロンビン経口・外用5千「化血研」、㉕献血トロンビン経口・外用1万「化血研」、㉖ボルヒール*、㉗ボルヒール組織接着用、㉘アンスロビンP500 注射用、㉙ヒスタグロビン皮下注用、㉚アルブミン 20%化血研*、㉛アルブミン 5%化血研*、㉜静注グロブリン*、㉝アンスロビンP1500 注射用、㉞バイクロット配合静注用、㉟ノバクトM静注用 500 単位、㊱ノバクトM静注用 1000 単位、㊲ノバクトM静注用 2000 単位
報告企業の意見	ライム病の病原体であるBorreliaは、全長約 10μm、直径 0.2-0.3μm の螺旋状のスピロヘータで、本病を引き起こすものが B. burgdorferi とされている。今回、ライム病が疑われる患者より、新たな原因菌として Borrelia mayonii(暫定的名称)が発見されたことが報告された。遺伝子検査で B. burgdorferi の近縁種であることが判明しているが、本病の新たな原因菌として注視していく必要がある。 弊所で製造している全ての血漿分画製剤の製造工程には、約 0.2μm の無菌ろ過工程およびウイルスの除去を目的としたウイルス除去膜ろ過工程が導入されているので、仮に製造原料に *Borrelia mayonii* が混入していたとしても、これらの工程により除去されるものと考えられる。更に、これまでに上記製剤によるライム病の報告例は無い。 以上の点から、上記製剤は Borrelia mayonii 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

INF2015－006

Identification of a novel pathogenic *Borrelia* species causing Lyme borreliosis with unusually high spirochaetaemia: a descriptive study

Bobbi S Pritt, Paul S Mead, Diep K Hoang Johnson, David F Neitzel, Laurel B Respicio-Kingry, Jeffrey P Davis, Elizabeth Schiffman, Lynne M Sloan, Martin E Schriefer, Adam J Replogle, Susan M Paskewitz, Julie A Ray, Jenna Bjork, Christopher R Steward, Alecia Deedon, Xia Lee, Luke C Kingry, Tracy K Miller, Michelle A Feist, Elitza S Theel, Robin Patel, Cole L Irish, Jeannine M Petersen

Summary

Background Lyme borreliosis is the most common tick-borne disease in the northern hemisphere. It is a multisystem disease caused by *Borrelia burgdorferi* sensu lato genospecies and characterised by tissue localisation and low spirochaetaemia. In this study we aimed to describe a novel *Borrelia* species causing Lyme borreliosis in the USA.

Methods At the Mayo clinic, from 2003 to 2014, we tested routine clinical diagnostic specimens from patients in the USA with PCR targeting the *oppA1* gene of *B burgdorferi* sensu lato. We identified positive specimens with an atypical PCR result (melting temperature outside of the expected range) by sequencing, microscopy, or culture. We collected *Ixodes scapularis* ticks from regions of suspected patient tick exposure and tested them by *oppA1* PCR.

Findings 100 545 specimens were submitted by physicians for routine PCR from Jan 1, 2003 to Sept 30, 2014. From these samples, six clinical specimens (five blood, one synovial fluid) yielded an atypical *oppA1* PCR product, but no atypical results were detected before 2012. Five of the six patients with atypical PCR results had presented with fever, four had diffuse or focal rash, three had symptoms suggestive of neurological inclusion, and two were admitted to hospital. The sixth patient presented with knee pain and swelling. Motile spirochaetes were seen in blood samples from one patient and cultured from blood samples from two patients. Among the five blood specimens, the median *oppA1* copy number was 180 times higher than that in 13 specimens that tested positive for *B burgdorferi* sensu stricto during the same time period. Multigene sequencing identified the spirochaete as a novel *B burgdorferi* sensu lato genospecies. This same genospecies was detected in ticks collected at a probable patient exposure site.

Interpretation We describe a new pathogenic *Borrelia burgdorferi* sensu lato genospecies (candidatus *Borrelia mayonii*) in the upper midwestern USA, which causes Lyme borreliosis with unusually high spirochaetaemia. Clinicians should be aware of this new *B burgdorferi* sensu lato genospecies, its distinct clinical features, and the usefulness of *oppA1* PCR for diagnosis.

Funding US Centers for Disease Control and Prevention Epidemiology and Laboratory Capacity for Infectious Diseases (ELC) Cooperative Agreement and Mayo Clinic Small Grant programme.

Lancet Infect Dis 2016
Published Online
February 5, 2016
http://dx.doi.org/10.1016/S1473-3099(15)00464-8

See Online/Comment
http://dx.doi.org/10.1016/S1473-3099(15)00483-1

Mayo Clinic, Rochester, MN, USA (B S Pritt MD, L M Sloan MT, E S Theel PhD, Prof R Patel MD, C L Irish BS, J A Ray MPH); Division of Vector Borne Diseases, US Centers for Disease Control and Prevention, Fort Collins, CO, USA (P S Mead MD, L B Respicio-Kingry MS, L C Kingry PhD, M E Schriefer PhD, A J Replogle BS, J M Petersen PhD); Wisconsin Department of Health Services, Madison, WI, USA (D K Hoang Johnson BS, Prof J P Davis MD, C R Steward MPH, A Deedon BS); Minnesota Department of Health, St Paul, MN, USA (D F Neitzel MS, E Schiffman MPH, J A Ray, J Bjork DVM); University of Wisconsin-Madison, Madison, WI, USA (Prof S M Paskewitz PhD, X Lee MS); and North Dakota Department of Health, Bismarck, ND, USA (T K Miller PhD, M A Feist BS)

Correspondence to:
Bobbi S Pritt, Mayo Clinic, Division of Clinical Microbiology, Rochester, MN 55905, USA
pritt.bobbi@mayo.edu

Introduction

Lyme borreliosis is a spirochaetal tick-borne disease caused by some genospecies of the *Borrelia burgdorferi* sensu lato complex.[1–4] With 85 000 cases estimated annually in Europe and 300 000 cases estimated annually in the USA, it is the most common tick-borne disease in the northern hemisphere.[5,6] Nearly all human infections are caused by three *B burgdorferi* sensu lato genospecies: *Borrelia garinii*, *Borrelia afzelii*, and *B burgdorferi* sensu stricto.[1] All three species cause Lyme borreliosis in Europe, whereas only *B burgdorferi* sensu stricto causes Lyme borreliosis in the USA.[7]

The clinical features of Lyme borreliosis are broad and seem to be associated with distinct tissue tropisms of specific *B burgdorferi* sensu lato genospecies.[8] Early localised infection typically results in erythema migrans rash, after which spirochaetes can disseminate to the nervous system, joints, and other organs. *B burgdorferi* sensu stricto is often associated with arthritis, *B garinii* with neurological effects, and *B afzelii* with acrodermatitis chronicum atrophicans.[8]

Lyme borreliosis is characterised by a low level of spirochaetaemia.[9] Spirochaetes are detectable by PCR in the peripheral blood of less than 50% of patients with erythema migrans, with average estimation of about 2330 genome copies per mL,[9,10] whereas the mean number of spirochaetes detected by culture of peripheral blood is only 0·1 spirochaetes per mL.[9] As expected, microscopic detection of *B burgdorferi* sensu lato spirochaetes has never been reported in peripheral blood, by marked contrast with relapsing fever borreliae, which have loads ranging from 10^5 spirochaetes per mL to more than 10^6 spirochaetes per mL, and are readily seen in peripheral blood.[11] We describe a new *B burgdorferi* sensu lato genospecies causing Lyme borreliosis with substantially elevated spirochaetaemia in acutely ill patients.

Research in context

Evidence before this study

Lyme borreliosis is a multisystem tick-borne disease of wide public health significance. It is the most frequently reported vector-borne disease in the temperate northern hemisphere and is caused by spirochaetes in the *Borrelia burgdorferi* sensu lato genospecies complex. There have been no previous descriptions of the pathogenic *Borrelia* species reported in this study in either ticks or human beings, or reports of Lyme borreliosis with high spirochaetaemia.

Added value of this study

The identification of a new *B burgdorferi* sensu lato genospecies causing Lyme borreliosis with substantially elevated spirochaetaemia and clinical features distinct from other *B burgdorferi* sensu lato genospecies has important implications for accurate diagnosis and treatment. In view of the differing clinical manifestations for patients infected with the novel *B burgdorferi* sensu lato genospecies, it is likely that Lyme borreliosis is not being considered in some patients with this infection. The medical and health-care community need to be aware of this new pathogen to recognise the infection and to treat patients appropriately.

Implications of all the available evidence

The discovery of a novel *B burgdorferi* sensu lato genospecies was attributable to the use of a diagnostic real-time PCR assay that detects and differentiates *B burgdorferi* sensu lato genospecies by melting temperature analysis. Those PCR assays designed specifically for detection of a single *B burgdorferi* sensu lato genospecies do not have the same ability to identify new or different genospecies. Since many tick-borne human pathogens have a global distribution (eg, *B burgdorferi*, *Babesia microti*, *Anaplasma phagocytophilum*, *Ehrlichia muris*, and *Borrelia miyamotoi*), the emergence of this pathogen highlights the need for widespread surveillance to look for emergence of this organism or related species in other parts of the world.

Methods

Patients

Mayo Medical Laboratories provides diagnostic PCR testing for Lyme borreliosis.[12] From Nov 1, 2003, to Sept 30, 2014, physicians throughout the USA submitted 100 545 specimens (synovial fluid, cerebrospinal fluid, EDTA [edetic acid]-anticoagulated whole blood, or fresh tissue) to our laboratory for routine clinical PCR testing. We interviewed patients with specimens yielding a PCR result that differed from that expected for *B burgdorferi* sensu stricto, *B garinii*, or *B afzelii*, to obtain clinical and epidemiological information. We reviewed medical records and requested additional samples. Patient follow-up and DNA sequencing of clinical specimens was approved by the Mayo Clinic institutional review board. Patients were interviewed by state public health officials as part of routine surveillance for a reportable condition.

See Online for appendix

Real-time PCR and DNA sequencing

We extracted DNA from diagnostic specimens using the MagNA Pure Instrument (Roche) and tested for *B burgdorferi* sensu stricto, *B afzelii*, and *B garinii* with a diagnostic real-time PCR assay that uses hybridisation probes and targets the chromosomal *oppA1* gene.[12,13] This assay is specific for *B burgdorferi* sensu lato and does not detect relapsing fever borreliae. We subjected PCR products to melting temperature analysis to differentiate *B burgdorferi* sensu lato genospecies (appendix). We established the number of *oppA1* copies with standard

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Demographic features						
Age (years)	10	65	11	21	67	51
Sex	Male	Male	Male	Female	Female	Male
Tick exposure	Probable	Probable	Known bite	Probable	Probable	Known bite
Symptoms						
Fever	Yes	Yes	Yes	No	Yes	Yes
Headache	Yes	Yes	Yes	No	Yes	Yes
Neck pain	Yes	Yes	Yes	No	No	No
Fatigue	Yes	Yes	Yes	No	Yes	No
Myalgia	Yes	Yes	No	No	Yes	Yes
Nausea or vomiting	Yes	No	Yes	No	Yes	Yes
Arthralgia (site)	No	No	No	Yes (left knee)	No	No
Other	Profound somnolence	..	Confused speech	..	Chills, abdominal and lumbar back pain, flashing lights	..

(Table 1 continues on next page)

www.thelancet.com/infection Published online February 5, 2016 http://dx.doi.org/10.1016/S1473-3099(15)00464-8

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
(Continued from previous page)						
Physical findings						
Measured temperature (°C)	40	NA	39·7	Afebrile	38·2	NA
Rash	Many erythematous macules on face, trunk, arms (figure 1A)*	NA	Initial macule, enlarged to erythema migrans; diffuse macular rash after single dose of doxycycline; many erythema migrans 28 days later	NA	Many erythematous macules on trunk and upper extremities	2 × 2 cm macule on leg at site of possible tick bite
Other	..	..	..	Swelling left knee	..	..
Laboratory results [normal range for adults and children aged 10–11 years combined]						
Days of illness before specimen collection for PCR	1	4	2	34	3	1
oppA1 PCR melting temperature (°C)	61·24	60·75	60·83	61·19	60·56	60·38
Crossing point	30·88	29·58	29·82	34·20	26·48	30·63
Estimated number of oppA1 copies per mL	$4{\cdot}2 \times 10^5$	$9{\cdot}4 \times 10^5$	$8{\cdot}1 \times 10^5$	Not determined	$6{\cdot}4 \times 10^6$	$4{\cdot}9 \times 10^5$
White blood cell count ($\times 10^{9}$/L) [3·4–10·5]	7·4	3·4	4·6	NA	12·4	5·3
Lymphocyte count ($\times 10^{9}$/L) [0·90–6·50]	0·74	0·31	0·44	NA	0·93	0·30
Platelet count ($\times 10^{9}$/L) [150–450]	184	113	122	NA	215	150
Haemoglobin (g/dL) [12·0–17·5]	14	NA	14·7	NA	9·6	15·5
Aspartate aminotransferase (U/L) [8–60]	46	NA	NA	NA	118	23
Alanine aminotransferase (U/L) [7–55]	33	NA	NA	NA	69	27
Treatment and outcome						
Antimicrobial therapy	Ceftriaxone (1 day), amoxicillin (21 days; dosage NA)	Doxycycline (dosage and duration NA)	Initial treatment: doxycycline (discontinued after 1 × 50 mg dose)†	Initial treatment: doxycycline (100 mg twice per day for 28 days)‡	Doxycycline (100 mg twice per day for 21 days)	Doxycycline (100 mg twice per day for 14 days)
Hospital admission	4 Days	No	No	No	1 day	No
Outcome	Recovered	Recovered	Recovered	Persistent joint pain	Improved, lingering fatigue (pre-existing anaemia)	Recovered

Clinical findings and symptoms were recorded by medical staff at time of initial patient presentation. NA=not available. *Rash was reported by patient's caregiver to involve the palms and soles, but this was not documented in the medical record. †Subsequent treatment for patient 3 was initiated 3 weeks after illness onset, and consisted of cefuroxime, 500 mg twice per day for 21 days. ‡For patient 4, subsequent treatment consisted of amoxicillin, 500 mg three times per day for 21 days.

Table 1: Demographic, clinical, and laboratory features in patients infected with suspected novel *B burgdorferi* sensu lato genospecies

curves that were prepared with genomic DNA from *B burgdorferi* sensu stricto B31 and the MN14-1420 isolate; *oppA1* is present on the chromosome in a single copy in both genospecies. We used the Wilcoxon rank-sum test (two-sided) for *oppA1* PCR crossing point comparison.

We amplified and sequenced portions of the 16S rDNA, *ospC*, *flaB*, *rrf-rrl*, *oppA1*, *uvrA*, *rplB*, *recG*, *pyrG*, *pepX*, *clpX*, *nifS*, and *clpA* genes using previously described primers.[12,14–17] We analysed, assembled, and trimmed sequences in Lasergene v9·0 (DNASTAR). Using BLAST, 16S rDNA, *ospC*, *flaB*, and *rrf-rrl* sequences were compared with *B burgdorferi* sensu lato and relapsing fever borreliae sequences in GenBank. For construction of phylogenetic trees, we obtained homologous *B burgdorferi* sensu lato and relapsing fever borreliae sequences from GenBank and PubMLST (appendix). We used MEGA 5 (ClustalW) to align sequences and trees constructed by maximum likelihood analysis using the generalised time-reversible nucleotide substitution model with gamma distribution (four categories) followed by bootstrap analysis (1000 replicates).[18] Housekeeping genes were concatenated in frame in the order *uvrA*, *rplB*, *recG*, *pyrG*, *pepX*, *clpX*, and *clpA*, with or without *nifS* and exported into MEGA 5 to calculate pairwise genetic distances using the Kimura-2 model.

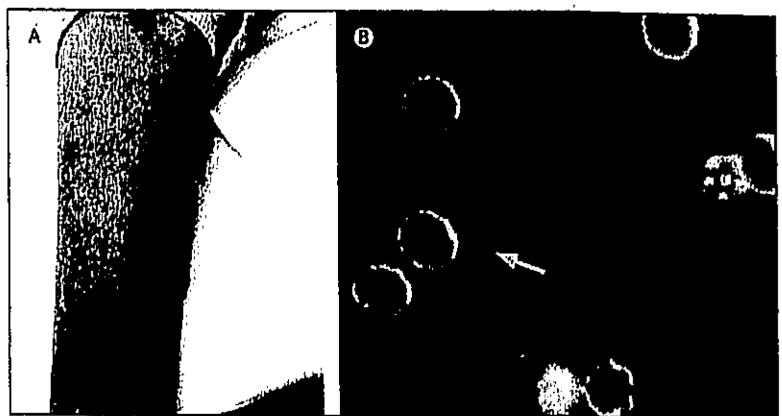


Figure 1: Diffuse macular rash in patient 1 and dark-field microscopic visualisation of a spirochaete in patient 6. (A) Diffuse macular rash seen 4 days after onset of symptoms in patient 1. Rash was reported by patient's caregiver to involve the palms and soles, but this was not documented in the medical record. (B) Dark-field microscopic visualisation (400× magnification) of a single spirochaete in diluted blood from patient 6.

For more on PubMLST see http://pubmlst.org/borrelia/

Microscopy and culture

Two clinical blood specimens with an atypical *oppA1* PCR melting temperature were available for microscopy and culture after storage at 4°C for either 5 days (one sample) or 39 days (one sample) (appendix). Other specimens were unavailable or previously frozen and not amenable to microscopy and culture. We examined wet mounts from patient samples and cultures with dark-field microscopy at 400 times magnification (appendix).

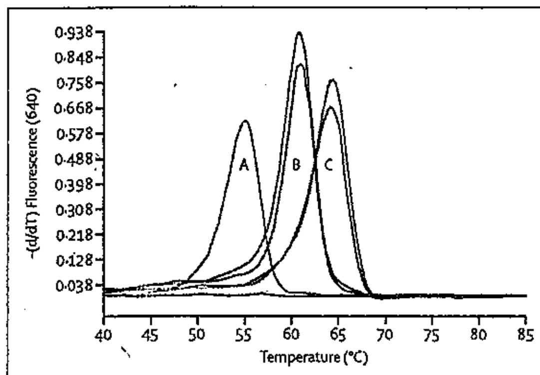


Figure 2: Representative *oppA1* PCR melting temperature peaks of *Borrelia* genospecies
Representative melting temperature peaks in °C for *B afzelii* (A peak; acceptable range 51·7–56·7°C), novel *B burgdorferi* sensu lato genospecies (B peaks; 60·38–61·24°C), and *B burgdorferi* sensu stricto (C peaks; 61·7–66·7°C). Y-axis represents the negative derivative of the ratio of the FRET signal (LC-Red640 flouresence) and background fluorescein fluorescence.

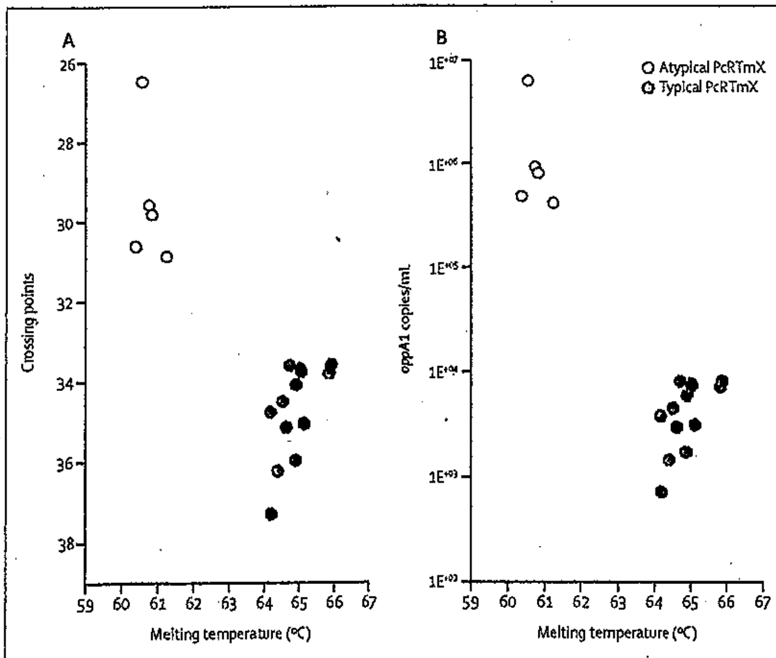


Figure 3: Comparison of *oppA1* PCR melting temperature, crossing points, and estimated *oppA1* copy number in *B burgdorferi*-positive blood specimens
Comparison of melting temperature and crossing point for the five atypical *oppA1* PCR positive blood specimens (open circles) and 13 *B burgdorferi* sensu stricto *oppA1* PCR positive blood specimens (closed circles). (B) Comparison of melting temperature and estimated *oppA1* copy number (genomes per mL of blood) for five atypical *oppA1* PCR positive blood specimens (open circles) and 13 *B burgdorferi* sensu stricto *oppA1* PCR positive blood specimens (closed circles).

Serological testing

We tested serum samples and plasma samples for antibodies reacting to *B burgdorferi* sensu stricto using FDA-cleared commercially available kits, following the recommended two-tiered algorithm (appendix).[19]

Tick collection and processing

We collected *Ixodes scapularis* ticks at approximate sites of possible patient exposure in Barron County, WI, USA, during 2013 and 2014 (appendix), and processed the ticks for PCR using a modification of a published protocol.[20] We also tested archived DNA from *I scapularis* collected in Eau Claire County, WI, USA, during 2009–10.

Role of the funding source

The funders of the study, Mayo Clinic and the US Centers for Disease Control and Prevention, had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

From Jan 1, 2012, to Sept 30, 2014, 9197 clinical specimens from residents of Minnesota, Wisconsin, and North Dakota were submitted to Mayo Clinic, Rochester, MN, USA, for routine diagnostic *B burgdorferi* sensu lato *oppA1* PCR testing. 3127 (34%) of 9197 specimens tested were blood samples, 1196 (13%) were synovial fluid, 4782 (52%) were cerebrospinal fluid, and 92 (1%) were tissue. 102 specimens were positive for *B burgdorferi*, including 13 blood, 81 synovial fluid, three cerebrospinal fluid, and five tissue samples. Six specimens (five blood, one synovial fluid) had positive *oppA1* PCR results with an atypical melting temperature (60·38–61·24°C), falling between the expected melting temperature for *B burgdorferi* sensu stricto (61·7–66·7°C) and *B afzelii* or *B garinii* (51·7–56·7°C) (table 1, figure 1A, figure 2, appendix).[12] No atypical melting temperatures were identified among 24786 clinical specimens tested from 44 other states during the same time period (Fisher-Exact, p=0·00039), or among more than 66562 clinical specimens from all states tested by the same method during 2003–11 (0 of 66562 *vs* six of 33983, p=0·00149). The five positive blood specimens with atypical melting temperatures were collected 1–4 days after onset of illness; the synovial fluid specimen was obtained 34 days after onset of illness (table 1). The median *oppA1* PCR crossing point was significantly lower (median 29·82, IQR 28·89–30·75) for the five blood specimens with atypical *oppA1* melting temperature compared with the 13 blood specimens that tested positive for *B burgdorferi* sensu stricto (median 34·51, IQR 33·73–35·55; p=0·0016; figure 3).

Sequence analysis of the atypical *oppA1* PCR products that were directly amplified from three patient specimens identified a *Borrelia* species with 89–95% similarity to *B burgdorferi* sensu lato genospecies (figure 4). Motile spirochaetes (two per 70 fields of diluted blood) of the *B burgdorferi* sensu lato genospecies were microscopically recorded in blood from patient 6, obtained 1 day after illness onset and analysed 6 days later (figure 1B). The number of spirochaetes was estimated at around $8 \cdot 5 \times 10^4$/mL (appendix). No spirochaetes were seen in the haemolysed blood specimen from patient 5. Cultures of the *B burgdorferi* sensu lato genospecies (MN14-1420 and MN14-1539) were established from both available blood specimens (patients 5 and 6) after incubation for about 16 days. Spirochaetes were seen in primary and blind passaged cultures; sustained growth was achieved after cryopreservation and additional passage.

Based on the high quantity of spirochaetes that were detected microscopically, we estimated the number of *oppA1* copies per mL of blood for all 18 specimens that tested positive for *oppA1* PCR identified in Minnesota, Wisconsin, and North Dakota during 2012–14 by comparison of *oppA1* crossing point values to standard curves prepared with *B burgdorferi* sensu stricto B31 or MN14-1420 (appendix). For the five atypical *oppA1* positives, the median *oppA1* copy number was 180 times higher (median $8 \cdot 1 \times 10^5$, IQR $4 \cdot 6 \times 10^5$–$3 \cdot 6 \times 10^6$) when compared with the 13 *B burgdorferi* sensu stricto positives (median $4 \cdot 5 \times 10^3$, IQR $2 \cdot 3 \times 10^3$–$7 \cdot 5 \times 10^3$; figure 3).

Sequence analysis of 16S rRNA (1327 nucleotides), *ospC* (561 base pairs), *flaB* (435 base pairs), and *rrf-rrl* (253 base pairs) amplified from the two blood isolates substantiated that the *Borrelia* species was not identical to any other *Borrelia* species in GenBank (appendix). The closest sequence identity was to *B burgdorferi* sensu lato genospecies at 99% for 16S RNA, 85% for *ospC*, 97% for *flaB*, and 95% for *rrf-rrl*. Multilocus sequence analysis of seven genes, *uvrA*, *rplB*, *recG*, *pyrG*, *pepX*, *clpX*, and *clpA* genes (3774 nucleotides), showed that the

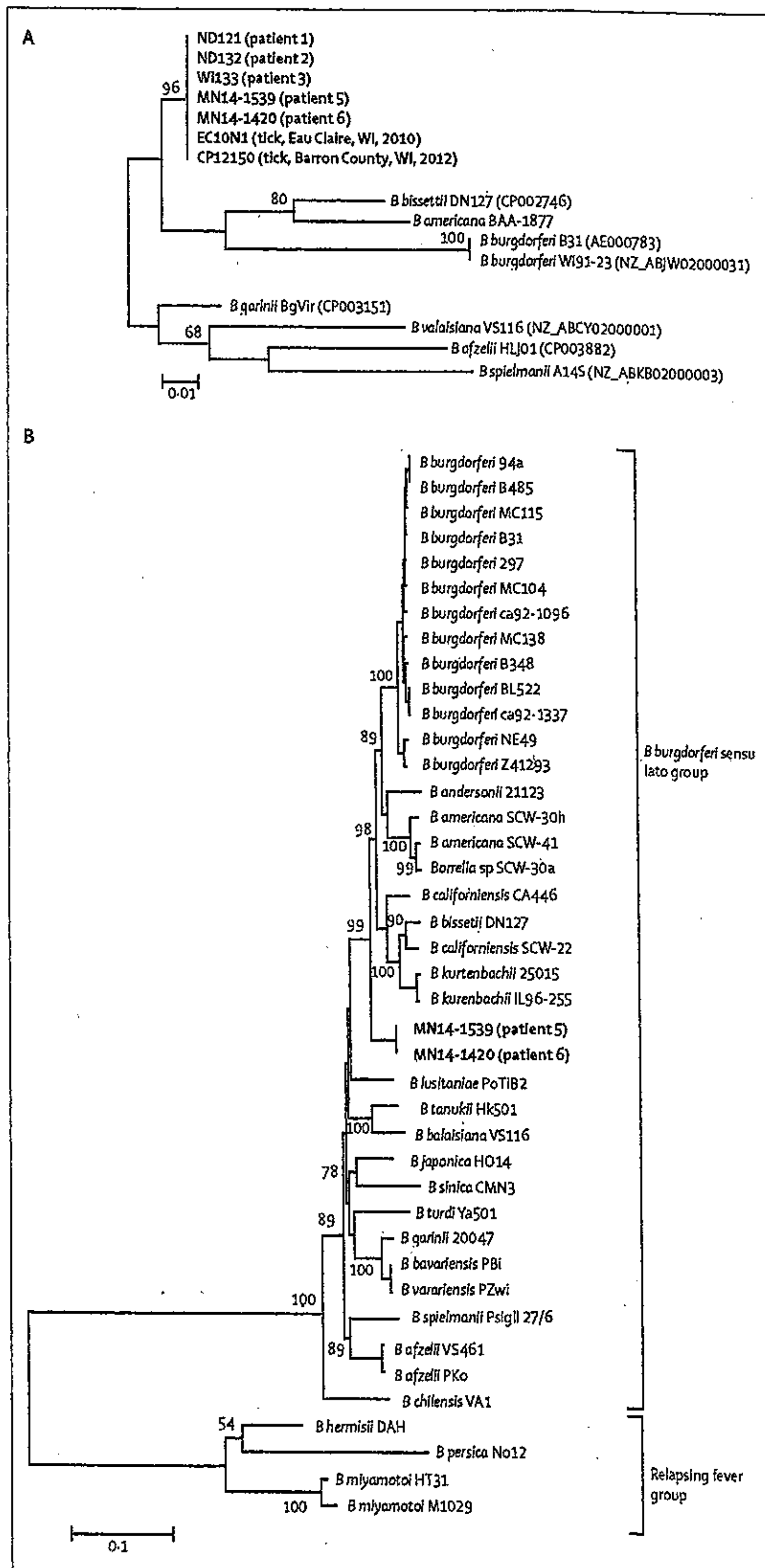


Figure 4: Phylogenetic analyses

(A) Phylogenetic analysis of a 149 base pair fragment of the *oppA1* gene amplified from patient specimens (MN14-1539, MN14-1420, WI133, ND132, and ND121) and tick specimens (CP12150 and EC10N1) compared with seven different species of the *B burgdorferi* sensu lato complex. There is no homologous sequence in relapsing fever borreliae. Bootstrap support values greater than 50% are shown. The scale bar corresponds to 0·01 substitutions per nucleotide position. Accession numbers are indicated for available *Borrelia* species *oppA1* sequences retrieved from GenBank. The *B americana* BAA-1877 *oppA1* gene sequence was generated in this study. GenBank does not allow deposition of sequences shorter than 200 bp; *oppA1* sequences generated in this study are available by request. (B) Phylogenetic analysis of eight concatenated housekeeping genes: *uvrA*, *rplB*, *recG*, *pyrG*, *pepX*, *clpX*, *clpA*, and *nifS*, amplified from patient isolates (MN14-1539, MN14-1420) compared with 18 different *B burgdorferi* sensu lato genospecies and three relapsing fever species. Bootstrap support values greater than 50% are shown. The scale bar corresponds to 0·1 substitutions per nucleotide position. The source of other *Borrelia* species gene sequences is shown in the supplemental methods. Sequence nomenclature (eg, MN14-1539, WI133) represents the state from which the diagnostic specimens were submitted for testing and does not necessarily show the patient's state of residence.

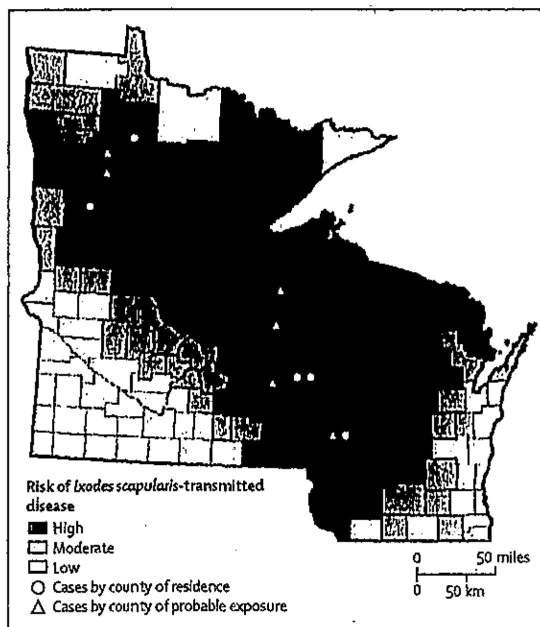


Figure 5: Probable counties of patient exposure to ticks in Minnesota and Wisconsin in relation to risk of diseases transmitted by *I scapularis*
I scapularis-transmitted diseases in the figure were Lyme borreliosis, babesiosis, and anaplasmosis. The county of residence for each patient (indicated with a circle) is deemed a county of potential exposure except for the patient from North Dakota, whose county of residence is not shown. Some patients had probable exposures in one or more county in addition to their county of residence (indicated with a triangle). The risk of disease transmitted by *I scapularis* is based on county-specific mean annual reported incidence of confirmed Lyme borreliosis and confirmed and probable human anaplasmosis and babesiosis in Minnesota and Wisconsin in 2007–13. Counties with 10·0 or fewer cases per 100 000 people were classified as low risk, counties with 10·1–24·9 cases per 100 000 people were classified as moderate risk, and counties with 25·0 or more cases per 100000 people were classified as high risk.

spirochaetes isolated from patients 5 and 6 fell within the *B burgdorferi* sensu lato genospecies complex and were the same *B burgdorferi* sensu lato genospecies amplified from the blood of patients 1, 2, and 3. The blood isolates were further compared with 18 *B burgdorferi* sensu lato genospecies and three relapsing fever borreliae using an eight-gene multilocus sequence analysis (*uvrA, rplB, recG, pyrG, pepX, clpX, clpA,* and *nifS*; 4335 nucleotides) previously described for defining *B burgdorferi* sensu lato genospecies[21] (figure 4B). The highest pairwise similarity was to *B burgdorferi* sensu stricto (94·9–95·2% similarity, genetic distance 0·051–0·048), well above the threshold defined for separating genospecies (98·3% similarity, genetic distance 0·017),[21] substantiating that the organism detected in the six patients is a novel *B burgdorferi* sensu lato genospecies, and not a relapsing fever borrelia (eg, *B miyamotoi*). For comparison, the genetic distance recorded between the novel *B burgdorferi* sensu lato genospecies and *B burgdorferi* sensu stricto B31/Z41293 is greater than that seen between other formally recognised *B burgdorferi* sensu lato genospecies, including *B bissetti* and *B kurtenbachii* (0·035) and *B garinii* and *B bavariensis* (0·018).[14,21]

All patients were residents of the upper midwest (Minnesota, North Dakota, or Wisconsin) (figure 5). Median patient age was 36 years (range 10–67 years); four patients were male (table 1). Five presented with acute febrile illnesses, including four with rash. The sixth patient was afebrile but had a 1-month history of unilateral knee pain and swelling. Two patients were admitted to hospital, but none had a known immunocompromising disorder. Testing showed lymphopenia (four of five tested), mild thrombocytopenia (two of five), and high hepatic transaminases (two of three). All patients reported onset of illness between May and July. Exposure to tick habitats in Minnesota or Wisconsin was reported by all patients and two recalled a tick bite less than 30 days before onset of illness. For one patient, the timing between tick bite and PCR sample acquisition was known to be 13 days.

Descriptions of illness-associated rash varied from diffuse macular rashes involving the face, trunk, and upper extremities (figure 1A, patients 1 and 5) to a single 2 cm diameter erythematous leg lesion at the tick bite location (patient 6; table 1). Patient 3 presented with a single annular erythematous leg lesion with central punctum consistent with erythema migrans, and developed fever, leg and arm pain, and diffuse macular rash on the trunk, upper and lower extremities, and face within 8 h of receiving doxycycline. Differential diagnosis included Jarisch-Herxheimer reaction, drug eruption, and erythema multiforme. Doxycycline was discontinued after one dose. The patient improved without additional treatment, but 3 weeks later developed three erythema migrans lesions on the back and leg, which resolved after treatment with cefuroxime.

All six patients were given antibiotics (table 1). All five patients with febrile illnesses recovered; one with pre-existing anaemia reported continuing fatigue. The patient with arthritis improved but reported persistent joint pain 6 months after treatment. Serum or plasma was available for five patients and was tested for reactivity to *B burgdorferi* sensu stricto antigens using the recommended two-tiered algorithm[19] (table 2). Patients 1, 3, and 4 were seropositive to *B burgdorferi* sensu stricto using this algorithm, including the 30 day cutoff for use of IgM immunoblot. Patient 5 had a positive EIA and IgM immunoblot in a sample obtained 32 days after onset of illness. All four seropositive patients had one or more samples positive using the first-tier C6 EIA; patients 3, 4, and 5 were positive using whole cell EIA. Two seropositive patients (3 and 5) had serial samples and seroconverted from a negative to positive IgM immunoblot. The only specimen from patient 6 was obtained 1 day after illness onset and was seronegative.

Among archived and prospectively collected ticks *I scapularis*, 19 (2·9%, range 0–5·2%) of 658 were *oppA1* PCR positive for the novel *B burgdorferi* sensu lato genospecies and 195 (29·6%, range 9·8–33·3%) of

	Days from onset of illness to collection of specimen	*B burgdorferi* EIA—whole cell	*B burgdorferi* EIA-C6	*B burgdorferi* IgM immunoblot (number of bands detected/number of possible bands); specific antigens detected	*B burgdorferi* IgG immunoblot (number of bands detected/number of possible bands); specific antigens detected
Patient 1	6	Not available	Positive	Positive (2/3); 23, 41	Negative (1/10); 41
Patient 3	2	Not available	Equivocal	Negative (0/3)	Negative (0/10)
Patient 3	29	Not available	Positive	Positive (3/3); 23, 39, 41	Negative (2/10); 23, 41
Patient 3	104	Positive	Positive	Negative (0/3)	Negative (4/10); 18, 23, 39, 41
Patient 4	266	Positive	Positive	Negative (1/3); 23	Positive (5/10); 23, 39, 41, 45, 58
Patient 5 (plasma)	3	Negative	Positive	Negative (0/3)	Negative (0/10)
Patient 5	32	Positive	Positive	Positive (2/3); 23, 39	Negative (2/10); 23, 41
Patient 6 (plasma)	1	Negative	Negative	Negative (0/3)	Negative (1/10); 41

Specimens from patient 2 were not available for testing. IgM immunoblot was deemed second tier positive for *B burgdorferi* if two or more of a possible three bands (21–25 kDA[OspC], 39 kDA[BmpA], and 41 kDA[Fla]) are detected within 30 days of onset.[19] IgG immunoblot was deemed second tier positive for *B burgdorferi* if five or more of a possible ten bands (18 kDa, 21 kDa[OspC], 28 kDa, 30 kDa, 39 kDa[BmpA], 41 kDa[Fla], 45 kDa, 58 kDa[not GroEL], 66 kDa, and 93 kDa) were detected.

Table 2: **Serological test results from patients infected with the novel *B burgdorferi* sensu lato genospecies**

658 were positive for *B burgdorferi* sensu stricto; two were positive for both. Sequence analysis of *oppA1* for two ticks (EC10N1 and CP12150) and seven-gene multilocus sequence analysis for one tick (EC10N1) substantiated that the *B burgdorferi* sensu lato genospecies detected in *I scapularis* was the same identified in patients (figure 4A, appendix).

Discussion

We have identified a new *B burgdorferi* sensu lato genospecies (candidatus *Borrelia mayonii*) among patients and *I scapularis* ticks from the upper midwestern USA. A causal role in the patients' illnesses was suggested by the detection of DNA from this genospecies in patient specimens during acute illness, detection of motile spirochaetes in one blood specimen, culture of the novel *B burgdorferi* sensu lato genospecies from two patient specimens, development of a patient antibody response after illness onset, and clinical improvement after antimicrobial therapy active against other *B burgdorferi* sensu lato genospecies. Failure to identify the organism in more than 90 000 clinical samples tested in previous years and from other states might suggest that this new species recently emerged in the upper midwestern USA.

Using an eight-gene multilocus sequence analysis and a published threshold for delineation of *B burgdorferi* sensu lato genospecies, we showed that the *Borrelia* species is a member of the *B burgdorferi* sensu lato group.

Spirochaetes were seen in the diluted blood of a patient who presented with a single erythema migrans lesion, estimated by microscopy at 10^5 genome copies per mL. The number of genomes in this specimen, based on the single-copy chromosomal gene *oppA1*, was estimated independently at 5×10^5 per mL. Importantly, the median *oppA1* copy number measured for the samples positive for *B burgdorferi* sensu stricto ($4 \cdot 5 \times 10^2$ per mL) agrees with that ($2 \cdot 3 \times 10^2$/mL) recorded previously using quantitative *flaB* PCR, thus supporting the use of the *oppA1* gene for *B burgdorferi* sensu lato quantitation.[10] For all five blood specimens from patients infected with the novel *B burgdorferi* sensu lato genospecies, the number of genomes was estimated to be 10^5–10^6 genome copies per mL. This number is similar to what has been reported for patients infected with relapsing fever borreliae and 50–8000 times higher than the blood specimens that tested positive for *B burgdorferi* sensu stricto during the same time period. The number of spirochaetes as estimated by both microscopy and PCR in blood from patients infected with the novel *B burgdorferi* sensu lato genospecies is greater than previously estimated for *Borrelia miyamotoi* (10^3–10^4 spirochaetes per mL of blood), a relapsing fever borreliae reported to cause human illness in the USA, Europe, and Russia.[22–25] Whether this high spirochaetaemia suggests a different tissue tropism for the new *B burgdorferi* sensu lato genospecies is an important question that needs to be further addressed; five of six novel *B burgdorferi* sensu lato genospecies PCR positives were blood specimens, whereas only 13 (13%) of the 102 *B burgdorferi* sensu stricto PCR positives detected during the same time period were blood, and 81 (79%) of 102 samples were synovial fluids.

Patients infected with the novel *B burgdorferi* sensu lato genospecies presented with differing clinical presentations when compared with patients infected with *B burgdorferi* sensu stricto. At least two patients presented with diffuse macular rash not typical of erythema migrans, including one rash that might have involved the palms and soles. Four patients presented with nausea or vomiting and two with fever over 39°C, symptoms not usually reported for Lyme borreliosis[7,26–28] but often reported among patients infected with relapsing fever borreliae.[29] Similarly, three patients had symptoms potentially consistent with neurological effects (confused speech, profound somnolence, visual difficulties) and two were admitted to hospital.

An important issue raised by identification of the novel *B burgdorferi* sensu lato genospecies is whether existing Lyme borreliosis diagnostic tests can detect infection with this organism. The six patients described here were fortuitously detected during routine clinical testing, because the diagnostic *oppA1* PCR used at Mayo Clinic detects and differentiates *B burgdorferi* sensu lato genospecies by melting temperature analysis. However, it is unknown if diagnostic PCR assays specific for *B burgdorferi* sensu stricto will detect the novel genospecies. Regarding serology, the *B burgdorferi* sensu stricto C6 EIA was positive in all four patients with specimens obtained 3 days or more after onset of illness and *B burgdorferi* sensu stricto IgM immunoblots of specimens obtained 6 to 32 days after onset were positive for three patients. The *B burgdorferi* sensu stricto IgG immunoblot, however, was positive only for the patient with more than 30 days of untreated illness. *B burgdorferi* sensu stricto serology was negative for three specimens drawn 1–3 days after onset of illness.

The patients' infections were probably acquired by the bite of *I scapularis*, which transmits *B burgdorferi* sensu stricto in the USA. Two patients recalled a tick bite before illness and *I scapularis* that tested positive for the new *B burgdorferi* sensu lato genospecies were collected at two Wisconsin locations, including one visited by two patients. Prevalence of the novel species in tested *I scapularis* ranged from 0·6–4·9%. Non-detection of the new *B burgdorferi* sensu lato genospecies in *I scapularis* collected in the midwestern USA during 2004–07 further suggests that it might have recently emerged in this region.[30,31]

The identification of a novel *B burgdorferi* sensu lato genospecies causing Lyme borreliosis with substantially elevated spirochaetaemia and clinical features distinct from other recognised *B burgdorferi* sensu lato genospecies has important implications for accurate diagnosis, treatment and disease reporting. In view of the differing clinical manifestations for patients infected with the novel *B burgdorferi* sensu lato genospecies, it is likely that Lyme borreliosis is not being considered—and therefore not diagnosed—in some patients with this infection. The clinical range of illness must be better defined in additional patients to ensure that physicians can recognise the infection and distinguish it from other tick-borne infections. Many tick-borne pathogens have global distribution, therefore studies are needed to establish the geographic distribution of human beings and ticks infected with the novel *B burgdorferi* sensu lato genopecies. Finally, clinicians should be aware of the potential role of *oppA1* PCR for diagnosing infection with this novel pathogen.

Contributors

BSP and JMP did the literature search, created tables and figures, participated in study design, data collection, analysis and interpretation, co-drafted the manuscript, and edited and approved the final report. PSM, LMS, MES, LBR-K, and AJR contributed to the literature search, helped to create figures, participated in study design, data collection, analysis and interpretation, and culture edited and approved the final manuscript. DKHJ, JPD, DFN, and ES contributed to literature search, helped to create figures, participated in data collection, analysis and interpretation, and edited and approved the final manuscript. SMP, JAR, JB, CRS, AD, XL, TKM, MAF, EST, RP, LCK, and CLI participated in data collection, analysis and interpretation, and edited and approved the final manuscript.

Declaration of interests

BSP, LMS, CLI, EST and RP are employed by Mayo Clinic, which provides commercial PCR and serologic laboratory testing for *Borrelia burgdorferi* and related species through its reference laboratory, Mayo Medical Laboratories. BSP received partial funding for this project from the Mayo Clinic Department of Laboratory Medicine and Pathology Small Grant Program, and all authors from the Minnesota Department of Health (DFN, ES, JAR, JB), Wisconsin Department of Health Services (DKHJ, JPD, CRS, AD), and North Dakota Department of Health (MAF and TKM) received funding from the Centers for Disease Control and Prevention through the Epidemiology and Laboratory Capacity Cooperative Agreement. SMP received funding from the State of Wisconsin through the CDC Epidemiology and Laboratory Capacity Cooperative Agreement. RP reports grant funding from Pfizer, Pradama, Tornier, Astellas, Procared, nanoMR, BioFire, Curetis, 3M, Cubist, Hutchinson Biofilm Medical Solutions, and Accelerate Diagnostics. She receives royalties from Up-To-Date and an editor's stipend and travel reimbursement from the American Society of Microbiology. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Acknowledgments

We would like to thank Marc Dolan, Division of Vector Borne Diseases, Centers for Disease Control and Prevention, Fort Collins, CO; Deke Haefner, Mayo Medical Laboratories, Rochester, MN; Joni J Franson, BS and Scott A Martin, MD, Mayo Clinic Health System–Eau Claire, WI; Amy L Livermore, Mayo Medical Laboratories New England, Andover, MA; Linda Machmueller, Wisconsin Department of Health Services, Madison, WI; Zachary Muehlenbein, St John's University, MN; Kayla Sippl, Jordan Dieckman, Jordan Mandli, Theoren Loo, Nathan Wong, and Ryan Swanson, Tick Surveillance Team at the University of Wisconsin-Madison, Medical Entomology Laboratory, Madison, Wisconsin, USA; and Lindsey Page, North Dakota Department of Health for their contributions to the collection and analysis of data described in this study.

References

1 Baranton G, Postic D, Saint Girons I, et al. Delineation of *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* sp. nov., and group VS461 associated with Lyme borreliosis. *Int J Syst Bacteriol* 1992; 42: 378–83.
2 Steere AC, Bartenhagen NH, Craft JE, et al. The early clinical manifestations of Lyme disease. *Ann Intern Med* 1983; 99: 76–82.
3 Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP. Lyme disease-a tick-borne spirochetosis? *Science* 1982; 216: 1317–19.
4 Johnson RC, Schmid GP, Hyde FW, Steigerwalt AG, Brenner DJ. *Borrelia burgdorferi* sp. nov.: etiologic agent of lyme disease. *Int J Syst Bacteriol* 1984; 34: 496–97.
5 Lindgren E, Jaenson TGT. Lyme borreliosis in Europe. Influences of climate and climate change, epidemiology, ecology and adaptation measures. Copenhagen, Denmark: World Health Organization, 2006.
6 Hinckley AF, Connally NP, Meek JI, et al. Lyme disease testing by large commercial laboratories in the United States. *Clin Infect Dis* 2014; 59: 676–81.
7 Stanek G, Wormser GP, Gray J, Strle F. Lyme borreliosis. *Lancet* 2012; 379: 461–73.
8 van Dam AP, Kuiper H, Vos K, et al. Different genospecies of *Borrelia burgdorferi* are associated with distinct clinical manifestations of Lyme borreliosis. *Clin Infect Dis* 1993; 17: 708–17.
9 Aguero-Rosenfeld ME, Wang G, Schwartz I, Wormser GP. Diagnosis of Lyme borreliosis. *Clin Microbiol Rev* 2005; 18: 484–509.
10 Liveris D, Schwartz I, McKenna D, et al. Quantitation of cell-associated borrelial DNA in the blood of Lyme disease patients with erythema migrans. *Eur J Clin Microbiol Infect Dis* 2012; 31: 791–95.

11 Dworkin MS, Schwan TG, Anderson DE Jr, Borchardt SM. Tick-borne relapsing fever. *Infect Dis Clin North Am* 2008; 22: 449–68, viii.

12 Babady NE, Sloan LM, Vetter EA, Patel R, Binnicker MJ. Percent positive rate of Lyme real-time polymerase chain reaction in blood, cerebrospinal fluid, synovial fluid, and tissue. *Diagn Microbiol Infect Dis* 2008; 62: 464–66.

13 Hu LT, Pratt SD, Perides G, Katz L, Rogers RA, Klempner MS. Isolation, cloning, and expression of a 70-kilodalton plasminogen binding protein of *Borrelia burgdorferi*. *Infect Immun* 1997; 65: 4989–95.

14 Margos G, Hojgaard A, Lane RS, et al. Multilocus sequence analysis of *Borrelia bissettii* strains from North America reveals a new *Borrelia* species, *Borrelia kurtenbachii*. *Ticks Tick Borne Dis* 2010; 1: 151–58.

15 Kugeler KJ, Gurfield N, Creek JG, Mahoney KS, Versage JL, Petersen JM. Discrimination between *Francisella tularensis* and *Francisella*-like endosymbionts when screening ticks by PCR. *Appl Environ Microbiol* 2005; 71: 7594–97.

16 Rudenko N, Golovchenko M, Lin T, Gao L, Grubhoffer L, Oliver JH Jr. Delineation of a new species of the *Borrelia burgdorferi* sensu lato complex, *Borrelia americana* sp. nov. *J Clin Microbiol* 2009; 47: 3875–80.

17 Wang G, Liveris D, Mukherjee P, Jungnick S, Margos G, Schwartz I. Molecular typing of *Borrelia burgdorferi*. *Curr Protoc Microbiol* 2014; 34: 12C.5.1-12C.5.31.

18 Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 2011; 28: 2731–39.

19 Centers for Disease Control and Prevention. Recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease. *MMWR MorB Mortal Wkly Rep* 1995; 44: 590–91.

20 Cao WC, Gao YM, Zhang PH, et al. Identification of *Ehrlichia chaffeensis* by nested PCR in ticks from southern China. *J Clin Microbiol* 2000; 38: 2778–80.

21 Margos G, Vollmer SA, Cornet M, et al. A new *Borrelia* species defined by multilocus sequence analysis of housekeeping genes. *Appl Environ Microbiol* 2009; 75: 5410–16.

22 Molloy PJ, Telford III SR, Chowdri HR, et al. *Borrelia miyamotoi* disease in the northeastern United States: a case series. *Ann Intern Med* 2015; 163: 91–98.

23 Krause PJ, Fish D, Narasimhan S, Barbour AG. *Borrelia miyamotoi* infection in nature and in humans. *Clin Microbiol Infect* 2015; 21: 631–39.

24 Hovius JW, de Wever B, Sohne M, et al. A case of meningoencephalitis by the relapsing fever spirochaete *Borrelia miyamotoi* in Europe. *Lancet* 2013; 382: 658.

25 Platonov AE, Karan LS, Kolyasnikova NM, et al. Humans infected with relapsing fever spirochete *Borrelia miyamotoi*, Russia. *Emerg Infect Dis* 2011; 17: 1816–23.

26 Strle F, Ruzic-Sabljic E, Cimperman J, Lotric-Furlan S, Maraspin V. Comparison of findings for patients with *Borrelia garinii* and *Borrelia afzelii* isolated from cerebrospinal fluid. *Clin Infect Dis* 2006; 43: 704–10.

27 Strle F, Ruzic-Sabljic E, Logar M, et al. Comparison of erythema migrans caused by *Borrelia burgdorferi* and *Borrelia garinii*. *Vector Borne Zoonotic Dis* 2011; 11: 1253–58.

28 Logar M, Ruzic-Sabljic E, Maraspin V, et al. Comparison of erythema migrans caused by *Borrelia afzelii* and *Borrelia garinii*. *Infection* 2004; 32: 15–19.

29 Dworkin MS, Anderson DE Jr, Schwan TG, et al. Tick-borne relapsing fever in the northwestern United States and southwestern Canada. *Clin Infect Dis* 1998; 26: 122–31.

30 Hamer SA, Hickling GJ, Walker ED, Tsao JI. Increased diversity of zoonotic pathogens and *Borrelia burgdorferi* strains in established versus incipient *Ixodes scapularis* populations across the midwestern United States. *Infect Genet Evol* 2014; 27: 531–42.

31 Hoen AG, Margos G, Bent SJ, et al. Phylogeography of *Borrelia burgdorferi* in the eastern United States reflects multiple independent Lyme disease emergence events. *Proc Natl Acad Sci USA* 2009; 106: 15013–38.

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。

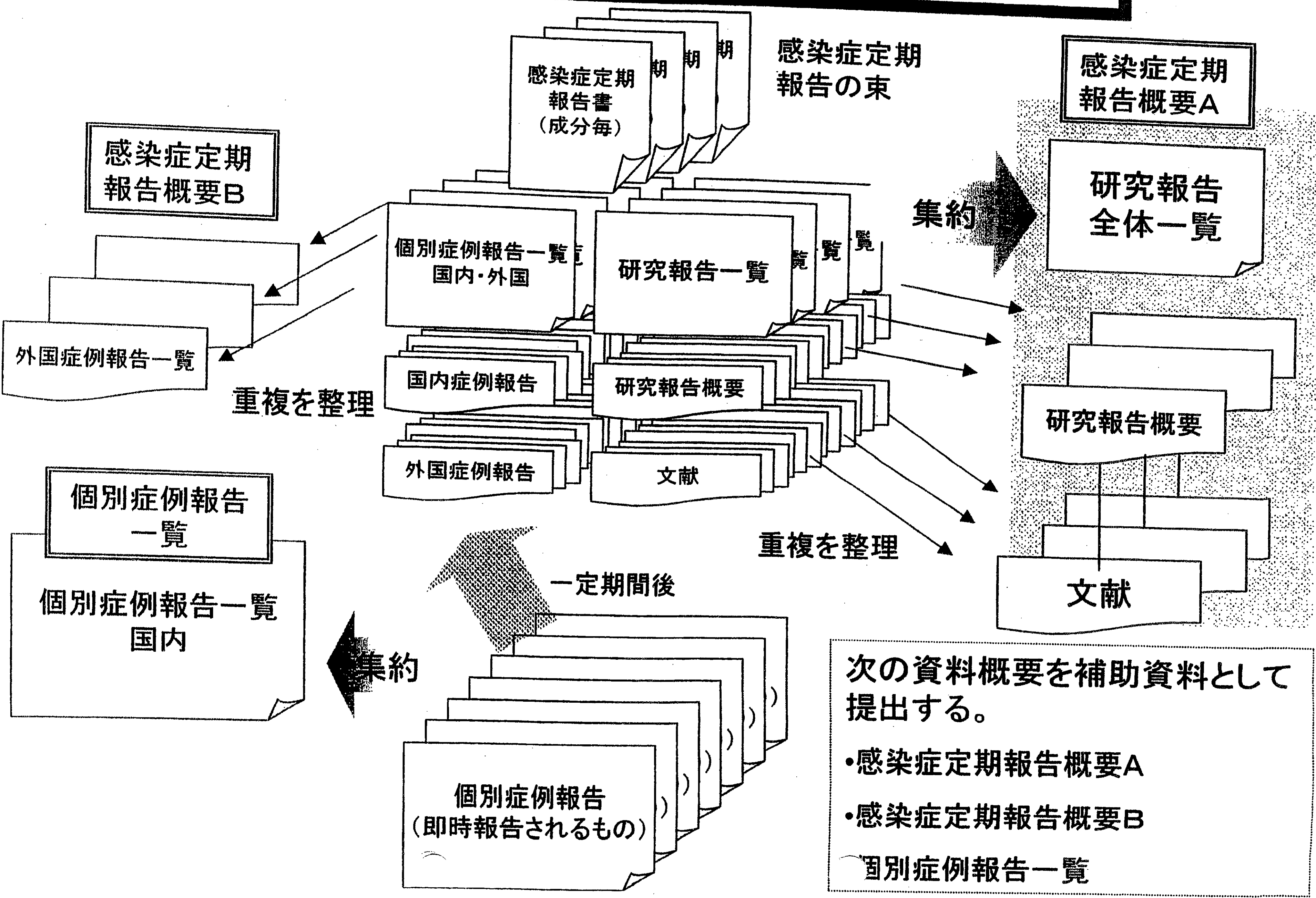
② 感染症発生症例報告のうち、発現国が「外国」の血漿分画製剤の使用による症例は、同一製品毎に報告期間を代表する感染症発生症例一覧(別紙様式第4)をまとめた**「資料概要B」**を事務局が作成し、送付する。

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