資料１－２

Ａ　研究報告（詳細版）

平成２８年９月１４日
（平成２８年５月～平成２８年７月受理分）

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. 4. 10	該当なし	

一般的名称	人全血液	研究報告の公表状況	Barzon L, Pacenti M, Berto A, et al. Euro Surveill. 2016;21(10). doi: 10.2807/1560-7917.ES.2016.21.10.30159.	公表国 イタリア
販売名(企業名)	人全血液-LR「日赤」(日本赤十字社) 照射人全血液-LR「日赤」(日本赤十字社)			

研究報告の概要

○ドミニカ共和国からイタリアに帰国した渡航者における唾液からの感染性ジカウイルス(ZIKV)の分離並びにウイルスRNAの長期排出(2016年1月)。
患者は2016年1月にドミニカ共和国からイタリアに帰国し、後に熱性疾患を発症した。唾液並びに尿中へのウイルスRNAの排出は遷延し、RNA量は血中量と比較して高値であった。ウイルスRNAは、血漿中に発症後10日目まで検出されたが、唾液と尿中への排出は発症後29日目まで認められた。IgM抗体は7日目に蛍光抗体法により、IgG抗体は10日目に酵素結合免疫吸着法によりそれぞれ検出された。ZIKVゲノムの配列決定により、ラテンアメリカで検出された株との関連が明らかになった。
この報告にて、発症後抗体が出現するまでの最初の7日間は唾液中から高濃度のウイルスが検出されたことから、唾液を介したヒト—ヒト感染の潜在的なリスクが提起される。

報告企業の意見	今後の対応
ジカウイルス流行国から帰国した渡航者において、ジカ熱発症後、血漿中よりも長い29日目まで唾液と尿からウイルスが検出されたという報告である。	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。

使用上の注意記載状況・その他参考事項等

人全血液-LR「日赤」
照射人全血液-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

RAPID COMMUNICATIONS

Isolation of infectious Zika virus from saliva and prolonged viral RNA shedding in a traveller returning from the Dominican Republic to Italy, January 2016

L Barzon [1,2], M Pacenti [2], A Berto [1], A Sinigaglia [3], E Franchin [1,2], E Lavezzo [1], P Brugnaro [4], G Palù [1,2]
1. Department of Molecular Medicine, University of Padova, Padova, Italy
2. Microbiology and Virology Unit, Padova University Hospital, Padova, Italy
3. Veneto Institute of Oncology IOV IRCCS, Padova, Italy
4. Infectious Disease Department, Venice City Hospital 'SS. Giovanni e Paolo', Venice, Italy

Correspondence: Luisa Barzon (luisa.barzon@unipd.it)

Citation style for this article:
Barzon L, Pacenti M, Berto A, Sinigaglia A, Franchin E, Lavezzo E, Brugnaro P, Palù G. Isolation of infectious Zika virus from saliva and prolonged viral RNA shedding in a traveller returning from the Dominican Republic to Italy, January 2016. Euro Surveill. 2016;21(10):pii=30159. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2016.21.10.30159

Article submitted on 03 March 2016 / accepted on 10 March 2016 / published on 10 March 2016

We report the isolation of infectious Zika virus (ZIKV) in cell culture from the saliva of a patient who developed a febrile illness after returning from the Dominican Republic to Italy, in January 2016. The patient had prolonged shedding of viral RNA in saliva and urine, at higher load than in blood, for up to 29 days after symptom onset. Sequencing of ZIKV genome showed relatedness with strains from Latin America.

Case report

A young woman in her 20s was admitted to the Infectious Disease Unit of Venice City Hospital in Italy because of persisting fever (38 °C) associated with arthralgia, myalgia, and macular cutaneous rash, that had developed four days before, upon return from a two-week stay in the Dominican Republic, in January 2016. Clinical examination was remarkable for a mild macular erythematous skin eruption on the arms and the abdomen, and for conjunctival hyperaemia. There was no lymph node, liver or spleen enlargement. The abdominal ultrasound did not reveal pathological findings. Fever disappeared on the second day of hospital stay, and the skin eruption faded away completely after three days. The patient had no underlying diseases or important medical history and was not taking any medication.

None of the household contacts reported suspected symptoms similar to that of the patient.

Laboratory findings

Upon hospital admission, laboratory tests showed blood cell count, haemoglobin, liver and kidney function tests in the normal range. Real-time RT-PCR tests for dengue virus (DENV) [1] and chikungunya virus (CHIKV) [2] were negative, while real-time RT-PCR for Zika virus (ZIKV) [3] was positive in plasma, urine, and saliva, with estimated ZIKV RNA loads of 30 copies/mL; 0.5×10^6 copies/mL; and 3×10^6 copies/mL, respectively; IgM and IgG antibodies against DENV (ELISA, Focus Diagnostics Inc., Cypress, CA), CHIKV (immunofluorescence assay, IFA, IgM and IgG, Euroimmun AG, Luebeck, Germany), and ZIKV (IFA Mosaic Arbovirus 2 IgM and IgG and ELISA Zika virus IgM and IgG; Euroimmun AG) were negative.

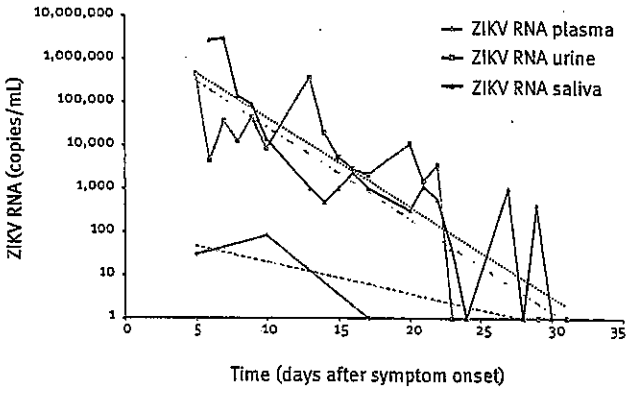
The patient was invited to collect saliva and urine samples daily and to return weekly for follow-up visits and blood sampling. Real-time RT-PCR testing of follow-up blood, urine, and saliva samples demonstrated persistent shedding of ZIKV RNA in saliva and urine for up to 29 days after symptom onset, while viral RNA was detectable in plasma up to day 10 after symptom onset. ZIKV RNA load in saliva and urine was higher than in blood also in follow-up samples (**Figure 1**). Anti-ZIKV IgM and IgG antibodies appeared on days 7 and 10, respectively, as demonstrated by IFA and ELISA.

Viral genome sequencing

Full ZIKV genome sequence was obtained with the Sanger method from nucleic acids purified from saliva and urine specimens collected on day 6 after symptom onset (GenBank KU853012). No nt sequence differences were observed between ZIKV in saliva and urine. Phylogenetic analysis demonstrated that the virus belonged to the Asian lineage and clustered with ZIKV strains from Latin America; it had >99.6% nt identity with ZIKV strains isolated in French Polynesia (2013) and Brazil (2015), 97.9% nt identity with a ZIKV strain isolated in Yap island in 2007, and 88.9% identity with the Uganda MR766 strain isolated in 1947 (**Figure 2**).

FIGURE 1

Kinetics of ZIKV RNA load measured by quantitative real-time RT-PCR in plasma, urine, and saliva samples of a patient with ZIKV infection, Italy, January 2016



ZIKV: Zika virus.

For real-time RT-PCR analysis, viral RNA was purified from 1 mL of plasma, saliva, or urine samples and eluted in a final volume of 50 µL by using a NucliSENS easyMag automated nucleic acid purification system (bioMérieux, Marcy-l'Étoile, France); 10 µL of purified nucleic acids were used for each real-time RT-PCR reaction, in a final volume of 30 µL. Real-time RT-PCR was performed using the primers and probe set 1086/1162c/1107-FAM developed by Lanciotti et al. [3] and AgPath-ID One-Step RT-PCR Reagents (Thermo Fisher Scientific, Waltham, MA) on a 7900HT Fast Real-Time PCR System (Thermo Fisher Scientific) for 45 cycles. ZIKV RNA load was estimated against a standard curve obtained by dilution of a plasmid in which the target sequence was cloned.

Viral isolation

Within the diagnostic workup for arboviral infections, viral isolation was attempted from serum, urine, and saliva specimens collected during the first week after symptom onset. In particular, ZIKV was isolated from a saliva sample collected on day 6 after symptom onset. For virus isolation, both Vero and Vero E6 cells were used, following the procedures described for WNV isolation, with slight modifications [4]. Briefly, saliva was diluted 1:3 in serum-free Dulbecco's modified Eagle's medium (DMEM), centrifuged at 1,200 x g for 10 minutes to separate cells from supernatant. Both saliva cells and supernatant were then inoculated into Vero and Vero E6 cells grown at 70% confluence in shell vials. After inoculation, shell vials were centrifuged at 290 x g for 30 minutes and incubated for 60 minutes at 37°C in 5% CO_2; then, DMEM with 2% fetal bovine serum was added, followed by cell culture at 37°C in 5% CO_2 for up to seven days. On day 4, a cytopathic effect appeared in all infection conditions, i.e. both Vero and Vero E6 cells infected with saliva cells or with saliva supernatant. Viral replication in cell culture was confirmed by increased ZIKV RNA load in cell supernatant (ca $330x10^6$ copies/mL). The ZIKV isolate was then propagated in Vero cells; a titre of $0.5x10^5$ TCID50 was obtained at the second passage in cell culture. Sequencing of the full ZIKV genome from the first passage of the viral cell culture (GenBank KU853013) identified only a G to A synonymous nt change in position 6971 in comparison with the ZIKV genome that was sequenced directly from urine and saliva specimens (Figure 2).

Background

ZIKV is a mosquito-borne flavivirus that generally causes asymptomatic infections in humans and, in an estimated 20% of cases, a mild and self-limited febrile illness associated with rash, arthralgia, and conjunctivitis. The virus, endemic in central and western Africa and in south and south-east Asia, was not considered a relevant human pathogen until outbreaks occurred in Yap, Federal States of Micronesia, in 2007 [5], in French Polynesia in 2013 [6], and in other countries in the Pacific Region in 2013–2014 [7]. In Brazil, the first cases of ZIKV infection were confirmed in March 2015 [8]; since then, the virus has spread exponentially also to other countries in South and Central America and has been estimated to have caused 0.5–1.5 million human infections [9].

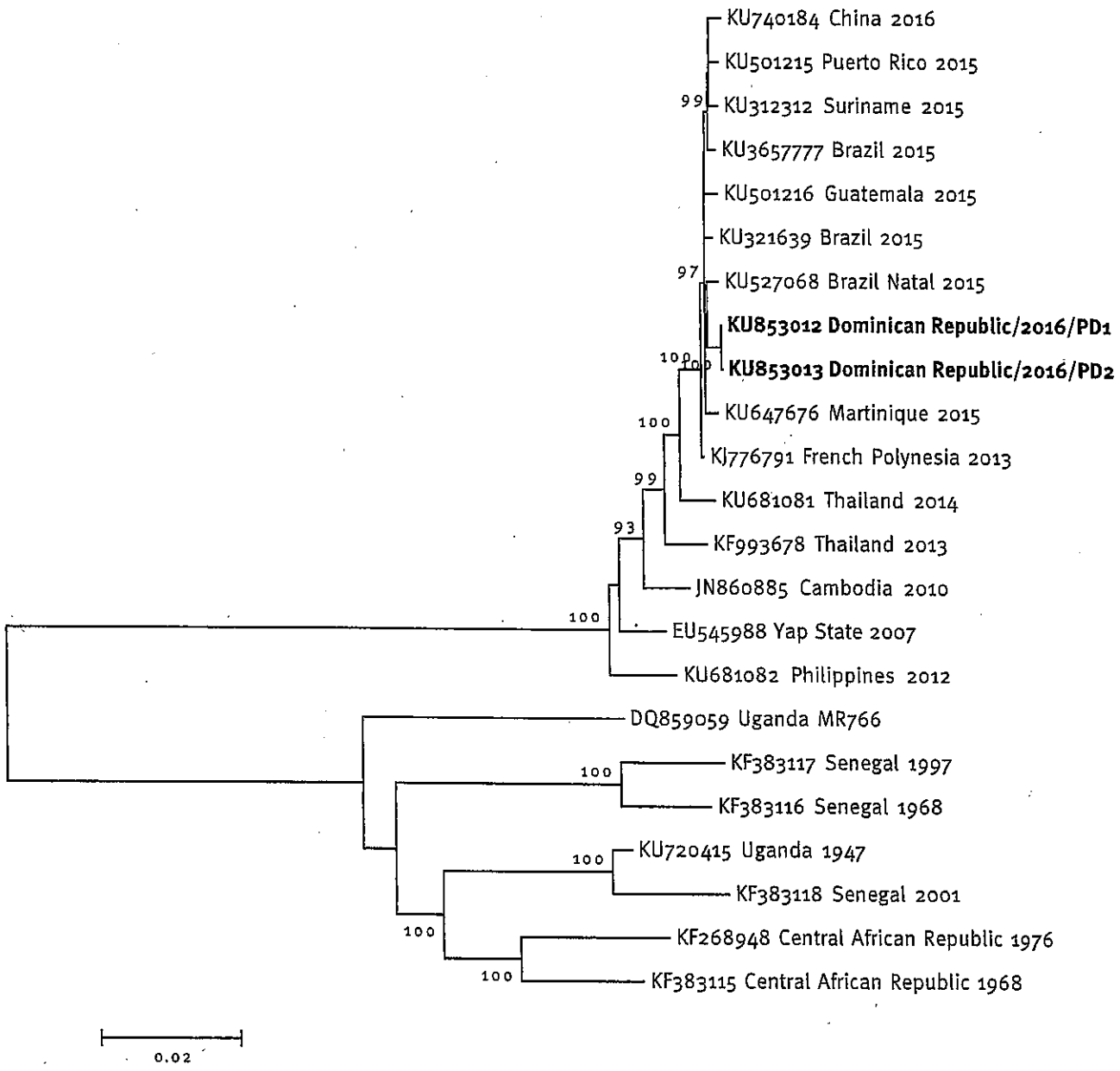
The association of the recent human epidemics of ZIKV infection in French Polynesia and Brazil with an increased incidence of Guillain–Barré syndrome and foetal microcephaly has led the World Health Organization (WHO) to declare a public health emergency of international concern on 1 February 2016 [9]. The aetiological link between foetal microcephaly and ZIKV infection has been recently supported by detection of the virus in the amniotic fluid [10] and in brain tissues of microcephalic foetuses [9,11,12], while the association with Guillain–Barré syndrome has been confirmed by a case–control study in French Polynesia [13].

ZIKV is transmitted between humans through *Aedes* spp. mosquito vectors, mainly the anthropophilic *Ae. aegypti* [14], which is widespread in tropical and subtropical regions in Africa, Asia, and Latin America, and is the main vector also for DENV and CHIKV. The virus has also been detected in *Ae. albopictus* [15], which has been shown to be a competent vector by experimental infection [16]. *Ae. albopictus* is established in Europe, especially in Mediterranean countries, including northern Italy [17], where the case reported in this study was imported. Due to the risk of emergence of outbreaks of vector-borne viruses following the introduction of a viraemic individual in areas where the vector is present [18], an integrated surveillance programme for imported dengue, chikungunya, and Zika virus infections has been implemented in Italy, along with veterinary and entomologic surveillance [17].

Although conceivably rare, non-vector-borne modes of ZIKV transmission may also occur, including trans-placental and perinatal transmission [11,19], blood-transfusion [20], and, potentially, organ donations. Unlike other arboviruses, sexual transmission of ZIKV is also possible and is of particular concern during pregnancy [21]. Actually, ZIKV has been detected and isolated in cell culture from semen samples of patients with infection and cases of probable sexual transmission of ZIKV

FIGURE 2

Phylogenetic tree of full genome sequences of Zika virus obtained directly from saliva and isolated in cell culture from saliva of a traveller returning from the Dominican Republic to Italy, January 2016



The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model [36]. The percentage of trees in which the associated taxa clustered together is shown next to the branches. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter=0.2745)). The analysis involved 23 nt sequences. All positions containing gaps and missing data were eliminated. There were a total of 10,092 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 [37].

infection from males to their female partners have been documented [22-24].

Discussion and conclusions

In this report, we described the isolation of infectious ZIKV in cell culture from saliva collected from a patient during acute ZIKV infection. This finding poses questions on the potential risk of human-to-human transmission of the virus through saliva.

In particular, the virus was isolated from saliva collected on day 6 after symptom onset. It is conceivable that viral isolation is more successful from saliva samples characterised by high viral load and collected during the first week after symptom onset, before the appearance of antibodies. However, further analyses in other patients are required to assess the infectivity of ZIKV in saliva.

Shedding of ZIKV RNA in saliva has been reported in the literature. In particular, it has been observed in 48% of patients tested during the first week after symptom onset, i.e. more frequently, although not for a longer time, than in plasma [25]. For this reason, testing ZIKV in saliva by RT-PCR has been recommended as a non-invasive and sensitive method for the direct diagnosis of ZIKV infection during the first week after symptom onset [25]. In the case reported here, ZIKV RNA was present at high titre during the first week after symptom onset and remained detectable for a relatively long period, up to 29 days after onset of symptoms. Viral RNA was also excreted in urine for a long-time, in agreement with previous reports on ZIKV detection in urine for more than 10 days after onset of disease [26,27]. Shedding in saliva and urine has also been demonstrated for other vector-borne flaviviruses, i.e. DENV [28,29] and West Nile virus [30,31], and these samples are used for direct diagnosis based on viral nucleic acid or antigen detection. While isolation of ZIKV in cell culture from urine, semen, and breast milk has been described [22,32,33], to our knowledge, isolation of ZIKV from saliva has not been reported so far. Epidemiological data and experimental studies are needed to assess the potential risk of ZIKV spread and transmission through saliva. Interestingly, a human case of ZIKV infection following a monkey bite has been reported [34]. In addition, CHIKV, a mosquito-borne alphavirus, has been isolated in oral fluids of patients with severe infection and in the saliva of experimentally infected mice and monkeys, and mouse-to-mouse transmission of CHIKV without an arthropod vector was demonstrated [35].

Finally, from the laboratory perspective, the results of this study showed that saliva is a useful sample not only for ZIKV nucleic acids detection, but also for virus isolation.

Acknowledgements

We thank the patient for collaborating in the sample collection; we also thank Dr Vittoria Lisi for technical support and Dr Erika Morelli for support in the management of the patient.

The study was approved by the local Ethics Committee and the patient provided written informed consent to participate in the study and for the publication of this case report.

Conflict of interest

None declared.

Authors' contributions

Coordinated the study: LB, MP, GP; managed the patient: PB; performed laboratory investigations: MP, EF, AS, AB, LB; performed bioinformatics analysis: EL; wrote the manuscript: LB, MP, PB, GP.

References

1. Santiago GA, Vergne E, Quiles Y, Cosme J, Vazquez J, Medina JF, et al. Analytical and clinical performance of the CDC real time RT-PCR assay for detection and typing of dengue virus. PLoS Negl Trop Dis. 2013;7(7):e2311. Available from: DOI: 10.1371/journal.pntd.0002311 PMID: 23875046
2. Pastorino B, Bessaud M, Grandadam M, Murri S, Tolou HJ, Peyrefitte CN. Development of a TaqMan RT-PCR assay without RNA extraction step for the detection and quantification of African Chikungunya viruses.J Virol Methods. 2005;124(1-2):65-71. Available from: DOI: 10.1016/j.jviromet.2004.11.002 PMID: 15664052
3. 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(8):1232-9. Available from: DOI: 10.3201/eid1408.080287 PMID: 18680646
4. Barzon L, Pacenti M, Franchin E, Squarzon L, Sinigaglia A, Ulbert S, et al. Isolation of West Nile virus from urine samples of patients with acute infection. J Clin Microbiol. 2014;52(9):3411-3. Available from: DOI: 10.1128/JCM.01328-14 PMID: 24951801
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;360(24):2536-43. Available from: DOI: 10.1056/NEJMoa0805715 PMID: 19516034
6. Cao-Lormeau VM, Roche C, Teissier A, Robin E, Berry AL, Mallet HP, et al. Zika virus, French Polynesia, South Pacific, 2013. Emerg Infect Dis. 2014;20(6):1085-6. Available from: DOI: 10.3201/eid2006.140138 PMID: 24856001
7. 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):20929. Available from: DOI: 10.2807/1560-7917.ES2014.19.41.20929 PMID: 25345518
8. Zammarchi L, Tappe D, Fortuna C, Remoli ME, Günther S, Venturi G, et al. Zika virus infection in a traveller returning to Europe from Brazil, March 2015. Euro Surveill. 2015;20(23):21153. Available from: DOI: 10.2807/1560-7917.ES2015.20.23.21153 PMID: 26084316
9. World Health Organization,. Zika virus infection: global update on epidemiology and potentially associated clinical manifestations.Wkly Epidemiol Rec. 2016;91(7):73-81.PMID: 26897760
10. Calvet G, Aguiar RS, Melo AS, Sampaio SA, de Filippis I, Fabri A, et al. Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: a case study. Lancet Infect Dis. 2016; pii: S1473-3099(16)00095-5.
11. Mlakar J, Korva M, Tul N, Popović M, Poljšak-Prijatelj M, Mraz J, et al. Zika virus associated with microcephaly. N Engl J Med. 2016. [Epub ahead of print].
12. Martines RB, Bhatnagar J, Keating MK, Silva-Flannery L, Muehlenbachs A, Gary J, et al. 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. MMWR Morb Mortal Wkly Rep. 2016;65(6):159-60. Available from: DOI: 10.15585/mmwr.mm6506e1 PMID: 26890059
13. Cao-Lormeau VM, Blake A, Mons S, Lastère S, Roche C, Vanhomwegen J, et al. Guillain-Barré Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. Lancet. 2016;S0140-6736(16)00562-6. [Epub ahead of print].PMID: 26948433
14. Li MI, Wong PS, Ng LC, Tan CH. Oral susceptibility of Singapore Aedes (Stegomyia) aegypti (Linnaeus) to Zika virus. PLoS Negl Trop Dis. 2012;6(8):e1792.
15. 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. Available from: DOI: 10.1371/journal.pntd.0002681 PMID: 24516683
16. 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;7(8):e2348. Available from: DOI: 10.1371/journal.pntd.0002348 PMID: 23936579
17. Summer Fever Study Group,Gobbi F, Capelli G, Angheben A, Giobbia M, Conforto M, Franzetti M, et al. . Human and entomological surveillance of West Nile fever, dengue and chikungunya in Veneto Region, Italy, 2010-2012.BMC Infect Dis. 2014;14(1):60. Available from: DOI: 10.1186/1471-2334-14-60 PMID: 24499011
18. CHIKV study group,Rezza G, Nicoletti L, Angelini R, Romi R, Finarelli AC, Panning M, et al. . Infection with chikungunya virus in Italy: an outbreak in a temperate region.Lancet.

2007;370(9602):1840-6. Available from: DOI: 10.1016/S0140-6736(07)61779-6 PMID: 18061059

19. 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):20751.DOI: 10.2807/1560-7917.ES2014.19.13.20751 PMID: 24721538
20. 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):20761. Available from: DOI: 10.2807/1560-7917.ES2014.19.14.20761 PMID: 24739982
21. Centers for Disease Control and Prevention (CDC). HAN Priority Professional and Media Partners Update. Update: Interim Guidelines for Prevention of Sexual Transmission of Zika Virus — United States, 2016. 23 Feb 2016. Available from: http://content.govdelivery.com/accounts/USCDC/bulletins/1383154.
22. Musso D, Roche C, Robin E, Nhan T, Teissier A, Cao-Lormeau VM. Potential sexual transmission of Zika virus.Emerg Infect Dis. 2015;21(2):359-61. Available from: DOI: 10.3201/eid2102.141363 PMID: 25625872
23. Hills SL, Russell K, Hennessey M, Williams C, Oster AM, Fischer M, et al. Transmission of Zika virus through sexual contact with travelers to areas of ongoing transmission — Continental United States, 2016. MMWR Morb Mortal Wkly Rep. 2016;65(8):215-6. Available from: DOI: 10.15585/mmwr.mm6508e2 PMID: 26937739
24. 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;17(5):880-2. Available from: DOI: 10.3201/eid1705.101939 PMID: 21529401
25. Musso D, Roche C, Nhan TX, Robin E, Teissier A, Cao-Lormeau VM. Detection of Zika virus in saliva.J Clin Virol. 2015;68:53-5. DOI: 10.1016/j.jcv.2015.04.021 PMID: 26071336
26. Gourinat AC, O'Connor O, Calvez E, Goarant C, Dupont-Rouzeyrol M. Detection of Zika virus in urine.Emerg Infect Dis. 2015;21(1):84-6. Available from: DOI: 10.3201/eid2101.140894 PMID: 25530324
27. de M Campos R. Cirne-Santos C,, Meira GL, Santos LL, de Meneses MD, Friedrich J, et al. Prolonged detection of Zika virus RNA in urine samples during the ongoing Zika virus epidemic in Brazil.J Clin Virol. 2016;77:69-70.
28. Andries AC, Duong V, Ly S, Cappelle J, Kim KS, Lorn Try P, et al. Value of routine dengue diagnostic tests in urine and saliva specimens. PLoS Negl Trop Dis. 2015;9(9):e0004100. Available from: DOI: 10.1371/journal.pntd.0004100 PMID: 26406240
29. Korhonen EM, Huhtamo E, Virtala AM, Kantele A, Vapalahti O. Approach to non-invasive sampling in dengue diagnostics: exploring virus and NS1 antigen detection in saliva and urine of travelers with dengue.J Clin Virol. 2014;61(3):353-8. Available from: DOI: 10.1016/j.jcv.2014.08.021 PMID: 25242312
30. Barzon L, Pacenti M, Franchin E, Pagni S, Martello T, Cattai M, et al. Excretion of West Nile virus in urine during acute infection. J Infect Dis. 2013;208(7):1086-92. Available from: DOI: 10.1093/infdis/jit290 PMID: 23821721
31. Barzon L, Pacenti M, Franchin E, Squarzon L, Lavezzo E, Toppo S, et al. Novel West Nile virus lineage 1a full genome sequences from human cases of infection in north-eastern Italy, 2011. Clin Microbiol Infect. 2012;18(12):E541-4. Available from: DOI: 10.1111/1469-0691.12001 PMID: 23004685
32. Fonseca K, Meatherall B, Zarra D, Drebot M, MacDonald J, Pabbaraju K, et al. First case of Zika virus infection in a returning Canadian traveler. Am J Trop Med Hyg. 2014;91(5):1035-8. Available from: DOI: 10.4269/ajtmh.14-0151 PMID: 25294619
33. Dupount-Rouzeyrol M, Biron A, O'Connor O, Huguan E, Descloux E. Infectious Zika viral particles in breastmilk. Lancet. 2016; S0140-6736(16)00624-3.
34. Leung GH, Baird RW, Druce J, Anstey NM. Zika virus infection in Australia following a monkey bite in Indonesia.Southeast Asian J Trop Med Public Health. 2015;46(3):460-4.PMID: 26521519
35. Gardner J, Rudd PA, Prow NA, Belarbi E, Roques P, Larcher T, et al. Infectious chikungunya virus in the saliva of mice, monkeys and humans. PLoS One. 2015;10(10):e0139481. Available from: DOI: 10.1371/journal.pone.0139481 PMID: 26447467
36. Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees.Mol Biol Evol. 1993;10(3):512-26. PMID: 8336541
37. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0.Mol Biol Evol. 2013;30(12):2725-9. Available from: DOI: 10.1093/molbev/mst197 PMID: 24132122

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. 4. 10	該当なし	
一般的名称	人全血液		研究報告の公表状況	Mlakar J, Korva M, Tul N, et al. N Engl J Med. 2016 Mar 10;374(10):951-8. doi: 10.1056/NEJMoa1600651. Epub 2016 Feb 10.	公表国 スロベニア	
販売名(企業名)	人全血液-LR「日赤」(日本赤十字社) 照射人全血液-LR「日赤」(日本赤十字社)					

研究報告の概要

○小頭症胎児脳組織からジカウイルス(ZIKV)検出。
ブラジルにてZIKVに感染した妊婦において、胎児に重症脳疾患、小頭症及び発育の遅れが確認された。中絶を希望したことから国と病院の倫理委員会の許可の下で、妊娠32週の間に人工妊娠中絶を実施した。中絶後の胎児を剖検したところ、外見的には顕著な小頭症以外に異常は認められなかった。頭囲は26cmしかなく、脳の重量は84gで、小脳と脳幹が小さかった。ほぼ完全な脳回欠損が認められ、脳水腫と皮質と白質には多巣性栄養障害性石灰化が見られた。皮質下核の発育は良好だった。
顕微鏡観察では、石灰化に関連する皮質の圧排と軽度の局所性炎症が検出された。小脳、脳幹、脊髄には、炎症も石灰化も見られなかった。胎盤の組織学的な観察でも巣状石灰化が確認されたが、炎症は認められなかった。胎児の他の組織、臍帯、胎児被膜に病理学的変化は見られなかった。マイクロアレイ技術を用いた胎児の核型分析結果は正常だった。
電子顕微鏡では、フラビウイルス属のウイルスと形態学的特徴が一致するウイルス粒子が確認された。
RT-PCRにより胎児の脳の組織にZIKVが検出された。他のフラビウイルス属のウイルス、サイトメガロウイルス、はしかウイルス等は、PCRでは陰性が確認された。
次世代シーケンシングにより、胎児の脳から、ZIKVの完全なゲノム配列が回収できた。系統発生解析により、2013年にフランス領ポリネシアで分離されたZIKV株およびブラジルのサンパウロで2015年に分離されたZIKV株との相同性は99.7%だった。

使用上の注意記載状況・その他参考事項等

人全血液-LR「日赤」
照射人全血液-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
ジカウイルス(ZIKV)感染した妊婦が中絶した小頭症胎児を剖検したところ、RT-PCRと電子顕微鏡により胎児脳組織にZIKVを確認し、垂直感染が生じることを報告した報告である。	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。

BRIEF REPORT

Zika Virus Associated with Microcephaly

Jernej Mlakar, M.D., Misa Korva, Ph.D., Nataša Tul, M.D., Ph.D., Mara Popović, M.D., Ph.D., Mateja Poljšak-Prijatelj, Ph.D., Jerica Mraz, M.Sc., Marko Kolenc, M.Sc., Katarina Resman Rus, M.Sc., Tina Vesnaver Vipotnik, M.D., Vesna Fabjan Vodušek, M.D., Alenka Vizjak, Ph.D., Jože Pižem, M.D., Ph.D., Miroslav Petrovec, M.D., Ph.D., and Tatjana Avšič Županc, Ph.D.

SUMMARY

A widespread epidemic of Zika virus (ZIKV) infection was reported in 2015 in South and Central America and the Caribbean. A major concern associated with this infection is the apparent increased incidence of microcephaly in fetuses born to mothers infected with ZIKV. In this report, we describe the case of an expectant mother who had a febrile illness with rash at the end of the first trimester of pregnancy while she was living in Brazil. Ultrasonography performed at 29 weeks of gestation revealed microcephaly with calcifications in the fetal brain and placenta. After the mother requested termination of the pregnancy, a fetal autopsy was performed. Micrencephaly (an abnormally small brain) was observed, with almost complete agyria, hydrocephalus, and multifocal dystrophic calcifications in the cortex and subcortical white matter, with associated cortical displacement and mild focal inflammation. ZIKV was found in the fetal brain tissue on reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay, with consistent findings on electron microscopy. The complete genome of ZIKV was recovered from the fetal brain.

ZIKV, AN EMERGING MOSQUITO-BORNE FLAVIVIRUS, WAS INITIALLY ISOlated from a rhesus monkey in the Zika forest in Uganda in 1947.[1] It is transmitted by various species of aedes mosquitoes. After the first human ZIKV infection, sporadic cases were reported in Southeast Asia and sub-Saharan Africa.[2] ZIKV was responsible for the outbreak in Yap Island of Micronesia in 2007 and for major epidemics in French Polynesia, New Caledonia, the Cook Islands, and Easter Island in 2013 and 2014.[3,4] In 2015, there was a dramatic increase in reports of ZIKV infection in the Americas. Brazil is the most affected country, with preliminary estimates of 440,000 to 1.3 million cases of autochthonous ZIKV infection reported through December 2015.[5]

The classic clinical picture of ZIKV infection resembles that of dengue fever and chikungunya and is manifested by fever, headache, arthralgia, myalgia, and maculopapular rash, a complex of symptoms that hampers differential diagnosis. Although the disease is self-limiting, cases of neurologic manifestations and the Guillain–Barré syndrome were described in French Polynesia and in Brazil during ZIKV epidemics.[5,6] Recent reports from the Ministry of Health of Brazil suggest that cases of microcephaly have increased by a factor of approximately 20 among newborns in the northeast region of the country, which indicates a possible association between ZIKV infection in pregnancy and fetal malformations.[5]

We present a case of vertical transmission of ZIKV in a woman who was prob-

From the Institute of Pathology, Faculty of Medicine (J. Mlakar, M. Popović, J. Mraz, A.V., J.P.), and the Institute of Microbiology and Immunology, Faculty of Medicine (M. Korva, M.P.-P., M. Kolenc, K.R.R., M. Petrovec, T.A.Z.), University of Ljubljana, and the Department of Perinatology, Division of Gynecology and Obstetrics (N.T., V.F.V.), and the Institute of Radiology (T.V.V.), University Medical Center Ljubljana — all in Ljubljana, Slovenia. Address reprint requests to Dr. Avšič Županc at the Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Zaloška 4, Ljubljana 1000, Slovenia, or at tatjana.avsic@mf.uni-lj.si.

This article was published on February 10, 2016, at NEJM.org.

N Engl J Med 2016;374:951-8.
DOI: 10.1056/NEJMoa1600651

ably infected with ZIKV in northeastern Brazil at the end of the first trimester of pregnancy. Our discussion includes details of fetal imaging and pathological and virologic analyses.

CASE REPORT

In mid-October 2015, a 25-year-old previously healthy European woman came to the Department of Perinatology at the University Medical Center in Ljubljana, Slovenia, because of assumed fetal anomalies. Since December 2013, she had lived and worked as a volunteer in Natal, the capital of Rio Grande do Norte state. She had become pregnant at the end of February 2015. During the 13th week of gestation, she had become ill with high fever, which was followed by severe musculoskeletal and retroocular pain and an itching, generalized maculopapular rash. Since there was a ZIKV epidemic in the community, infection with the virus was suspected, but no virologic diagnostic testing was performed. Ultrasonography that was performed at 14 and 20 weeks of gestation showed normal fetal growth and anatomy.

The patient returned to Europe at 28 weeks of gestation. Ultrasonographic examination that was performed at 29 weeks of gestation showed the first signs of fetal anomalies, and she was referred to the Department of Perinatology. At that time, she also noticed reduced fetal movements. Ultrasonography that was performed at 32 weeks of gestation confirmed intrauterine growth retardation (estimated third percentile of fetal weight) with normal amniotic fluid, a placenta measuring 3.5 cm in thickness (normal size) with numerous calcifications, a head circumference below the second percentile for gestation (microcephaly), moderate ventriculomegaly, and a transcerebellar diameter below the second percentile. Brain structures were blurred, and there were numerous calcifications in various parts of the brain (Fig. 1A and 1B). There were no other obvious fetal structural abnormalities. Fetal, umbilical, and uterine blood flows were normal on Doppler ultrasonography.

The clinical presentation raised suspicion of fetal viral infection. Because of severe brain disease and microcephaly, the fetus was given a poor prognosis for neonatal health. The mother requested that the pregnancy be terminated, and the procedure was subsequently approved by national and hospital ethics committees. Medical termination of the pregnancy was performed at 32 weeks of gestation. At the delivery, the only morphologic anomaly was the prominent microcephaly. Genetic consultation that included a detailed maternal family history revealed no suspicion of genetic syndromes or diseases. An autopsy was performed, as is mandatory in all cases of termination of pregnancy. The mother provided written informed consent for the publication of this case report.

METHODS

AUTOPSY AND CENTRAL NERVOUS SYSTEM (CNS) EXAMINATION

An autopsy of the fetus and placenta was performed 3 days after termination of the pregnancy, with an extensive sampling of all organs, placenta, and umbilical cord. Samples were fixed in 10% buffered formalin and embedded in paraffin. Fresh tissue samples were collected for microbiologic investigations. Brain and spinal cord were fixed in 27% buffered formalin for 3 weeks, after which a neuropathological examination was performed with extensive sampling of the brain and spinal cord. Sections of all tissue samples were stained with hematoxylin and eosin. Immunostaining for glial fibrillary acid protein, neurofilament, human leukocyte antigen DR (HLA-DR), CD3 (to highlight T cells), and CD20 (to highlight B cells) was performed on representative CNS samples.

ELECTRON MICROSCOPY

Tissue was collected from formalin-fixed brain and underwent fixation in 1% osmium tetroxide and dehydration in increasing concentrations of ethanol. The sample was then embedded in Epon. Semithin sections (1.4 μm) were made, stained with Azur II, and analyzed by means of light microscopy. Ultrathin sections (60 nm) were stained with uranyl acetate and lead citrate. In addition, a small piece of brain (5 mm^3) was homogenized in buffer. The suspension was then cleared by low-speed centrifugation, and the obtained supernatant was ultracentrifuged directly onto an electron microscopic grid with the use of an Airfuge (Beckman Coulter). Negative staining was performed with 1% phosphotungstic

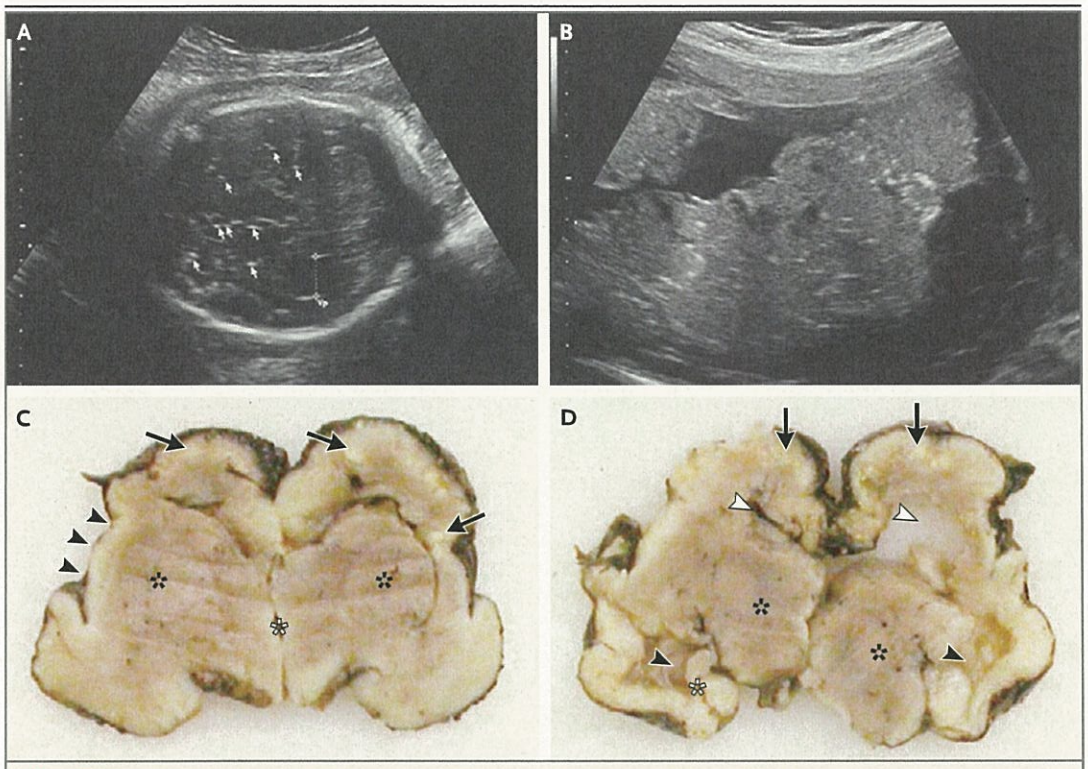


Figure 1. Prenatal Ultrasonographic Images and Photographs of Coronal Slices of Brain.

Panel A shows numerous calcifications in various parts of the brain (some marked with arrows) and the dilated occipital horn of the lateral ventricle (Vp, marked with a measurement bar) as seen on transverse ultrasonography. Panel B shows numerous calcifications in the placenta. Panel C shows multifocal cortical and subcortical white calcifications (arrows) and almost complete loss of gyration of the cortex. The basal ganglia are developed but poorly delineated (black asterisks), and the sylvian fissures are widely open on both sides (arrowheads on the left). The third ventricle is not dilated (white asterisk). Panel D shows dilated body of the lateral ventricles (white arrowheads); the left is collapsed. Temporal horns of the lateral ventricles (black arrowheads) are also dilated. The thalami (black asterisks) and the left hippocampus (white asterisk) are well developed, whereas the contralateral structure is not recognizable owing to autolysis.

acid. Imaging of the ultrathin sections and brain homogenate was performed with the use of a 120-kV JEM-1400Plus transmission electron microscope (JEOL).

INDIRECT IMMUNOFLUORESCENCE

Paraffin-embedded sections of the fetal brain tissue and brain tissue of an autopsied man as a negative control were incubated with serum obtained from the mother of the fetus (dilution, 1:10), followed by antihuman IgG antibodies labeled with fluorescein isothiocyanate (FITC) (dilution, 1:50). In addition, fetal brain tissue was incubated with a serum obtained from a healthy blood donor, as well as with FITC-labeled antihuman IgG antibodies only.

MICROBIOLOGIC INVESTIGATION

RNA was extracted from 10 mg of the placenta, lungs, heart, skin, spleen, thymus, liver, kidneys, and cerebral cortex with the use of a TRIzol Plus RNA purification kit (Thermo Fisher Scientific). Real-time RT-PCR for the detection of ZIKV RNA (NS5) and one-step RT-PCR for the detection of the envelope-protein coding region (360 bp) were performed as described previously.[7,8] In addition, next-generation sequencing was performed in samples of fetal brain tissue with the use of Ion Torrent (Thermo Fisher Scientific) and Geneious software, version 9.0.6. Reads from both runs were combined and mapped to the reference sequence (ZIKV MR766; LC002520) with the use of default measures. For phylogenetic analysis,

complete-genome ZIKV sequences were used, and multiple sequence alignments (ClustalW) were performed. A neighbor-joining phylogenetic tree (GTR+G+I model) was constructed, with the use of the MEGA6 software system,[9] to show the phylogenetic relationships. The nucleotide sequence of ZIKV that was obtained in this study has been deposited in GenBank under accession number KU527068. A detailed description of the molecular methods is provided in the Supplementary Appendix, available with the full text of this article at NEJM.org. The results of comprehensive serologic analyses of maternal serum and a description of the molecular differential diagnostic procedures used with fetal tissue samples are provided in Tables S1 and S2 in the Supplementary Appendix. All the authors vouch for the completeness and accuracy of the data and analyses presented.

RESULTS

AUTOPSY AND NEUROPATHOLOGICAL FINDINGS

The fetal body weight was 1470 g (5th percentile), the length 42 cm (10th percentile), and the head circumference 26 cm (1st percentile). The only external anomaly that was noted was microcephaly. The placenta weighed 200 g, resulting in a placental–fetal weight ratio of 0.136 (<3rd percentile). Macroscopic examination of the CNS revealed micrencephaly with a whole-brain weight of 84 g (4 SD below average), widely open sylvian fissures, and a small cerebellum and brain stem. Almost complete agyria and internal hydrocephalus of the lateral ventricles were observed. There were numerous variable-sized calcifications in the cortex and subcortical white matter in the frontal, parietal, and occipital lobes. The subcortical nuclei were quite well developed (Fig. 1C and 1D). In spite of some autolysis, microscopic examination revealed appropriate cytoarchitecture of the fetal brain. The most prominent histopathological features were multifocal collections of filamentous, granular, and neuron-shaped calcifications in the cortex and subcortical white matter with focal involvement of the whole cortical ribbon, occasionally associated with cortical displacement (Fig. 2A and 2B). Diffuse astrogliosis was present with focal astrocytic outburst into the subarachnoid space, mostly on the convexity of the cerebral hemispheres (Fig. 2C). Activated microglial cells and some macrophages expressing HLA-DR were present throughout most of the cerebral gray and white matter (Fig. 2D). Scattered mild perivascular infiltrates composed of T cells and some B cells were present in the subcortical white matter (Fig. S1 in the Supplementary Appendix). The cerebellum, brain stem, and spinal cord showed neither inflammation nor dystrophic calcifications. The brain stem and spinal cord showed Wallerian degeneration of the long descending tracts, especially the lateral corticospinal tract, whereas ascending dorsal columns were well preserved (Fig. 2E). Indirect immunofluorescence revealed granular intracytoplasmic reaction in destroyed neuronal structures, which pointed to a possible location of the virus in neurons (Fig. 2F, and Fig. S1 in the Supplementary Appendix). Histologic examination of the placenta confirmed focal calcifications in villi and decidua, but no inflammation was found. There were no relevant pathological changes in other fetal organs or in the umbilical cord or fetal membranes. Fetal karyotyping with the use of microarray technology showed a normal 46XY (male) profile.

ELECTRON MICROSCOPY

Although analysis of the ultrathin sections of the brain showed poorly preserved brain tissue with ruptured and lysed cells, clusters of dense virus-like particles of approximately 50 nm in size were found in damaged cytoplasmic vesicles. Groups of enveloped structures with a bright interior were also detected. At the periphery of such groups, the remains of membranes could be seen. Negative staining of homogenized brain revealed spherical virus particles measuring 42 to 54 nm with morphologic characteristics consistent with viruses of the Flaviviridae family (Fig. 3).

MICROBIOLOGIC INVESTIGATION

Positive results for ZIKV were obtained on RT-PCR assay only in the fetal brain sample, where 6.5×10^7 viral RNA copies per milligram of tissue were detected. In addition, all autopsy samples were tested on PCR assay and were found to be negative for other flaviviruses (dengue virus, yellow fever virus, West Nile virus, and tick-borne encephalitis virus), along with chikungunya virus, lymphocytic choriomeningitis, cytomegalovirus, rubella virus, varicella–zoster virus, herpes simplex virus, parvovirus B19, enteroviruses, and

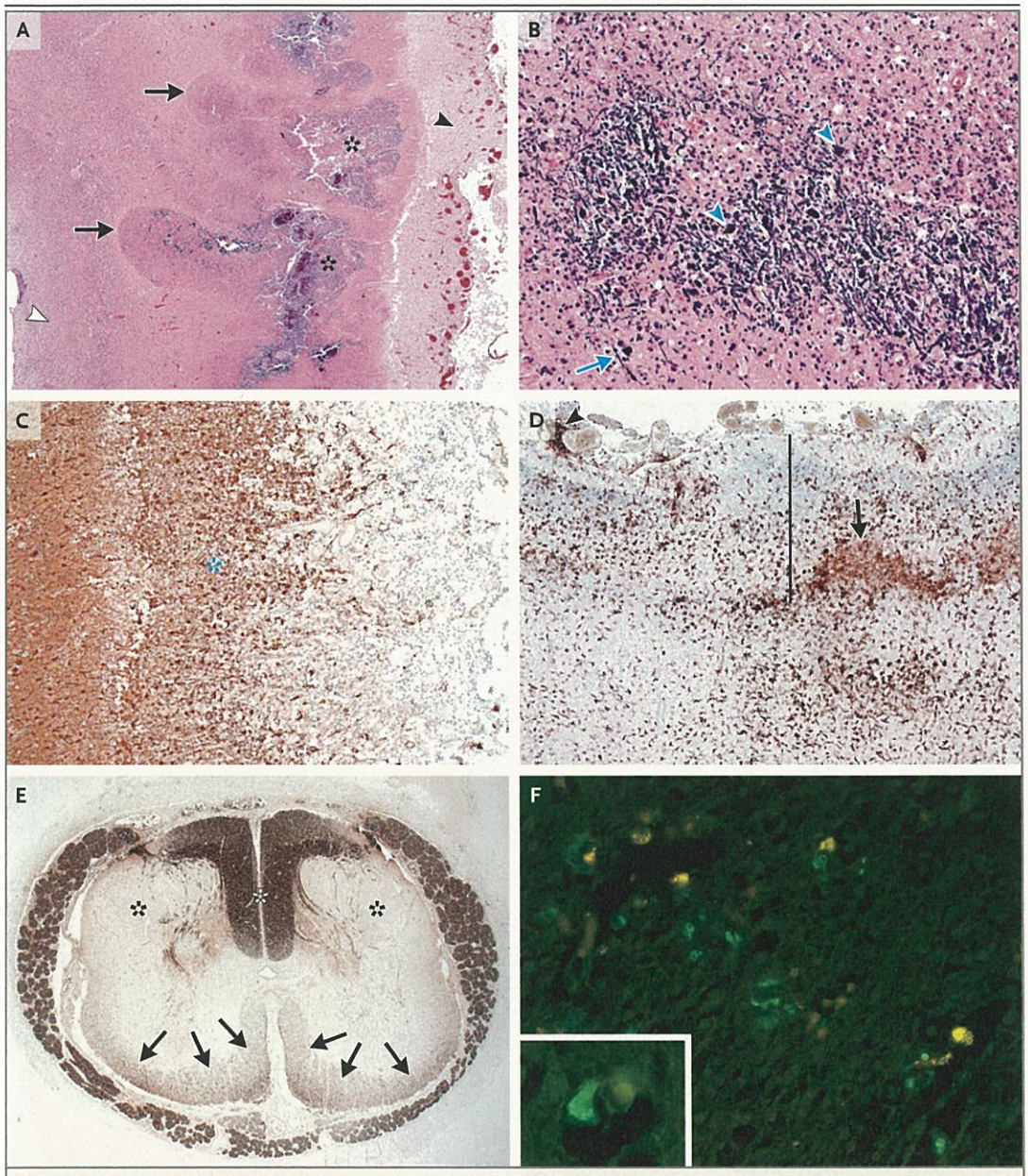


Figure 2. Microscopic Analysis of Brain Tissue.

Panel A shows thickened leptomeninges (black arrowhead) and irregular cortical and subcortical calcifications (asterisks) associated with cortical displacement (arrows), with preserved germinative matrix (white arrowhead); gyration is absent. Panel B shows higher magnification of calcifications with filamentous structures (arrow), possibly representing encrusted, damaged axons and dendrites, and oval and polygonal structures (arrowheads), possibly representing encrusted, damaged neuronal-cell bodies (hematoxylin and eosin staining in Panels A and B). Panel C shows immunohistochemical labeling of proliferated reactive astrocytes that extend into the subarachnoid space (asterisk) (glial fibrillary acid protein, clone 6F2 [Dako]). Panel D shows immunohistochemical labeling of numerous activated microglial cells and macrophages in the cortex (full thickness marked with a line) and subcortical white matter (lower part of the figure). Nonspecific staining of the calcifications is present (arrow). Focal leptomeningeal infiltrates of macrophages are seen (arrowhead) (HLA-DR, clone TAL 1B5 [Dako]). Panel E shows neurofilament immunohistochemical staining of axons in a cross-section of the lumbar spinal cord with severe Wallerian degeneration of the lateral corticospinal tracts (black asterisks), moderate involvement of other descending tracts (arrows), and well-preserved ascending tracts in the dorsal columns (white asterisk) (neurofilament, clone 2F11 [Dako]). Panel F shows indirect immunofluorescence of fetal brain tissue, revealing a green granular intracytoplasmic reaction (see also inset). The yellow signals adjacent to the green granules indicate autofluorescence of lipofuscin, suggesting that viral particles are located in the cytoplasm of neurons.

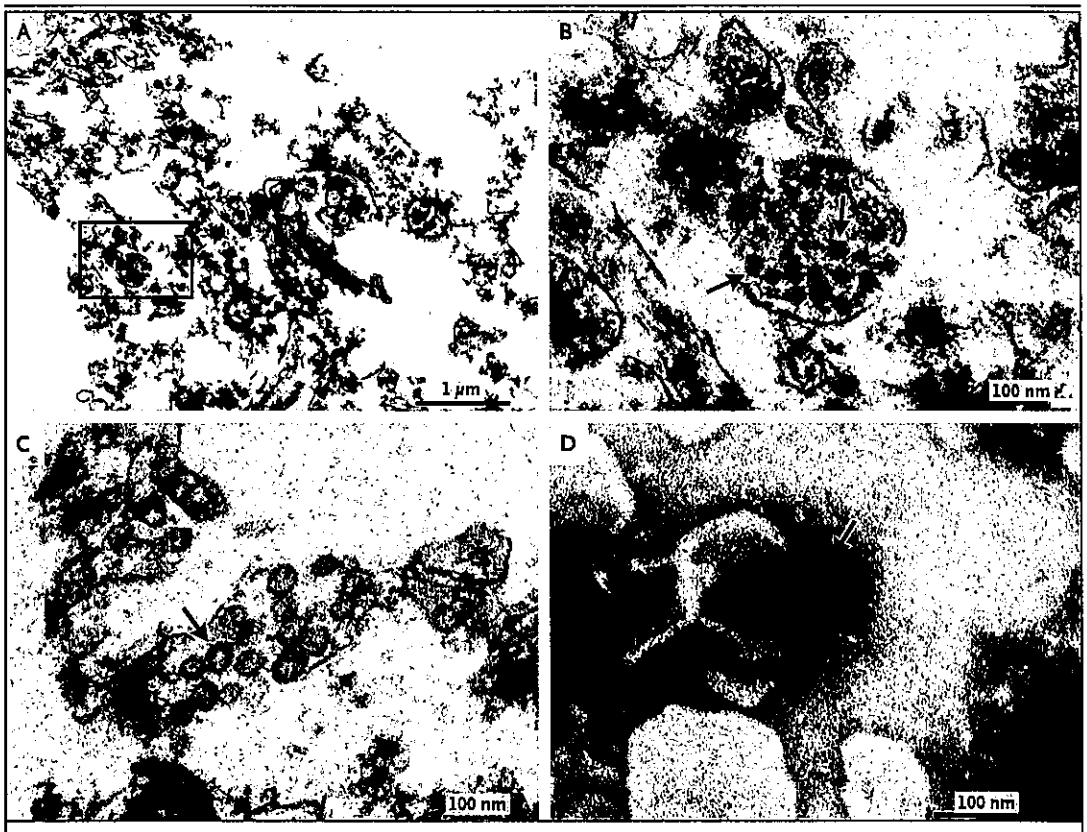


Figure 3. Electron Microscopy of Ultrathin Sections of Fetal Brain and Staining of a Flavivirus-like Particle.

Panel A shows a damaged brain cell with a cluster of dense virions located in the disrupted endoplasmic reticulum. Remains of membranes derived from different cellular compartments and filamentous structures are also seen. A magnified view of the boxed area with virions clearly visible (arrows) is shown in Panel B. Panel C shows a group of enveloped structures with a bright interior, presumably indicating viral replication (arrow). Panel D shows a negatively stained viral particle with morphologic characteristics consistent with those of Flaviviridae viruses (arrow).

Toxoplasma gondii (Table S2 in the Supplementary Appendix).

A complete ZIKV genome sequence (10,808 nucelotides) was recovered from brain tissue. Phylogenetic analysis showed the highest identity (99.7%) with the ZIKV strain isolated from a patient from French Polynesia in 2013 (KJ776791) and ZIKV detected in Sao Paolo, Brazil, in 2015 (KU321639), followed by a strain isolated in Cambodia in 2010 (JN860885, with 98.3% identity) and with a strain from the outbreak in Micronesia in 2007 (EU545988, with 98% identity) (Fig. 4). In the ZIKV polyprotein, 23 polymorphisms were detected in comparison with the strain from Micronesia and 5 polymorphisms in comparison with the isolate from French Polynesia; three amino acid changes were found in the NS1 region (K940E, T1027A, and M1143V), one in the NS4B region (T2509I), and one in the FtsJ-like methyltransferase region (M2634V).

DISCUSSION

This case shows severe fetal brain injury associated with ZIKV infection with vertical transmission. Recently, ZIKV was found in amniotic fluid of two fetuses that were found to have microcephaly, which was consistent with intrauterine transmission of the virus.[10] Described cases are similar to the case presented here and were characterized by severely affected CNS and gross intrauterine growth retardation. Calcifications in the placenta and a low placental–fetal weight ratio,[11] which were seen in this case, indicate potential damage to the placenta by the virus. Among the few reports of teratogenic effects of

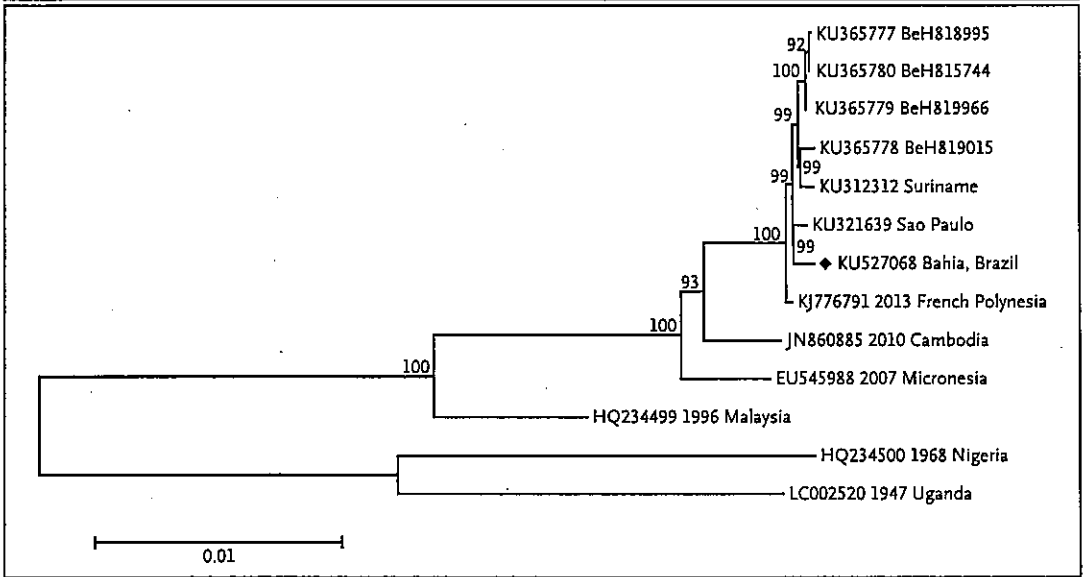


Figure 4. Phylogenetic Analysis of the Complete Genome of Zika Virus.

The evolutionary history was inferred by means of the neighbor-joining method under a GTR+G+I substitution model. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (2000 replicates) is shown next to the branches. The GenBank accession number, year of isolation, and country of origin are indicated on the ZIKV branches for all strains except for those identified in 2015 and 2016. ZIKV strain Bahia, Brazil (KU527068), was obtained in this study. The complete genome sequence was recovered from fetal brain tissue. The 0.01 scale bar denotes the genetic distance in nucleotide substitutions per site.

flaviviruses, investigators described the brain and eyes as the main targets.[12,13] No presence of virus and no pathological changes were detected in any other fetal organs apart from the brain, which suggests a strong neurotropism of the virus.

The localization of immunofluorescence signal and the morphologic appearance of the calcifications, which resembled destroyed neuronal structures, indicate a possible location of the virus in neurons. The consequent damage might cause arrested development of the cerebral cortex at the embryonic age of approximately 20 weeks.[14] The mechanism involved in the neurotropism of ZIKV is currently not clear. The association between ZIKV infection and fetal brain anomalies was also noted by findings on electron microscopy that were consistent with ZIKV detection in the fetal brain. Dense particles consistent with ZIKV were seen in damaged endoplasmic reticulum. Groups of enveloped structures with a bright interior resembling the remains of replication complexes that are characteristic of flaviviruses[15,16] indicate viral replication in the brain. The findings on electron microscopy suggest a possible persistence of ZIKV in the fetal brain, possibly because of the immunologically secure milieu for the virus. The number of viral copies that were detected in the fetal brain were substantially higher than those reported in the serum obtained from adult ZIKV-infected patients[17] but similar to those reported in semen samples.[18]

The complete genome sequence of ZIKV that was recovered in this study is consistent with the observation that the present strain in Brazil has emerged from the Asian lineage.[19] The presence of two major amino acid substitutions positioned in nonstructural proteins NS1 and NS4B probably represents an accidental event or indicates a process of eventual adaptation of the virus to a new environment. Further research is needed to better understand the potential implications of these observations. It is likely that the rapid spread of ZIKV around the globe will be a strong impetus for collaborative research on the biologic properties of the virus, particularly since the risk of neurotropic and teratogenic virus infections places a high emotional and economic burden on society.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank the patient in this case for her willingness to provide detailed medical and immunologic data; Miha Juvan for processing of brain photographs; Peter Štrafela for his assistance with the neuropathological analyses; Martin Sagadin, Tina Uršič, Nataša Toplak, Simon Koren, and Andrej Steyer for their assistance in virus detection, sequencing, and analysis of next-generation sequencing data; Mateja Jelovšek for her assistance in comprehensive serologic investigations, and Luca Lovrečič and Marija Volk for their assistance in molecular karyotyping with microarray testing.

REFERENCES

1. Dick GW, Kitchen SF, Haddow AJ. Zika virus. I. Isolations and serological specificity. Trans R Soc Trop Med Hyg 1952;46:509-20.
2. Hayes EB. Zika virus outside Africa. Emerg Infect Dis 2009;15:1347-50.
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.
4. Cao-Lormeau VM, Roche C, Teissier A, et al. Zika virus, French Polynesia, South Pacific, 2013. Emerg Infect Dis 2014;20:1085-6.
5. Rapid risk assessment: Zika virus epidemic in the Americas: potential association with microcephaly and Guillain-Barré syndrome. Stockholm: European Centre for Disease Prevention and Control, December 10, 2015 (http://ecdc.europa.eu/en/publications/Publications/zika-virus-americas-association-with-microcephaly-rapid-risk-assessment.pdf).
6. Ioos S, Mallet HP, Leparc Goffart I, Gauthier V, Cardoso T, Herida M. Current Zika virus epidemiology and recent epidemics. Med Mal Infect 2014;44:302-7.
7. Faye O, Faye O, Diallo D, Diallo M, Weidmann M, Sall AA. Quantitative real-time PCR detection of Zika virus and evaluation with field-caught mosquitoes. Virol J 2013;10:311.
8. Faye O, Faye O, Dupressoir A, Weidmann M, Ndiaye M, Alpha Sall A. One-step RT-PCR for detection of Zika virus. J Clin Virol 2008;43:96-101.
9. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol 2013;30:2725-29.
10. 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.
11. Macdonald EM, Koval JJ, Natale R, Regnault T, Campbell MK. Population-based placental weight ratio distributions. Int J Pediatr 2014;2014:291846.
12. Alpert SG, Fergerson J, Noël LP. Intrauterine West Nile virus: ocular and systemic findings. Am J Ophthalmol 2003;136:733-5.
13. Tsai TF. Congenital arboviral infections: something new, something old. Pediatrics 2006;117:936-9.
14. Chi JG, Dooling EC, Gilles FH. Gyral development of the human brain. Ann Neurol 1977;1:86-93.
15. Goldsmith CS, Ksiazek TG, Rollin PE, et al. Cell culture and electron microscopy for identifying viruses in diseases of unknown cause. Emerg Infect Dis 2013;19:886-91.
16. Gillespie LK, Hoenen A, Morgan G, Mackenzie JM. The endoplasmic reticulum provides the membrane platform for biogenesis of the flavivirus replication complex. J Virol 2010;84:10438-47.
17. 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.
18. 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.
19. Faye O, Freire CC, Iamarino A, et al. Molecular evolution of Zika virus during its emergence in the 20th century. PLoS Negl Trop Dis 2014;8(1):e2636.

SHARING DATA IN A PUBLIC HEALTH EMERGENCY

The case for sharing data, and the consequences of not doing so, have been brought into stark relief by the Ebola and Zika outbreaks. In response, the *New England Journal of Medicine* has become a journal signatory to the following statement.

"In the context of a public health emergency of international concern, it is imperative that all parties make available any information that might have value in combatting the crisis. As research funders and journals, we are committed to working in partnership to ensure that the global response to public health emergencies is informed by the best available research evidence and data.

Journal signatories will make all content concerning the Zika virus free access. Any data or preprint deposited for unrestricted dissemination ahead of submission of any paper will not preempt later publication in these journals.

Funder signatories will require researchers undertaking work relevant to public health emergencies to establish mechanisms to share quality-assured interim and final data as rapidly and widely as possible, including with public health and research communities and the World Health Organization.

We urge other journals and research funders to make the same commitments."

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 04 月 25 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 ポリエチレングリコール処理抗破傷風人免疫グロブリン ③ 乾燥抗破傷風人免疫グロブリン	研究報告の公表状況	http://www.cdc.gov/mmwr/volumes/65/wr/mm6515e1.htm?s_cid=mm6515e1_w/2016/04/15	公表国 アメリカ
販売名 (企業名)	① テタノブリン IH 静注 250 単位　(日本血液製剤機構) ② テタノブリン IH 静注 1500 単位　(日本血液製剤機構) ③ テタノブリン筋注用 250 単位　(日本血液製剤機構)			

研究報告の概要

症状と妊娠の状況の報告からのジカウイルス検査と感染のパターン−米国，2016 年 1 月 3 日〜3 月 5 日

2016 年 1 月 3 日〜3 月 5 日，米国の州および District of Columbia (DC) でジカウイルス感染の活発な地域へ旅行または移動したヒト 4534 人（うち妊婦 3335 人（73.6%））に対し，ジカウイルス検査が実施された。ジカウイルス関連の症状（例えば発熱、発疹、関節痛、結膜炎）を 1 回以上発現し検査を受けた 1541 例のうち，182 例（11.8%）はジカウイルス感染が確認された。検査を受けた無症候の 2557 人（うち妊婦 2425 人）のうち 7 例（0.3%）がジカウイルス感染と確認された。

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

代表としてテタノブリン IH 静注 250 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破傷風抗毒素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破傷風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。

Morbidity and Mortality Weekly Report

Patterns in Zika Virus Testing and Infection, by Report of Symptoms and Pregnancy Status — United States, January 3–March 5, 2016

Sharoda Dasgupta, PhD[1,2]; Sarah Reagan-Steiner, MD[3]; Dana Goodenough, MPH[4]; Kate Russell, MD[1,5]; Mary Tanner, MD[1,2]; Lillianne Lewis, MD[1,6]; Emily E. Petersen, MD[7]; Ann M. Powers, PhD[8]; Krista Kniss, MPH[5]; Dana Meaney-Delman, MD[9]; Titilope Oduyebo, MD[1,7]; Dan O'Leary, DVM[10]; Sophia Chiu, MD[1,11]; Pamela Talley, MD[1,12]; Morgan Hennessey, DVM[1,8]; Susan Hills, MBBS[8]; Amanda Cohn, MD[13]; Christopher Gregory, MD[14]; The Zika Virus Response Epidemiology and Laboratory Team

On April 15, 2016 this report was posted as an MMWR Early Release on the MMWR website (http://www.cdc.gov/mmwr).

CDC recommends Zika virus testing for potentially exposed persons with signs or symptoms consistent with Zika virus disease, and recommends that health care providers offer testing to asymptomatic pregnant women within 12 weeks of exposure. During January 3–March 5, 2016, Zika virus testing was performed for 4,534 persons who traveled to or moved from areas with active Zika virus transmission; 3,335 (73.6%) were pregnant women. Among persons who received testing, 1,541 (34.0%) reported at least one Zika virus-associated sign or symptom (e.g., fever, rash, arthralgia, or conjunctivitis), 436 (9.6%) reported at least one other clinical sign or symptom only, and 2,557 (56.4%) reported no signs or symptoms. Among 1,541 persons with one or more Zika virus-associated symptoms who received testing, 182 (11.8%) had confirmed Zika virus infection. Among the 2,557 asymptomatic persons who received testing, 2,425 (94.8%) were pregnant women, seven (0.3%) of whom had confirmed Zika virus infection. Although risk for Zika virus infection might vary based on exposure-related factors (e.g., location and duration of travel), in the current setting in U.S. states, where there is no local transmission, most asymptomatic pregnant women who receive testing do not have Zika virus infection.

Zika virus is a flavivirus primarily transmitted by *Aedes* species mosquitoes (*1*,*2*) that has recently spread in the Region of the Americas (*2*). From January 1, 2015 to April 13, 2016, a total of 358 travel-associated cases of Zika virus disease were reported from U.S. states, 351 of which were in persons who traveled to or moved from areas with active Zika virus transmission (http://www.cdc.gov/zika/index.html). Most Zika virus infections are asymptomatic or cause mild clinical disease (*3*). Among persons with clinical illness, signs and symptoms commonly include one or more of the following: fever, rash, arthralgia, and conjunctivitis (*3*,*4*). Zika virus infection during pregnancy has been causally linked to congenital microcephaly and has been associated with other adverse pregnancy outcomes, including pregnancy loss (*5*–*8*). CDC recommends that persons with possible exposure to Zika virus receive testing if they have symptoms of Zika virus disease within 2 weeks of exposure. On February 12, 2016, CDC recommended that health care providers offer testing to asymptomatic pregnant women with possible exposure to Zika virus (*9*).

CDC calculated the number of persons in the 50 U.S. states and District of Columbia (DC) who traveled to or moved from areas of active Zika virus transmission and received testing for Zika virus infection in early 2016, and the proportion of tested persons who had evidence of confirmed Zika virus infection or recent unspecified flavivirus infection, by pregnancy status and presence of reported signs and symptoms. This analysis included specimens that were received for Zika virus testing at CDC's Arboviral Diseases Branch during January 3–March 5, 2016, corresponding to epidemiologic weeks 1–9. Confirmed Zika virus infection was defined as detection of 1) Zika virus RNA by reverse transcription-polymerase chain reaction (RT-PCR) or 2) anti-Zika immunoglobulin M (IgM) antibodies by enzyme-linked immunosorbent assay (ELISA) with neutralizing antibody titers against Zika virus, at levels ≥4-fold higher than those against dengue virus. Recent unspecified flavivirus infection was defined as detection of anti-Zika or anti-dengue virus IgM antibodies by ELISA with <4-fold difference in neutralizing antibody titers between Zika and dengue viruses. State and local health departments collected information on clinical signs and symptoms. Zika virus-associated signs and symptoms were defined as at least one of the following: fever, rash, arthralgia, or conjunctivitis (*5*). Other signs and symptoms not necessarily associated with Zika virus disease were defined as one or more of the following: headache, myalgia, vomiting, diarrhea, edema, oral ulcers, chills, influenza-like illness, or malaise. Persons who reported no symptoms were considered to be asymptomatic. All persons tested in this analysis had traveled to or moved from areas with active Zika virus transmission. Suspected cases of sexually transmitted and congenital Zika virus disease were excluded from the analysis.

During January 3–March 5, 2016, Zika virus testing was performed for 4,534 persons (Table), among whom 3,335 (73.6%) were pregnant women. Among all persons receiving testing, 197 (4.3%) had confirmed Zika virus infection, 55 (1.2%) had recent unspecified flavivirus infection, and 4,282 (94.4%) had no evidence of recent Zika virus infection. Among all persons receiving testing, 1,541 (34.0%) reported one or more Zika

virus-associated symptoms, 436 (9.6%) reported at least one other symptom only, and 2,557 (56.4%) were asymptomatic. Among persons with at least one Zika virus-associated symptom, 620 (40.2%) were pregnant women; among persons with at least one other symptom only, 290 (66.5%) were pregnant women; and among persons with no symptoms, 2,425 (94.8%) were pregnant women.

During epidemiologic weeks 1–5 (weeks ending January 9–February 6, 2016), <10% of persons receiving testing were asymptomatic (Figure). After the recommendation to offer serologic testing to asymptomatic pregnant women was published on February 12, 2016 (*9*), the proportion of persons receiving testing for Zika virus infection who were asymptomatic increased, ranging from 26.1% to 75.9% during epidemiologic weeks 6–9. The proportion of persons who received testing who had confirmed Zika virus infection decreased from 33.3% (epidemiologic week 1) to 1.5% (week 9).

Among all persons with one or more Zika virus-associated symptoms, 182 (11.8%) had confirmed Zika virus infection and 41 (2.7%) had recent unspecified flavivirus infection (Table). Among persons who reported one or more other symptoms only, eight (1.8%) had confirmed Zika virus and three (0.7%) had recent unspecified flavivirus infection. Among asymptomatic persons, seven (0.3%) had confirmed Zika virus and 11 (0.4%) had recent unspecified flavivirus infection.

Among 3,335 pregnant women receiving testing, 28 (0.8%) had confirmed Zika virus infection and 19 (0.6%) had recent unspecified flavivirus infection. Among pregnant women with at least one Zika virus-associated symptom, 18 (2.9%) had confirmed Zika virus infection, and nine (1.5%) had recent unspecified flavivirus infection. Among 2,425 asymptomatic pregnant women, only seven (0.3%) had confirmed Zika virus infection, and 10 (0.4%) had recent unspecified flavivirus infection. Among pregnant women tested after guidelines were expanded to recommend testing of asymptomatic pregnant women (epidemiologic weeks 6–9), seven (35%) of 20 pregnant women with confirmed Zika virus infection were asymptomatic. Among the seven asymptomatic pregnant women with confirmed Zika virus infection, five were residing in areas with active Zika virus transmission at some time during their pregnancy and two were short-term travelers.

Discussion

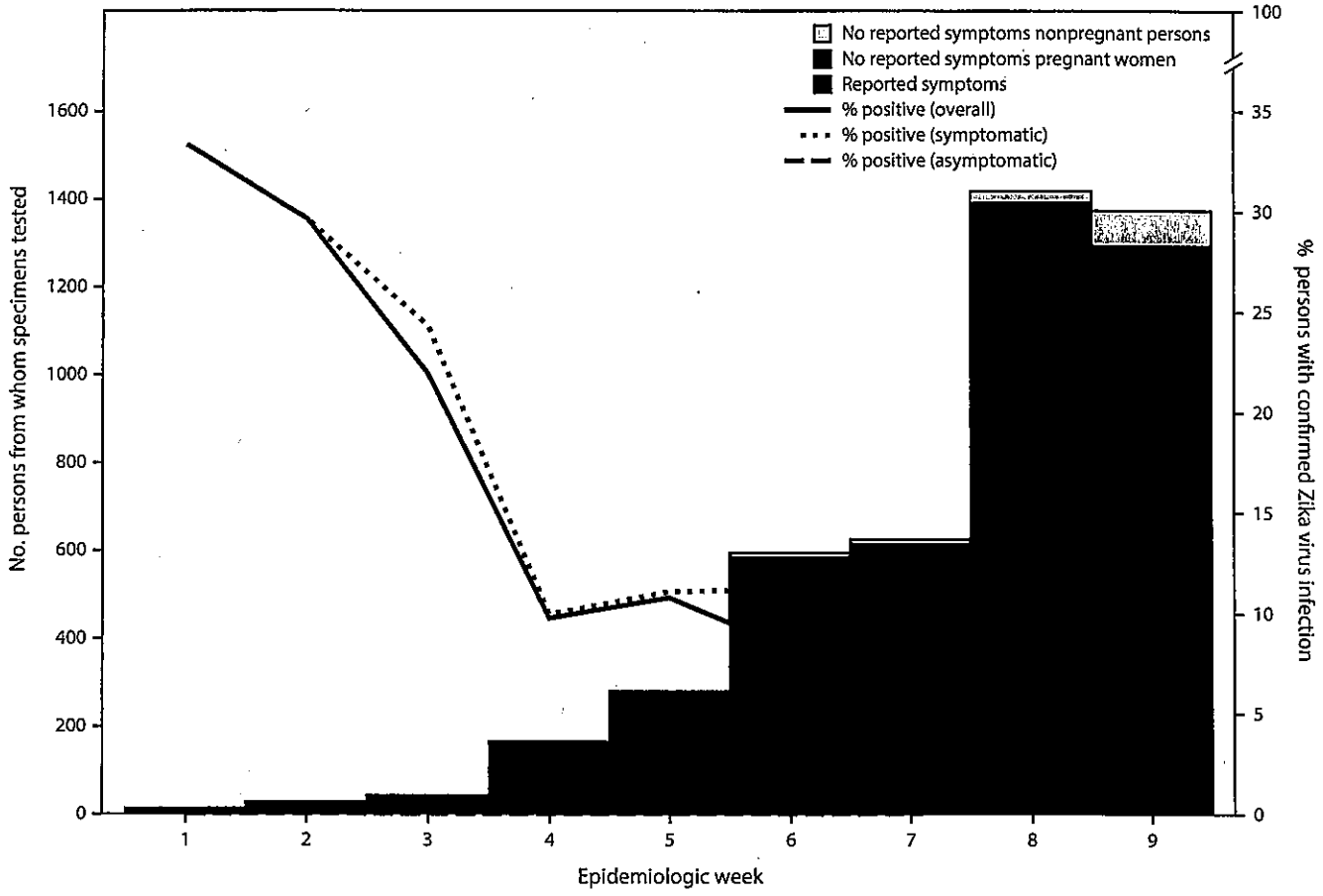
Overall, relatively few persons receiving testing for Zika virus at CDC had confirmed Zika virus infection, and the proportion with confirmed Zika virus infection was higher among persons who reported at least one Zika virus-associated symptom than among persons with other symptoms only or asymptomatic persons. These results reflect the current situation in U.S. states and DC, where there is no local mosquito-borne transmission; results of testing in areas with active Zika virus transmission might be different. Although confirmed Zika virus infection was identified in seven (0.3%) asymptomatic pregnant women who received testing, it is reassuring that the proportion of asymptomatic pregnant women with confirmed Zika virus infection in this report was low. However, because

TABLE. Zika virus testing outcomes among persons with specimens tested at CDC's Arboviral Diseases Branch, by Zika virus infection status, reported symptoms, and pregnancy status* — United States, January 3–March 5, 2016†

Testing outcome	≥1 Zika virus-associated symptom§ No. (%)	≥1 other symptom only¶ No. (%)	No symptoms No. (%)	Total No. (%)
All persons tested				
Confirmed Zika virus infection	182 (11.8)	8 (1.8)	7 (0.3)	197 (4.3)
Recent unspecified flavivirus infection	41 (2.7)	3 (0.7)	11 (0.4)	55 (1.2)
No Zika virus infection	1,318 (85.5)	425 (97.5)	2,539 (99.3)	4,282 (94.4)
Total	**1,541 (100)**	**436 (100)**	**2,557 (100)**	**4,534 (100)**
Pregnant women*				
Confirmed Zika virus infection	18 (2.9)	3 (1.0)	7 (0.3)	28 (0.8)
Recent unspecified flavivirus infection	9 (1.5)	0 (0)	10 (0.4)	19 (0.6)
No Zika virus infection	593 (95.7)	287 (99.0)	2,408 (99.3)	3,288 (98.6)
Total	**620 (100)**	**290 (100)**	**2,425 (100)**	**3,335 (100)**
Other persons (excluding pregnant women)				
Confirmed Zika virus infection	164 (17.8)	5 (3.4)	0 (0)	169 (14.1)
Recent unspecified flavivirus infection	32 (3.5)	3 (2.1)	1 (0.8)	36 (3.0)
No Zika virus infection	725 (78.7)	138 (94.5)	131 (99.2)	994 (82.9)
Total	**921 (100)**	**146 (100)**	**132 (100)**	**1,199 (100)**

* Determined at the time of illness onset (or date of specimen collection, among asymptomatic persons).
† As of April 11, 2016.
§ Fever, rash, arthralgia, or conjunctivitis.
¶ Headache, myalgia, vomiting, diarrhea, edema, oral ulcers, chills, influenza-like illness, or malaise.

FIGURE. Number of symptomatic and asymptomatic persons who received Zika virus testing,* by pregnancy status, and percentage of positive results among all persons tested, persons with symptoms, and asymptomatic persons, by epidemiologic week — United States, January 3–March 5, 2016†



* Testing performed at CDC's Arboviral Diseases Branch.
† As of April 11, 2016.

of the potential serious adverse pregnancy and neonatal outcomes associated with maternal Zika virus infection, health care providers should continue to offer testing to pregnant women with potential exposure to Zika virus, even if they do not have symptoms (*9*). Follow-up of women with confirmed Zika virus infection or recent unspecified flavivirus infection during pregnancy is important to identify congenital Zika virus infection and other possible adverse pregnancy outcomes.

The findings in this report are subject to at least five limitations. First, because testing might have been performed weeks after potential exposure, persons might not recall symptoms, particularly if they were mild. Second, only tests performed at CDC's Arboviral Diseases Branch were included in this analysis. Some state health departments were testing for Zika virus during this time and the total number of cases reported in this period from U.S. states (http://www.cdc.gov/zika/index.html) exceeds the number of cases described in this analysis. Third, this study did not account for heterogeneous exposure risk among persons receiving testing. Travel-associated exposure can vary by location, duration, accommodations, and activities during travel. Fourth, findings in this report are not generalizable to residents of areas with active Zika virus transmission. Finally, patients with unspecified flavivirus infection likely experienced a previous infection with or had been vaccinated against other related flaviviruses making results difficult to interpret. In the setting of the current Zika virus outbreak and because of the concern for adverse fetal effects, pregnant women with unspecified flavivirus infection should follow CDC guidance for pregnant women with possible Zika virus infection (*10*).

In the U.S. states and DC, the proportion of persons who traveled to or moved from areas with active Zika virus

Summary

What is already known about this topic?

Zika virus is an emerging mosquito-borne flavivirus. Travel-associated cases of Zika virus disease have been reported in the United States. Zika virus infection during pregnancy has been causally linked to congenital microcephaly and has been associated with other adverse pregnancy outcomes, including pregnancy loss. On February 1, 2016, CDC recommended that health care providers offer testing for Zika virus disease to asymptomatic pregnant women with possible exposure to Zika virus.

What is added by this report?

During January 3–March 5, 2016, Zika virus testing was performed for 4,534 persons from the U.S. states and District of Columbia (DC), among whom 3,335 (73.6%) were pregnant women. Among 1,541 persons with one or more Zika-virus associated symptoms who received testing and reported symptoms, 182 (11.8%) had confirmed Zika virus infection. Only seven (0.3%) of 2,425 asymptomatic pregnant women who received testing had confirmed Zika virus infection.

What are the implications for public health practice?

Among persons from U.S. states and DC receiving testing for Zika virus, few persons had confirmed Zika virus infection. Approximately 99% of asymptomatic pregnant women who received testing did not have Zika virus infection. In the current U.S. setting, where most exposure is travel-associated, the likelihood of Zika virus infection among asymptomatic persons is low. Given the potential for adverse pregnancy and infant outcomes associated with Zika virus infection, health care providers should continue to offer Zika virus testing to asymptomatic pregnant women with potential exposure.

transmission, who received testing, and who had confirmed Zika virus infection was substantially higher in symptomatic than asymptomatic persons. Furthermore, 64% of pregnant women with confirmed Zika virus infection had at least one Zika virus-associated symptom, and approximately 99% of asymptomatic pregnant women who received testing did not have Zika virus infection. Because of the potential for adverse outcomes associated with Zika virus infection during pregnancy and the lack of current understanding of the risks for infection in asymptomatic pregnant women, health care providers should continue to offer Zika virus testing to asymptomatic pregnant women with potential exposure (*9*). Although individual risk for Zika virus infection will differ on the basis of exposure, these data suggest that in the current setting in U.S. states, where most exposure is travel-associated, most asymptomatic persons do not have Zika virus infection.

[1]Epidemic Intelligence Service, CDC; [2]Division of HIV/AIDS Prevention, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, CDC; [3]Immunization Services Division, National Center for Immunization and Respiratory Diseases, CDC; [4]Oak Ridge Institute for Science and Education (ORISE) Fellow, Division of Human Development and Disability, National Center on Birth Defects and Developmental Disabilities, CDC; [5]Influenza Division, National Center for Immunization and Respiratory Diseases, CDC; [6]National Center for Environmental Health, Division of Environmental Hazards and Health Effects, CDC; [7]Division of Reproductive Health, National Center for Chronic Disease Prevention and Health Promotion, CDC; [8]Division of Vector-Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, CDC; [9]Office of the Director, National Center for Emerging and Zoonotic Infectious Diseases, CDC; [10]Division of State and Local Readiness, Office of Public Health Preparedness and Response, CDC; [11]National Institute for Occupational Safety and Health, Division of Surveillance, Hazard Evaluations, and Field Studies; [12]Minnesota Department of Health; [13]Office of the Director, National Center for Immunization and Respiratory Diseases, CDC; [14]Division of Global Health Protection, Center for Global Health, CDC.

Corresponding author: Sarah Reagan-Steiner, sor1@cdc.gov, 404-639-8205.

The Zika Virus Response Epidemiology and Laboratory Team

Janeen Laven, Olga Kosoy, Amanda Panella, Marc Fischer, Amanda Calvert, Jane Basile, Christin Goodman, Robert Lanciotti, Jeremy Ledermann, Jennifer Lehman, Eric Mossel, Katherine Chu, Charles Futoran, Paul Burns, Sandor Karpathy, Joseph Singleton, Aubree Kelly, Michelle Allerdice, Rachael Priestley, Kelly Fitzpatrick, Sher'i Brooks, Avi Stein, Molly Lauterbach, Ryan Pappert, Adam Replogle, Brook Yockey, Chris Sexton, John Young (all these individuals meet collaborator criteria).

References

1. Hayes EB. Zika virus outside Africa. Emerg Infect Dis 2009;15:1347–50. http://dx.doi.org/10.3201/eid1509.090442
2. CDC. Areas with Zika. Atlanta, GA: US Department of Health and Human Services, CDC; 2016. http://www.cdc.gov/zika/geo/
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. http://dx.doi.org/10.1056/NEJMoa0805715
4. Armstrong P, Hennessey M, Adams M, et al. Travel-associated Zika virus disease cases among US residents—United States, January 2015–February 2016. MMWR Morb Mortal Wkly Rep 2016;65:286–9. http://dx.doi.org/10.15585/mmwr.mm6511e1
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. http://dx.doi.org/10.1002/uog.15831
6. Martines RB, Bhatnagar J, Keating MK, et al. 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. MMWR Morb Mortal Wkly Rep 2016;65:159–60. http://dx.doi.org/10.15585/mmwr.mm6506e1
7. Mlakar J, Korva M, Tul N, et al. Zika virus associated with microcephaly. N Engl J Med 2016;374:951–8. http://dx.doi.org/10.1056/NEJMoa1600651

8. Rasmussen SA, Jamieson DJ, Honein MA, Petersen LR. Zika virus and birth defects—reviewing the evidence for causality. N Engl J Med 2016. E-pub April 13, 2016. http://dx.doi.org/10.1056/NEJMsr1604338
9. 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;65:122–7. http://dx.doi.org/10.15585/mmwr.mm6505e2
10. Petersen EE, Polen KN, Meaney-Delman D, et al. Update: interim guidance for health care providers caring for women of reproductive age with possible Zika virus exposure—United States, 2016. MMWR Morb Mortal Wkly Rep 2016;65:315–22. http://dx.doi.org/10.15585/mmwr.mm6512e2

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 04 月 25 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 ポリエチレングリコール処理抗破傷風人免疫グロブリン ③ 乾燥抗破傷風人免疫グロブリン		研究報告の公表状況	http://www.aabb.org/programs/publications/weeklyreport/Pages/default.aspx/2016/04/15	公表国 プエルトリコ	
販売名 (企業名)	① テタノブリン IH 静注 250 単位　(日本血液製剤機構) ② テタノブリン IH 静注 1500 単位　(日本血液製剤機構) ③ テタノブリン筋注用 250 単位　(日本血液製剤機構)					

研究報告の概要

Creative Testing Solutions (CTS) は, Puerto Rico における献血のジカウイルス検査を開始する：

Creative Testing Solutions (CTS) は, Puerto Rico の数ヵ所の血液センターで採血された血液に対するジカウイルス検査を開始した。この検査は, HIV, B 型および C 型肝炎, ウエストナイルウイルスのスクリーニングで既に使用されている検査と同様のプラットフォームを使用している。米 FDA は, 治験許可申請 (IND) 受領後 6 週間以内の検査をレビューし, IND プロトコールに基づく Puerto Rico での血液のジカウイルス検査を承認した。CTS は, この検査を IND に従い米国本土でも使用する予定である。
ここ数週間, Puerto Rico は, 蚊媒介によるジカウイルス感染の活動性がない米国本土で採取された血液製剤を輸入しなければならなかった。U.S. Department of Health and Human Services は、本日期限切れとなった契約に基づき, Puerto Rico に対して血液成分輸入のための資金援助を行っていた。

使用上の注意記載状況・その他参考事項等

代表としてテタノブリン IH 静注 250 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破傷風抗毒素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破傷風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。

AABB Weekly Report - April 15, 2016

AABB Weekly Report

Visit aabb.org Unsubscribe to this Newsletter April 15, 2016 | Vol. 22 | No. 13

IN THIS ISSUE

ZIKA UPDATE

CDC Confirms Causal Relationship Between Prenatal Zika Virus Infection, Microcephaly

Based on an analysis by the Centers for Disease Control and Prevention, researchers determined that there is sufficient evidence to infer a causal relationship between prenatal Zika virus infection and microcephaly, and other severe brain abnormalities. Scientists reviewed available data on the effect of Zika virus infection during pregnancy using a framework based on seven proposed criteria for establishing proof of teratogenicity and other criteria to assess causation. The determination is expected to spur additional prevention efforts, such as increased vector control strategies,

Event Calendar

April 27 — AABB Audioconference: HOT TOPIC: Implementation of Day 7 Platelets — You Can Do It! Here's How read more »

May 9 — AABB CT Audioconference: Expansion of Umbilical Cord Blood Stem Cells for Transplantation read more »

May 12 — AABB CT Webinar: Perinatal Cells and the

and to accelerate the development of a Zika virus vaccine, improved diagnostic tests and therapeutic measures. The review appears in "The New England Journal of Medicine."

CTS Begins Screening Donated Blood for Zika Virus in Puerto Rico

Creative Testing Solutions (CTS) has begun screening blood collected in several blood centers in Puerto Rico for Zika virus. The test uses the same platform as a test already in use to screen for HIV, hepatitis B and C, and West Nile virus. The Food and Drug Administration reviewed the test within six weeks of receiving an investigational new drug application (IND) for its use and approved it to screen blood for Zika virus in Puerto Rico under the IND protocol. CTS plans to use the test under the IND in the continental United States, as well.

Puerto Rico has had to import blood products in recent weeks that were collected in mainland locations without active mosquito-borne Zika virus transmission. The U.S. Department of Health and Human Services provided financial assistance to Puerto Rico for the imported components under contracts that expired today.

CDC Collaborates With Puerto Rico DOH on Rapid Assessment of Blood Collection and Use

All of Puerto Rico's blood centers and 91 percent of its hospital transfusion services indicated that they collected more whole blood units locally than they imported from the continental U.S. in 2015, according to a survey conducted by CDC and the Puerto Rico Department of Health (DOH). The survey shows that 82,381 units were collected locally and 52,411 units were imported from areas in the continental U.S. without active Zika virus transmission. Also, approximately 25 percent of apheresis platelets were treated with pathogen reduction technologies (PRT), and 36.4 percent of the treated platelets were transfused to patients.

CDC and the Puerto Rico DOH surveyed 12 blood centers and 56 hospital transfusion services that collected or transfused whole blood or components during 2015. The survey asked respondants about collection methods, product types, importation of blood products, blood use and extent of PRT used for plasma and platelets. The survey, which was open Feb. 10-24, 2016, was the first effort to estimate blood collection and use in Puerto Rico.

There have been no confirmed cases of transfusion-transmitted Zika virus in Puerto Rico or the continental U.S. at present. Based on the likelihood of local mosquito-borne Zika virus transmission and resulting asymptomatic infections, CDC suggests that blood establishments and public health agencies collaborate to develop plans to ensure local blood safety and adequacy in the continental U.S.

Landscape of Ancillary Cord Blood Banking Services read more »

May 17 — AABB Audioconference: HOT TOPIC: Anti-CD38 Interference with Serologic Testing read more »

May 18 — AABB Audioconference: Universal Donor Products — Group O Negative Blood and Group AB Plasma — Where Do They Go? read more »

June 1 — AABB Audioconference: Customer Care — Building Trust, Establishing Rapport and Making Donors Feel Valued read more »

Full Calendar read more »

New Web Resources

FDA Corrects Docket Number of Guidance on Donor Screening Recommendations to Reduce Risk of Transmission of Zika by HCT/Ps read more »

FDA Approves Venclexta (Venetoclax) to Treat Patients With Chromosomal Abnormality Suffering From Chronic Lymphocytic Leukemia read more »

別紙様式第2-1
番号15

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05月18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	人ハプトグロビン	研究報告の公表状況	http://biorxiv.org/content/early/2016/04/25/050112/2016/04/25	公表国 アメリカ
販売名 (企業名)	ハプトグロビン静注2000単位「JB」（日本血液製剤機構）			

研究報告の概要

数十年にわたり、蚊により伝播するフラビウイルスであるジカウイルスのヒトへの感染は、軽度で散発的であった。同じ地域で流行する急性熱性疾患に類似した症状であったことから実際よりも報告が少なかった。近年ギラン・バレー症候群と重症胎児異常に関連した重症なジカウイルス感染は、大きな関心事となってきた。免疫のない人々への急速な広がりを見せている近年の経過を考えると、ジカウイルス（ZIKV）はアメリカ大陸と世界的にも伝播力があるヒトスジシマカが見つかる地域において広がり続けるであろう。
デングウイルス（DENV）は、蚊によりヒトへ伝播する世界的に最も知られたフラビウイルスであり、また、最近ジカウイルスが確認された地域のアウトブレイクの感染源であり、何れもよく知られている。DENVとZIKVは密接にかなりの抗原性の重複が生じ、関連している。抗体依存性増強（ADE）として知られているメカニズムにより、抗DENV抗体は、ある特定の免疫細胞へのDENVの感染性を増強し、結果的に重篤な転帰となる疾患に関連したウイルス生成を増強する。同様に、ZIKVは、他のフラビウイルスによって生成される抗体によるADEを受けることが示されている。しかしながら、DENV抗体に対する反応はまだ調査されていない。
我々は、中和試験とADEアッセイを用いて、ZIKVに対するヒトDENV血清と、よく理解され広い中和作用のあるヒト抗DENVモノクローナル抗体（HMAb）の中和試験と感染増強の可能性を試験した。抗DENV HMAbsは、交差反応、即ち中和せず、大幅にin vitroでZIKV感染を増強する。DENV免疫血清も同様に、ZIKV感染を増強し、ZIKVに対する中和作用の程度の変化があった。
我々の結果は、既存のDENV免疫が、in vivoでZIKV感染を増強し、疾患の重症度を悪化させることを示唆している。
ZIKVとDENVの間の相互作用を明確に理解することは、これらのウイルスが共に流行している地域において公衆衛生に対する的確な情報提供を行う上で重要であり、ZIKVとDENVのワクチンの設計と対策の実行のために特に有益であろう。

使用上の注意記載状況・その他参考事項等

2. 重要な基本的注意
(1)本剤の原材料となる献血者の血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体、抗HTLV-1抗体陰性で、かつALT（GPT）値でスクリーニングを実施している。更に、HBV、HCV及びHIVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は,以上の検査に適合した血漿を原料として、Cohnの低温エタノール分画で得た画分から人ハプトグロビンを濃縮・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

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。

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。

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。

Dengue Virus Antibodies Enhance Zika Virus Infection

Short Title: Dengue gives Zika a boost

Lauren M. Paul[1], Eric R. Carlin[1], Meagan M. Jenkins[1], Amanda L. Tan[1], Carolyn M. Barcellona[1], Cindo O. Nicholson[1,#], Lydie Trautmann[2,3], Scott F. Michael[1¶], and Sharon Isern[1¶*]

[1]Department of Biological Sciences, College of Arts and Sciences, Florida Gulf Coast University, Fort Myers, Florida, United States of America

[2]U.S. Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, Maryland, United States of America

[3]Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, MD, USA

[#]Current Address: Department of Molecular Genetics and Microbiology, Duke University School of Medicine, Durham, North Carolina, United States of America

*Corresponding author

E-mail: sisern@fgcu.edu (SI)

[¶]SI and SFM are Joint Senior Authors.

Abstract:

Background

For decades, human infections with Zika virus (ZIKV), a mosquito-transmitted flavivirus, were sporadic, associated with mild disease, and went underreported since symptoms were similar to other acute febrile diseases endemic in the same regions. Recent reports of severe disease associated with ZIKV, including Guillain-Barré syndrome and severe fetal abnormalities, have greatly heightened awareness. Given its recent history of rapid spread in immune naïve populations, it is anticipated that ZIKV will continue to spread in the Americas and globally in regions where competent *Aedes* mosquito vectors are found. Globally, dengue virus (DENV) is the most common mosquito-transmitted human flavivirus and is both well-established and the source of outbreaks in areas of recent ZIKV introduction. DENV and ZIKV are closely related, resulting in substantial antigenic overlap. Through a mechanism known as antibody-dependent enhancement (ADE), anti-DENV antibodies can enhance the infectivity of DENV for certain classes of immune cells, causing increased viral production that correlates with severe disease outcomes. Similarly, ZIKV has been shown to undergo ADE in response to antibodies generated by other flaviviruses. However, response to DENV antibodies has not yet been investigated.

Methodology / Principal Findings

We tested the neutralizing and enhancing potential of well-characterized broadly neutralizing human anti-DENV monoclonal antibodies (HMAbs) and human DENV immune sera against ZIKV using neutralization and ADE assays. We show that anti-

DENV HMAbs, cross-react, do not neutralize, and greatly enhance ZIKV infection *in vitro*. DENV immune sera had varying degrees of neutralization against ZIKV and similarly enhanced ZIKV infection.

Conclusions / Significance

Our results suggest that pre-existing DENV immunity will enhance ZIKV infection *in vivo* and may increase disease severity. A clear understanding of the interplay between ZIKV and DENV will be critical in informing public health responses in regions where these viruses co-circulate and will be particularly valuable for ZIKV and DENV vaccine design and implementation strategies.

Author Summary:

Recent reports of severe disease, including developmental problems in newborns, have greatly heightened public health awareness of Zika virus (ZIKV), a mosquito-transmitted virus for which there is no vaccine or treatment. It is anticipated that ZIKV will continue to spread in the Americas and globally in regions where competent mosquitoes are found. Dengue virus (DENV), a closely related mosquito-transmitted virus is well-established in regions of recent ZIKV introduction and spread. It is increasingly common that individuals living in these regions may have had a prior DENV infection or may be infected with DENV and ZIKV at the same time. However, very little is known about the impact of DENV infections on ZIKV disease severity. In this study, we tested the ability of antibodies against DENV to prevent or enhance ZIKV infection in cell culture-based assays. We found that DENV antibodies can greatly enhance ZIKV infection in cells.

Our results suggest that ZIKV infection in individuals that had a prior DENV infection may experience more severe clinical manifestations. The results of this study provide a better understanding of the interplay between ZIKV and DENV infections that can serve to inform public health responses and vaccine strategies.

Introduction:

Zika virus (ZIKV), a mosquito-transmitted flavivirus, was first isolated in a sentinel rhesus monkey and *Aedes africanus* mosquitoes in the Zika Forest near Entebbe, Uganda in 1947 during routine arbovirus surveillance by the Virus Research Institute in Entebbe [1]. A subsequent survey of human sera for ZIKV neutralizing antibodies in localities in Uganda including Zika, Kampala and Bwamba concluded that 6.1% of individuals tested were ZIKV seropositive [2]. Although no human disease had been associated with ZIKV at the time, it was speculated that ZIKV infection was not necessarily rare or unimportant. Neutralizing anti-ZIKV activity was found in serum collected between 1945 and 1948 from individuals residing in East Africa including Uganda and then northern Tanganyika south of Lake Victoria. Over 12% of individuals tested had ZIKV neutralizing activity though at the time ZIKV was an agent of unknown disease [3]. Simpson described the first well-documented case of ZIKV disease and virus isolation in humans [4]. He became infected while working in the Zika Forest in 1963, and his mild disease symptoms, that lasted for 5 days, included low-grade fever, headache, body aches, and a maculopapular rash. These symptoms have since become hallmark features of ZIKV human disease. In 1968, ZIKV was isolated from 3 non-hospitalized children in Ibadan, Nigeria indicating that ZIKV was not restricted to

East Africa [5]. A 1953 and 1954 serological survey in South East Asia that included individuals from Malaysia near Kuala Lumpur, Thailand, and North Vietnam found ZIKV protective sera in individuals residing in these regions ranging from 75% positive in Malayans, 8% in Thailand, and 2% in North Vietnam [6]. An early 1980s serologic study of human volunteers in Lombok, Indonesia reported that 13% had neutralizing antibodies to ZIKV [7]. These studies illustrated that ZIKV had spread beyond Africa and at some point became endemic in Asia [8].

For decades, human ZIKV infections were sporadic, spread in geographic location, remained associated with mild disease, and perhaps went underreported since its symptoms were similar to other acute febrile diseases endemic in the same regions. As is the case with other flaviviruses, it is known that ZIKV antibodies cross-react with other flavivirus antigens including dengue virus (DENV) as was illustrated in the Yap State, Micronesia ZIKV outbreak in 2007. Initial serologic testing by IgM capture ELISA with DENV antigen was positive which led physicians to initially conclude that the causative agent for the outbreak was DENV, though the epidemic was characterized by a rash, conjunctivitis and arthralgia symptoms clinically distinct from DENV [9]. Subsequent testing using a ZIKV-specific reverse transcriptase polymerase chain reaction (RT-PCR) assay revealed that ZIKV was the causative agent [10]. Sequencing and phylogenetic analysis indicated that only one ZIKV strain circulated in the epidemic and that it had a 88.7% nucleotide and 96.5% amino acid identity to the African 1947 ZIKV strain MR766. A 12-nucleotide sequence was found in the envelope gene that was absent in the ZIKV African prototype. The consequence of this addition with regards to virus replication, fitness, and disease outcome is not yet known. No further transmission

was reported in the Pacific until 2013 when French Polynesia reported an explosive ZIKV outbreak with 11% of the population seeking medical care [11]. Phylogenetic analysis revealed that the outbreak strain was most closely related to a Cambodia 2010 strain and the Yap State 2007 strain corroborating expansion of the Asian ZIKV lineage. Perinatal ZIKV transmission was also reported in French Polynesia [12]. In addition, 3% of blood bank samples tested positive for ZIKV by RT-PCR even though the donors were asymptomatic when they donated, underscoring the potential risk of ZIKV transmission through blood transfusions [13]. ZIKV transmission and spread maintained a solid foothold in the Pacific [14] and continued its spread in 2014 with confirmed outbreaks in French Polynesia, New Caledonia, Easter Island, and the Cook Islands [15-18].

The first report of local transmission of ZIKV in the Americas occurred in the city of Natal in Northern Brazil in 2015 [19]. Natal patients reported intense pain resembling Chikungunya virus (CHIKV) infection but with a shorter clinical course, in addition to maculopapular rash. No deaths or complications were reported at the time, though given the naïve immunological status of the Brazilian population, ZIKV expansion was predicted. Several theories arose to explain the probable introduction of ZIKV into Brazil. These included the soccer World Cup in 2014, though no ZIKV endemic countries competed [19], the 2014 Va'a World Sprint Championships canoe race held in Rio de Janeiro with participants from French Polynesia, New Caledonia, Cook Islands, and Easter Island [20], and the 2013 Confederations Cup soccer tournament which included competitors from French Polynesia [21]. Molecular clock analysis of various Brazilian ZIKV strains estimated that the most recent common ancestor dated back to

2013 making the first two theories less likely [21]. By mid-January 2016, ZIKV transmission had occurred in 20 countries or territories in the Americas as reported to the Pan American Health Organization [22]. The primary mode of ZIKV transmission appeared to be through mosquito vectors, although cases of perinatal and sexual transmission were also reported [12,23]. Given its recent history of rapid spread in immune naïve populations, it is anticipated that ZIKV will continue to spread for the foreseeable future in the Americas and globally in regions where competent *Aedes* mosquito vectors are present. Kindhauser et al. 2016 can serve as a comprehensive account of the world-wide temporal and geographic distribution of ZIKV from 1947 to present day [24].

Until relatively recently, due to its mild clinical outcome, ZIKV disease had not been a critical public health problem. As a result, compared to other related viruses, it remained understudied. However, recent reports of severe ZIKV disease including Guillain-Barré syndrome in French Polynesia [14,25] and associations between ZIKV and microcephaly and other severe fetal abnormalities in Brazil [26-30] have greatly heightened awareness of ZIKV. Retrospectively, the incidence of Guillain-Barré syndrome during the 2014 ZIKV French Polynesia outbreak and the incidence of microcephaly in Brazil in 2015 were both 20 times higher than in previous years. The cause of these severe ZIKV disease outcomes remains an open question. Recent ZIKV outbreaks in the Pacific and the Americas have been explosive and associated with severe disease, yet earlier expansions in Africa and Asia were gradual, continuous and associated with mild clinical outcomes. Much of the difference may lie in the age of exposure. In ZIKV endemic areas, most adults have pre-existing ZIKV immunity and

new cases primarily occur in children. Introduction of ZIKV into immune naïve populations where all ages are susceptible to infection, including women of child-bearing age, is the new scenario for ZIKV expansion. However, we are still left without an understanding of why certain individuals develop severe disease such as Guillain-Barré syndrome, and why some expectant mothers transmit ZIKV to their developing offspring *in utero*, resulting in fetal infection and developmental abnormalities, whereas others do not. A possible explanation could be the impact of pre-existing immunity to co-circulating flaviviruses.

Globally, DENV is the most common mosquito-transmitted human flavivirus [31] and is both well-established and the source of new outbreaks in many areas of recent ZIKV introduction [15,16]. DENV and ZIKV are very closely related resulting in substantial antigenic overlap. The four serotypes of DENV (DENV-1, DENV-2, DENV-3, and DENV-4) have an antigenic relationship that impacts disease severity. Infection with one serotype typically results in a life-long neutralizing antibody response to that serotype, but yields cross-reactive, non-neutralizing antibodies against the other serotypes. These cross-reactive, non-neutralizing antibodies are responsible for antibody-dependent enhancement (ADE), a phenomenon where DENV particles are bound (opsonized) by these antibodies, which allows the infection of antibody Fc receptor (FcR) bearing cells, such as macrophages, dendrocytes, and monocytes, that are normally not infected. The presence of enhancing antibodies correlates with increased DENV viremia and disease severity [32-34]. Similarly, ZIKV has also been shown to undergo ADE in response to sub-neutralizing concentrations of homologous anti-serum, and in response to heterologous anti-serum from several different

185 flaviviruses [35]. In addition, anti-ZIKV sera has been shown to enhance infectivity of
186 related viruses [36]. In one study, immune mouse ascites against various flaviviruses
187 including ZIKV, West Nile virus (WNV), Yellow Fever-17D (YF17D), Wesselsbron virus,
188 Potiskum, Dakar Bat, and Uganda S were tested for ZIKV ADE in $P388D_1$, a mouse
189 macrophage Fc receptor cell line [35]. All heterologous immune mouse ascites, as well
190 as homologous immune ascites, enhanced ZIKV in culture. Of note, the fold-
191 enhancement was greater for ZIKV compared to peak enhancement of other
192 flaviviruses tested against their heterologous immune ascites. Given the incidence of
193 co-circulating flaviviruses, the study authors alluded to the importance of testing human
194 sera for ADE potential of circulating flaviviruses. In a subsequent study, human cord
195 blood and sera of newborns and adults collected in Ibadan, Nigeria, was tested for ADE
196 of DENV-2, YF17D and WNV in $P388D_1$, but the ADE potential of ZIKV was not tested
197 [37]. To our knowledge, only mouse sera and mouse cells have been used to date for *in*
198 *vitro* ZIKV ADE assays. In addition, anti-DENV immune serum has never been tested
199 for ZIKV enhancement activity. Curiously, the 2013-14 French Polynesia ZIKV outbreak
200 demonstrated that all the patients with Guillain-Barré syndrome had pre-existing DENV
201 immunity [25].

202 In this study, we investigated the role that pre-existing DENV immunity plays
203 during ZIKV infection. Here we report that human anti-DENV serum and well-
204 characterized human anti-DENV monoclonal antibodies (HMAbs) cause substantial
205 ZIKV ADE in a human Fc receptor bearing cell line. Our results suggest that pre-existing
206 antibodies from a prior DENV infection will enhance ZIKV infection *in vivo* and may
207 increase disease severity.

208

209 **Methods:**

210 Human Sera and Monoclonal Antibodies

211 The collection of human blood samples was reviewed and approved by the
212 institutional review board of Florida Gulf Coast University (protocols 2007-08 and 2007-
213 12) and the research ethics committee of the Centre Hospitalier de l'Université de
214 Montréal. Informed written consent was obtained from all subjects. Jamaica 1, and
215 Singapore 1 sera have been previously described, from subject 8C and subject DA003,
216 respectively [38]. Subject Jamaica 1 (8C) was infected with DENV in Jamaica in 2007
217 and had blood drawn in 2008, approximately 3 months post-recovery. The subject had
218 fever for 12 days, headache, retro-orbital pain, and blood in sputum. Subject Jamaica 2
219 (10E) was infected with DENV in Jamaica in 2007 with severe symptoms and had blood
220 drawn in 2008, 3 months after recovery. Subject Singapore 1 (DA003) was hospitalized
221 in Singapore in 2008 for complications due to DENV infection and had blood drawn
222 approximately 4 weeks post-recovery. No hemoconcentration or bleeding was present.
223 Subject Singapore 2 (PHC) was infected with DENV and hospitalized in Singapore in
224 2008 and had blood drawn approximately 4 weeks after recovery. A healthy subject
225 from Montreal, Canada provided control serum that was collected in 2003 prior to
226 vaccination with yellow fever 17D vaccine. Travel history confirmed that the subject had
227 not travelled to regions outside North America and had no previous exposure to DENV
228 or ZIKV. Sera were heat inactivated for 30 min at 56°C prior to use. Anti-DENV HMAbs
229 1.6D and D11C isolated from subject Jamaica 1 and Singapore 1, respectively, were

kindly provided by J. S. Schieffelin from Tulane University and have been well-characterized and described previously [38].

Viruses and Cell Culture

The 1947 Ugandan isolate, ZIKV MR766, and DENV-1 strain HI-1, DENV-2 strain NG-2, DENV-3 strain H-78, and DENV-4 strain H-42, were kindly provided by R. B. Tesh at the University of Texas at Galveston through the World Reference Center for Emerging Viruses and Arboviruses. ZIKV stock was propagated by single passage in African green monkey (*Cercopithecus aethiops*) kidney epithelial cells, Vero (ATCC CCL-81, American Type Culture Collection, Manassas, VA), cultured in Eagle's Minimum Essential Medium supplemented with 10% (v/v) fetal bovine serum (FBS), 2mM Glutamax, 100U/mL penicillin G, 100ug/mL streptomycin, and 0.25ug/mL amphotericin B at 37°C with 5% (v/v) CO_2. Rhesus macaque (*Macaca mulatta*) kidney epithelial cells, LLC-MK2 (ATCC CCL-7) used to propagate DENV and titer and perform focus-forming unit reduction neutralization assays, were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) FBS, 2mM Glutamax, 100U/mL penicillin G, 100ug/mL streptomycin, and 0.25ug/mL amphotericin B at 37°C with 5% (v/v) CO_2. Human bone-marrow lymphoblast cells bearing FcRII, K-562 (ATCC CCL-243) used to perform antibody-dependent enhancement assays (ADE), were cultured in RPMI-1640 (Hyclone, Logan, UT) supplemented with 10% (v/v) FBS, 2mM Glutamax, 100U/mL penicillin G, 100ug/mL streptomycin, and 0.25ug/mL amphotericin B at 37°C with 5% (v/v) CO_2. All reagents were purchased from ThermoFisher, Waltham, MA unless otherwise noted.

253

254 Enzyme-linked Immunosorbent Assay

255 Enzyme-linked immunosorbent assays (ELISA) were performed as follows. Corning
256 brand high-bind 96-well plates (ThermoFisher, Waltham, MA) were coated with 100uL
257 Concanavalin A (ConA) (Vector Laboratories, Burlingame, CA) at 25ug/mL in 0.01M
258 HEPES (Sigma, Saint Louis, MO) and incubated for 1 hr at room temperature. Wells
259 were washed with phosphate buffered saline (PBS) with 0.1% (v/v) Tween 20 (Sigma)
260 and incubated for 1 hr at room temperature with 100uL of filtered ZIKV or DENV-2
261 culture supernatant inactivated with 0.1% (v/v) Triton-X100 (Sigma). After a wash step
262 with PBS containing 0.1% (v/v) Tween 20, wells were blocked with 200uL PBS
263 containing 0.5% (v/v) Tween 20 and 5% (w/v) non-fat dry milk for 30 min. Primary
264 HMAbs D11C and 1.6D in PBS containing 0.5% (v/v) Tween 20 were incubated for 30
265 min at room temperature. After a wash step, 100uL of a peroxidase-conjugated affinity
266 purified anti-human IgG (Pierce, Rockford, IL) diluted to 1ug/mL in PBS-0.5% (v/v)
267 Tween 20 was incubated at room temperature for 30 min to detect the primary antibody.
268 After a final wash step, color was developed with tetramethylbenzidineperoxide
269 (ProMega, Madison, WI) as the substrate for peroxidase. The reaction was stopped
270 after 3 min by adding 100uL1M phosphoric acid (Sigma), and the absorbance was read
271 at 450 nm. Negative controls included media without virus, ConA only, and ConA
272 without primary or secondary antibodies.

273

274 Focus-forming Assay

Focus-forming assays were performed essentially as previously described [38]. LLC-MK2 target cells were seeded at a density of approximately 500,000 cells in each well of a 12-well plate 24-48 hrs prior to infection. For titer assays, 10-fold serial dilutions of virus were prepared. For neutralization assays, approximately 100 focus-forming units of virus were incubated with dilutions of heat-inactivated serum or purified HMAbs in serum-free DMEM for 1 hr at 37°C. Mixtures were allowed to infect confluent target cell monolayers for 1 hr at 37°C, with rocking every 15 min, after which the inoculum was aspirated and cells were overlaid with fresh Minimum Essential Medium (MEM) supplemented with 10% (v/v) FBS, 2mM Glutamax, 100U/mL penicillin G, 100ug/mL streptomycin, and 0.25ug/mL amphotericin B containing 1.2% (w/v) microcrystalline cellulose Avicel (FMC BioPolymer, Philadelphia, PA). The infected cells were then incubated at 37°C with 5% (v/v) CO_2 for 48 hr (DENV-4), 60 hr (ZIKV), or 72 hr (DENV-1, -2, and -3). Cells were fixed in Formalde-Fresh Solution (ThermoFisher), either overnight at 4°C or for 1 hr at room temperature and permeabilized with 70% (v/v) ethanol for 30 min. Foci were detected using primary HMAbs 1.6D or D11C incubated for 8 hr at room temperature, followed by secondary horseradish peroxidase-conjugated goat anti-human IgG (H+L) (Pierce, Rockford, IL) incubated for 8 hr at room temperature. Foci were visualized by the addition of 3,3-diaminobenzidine tetrahydrochloride (Sigma-Aldrich, St. Louis, MO).

Antibody-dependent Enhancement Assay

Antibody-dependent enhancement assays were performed as previously described [38,39]. Briefly, 250 focus-forming units of ZIKV were mixed with human sera or HMAbs

and RPMI medium in a 200ul volume and incubated for 1 hr at 37°C. Mixtures were added to 80,000 K562 cells in 300ul of complete RPMI medium and incubated for 3 days at 37°C, 5% (v/v) CO_2. Control experiments were performed by pre-incubating cells for 1 hr at 37°C with a mouse anti-human FcRII MAb (anti-CD32) (Biolegend, San Diego, CA). Cells were collected by centrifugation and total RNA was isolated using an RNeasy Mini-kit (Qiagen, Valencia, CA) following the manufacturer's protocol. Quantitative reverse transcription (qRT-PCR) was performed on isolated RNA using ZIKV-specific forward (CTGCTGGCTGGGACACCCGC) and reverse (CGGCCAACGCCAGAGTTCTGTGC) primers to amplify a 99bp product from the ZIKV NS5 region. A Roche LightCycler 480 II was used to run qRT-PCR using a LightCycler RNA Master SYBR Green I kit (Roche, Indianapolis, IN). Amplification conditions were as follows: reverse transcription at 61°C for 40 min, denaturation at 95°C for 30 sec, followed by 45 cycles of denaturing at 95°C for 5 sec, annealing at 47°C for 10 sec, and extension at 72°C for 15 sec.

Results:

Cross-recognition of ZIKV E protein by human anti-DENV antibodies

It is well known that infection with closely related flaviviruses often results in a cross-reactive serum antibody response. The primary neutralizing epitopes targeted by human antibodies during a flavivirus infection are found in the envelope glycoprotein (E protein) [38,40-46]. The role of the E protein is to facilitate virus entry by binding and mediating the fusion of the virus membrane and cellular membrane in target cells. The

E protein of ZIKV and the four serotypes of DENV have a high degree of genetic similarity and the amino acid sequence of fusion loop region of these viruses is identical. In a previous study, we characterized broadly neutralizing anti-DENV human monoclonal antibodies (HMAbs) derived from patients that had recovered from DENV infection [38]. These HMAbs recognized the E protein with high affinity, neutralized the four DENV serotypes, and mediated ADE *in vitro* at subneutralizing concentrations. Their neutralization activities correlated with a strong inhibition of intracellular fusion, rather than virus-cell binding. Additionally, we mapped epitopes of these HMAbs to the highly conserved fusion loop region of the E protein.

Given the high degree of similarity between the DENV E protein and the ZIKV E protein, we thus tested the ability of two of these well-characterized anti-DENV HMAbs, 1.6D and D11C, to recognize the glycosylated ZIKV E surface protein using a conA capture assay [38]. In this assay, the glycoprotein-binding lectin, conA, is used to capture ZIKV MR766 E glycoprotein, which is then recognized by anti-DENV HMAbs that recognize the DENV E protein fusion loop. The HMAb is then detected with an anti-human IgG HRP-conjugated secondary antibody and an HRP colorimetric substrate. Our results show that anti-DENV HMAbs, 1.6D and D11C, strongly recognize the ZIKV E surface glycoprotein (**Fig 1A, B**). In addition, we tested the ability of these HMAbs to recognize ZIKV-infected cells in an immunostained focus forming assay (**Fig 1C, D**). This result confirms that anti-DENV E fusion loop HMAbs cross-react with ZIKV.

Fig 1. Cross-reactivity of anti-DENV HMAbs against ZIKV. Anti-DENV HMAbs 1.6D and D11C that recognize the DENV E protein fusion loop cross-react with ZIKV MR766

We tested two human anti-DENV sera from Singapore and two from Jamaica, in addition to serum from a DENV-negative donor from Canada. The Singapore patient sera were collected in 2008 during which time ZIKV was endemic in Southeast Asia and after its expansion in the Yap State in Micronesia in the Pacific in 2007. The Canada donor serum was collected in 2003 and the Jamaica sera were collected in 2008 prior to documented introduction of ZIKV in the Americas. Additionally, the Jamaica and Canada subjects had no travel history to ZIKV endemic countries. We purposely selected Singapore 1 and Jamaica 1 sera for these studies since subject Singapore 1 was the source of HMAb D11C and subject Jamaica 1 was the source of HMAb 1.6D [38]. We wanted to determine whether the antibody repertoire of the same individuals contained DENV antibodies that could also neutralize ZIKV infection. Singapore 2 and Jamaica 2 sera were selected based on their broadly neutralizing activity against DENV. As shown in **Fig 4**, the Singapore (1 and 2) and Jamaica (1 and 2) sera showed broadly neutralizing activity against all four serotypes of DENV [38], indicating that they were likely from subjects with secondary DENV infections.

Fig 4. Neutralizing activity of anti-DENV human sera against DENV. All anti-DENV human sera showed broad neutralizing activity against multiple DENV serotypes 1-4. (**A**) Singapore 1, (**B**) Singapore 2, (**C**) Jamaica 1, (**D**) Jamaica 2. DENV-1, -2, -3, and -4 neutralizing activity of Singapore 1 and Jamaica 1 sera has previously been described and is shown here for clarity [38].

412 The results of the ZIKV neutralization assays with human anti-DENV sera are
413 shown in **Fig 5.** We found that Singapore 1 serum strongly neutralized ZIKV, even at
414 high dilutions (1:10,000 dilution), while Singapore 2 had no ZIKV neutralizing activity.
415 Jamaica 1 serum neutralized ZIKV at the highest serum concentrations tested (1:100,
416 1:50), while Jamaica 2 serum did not. We suspect that the strongly ZIKV neutralizing
417 Singapore 1 serum may be the result of a prior undiagnosed ZIKV infection, as ZIKV
418 has been present in Southeast Asia for decades [6,7,24]. However, the less potent
419 neutralizing activity from Jamaica 1 serum is very likely due to cross-neutralization from
420 prior DENV infection, or infections, as ZIKV was unknown in the Americas at the time
421 the serum was collected. Serum from Canada with no exposure to DENV or ZIKV was
422 used as a negative control and had no ZIKV neutralizing activity [48].
423
424 **Fig 5. Neutralizing activity of anti-DENV human sera against ZIKV.** Human anti-
425 DENV sera from Singapore and Jamaica show both non-neutralizing and neutralizing
426 activity against ZIKV MR766. Singapore 1 serum strongly neutralizes ZIKV MR766,
427 suggesting prior ZIKV infection, while Singapore 2 serum has no neutralizing activity.
428 Jamaica 1 serum neutralizes ZIKV MR766 at high serum concentrations, while Jamaica
429 2 serum shows no neutralizing activity at the dilutions tested. Control serum from
430 Canada shows no ZIKV neutralizing activity. The results shown are the average +/- the
431 standard deviation of 6 replicates.
432
433 ***In vitro* ZIKV enhancement activity of human anti-DENV serum**

We then tested whether human DENV immune sera could mediate ADE *in vitro*. We show that ZIKV infection of FcRII bearing K562 cells can be strongly enhanced (up to 200 fold) by all human anti-DENV sera tested (**Fig 6**). In comparison, the control serum from Canada showed no enhancement. The highly neutralizing Singapore 1 serum showed strong ZIKV enhancement at intermediate dilutions (1:100,000 to 1:10,000) that diminished at lower dilutions (1:5,000 to 1:100), indicating that highly neutralizing antibodies can overcome ZIKV infection enhancement at sufficiently high concentrations. To confirm that the mechanism of enhancement involved entry of antibody-bound ZIKV particles through the K562 FcRII pathway, we pre-incubated K562 cells with a mouse anti-FcRII MAb prior to infection with ZIKV that had been pre-incubated with a highly enhancing dilution (1:50,000) of the ZIKV-neutralizing Singapore 1 serum. Our results demonstrate that the ZIKV enhancement effect can be effectively blocked in a dose-responsive manner with an anti-FcRII MAb (**Fig 7**).

Fig 6. Enhancing activity of anti-DENV human sera against ZIKV. The effect of anti-DENV human sera on enhancement of ZIKV infection was determined in the human macrophage-like FcRII bearing cell line K562. All human anti-DENV sera tested showed strong infection enhancing activity of ZIKV MR766. At high serum concentrations, Singapore 1 serum blocked enhancement due to its strong neutralizing activity. Independent assays were repeated twice in triplicate.

Fig 7. Anti-FcRII antibody blocks ZIKV enhancement activity of anti-DENV serum. K562 cells were pre-incubated with increasing concentrations of mouse anti-FcRII MAb

457 prior to infection with ZIKV MR766 that had been pre-incubated with a highly enhancing
458 dilution (1:50,000) of Singapore 1 serum. The results indicate that the ZIKV
459 enhancement effect can be effectively blocked in a dose-responsive manner with an
460 anti-FcRII MAb.
461
462 **Discussion:**
463 The present scenario of ZIKV introduction and spread in the Pacific and the
464 Americas is complicated by pre-existing immunity to DENV. A recent serological survey
465 of women giving birth in 2009-2010 in central Brazil documented that 53% of the new
466 mothers were IgG positive for DENV [49]. ZIKV enhancement has been previously
467 described to occur in the presence of cross-reactive sera raised against other
468 flaviviruses. However, previous studies of ZIKV enhancement have not reported the
469 effect of anti-DENV sera or antibodies or used human sera and cells [35,36]. Here we
470 demonstrate that broadly neutralizing anti-DENV E protein fusion loop HMAbs cross-
471 react with ZIKV, do not neutralize ZIKV, and greatly enhance ZIKV infection *in vitro*.
472 Although the 10 amino acid E protein fusion loop region itself is identical between DENV
473 and ZIKV, the binding epitope for these HMAbs is likely to be much larger and include
474 important interactions with other variable portions of the E proteins that impact
475 neutralization activity. We noted previously that these two HMAbs show little or no
476 neutralizing activity against YFV or WNV [38].
477 In this study, we also investigated the role of secondary anti-DENV sera that
478 might be considered as the worst-case scenario in DENV endemic regions. Our results
479 show that human sera from secondary DENV infections can show varying degrees of

480 neutralization, from neutralizing to non-neutralizing, and similarly enhance ZIKV
481 infection. We have confirmed that the *in vitro* mechanism of ZIKV enhancement occurs
482 through an FcRII-dependent process in human K562 cells in a manner very similar to
483 DENV. If ZIKV ADE is fundamentally similar to DENV ADE, it is highly likely that pre-
484 existing anti-DENV antibodies will increase ZIKV viremia in humans and lead to more
485 severe disease *in vivo*. This correlation will need to be confirmed clinically.
486 These results have implications for our understanding of ZIKV spread and
487 persistence. In areas where DENV is endemic, ZIKV may transmit more readily and
488 persist more strongly than expected from epidemiological transmission models of ZIKV
489 alone, as has been observed in the recent ZIKV expansion in the Pacific and the
490 Americas. How this plays out as ZIKV continues to spread in the Americas and other
491 parts of the world where competent *Aedes* mosquito vectors are present, remains to be
492 seen. One hopeful possibility is that ZIKV spread may be slower in areas where DENV
493 immunity is low.
494 These results also have consequences for DENV and ZIKV vaccine design and
495 use. We identified two serum samples that showed neutralizing activity against both
496 DENV and ZIKV. The activity of highly neutralizing Singapore 1 serum is most likely
497 explained by prior, undiagnosed ZIKV infection, whereas the Jamaica 1 serum
498 neutralizing activity is likely not due to prior ZIKV infection, but may be a combined
499 response against multiple DENV infections. In any case, this raises the possibility of
500 inducing dual ZIKV and DENV immunity, perhaps with a single vaccine. Although the
501 broadly neutralizing, anti-DENV HMAbs we tested did not neutralize ZIKV, there may be
502 other human antibodies that may recognize and neutralize both ZIKV and DENV.

503 However, DENV vaccines that induce a broadly reactive antibody response against viral
504 surface envelope proteins with a large non-neutralizing antibody component may result
505 in a cross-reactive, enhancing response against ZIKV, especially as the vaccine
506 response wanes over time. Additionally, we know little about the reciprocal response of
507 anti-ZIKV antibodies and their capacity to enhance DENV infections, although it would
508 seem plausible that anti-ZIKV antibodies might similarly enhance DENV. A clear
509 understanding of the interplay between ZIKV and DENV infections will be critical to
510 ZIKV planning and response efforts in regions where ZIKV and DENV co-circulate, and
511 particularly valuable for vaccine design and implementation strategies for both ZIKV and
512 DENV.
513
514 **Acknowledgments:**
515 The authors would like to thank John S. Schieffelin at Tulane Univeristy for providing
516 HMAbs 1.6D and D11C and Robert B. Tesh at the University of Texas at Galveston for
517 providing virus strains through the World Reference Center for Emerging Viruses and
518 Arboviruses.
519
520 **References:**
521 1. Dick GW (1952) Zika virus. II. Pathogenicity and physical properties. Trans R Soc
522 Trop Med Hyg 46: 521-534.
523 2. Dick GW, Kitchen SF, Haddow AJ (1952) Zika virus. I. Isolations and serological
524 specificity. Trans R Soc Trop Med Hyg 46: 509-520.

525 3. Smithburn KC (1952) Neutralizing antibodies against certain recently isolated viruses
526 in the sera of human beings residing in East Africa. J Immunol 69: 223-234.
527 4. Simpson DI (1964) Zika Virus Infection in Man. Trans R Soc Trop Med Hyg 58: 335-
528 338.
529 5. Moore DL, Causey OR, Carey DE, Reddy S, Cooke AR, et al. (1975) Arthropod-
530 borne viral infections of man in Nigeria, 1964-1970. Ann Trop Med Parasitol 69:
531 49-64.
532 6. Pond WL (1963) Arthropod-Borne Virus Antibodies in Sera from Residents of South-
533 East Asia. Trans R Soc Trop Med Hyg 57: 364-371.
534 7. Olson JG, Ksiazek TG, Gubler DJ, Lubis SI, Simanjuntak G, et al. (1983) A survey for
535 arboviral antibodies in sera of humans and animals in Lombok, Republic of
536 Indonesia. Ann Trop Med Parasitol 77: 131-137.
537 8. Hayes EB (2009) Zika virus outside Africa. Emerg Infect Dis 15: 1347-1350.
538 9. Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL, et al. (2009) Zika virus
539 outbreak on Yap Island, Federated States of Micronesia. N Engl J Med 360:
540 2536-2543.
541 10. Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, et al. (2008) Genetic and
542 serologic properties of Zika virus associated with an epidemic, Yap State,
543 Micronesia, 2007. Emerg Infect Dis 14: 1232-1239.
544 11. Cao-Lormeau VM, Roche C, Teissier A, Robin E, Berry AL, et al. (2014) Zika virus,
545 French polynesia, South pacific, 2013. Emerg Infect Dis 20: 1085-1086.

546 12. Besnard M, Lastere S, Teissier A, Cao-Lormeau V, Musso D (2014) Evidence of
547 perinatal transmission of Zika virus, French Polynesia, December 2013 and
548 February 2014. Euro Surveill 19.
549 13. Musso D, Nhan T, Robin E, Roche C, Bierlaire D, et al. (2014) Potential for Zika
550 virus transmission through blood transfusion demonstrated during an outbreak in
551 French Polynesia, November 2013 to February 2014. Euro Surveill 19.
552 14. Oehler E, Watrin L, Larre P, Leparc-Goffart I, Lastere S, et al. (2014) Zika virus
553 infection complicated by Guillain-Barre syndrome--case report, French Polynesia,
554 December 2013. Euro Surveill 19.
555 15. Roth A, Mercier A, Lepers C, Hoy D, Duituturaga S, et al. (2014) Concurrent
556 outbreaks of dengue, chikungunya and Zika virus infections - an unprecedented
557 epidemic wave of mosquito-borne viruses in the Pacific 2012-2014. Euro Surveill
558 19.
559 16. Dupont-Rouzeyrol M, O'Connor O, Calvez E, Daures M, John M, et al. (2015) Co-
560 infection with Zika and dengue viruses in 2 patients, New Caledonia, 2014.
561 Emerg Infect Dis 21: 381-382.
562 17. Tognarelli J, Ulloa S, Villagra E, Lagos J, Aguayo C, et al. (2016) A report on the
563 outbreak of Zika virus on Easter Island, South Pacific, 2014. Arch Virol 161: 665-
564 668.
565 18. Pyke AT, Daly MT, Cameron JN, Moore PR, Taylor CT, et al. (2014) Imported zika
566 virus infection from the cook islands into australia, 2014. PLoS Curr 6.

19. Zanluca C, de Melo VC, Mosimann AL, Dos Santos GI, Dos Santos CN, et al. (2015) First report of autochthonous transmission of Zika virus in Brazil. Mem Inst Oswaldo Cruz 110: 569-572.

20. Musso D (2015) Zika Virus Transmission from French Polynesia to Brazil. Emerg Infect Dis 21: 1887.

21. Faria NR, Azevedo RD, Kraemer MU, Souza R, Cunha MS, et al. (2016) Zika virus in the Americas: Early epidemiological and genetic findings. Science.

22. Hennessey M, Fischer M, Staples JE (2016) Zika Virus Spreads to New Areas - Region of the Americas, May 2015-January 2016. MMWR Morb Mortal Wkly Rep 65: 55-58.

23. Foy BD, Kobylinski KC, Chilson Foy JL, Blitvich BJ, Travassos da Rosa A, et al. (2011) Probable non-vector-borne transmission of Zika virus, Colorado, USA. Emerg Infect Dis 17: 880-882.

24. Kindhauser MKA, T.; Frank, V.; Santhana, R.; Dye, C. (2016) Zika: The Origin and Spread of a Mosquito-borne Virus. Bulletin of the World Health Organization

25. Cao-Lormeau VM, Blake A, Mons S, Lastere S, Roche C, et al. (2016) Guillain-Barre Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. Lancet.

26. Mlakar J, Korva M, Tul N, Popovic M, Poljsak-Prijatelj M, et al. (2016) Zika Virus Associated with Microcephaly. N Engl J Med.

27. Martines RB, Bhatnagar J, Keating MK, Silva-Flannery L, Muehlenbachs A, et al. (2016) Notes from the Field: Evidence of Zika Virus Infection in Brain and

589 Placental Tissues from Two Congenitally Infected Newborns and Two Fetal
590 Losses - Brazil, 2015. MMWR Morb Mortal Wkly Rep 65: 159-160.
591 28. Calvet G, Aguiar RS, Melo AS, Sampaio SA, de Filippis I, et al. (2016) Detection
592 and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in
593 Brazil: a case study. Lancet Infect Dis.
594 29. Sarno M, Sacramento GA, Khouri R, do Rosario MS, Costa F, et al. (2016) Zika
595 Virus Infection and Stillbirths: A Case of Hydrops Fetalis, Hydranencephaly and
596 Fetal Demise. PLoS Negl Trop Dis 10: e0004517.
597 30. de Paula Freitas B, de Oliveira Dias JR, Prazeres J, Sacramento GA, Ko AI, et al.
598 (2016) Ocular Findings in Infants With Microcephaly Associated With Presumed
599 Zika Virus Congenital Infection in Salvador, Brazil. JAMA Ophthalmol.
600 31. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, et al. (2013) The global
601 distribution and burden of dengue. Nature 496: 504-507.
602 32. Halstead SB (1988) Pathogenesis of dengue: challenges to molecular biology.
603 Science 239: 476-481.
604 33. Kliks SC, Nimmanitya S, Nisalak A, Burke DS (1988) Evidence that maternal
605 dengue antibodies are important in the development of dengue hemorrhagic
606 fever in infants. Am J Trop Med Hyg 38: 411-419.
607 34. Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, et al. (2000)
608 Dengue viremia titer, antibody response pattern, and virus serotype correlate
609 with disease severity. J Infect Dis 181: 2-9.

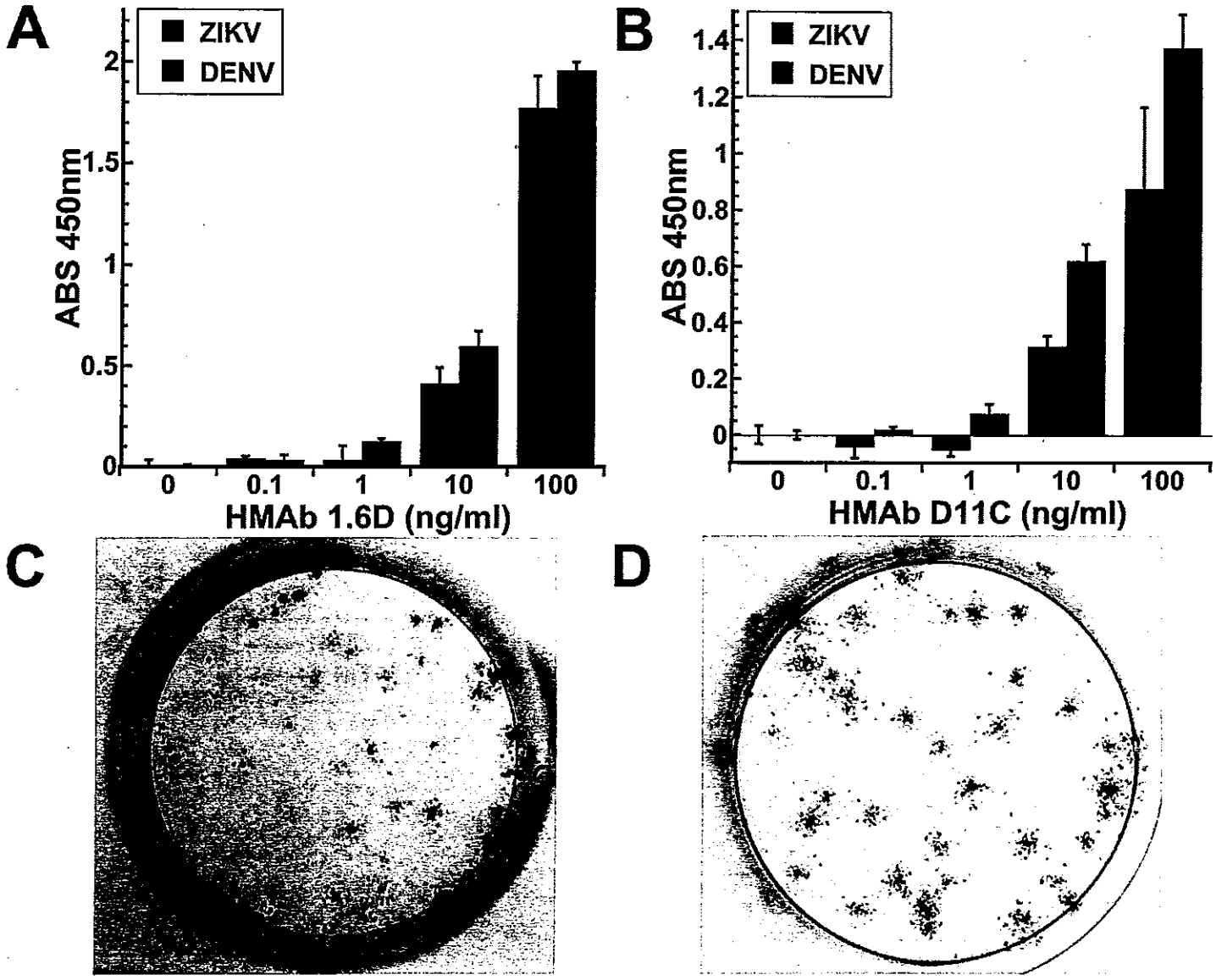
610 35. Fagbami AH, Halstead SB, Marchette NJ, Larsen K (1987) Cross-infection
611 enhancement among African flaviviruses by immune mouse ascitic fluids.
612 Cytobios 49: 49-55.
613 36. Fagbami AH, Halstead SB (1986) Antibody-mediated enhancement of Wesselsbron
614 virus in P388D1 cells. Afr J Med Med Sci 15: 103-107.
615 37. Fagbami A, Halstead SB, Marchette N, Larsen K (1988) Heterologous flavivirus
616 infection-enhancing antibodies in sera of Nigerians. Am J Trop Med Hyg 38: 205-
617 207.
618 38. Costin JM, Zaitseva E, Kahle KM, Nicholson CO, Rowe DK, et al. (2013)
619 Mechanistic study of broadly neutralizing human monoclonal antibodies against
620 dengue virus that target the fusion loop. J Virol 87: 52-66.
621 39. Nicholson CO, Costin JM, Rowe DK, Lin L, Jenwitheesuk E, et al. (2011) Viral entry
622 inhibitors block dengue antibody-dependent enhancement in vitro. Antiviral Res
623 89: 71-74.
624 40. Lai CY, Tsai WY, Lin SR, Kao CL, Hu HP, et al. (2008) Antibodies to envelope
625 glycoprotein of dengue virus during the natural course of infection are
626 predominantly cross-reactive and recognize epitopes containing highly
627 conserved residues at the fusion loop of domain II. J Virol 82: 6631-6643.
628 41. Lin HE, Tsai WY, Liu IJ, Li PC, Liao MY, et al. (2012) Analysis of epitopes on
629 dengue virus envelope protein recognized by monoclonal antibodies and
630 polyclonal human sera by a high throughput assay. PLoS Negl Trop Dis 6:
631 e1447.

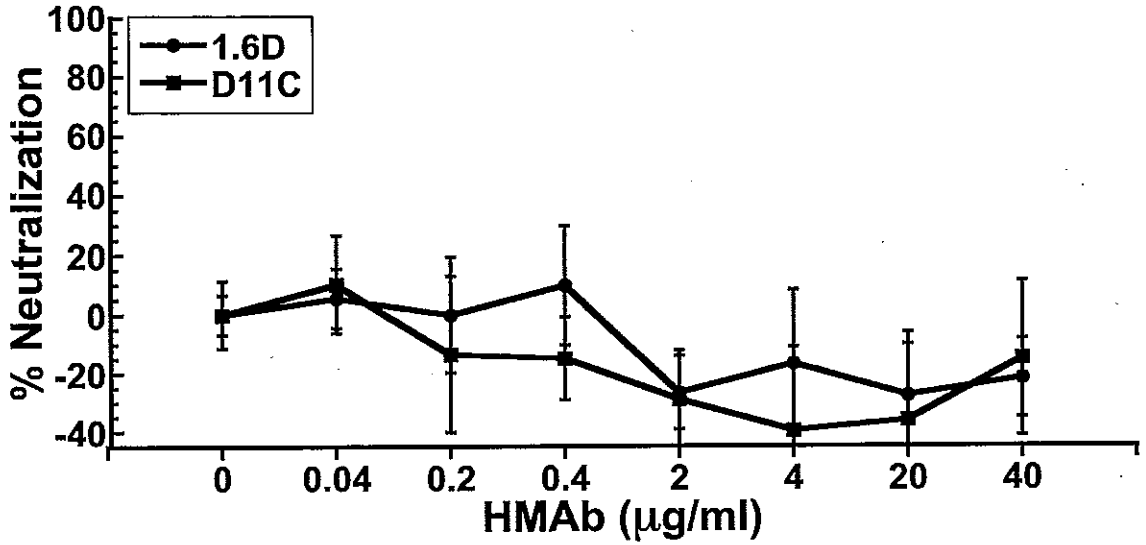
632 42. Wahala WM, Kraus AA, Haymore LB, Accavitti-Loper MA, de Silva AM (2009)
633 Dengue virus neutralization by human immune sera: role of envelope protein
634 domain III-reactive antibody. Virology 392: 103-113.
635 43. Dejnirattisai W, Jumnainsong A, Onsirisakul N, Fitton P, Vasanawathana S, et al.
636 (2010) Cross-reacting antibodies enhance dengue virus infection in humans.
637 Science 328: 745-748.
638 44. Beltramello M, Williams KL, Simmons CP, Macagno A, Simonelli L, et al. (2010) The
639 human immune response to Dengue virus is dominated by highly cross-reactive
640 antibodies endowed with neutralizing and enhancing activity. Cell Host Microbe
641 8: 271-283.
642 45. de Alwis R, Beltramello M, Messer WB, Sukupolvi-Petty S, Wahala WM, et al.
643 (2011) In-depth analysis of the antibody response of individuals exposed to
644 primary dengue virus infection. PLoS Negl Trop Dis 5: e1188.
645 46. Schieffelin JS, Costin JM, Nicholson CO, Orgeron NM, Fontaine KA, et al. (2010)
646 Neutralizing and non-neutralizing monoclonal antibodies against dengue virus E
647 protein derived from a naturally infected patient. Virol J 7: 28.
648 47. Kliks SC, Nisalak A, Brandt WE, Wahl L, Burke DS (1989) Antibody-dependent
649 enhancement of dengue virus growth in human monocytes as a risk factor for
650 dengue hemorrhagic fever. Am J Trop Med Hyg 40: 444-451.
651 48. Muyanja E, Ssemaganda A, Ngauv P, Cubas R, Perrin H, et al. (2014) Immune
652 activation alters cellular and humoral responses to yellow fever 17D vaccine. J
653 Clin Invest 124: 3147-3158.

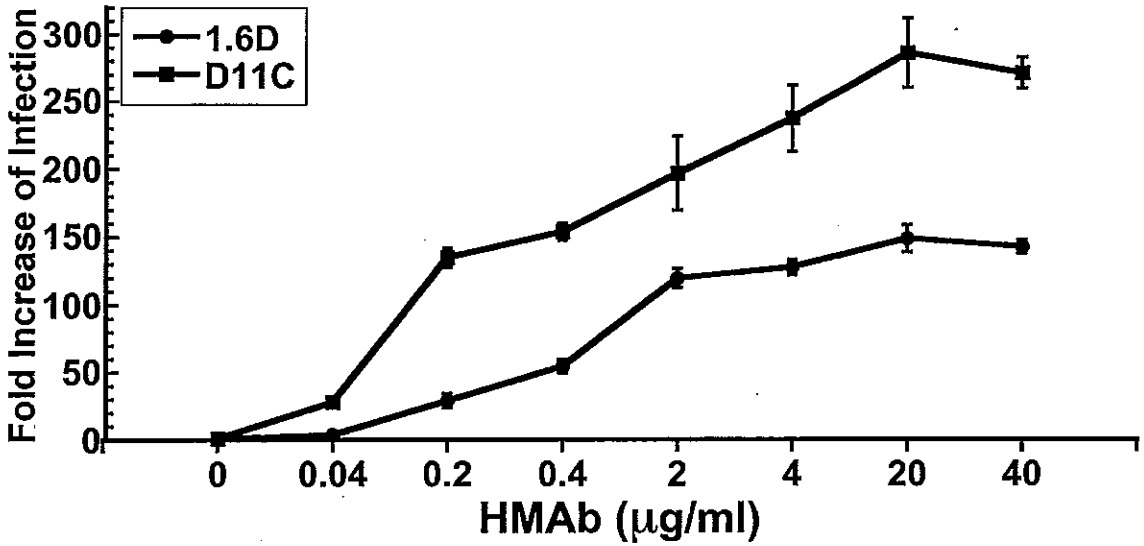
654 49. Argolo AF, Feres VC, Silveira LA, Oliveira AC, Pereira LA, et al. (2013) Prevalence
655 and incidence of dengue virus and antibody placental transfer during late
656 pregnancy in central Brazil. BMC Infect Dis 13: 254.

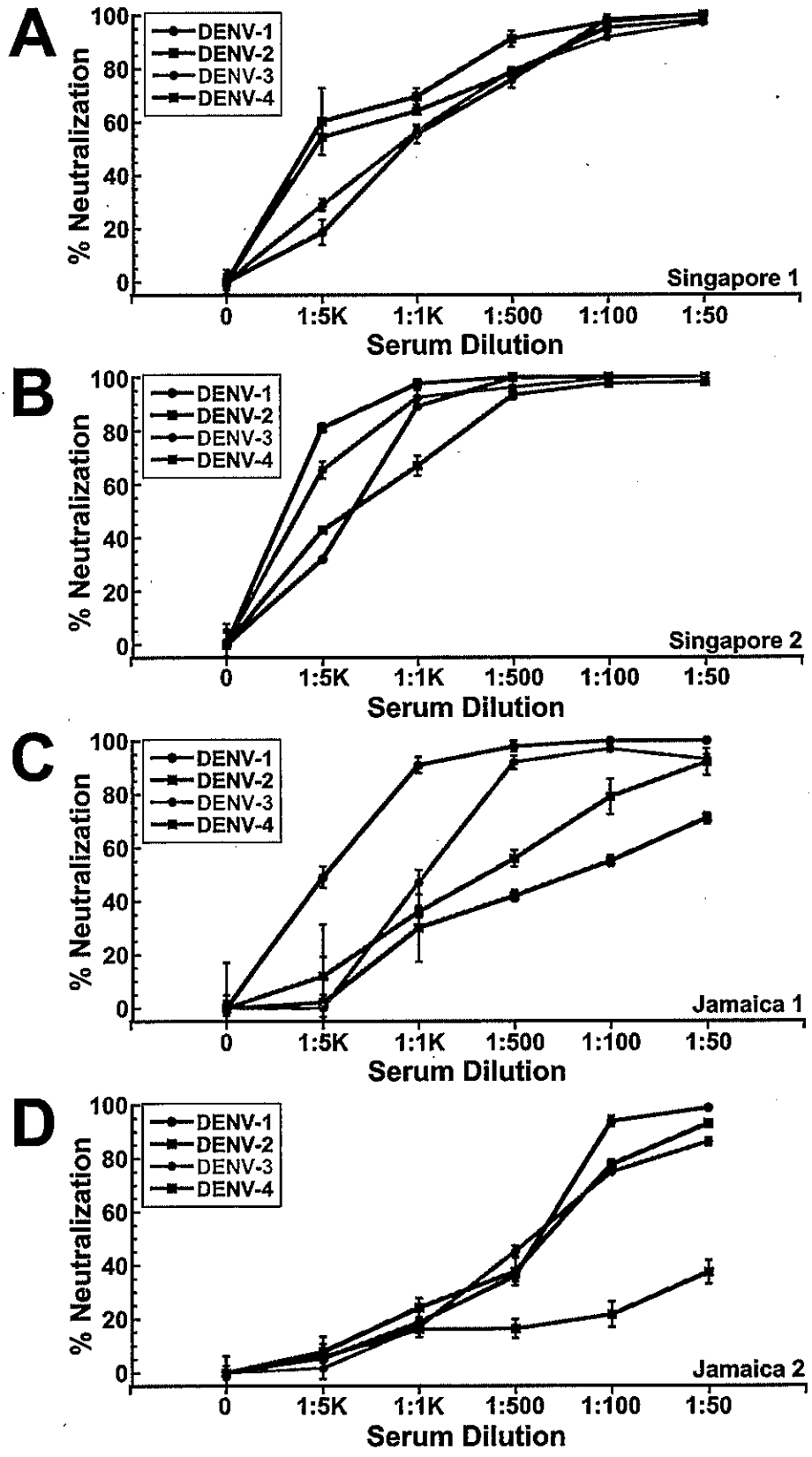
657

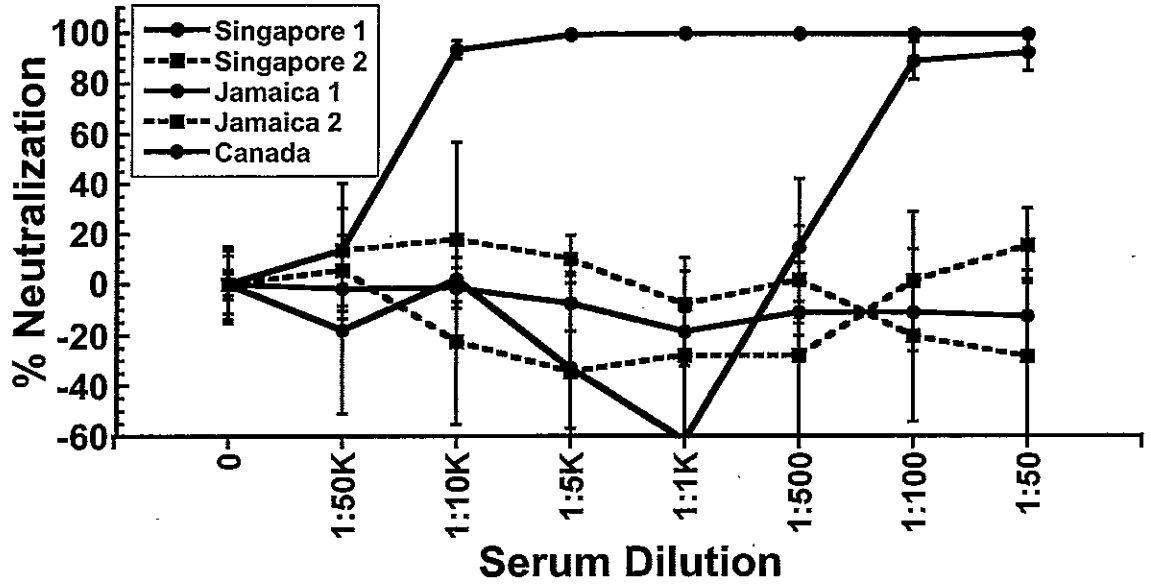
658

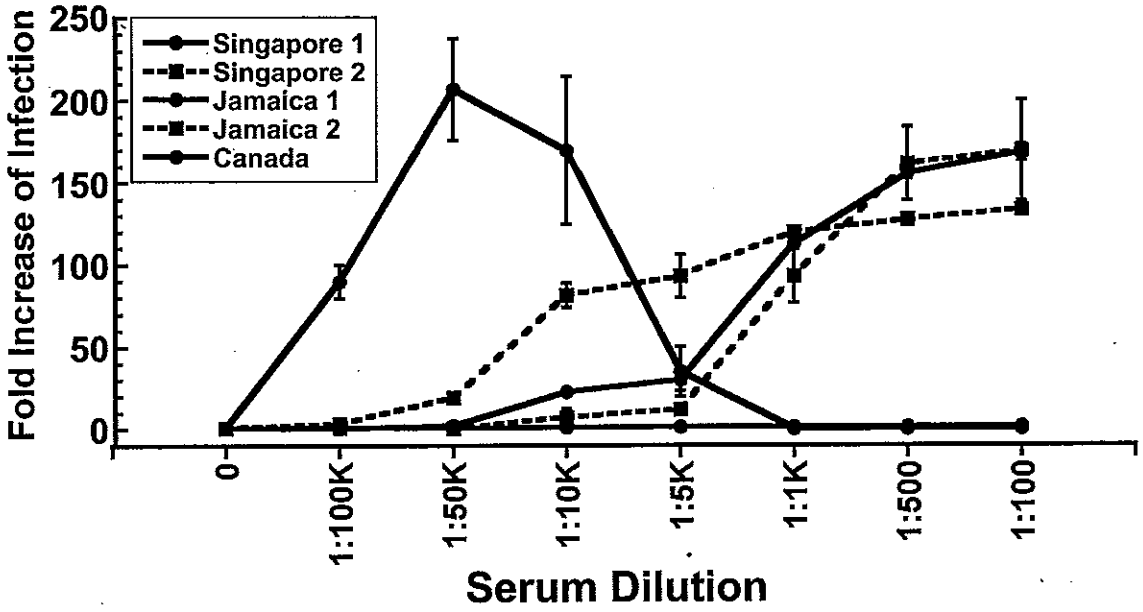

A
ZIKV
DENV
ABS 450nm
2
1.5
1
0.5
0
0
0.1
1
10
100
HMAb 1.6D (ng/ml)
B
ZIKV
DENV
ABS 450nm
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
0.1
1
10
100
HMAb D11C (ng/ml)
C
D

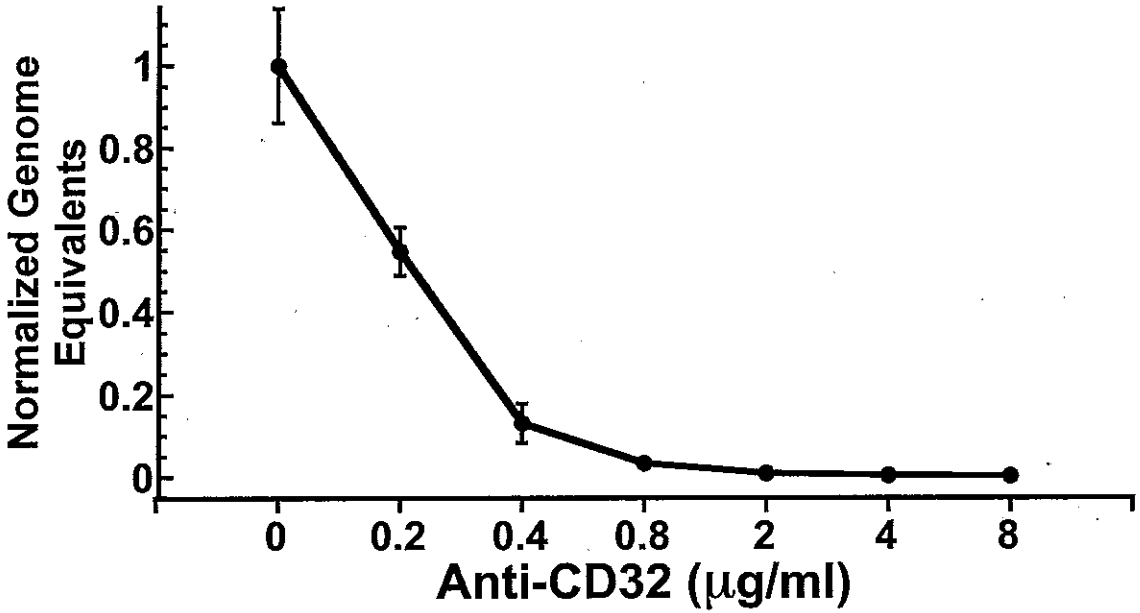

100
80
60
40
20
0
-20
-40
% Neutralization
1.6D
D11C
0
0.04
0.2
0.4
2
4
20
40
HMAb (µg/ml)


1.6D
D11C
Fold Increase of Infection
300
250
200
150
100
50
0
0
0.04
0.2
0.4
2
4
20
40
HMAb (μg/ml)


A
B
C
D
DENV-1
DENV-2
DENV-3
DENV-4
% Neutralization
Serum Dilution
0
1:5K
1:1K
1:500
1:100
1:50
Singapore 1
Singapore 2
Jamaica 1
Jamaica 2


Singapore 1
Singapore 2
Jamaica 1
Jamaica 2
Canada
% Neutralization
100
80
60
40
20
0
-20
-40
-60
0
1:50K
1:10K
1:5K
1:1K
1:500
1:100
1:50
Serum Dilution


Fold Increase of Infection
250
200
150
100
50
0
Singapore 1
Singapore 2
Jamaica 1
Jamaica 2
Canada
0
1:100K
1:50K
1:10K
1:5K
1:1K
1:500
1:100
Serum Dilution


Normalized Genome Equivalents
1
0.8
0.6
0.4
0.2
0
0
0.2
0.4
0.8
2
4
8
Anti-CD32 (μg/ml)

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. 3. 23	該当なし	

一般的名称	人全血液	研究報告の公表状況	Aubry M, Richard V, Green J, Broult J, Musso D Transfusion. 2016 Jan;56(1):33-40. Epub 2015 Aug 18.	公表国 フランス領ポリネシア
販売名(企業名)	人全血液-LR「日赤」(日本赤十字社) 照射人全血液-LR「日赤」(日本赤十字社)			

研究報告の概要

○アモトサレンと紫外線A波(UVA)照射による血漿中ジカウイルス(ZIKV)の不活化。
背景: ZIKVは蚊によって伝播される節足動物媒介性ウイルス(アルボウイルス)である。フランス領ポリネシアにおいて2013年10月から2014年4月に発生したジカウイルスのアウトブレイク時に、輸血による伝播の可能性が示されていた。血液製剤の病原体不活化は、輸血感染症低減の可能性を提供する積極的な対策である。アモトサレンとUVA照射によるアルボウイルスの不活化については、チクングニヤウイルス、ウエストナイルウイルス並びにデングウイルスの不活化が既に実証されている。我々は本稿において、ヒト血漿中のZIKVの不活化における当該処理の有効性について報告する。
研究デザイン及び方法:血漿製剤にZIKVを添加し、アモトサレンとUVAによる光化学処理の前後に、Vero細胞を用いたin vitro感染実験の結果において血漿中の感染力価とRNA量を測定した。
結果:不活化処理前の血漿中感染力価の平均値は6.57 log $TCID_{50}$/mLであり、RNA量の平均値は10.25 log copies/mLであった。不活化処理後のRNA量の平均値は9.51 log copies/mLであったが、不活化処理血漿を接種した細胞を培養した結果、感染細胞は検出されなかった。また1継代後に複製ウイルスは産生されず、2継代以降にはウイルスRNAも検出不能となった。
結論:我々は本研究において、アモトサレンとUVAの組み合わせにより、新鮮凍結血漿中のZIKVが不活化されることを実証した。フランス領ポリネシア等の地域では複数のアルボウイルスが同時に循環している。こうした地域では、当該不活化処理が、血漿輸血によるZIKV感染を防ぐ上での特別な関心事となっている。

報告企業の意見	今後の対応
アモトサレンとUVA照射の組み合わせによる血液製剤の病原体不活化法は、血漿製剤に混入したジカウイルスの不活化にin vitroで有効であったという報告である。	今後も輸血用血液中の細菌やウイルス等の検出や不活化策等の安全対策についての情報の収集に努める。

使用上の注意記載状況・その他参考事項等

人全血液-LR「日赤」
照射人全血液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

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

Maite Aubry,[1] Vaea Richard,[1] Jennifer Green,[2] Julien Broult,[3] and Didier Musso[1]

BACKGROUND: Zika virus (ZIKV) is an arthropod-borne virus (arbovirus) transmitted by mosquitoes. The potential for ZIKV transmission through blood transfusion was demonstrated during the ZIKV outbreak that occurred in French Polynesia from October 2013 to April 2014. Pathogen inactivation of blood products is a proactive strategy that provides the potential to reduce transfusion-transmitted diseases. Inactivation of arboviruses by amotosalen and ultraviolet A (UVA) illumination was previously demonstrated for chikungunya, West Nile, and dengue viruses. We report here the efficiency of this process for ZIKV inactivation of human plasma.

STUDY DESIGN AND METHODS: Plasma units were spiked with ZIKV. Viral titers and RNA loads were measured in plasma before and after amotosalen and UVA photochemical treatment.

RESULTS: The mean ZIKV titers and RNA loads in plasma before inactivation were respectively 6.57 log $TCID_{50}$/mL and 10.25 log copies/mL. After inactivation, the mean ZIKV RNA loads was 9.51 log copies/mL, but cell cultures inoculated with inactivated plasma did not result in infected cells and did not produce any replicative virus after one passage, nor detectable viral RNA from the second passage.

CONCLUSION: In this study we demonstrate that amotosalen combined with UVA light inactivates ZIKV in fresh-frozen plasma. This inactivation process is of particular interest to prevent plasma transfusion-transmitted ZIKV infections in areas such as French Polynesia, where several arboviruses are cocirculating.

Zika virus (ZIKV) is an arthropod-borne virus (arbovirus) of the genus *Flavivirus*, family *Flaviviridae*. ZIKV was first isolated in 1947 from a Rhesus monkey from the Zika forest in Uganda.[1] Until 2007, only sporadic cases have been recorded in Africa and Asia.[2-6] The first reported outbreak of ZIKV outside Africa and Asia occurred in 2007 on the North Pacific island country of Yap Island (Federated States of Micronesia).[7] ZIKV then caused the largest outbreak ever recorded from October 2013 to April 2014 in French Polynesia, South Pacific, with an estimated 28,000 cases.[8-10] In 2015, the first documented outbreak of ZIKV in the Americas occurred in Brazil[11] in which dengue (DENV) and chikungunya (CHIKV) viruses also circulate. The most common clinical manifestations of ZIKV infections are rash, conjunctivitis, fever, and arthralgia,[7] but severe neurologic complications have also been reported in French Polynesian patients.[12]

ABBREVIATIONS: CHIKV = chikungunya virus; DENV = dengue virus; $TCID_{50}$ = 50% tissue culture infectious dose; WNV = West Nile virus; ZIKV = Zika virus.

From the [1]Pôle de Recherche et de Veille sur les Maladies Infectieuses Émergentes, Institut Louis Malardé, Tahiti, French Polynesia; [2]Cerus Corporation, Concord, California; and the [3]Centre de Transfusion Sanguine de la Polynésie Française, Hôpital Du Taaone, Tahiti, French Polynesia.

Address reprint requests to: Maite Aubry, Institut Louis Malardé, PO Box 30, 98713 Papeete, Tahiti, French Polynesia; e-mail: maubry@ilm.pf.

This study was partially funded by Cerus Corporation.

Received for publication March 30, 2015; revision received June 26, 2015; and accepted July 8, 2015.

doi:10.1111/trf.13271

TRANSFUSION 2016;56;33–40

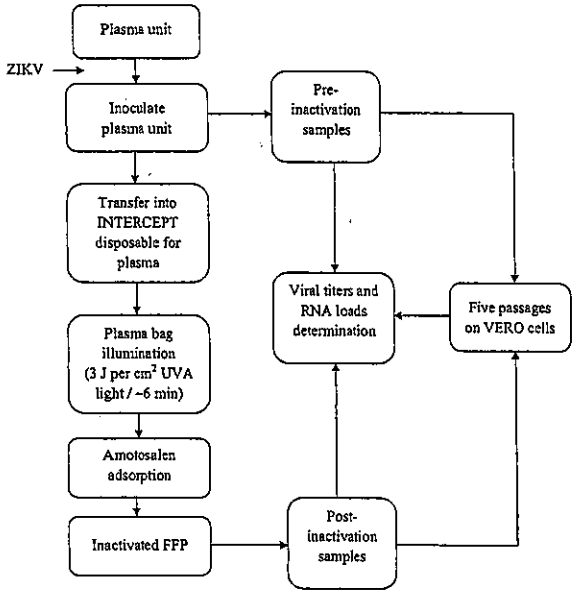


Fig. 1. Schematic flow diagram of the experimental design.

The ZIKV natural transmission cycle involves mosquitoes. ZIKV has been isolated from several *Aedes* (*Ae.*) mosquito species, with *Ae.*[13] and *Ae.*[14] being confirmed as competent vectors. In French Polynesia, *Ae. aegypti* and *Ae. polynesiensis* are suspected to have contributed to ZIKV transmission during the 2013 to 2014 outbreak.[8] Non–vector-borne transmissions of ZIKV by sexual intercourse[15,16] and perinatal infection[17] have also been reported. The potential for ZIKV transmission through blood transfusion has been demonstrated during the French Polynesian outbreak as 2.8% of blood donors, who were asymptomatic at the time of donation, were found positive for acute ZIKV infection using specific reverse transcription–polymerase chain reaction (RT-PCR).[18]

Prevention of transfusion-transmitted ZIKV infections is challenging because most of the cases are asymptomatic and are not detected during medical questionnaire,[18] and nucleic acid testing (NAT) for ZIKV is often not routinely available. An alternative strategy to NAT is pathogen inactivation, a proactive method designed to reduce or abolish infectivity of pathogens in blood products.[19] Several processes have been developed for the inactivation of pathogens during the preparation of fresh-frozen plasma (FFP) and platelet (PLT) concentrates.[19] Among them, a photochemical treatment using a psoralen (amotosalen, S-59), in combination with ultraviolet A (UVA) illumination, has been shown to inactivate a broad range of viruses, bacteria, and protozoans.[20-24] Amotosalen intercalates into double-helical structures of DNA and RNA and forms covalent adducts with pyrimidine bases upon UVA illumination. These adducts prevent nucleic acid replication and transcription to occur and also inhibits DNA repair mechanisms.

Inactivation of arboviruses in plasma by treatment with amotosalen and UVA light has been previously demonstrated for CHIKV,[25] arbovirus of the *Alphavirus* genus, and for West Nile virus (WNV)[25] and DENV,[26] arboviruses of the *Flavivirus* genus. However, to our knowledge, the use of this process to inactivate ZIKV in plasma has never been investigated.

To evaluate the efficacy of amotosalen and UVA light treatment for inactivation of ZIKV in human plasma, we performed a spiking experiment of plasma units with ZIKV and compared the viral titers and viral RNA loads before and after inactivation, in accordance with the recommendations for evaluation of pathogen reduction efficacy.[27] Moreover, we measured the reduction of viral RNA loads induced by inactivation and compared it to the viral RNA loads of sera collected in French Polynesia in 2013 and 2014 from ZIKV-infected, but asymptomatic blood donors[18] to validate the use of amotosalen and UVA light to prevent transfusion-transmitted ZIKV infections.

MATERIALS AND METHODS

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

Virus

The ZIKV strain (PF13/251013-18) was isolated from the serum of a French Polynesian patient infected in 2013. ZIKV was propagated in African green monkey kidney cells (VERO) grown at 37°C in an atmosphere of 5% CO_2 in minimum essential medium supplemented with 2% fetal bovine serum (FBS), 1% penicillin-streptomycin, and 3% sodium bicarbonate 7.5% (Life Technologies, Carlsbad, CA). From the cell culture supernatants, four viral concentrates (between 1.25 and 7 mL each) were obtained as previously described[26] and stored at −80°C with 20% FBS.

Plasma and serum collection

Plasma units were collected from American blood donors by the Interstate Blood Bank of Chicago (Chicago, IL) to eliminate risk of ZIKV antibodies. To assess the absence of previous infections by *Flaviviruses* known to circulate in the United States (DENV and WNV), each plasma unit was tested with a dengue immunoglobulin (Ig)G capture kit (Platelia, Bio-Rad, Hercules, CA) and a classic WNV IgG kit (Serion Elisa, Abcam, Cambridge, UK), to detect IgG antibodies against DENV and WNV, respectively. Only plasma units found negative for both DENV and WNV IgG were selected for the study. The 26 sera of French Polynesian

ZIKV-infected and asymptomatic blood donors were obtained from the blood bank center of French Polynesia as previously reported.[18]

Infection of plasma units with ZIKV

Four plasma units (A, B, C, and D) were inoculated with ZIKV as previously described.[26] A sample from each infected plasma unit (preinactivation sample) was then collected and stored at −80°C until the determination of viral titers and RNA loads.

Inactivation process

Inoculated Plasma Units A, B, and C were treated with amotosalen combined with UVA illumination as previously described,[26] whereas inoculated Plasma Unit D was not inactivated and was the positive control. After transfer into a container with a compound absorption device that removes the residual amotosalen and the free photoproducts, samples from each inactivated plasma unit (inactivated samples) and from the positive control (noninactivated sample) were collected and stored at −80°C until the determination of viral titers and RNA loads.

Detection of replicative ZIKV and viral titration

For the detection of replicative ZIKV, all pre-, post-, and noninactivated samples were inoculated in triplicate on VERO cells in 24-well plates, and five successive passages were performed as previously described.[26] For each passage, inoculated cells were maintained at 37°C in an atmosphere of 5% CO_2 for 4 to 6 days. After each passage, indirect immunofluorescence assay was performed as previously reported[26] to detect ZIKV inoculated cells, using anti-flavivirus mouse antibodies 4G2 (Institut Pasteur, Paris, France) at a dilution of 1:50 in phosphate-buffered saline (PBS, Biomérieux, Marcy l'Etoile, France).

For viral titration, triplicate 10-fold dilutions of preinactivation, postinactivation, and control samples were inoculated on VERO cells in 96-well plates. Inoculated cells were maintained at 37°C in an atmosphere of 5% CO_2 for 6 days and then were washed twice with 400 µL of PBS using a microplate washer (Bio-Rad). Cells were fixed in cold acetone for 10 minutes at room temperature and air-dried. Fifty microliters of antibodies 4G2 diluted 1:2000 in diluent buffer containing PBS with 5% skimmed milk (Régilait, Saint-Martin-Belle-Roche, France) and 0.3% Tween 20 (Merck, Darmstadt, Germany) were distributed in all wells. Plates were stored at 30°C for 1 hour, and then cells were washed four times with 400 µL of wash buffer (PBS with 0.3% Tween 20). Fifty microliters of goat anti-mouse IgG conjugated to horseradish peroxidase (Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:2000 in diluent buffer were distributed in all wells. Plates were stored at 30°C for 1 hour, and then cells were washed four times with 400 µL of wash buffer. Cells were incubated at room temperature in the dark for 5 minutes with 50 µL of peroxidase substrate tetramethylbenzidine before the addition of 50 µL of tetramethylbenzidine stop solution (KPL, Inc., Gaithersburg, MD). Absorbance was read at a wavelength of 450 nm (OD_{450}) using a microplate photometer (Thermo Scientific, Waltham, MA). Infectious wells were counted for each dilution and viral titers were expressed as 50% tissue culture infectious dose ($TCID_{50}$/mL) using the method of Reed and Muench.[28]

ZIKV RNA quantitation

For all plasma (pre-, post-, and noninactivated samples), cell supernatant, and serum samples, RNA extraction was performed from 200 µL of each sample using an extraction system (easyMAG, bioMérieux), and real-time RT-PCR was performed in a thermocycler (CFX96, Bio-Rad) as previously described.[29] A standard curve using 10-fold serial dilutions of a ZIKV RNA transcript, obtained from the strain PF13/251013-18 with an in vitro transcription system (Riboprobe System-T7, Promega, Madison, WI) and quantitated using a RNA assay kit with a fluorometer (Quant-iT and Qubit, respectively, Invitrogen, Carlsbad, CA), was included within the RT-PCR run to estimate the copy number in samples. The limit of detection for this assay is 25 to 100 viral RNA copies.[29] Results were expressed in log copies/mL.

RESULTS

Detection of replicative ZIKV and ZIKV titration

Viral titers in preinactivation samples (A, B, and C) ranged from 6.46 to 6.63 log $TCID_{50}$/mL (mean, 6.57 log $TCID_{50}$/mL; Table 1). The culture of preinactivation and noninactivated (D) samples produced replicative viruses during successive passages, whereas no replicative virus was detected in inactivated samples, even after five passages.

ZIKV RNA quantitation

Viral RNA loads in preinactivation and inactivated samples, respectively, ranged from 10.11 to 10.41 log copies/mL (mean, 10.25 log copies/mL) and from 9.41 to 9.67 log copies/mL (mean, 9.51 log copies/mL; Table 2). Viral RNA load (copies/mL) and viral infectivity ($TCID_{50}$/mL) values are different because not all virus particles are infectious. After the first passage of inactivated samples on VERO cells, ZIKV RNA loads ranged from 3.63 to 4.07 log copies/mL (mean, 3.86 log copies/mL), and from the second to the fifth passage, viral RNA remained undetectable indicating the absence of replicative virus following inactivation. The 26 sera from French Polynesian blood donors showed ZIKV RNA loads ranging from 3.40 to 6.91 log copies/mL (mean, 4.85 log copies/mL; Table 3).

TABLE 1. Detection of replicative ZIKV and ZIKV titration (log $TCID_{50}$/mL) in plasma samples before and after inactivation

Plasma samples	Initial viral titers	Replicative ZIKV after First passage	Second passage	Third passage	Fourth passage	Fifth passage	Log reduction
A							
Preinactivation sample	6.46	+*	+	+	+	+	>6.46
Inactivated sample	−†	−	−	−	−	−	
B							
Preinactivation sample	6.63	+	+	+	+	+	>6.63
Inactivated sample	−	−	−	−	−	−	
C							
Preinactivation sample	6.61	+	+	+	+	+	>6.61
Inactivated sample	−	−	−	−	−	−	
D (control)							
Preinactivation sample	6.14	+	+	+	+	+	
Noninactivated sample	6.28	+	+	+	+	+	

* Positive immunofluorescence.
† Negative immunofluorescence.

TABLE 2. ZIKV RNA quantitation (log copies/mL) in plasma samples before and after inactivation

Plasma sample	Initial RNA loads	RNA loads after First passage	Second passage	Third passage	Fourth passage	Fifth passage
A						
Preinactivation sample	10.22	10.31	10.08	10.36	10.17	10.48
Inactivated sample	9.67	4.07	ND*	ND	ND	ND
B						
Preinactivation sample	10.41	10.30	10.17	10.26	10.22	10.42
Inactivated sample	9.44	3.63	ND	ND	ND	ND
C						
Preinactivation sample	10.11	10.43	10.19	10.30	10.20	10.65
Inactivated sample	9.41	3.87	ND	ND	ND	ND
D (control)						
Preinactivation sample	10.36	10.26	10.02	10.32	10.02	10.28
Noninactivated sample	9.90	9.89	9.99	9.95	10.18	10.01

* ZIKV RNA not detected.

DISCUSSION

Members of the AABB's Transfusion Transmitted Diseases Committee identified emerging infectious disease agents that pose a real or theoretical threat to transfusion safety, due to their presence in blood during the donor's asymptomatic phase; their survival or persistence in blood during processing and storage; and the fact they must be recognized as responsible for a clinically apparent outcome in some recipients who become infected.[30,31] Arboviruses are considered as threats for the blood supply since evidence of their transfusion transmissibility has been found. WNV is the best documented transfusion-transmitted arbovirus with 23 patients that were confirmed to have been infected in 2002 in the United States through transfused red blood cells (RBCs), PLTs, and FFP.[32] DENV infections after transfusion with RBCs or FFP have been reported in 2002 in Hong Kong,[33] and in 2007 in Puerto Rico[34] and Singapore,[35] the latter two resulting in cases of DENV hemorrhagic fever. In addition, the potential for CHIKV transmission by blood transfusion has been demonstrated in the Caribbean in 2014.[36]

ZIKV is an emerging pathogen that has caused several outbreaks in the Pacific region since 2007,[9,37] and the potential for ZIKV transfusion-transmitted infection has been demonstrated in French Polynesia.[18] Symptoms of ZIKV infections are typically mild, but severe neurologic complications can occur[12] and raise the question of the threat posed by ZIKV for the blood supply. Although no posttransfusion ZIKV infection has ever been reported, the detection of a high number of ZIKV asymptomatic infections among blood donors (42/1,505) during the 2013 to 2014 outbreak in French Polynesia[18] revealed the risk of

TABLE 3. ZIKV RNA quantitation (log copies/mL) in sera of French Polynesian asymptomatic blood donors

Sample IDs	RNA loads
27/11/13-66 P2	5.70
27/11/13-71 P1	5.25
28/11/13-86 P1	3.95
28/11/13-86 P2	3.76
28/11/13-95 P2	5.33
28/11/13-95 P3	4.15
29/11/13-59 P3	4.96
04/12/13-106 P1	5.65
06/12/13-79 P1	4.06
09/12/13-117 P2	6.91
09/12/13-121 P2	5.54
09/12/13-193 P1	6.72
10/12/13-36 P2	6.16
20/12/13-59 P2	4.63
20/12/13-63 P2	3.73
20/12/13-70 P2	3.47
20/12/13-78 P1	5.35
23/12/13-38 P1	5.15
24/12/13-43 P2	5.34
31/12/13-34 P1	4.49
03/01/14-34 P3	6.19
03/01/14-38 P1	3.40
09/01/14-116 P3	4.24
09/01/14-122 P1	3.45
24/01/14-44 P2	4.71
13/02/14-128 P2	3.90

transfusion-associated transmission of this virus. Subsequently, the European Center for Disease Control recommended that the blood safety authorities be vigilant regarding the risk of Zika fever, including deferral of blood donors with travel history in areas with ongoing circulation of ZIKV.[38]

During outbreaks, several strategies have been implemented to prevent transfusion-associated transmission of arboviruses. During the CHIKV outbreaks in 2005 to 2007 on Reunion Island[39] and in 2007 in Italy,[40] local blood donations were interrupted, and blood products were imported from blood bank centers elsewhere. In geographically isolated areas such as French Polynesia, importation of blood products from foreign blood bank centers is not routinely possible. In addition, the deferral of blood donors that have spent time in epidemic areas, as was recommended in Europe, is impossible. During the French Polynesian outbreak, blood products were kept in quarantine during 1 week, and blood donors were asked to contact the blood bank center in case of Zika fever symptoms. However, this procedure was not effective for asymptomatic infected blood donors.

Specific ZIKV NAT was implemented in routine practice during the French Polynesian outbreak,[18] on the basis of protocols implemented to prevent WNV transmission by transfusion in North America. In the United States, NAT has been routinely used since 2003 for the detection of WNV in donated blood products, after cases of post-transfusion infections that occurred in 2002.[41] Some limitations are to be considered when using NAT: first, it does not detect all infected blood donations, especially when nucleic acid loads are low and when sera are tested in large pools; second, due to its nucleotide sequence specificity, NAT cannot be used to screen a wide range of pathogens with one run, necessitating the use of multiple assays if several pathogens are cocirculating in the same area; third, it requires molecular biology expertise or access to a validated screening facility; and fourth, testing several pathogens by NAT is very expensive and time-consuming. Failures of NAT have also been reported. Indeed, since implementation of systematic screening of blood donations in the United States for the detection of WNV, several cases of transfusion-associated transmission of WNV have been documented.[42-44] In addition, one fatal case of WNV infection after probable transfusion-associated transmission, with a blood donation that was nonreactive by individual NAT, was reported in 2012.[45]

In contrast to NAT, the pathogen inactivation process is nonspecific and it can inactivate a broad spectrum of pathogens including bacteria, viruses, and protozoan.[20-24] This process is particularly suitable in areas with endemic circulation of pathogens and/or with cocirculation of multiple pathogens. Photochemical treatment is efficient for pathogen inactivation in PLT and plasma blood components, but it cannot be used on RBCs because UVA light is absorbed by hemoglobin and poorly penetrates through RBCs.[19] Thus, until the introduction of a new pathogen inactivation technology that can be used in RBCs concentrates, both NAT and inactivation strategies should be considered to reduce the risk of transfusion-associated transmission diseases, depending on the pathogen targeted, its mode of circulation, and the type of blood product used for transfusion.

Photochemical treatment of plasma with amotosalen and UVA light has been previously shown to inactivate more than 6.8 logs of WNV[25] and more than 5.61 logs of DENV.[26] The efficacy of the treatment of plasma with amotosalen and UVA light to inactivate ZIKV should be specifically demonstrated, even though other members of the *Flaviviridae* family have been shown to be inactivated by the same process.

In our study, we inoculated plasma units with ZIKV and monitored the ZIKV inactivation by both viral culture and RT-PCR. As a large part of the French Polynesian population had been immunized against ZIKV during the 2013 to 2014 outbreak,[9] the plasma samples used for our experiments were collected from American blood donors to avoid virus neutralization by existing ZIKV IgG antibodies. In addition, because cross-reactions exist within the *Flavivirus* genus,[46] DENV and WNV IgG-negative plasma units were selected for the experiments.

Immediately after inactivation, we found no replicative viruses in plasma samples. To ensure that there were no remaining infectious viral particles, we performed

successive passages on VERO cells and, even after five passages, we detected no replicative viruses. According to the recommendations of the Food and Drug Administration (FDA) for evaluation of pathogen reduction efficacy, the pathogen inactivation process should, ideally, have the ability to reduce the pathogen load in a blood product by 6 to 10 log copies/mL.[27] Our results showed that amotosalen and UVA light treatment of ZIKV inoculated plasma samples was able to inactivate a mean viral RNA load of 10.25 log copies/mL, which meets the criteria set by the FDA.

After inactivation, we still detected high viral RNA loads in plasma samples. It was previously demonstrated that amotosalen and UVA light treatment, although abolishing viral infectivity by blocking viral RNA replication and transcription via adduct formation on nucleic acids, does not impair RT-PCR detection of DENV and viruses belonging to alpha- and poxvirus genera.[26,47] Similarly, even though ZIKV particles became noninfectious after photochemical inactivation, amotosalen-modified viral RNAs could still be detected by RT-PCR. Indeed, it has been shown that RT-PCR amplification of nucleic acid fragments of less than 300 bp are not inhibited[48] and our RT-PCR protocol generated amplicons of 76 bp.[29] After one passage on VERO cells, the mean viral RNA load dramatically decreased, and from the second passage, ZIKV RNA was no longer detected, confirming the absence of viral replication due to the lack of infectious particles after inactivation with amotosalen and UVA light. The fact that replicative viruses were still detected in the noninactivated sample (positive control) confirmed that the inactivation of ZIKV was due to the amotosalen and UVA treatment and not to the compound absorption device that removed residual amotosalen.

When testing the blood samples of asymptomatic blood donors from French Polynesia by RT-PCR, we measured ZIKV RNA loads ranging from 3.40 to 6.91 log copies/mL (mean, 4.85 log copies/mL). Equivalent ZIKV RNA loads, ranging from 930 to 728,800 copies/mL (i.e., 2.97 to 5.86 log copies/mL; mean, 4.40 log copies/mL) have been previously found from blood samples of 17 patients infected during the ZIKV outbreak in Yap Island in 2007.[29] In our experiments, amotosalen and UVA light treatment induced a reduction in ZIKV RNA loads of 10.25 log copies/mL on average, that is, at least 10^4 times higher than the mean viral RNA loads and at least 10^3 times higher than the upper viral RNA loads measured in blood samples of infected patients and asymptomatic blood donors detected positive respectively during Yap's and French Polynesia's epidemics. These results suggest that the amotosalen and UVA light treatment may be used to inactivate ZIKV in blood products collected in ZIKV epidemic areas.

French Polynesia is a highly endemic area for DENV and has also recently experienced two large outbreaks of ZIKV and CHIKV. Amotosalen and UVA light treatment has been already shown to inactivate DENV and CHIKV in FFP.[25,26] Our study demonstrates that this photochemical process also inactivates ZIKV in plasma and induces a decrease of viral RNA loads higher than those found in ZIKV-infected French Polynesian blood donors. Based on our results, the amotosalen and UVA light inactivation process appears suitable to reduce the risk of plasma transfusion-transmitted ZIKV infections. This procedure is of particular interest in areas, such as French Polynesia or Brazil, in which several arboviruses are cocirculating.

ACKNOWLEDGMENTS

We are grateful to Philippe Desprès (Institut Pasteur, Paris, France) for providing flavivirus antibodies for immunofluorescence and titration assays. We thank Allison Imrie (University of Western Australia, Perth, Australia) who provided the titration protocol.

CONFLICT OF INTEREST

JG is an employee and owns stock of Cerus Corporation. The other authors have disclosed no conflicts of interest.

REFERENCES

1. Dick GW, Kitchen SF, Haddow AJ. Zika virus. I. Isolations and serological specificity. Trans R Soc Trop Med Hyg 1952;46:509-20.
2. Simpson DI. Zika virus infection in man. Trans R Soc Trop Med Hyg 1964;58:335-8.
3. Moore DL, Causey OR, Carey DE, et al. Arthropod-borne viral infections of man in Nigeria, 1964-1970. Ann Trop Med Parasitol 1975;69:49-64.
4. Fagbami AH. Zika virus infections in Nigeria: virological and seroepidemiological investigations in Oyo State. J Hyg (Lond) 1979;83:213-9.
5. Filipe AR, Martins CM, Rocha H. Laboratory infection with Zika virus after vaccination against yellow fever. Arch Gesamte Virusforsch 1973;43:315-9.
6. Olson JG, Ksiazek TG, Suhandiman, et al. Zika virus, a cause of fever in Central Java, Indonesia. Trans R Soc Trop Med Hyg 1981;75:389-93.
7. 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.
8. Cao-Lormeau VM, Roche C, Teissier A, et al. Zika virus, French Polynesia, South Pacific, 2013. Emerg Infect Dis 2014;20:1085-6.
9. Musso D, Nilles EJ, Cao-Lormeau VM. Rapid spread of emerging Zika virus in the Pacific area. Clin Microbiol Infect 2014;20:O595-6.
10. Cao-Lormeau VM, Musso D. Emerging arboviruses in the Pacific. Lancet 2014;384:1571-2.

11. Campos GS, Bandeira AC, Sardi SI. Zika virus outbreak, Bahia, Brazil. Emerg Infect Dis 2015;21.
12. Oehler E, Watrin L, Larre P, et al. Zika virus infection complicated by Guillain-Barre syndrome-case report, French Polynesia, December 2013. Euro Surveill 2014;19(9).
13. Marchette NJ, Garcia R, Rudnick A. Isolation of Zika virus from Aedes aegypti mosquitoes in Malaysia. Am J Trop Med Hyg 1969;18:411-5.
14. Grard G, Caron M, Mombo IM, et al. Zika virus in Gabon (Central Africa)—2007: a new threat from Aedes albopictus? PLoS Negl Trop Dis 2014;8:e2681-
15. 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.
16. Musso D, Roche C, Robin E, et al. Potential sexual transmission of zika virus. Emerg Infect Dis 2015;21:359-61.
17. Besnard M, Lastere S, Teissier A, et al. Evidence of perinatal transmission of Zika virus, French Polynesia, December 2013 and February 2014. Euro Surveill 2014;19.
18. Musso D, Nhan T, Robin E, 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.
19. Allain JP, Bianco C, Blajchman MA, et al. Protecting the blood supply from emerging pathogens: the role of pathogen inactivation. Transfus Med Rev 2005;19:110-26.
20. Lin L, Cook DN, Wiesehahn GP, et al. Photochemical inactivation of viruses and bacteria in platelet concentrates by use of a novel psoralen and long-wavelength ultraviolet light. Transfusion 1997;37:423-35.
21. Irsch J, Lin L. Pathogen Inactivation of Platelet and Plasma Blood Components for Transfusion Using the INTERCEPT Blood System™. Transfus Med Hemother 2011;38:19-31.
22. Singh Y, Sawyer LS, Pinkoski LS, et al. Photochemical treatment of plasma with amotosalen and long-wavelength ultraviolet light inactivates pathogens while retaining coagulation function. Transfusion 2006;46:1168-77.
23. Tan LK. Evaluation of pathogen reduction systems to inactivate Dengue and Chikungunya viruses in apheresis platelets suspended in plasma. Adv Infect Dis 2013;03:1-9.
24. Dupuis K, Arnold D, Sawyer L. High titers of dengue virus in platelet concentrates are inactivated by treatment with amotosalen and UVA light [abstract]. Transfusion 2012;52(Suppl): 225A.
25. Corten L, Sawyer L, Liu W, et al. Third International Meeting on Emerging Diseases and Surveillance. Vienna, Austria. Pathogen inactivation of blood components for prevention of transfusion-transmitted emerging infectious diseases?: the INTERCEPT Blood System [Internet]. Concord (CA): Cerus Corp.; 2011 [cited 2015 Feb 18]. Available from: http://www.interceptbloodsystem.com/resource-center/scientific-publications/intercept-conference-abstracts/2011.
26. Musso D, Richard V, Broult J, et al. Inactivation of dengue virus in plasma with amotosalen and ultraviolet A illumination. Transfusion 2014;54:2924-30.
27. Epstein JS, Vostal JG. FDA approach to evaluation of pathogen reduction technology. Transfusion 2003;43:1347-50.
28. Reed L, Muench H. A simple method of estimating fifty per cent endpoints. Am J Epidemiol 1938;27:493-7.
29. 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.
30. Stramer SL, Hollinger FB, Katz LM, et al. Emerging infectious disease agents and their potential threat to transfusion safety. Transfusion 2009;49 Suppl 2:1S-29S.
31. Stramer SL. Current perspectives in transfusion-transmitted infectious diseases: emerging and re-emerging infections. ISBT Sci Ser 2014;9:30-6.
32. Pealer LN, Marfin AA, Petersen LR, et al. Transmission of West Nile virus through blood transfusion in the United States in 2002. N Engl J Med 2003;349:1236-45.
33. Chuang VW, Wong TY, Leung YH, et al. Review of dengue fever cases in Hong Kong during 1998 to 2005. Hong Kong Med J 2008;14:170-7.
34. Stramer SL, Linnen JM, Carrick JM, et al. Dengue viremia in blood donors identified by RNA and detection of dengue transfusion transmission during the 2007 dengue outbreak in Puerto Rico. Transfusion 2012;52:1657-66.
35. Tambyah PA, Koay ES, Poon ML, et al. Dengue hemorrhagic fever transmitted by blood transfusion. N Engl J Med 2008; 359:1526-7.
36. Gallian P, de Lamballerie X, Salez N, et al. Prospective detection of chikungunya virus in blood donors, Caribbean 2014. Blood 2014;123:3679-81.
37. Roth A, Mercier A, Lepers C, 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).
38. European Centre for Disease Prevention and Control. Zika virus infection outbreak, French Polynesia [Internet]. Stockholm: ECDC; 2014 [cited 2015 Mar 4]. Available from: http://www.ecdc.europa.eu/en/publications/Publications/Zika-virus-French-Polynesia-rapid-risk-assessment.pdf.
39. Brouard C, Bernillon P, Quatresous I, et al. Estimated risk of Chikungunya viremic blood donation during an epidemic on Reunion Island in the Indian Ocean, 2005 to 2007. Transfusion 2008;48:1333-41.
40. Liumbruno GM, Calteri D, Petropulacos K, et al. The Chikungunya epidemic in Italy and its repercussion on the blood system. Blood Transfus 2008;6:199-210.
41. Centers for Disease Control and Prevention (CDC). Detection of West Nile Virus in blood donations–United States, 2003. MMWR Morb Mortal Wkly Rep 2003;52:769-72.
42. Montgomery SP, Brown JA, Kuehnert M, et al. Transfusion-associated transmission of West Nile virus, United States 2003 through 2005. Transfusion 2006;46:2038-46.
43. Petersen LR, Busch MP. Transfusion-transmitted arboviruses. Vox Sang 2010;98:495-503.

44. Meny GM, Santos-Zabala L, Szallasi A, et al. West Nile virus infection transmitted by granulocyte transfusion. Blood 2011;117:5778-9.
45. Centers for Disease Control and Prevention (CDC). Fatal West Nile virus infection after probable transfusion-associated transmission–Colorado, 2012. MMWR Morb Mortal Wkly Rep 2013;62:622-4.
46. Calisher CH, Karabatsos N, Dalrymple JM, et al. Antigenic relationships between flaviviruses as determined by cross-neutralization tests with polyclonal antisera. J Gen Virol 1989;70(Pt 1):37-43.
47. Sagripanti JL, Marschall HJ, Voss L, et al. Photochemical inactivation of alpha- and poxviruses. Photochem Photobiol 2011;87:1369-78.
48. Bruchmüller I, Janetzko K, Bugert P, et al. Polymerase chain reaction inhibition assay documenting the amotosalen-based photochemical pathogen inactivation process of platelet concentrates. Transfusion 2005;45:1464-72.

別紙様式第2-1
番号14

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	新医薬品等の区分	厚生労働省処理欄
				2016年05月12日	該当なし	

一般的名称	人ハプトグロビン	研究報告の公表状況	http://www.who.int/csr/don/3-may-2016-ah7n9-china/en/#/2016/05/03	公表国 中国
販売名（企業名）	ハプトグロビン静注2000単位「JB」（日本血液製剤機構）			

研究報告の概要

トリインフルエンザA（H7N9）ウイルスのヒト感染-中国：

2016年4月18日，中国のNational Health and Family Planning Commission（NHFPC）は，死亡5例を含むトリインフルエンザA（H7N9）ウイルスのヒト感染症の追加の検査確定例17例をWHOに報告した。
発症日は2月21日～3月20日に分布しており，感染者の年齢は26歳～86歳（中央値：60歳）であった。17例のうち，11例（65%）が男性であった。症例の多く（15例，88%）は生きた家禽，食肉処理された家禽，または生きた家禽の市場への曝露を報告した。1例は過去にWHOへ報告された症例2例のクラスターに関連している。
症例は以下の6つの省と直轄市から報告された：安徽省（4例），江蘇省（4例），福建省（3例），広東省（3例），浙江省（2例），湖北省（1例）。
浙江省出身の女性（85歳）を含む1つのクラスターが報告された。この女性は3月1日に症状を呈し，3月8日に死亡した。2月22日～23日に，他の確定例と同じ病院の病棟に入院していた。親族によると，女性は生きた家禽や生きた家禽の市場への曝露はなかった。
同じ病棟に入院していた他の確定例は，2月15日に症状を呈した浙江省出身の男性（29歳）であった。この男性は生きた家禽の市場と，確定例である家庭内接触者に曝露していた。福建省出身の接触者は生きた家禽の市場に曝露しており，2月4日に症状を呈した。
29歳男性と85歳女性との間のヒトーヒト感染の可能性は除外できず，さらなるウイルス学的情報が必要である。

使用上の注意記載状況・その他参考事項等

2. 重要な基本的注意
(1)本剤の原材料となる献血者の血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体、抗HTLV-1抗体陰性で、かつALT（GPT）値でスクリーニングを実施している。更に、HBV、HCV及びHIVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は，以上の検査に適合した血漿を原料として、Cohnの低温エタノール分画で得た画分から人ハプトグロビンを濃縮・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	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あり、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ウイルスであり、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。

Emergencies preparedness, response

Human infection with avian influenza A (H7N9) virus – China

Disease outbreak news
3 May 2016

On 18 April 2016, the National Health and Family Planning Commission (NHFPC) of China notified WHO of 17 additional laboratory-confirmed cases of human infection with avian influenza A(H7N9) virus, including 5 deaths.

Onset dates range from 21 February to 20 March. Cases range in age from 26 to 86 years, with a median age of 60 years. Of these 17 cases, 11 (65%) are male. The majority (15 cases, 88%) reported exposure to live poultry, slaughtered poultry, or live poultry markets. The exposure history of one (1) case is unknown. One (1) case is linked to a cluster of two (2) cases reported earlier to WHO (see below).

Cases were reported from 6 provinces and municipalities: Anhui (4), Jiangsu (4), Fujian (3), Guangdong (3), Zhejiang (2) and Hubei (1).

One cluster was reported. The cluster includes an 85-year-old female from Zhejiang Province. She had onset of symptoms on 1 March and passed away on 8 March. She had been admitted to the same hospital and shared the ward with a confirmed case between 22 and 23 February. She was not exposed to live poultry or live poultry market, according to her relatives.

The confirmed case admitted at the same ward was a 29-year-old male from Zhejiang Province who developed symptoms on 15 February. He had exposure to a live poultry market and a household contact who was also a confirmed case. The contact from Fujian Province developed symptoms on 4 February and had exposure to a live poultry market.

Human to human transmission between the 29-year-old male and the 85-year-old female cannot be ruled out. Further virological information is awaited.

Public health response

The Chinese Government has taken the following surveillance and control measures:

- strengthening outbreak surveillance and situation analysis;
- reinforcing all efforts on medical treatment; and
- conducting risk communication with the public and dissemination of information.

WHO risk assessment

Most human cases are exposed to the A(H7N9) virus through contact with infected poultry or contaminated environments, including live poultry markets. Since the virus continues to be detected in animals and environments, further human cases can be expected. Although small clusters of human cases with influenza A(H7N9) viruses have been reported including those involving healthcare workers, current epidemiological and virological evidence suggests that this virus has not acquired the ability of sustained transmission among humans. Therefore further community level spread is considered unlikely.

Human infections with the A(H7N9) virus are unusual and need to be monitored closely in order to identify changes in the virus and/or its transmission behaviour to humans as it may have a serious public health impact.

WHO advice

WHO advises that travellers to countries with known outbreaks of avian influenza should avoid poultry farms, contact with animals in live bird markets, entering areas where poultry may be slaughtered, or contact with any surfaces that appear to be contaminated with faeces from poultry or other animals. Travellers should also wash their hands often with soap and water. Travellers should follow good food safety and good food hygiene practices.

WHO does not advise special screening at points of entry with regard to this event, nor does it currently recommend any travel or trade restrictions. As always, a diagnosis of infection with an avian influenza virus should be considered in individuals who develop severe acute respiratory symptoms while travelling or soon after returning from an area where avian influenza is a concern.

WHO encourages countries to continue strengthening influenza surveillance, including surveillance for severe acute respiratory infections (SARI) and to carefully review any unusual patterns, in order to ensure reporting of human infections under the IHR (2005), and continue national health preparedness actions.

別紙様式第2-1

No. 12

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2016. 3. 23	該当なし	

一般的名称	人全血液	研究報告の公表状況		公表国
販売名(企業名)	人全血液-LR「日赤」(日本赤十字社) 照射人全血液-LR「日赤」(日本赤十字社)		Tanaka T, Hirata T, Parrott G, et al. Am J Trop Med Hyg. 2016 Feb 3;94(2):365-70.	日本

研究報告の概要

○糞線虫感染またはヒトT細胞白血病ウイルス1型(HTLV-1)感染と癌との関連性:沖縄県における24年にわたる入院患者コホート研究。
琉球大学病院入院患者に対して、糞線虫感染及びHTLV-1感染の罹患率を求めた後ろ向きコホート研究であり、これらの罹患率と癌の発症リスクとの関連性について検討を行った。全患者5,209例における糞線虫感染の罹患率は5.2%であり、男性(6.3%)は女性(3.6%)より有意に高かった(P < 0.001)。当該集団におけるHTLV-1感染の罹患率は13.6%であり、女性(15.5%)は男性(12.3%)より有意に高かった(P < 0.001)。HTLV-1の血清陽性率については、腫瘍を有さない患者との比較において、肝臓癌患者の陽性率はより高く(P = 0.003、オッズ比[OR]: 1.91、95%信頼区間[CI]: 1.24、2.95)、また成人T細胞白血病/リンパ腫(ATLL)以外のリンパ腫を有する患者の陽性率もより高くなった(P = 0.005、補正OR: 2.76、95% CI: 1.36、5.62)。沖縄県住民における糞線虫感染及びHTLV-1感染の罹患率は、過去24年間で着実に減少してきた。HTLV-1感染は、肝臓癌並びにATLL以外のリンパ腫の発症の確率を有意に上昇させる。

報告企業の意見	今後の対応
沖縄県住民における糞線虫感染及びヒトT細胞白血病ウイルス1型(HTLV-1)感染の罹患率は、過去24年間で減少してきたが、HTLV-1感染が肝臓癌並びに成人T細胞白血病/リンパ腫(ATLL)以外のリンパ腫の発症率を有意に上昇させることが明らかとなったという報告である。	日本赤十字社では、HTLV-1抗体のスクリーニング検査を行っている。今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

人全血液-LR「日赤」
照射人全血液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

Am. J. Trop. Med. Hyg., 94(2), 2016, pp. 365–370
doi:10.4269/ajtmh.15-0556

JRC2016T-008

Relationship among *Strongyloides stercoralis* Infection, Human T-Cell Lymphotropic Virus Type 1 Infection, and Cancer: A 24-Year Cohort Inpatient Study in Okinawa, Japan

Teruhisa Tanaka,* Tetsuo Hirata, Gretchen Parrott, Miwa Higashiarakawa, Takeshi Kinjo, Tetsu Kinjo, Akira Hokama, and Jiro Fujita

Department of Infectious, Respiratory, and Digestive Medicine, Faculty of Medicine, University of the Ryukyus, Okinawa, Japan; Department of Endoscopy, University of Ryukyus Hospital, Okinawa, Japan

Abstract. This study evaluated the prevalence of *Strongyloides stercoralis* infection and human T-cell lymphotropic virus type 1 (HTLV-1) infection in the population. In addition, this study investigated the relationship between *S. stercoralis* infection or HTLV-1 infection and a patient's risk of developing related cancers. This is a retrospective cohort study of 5,209 patients. The prevalence of *S. stercoralis* infection was 5.2% among all patients. The prevalence among men (6.3%) was significantly higher than among women (3.6%, $P < 0.001$). The prevalence of HTLV-1 infection among this population was 13.6% and the prevalence among women (15.5%) was significantly higher than that of men (12.3%, $P < 0.001$). HTLV-1 seroprevalence was higher in patients with liver cancer ($P = 0.003$, odds ratio [OR]: 1.91, 95% confidence interval [CI]: 1.24, 2.95) and in those with lymphoma other than adult T-cell leukemia/lymphoma (ATLL) ($P = 0.005$, adjusted OR: 2.76, 95% CI: 1.36, 5.62) if compared with patients without any neoplasm. The prevalence of both *S. stercoralis* and HTLV-1 in the Okinawan population has been steadily decreasing over the past 24 years. HTLV-1 infection significantly increases the odds of developing liver cancer and lymphomas other than ATLL.

INTRODUCTION

Strongyloides stercoralis is one of the most common human gastrointestinal parasites in the world. The Okinawa Prefecture of Japan is located in a subtropical region, which is endemic for *S. stercoralis*.[1,2] With humid and warm soil, subtropical regions provide the preferred external environment for *S. stercoralis*. The filariform larvae, which inhabit the soil, usually infect humans via skin penetration. After infection, the larvae travel to the duodenum to become adult females. Rhabditiform larvae, hatched from eggs produced by the females, are excreted from the human host. However, some larvae reinfect the host through the intestinal mucosa or perianal skin, using a process called autoinfection, which is unique to only a few parasites, allowing *S. stercoralis* to complete its life cycle and proliferate successfully within a single host.[3]

Okinawa Prefecture is also endemic for human T-cell lymphotropic virus type 1 (HTLV-1), a virus associated with adult T-cell leukemia/lymphoma (ATLL).[4–6] There are three possible transmission routes for HTLV-1: sexual transmission, mother to child transmission via breast milk, and exposure to contaminated blood. In Japan, the virus is most commonly transmitted from mother to child.[7] It is well known that infection of HTLV-1 early in life may increase the risk for subsequent diseases, particularly ATLL.[8]

Infectious agents, including parasites, often have oncogenic potential. Infection can initiate or promote carcinogenesis by any of three main mechanisms: 1) chronic inflammation due to a prolonged persistence of infectious agents within the host tissue, 2) insertion of active oncogenes into the host genome, and 3) reduced immunosurveillance as a result of immunosuppression.[9] Similarly, the autoinfection route of *S. stercoralis* in host gastrointestinal and lung tissue also has the potential to cause chronic inflammation and promote subsequent carcinogenesis. Some studies have reported an association between HTLV-1 infection and carcinomas other than ATLL; however, this link is still controversial.[10–12]

With this foundational evidence, we conducted an inpatient study to investigate the prevalence of *S. stercoralis* and HTLV-1 infections, as well as the relationship between these two infections. Within the same cohort, we also conducted a retrospective cohort study to investigate the relationship between a history of *S. stercoralis* or HTLV-1 infection and a potentially increased risk of developing various cancers.

MATERIAL AND METHODS

Study population. This retrospective cohort study included 5,209 patients (3,154 men and 2,055 women) who were admitted to the First Department of Internal Medicine for Infectious, Respiratory, and Digestive Medicine at University of Ryukyus Hospital in Okinawa between 1991 and 2014 (Table 1).

Controls, included for the investigation of *S. stercoralis* infection and its association with the development of cancer, were composed of all patients born before 1960 without cancer or a history of cancer. The controls used in the HTLV-1 infection analysis included all patients born before 1990 without cancer or a history of cancer. All patients were admitted as inpatients to the First Department of Internal Medicine at the University of Ryukyus Hospital during the same period.

Evaluation of *S. stercoralis* and HTLV-1 infections. Infection of *S. stercoralis* was diagnosed in all patients using the stool agar plate culture method.[13] Serum antibody to HTLV-1 was measured in all patients using the gelatin particle agglutination method.[14]

Cancer diagnosis. The diagnosis of cancer was based on histology, cytology, and radiological findings. Patients diagnosed with metastatic cancer were excluded because the source of primary cancer could not be determined within reasonable time constraints.

Statistical analyses. The χ^2 test was used to compare the prevalence of *S. stercoralis* or HTLV-1 infection between

*Address correspondence to Teruhisa Tanaka, Department of Infectious, Respiratory, and Digestive Medicine, Faculty of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-0215, Japan. E-mail: teruhisa.t@gmail.com

TABLE 1
Patient characteristics ($N = 5,209$)

Men	3,154 (60.5%)
Age	56.4 (SD: 17.9) range: 11–101 years
Cancers	
Esophagus	114 (2.2%)
Stomach	262 (5.0%)
Biliary tract	71 (1.4%)
Liver	143 (2.7%)
Colon and rectum	200 (3.8%)
Lung	444 (8.5%)
Pancreas	38 (0.7%)
Lymphoma without ATLL	42 (0.8%)
Others*	171 (3.3%)

ATLL = adult T-cell leukemia/lymphoma; SD = standard deviation.
*Other cancers include breast cancer, uterine cancer, kidney cancer, pharyngeal and laryngeal cancer, and ATLL, among others.

sexes. The χ^2 test was also used to compare the prevalence of each cancer in a crude analysis with a history of *S. stercoralis* or HTLV-1 infection. Logistic regression analyses adjusted for age and sex were used to examine the odds of developing each cancer considering the incidence of *S. stercoralis* or HTLV-1 infection. All statistical analyses and graphical representations were performed using SPSS (version 21.0; IBM Corp., Armonk, NY) software packages. The P values reported here are two sided.

RESULTS

Prevalence of *S. stercoralis* and HTLV-1 infection. The study population was composed of 3,154 men and 2,055 women, with a mean age of 56.4 ± 17.9 (standard deviation [SD]) years (range: 11–101 years). The total prevalence of *S. stercoralis* infection in our study population was 5.2% (Table 2, Figure 1A). The prevalence of *S. stercoralis* in the male population (6.3%) was significantly higher than that in the female population (3.6%, $P < 0.001$). There were no patients with a *S. stercoralis* infection that were born after 1960. The total prevalence of HTLV-1 infection was 13.6% (Table 2, Figure 1B). The prevalence of HTLV-1 infection in men and women was 12.3% and 15.5%, respectively. HTLV-1 infection was significantly more prevalent in women than in men ($P < 0.001$). The number of *S. stercoralis* and HTLV-1 infections steadily decreased for both sexes in each successive generation.

To evaluate the relationship between *S. stercoralis* infection and HTLV-1 infection, we compared only patients born before 1960. The total number of patients born before 1960 was 4,056 (2,459 men and 1,597 women). Within this population, the prevalence of *S. stercoralis* infection was significantly higher in patients with HTLV-1 infection compared with that in patients without HTLV-1 infection (Tables 3 and 4). The

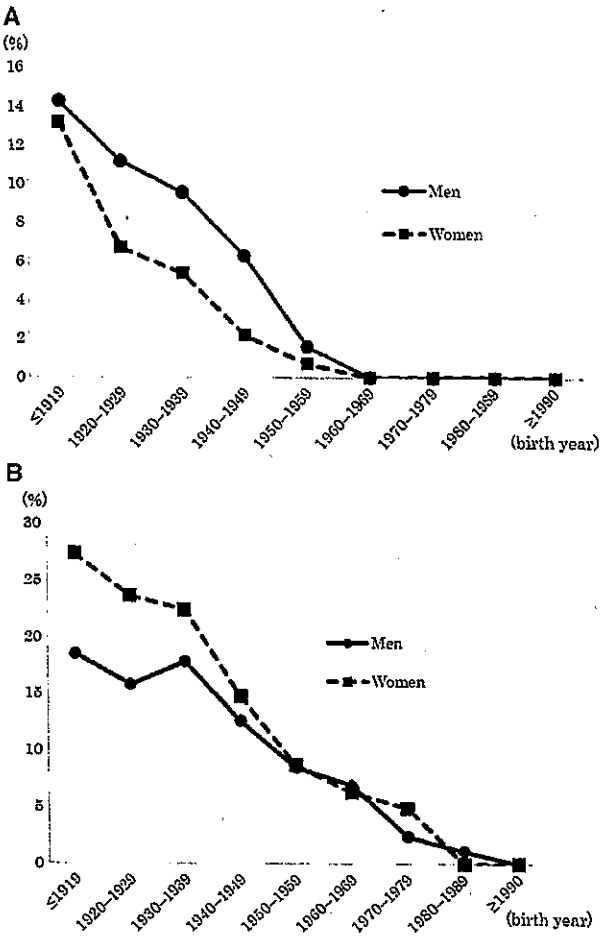


FIGURE 1. The study included 5,209 patients who were admitted to the First Department of Internal Medicine for Infectious, Respiratory, and Digestive Medicine at the University of Ryukyus Hospital in Okinawa, Japan, between 1991 and 2014. (**A**) The prevalence of *Strongyloides stercoralis* infection in men (circles) and women (squares) by age. (**B**) The prevalence of human T-cell lymphotropic virus type 1 infection in the men (circles) and women (squares) by age.

TABLE 2
Prevalences of *Strongyloides stercoralis* infection and HTLV-1 infection

	Number of *S. stercoralis*-positive patients/number of tested patients (%)			Number of HTLV-1 positive patients/number of tested patients (%)		
Birth year	Men	Women	Total	Men	Women	Total
≤ 1919	24/168 (14.8)	14/106 (13.2)	38/274 (13.9)	31/168 (18.5)	29/106 (27.3)	60/274 (21.9)
1920–1929	59/526 (11.2)	22/326 (6.7)	81/852 (9.5)	83/526 (15.8)	77/326 (23.6)	160/852 (18.8)
1930–1939	76/794 (9.6)	27/500 (5.4)	103/1,294 (8.0)	141/794 (17.8)	112/500 (22.4)	253/1,294 (19.6)
1940–1949	33/522 (6.3)	8/365 (2.2)	41/887 (4.6)	66/522 (12.6)	54/365 (14.8)	120/887 (13.5)
1950–1959	7/449 (1.6)	2/300 (0.7)	9/749 (1.2)	38/449 (8.5)	26/300 (8.7)	64/749 (8.5)
1960–1969	0/331 (0.0)	0/207 (0.0)	0/538 (0.0)	23/331 (6.9)	13/207 (6.3)	36/538 (6.7)
1970–1979	0/251 (0.0)	0/163 (0.0)	0/414 (0.0)	6/251 (2.4)	8/163 (4.9)	14/414 (3.4)
1980–1989	0/88 (0.0)	0/72 (0.0)	0/160 (0.0)	1/88 (1.1)	0/72 (0.0)	1/160 (0.6)
≥ 1990	0/25 (0.0)	0/16 (0.0)	0/41 (0.0)	0/25 (0.0)	0/16 (0.0)	0/41 (0.0)
Total	199/3,154 (6.3)*	73/2,055 (3.6)	272/5,209 (5.2)	389/3,154 (12.3)	319/2,055 (15.5)*	708/5,209 (13.6)

HTLV-1 = human T-cell lymphotropic virus type 1.
*$P < 0.001$ for male vs. female by χ^2 analysis.

TABLE 3
Crude analysis (all cases and controls born before 1960)

		HTLV-1		
		Positive	Negative	Total
Strongyloides stercoralis	Positive	82 (2.0%)	190 (4.7%)	272
	Negative	575 (14.2%)	3,209 (79.1%)	3,784
	Total	657	3,399	4,056

HTLV-1 = human T-cell lymphotropic virus type 1.
Odds ratio = 2.41 (95% confidence interval = 1.83, 3.17; $P < 0.001$) by χ^2 analysis.

odds ratio (OR) of this comparison was higher in female patients than in male patients.

Association between *S. stercoralis* infection and each type of cancer. Within the 4,056 patients born before 1960, we identified 1,352 patients with diagnostically confirmed cancer. The cancer patients consisted of 953 men and 399 women, with a mean age of 67.0 ± 10.2 (SD) years. The cancer-free control group consisted of 1,446 men and 1,150 women with a mean age of 61.8 ± 12.9 (SD) years.

Table 5 presents the prevalence and association of *S. stercoralis* infection among cancer patients and controls. The prevalence of *S. stercoralis* infection in controls and in cancer patients were significantly different from one another at 5.7% and 8.7% ($P < 0.001$), respectively. Using a logistic regression model adjusted for age and sex, we calculated the OR stratified for each cancer. Although our data suggest that *S. stercoralis* patients are more likely to develop cancer ($P < 0.001$), *S. stercoralis* infection was not found to be significantly associated with any specific type of cancer.

Association between HTLV-1 infection and cancer. Within the 5,168 patients born before 1990, we identified 1,437 patients with diagnostically confirmed cancer. In this population, the cancer patients consisted of 1,005 men and 432 women with a mean age of 65.4 ± 11.9 (SD) years. The control group consisted of 2,056 men and 1,556 women with a mean age of 51.4 ± 17.9 (SD) years.

Table 6 presents the prevalence and association between HTLV-1 infection among controls and cancer patients. The prevalence of HTLV-1 infection in controls and in cancer patients were significantly different from one another at 12.9% and 15.2% ($P = 0.03$), respectively. In addition, the prevalence of HTLV-1 infection in patients with liver cancer ($P = 0.01$) or with lymphomas other than ATLL were significantly higher than that in patients with other types of cancer ($P = 0.03$).

TABLE 4
Gender-stratified analyses

		HTLV-1		
		Positive	Negative	Total
Men*				
Strongyloides stercoralis	Positive	48 (2.0%)	151 (6.1%)	199
	Negative	311 (12.6%)	1,949 (79.3%)	2,260
	Total	359	2,100	2,459
Women†				
S. stercoralis	Positive	34 (2.1%)	39 (2.4%)	73
	Negative	264 (16.5%)	1,260 (78.9%)	1,524
	Total	298	1,299	1,597

HTLV-1 = human T-cell lymphotropic virus type 1.
*Odds ratio (OR) = 1.99 (95% confidence interval (CI) = 1.41, 2.82; $P < 0.001$) by χ^2 analysis.
†OR = 4.16 (95% CI = 2.58, 6.72; $P < 0.001$) by χ^2 analysis.

Using a logistic regression model adjust for age and sex, we calculated the OR stratified for each cancer. HTLV-1 infection was not shown to significantly increase the odds of developing most types of cancer, except for liver cancer and lymphomas other than ATLL. Patients with an HTLV-1 infection in our cohort were approximately twice as likely to develop liver cancer (OR: 1.91, 95% confidence interval [CI]: 1.24, 2.95) and approximately three times more likely to develop lymphoma other than ATLL (OR: 2.76, 95% CI: 1.36, 5.62) compared with patients without HTLV-1.

DISCUSSION

Our results show that there were no patients born after 1960 with *S. stercoralis* infection in our cohort. Although some publications report younger patients with *S. stercoralis* infection who have never traveled outside of Japan,[15,16] the overall prevalence of *S. stercoralis* infection has markedly decreased since 1960. This change is most attributed to improvements in public health and sanitation. After World War II, intestinal parasitic infections were common in Okinawa because of poverty, poor sanitation, the use of human waste as fertilizer, and the common practice of barefoot agricultural work.[15] At that time, public health centers also lacked the ability to detect, treat, or provide prevention for parasites.[17] In Ozato village, Okinawa, in 1957 the recorded prevalences of hookworm and *S. stercoralis* infections were 78.9% and 10.3%, respectively.[17] After implementation of the "Zero Parasite Campaign" from 1965 to 1969, the infection rate of parasites was drastically reduced and soil sanitation was improved.

Our study also shows that the prevalence of HTLV-1 infection is decreasing steadily, which supports existing literature from Japan and Okinawa.[18,19] Satake and others suggested this reduction might be called the "birth cohort effect" whereby the high-prevalence cohort (those born 1930–1960) ages while younger cohorts (those born after 1960) have lower prevalence rates.[20] These findings may be the result of increased knowledge regarding HTLV-1 and its transmission routes.[18] In Japan, the transmission of virus via transfusion has been eliminated since the implementation of HTLV-1 screening of donated blood in 1986. Japanese mothers have increased the number of bottle-fed babies,[21–23] thereby decreasing the vertical infection of HTLV-1. In 2011, the Japanese Ministry of Health, Labour and Welfare initiated a nationwide program to prevent mother-to-child infection by screening all pregnant women for HTLV-1 infection and recommending bottle feeding for women with positive results.[24]

The data suggest a strong correlation between *S. stercoralis* and HTLV-1 infections. The prevalence of *S. stercoralis* infection was significantly higher ($P < 0.001$) in patients with HTLV-1 infection compared with that in patients without HTLV-1 infection. Patients infected with HTVL-1 developed *S. stercoralis* infection 2.4 times more often than noninfected patients. Multiple studies in Okinawa have showed an increased risk for *S. stercoralis* infection when the host is immunocompromised,[19,25,26] and similar findings were reported in studies conducted in other regions, such as South America.[27–29] Furthermore, when the data were stratified for sex, we also found that females were four times more likely to have concurrent infections of *S. stercoralis* and HTLV-1. This altered susceptibility is most likely due to the difference in effectiveness of HTLV-1 transmission. It has been documented that

TABLE 5
Association between *Strongyloides stercoralis* infection and each cancer type (patients born before 1960, $N = 4,056$)

	Stratified analysis		Multivariate analysis		
	S. stercoralis infection rate	*P* value	OR	95% CI	*P* value
Control	5.7% (147/2,596)				
Total cancer	8.7% (117/1,352)	< 0.001*	1.28	0.98–1.66	0.06‡
Esophagus	6.4% (7/109)	0.48†	0.65	0.29–1.45	0.29§
Stomach	9.9% (24/242)	0.45†	1.22	0.76–1.97	0.42§
Biliary tract	14.5% (10/69)	0.05†	1.90	0.93–3.87	0.08§
Liver	6.4% (9/140)	0.43†	0.72	0.35–1.47	0.37§
Colon and rectum	7.7% (15/194)	0.68†	0.94	0.53–1.66	0.82§
Lung	9.6% (40/418)	0.46†	1.09	0.73–1.64	0.68§
Pancreas	5.4% (2/37)	0.77†	0.83	0.19–3.55	0.80§
Lymphoma without ATLL	2.7% (1/37)	0.37†	0.28	0.28–2.08	0.21§

ATLL = adult T-cell leukemia/lymphoma; CI = confidence interval; OR = odds ratio.
*A χ^2 analysis was used to compare *S. stercoralis* infection between patients with cancer (total cancer) and control patients (control).
†A χ^2 analysis was used to compare *S. stercoralis* infection between patients with each type of cancer and patients with other types of cancer.
‡A logistic regression analysis, adjusted for age and sex, was used to compare *S. stercoralis* infection between patients with cancer (total cancer) and control patients (control).
§A logistic regression analysis, adjusted for age and sex, was used to compare *S. stercoralis* infection between patients with each type of cancer and patients with other types of cancer.

male-to-female sexual transmission is more efficient than female-to-male sexual transmission.[30] Sexual transmission requires intimate and prolonged contact between partners.[31] Several studies have also suggested a correlation between older age and risk of infection, particularly for women, whose increased susceptibility may be due to the thinning of vaginal epithelia tissue after menopause.[30,32,33] However, some studies have shown that there are no correlations between the prevalence of *S. stercoralis* and HTLV-1 infections.[27,34] Carvalho and others suggested that the controversial results were due to the type of technique used to determine *S. stercoralis* infection: stool examination or serological test.[27] In our study, results show a strong correlation between *S. stercoralis* and HTLV-1 infections because only stool examinations were used for determining *S. stercoralis* infection.

No statistically significant associations between *S. stercoralis* infection and the development of any specific types of cancer were found in our data. One study from Okinawa shows a significantly high prevalence of *S. stercoralis* infection in patients with biliary tract cancer.[35] Adult *S. stercoralis* persist in human duodenum and upper jejunum, and the nematodes often migrate via the biliary tract. The resulting damage could cause cholangitis or pancreatitis or it could initiate and promote carcinogenesis.[36–39] Although our study shows that patients with biliary tract cancer may be almost twice as likely to have evidence of *S. stercoralis* infection as control patients (OR: 1.90, 95% CI: 0.93, 3.87), the evidence for this association is not statistically significant ($P = 0.08$). This result may be due to low statistical power, as only 69 cases of biliary tract cancers were included in our cohort.

Some studies suggest that HTLV-1 infection is associated with many types of cancer, mainly liver and other blood cancers.[12,40–42] Other reports showed that HTLV-1 infection may have a protective effect against gastric cancers.[10,11,43] Our data show that HTLV-1 infection is not associated with cancer development apart from liver cancer and lymphomas other than ATLL. In addition, although our study found that patients with gastric cancer might be less likely to have evidence of HTLV-1 infection than patients with other types of cancer (OR: 0.75, 95% CI: 0.50, 1.12), the data are not statistically significant ($P = 0.16$). Similarly, we saw a trend that patients with esophageal cancer might be less likely to have evidence of HTLV-1 infection than patients with other types of cancer (OR: 0.56, 95% CI: 0.29, 1.11), but this difference also failed to reach statistical significance ($P = 0.10$). A report from Iran also described a trend toward an association of HTLV-1 infection and esophageal squamous cell carcinoma, but their data similarly failed to reach statistical significance.[44]

This study found that HTLV-1 infection is associated with the development of liver cancer (OR: 1.91, 95% CI: 1.24, 2.95, $P = 0.003$). Similarly, a previous report showed a high association of HTLV-1 infection with the incidence of liver

TABLE 6
Association between HTLV-1 infection and each cancer type (patients born before 1990, $N = 5,168$)

	Stratified analysis		Multivariate analysis		
	HTLV-1 infection rate	*P* value	OR	95% CI	*P* value
Control	12.9% (467/3,612)	–	–	–	–
Total cancer	15.2% (219/1,437)	0.03*	0.90	0.75–1.09	0.28‡
Esophagus	8.8% (10/114)	0.06†	0.56	0.29–1.11	0.10§
Stomach	12.2% (32/262)	0.15†	0.75	0.50–1.12	0.16§
Biliary tract	16.9% (12/71)	0.73†	0.96	0.53–1.84	0.90§
Liver	22.3% (32/143)	0.01†	1.91	1.24–2.95	0.003§
Colon and rectum	15.0% (30/200)	1.00†	0.91	0.60–1.40	0.68§
Lung	13.5% (60/444)	0.23†	0.81	0.58–1.12	0.19§
Pancreas	7.8% (3/38)	0.26†	0.45	0.14–1.49	0.19§
Lymphoma without ATLL	28.5% (12/42)	0.03†	2.76	1.36–5.62	0.005§

ATLL = adult T-cell leukemia/lymphoma; HTLV-1 = human T-cell lymphotropic virus type 1; CI = confidence interval; OR = odds ratio.
*A χ^2 analysis was used to compare HTLV-1 infection between patients with cancer (total cancer) and control patients (control).
†A χ^2 analysis was used to compare HTLV-1 infection between patients with each type of cancer and patients with other types of cancer.
‡A logistic regression analysis, adjusted for age and sex, was used to compare HTLV-1 infection between patients with cancer (total cancer) and control patients (control).
§A logistic regression analysis, adjusted for age and sex, was used to compare HTLV-1 infection between patients with each type of cancer and patients with other types of cancer.

cancer.[12] Here, we also showed that HTLV-1 infection in patients with non-ATLL lymphoma was significantly higher than that in patients with other types of cancer (OR: 2.76, 95% CI: 1.36, 5.62, $P = 0.005$). Although HTLV-1 has not been previously associated with the occurrence of lymphoma other than ATLL, some reports have suggested that HTLV-1 carriers with B-cell lymphoma tend to have worse prognosis or that the frequency of primary malignant neoplasms in HTLV-1 carriers is higher than that in seronegative cases.[40,41] Another report also suggested that the interaction between Epstein–Barr virus and HTLV-1 could promote T- and B-cell dysfunctions and cell proliferation and inhibit apoptosis, favoring lymphomagenesis.[42]

Some limitations exist in this study. First, only the patients that were admitted to the Department of Infectious, Respiratory, and Digestive Medicine University of the Ryukyus Hospital and tested for HTLV-1 or *S. stercoralis* were included. The use of this population may introduce a selection bias in our results. Second, we did not examine the effect of confounding variables in our logistic regression, including other known carcinogens, such as smoking, drinking, parasitic infections other than *S. stercoralis*, and viral infections other than HTLV-1 (hepatitis B/C virus, Epstein–Barr virus, etc.). All patients with HTLV-1 carrier status were included in this study regardless of age. The number of young patients that were included in the HTLV-1-associated cancer development sub-analysis may have skewed the results in the opposite direction. To help normalize the results, age and sex were included in the logistic regression model to eliminate those biases.

CONCLUSIONS

Our study indicates that the prevalence of *S. stercoralis* and HTLV-1 infections have been decreasing in recent years. *Strongyloides stercoralis* infection was 2.4 times more likely in patients with HTLV-1 infection than in patients without it. Diligence toward the prevention of these diseases through decreased poverty and increased sanitation has proven effective. Continued improvements in education, testing, and treatment could easily eliminate *S. stercoralis* infections and drastically reduce the prevalence of HTLV-1 infections.

In addition, HTLV-1 infection in patients with hepatic cancer or lymphomas other than ATLL appears to be significantly higher than that in patients with other types of cancer. Further investigation regarding the possible mechanisms behind these associations is needed.

Received July 31, 2015. Accepted for publication October 19, 2015.

Published online November 30, 2015.

Authors' addresses: Teruhisa Tanaka, Tetsuo Hirata, Gretchen Parrott, Miwa Higashiarakawa, Takeshi Kinjo, Tetsu Kinjo, and Jiro Fujita, Department of Infectious, Respiratory, and Digestive Medicine, Faculty of Medicine, University of the Ryukyus, Okinawa, Japan, E-mails: teruhisa.t@gmail.com, h400314@med.u-ryukyu.ac.jp, groseler1@gmail.com, higasiarakawa@mail.goo.ne.jp, kumanomis6@yahoo.co.jp, tetsu19761976@yahoo.co.jp, and fujita@med.u-ryukyu.ac.jp. Akira Hokama, Department of Endoscopy, University of Ryukyus Hospital, Okinawa, Japan, E-mail: hokama-a@med.u-ryukyu.ac.jp.

REFERENCES

1. Asato R, Nakasone T, Yoshida C, Arakakai T, Ikeshiro T, Murakami H, 1992. Current status of *Strongyloides* infection in Okinawa, Japan. *Jpn J Trop Med Hgy 20:* 169–173.
2. Toma H, Shimabukuro I, Kobayashi J, Tasaki T, Takara M, Sato Y, 2000. Community control studies on *Strongyloides* infection in a model island of Okinawa, Japan. *Southeast Asian J Trop Med Public Health 31:* 383–387.
3. Zaha O, Hirata T, Kinjo F, Saito A, 2000. Strongyloidiasis—progress in diagnosis and treatment. *Intern Med 39:* 695–700.
4. Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC, 1980. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc Natl Acad Sci USA 77:* 7415–7419.
5. Yoshida M, Miyoshi I, Hinuma Y, 1982. Isolation and characterization of retrovirus from cell lines of human T-cell leukemia and its implication in the disease. *Proc Natl Acad Sci USA 79:* 2031–2035.
6. Proietti FA, Carneiro-Proietti AB, Catalan-Soares BC, Murphy EL, 2005. Global epidemiology of HTLV-1 infection and associated diseases. *Oncogene 24:* 6058–6068.
7. Tajima K, 1988. The third nation-wide study on adult T-cell leukemia/lymphoma (ATL) in Japan: characteristic patterns of HLA antigen and HTLV-1 infection in ATL patients and their relatives. *Int J Cancer 41:* 505–512.
8. Wilks R, Hanchard B, Morgan O, Williams E, Cranston B, Smith ML, Rodgers-Johnson P, Manns A, 1996. Patterns of HTLV-1 infection among family members of patients with adult T-cell leukemia/lymphoma and HTLV-1 associated myelopathy/tropical spastic paraparesis. *Int J Cancer 65:* 272–273.
9. Khurana S, Dubey ML, Malla N, 2005. Association of parasitic infections and cancers. *Indian J Med Microbiol 23:* 74–79.
10. Arisawa K, Sobue T, Yoshimi I, Soda M, Shirahama S, Doi H, Katamine S, Saito H, Urata M, 2003. Human T-lymphotropic virus type-1 infection, survival and cancer risk in southwestern Japan: a prospective cohort study. *Cancer Causes Control 14:* 889–896.
11. Hirata T, Nakamoto M, Nakamura M, Kinjo N, Hokama A, Kinjo F, Fujita J, 2007. Low prevalence of human T cell lymphotropic virus type 1 infection in patients with gastric cancer. *J Gastroenterol Hepatol 22:* 2238–2241.
12. Arisawa K, Soda M, Akahoshi M, Fujiwara S, Uemura H, Hiyoshi M, Takeda H, Kashino W, Suyama A, 2006. Human T-cell lymphotropic virus type-1 infection and risk of cancer: 15.4 year longitudinal study among atomic bomb survivors in Nagasaki, Japan. *Cancer Sci 97:* 535–539.
13. Arakaki T, Iwanaga M, Kinjo F, Saito A, Asato R, Ikeshiro T, 1990. Efficacy of agar-plate culture in detection of *Strongyloides stercoralis* infection. *J Parasitol 76:* 425–428.
14. Fujino R, Kawato K, Ikeda M, Miyakoshi H, Mizukoshi M, Imai J, 1991. Improvement of gelatin particle agglutination test for detection of anti-HTLV-1 antibody. *Jpn J Cancer Res 82:* 367–370.
15. Aoyama H, Hirata T, Sakugawa H, Watanabe T, Miyagi S, Maeshiro T, Chinen T, Kawane M, Zaha O, Nakayoshi T, Kinjo F, Fujita J, 2007. An inverse relationship between autoimmune liver diseases and *Strongyloides stercoralis* infection. *Am J Trop Med Hyg 76:* 972–976.
16. Yoshikawa M, Ouji Y, Nakamura-Uchiyama F, Nawa Y, 2014. Analysis of strongyloidiasis cases reported in Japan in the recent period, 2000–2013 [in Japanese]. *Clin Parasitol 25:* 20–26.
17. Ogawa S, Boostrom E, 2009. *Okinawa's Post-War Health Recovery and Development: Relevance for International Health.* Kanagawa, Japan: Seizansha.
18. Sataka M, Yamaguchi K, Tadokoro K, 2012. Current prevalence of HTLV-1 in Japan as determined by screening of blood donors. *J Med Virol 84:* 327–335.
19. Hirata T, Uchima N, Kishimoto K, Zaha O, Kinjo N, Hokama A, Sakugawa H, Kinjo F, Fujita J, 2006. Impairment of host immune response against *Strongyloides stercoralis* by human T cell lymphotropic virus type 1 infection. *Am J Trop Med Hyg 74:* 246–249.
20. Inaba S, Sato H, Okochi K, Fukuda K, Takakura F, Tokunaga K, 1989. Prevention of transmission of human T-lymphotropic

virus type 1 (HTLV-1) through transfusion, by donor screening with antibody to the virus. One-year experience. *Transfusion 29:* 7–11.
21. Ando Y, Saito K, Nakano S, Kakimoto K, Furuki K, Tanigawa T, Hashimoto H, Moriyama I, Ichijo M, Toyama T, 1989. Bottle-feeding can prevent transmission of HTLV-1 from mothers to their babies. *J Infect 19:* 25–29.
22. Kinoshita K, Hino S, Amagasaki T, Ikeda S, Yamada Y, Suzuyama J, Momita S, Toriya K, Kamihira S, Ichimaru M, 1984. Demonstration of adult T-cell leukemia virus antigen in milk from three sero-positive mothers. *Gan 75:* 103–105.
23. Nakano S, Ando Y, Ichijo M, Moriyama I, Saito S, Sugamura K, Hinuma Y, 1984. Search for possible routes of vertical and horizontal transmission of adult T-cell leukemia virus. *Gan 75:* 1044–1045.
24. The Japanese Ministry of Health, Labour and Welfare study group, 2011. HTLV-1 Carrier, Guide of the Instruction. Japan. [In Japanese].
25. Nakada K, Kohakura M, Komoda H, Hinuma K, 1984. High incidence of HTLV antibody in carriers of *Strongyloides stercoralis. Lancet 1:* 633.
26. Hayashi J, Kishihara Y, Yoshimura E, Furusyo N, Yamaji K, Kawakami K, Murakami H, Kashiwagi S, 1997. Correlation between human T cell lymphotropic virus type-1 and *Strongyloides stercoralis* infections and serum immunoglobulin E responses in residents of Okinawa, Japan. *Am J Trop Med Hyg 56:* 71–75.
27. Carvalho EM, Da Fronseca Porto A, 2004. Epidemiological and clinical interaction between HTLV-1 and *Strongyloides stercoralis. Parasite Immunol 26:* 487–497.
28. Gotuzzo E, Terashima A, Alvarez H, Tello R, Infante R, Watts DM, Freedman DO, 1999. *Strongyloides stercoralis* hyperinfection associated with human T cell lymphotropic virus type-1 infection in Peru. *Am J Trop Med Hyg 60:* 146–149.
29. Montes M, Sanchez C, Verdonck K, Lake JE, Gonzalez E, Lopez G, Terashima A, Nolan T, Lewis DE, Gotuzzo E, White AC Jr, 2009. Regulatory T cell expansion in HTLV-1 and strongyloidiasis co-infection is associated with reduced IL-5 responses to *Strongyloides stercoralis* antigen. *PLoS Negl Trop Dis 3:* e456.
30. Stuver SO, Tachibana N, Okayama A, Shoiri S, Tsunetoshi Y, Tsuda K, Mueller NE, 1993. Heterosexual transmission of human T cell leukemia/lymphoma virus type I among married couples in southwestern Japan: an initial report from the Miyazaki Cohort Study. *J Infect Dis 167:* 57–65.
31. Moriuchi H, Masuzaki H, Doi H, Katamine S, 2013. Mother-to-child transmission of human T-cell lymphotropic virus type 1. *Pediatr Infect Dis J 32:* 175–177.
32. Roucoux DF, Wang B, Smith D, Nass CC, Smith J, Hutching ST, Newman B, Lee TH, Chafets DM, Murphy EL, HTLV Outcomes Study Investigators, 2005. A prospective study of sexual transmission of human T lymphotropic virus (HTLV)-I and HTLV-II. *J Infect Dis 191:* 1490–1497.
33. Melbye M, Poulsen AG, Gallo D, Pedersen JB, Biggar RJ, Larsen O, Dias F, Aaby P, 1998. HTLV-1 infection in a population-based cohort of older persons in Guinea-Bissau, West Africa: risk factors and impact on survival. *Int J Cancer 76:* 293–298.
34. Buonfrate D, Formenti F, Perandin F, Bisoffi Z, 2015. Novel approaches to the diagnosis of *Strongyloides stercoralis* infection. *Clin Microbiol Infect 21:* 543–552.
35. Hirata T, Kishimoto K, Kinjo N, Hokama A, Kinjo F, Fujita J, 2007. Association between *Strongyloides stercoralis* infection and biliary tract cancer. *Parasitol Res 101:* 1345–1348.
36. Hokama A, Adaniya H, Saito A, 1998. Image of the month. *Strongyroides stercoralis* larvae. *Gastroenterology 114: 6,* 230.
37. Hennequin C, Pialoux G, Taillet BC, Caujolle B, Petite JP, 1991. Anguilluliasis hyperinfection in a patient receiving corticosteroids: hepatic and biliary involvement. *Gastroenterol Clin Biol 15:* 87–88.
38. Delarocque AE, Hadengue A, Degott C, Vilgrain V, Erlinger S, Benhamou JP, 1994. Biliary obstruction resulting from *Strongyloides stercoralis* infection. Report of a case. *Gut 35:* 705–706.
39. Jones N, Cocchiarella A, Faris K, Heard SO, 2010. Pancreatitis associated with *Strongyloides stercoralis* infection in a patient chronically treated with corticosteroids. *J Intensive Care Med 25:* 172–174.
40. Suefuji H, Ohshima K, Hayabuchi N, Nakamura K, Kikuchi M, 2003. HTLV-1 carriers with B-cell lymphoma of localized stage head and neck: prognosis, clinical and immunophathological features. *Br J Haematol 123:* 606–612.
41. Kozuru M, Uike N, Muta K, Goto T, Suehiro Y, Nagano M, 1996. High occurrence of primary malignant neoplasms in patients with adult T-cell leukemia/lymphoma, their siblings, and their mothers. *Cancer 78:* 1119–1124.
42. Beltran BE, Quiñones P, Morales D, Revilla JC, Alva JC, Castillo JJ, 2012. Diffuse large B-cell lymphoma in human T-lymphotropic virus type 1 carriers. *Leukemia Res Treat 2012:* 262363.
43. Tahaei SM, Mohebbi SR, Fatemi SR, Mohammadi P, Malek FN, Azimzadeh P, Almasi S, Mirsattari D, Zali MR, 2011. Low frequency of human T-cell lymphotropic virus 1 antibodies in Iranian gastric cancer patients in comparison to controls. *Asian Pac J Cancer Prev 12:* 2447–2450.
44. Mirsadraee M, Kalantari MR, Saffari A, Mahmoudi M, 2007. Association of HTLV-1 infection and esophageal squamous cell carcinoma. *J Gastrointest Cancer 38:* 15–18.

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 03 月 07 日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	乾燥濃縮人アンチトロンビンⅢ		研究報告の公表状況	http://www.who.int/mediacentre/news/statements/2016/8th-IHR-emergency-committee-polio/en//2016/03/01	公表国 スイス	
販売名 (企業名)	①ノイアート静注用 500 単位　(日本血液製剤機構) ②ノイアート静注用 1500 単位　(日本血液製剤機構)					

研究報告の概要

ポリオウイルスの国際的拡大に関する International Health Regulations Emergency Committee の第 8 回会議の声明：
2016 年 2 月 12 日，ポリオウイルスの国際的拡大に関連し，International Health Regulations (IHR) の Emergency Committee の第 8 回会議が開催された。
ポリオの国際的拡大は国際的に懸念される公衆の保健上の緊急事態 (PHEIC) として声明が 2014 年 5 月に出されてから，野生型ポリオウイルスの伝播の遮断，事務局長が発出した暫定的な勧告の実施により力強い前進が各国で達成されていることを Committee は認識している。野生型ポリオウイルスの国際的拡大の発生は全体的に減少しているものの継続しており，2015 年 10 月と 11 月に発生したパキスタンからアフガニスタンへの新規輸出が 2 件報告されている。
2015 年には循環型ワクチン由来ポリオウイルス (cVDPV) のアウトブレイクが 6 件発生した。3 件はウクライナ，マダガスカル，ラオス人民民主共和国における循環型ワクチン由来ポリオウイルス 1 型 (cVDPV1) のアウトブレイクであり，3 件はミャンマー，ナイジェリア，ギニアにおける循環型ワクチン由来ポリオウイルス 2 型 (cVDPV2) のアウトブレイクであった。前回の会議以降，さらに 6 例の cVDPV2 症例がギニアで報告された。
現時点で野生型ポリオウイルスまたは cVDPV を輸出しているのは，パキスタン (野生型ポリオウイルスの最終輸出：2015 年 11 月 3 日) およびアフガニスタン (野生型ポリオウイルスの最終輸出：2015 年 6 月 6 日) である。
現時点で野生型ポリオウイルスまたは cVDPV を輸出していないが感染している国はナイジェリア，ギニア，マダガスカル，ウクライナ，ラオス人民民主共和国，ミャンマーであることが報告された。

報告企業の意見	今後の対応
ポリオウイルス (Poliovirus) はピコルナウイルス科 (Picorna) エンテロウイルス属 (Enterovirus) に属する RNA ウイルスで、形状は直径 25～30nm の正 20 面体の形状で、抗原性の異なる 3 種類がある。万一原料血漿にポリオ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。

使用上の注意記載状況・その他参考事項等

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

Me

Statement on the 8th IHR Emergency Committee meeting regarding the international spread of poliovirus

WHO statement
1 March 2016

The eighth meeting of the Emergency Committee under the International Health Regulations (2005) (IHR) regarding the international spread of poliovirus was convened via teleconference by the Director-General on 12 February 2016. As with the seventh meeting, the Emergency Committee reviewed the data on circulating wild poliovirus as well as circulating vaccine-derived polioviruses (cVDPV). The latter is particularly important as cVDPVs reflect serious gaps in immunity to poliovirus due to weaknesses in routine immunization coverage in otherwise polio-free countries. In addition, it is essential to stop type 2 cVDPVs in advance of the globally synchronized withdrawal of type 2 OPV in April 2016.

The following IHR States Parties submitted an update on the implementation of the Temporary Recommendations since the Committee last met on 10 November 2015: Afghanistan, Pakistan and Guinea.

Wild polio

The Committee noted that since the declaration that the international spread of polio constituted a Public Health Emergency of International Concern (PHEIC) in May 2014, strong progress has been made by countries toward interruption of wild poliovirus transmission and implementation of Temporary Recommendations issued by the Director-General. There has been an overall decline in the occurrence of international spread of wild poliovirus. The Committee was particularly encouraged by the intensified efforts and progress toward interruption of poliovirus transmission in Pakistan and Afghanistan despite challenging circumstances, and the renewed emphasis on cooperation along the long international border between the two countries.

The Committee noted however that the international spread of wild poliovirus has continued, with two new recent reports of exportations from Pakistan into Afghanistan which occurred in October and November 2015. These cases occurred in Nangarhar and Kunar Provinces, in the eastern region, adjoining the Pakistan border. While there has been no new exportation from Afghanistan to Pakistan, ongoing transmission particularly in inaccessible parts of the Eastern Region of Afghanistan close to the international border presents an ongoing risk.

The Committee noted that while Pakistan and Afghanistan have historically shared a vast common zone of poliovirus transmission, the ongoing spread between the two countries is occurring from discrete zones of persistent transmission in each country. Strong programmatic action in these zones should interrupt such cross-border transmission, as illustrated by the experience in regions that were previously polio-endemic.

The committee re-emphasized that under the IHR, spread of poliovirus between two Member States can constitute international spread. The Committee acknowledged that cross border collaboration efforts have continued to be strengthened. Whilst border vaccination between these two countries is limited to children under ten years of age, efforts are being made to vaccinate departing travellers of all age groups from airports when leaving this epidemiological block formed by the two countries. The committee was particularly pleased that the Temporary Recommendations for international travellers of all ages are now being implemented in Afghanistan at the international airport in Kabul. In this respect, it noted that all countries, and particularly those with embassies in Afghanistan and Pakistan, should facilitate implementation of Temporary Recommendations through adopting procedures that include proof of polio vaccination as part of visa application processes for travellers departing from Afghanistan or Pakistan.

The committee noted that globally there are still significant vulnerable areas and populations that are inadequately immunized due to conflict, insecurity and poor coverage associated with weak immunization programmes. Such vulnerable areas include countries in the Middle East, the Horn of Africa, central Africa and parts of Europe. The hard-earned gains of the GPEI can be quickly lost if there is re-introduction of poliovirus in settings of disrupted health systems and complex humanitarian emergencies. The large population movements across the Middle East and from Afghanistan and Pakistan create a heightened risk of international spread of polio. There is a risk of missing polio vaccination among refugee and mobile populations, adding to missed and under vaccinated populations in Europe, the Middle East and Africa. An estimated three to four million people have been displaced to Turkey, Lebanon, and Jordan and are at the centre of a mass migration across Europe.

The committee was very concerned by the weakening of AFP surveillance in Equatorial Guinea, and urged renewed efforts to strengthen surveillance and routine immunization there. Insecurity in Africa, notably in parts of Cameroon and Somalia, continues to pose a threat to polio eradication in that continent.

Vaccine derived poliovirus

The current circulating vaccine-derived poliovirus (cVDPV) outbreaks across four WHO regions illustrate serious gaps in routine immunization programs, leading to significant pockets of vulnerability to polio outbreaks. In 2015, six outbreaks of circulating vaccine derived poliovirus have occurred – three cVDPV type 1 outbreaks (Ukraine, Madagascar and Lao People's Democratic Republic) and three cVDPV type 2 outbreaks (Myanmar, Nigeria and Guinea).

Six additional cases of cVDPV type 2 have been reported in Guinea since the last meeting. This increases the threat of international spread, particularly to neighbouring countries, where the Ebola epidemic has weakened health systems including routine immunization. This is of particular concern given the imminent global withdrawal of type 2 oral polio vaccine (OPV2) in April 2016. The committee noted with concern that AFP surveillance does not meet international standards in parts of Guinea, heightening concern about whether circulation could be missed. Post-Ebola there was a new community reluctance to accept vaccination, and this needs to be urgently addressed. The committee acknowledged the efforts to improve the quality of supplementary immunization activities (SIAs), and urged that this continue.

The committee noted that in Lao People's Democratic Republic and Myanmar there was ongoing circulation of vaccine derived polioviruses, particularly in hard to reach populations in both countries, underlining the importance of communication to counteract vaccine hesitancy.

While there have been no new cases of cVDPV in Ukraine, Madagascar, South Sudan or Nigeria since the last committee meeting, threats remain. More needs to be done in each of these countries to improve routine coverage and AFP surveillance. In Ukraine, the committee was concerned by the restricted availability of polio vaccines (including non-availability to persons >10 years of age) and suboptimal routine immunization, and reports of lack of community acceptance of polio vaccines. This reluctance to be vaccinated needs to be addressed through well-crafted communications. In South Sudan and Nigeria, there was heightened risk of further circulation in areas affected by conflict and insecurity. Complacency is another risk in Nigeria, and as the number of SIAs decreases, the strengthening of routine immunization needs to be a high priority.

Conclusion

The Committee unanimously agreed that the international spread of polio remains a Public Health Emergency of International Concern (PHEIC) and recommended the extension of the Temporary Recommendations for a further three months. The Committee considered the factors expressed in reaching this conclusion at the seventh meeting still applied:

- The continued international spread of wild poliovirus during 2015 involving Pakistan and Afghanistan.
- The risk and consequent costs of failure to eradicate globally one of the world's most serious vaccine preventable diseases.
- The continued necessity of a coordinated international response to improve immunization and surveillance for wild poliovirus, stop its international spread and reduce the risk of new spread.
- The serious consequences of further international spread for the increasing number of countries in which immunization systems have been weakened or disrupted by conflict and complex emergencies. Populations in these fragile states are vulnerable to outbreaks of polio. Outbreaks in fragile states are exceedingly difficult to control and threaten the completion of global polio eradication during its end stage.
- The importance of a regional approach and strong cross-border cooperation, as much international spread of polio occurs over land

borders, while recognizing that the risk of distant international spread remains from zones with active poliovirus transmission.

- Additionally with respect to cVDPV:
    - cVDPVs also pose a risk for international spread, and if there is no urgent response with appropriate measures, particularly threaten vulnerable populations as noted above;
    - The emergence and circulation of VDPVs in four WHO regions demonstrates significant gaps in population immunity at a critical time in the polio endgame;
    - There is a particular urgency of stopping type 2 cVDPVs in advance of the globally synchronized withdrawal of type 2 component of the oral poliovirus vaccine in April 2016.

Risk categories

The Committee provided the Director-General with the following advice aimed at reducing the risk of international spread of wild poliovirus and cVDPVs, based on the risk stratification as follows:

Wild poliovirus

- States currently exporting wild poliovirus;
- States infected with wild poliovirus but not currently exporting;
- States no longer infected by wild poliovirus, but which remain vulnerable to international spread.

Circulating vaccine derived poliovirus

- States currently exporting cVDPV;
- States infected with cVDPV but not currently exporting;
- States no longer infected by cVDPV, but which remain vulnerable to the emergence and circulation of VDPV.

The Committee applied the following criteria to assess the period for detection of no new exportations and the period for detection of no new cases or environmental isolates of wild poliovirus or cVDPV:

Criteria to assess States no longer exporting (detection of no new wild poliovirus or cVDPV exportation)

- Poliovirus Case: 12 months after the onset date of the first case caused by the most recent exportation PLUS one month to account for case detection, investigation, laboratory testing and reporting period, OR when all reported AFP cases with onset within 12 months of the first case caused by the most recent importation have been tested for polio and excluded for newly imported WPV1 or cVDPV, and environmental samples collected within 12 months of the first case have also tested negative, whichever is the longer.
- Environmental isolation of exported poliovirus: 12 months after collection of the first positive environmental sample in the country that received the new exportation PLUS one month to account for the laboratory testing and reporting period.

Criteria to assess States no longer infected (detection of no new wild poliovirus or cVDPV)

- Poliovirus Case: 12 months after the onset date of the most recent case PLUS one month to account for case detection, investigation, laboratory testing and reporting period OR when all reported AFP cases with onset within 12 months of last case have been tested for polio and excluded for WPV1 or cVDPV, and environmental samples

collected within 12 months of the last case have also tested negative, whichever is the longer.

- Environmental isolation of wild poliovirus or cVDPV (no poliovirus case): 12 months after collection of the most recent positive environmental sample PLUS one month to account for the laboratory testing and reporting period

Temporary recommendations

States currently exporting wild poliovirus or cVDPV

(Currently Pakistan (last wild poliovirus exportation: 3rd November 2015) and Afghanistan (last wild poliovirus exportation: 6 June 2015).

Exporting countries should:

- Officially declare, if not already done, at the level of head of state or government, that the interruption of poliovirus transmission is a national public health emergency; where such declaration has already been made, this emergency status should be maintained.
- Ensure that all residents and long-term visitors (i.e. > four weeks) of all ages, receive a dose of oral poliovirus vaccine (OPV) or inactivated poliovirus vaccine (IPV) between four weeks and 12 months prior to international travel.
- Ensure that those undertaking urgent travel (i.e. within four weeks), who have not received a dose of OPV or IPV in the previous four weeks to 12 months, receive a dose of polio vaccine at least by the time of departure as this will still provide benefit, particularly for frequent travellers.
- Ensure that such travellers are provided with an International Certificate of Vaccination or Prophylaxis in the form specified in Annex 6 of the IHR to record their polio vaccination and serve as proof of vaccination.
- Restrict at the point of departure the international travel of any resident lacking documentation of appropriate polio vaccination. These recommendations apply to international travellers from all points of departure, irrespective of the means of conveyance (e.g. road, air, sea).
- Recognising that the movement of people across the border between Pakistan and Afghanistan continues to facilitate exportation of wild poliovirus, both countries should further intensify cross-border efforts by significantly improving coordination at the national, regional and local levels to substantially increase vaccination coverage of travellers crossing the border and of high risk cross-border populations. Both countries have maintained permanent vaccination teams at the main border crossings for many years. Improved coordination of cross-border efforts should include closer supervision and monitoring of the quality of vaccination at border transit points, as well as tracking of the proportion of travellers that are identified as unvaccinated after they have crossed the border.
- Maintain these measures until the following criteria have been met: (i) at least six months have passed without new exportations and (ii) there is documentation of full application of high quality eradication activities in all infected and high risk areas; in the absence of such documentation these measures should be maintained until the state meets the above criteria of a 'state no longer exporting'.
- Provide to the Director-General a monthly report on the implementation of the Temporary Recommendations on international travel, including the number of residents whose travel was restricted and the number of travellers who were vaccinated and provided appropriate documentation at the point of departure.

States infected with wild poliovirus or cVDPVs but not currently exporting

(Currently Nigeria, Guinea, Madagascar, Ukraine, Lao People's Democratic Republic and Myanmar)

Country	Virus type	# cases since outbreak began	Most recent onset
Nigeria	cVDPV2	1	16th May 2015
Ukraine	cVDPV1	2	7th July 2015
Guinea	cVDPV2	8	14th December 2015
Madagascar	cVDPV1	11	22nd August 2015
Lao People's Democratic Republic	cVDPV1	10	11th January 2016
Myanmar	cVDPV2	2	5th October 2015

These countries should:

- Officially declare, if not already done, at the level of head of state or government, that the interruption of poliovirus transmission is a national public health emergency; where such declaration has already been made, this emergency status should be maintained.
- Encourage residents and long-term visitors to receive a dose of OPV or IPV four weeks to 12 months prior to international travel; those undertaking urgent travel (i.e. within four weeks) should be encouraged to receive a dose at least by the time of departure.
- Ensure that travellers who receive such vaccination have access to an appropriate document to record their polio vaccination status.
- Intensify regional cooperation and cross-border coordination to enhance surveillance for prompt detection of poliovirus and substantially increase vaccination coverage among refugees, travellers and cross-border populations.
- Maintain these measures until the following criteria have been met: (i) at least six months have passed without the detection of wild poliovirus transmission or circulation of VDPV in the country from any source, and (ii) there is documentation of full application of high quality eradication activities in all infected and high risk areas; in the absence of such documentation these measures should be maintained until the state meets the criteria of a 'state no longer infected'.
- At the end of 12 months without evidence of transmission, provide a report to the Director-General on measures taken to implement the Temporary Recommendations.

States no longer infected by wild poliovirus or cVDPV, but which remain vulnerable to international spread, and states that are vulnerable to the emergence and circulation of VDPV

(Currently Somalia, Iraq, Israel, Equatorial Guinea, Cameroon and South Sudan)

These countries should:

- Urgently strengthen routine immunization to boost population immunity.
- Enhance surveillance quality to reduce the risk of undetected wild poliovirus and cVDPV transmission, particularly among high risk mobile and vulnerable populations.
- Intensify efforts to ensure vaccination of mobile and cross-border populations, Internally Displaced Persons, refugees and other vulnerable groups.
- Enhance regional cooperation and cross border coordination to ensure prompt detection of wild poliovirus and cVDPV, and vaccination of high risk population groups.
- Maintain these measures with documentation of full application of high quality surveillance and vaccination activities.
- At the end of 12 months without evidence of reintroduction of wild poliovirus or new emergence and circulation of cVDPV, provide a report to the Director General on measures taken to implement the Temporary Recommendations.

GPEI and other international organizations, particularly Gavi, should provide all necessary support to reduce the risk of emergence and circulation of VDPV.

These countries should provide a final report as per the table below:

Country	Most recent case onset / +ve environmental isolate	Final Report due
Israel	30-Mar-14	Apr-16
Iraq	7-Apr-14	May-16
South Sudan	19-Apr-15	May-16
Equatorial Guinea	3-May-14	Jun-16
Cameroon	9-Jul-14	Aug-16
Nigeria	16-May-2015*	Aug-16
Somalia	11-Aug-14	Sep-16
* most recent cVDPV2 in Nigeria		

Additional considerations for all infected countries

The Committee strongly urged global partners in polio eradication to provide optimal support to all infected countries at this critical time in the polio eradication program for implementation of the Temporary Recommendations under the IHR. The Committee advised that in view of the evolving situation, periodic review and assessment of the risk of international spread and measures to mitigate these risks are warranted.

The Committee recommended that international partners assist countries affected by cVDPV with development of appropriate communications strategies and materials to ensure clear public understanding of cVDPV, their distinction from wild poliovirus, and maintenance of confidence in the effectiveness, safety and necessity of polio vaccines during the polio endgame. Recognizing that cVDPV illustrates serious gaps in routine immunization programs in otherwise polio free countries, the Committee

recommended that the international partners in routine immunization, for example Gavi, should urgently assist affected countries to improve the national immunization program.

The Committee again requested the Secretariat to conduct an analysis of the public health benefits and costs of implementing the temporary recommendation requiring exporting countries to vaccinate all international travellers before departure.

Based on the advice concerning wild poliovirus and cVDPV, and the reports made by Afghanistan, Pakistan, and Guinea, the Director-General accepted the Committee's assessment and on 26 February 2016 determined that the events relating to poliovirus continue to constitute a PHEIC, with respect to wild poliovirus and cVDPV. The Director-General endorsed the Committee's recommendations for 'States currently exporting wild polioviruses or cVDPV', for 'States infected with wild poliovirus or cVDPV but not currently exporting' and for 'States no longer infected by wild poliovirus, but which remain vulnerable to international spread, and states that are vulnerable to the emergence and circulation of VDPV' and extended the Temporary Recommendations as revised by the Committee under the IHR to reduce the international spread of poliovirus, effective 26 February 2016.

The Director-General thanked the Committee Members and Advisors for their advice and requested their reassessment of this situation within the next three months.

Related links

Fact sheet on poliomyelitis

IHR Emergency Committee concerning ongoing events and context involving transmission and international spread of poliovirus

More on the IHR Emergency Committee and Members

別紙様式第2-1

No. 20

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2016. 3. 23	該当なし	

一般的名称	人全血液	研究報告の公表状況	Hong H, Xiao W, Lazarus HM, Good CE, Maitta RW, Jacobs MR. Blood. 2016 Jan 28;127(4):496-502.	公表国 米国
販売名(企業名)	人全血液-LR「日赤」(日本赤十字社) 照射人全血液-LR「日赤」(日本赤十字社)			

研究報告の概要

○積極的及び受動的サーベイランスによる血小板輸血に対する敗血症反応(STR)の検出。
血小板製剤の細菌汚染に対して、血小板製剤の一部分割液を培養する積極的サーベイランス並びに医療機関から報告される輸血反応を再検討する受動的サーベイランスが、7年間(2007年～2013年)にわたり実施されていた。全ての血小板製剤が採血後の24時間培養の対象となっており、陰性製剤として出荷されていた。AABBの新たなSTR基準を含む5種類のSTR基準の感度及び特異度、並びに積極的及び受動的サーベイランスによる検出について検討を行った。積極的サーベイランスにより、輸血された51,440本の血小板製剤のうち、20本(0.004%*、100万本当たり389本)が細菌に汚染されていたことが判明した。結果的には、5例が輸血後9時間から24時間までの間にSTRを発症していたが、受動的サーベイランスによって報告されたものはなかった。STRは、高濃度の細菌汚染した製剤を輸血された好中球減少症患者にのみ発症していた。受動的サーベイランスによって報告された総数284例(0.55%)の輸血反応例に、細菌汚染した血小板の輸血を受けた患者は含まれていなかった。しかし、その284例のうち6例～93例(2.1%～32.7%)は5種類のSTR基準のうちの1つ以上の基準を満たしており、STR基準の感度については、5.1%から45.5%までの幅があった。これらの結果は、受動的サーベイランスではSTRを検出できないこと、並びにSTR基準の特異度の欠如を実証している。これらの知見は、受動的サーベイランスに基づいて報告されたSTRの全国データの限界、並びにこの問題への対処を目的とする、2次検査や病原体低減技術の使用といった更なる措置を実施する必要性を強調している。

*訳者注:0.004%は、計算(100万本当たり389本)すると0.04%の誤り

使用上の注意記載状況・その他参考事項等

人全血液-LR「日赤」
照射人全血液-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見

米国にて7年間に輸血された血小板製剤51,440本に対して、医療機関から輸血反応報告が284件(0.55%)報告されたが、血液培養にて細菌汚染が確認された製剤20本に起因する輸血反応は報告されなかったという報告である。

今後の対応

日本赤十字社では全献血者に問診を実施しているほか、輸血による細菌感染予防対策として全輸血用血液製剤を対象に初流血除去及び保存前白血球除去を行っている。なお、PCの有効期間は採血後3日間である(採血日=0日)。また、医薬品医療機器等法に基づき及び関連法令に従い輸血副作用・感染症情報を収集し、医薬品医療機器総合機構を通じて国に報告している。今後も引き続き輸血副作用・感染症に関する情報の収集に努める。

Regular Article JRC2016T-009

TRANSFUSION MEDICINE

Detection of septic transfusion reactions to platelet transfusions by active and passive surveillance

Hong Hong,* Wenbin Xiao,* Hillard M. Lazarus, Caryn E. Good, Robert W. Maitta, and Michael R. Jacobs

Departments of Pathology and Medicine, University Hospitals Case Medical Center and Case Western Reserve University School of Medicine, Cleveland, OH

Key Points

- Bacterial sepsis from contaminated platelet transfusions continues to occur despite recent interventions; additional measures are needed.
- STR to platelet transfusion is frequently not recognized or reported; use of recent AABB criteria showed highest diagnostic sensitivity.

Septic transfusion reactions (STRs) resulting from transfusion of bacterially contaminated platelets are a major hazard of platelet transfusion despite recent interventions. Active and passive surveillance for bacterially contaminated platelets was performed over 7 years (2007-2013) by culture of platelet aliquots at time of transfusion and review of reported transfusion reactions. All platelet units had been cultured 24 hours after collection and released as negative. Five sets of STR criteria were evaluated, including recent AABB criteria; sensitivity and specificity of these criteria, as well as detection by active and passive surveillance, were determined. Twenty of 51 440 platelet units transfused (0.004%; 389 per million) were bacterially contaminated by active surveillance and resulted in 5 STRs occurring 9 to 24 hours posttransfusion; none of these STRs had been reported by passive surveillance. STR occurred only in neutropenic patients transfused with high bacterial loads. A total of 284 transfusion reactions (0.55%) were reported by passive surveillance. None of these patients had received contaminated platelets. However, 6 to 93 (2.1%-32.7%) of these 284 reactions met 1 or more STR criteria, and sensitivity of STR criteria varied from 5.1% to 45.5%. These results document the continued occurrence of bacterial contamination of platelets resulting in STR in neutropenic patients, failure of passive surveillance to detect STR, and lack of specificity of STR criteria. These findings highlight the limitations of reported national STR data based on passive surveillance and the need to implement further measures to address this problem such as secondary testing or use of pathogen reduction technologies. (*Blood.* 2016;127(4):496-502)

Introduction

Platelet transfusions are important in the prevention or treatment of bleeding in patients with thrombocytopenia or impaired platelet function.[1] Approximately 2.2 million platelet transfusions were administered in the United States in 2011, with 2 million in the form of apheresis units and 200 000 as pooled whole-blood–derived units.[2] Bacterial contamination of platelets is currently the leading infectious risk of platelet transfusion therapy despite recent interventions to reduce this risk.[3] Over the 5-year period from 2009 to 2013, 13 fatalities associated with bacterial contamination of platelet products were recorded by the US Food and Drug Administration (FDA), a rate of 2.6 per year or ~1.3 per million platelet transfusions.[4] Regrettably, this situation is underappreciated or underrecognized by health care providers caring for patients receiving platelets, as well as by regulatory agencies, which have not yet mandated available additional measures to address this problem.[5,6]

Further confounding our understanding of this problem is the recognition of septic transfusion reactions (STRs) resulting from transfusion of bacterially contaminated platelets.[7] Criteria for the diagnosis of STRs caused by bacterial contamination of platelets have been established to trigger recognition, management, and further investigation.[8] These criteria, however, broadly overlap with the diagnostic criteria for other noninfectious transfusion reactions such as febrile-like/febrile nonhemolytic transfusion reactions (FNHTRs) and hypotensive transfusion reactions. Moreover, it has been estimated that reported STR rates have been underestimated by as much as 10-fold due to limitations of passive reporting mechanisms.[9]

In this study, we retrospectively evaluated the records of patients who received bacterially contaminated platelet transfusions at our institution over 7 years, as well as the records of patients with reported transfusion reactions. We analyzed the associations between bacterial species, bacterial load, and transfusion reactions, as well as the sensitivity and specificity of various STR criteria in identifying patients with true transfusion-transmitted bacterial contamination of platelets.

Patients and methods

Patients

The study was conducted at University Hospitals Case Medical Center (UHCMC), a 947-bed tertiary care academic medical center affiliated with Case Western Reserve University. Patients receiving prepooled, whole-blood–derived,

Submitted July 6, 2015; accepted November 17, 2015. Prepublished online as *Blood* First Edition paper, November 23, 2015; DOI 10.1182/blood-2015-07-655944.

*H.H. and W.X. contributed equally.

The online version of this article contains a data supplement.

There is an Inside *Blood* Commentary on this article in this issue.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

Table 1. STR definitions and criteria used to analyze study findings

STR criterion	STR definition (observation period)				
	CDC[11] (4 h)	SJCRH[4] (24 h)	ARC[8,12] (4 h)	PHAC[13] (6 h)	AABB[6] (24 h)
A	Fever ≥39°C	Fever ≥40°C	Fever ≥39°C	Fever ≥39°C with a rise of ≥1°C	Fever ≥38°C with a rise of ≥1°C PLUS any of: rigors, dyspnea, nausea/vomiting, tachycardia, hypotension, shock
B	A rise of ≥2°C PLUS any of: rigors, dyspnea, nausea/vomiting, tachycardia, hypotension, shock	Cardiovascular collapse	A rise of ≥2°C	Fever ≥38°C with a rise of ≥1°C PLUS any of: rigors, dyspnea, nausea/vomiting, tachycardia, hypotension, shock	Any sepsis like syncope/hypotension
C			Any of: rigors, tachycardia, hypotension, shock	Any other suspicious clinical changes	

Observation period refers to the time period following transfusion during which changes in signs and symptoms should be regarded as associated with the transfusion. Patients meeting any criterion (A, B, or C) during the observation period are deemed to have STR.

random-donor platelet units (usually in pools of 5 units), and single-donor apheresis platelet units from January 1, 2007 through December 31, 2013 comprised the study population. At our institution, 75% to 80% of platelets are transfused in inpatients and 20% to 25% in outpatients. H.H., W.X., C.E.G., and R.W.M. extracted and analyzed the data from clinical and other records, and all authors had access to primary clinical data.

Platelet units

Leukoreduced apheresis and prepooled whole-blood–derived platelet units were obtained primarily from local blood suppliers, the American Red Cross (ARC) of Northeast Ohio (Cleveland, OH), and LifeShare Community Blood Services (Elyria, OH). Both suppliers diverted the first 5 to 10 mL of each collection, cultured all platelet units 24 hours after collection, and released units if culture was negative 24 hours later. Culture was performed by inoculation of 8 to 10 mL of platelets into BacT/ALERT BPA culture bottles (ARC) or 3 to 4 mL into eBDS culture pouches (LifeShare).

Surveillance for bacterial contamination of platelet units and STRs

Active and passive surveillance were performed on all platelet products issued and patients receiving platelet products during the study period.[7] Active surveillance was performed by collecting 1- to 2-mL aliquots of apheresis or pooled platelet units at the time of issue for culture (see "Microbiology methods" below). Patients were evaluated for evidence of transfusion reactions as soon as positive culture results were obtained. Passive surveillance in our institution depends on reporting of transfusion reactions by clinicians in outpatient and inpatient settings to the transfusion medicine service; no institutional transfusion safety officer has been appointed. Detailed instructions (kept updated with the most recent FDA hemovigilance guidelines, providing guidance on diagnosis, timing of reporting, laboratory workup, sample collection, and treatment according to the type of transfusion reaction encountered) have been developed and are available on the hospital's intranet service. Additionally, these instructions include educational materials and forms that are required to accompany all samples sent to the blood bank for testing. Further assistance is provided by the transfusion medicine service once notification has occurred. All reactions are then investigated by the transfusion medicine service, which follows up the patient's clinical status to determine whether resolution of the reaction occurred following treatment and analyzes applicable laboratory results. Transfusion reaction investigations are not considered complete until a transfusion medicine physician writes a comprehensive note verifying that the investigation has been completed and determines that all appropriate testing has been performed. Investigation of transfusion reactions to platelets includes patient evaluation and Gram stain and culture of the remains of implicated platelet units. Patients' transfusion reactions, documented by active or passive surveillance, were classified by severity as previously described[7] and correlated with platelet and posttransfusion blood cultures and other investigations.

Microbiology methods

For active surveillance, bacterial culture was performed by plating 0.1-mL aliquots of platelet samples obtained at time of issue onto blood agar plates, which were incubated for up to 48 hours in 5% CO_2 at 35°C; isolates recovered were identified, and antimicrobial susceptibility testing performed according to standard procedures, and preserved at −70°C.[10] Culture was repeated on platelet specimens with initial positive culture results from samples retained at 4°C to determine whether results were true or false positives. True positives were defined as isolation of the same bacterial species from the repeat culture. Quantitative cultures to determine bacterial load were also performed on initial positive cultures by plating 0.1 mL of serial 10-fold dilutions of platelet specimens (retained at 4°C to prevent further bacterial replication) onto blood agar plates, which were incubated aerobically for up to 48 hours in 5% CO_2 at 35°C. Culture results were recorded as colony-forming units (cfu) per mL of platelet product. Bacterial loads were interpreted as high if $\geq 10^5$ cfu/mL and low if $<10^5$ cfu/mL, as previously described.[7]

For passive surveillance, Gram stain and plate culture of the remains of implicated platelet units were performed. The culture method was the same as used for active surveillance with the addition of a second blood agar plate, which was incubated anaerobically for up to 48 hours at 35°C.

Blood cultures on patients were performed according to standard procedures using SA aerobic and SN anaerobic blood culture bottles (BacT/ALERT; Biomerieux).

STR criteria

The records of patients who received bacterially contaminated platelets (ie, detected by active surveillance) as well as those with reported transfusion reactions after platelet transfusion (ie, detected by passive surveillance) were evaluated and reactions classified according to reaction type as previously described.[7,11] Reactions were also classified using 5 sets of STR criteria, including those from the Centers for Disease Control and Prevention (CDC) Biovigilance Guidelines[12] and recommendations from Sanders et al/St. Jude Children's Research Hospital (SJCRH),[13] ARC,[8,14] Blood Safety Surveillance and Health Care Acquired Infections Division, Public Health Agency of Canada (PHAC),[15] and AABB[5] (Table 1). These criteria are based on the clinical features of transfusion reactions, including symptoms, observation period for onset of symptoms after platelet transfusion, and changes in vital signs. The length of the observation period for onset of symptoms after transfusion in these criteria varies from 4 to 24 hours. Patient records were also reviewed for evidence of delayed sequela.[16]

Data sources

After approval from the UHCMC Institutional Review Board, data were extracted from available sources, including surveillance records, clinical charts, blood bank investigation logs, transfusion reaction reports, and laboratory records.

Table 2. Bacterial culture results of bacterially contaminated platelets administered to patients, with patient peripheral blood leukocyte count, transfusion reactions, and posttransfusion blood culture results

Case no.	Platelet type	Organism	WBC, $10^3/\mu L$	Load, cfu/mL	Transfusion reaction	Posttransfusion blood culture
55	Prepooled	*S epidermidis*	9.4	2.0×10^4	No	ND
56	Apheresis	*S epidermidis*	0.7	6.0×10^6	No	Negative
57	Apheresis	*S epidermidis*	27.7	3.0×10^6	No	Negative
58	Apheresis	*Staphylococcus warneri*	0.3	2.2×10^2	No	Negative
59	Apheresis	*S epidermidis*	16.9	1.5×10^7	No	ND
60	Apheresis	*A baumannii*	2.1	4.0×10^2	No	Negative
61	Apheresis	*S epidermidis*	4.1	1.0×10^6	No	Negative
62	Apheresis	*Streptococcus sanguinis*	0.1	6.0×10^7	No	Negative
63	Prepooled	*S aureus*	1.4	1.4×10^5	Yes	Positive*
64	Apheresis	*S gallolyticus*	3	1.0×10^2	No	Negative
65	Apheresis	*S gallolyticus*	6.8	1.0×10^2	No	Negative
66	Apheresis	*S epidermidis*	11	8.5×10^3	No	Negative
67	Prepooled	*S epidermidis*	10.8	1.0×10^5	No	Negative
68	Apheresis	*S warneri*	0.5	5.0×10^6	Yes	Positive*
70	Apheresis	*S oralis*	0.7	7.0×10^6	Yes	Positive*
71	Apheresis	*S epidermidis*	7.4	4.0×10^2	No	Negative
72	Apheresis	*S epidermidis*	2.6	5.0×10^6	Yes	Negative
74	Prepooled	*S epidermidis*	3.9	5.0×10^4	No	Negative
75	Apheresis	*S epidermidis*	10.8	4.0×10^2	No	ND
76	Apheresis	*S oralis*	1.1	1.1×10^6	Yes	Positive*

ND, not done.
*Same organism as in platelet product.

Statistical analysis

All statistics were performed using Prism 6 (GraphPad Software Inc). Results are presented as mean ± standard deviation. The intergroup data comparisons were performed using the Student *t* test or χ^2 test. All reported *P* values are 2-sided with a type I error rate of 5% and a $P < .05$ set for significance.

Results

A total of 51 440 leukoreduced platelet products were transfused during the 7-year study period, of which 38 692 (75.2%) were apheresis units and 12 748 (24.8%) were pooled units. A total of 284 transfusion reactions were reported by passive surveillance during the observation period. The rate of transfusion reactions was not significantly different between inpatients and outpatients.

Bacterial culture results of platelet units

Twenty of the 51 440 platelet products (389 per million) transfused during the study period were detected as bacterially contaminated by active surveillance. The incidence of bacterial contamination was 4 of 12 748 (314 per million) in prepooled products and 16 of 38 692 (414 per million) in apheresis units (χ^2 test, $P = .62$). Contaminants included 19 gram-positive species, predominantly coagulase-negative staphylococci (n = 13) and streptococci (n = 5), and only 1 gram-negative species, *Acinetobacter baumannii* (Table 2). Two pairs of isolates, *Staphylococcus epidermidis* (cases 56 and 57) and *Streptococcus gallolyticus* (*Streptococcus bovis*) (cases 64 and 65), were from split apheresis units from the same collections. Bacterial loads ranged from 4×10^2 cfu/mL to 6×10^7 cfu/mL, with 11 units having loads of $\geq 10^5$ cfu/mL. Demographic information on the 20 patients who received bacterially contaminated platelet products is shown in supplemental Table 1 (see supplemental Data, available on the *Blood* Web site).

No additional contaminated units were detected among the 284 units recultured following reported transfusion reactions (passive surveillance), which included febrile-like/FNHTR, allergic, and other transfusion reactions.

STR in patients receiving bacterially contaminated platelet units

Ten patients who received contaminated platelets had hematologic malignancies: 2 had other malignancies and 8 had nonneoplastic thrombocytopenia (supplemental Table 1). Four were undergoing hematopoietic stem cell transplants. None of the patients were receiving antimicrobial therapy on day of transfusion. Five of the 20 patients who received contaminated platelets developed signs and symptoms that should have been reported to the transfusion service as transfusion reactions according to transfusion policies (Table 3). However, none of these reactions had been reported to the transfusion service and were only revealed during investigation of positive platelet cultures. Four of these patients received their transfusions in outpatient clinics and the

Table 3. Clinical presentations of patients with confirmed STR

Case no.	Age, y/sex	Diagnosis	In/out patient	Onset, h	Presentation	Severity
63	56/M	MM PBSCT	Out	24	Hypotension syncope	Moderate
68	62/M	AML BMT	In	12	Cardiac arrest multiorgan failure	Fatal
70	78/M	NHL	Out	9	Fever (39.5°C), rigors	Life-threatening
72*	22/F	AA	Out	18	Hypotension	Moderate
76	7/M	ALL	Out	16	Fever (39.5°C), rigors	Severe

AA, aplastic anemia; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BMT, bone marrow transplant; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; PBSCT, peripheral blood stem cell transplant.
*Posttransfusion blood culture was negative.

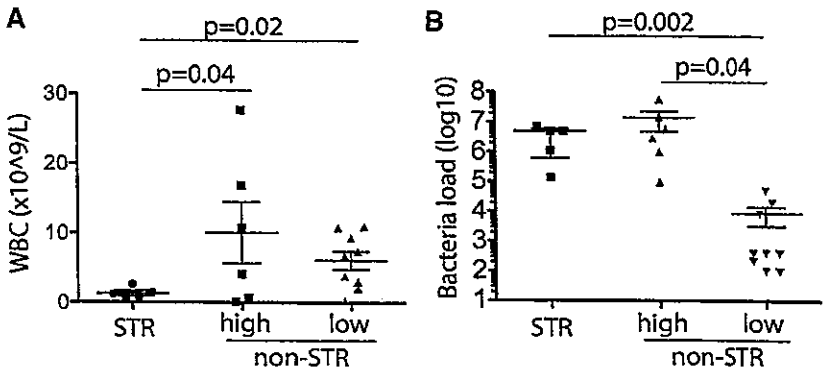


Figure 1. Relationships between WBC and STR and bacterial load and STR. Correlation of WBC count (A) and bacterial load (B) in patients who received bacterially contaminated platelets with presence or absence of STR following transfusion. Bacterial load is shown in cfu/mL. Of the 11 patients who received platelet units with high bacterial loads, 5 developed STRs whereas 6 did not; none of the 9 patients who received platelet units with low bacterial loads developed STRs. STRs were significantly associated with low WBC count (A) and high bacterial loads (B).

fifth was an inpatient at the time of transfusion. These 5 patients had no symptoms or signs of sepsis prior to transfusion. All 5 patients had delayed-onset reactions, occurring 9 to 24 hours posttransfusion. Transfusion reactions were recognized and patients treated appropriately in 3 of these cases (all outpatients). The reaction in another outpatient was only recognized when the positive culture was reported and the patient was called in for treatment. The remaining reaction occurred in an inpatient, where the patient presented in cardiac arrest. Bacterial species involved were *Staphylococcus aureus* (n = 1), coagulase-negative staphylococci (n = 2), and *Streptococcus oralis* (n = 2). Posttransfusion blood culture yielded the same bacterial species as detected in the product in 4 of the 5 patients. Of the 15 patients who had no reaction, 13 had blood cultures performed within 48 hours of receipt of contaminated platelets which were negative; the remaining 2 patients did not have posttransfusion blood cultures performed within this time period.

The clinical presentations of the 5 patients with STR included fever/rigors (n = 2), hypotension (n = 1), hypotension and fever (n = 1), and cardiac arrest (n = 1) (Table 3). These reactions were not initially identified as STR, but were recorded as FNHTR or hypotensive transfusion reactions in patients' medical records. After chart review, communication with clinical teams, and correlation with culture results, these 5 reactions were clinically deemed to be STR: 1 fatal, 1 life-threatening, 1 severe, and 2 moderate. In the fatal case, the patient developed cardiac arrest 12 hours posttransfusion, which progressed to multiorgan failure, and the patient died 10 days later. No other adverse events or delayed sequela to receipt of contaminated platelets such as central venous line infections with the same bacterial species present in contaminated units were detected on review of long-term patient records.

Bacterial load and leukocyte count at time of transfusion in patients transfused with bacterially contaminated platelets

Bacterial loads were high ($\geq 10^5$ cfu/mL) in 11 of the contaminated units transfused and low ($<10^5$ cfu/mL) in the other 9 transfused units (Table 2). Of the 11 patients transfused with high bacterial load products, 5 developed STR. In contrast, none of the 9 patients receiving low bacterial loads developed STR (Figure 1A; supplemental Table 2). The median bacterial load was 2 $\log_{10}$ higher in products transfused to patients who developed STR than that in products not resulting in transfusion reactions (5×10^6 vs 2×10^4 cfu/mL, $P = .005$) (Figure 1B). Leukocyte counts at time of transfusion were eightfold lower in the 5 patients who had STR than in the 6 who did not but were transfused with high-load contaminated products ($1.26 \pm 0.37 \times 10^9$/L vs $10.1 \pm 4.4 \times 10^9$/L, $P = .04$ by t test) (Figure 1A).

Sensitivity and specificity of active vs passive surveillance in detecting any transfusion reaction

As noted under "STR in patients receiving bacterially contaminated platelet units," no reactions were reported from the 20 patients who received contaminated platelets, although reactions had occurred and had been documented in patients' medical records in 5 cases, whereas 284 reactions were reported on patients who received sterile platelets. Demographic information on the 284 patients who did not receive bacterially contaminated platelet products was similar to that of patients who received contaminated platelet products and is shown in supplemental Table 3. Although the specificity of passive surveillance to detect transfusion of bacterially contaminated platelets was high (51 156 of 51 420, 99.5%), sensitivity was zero (0 of 20) (supplemental Table 4). In contrast, sensitivity of active surveillance was 25% (5 of 20), whereas specificity was the same as that for passive surveillance.

Sensitivity and specificity of STR criteria

The sensitivity and efficiency of the diagnostic criteria currently available for detecting STR was determined for the 5 patients who developed reactions after receiving bacterially contaminated platelets. All 5 patients met AABB criteria, whereas only 3 met SJCRH criteria and 2 PHAC criteria; none met CDC or ARC criteria (Table 4). The single most important reason for patients not meeting STR criteria was the short observation time of 4 to 6 hours used by CDC, ARC, and PHAC (Table 1). For SJCRH criteria, the requirement for fever of ≥40°C resulted in 2 patients not meeting criteria. The AABB criteria therefore showed the highest sensitivity (100%) whereas the sensitivities of the CDC, ARC, SJCRH, and PHAC criteria varied from 0% to 60%.

To examine the specificity of these STR criteria, we analyzed reported transfusion reactions in patients receiving culture-negative platelet products (n = 51 420). A total of 284 reactions were reported (incidence: 0.55%; apheresis 199 of 38 675 [0.51%] vs prepooled 85 of 12 744 [0.67%]), with similar rates of reaction types to apheresis and prepooled units. The demographic information for these patients was also similar to that of patients who received contaminated platelets (supplemental Table 3). Of these reactions, 127 (44.7%)

Table 4. Number of patients with clinically confirmed STR meeting various STR criteria

Total TR (n = 5)	CDC	SJCRH	ARC	PHAC	AABB
Cases meeting STR criteria	0	3	0	2	5
Reasons for not meeting various STR criteria					
Long latency (>6 h)	5	0	5	3	0
Temperature did not reach ≥40°C	0	2	0	0	0

Table 5. Details of 284 transfusion reactions in patients receiving 51 420 culture-negative platelet units from 2007 to 2013

Reaction type	No.	Reaction rate, %
Febrile/FNHTR	127	0.29
Allergic transfusion reaction	111	0.26
Other	46	0.11
Total	284	0.66

were categorized as FNHTR/febrile-like transfusion reactions (TR), 111 (39.1%) as allergic TR, and 46 (16.2%) as other TR (Table 5). Overall, 36 of these 284 reactions met AABB STR criteria, 52 met PHAC criteria, and 93 met CDC and ARC criteria; only 6 met SJCRH criteria (Table 6). Of 127 patients with FNHTR/febrile-like transfusion reactions, 30 met AABB STR criteria, 48 PHAC criteria, and 81 CDC and ARC criteria; none met SJCRH criteria. Of 46 other transfusion reactions, 6 met AABB STR criteria, 4 PHAC criteria, 12 CDC and ARC criteria, and 6 SJCRH criteria. None of 111 allergic TR met any of the STR criteria.

Sensitivity, specificity, and positive and negative predictive values of the 5 STR sets of criteria are shown in Table 7. Specificity and negative predictive values were high (>99%) for all STR criteria sets, but sensitivity varied from 0% to 100% and positive predictive value from 0% to 33.3%. Overall, AABB criteria provided the best predictive values. As none of the 5 STRs had been reported to the transfusion service and were only detected by active surveillance, sensitivity and positive predictive value of STRs reported by passive surveillance was 0%. Overall sensitivity of STR detection by combined active and passive surveillance varied from 5 of 98 (5.1%) to 5 of 11 (45.5%); sensitivity of AABB criteria was 5 of 41 (35.7%).

Discussion

STR is one of the most commonly recognized causes of transfusion-related fatalities following transfusion-associated acute lung injury, hemolytic TR, and transfusion-associated circulatory overload.[4,5,17-20] Despite the recognition of these complications of platelet transfusion, their true incidence is unknown as a result of underdetection and underreporting, notwithstanding improved hemovigilance programs. A recent report on transfusion-associated circulatory overload showed considerable underreporting, with a 36-fold difference in detection between active and passive surveillance: 1:5997 cases reported by passive surveillance vs 1:167 by active surveillance.[21] Furthermore, there is considerable variation in the reported incidence of transfusion reactions to platelets, with recent reports ranging from 0.01% to 10.0%, with considerable variation in reaction severity definitions and surveillance methods likely accounting for this wide range.[22-24]

Platelet components are particularly vulnerable to bacterial contamination, mainly due to their storage at room temperature, which allows growth of small inocula to very high titers and limits the shelf-life of these products.[12,25] For several decades, efforts have been made at multiple levels to reduce STR and fatalities from bacterially contaminated platelet transfusions, including prevention of contamination during collection and processing,[8,26] culture of platelet products 24 hours after collection,[3,27-29] point-of-issue testing,[27,30,31] as well as pathogen-reduction technologies.[32,33] Introduction of culture of platelet products in 2004 led to a decrease in gram-negative contaminants, which had accounted for one-third of contaminants and two-thirds of fatalities.[34] However, although considerable progress has been made, pathogen transmission through transfusion has continued and STRs continue to occur.[6] In the United States, 13 fatalities were reported to the FDA associated with platelet transfusion-transmitted bacterial sepsis during the previous 5 fiscal years (2009-2013), with 4 associated with pooled platelets and 9 with apheresis units.[4] The fatality rate associated with gram-positive sepsis in our study is similar to that reported in the literature.[34]

Our study highlights these issues and extends previous findings at our institution that bacterial contamination of platelets is an ongoing problem and that detection by passive surveillance continues to be poor.[7,10] Specifically, none of the 5 STRs that occurred during the 7-year study period had been reported to the transfusion service, and cases were only documented as a result of our active surveillance program. This finding is similar to our experience in 1991 to 2006, where active surveillance detected 32-fold more bacterially contaminated platelet units and 10.6-fold more septic reactions than did passive surveillance.[7] During this previous period, 54 bacterial contaminants included 4 gram-negative bacilli, 3 of which were associated with fatal reactions, 44 staphylococci, 4 streptococci, and 2 *Bacillus cereus*. This contrasts with the current study (2007-2013), in which staphylococci and streptococci accounted for 19 of 20 contaminants; the decrease in gram-negative bacilli was likely the result of introduction of early culture in 2004, with improved detection of these rapidly growing species. This decrease in virulent gram-negative contaminants is also reflected in reported national fatality data, where fatalities from gram-negative species decreased from 3.7 per year prior to introduction of early culture to 1.2 per year following use of early culture vs 2.3 and 2.0 per year, respectively, for gram-positive species.[4] These findings and trends emphasize the importance of monitoring patients receiving platelet transfusions closely for at least 24 hours, investigating all reactions for STR by culture of platelet products regardless of severity of reactions, and reporting all reactions to the transfusion service. Although the yield from culture of products administered to patients who develop any reaction is low, culture or other microbiologic testing is the only practical method of differentiating STRs from other reaction types due to overlap of signs and symptoms. Use of active surveillance of patients by dedicated hemovigilance personnel has been shown to improve recognition of STRs.[19] We also noted that STRs occurred with high bacterial loads only when patients were neutropenic (white blood cell [WBC] count $<2 \times 10^9$/L), likely related to the relatively low virulence of the gram-positive contaminants transfused. However, we did not find any evidence of delayed sequela resulting from transfusion of contaminated platelets. In particular, no cases of central line infections caused by bacterial isolates present in contaminated platelets were found. Although central line infections did occur, causative pathogens were very different from those associated with platelet contaminants. Specifically, strains of *Staphylococcus epidermidis* and other coagulase-negative staphylococci associated with contaminated platelets were very susceptible to antimicrobial agents, consistent with their origin from the skin of healthy donors. In contrast, coagulase-negative staphylococci associated with central line infections were methicillin and multidrug resistant, consistent with

Table 6. Numbers of patients with transfusion reactions meeting various STR criteria who received culture-negative platelets (n = 51 420) according to reaction type

Reaction type	Cases meeting STR criteria				
	CDC[11]	SJCRH[4]	ARC[5,12]	PHAC[13]	AABB[6]
Allergic TR (n = 111)	0	0	0	0	0
FNHTR/Febrile-like TR (n = 127)	81	0	81	48	30
Other TR (n = 46)	12	6	12	4	6
Total (n = 284)	93	6	93	52	36

Table 7. Evaluation of sensitivity, specificity, and positive and negative predictive values of 5 sets of STR criteria in identifying patients with true septic reactions

	Results of analysis using specified STR criteria (% values with 95% CI in parentheses)				
	CDC	SJCRH	ARC	PHAC	AABB
Sensitivity (n = 5)	0 (0-52.2)	60 (14.7-94.7)	0 (0-52.2)	40 (5.3-85.3)	100 (47.8-100)
Specificity (excluding allergic TR) (n = 173)	99.82 (97.78-99.85)	99.99 (99.97-100)	99.82 (97.78-99.85)	99.90 (99.87-99.92)	99.93 (99.90-99.95)
Positive predictive value	0 (0-3.89)	33.33 (7.5-70.1)	0 (0-3.89)	3.7 (0.45-12.75)	12.20 (4.1-26.2)
Negative predictive value	99.99 (99.98-100)	100 (99.99-100)	99.99 (99.98-100)	99.99 (99.98-100)	100 (99.99-100)

CI, confidence interval.

their origin from the skin of patients exposed to antimicrobial agents and the hospital environment.

The need to take additional measures to address the problem of bacterial contamination of platelets has recently been acknowledged by the AABB and the FDA.[5,6] An AABB Association Bulletin in 2012 recommended that blood establishments develop a policy or policies to further reduce the residual risk of bacterial contamination of apheresis platelets, improve the recognition of and monitoring for STRs to all platelet components, and optimize appropriate transfusion practice for all platelet components.[35] The AABB subsequently issued a bulletin in 2014 emphasizing the need to recognize and provide a timely response to suspected STRs and to protect other patients from receiving contaminated co-components.[5] The FDA has also recently issued a draft guidance document (*Bacterial Detection Testing by Blood Collection Establishments and Transfusion Services to Enhance the Safety and Availability of Platelets for Transfusion*) recommending additional testing by culture or rapid test during the storage period, or use of pathogen reduction technologies at time of production to further reduce the risk of bacterial contamination.[6]

As documented in this Discussion, the problems of bacterial contamination of platelets and resultant STRs are well recognized and the means to address them are available, including delayed primary testing, use of secondary testing by culture or rapid device during storage, and use of pathogen reduction systems; regulatory agencies strongly recommend use of such systems.[5,6] Another issue that needs to be addressed is improving both the sensitivity and specificity of diagnosing STRs. The recent AABB criteria[5] provided the best combination of sensitivity and specificity, but were compromised in our series by the fact that none of these reactions were reported to the transfusion service. Lack of reporting was, in part, associated with delayed reactions to transfusions administered in satellite outpatient clinics, with different clinical teams seeing patients in acute care settings to those administering the transfusions. Additionally, overlap in signs and symptoms of other more common TR further limits clinical diagnosis, and no specific temperature threshold or clinical feature was shown to be highly predictive of STRs.

In conclusion, our findings document the continued occurrence of bacterially contaminated platelets leading to STRs and highlight the need for implementation of additional, available measures such as secondary testing or pathogen reduction to further reduce these reactions and fatalities.

Acknowledgments

The contribution of the late Roslyn A. Yomtovian, MD, Medical Director, Transfusion Service, University Hospitals Case Medical Center and Case Western Reserve University School of Medicine, Cleveland, OH, 1988-2007, to the field of bacterial contamination of platelets is recognized with appreciation for her early recognition of this problem and her contributions to its study and prevention.

Authorship

Contribution: H.H., W.X., C.E.G., R.W.M., and M.R.J. extracted data from patient and laboratory records and analyzed results; W.X. made the figures; R.W.M. and M.R.J. designed the research; and H.H., W.X., H.M.L., C.E.G., R.W.M., and M.R.J. wrote the paper.

Conflict-of-interest disclosure: M.R.J. has received research support and/or honoraria from Verax, Pall, Gambro, Hemosystem, Immunetics, Genprime, Fenwal, and Charles River Labs, has been a consultant for BioSense Technologies and Lynntech, Inc, and is a member of the Bacterial Contamination Task Forces of the AABB and the International Society of Blood Transfusion (ISBT). The remaining authors declare no competing financial interests.

Correspondence: Michael R. Jacobs, Department of Pathology, University Hospitals Case Medical Center, 11100 Euclid Ave, Cleveland, OH 44106; e-mail: michael.jacobs@case.edu; and Robert W. Maitta, Department of Pathology, University Hospitals Case Medical Center, 11100 Euclid Ave, Cleveland, OH 44106; e-mail: robert.maitta@case.edu.

References

1. Kaufman RM, Djulbegovic B, Gernsheimer T, et al; AABB. Platelet transfusion: a clinical practice guideline from the AABB. *Ann Intern Med*. 2015;162(3):205-213.
2. US Department of Health and Human Services. The 2011 National Blood Collection and Utilization Survey Report. Available at: http://www.hhs.gov/ash/bloodsafety/2011-nbcus.pdf. Accessed November 20, 2015.
3. Brecher ME, Blajchman MA, Yomtovian R, Ness P, AuBuchon JP. Addressing the risk of bacterial contamination of platelets within the United States: a history to help illuminate the future. *Transfusion*. 2013;53(1):221-231.
4. US Food and Drug Administration. Fatalities Reported to FDA Following Blood Collection and Transfusion: Annual Summary for Fiscal Year 2013. http://www.fda.gov/BiologicsBloodVaccines/SafetyAvailability/ReportaProblem/TransfusionDonationFatalities/ucm391574.htm. Accessed November 20, 2015.
5. AABB. Clinical Recognition and Investigation of Suspected Bacterial Contamination of Platelets. AABB Association Bulletin #14-04. http://www.aabb.org/programs/publications/bulletins/Documents/ab14-04.pdf. Accessed November 20, 2015.
6. US Food and Drug Administration. Bacterial Detection Testing by Blood Collection Establishments and Transfusion Services to Enhance the Safety and Availability of Platelets for Transfusion. http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/UCM425952.pdf. Accessed November 20, 2015.
7. Jacobs MR, Good CE, Lazarus HM, Yomtovian RA. Relationship between bacterial load,

species virulence, and transfusion reaction with transfusion of bacterially contaminated platelets. *Clin Infect Dis.* 2008;46(8):1214-1220.

8. Benjamin RJ, Kline L, Dy BA, et al. Bacterial contamination of whole-blood-derived platelets: the introduction of sample diversion and prestorage pooling with culture testing in the American Red Cross. *Transfusion.* 2008;48(11):2348-2355.
9. Hamburg M. Bacterial Contamination of Platelets: Report to Congress in response to House of Representatives Report 112-542. Washington, DC. June 19, 2012.
10. Yomtovian RA, Palavecino EL, Dyskstra AH, et al. Evolution of surveillance methods for detection of bacterial contamination of platelets in a university hospital, 1991 through 2004. *Transfusion.* 2006;46(5):719-730.
11. Xiao W, Tormey CA, Capetillo A, Maitta RW. Allergic transfusion reactions to platelets are more commonly associated with prepooled than apheresis components. *Vox Sang.* 2013;105(4):334-340.
12. Kuehnert MJ, Roth VR, Haley NR, et al. Transfusion-transmitted bacterial infection in the United States, 1998 through 2000. *Transfusion.* 2001;41(12):1493-1499.
13. Sanders RP, Geiger TL, Heddle N, Pui CH, Howard SC. A revised classification scheme for acute transfusion reactions. *Transfusion.* 2007;47(4):621-628.
14. Eder AF, Goldman M. How do I investigate septic transfusion reactions and blood donors with culture-positive platelet donations? *Transfusion.* 2011;51(8):1662-1668.
15. Public Health Agency of Canada. Guideline for Investigation of Suspected Transfusion Transmitted Bacterial Contamination. *CCDR.* 2008;34S1:1-8. http://www.phac-aspc.gc.ca/publicat/ccdr-rmtc/08vol34/34s1/34s1-eng.php. Accessed November 20, 2015.
16. Rhame FS, Root RK, MacLowry JD, Dadisman TA, Bennett JV. Salmonella septicemia from platelet transfusions. Study of an outbreak traced to a hematogenous carrier of Salmonella cholerae-suis. *Ann Intern Med.* 1973;78(5):633-641.
17. Bolton-Maggs PH, Cohen H. Serious Hazards of Transfusion (SHOT) haemovigilance and progress is improving transfusion safety. *Br J Haematol.* 2013;163(3):303-314.
18. Funk MB, Lohmann A, Guenay S, et al. Transfusion-transmitted bacterial infections - haemovigilance data of German blood establishments (1997-2010). *Transfus Med Hemother.* 2011;38(4):266-271.
19. Lafeuillade B, Eb F, Ounnoughene N, et al. Residual risk and retrospective analysis of transfusion-transmitted bacterial infection reported by the French National Hemovigilance Network from 2000 to 2008. *Transfusion.* 2015;55(3):636-646.
20. Robillard P, Delage G, Itaj NK, Goldman M. Use of hemovigilance data to evaluate the effectiveness of diversion and bacterial detection. *Transfusion.* 2011;51(7):1405-1411.
21. Raval JS, Mazepa MA, Russell SL, Immel CC, Whinna HC, Park YA. Passive reporting greatly underestimates the rate of transfusion-associated circulatory overload after platelet transfusion. *Vox Sang.* 2015;108(4):387-392.
22. Kaufman RM, Assmann SF, Triulzi DJ, et al. Transfusion-related adverse events in the Platelet Dose study. *Transfusion.* 2015;55(1):144-153.
23. Tormey CA, Sweeney JD, Champion MH, Pisciotto PT, Snyder EL, Wu Y. Analysis of transfusion reactions associated with prestorage-pooled platelet components. *Transfusion.* 2009;49(6):1242-1247.
24. Poles D, Watt A, Thomas D, Cohen H; on behalf of the Serious Hazards of Transfusion (SHOT) Steering Group. The 2012 Annual SHOT Report (2013). In: Bolton-Maggs PBH, ed. http://www.shotuk.org/wp-content/uploads/2013/08/SHOT-Annual-Report-2012.pdf. Accessed November 20, 2015.
25. Yomtovian R, Tomasulo P, Jacobs MR. Platelet bacterial contamination: assessing progress and identifying quandaries in a rapidly evolving field. *Transfusion.* 2007;47(8):1340-1346.
26. Benjamin RJ, Dy B, Warren R, Lischka M, Eder AF. Skin disinfection with a single-step 2% chlorhexidine swab is more effective than a two-step povidone-iodine method in preventing bacterial contamination of apheresis platelets. *Transfusion.* 2011;51(3):531-538.
27. Brecher ME, Jacobs MR, Katz LM, et al; AABB Bacterial Contamination Task Force. Survey of methods used to detect bacterial contamination of platelet products in the United States in 2011. *Transfusion.* 2013;53(4):911-918.
28. Eder AF, Kennedy JM, Dy BA, et al; American Red Cross Regional Blood Centers. Bacterial screening of apheresis platelets and the residual risk of septic transfusion reactions: the American Red Cross experience (2004-2006). *Transfusion.* 2007;47(7):1134-1142.
29. Ezuki S, Kawabata K, Kanno T, Ohto H. Culture-based bacterial detection systems for platelets: the effect of time prior to sampling and duration of incubation required for detection with aerobic culture. *Transfusion.* 2007;47(11):2044-2049.
30. Heaton WA, Good CE, Galloway-Haskins R, Yomtovian RA, Jacobs MR. Evaluation of a rapid colorimetric assay for detection of bacterial contamination in apheresis and pooled random-donor platelet units. *Transfusion.* 2014;54(6):1634-1641.
31. Jacobs MR, Smith D, Heaton WA, Zantek ND, Good CE; PGD Study Group. Detection of bacterial contamination in prestorage culture-negative apheresis platelets on day of issue with the Pan Genera Detection test. *Transfusion.* 2011;51(12):2573-2582.
32. Salunkhe V, van der Meer PF, de Korte D, Seghatchian J, Gutiérrez L. Development of blood transfusion product pathogen reduction treatments: a review of methods, current applications and demands. *Transfus Apheresis Sci.* 2015;52(1):19-34.
33. Snyder EL, Stramer SL, Benjamin RJ. The safety of the blood supply–time to raise the bar. *N Engl J Med.* 2015;372(20):1882-1885.
34. Brecher ME, Hay SN. Bacterial contamination of blood components. *Clin Microbiol Rev.* 2005;18(1):195-204.
35. AABB. Recommendations to Address Residual Risk of Bacterial Contamination of Platelets. AABB Association Bulletin #12-04.

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04月04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 ポリエチレングリコール処理抗破傷風人免疫グロブリン ③ 乾燥抗破傷風人免疫グロブリン	研究報告の公表状況	http://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=19974/2016/03/31	公表国 フランス	
販売名 (企業名)	① テタノブリンIH静注250単位　(日本血液製剤機構) ② テタノブリンIH静注1500単位　(日本血液製剤機構) ③ テタノブリン筋注用250単位　(日本血液製剤機構)				

研究報告の概要

アウトブレイクの発生日-2016年3月1日
アウトブレイクの確定日-2016年3月23日
報告日-2016年3月25日
前回の発生日-2014年
病原体-病原性プリオンタンパク質
新たなアウトブレイク-Ardennes, Givron, Givronの農場における1件（アウトブレイクの発生日2016年3月1日）：ウシにおいて疑い例394例，症例1例，処分1例
感染源-不明もしくは結論に到達していない

古典的BSEの最後の症例は2011年に確認された。2014年に検出されたケースは、非定型BSEであった。法的な感染宣言が発行され、消毒が終わるまでの間、あらゆる行動が禁止された。屠畜された動物の中でリスクの可能性がある動物を特定するめの調査が進められている。疫学的調査は、感染が起こった潜在的な要因を特定することを目的としている。フランスは3月25日より、「管理されたBSEリスク」の国のカテゴリーが適用された。

使用上の注意記載状況・その他参考事項等

代表としてテタノブリンIH静注250単位の記載を示す。
2. 重要な基本的注意
(1)略
1)略
2)現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

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

Bovine spongiform encephalopathy, France

Information received on 25/03/2016 from Dr Loic Evain, Directeur G鈥攔al adjoint, CVO, Direction g鈥攔ale de l'alimentation, Minist鈥攔e de l'Agriculture, de l'Agroalimentaire et de la For锛, Paris, France

Summary

Report type	Immediate notification
Date of start of the event	01/03/2016
Date of confirmation of the event	23/03/2016
Report date	25/03/2016
Date submitted to OIE	25/03/2016
Reason for notification	Reoccurrence of a listed disease
Date of previous occurrence	2014
Manifestation of disease	Clinical disease
Causal agent	Pathological prion protein
Nature of diagnosis	Laboratory (advanced)
This event pertains to	a defined zone within the country

New outbreaks (1)

Outbreak 1 (1)	**GIVRON, GIVRON, ARDENNES**
Date of start of the outbreak	01/03/2016
Outbreak status	Continuing (or date resolved not provided)
Epidemiological unit	Farm

Affected animals

Species	Susceptible	Cases	Deaths	Destroyed	Slaughtered
Cattle	394	1	0	1	0

Affected population

The affected bovine, a Salers female born on April, 8th 2011, showed paresis and was euthanized on March, 1st 2016. Samples made on March, 4th 2016 during rendering were analyzed at the Department Laboratory of La Somme. The rapid test proved positive on March, 8th 2016 and the samples were then sent for further analysis to the National Reference Laboratory, ANSES, which confirmed a case of classical BSE on March, 21st 2016. The European Union Reference Laboratory confirmed those results on the basis of documentation on March, 23rd 2016.

Summary of outbreaks

Total outbreaks: 1

Total animals affected

Species	Susceptible	Cases	Deaths	Destroyed	Slaughtered
Cattle	394	1	0	1	0

Outbreak statistics

Species	Apparent morbidity rate	Apparent mortality rate	Apparent case fatality rate	Proportion susceptible animals lost*
Cattle	0.25%	0.00%	0.00%	0.25%

*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 last case of classical BSE was detected in 2011. The case detected in 2014 was atypical BSE. A by-law declaring infection was issued and prohibited any movements until sanitation. A traceability investigation is on-going to identify animals potentially at risk (cohort and offspring) that will be destroyed. An epidemiological investigation aimed at identifying the potential causes of infection was launched. The requirements applicable to the category of countries with a controlled BSE risk (SRM list) enter into force as of the date of March, 25th.

Control measures

Measures applied	Screening Traceability No vaccination

	No treatment of affected animals
Measures to be applied	Stamping out Official disposal of carcasses, by-products and waste

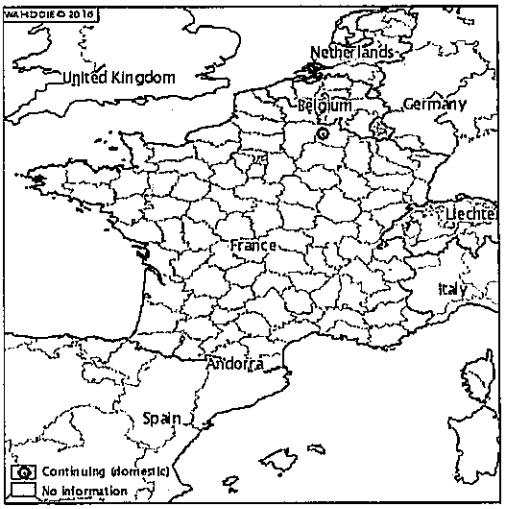
Diagnostic test results

Laboratory name and type	Species	Test	Test date	Result
ANSES TSE National Reference Laboratory, Lyon (National laboratory)	Cattle	western blot	21/03/2016	Positive
TSE Molecular Team, Animal and Plant Health Agency, United Kingdom (OIE Reference Laboratory)	Cattle	western blot	23/03/2016	Positive

Future Reporting

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

Map of outbreak locations



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 05 月 30 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	人ハプトグロビン		研究報告の公表状況	http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/UCM273685.pdf	公表国 アメリカ	
販売名 （企業名）	ハプトグロビン静注 2000 単位「JB」（日本血液製剤機構）					

研究報告の概要

2016 年 5 月付けで「業界向けガイダンス：血液および血液成分のドナースクリーニングに使用する容認可能な完全版および簡易版ドナー履歴問診と附属資料の導入」最新版が公表された。
本ガイダンスは、FDA の要件と勧告を満たした血液および血液成分のドナー履歴情報の収集のための有効な方法として、AABB ドナー病歴特別専門委員会により作成された標準的全文ドナー履歴問診票、簡略版ドナー履歴問診票（それぞれ FL-DHQ と aDHQ と略す）および付随文書、2016 年 2 月付 Version 2.0、が承認されたことを示している。
本ガイダンスは、2006 年 10 月及び 2013 年 5 月に発行された同じ名称のガイダンスの更新版で、これらは「血液及び血液成分」を対象としたガイダンスである。同様のドナー履歴問診票についてのガイダンスの原料血漿（Source Plasma）は 2013 年 2 月に発行されているが、こちらについては更新されていない。

報告企業の意見

血液媒介ウイルス（blood mediated virus）は、B 型肝炎ウイルス（HBV）、C 型肝炎ウイルス（HCV）、ヒト免疫不全ウイルス（HIV）、サイトメガロウイルス（CMV）、成人 T 細胞白血病ウイルス（HTLV-1）、パルボウイルス B19（B19）などの血液を媒介して感染を起こし得る病原体ウイルスである。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、HBV、HCV 及び HIV 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。万一、原料血漿に血液媒介ウイルスが混入したとしても、各種モデルウイルスを用いたウイルスクリアランス試験成績から、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

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、HBV、HCV 及び HIV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は,以上の検査に適合した血漿を原料として,Cohn の低温エタノール分画で得た画分から人ハプトグロビンを濃縮・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。

Implementation of Acceptable Full-Length and Abbreviated Donor History Questionnaires and Accompanying Materials for Use in Screening Donors of Blood and Blood Components

---

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)(4)(i). Submit one set of either electronic or written comments on this guidance at anytime. Submit electronic comments to http://www.regulations.gov. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. You should identify all comments with docket number FDA-2016-D-1342.

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 e-mail address listed above.

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

Contains Nonbinding Recommendations

Table of Contents

Contains Nonbinding Recommendations

Implementation of Acceptable Full-Length and Abbreviated Donor History Questionnaires and Accompanying Materials for Use in Screening Donors of Blood and Blood Components

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 recognizes the standardized full-length and abbreviated donor history questionnaires (FL-DHQ and aDHQ, respectively) and accompanying materials, version 2.0 dated February 2016, prepared by the AABB Donor History Task Force (referred to as "task force"), as an acceptable mechanism for collecting blood donor history information from donors of blood and blood components that is consistent with the FDA requirements and recommendations.[1] The FL-DHQ documents are being updated to align with the requirements promulgated in the final rule published in the *Federal Register* of May 22, 2015 entitled "Requirements for Blood and Blood Components Intended for Transfusion or for Further Manufacturing Use" (80 FR 29842), which became effective May 23, 2016, and incorporate the recommendations provided in the document entitled "Revised Recommendations for Reducing the Risk of Human Immunodeficiency Virus Transmission by Blood and Blood Products; Guidance for Industry" dated December 2015 (Ref. 1). In the future, we may recognize other AABB donor history questionnaires and accompanying materials (referred to as "DHQ documents") as acceptable.

This guidance supersedes the following documents entitled:

- "Guidance for Industry: Implementation of Acceptable Full-Length Donor History Questionnaire and Accompanying Materials for Use in Screening Donors of Blood and Blood Components" dated October 2006, which accepted version 1.3 of the AABB full-length DHQ (Ref. 2); and
- "Guidance for Industry: Implementation of an Acceptable Abbreviated Donor History Questionnaire and Accompanying Materials for Use in Screening Frequent Donors of Blood and Blood Components" dated May 2013 (Ref. 3), which accepted version 1.3 of the AABB abbreviated DHQ.

[1] See section III of this guidance for certain exceptions.

Additionally, this guidance supersedes FDA's acceptance of the referenced donor history questionnaires and accompanying materials in the documents 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 (Ref. 4), which accepted revisions to version 1.3 of the AABB full-length DHQ[2]; and
- "Guidance for Industry: Recommendations for Donor Questioning, Deferral, Reentry and Product Management to Reduce the Risk of Transfusion-Transmitted Malaria" dated August 2014 (Ref. 5), which accepted revisions to versions 1.3 of the AABB full-length and abbreviated DHQ's, respectively.

The DHQ documents provides blood establishments that collect blood and blood components (referred to as "manufacturers" or "you") with a specific process for administering questions to donors of blood and blood components (referred to as "blood donors") to determine their eligibility to donate. (In this guidance, the term "eligibility" refers to the donor eligibility requirements described in Title 21 of the Code of Federal Regulations 630.10 and 630.15 (21 CFR 630.10 and 630.15)). Acceptable DHQ documents are those documents that FDA has determined provide manufacturers with one means of obtaining donor history information from a blood donor to determine if the donor is eligible, consistent with the requirements in 21 CFR 630.10 and 630.15.

This guidance also advises licensed manufacturers who choose to implement the acceptable DHQ documents on how to report the manufacturing change consisting of the implementation of the DHQ documents under 21 CFR 601.12 (§ 601.12).

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, these 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

Section 630.10(c) requires the eligibility of all blood donors to be determined on the day of donation and before collection, with certain exceptions (21 CFR 630.10(c)(1)-(2)). Such determination is intended to ensure a donor's overall good health and that the donor is free from transfusion-transmitted infections (21 CFR 630.10(a)(1)-(2)). A donor's eligibility to donate blood and blood components is determined in part by a physical assessment and the donor's answers to questions concerning medical history and risk factors associated with exposure to, or clinical evidence of a relevant transfusion-transmitted infection and other conditions that may adversely affect the health of the donor or the safety, purity, or potency of the blood or blood components or any product manufactured from the blood or blood components

[2] This guidance was updated in January 2016.

(21 CFR 630.10(e)-(f)). The donor screening interview is especially important in identifying risks for diseases and conditions for which there are no adequate laboratory tests or for which tests are unable to identify early stage or window period infection.

The first formal uniform questionnaire developed for the purpose of blood donor screening was implemented nearly sixty years ago (Ref. 6). Though the donor interview process is helpful in excluding ineligible donors, errors in this process do occur because some information may not be understood or captured during the screening process (Ref. 7). As noted during public meetings sponsored by FDA to discuss this issue, the blood donor screening process should consider such factors as question complexity, donor recall ability, donor health and safety, donor satisfaction and willingness to return, any further processing which a product may undergo prior to use, and risk to the end user/recipient of blood and blood components (Refs. 8 through 10). Strategies such as using self-administered computer-assisted and abbreviated questionnaires have been implemented as approaches to improve donor understanding and satisfaction over what some view as a lengthy and time-consuming process, particularly for frequent donors (Refs. 2, 3, and 11).

A. Abbreviated Donor History Questionnaire

In the Abbreviated Donor History Questionnaire User Brochure (User Brochure), AABB defines a frequent donor as "[a] donor who has previously donated two times using the full-length Donor History Questionnaire, one donation of which occurred within the previous 6 months." The User Brochure contains additional instructions that delineate when the aDHQ documents and FL-DHQ should be administered.

During the Blood Products Advisory Committee meeting held on March 18, 2005, the task force presented a study design for evaluating the abbreviated questionnaire post-implementation (Ref. 12). The task force also proposed to assess inappropriate use of the aDHQ documents instead of the FL-DHQ for donor screening. This study will include a review of post-donation information and data about inappropriate use of the aDHQ documents. The task force has agreed to submit the summary data to FDA once the study has been completed. Based on the outcome of the post-implementation evaluation, FDA may revise its recommendations for the use of the aDHQ.

B. DHQ Documents

The DHQ documents include the following materials and are intended to be used in their entirety, with the exceptions noted in sections III and IV.A.2:

- Full-Length Donor History Questionnaire
- Full-Length Donor History Questionnaire User Brochure – includes glossary; describes how questions can be administered.
- Full-Length Donor History Questionnaire Flow Charts – contain follow-up questions as a method to obtain additional information to further evaluate a potential donor's response to capture questions. ("Capture" questions ask a

general question about a donor's history or behavior and are followed up by obtaining additional information about the donor if needed.)

- Abbreviated Donor History Questionnaire
- Abbreviated Donor History Questionnaire User Brochure – includes glossary and references; describes which donors may complete the aDHQ questionnaire and how questions can be administered.
- Abbreviated Donor History Questionnaire Flow Charts – contain follow-up questions as a method to obtain additional information to further evaluate a potential donor's response to capture questions.
- Medication Deferral List – contains a list of medications that may serve as a basis for donor deferral.
- Blood Donor Educational Material – educates the donor about certain risks and conditions that are a basis for deferral.
- Variant Creutzfeldt-Jakob Disease (vCJD) Countries of Risk – contains a list of countries identified as being at risk for vCJD.
- References

The FL-DHQ and aDHQ questionnaires are designed to be implemented together. For example, if you choose to implement the AABB Abbreviated Donor History Questionnaire, you should also implement the AABB Full-Length Donor History Questionnaire as described in the User Brochure. Both the full-length and abbreviated questionnaires are designed to be administered either by blood establishment personnel or self-administered with follow-up by establishment personnel.

III. RECOGNITION OF DHQ DOCUMENTS

We find the DHQ documents version 2.0 dated February 2016 to be acceptable for use in screening donors of blood and blood components. These documents are consistent with FDA requirements and recommendations related to donor eligibility interviews, subject to the following exception: the acceptable DHQ documents do not contain the donor questions and donor educational material related to Zika virus recommended by FDA in the document titled, "Recommendations for Donor Screening, Deferral, and Product Management to Reduce the Risk of Transfusion-Transmission of Zika Virus: Guidance for Industry," dated February 2016. Blood establishments should revise the acceptable DHQ documents to address the recommendations for Zika virus, as appropriate.

In addition, the DHQ documents contain questions related to the following donor medical history issues for which we currently do not have requirements or recommendations: cancer; certain organ, tissue, or bone marrow transplant; and bone or skin graft. By recognizing the acceptable DHQ documents as one way to satisfy FDA's regulatory requirements, we are not requiring or recommending that donors be screened or deferred for these issues. If you choose to implement the acceptable DHQ documents and omit these questions, you would still be in compliance with FDA requirements.

While we recognize that the acceptable DHQ documents provide an effective tool for screening blood donors, we do not require that you implement the acceptable DHQ documents. You may continue to use any FL-DHQ and aDHQ and accompanying materials developed by your establishment and for licensed establishments, approved by FDA. These materials may include procedures and wording that are different from those in the DHQ documents. In the future, you may implement, consistent with § 601.12, new procedures and materials that differ from those in DHQ documents (Ref. 13).

IV. REPORTING TO FDA THE IMPLEMENTATION OF ACCEPTABLE FULL-LENGTH AND ABBREVIATED DONOR HISTORY QUESTIONNAIRE AND ACCOMPANYING MATERIALS

As discussed in section II of this guidance, we recommend that the FL-DHQ and aDHQ be used together. For example, if you choose to implement the FL-DHQ, we recommend that you also implement the aDHQ.

A. Implementation of Acceptable DHQ Documents

Licensed manufacturers must report the implementation of the acceptable DHQ documents to FDA under § 601.12 as follows:

1. If the acceptable DHQ documents are implemented without modifications and in their entirety, except as described below, as a complete process for administering questions to blood donors, the change is considered to be minor, with a minimal potential to have an adverse effect on the identity, strength, quality, purity, or potency of the product as they may relate to the safety or effectiveness of the product. You must report such changes to FDA in your annual report under § 601.12(d), noting the date the process was implemented. If donors will be allowed to self-administer acceptable DHQ documents, see section IV.B of this guidance.

2. If the acceptable DHQ documents are implemented in their entirety, but modified by: (a) revising the DHQ documents to include FDA recommendations related to Zika virus; (b) adding additional, more restrictive selection criteria that are specific to your establishment; or (c) omitting questions related to cancer; organ, tissue, or bone marrow transplant, except for xenotransplantation; bone or skin graft, which FDA has not required or recommended for determining donor eligibility, the changes are considered to be minor. You must report such changes to FDA in your annual report under § 601.12(d), noting the date the process was implemented and describing the additional criteria or questions that were omitted from your questionnaire.

3. If the acceptable DHQ documents are implemented in their entirety but modified by displaying the flow charts in another format that is compatible with your current process, the changes are considered minor, provided there is no change to

the content in the flow charts, other than changes incorporating donor deferral criteria that are stricter than the FDA required/recommended donor deferral criteria. You must report such changes to FDA in your annual report under § 601.12(d), noting the date the process was implemented and describing how you modified the acceptable DHQ documents.

4. If the acceptable DHQ documents are implemented in their entirety, but modified by reformatting any of the acceptable DHQ documents (other than the flow charts) to be consistent with your current process, the changes are considered to be minor provided you do not change the wording and the order of content in the acceptable DHQ documents. You must report such changes to FDA in your annual report under § 601.12(d), noting the date the process was implemented and describing how you modified the acceptable DHQ documents.

5. Donor screening procedures have a substantial potential to have an adverse effect on the identity, strength, quality, purity, or potency of blood and blood components, as they may relate to the safety or effectiveness of the product. Therefore, the implementation of the acceptable DHQ documents that have been modified other than as specifically described in sections IV.A.2-4 of this guidance is considered a major change. If you wish to implement the acceptable DHQ documents modified in a manner other than as described in sections IV.A.2-4 of this guidance, you must report such changes as a Prior Approval Supplement (PAS) under § 601.12(b). We recommend that you include the following in the submission:

    a. FDA Form 356h "Application to Market a New Drug, Biologic or an Antibiotic Drug for Human Use" which may be obtained at http://www.fda.gov/AboutFDA/ReportsManualsForms/Forms/default.htm.
    b. A cover letter describing the request and the contents of the submission.
    c. A written standard operating procedure (SOP) describing the donor questions and questionnaire process.
    d. The donor history questionnaires and accompanying document(s). Please highlight the modifications.

For assistance in preparing the supplement, please refer to the document entitled "Guidance for Industry: For the Submission of Chemistry, Manufacturing and Controls and Establishment Description Information for Human Blood and Blood Components Intended for Transfusion or for Further Manufacture and for the Completion of the Form FDA 356h 'Application to Market a New Drug, Biologic or an Antibiotic Drug for Human Use'" dated May 1999 (Ref. 14).

B. Implementation of Self-Administered Acceptable DHQ Documents

In July 2003, we issued a document entitled "Guidance for Industry: Streamlining the Donor Interview Process: Recommendations for Self-Administered Questionnaires" (Streamlining Donor Interview guidance) (Ref. 11) advising licensed blood

establishments to submit procedures for self-administering the donor history questionnaire to FDA as a Changes Being Effected in 30 days supplement (CBE30) under § 601.12(c). We determined in the Streamlining Donor Interview guidance that a CBE30 was an appropriate supplement to ensure that controls were in place to manage this process. However, we have since determined that when acceptable DHQ documents include instructions for controlling the self-administration process, such as in the User Brochure, this change may be reported in an annual report or in some situations as a CBE30, as described in sections IV.B.1 and IV.B.2 of this guidance. These recommendations modify those in the Streamlining Donor Interview guidance. Licensed manufacturers planning to implement self-administration of a questionnaire other than the acceptable DHQ documents should continue to consult the Streamlining Donor Interview guidance (Ref. 11).

Licensed manufacturers must report implementation of self-administered acceptable DHQ documents under § 601.12 as follows:

1. If you choose to implement self-administration of the acceptable DHQ documents using the written form or audio/visual presentation methods described in the acceptable DHQ documents, this is considered a minor change. You must report such a change to FDA in your annual report under § 601.12(d), noting the date the process was implemented.

2. If you choose to implement the acceptable DHQ documents using a computer-assisted interactive interview procedure, you must report this change to FDA as a CBE30 under § 601.12(c). This change presents a moderate potential to adversely affect the identity, strength, quality, purity, or potency of blood and blood components, as they may relate to the safety or effectiveness of the product, because of concerns that the presentation of the questions and information may not be easily readable in all conditions and by all potential users. Additionally, implementation for the first time of a computer-assisted interactive interview procedure may raise new issues that should be evaluated, such as the management of electronic records. Therefore, we cannot conclude at this time that the implementation of a computer-assisted interactive interview procedure will be a minor change.

For recommendations on the implementation and reporting of the use of self-administered questionnaires other than as described above, and for preparing the CBE30 for the computer-assisted interactive interview procedure, see the Streamlining Donor Interview guidance (Ref. 11).

Unlicensed blood establishments do not need to report implementation of the DHQ (as described in sections IV.A and IV.B of this guidance) to FDA.

V. RECOGNITION AND IMPLEMENTATION OF FUTURE ACCEPTABLE DHQ DOCUMENTS

In the future, we may issue regulations or guidance documents concerning donor eligibility. For example, we may recommend revised eligibility criteria with respect to transfusion-transmitted infections, medical conditions, behaviors, geographic exposures or medications. Implementation of new eligibility criteria would change your donor interview SOPs, and involve amending the accepted DHQ documents (typically by adding a question at the end of the questionnaire in the area designated for additional questions or by implementing new or revised DHQ documents)[3]. We note that the User Brochure describes how to add and administer revised DHQ documents.

We anticipate that in the event we recommend a new donor eligibility deferral criterion, we will, in the same guidance, provide recommendations concerning implementing and reporting to FDA the manufacturing changes associated with this change in procedure. If the revised DHQ documents are available and found acceptable, we also intend to recognize those DHQ documents as acceptable in the guidance document addressing the new criterion.

We recommend that you have a procedure in place for implementing updated acceptable DHQ documents in all of your facilities.

VI. FOR MORE INFORMATION

If you have questions regarding this guidance and FDA policies for implementing acceptable DHQ documents, contact OCOD at the phone numbers or email address provided in this guidance.

If you have questions regarding the DHQ documents, contact AABB by phone at 301-907-6977, by fax at 301-907-6895 or by email at aabb@aabb.org to the attention of the AABB Donor History Task Force.

The acceptable DHQ documents can be accessed on the AABB website at http://www.aabb.org/tm/questionnaires/Pages/dhqaabb.aspx.

[3] If you do not use the acceptable DHQ documents, this would involve amending your own donor history questionnaire.

VII. REFERENCES

1. Revised Recommendations for Reducing the Risk of Human Immunodeficiency Virus Transmission by Blood and Blood Products; Guidance for Industry, December 2015. Available at http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/UCM446580.pdf.
2. Guidance for Industry: Implementation of Acceptable Full-Length Donor History Questionnaire and Accompanying Materials for Use in Screening Donors of Blood and Blood Components, 71 FR 63327 (October 30, 2006).
3. Guidance for Industry: Implementation of an Acceptable Abbreviated Donor History Questionnaire and Accompanying Materials for Use in Screening Frequent Donors of Blood and Blood Components," 78 FR 26785 (May 8, 2013).
4. 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, 75 FR 29768 (May 27, 2010).
5. Guidance for Industry: Recommendations for Donor Questioning, Deferral, Reentry and Product Management to Reduce the Risk of Transfusion-Transmitted Malaria, August 2014. Available at http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/UCM080784.pdf.
6. American Association of Blood Banks, *Technical Methods and Procedures of the American Association of Blood Banks*, pp. 3-5, Minneapolis: Burgess Publishing Co., 1953.
7. Biological Product Deviation Reports Annual Summaries, CBER. Available at http://www.fda.gov/BiologicsBloodVaccines/SafetyAvailability/ReportaProblem/BiologicalProductDeviations/default.htm.
8. FDA/AABB Workshop on "Streamlining the Blood Donor History Questionnaire" Transcript, October 16, 2000.
9. Orton S, Virvos V, Williams A. Validation of selected donor-screening questions: structure, content and comprehension. Transfusion 2000; 40:1407-1413.
10. Blood Products Advisory Committee Meeting Transcript, December 11, 2003. Available at http://www.fda.gov/ohrms/dockets/ac/03/transcripts/4014t1.htm.
11. Guidance for Industry: Streamlining the Donor Interview Process: Recommendations for Self-Administered Questionnaires, July 2003. Available at http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/ucm075086.htm.
12. Blood Products Advisory Committee Meeting Transcript, March 18, 2005. Available at http://www.fda.gov/ohrms/dockets/ac/05/transcripts/2005-4096t2.htm.
13. Guidance for Industry: Changes to an Approved Application: Biological Products: Human Blood and Blood Components Intended for Transfusion or for Further Manufacture, November 2014. Available at http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/ucm354559.htm.
14. Guidance for Industry: For the Submission of Chemistry, Manufacturing and Controls and Establishment Description Information for Human Blood and Blood Components Intended for Transfusion or for Further Manufacture and For the Completion of the Form FDA 356h

"Application to Market a New Drug, Biologic or an Antibiotic Drug for Human Use," May 1999. Available at http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/ucm077087.htm.

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。

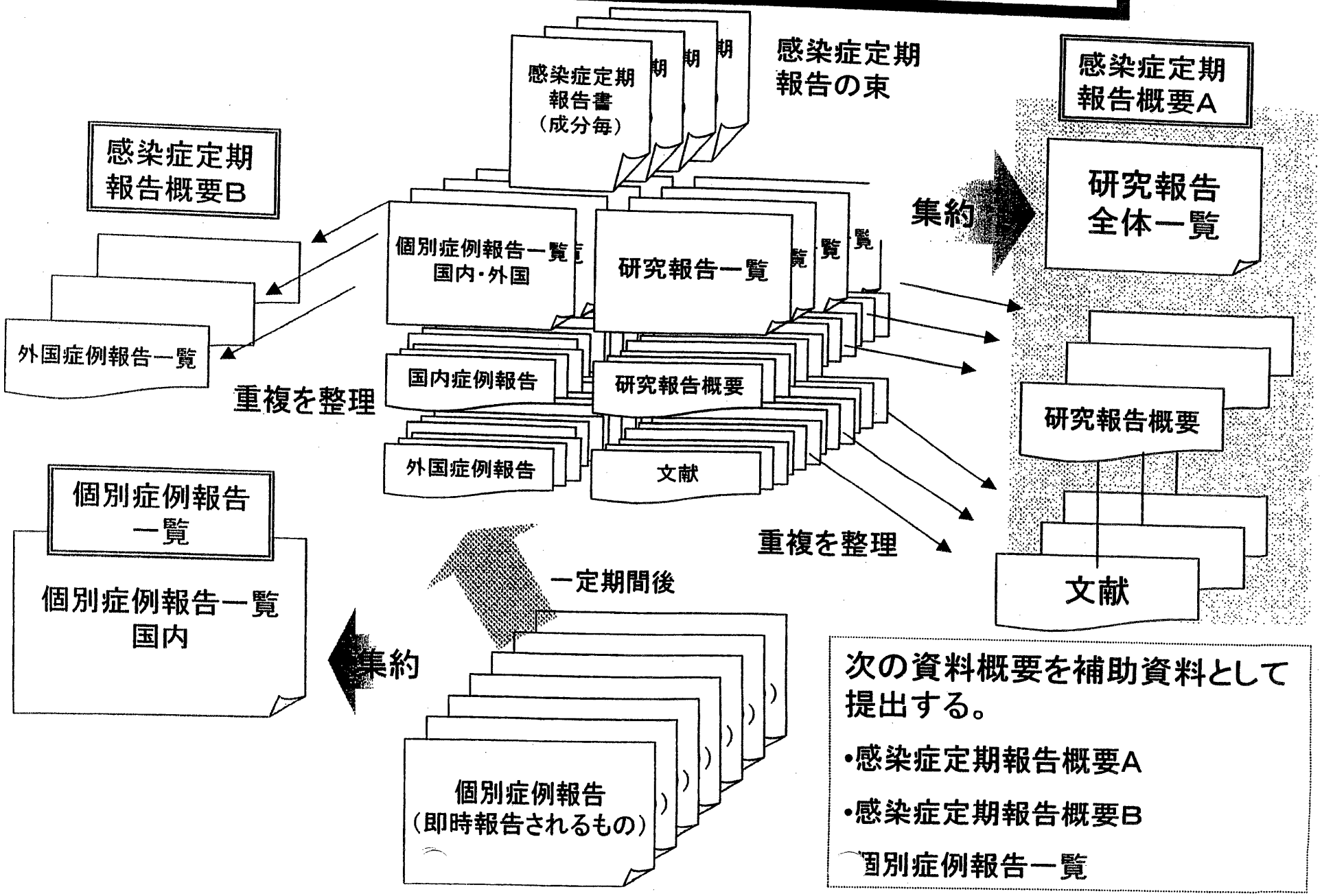
② 感染症発生症例報告のうち、発現国が「外国」の血漿分画製剤の使用による症例は、同一製品毎に報告期間を代表する感染症発生症例一覧（別紙様式第4）をまとめた**「資料概要B」**を事務局が作成し、送付する。

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