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研究報告の概要

○ C型肝炎ウイルス(HCV)コア特異的抗体の検出は供血者におけるオカルトHCV感染を示唆している。
研究デザイン及び方法:スクリーニング検査でHCV抗体及びHCV RNAが陰性で、無作為に抽出された初回供血者2,007名の血液を対象として、HCVコア特異的抗体検査という新たな検査を実施した。倫理的理由により、供血血液と供血者を結び付けるコードは、検体を回収する際に無効化された。
結果:新たな検査では、42名の供血血液(2.1%)が陽性となった。特異性は、ペプチド抑制試験並びに重複しないHCVコアペプチドのエピトープと他のHCV抗原による追加検査により評価した。上記の追加検査を実施した後、6名の供血血液(14.3%、全体の0.30%)にHCVコア抗体が含まれていると判断した。更に、超遠心分離法によってウイルスを濃縮した後、陽性供血者の末梢血単核細胞(PBMNC)及び血清(または血漿)を検体として、HCV RNAの検出を行ったところ、PBMNCを用いた検査の結果は全て陰性となった。3名の濃縮血清(または濃縮血漿)からは極少量のウイルスゲノムが検出されたが、無作為に抽出された100例の陰性供血者については、濃縮血清(または濃縮血漿)からの検出はなかった。これらのPCR産物の配列決定により、株間の差異が明らかになった。この結果は、共通の汚染源による検体汚染がなかったことを裏付ける事実の一つであった。
結論:これらの知見は、上記の供血者におけるオカルトHCV感染を支持するものであり、このような供血者の血液における、かなり低いとはいえ無視はできないと考えられる感染のリスクを説明するものである。追跡可能な供血者の血液を対象とした、より規模の大きい今後の研究によって、血液供給におけるウイルス関連の安全性という問題における上記知見の重要性が明らかになるであろう。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
スクリーニング検査でHCV抗体及びHCV RNAが陰性の2,007名に行ったHCVコア抗体検査により、42名が陽性反応を示し、この中から最終的に6名をHCVコア抗体陽性者と判定した。また、この42名の検体をウイルス濃縮して3名からHCV RNAを検出した。スクリーニング検査合格者からオカルトHCV感染者を検出したという報告である。	日本赤十字社では、HCV抗体検査を行い、更に全検体に対する個別検体によるNAT(個別NAT)スクリーニングを開始し、陽性血液を排除している。HCV感染に関する新たな知見等について、今後も情報の収集に努める。

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Detection of hepatitis C virus (HCV) core–specific antibody suggests occult HCV infection among blood donors

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BACKGROUND: Blood transfusion safety is based on reliable donor screening for transmissible infections such as the hepatitis C virus (HCV) infection.

STUDY DESIGN AND METHODS: A novel HCV core–specific antibody was assayed on random single donations from 2007 first-time blood donors who tested negative for anti-HCV and HCV RNA on routine screening. Sample collection broke the code between donations and donors for ethical reasons.

RESULTS: Forty-two donations (2.1%) displayed reactivity in the novel test. The specificity of the reactivity was evaluated by a peptide inhibition assay, and testing against additional nonoverlapping HCV core peptide epitopes and other HCV antigens was performed on these samples. Six donations (14.3%; 0.30% from the total) were considered to contain anti-HCV after such supplemental testing. HCV RNA detection was also performed in peripheral blood mononuclear cells (PBMNCs) and serum or plasma samples from reactive donors after virus concentration by ultracentrifugation. HCV RNA tested negative in all PBMNCs samples, and a very low amount of viral genome was detected in serum or plasma concentrates from three anti-HCV core–reactive donors (7.1%) but not among concentrates from 100 randomly selected nonreactive donors. Sequencing of these polymerase chain reaction products revealed differences between the isolates that excluded partially sample contamination from a common source.

CONCLUSION: These findings argue in favor of an ongoing occult HCV infection among these blood donors and account for some rather low, but perhaps not negligible, infection risk for such donations. Future studies involving larger samples of donations from traceable donors would enlighten the significance of these findings for the viral safety of the blood supply.

Chronic infection caused by hepatitis C virus (HCV) is characterized by the presence of both specific antibody to HCV (anti-HCV) and HCV RNA in serum or plasma.[1,2] Anti-HCV in the absence of viremia presumably reflects immune memory after a past, resolved infection. Anti-HCV and HCV RNA

ABBREVIATIONS: AI = absorbance index; CNM = Centro Nacional de Microbiología; Ct = threshold cycle; DEPC = diethylpyrocarbonate; FEHV = Fundación para el Estudio de las Hepatitis Virales; GGTP = γ-glutamyl transpeptidase; NC = noncoding; OCI = occult hepatitis C virus infection; PI = percentage of inhibition.

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testing are mandatory as part of blood donor screening, and both markers must test negative for the donor to be accepted. However, the risk of HCV transmission, although very low, is not zero.[3-5]

Occult HCV infection (OCI) has been reported and was characterized by the presence of HCV RNA in peripheral blood mononuclear cells (PBMNCs) and/or liver tissue among patients who tested negative for both anti-HCV and HCV RNA in serum or plasma.[6] HCV RNA was also detected in serum after concentrating viral particles by ultracentrifugation, which increases the sensitivity of the testing.[7] OCI has been found among patients with cryptogenic hepatitis and chronic renal diseases, but also in 3.3% of individuals from the general population displaying normal liver function tests.[6,8,9] A proportion of these cases tested in addition positive by a novel anti-HCV core–specific antibody test,[10] but this test has not been yet compared with conventional screening assays among blood donors. Actually, the occurrence of OCI among first time blood donors has never been communicated.

The aims of this prospective, collaborative study were: 1) to investigate the frequency and the significance of the finding of HCV core–specific antibody reactivity among blood donors testing negative for anti-HCV and HCV RNA in screening and 2) to assess whether such reactivity correlates with the finding of HCV RNA sequences in PBMNCs and/or serum or plasma concentrated by ultracentrifugation.

PATIENTS AND METHODS

Study population

A total of 2012 consecutive, unselected, first-time unpaid blood donors admitted for screening of blood donation markers were prospectively enrolled from October 2014 to July 2015 at three transfusion centres: Red Cross Spain (n = 823) and the transfusion centers from the communities of Madrid (n = 1094) and Castilla y León (n = 95). The inclusion criteria were: 1) first-time blood donors who agreed to sign the informed consent and 2) availability of sufficient serum, plasma, and PBMNC samples collected at the time of study entry. Units testing positive for markers of HCV infection (anti-HCV and/or HCV RNA), human immunodeficiency virus (HIV; anti-HIV and/or HIV RNA), hepatitis B virus (HBV) infection (HBV surface antigen and/or DNA), or other infections (syphilis, malaria) were not excluded. Five donations were excluded because the lack of PBMNC samples, and the final population consisted of 2007 donors of whom 1104 were males and 903 females. No other demographic data were provided to the investigators, and the link between unit and donor identification was broken before sending the materials to the laboratories involved in the study. This procedure prevented communication of results to the donors, although also the obtaining of additional samples and the design of follow-up studies. The study was approved by the Ethic Committee of the Hospital Clínico San Carlos (Madrid, Spain) and was conducted according to the Helsinki Declaration of the World Medical Association.

Screening tests

Participating centers performed anti-HCV screening by either PRISM or ARCHITECT chemiluminescent immunoassays (Abbott Laboratories), and individual HCV nucleic acid test (NAT) by Procleix Ultrio Elite (Grifols Diagnostic Solutions Inc.) or Cobas MPX Multiplex on the Cobas 6800/8800 platform (Roche Molecular Diagnostics). Anti-HCV–reactive, HCV NAT–nonreactive donations were confirmed by immunoblot (INNO-LIA HCV-Score, Fujirebio Europe). Liver function tests (aspartic aminotransferase [AST], alanine aminotransferase [ALT], and γ-glutamyl transpeptidase [GGTP]) were determined centrally at the Fundación para el Estudio de las Hepatitis Virales (FEHV) upon donor inclusion in the study by the Roche Cobas Mira analyzer (normal values for AST, ≤34 IU/L; ALT, males ≤40, females ≤32 IU/L; GGTP, males ≤50, females ≤30 IU/L).

Testing of anti-HCV core–specific antibody

HCV core–specific antibody was tested by an immunoassay with enhanced sensitivity (anti-hcv core high-sensitivity enzyme-linked immunosorbent assa (ELISA kit, DIATER Laboratories) using as antigen a single peptide from a conserved HCV core region (Amino Acids 5-19). The test is based on an investigational anti-HCV core immunoassay.[10] According to current CE labeling regulations, calculation of the cutoff value was referred to the formula $CO = 0.5 \times (NC + 0.1 \times PC)$, where NC is the mean value for the negative control (six replicas) and PC is the mean value for the positive control (two replicas) after assaying more than 1000 positive, negative, and interfering samples in parallel with two CE-marked methods. Testing was performed in prediluted samples (1/10) according to supplier's instructions, and sample-to-cutoff absorbance ratios (absorbance index [AI]) of at least 1.2 were considered reactive. The assay has shown 100% diagnostic sensitivity in chronic infections by HCV Genotypes 1 to 6, and specificities of 100 and 99.7% among blood donors and clinical specimens, respectively.[11]

Anti-HCV core–reactive samples were further analyzed by: 1) peptide inhibition assay,[10] the percentage of inhibition (PI) being calculated by the formula PI = 1 – [(absorbance of sample with 100 μg/mL of peptide)/(absorbance of sample without peptide)] × 100; 2) a supplemental anti-HCV core peptide immunoassay based on peptides spanning Amino Acids 21 to 40 and 101 to 120 of the HCV core sequence (European Patent EP2258714B1), the testing procedure being exactly as described for Peptides 5 to 19;[10-12] and 3) conventional confirmatory testing by a commercial immunoblot assay (INNO-LIA HCV-Score, Fujirebio Europe).

Reactivity for anti-HCV core was thought specific when the PI was at least 50%. Concurrent reactivity in the

TABLE 1. Primers and probes used for HCV RNA amplification*

Name	Nucleotide sequence (5′ to 3′)	Position
Real-time PCR		
Forward primer	CTGTGAGGAACTACTGTCTT	36-55
Reverse primer	CTCGCAAGCACCCTATCAGG	283-302
Anchor probe	GCAGCCTCCAGGACCCCCC-Fl	98-116
Sensor probe	LC640-CCCGGGAGAGCCATAGTGGTCTG-ph	119-141
Nested PCR		
Outer forward primer	CCCTGTGAGGAACTWCTGTCTTCACGC	38-62
Outer reverse primer	GCTCATGRTGCACGGTCTACGAGACCT	312-338
Inner forward primer	TCTAGCCATGGCGTTAGTAYGAGTG	71-95
Inner reverse primer	CACTCGCAAGCACCCTATCAGGCAGT	279-304

* Nucleotide designation according to Takamizawa et al.[14]

supplemental anti-HCV core peptide test and the finding of reactivity (positive or indeterminate results) by immunoblot were also taken into account for the final interpretation. Samples testing positive by at least two of the three supplemental tests were considered positive for anti-HCV.

HCV RNA detection in PBMNCs and in ultracentrifuged serum and plasma samples

Nonrefrigerated blood samples were received at FEHV within 24 hours after extraction. Upon arrival, serum samples were obtained from coagulated blood, made into aliquots, and stored at −30°C. PBMNCs and plasma were isolated from anticoagulated blood by density gradient centrifugation (Biocoll, Biochrom). Plasma was aliquoted and stored at −30°C, while cells were washed three times in phosphate-buffered saline and thereafter stored in solution (RNAlater, Ambion) also at −30°C.

HCV RNA detection was performed by laboratory personnel who were blinded to the serologic status of the donors. Each polymerase chain reaction (PCR) run included a maximum number of 10 samples along with negative controls (repeatedly HCV RNA–negative sera and PBMNCs samples) and reagent blanks in which total RNA was replaced with PCR-grade water. Negative controls and blanks were coprepared with the samples and accompanied them through the entire PCR process. As positive controls, HCV RNA–positive sera and PBMNCs from patients with chronic HCV infection were used. The guidelines of Kwok and Higuchi[13] were strictly observed for avoiding contaminations.

Total RNA was isolated from PBMNCs with an RNA isolation system (SV Total, Promega). After precipitation, pellets were dissolved in diethylpyrocarbonate (DEPC)-treated water and RNA concentration was determined by spectrophotometry. Total RNA was extracted from 250 μL of serum using reagent (Trizol LS, Invitrogen), and the pellet was dissolved in 10 μL of DEPC-water. In addition, 2 mL of serum and 2 mL of plasma were ultracentrifuged[7] over a 10% sucrose cushion at 100,000 × *g* for 17 hours at 4°C. Pellets were dissolved in 250 μL of TE buffer (10 mmol/L tris(hydroxymethyl)aminomethane-HCl, 10 mmol/L ethylenediaminetetraacetic acid; pH 7.5) and total RNA was isolated with reagent (Trizol LS, Invitrogen) and precipitated, and the pellet was dissolved in 10 μL of DEPC-water.

Amplification of the HCV RNA 5′ noncoding (NC) region (226 bp) was performed by quantitative real-time reverse transcription (RT)-PCR with fluorescence resonance energy transfer probes. Two microliters of total RNA isolated from 250 μL of serum or from 2 mL of ultracentrifuged serum or plasma, or 0.5 μg of total RNA from PBMNCs was retrotranscribed and amplified in a single-tube reaction containing RNA reaction mix (LightCycler Master Hybprobe, Roche Diagnostics), 3.25 μmol/L $Mn(OAc)_2$, 0.5 μmol/L of each primer, 0.2 μmol/L of the anchor probe, and 0.4 μmol/L of the sensor probe. RT was performed at 61°C for 30 minutes; the mixture was then heated at 95°C for 2 minutes; and then PCR was performed for 50 cycles of 95°C for 0 seconds (denaturation), 55°C for 12 seconds (annealing), and 72°C for 10 seconds (extension). The fluorescence was measured at the end of each annealing step. A standard curve constructed with 10-fold dilutions of a synthetic HCV RNA was used for quantification. The lower limit of HCV RNA detection of the assay was 10 IU/mL (mean threshold cycle [Ct], 39.32) with a lower limit of quantification of 100 IU/mL (mean Ct, 35.64), as determined by testing serial dilutions of HCV RNA–positive serum sample, in which HCV RNA quantification was previously assessed by an HCV test (Cobas TaqMan, Roche Diagnostics). PCR testing was considered positive if the Ct level was obtained at no more than 39 cycles, and quantification was achieved by reference to the standard curve.

To further confirm the results, the remaining total RNA extracted from positive samples was sent to a second laboratory (Centro Nacional de Microbiología [CNM]) and used to perform an additional amplification of the 5′ NC region of the HCV genome by nested RT-PCR.[14] The PCR products obtained (235 bp) were also purified and sequenced as described below. The sequences of the primers used in these tests are given in Table 1.

Sequencing and sequence analysis

PCR products from positive samples obtained either at the FEHV or at the CNM were purified by PCR clean-up

technology (Illustra Exoprostar 1 step, VWR International Eurolab), and both strands were directly sequenced using Sanger sequencing. Sequence analysis was performed on the fragment comprised between Nucleotide 104 and Nucleotide 287, which were present in all PCR products.

Statistical analysis

Categorical variables were compared using the chi-square test (or Fisher's exact test when applicable). Continuous variables were compared using the nonparametric Mann-Whitney's U test. All p values reported are two-tailed.

RESULTS

Infectious testing at the transfusion centers

Anti-HCV reactivity was recorded in two donors (0.1%), but none of them coud be confirmed on supplemental immunoblot testing. Therefore, all donors finally tested negative for anti-HCV. Markers of other infectious diseases were detected in six donors (0.3%): HBV DNA in two (0.1%), malaria in two (0.1%), syphilis in one, and cytomegalovirus in one. None of the 2007 donors gave a HCV NAT yield.

Anti-HCV testing

Forty-two samples (2.1%) displayed reactivity in the anti-HCV core test, and the eight samples reacting for any infectious marker in the blood bank screening did not account among them. Table 2 shows the AI values obtained for each sample (mean, 1.88; range, 1.20-6.20) and the individual results obtained from supplemental testing. Twelve reactive samples (28.6%) rendered PI of more than 50% in the inhibition test, which led us to consider the reactivity as due to a specific reaction involving some antibody species and some epitope represented in the linear peptide spanning Amino Acids 5 to 19 of the HCV core antigen. Reactivity to epitopes spanning Amino Acids 21 to 40 or 101 to 120 was found in 15 samples (35.7%). Seven samples (16.7%) reacted with at least one antigen in immunoblot: six samples were indeterminate (C1, E2, or NS4 lines; Samples 2, 19, 21, 26, 30, and 33) and one was positive (C1 and C2 lines; Sample 40). Criteria for anti-HCV positivity were satisfied by six samples (14.3%, 0.30% from the total; Samples 2, 19, 21, 30, 33, and 40), and one sample was considered indeterminate because of isolated reactivity to NS4 antigen in immunoblot (Sample 26).

HCV RNA detection

First, HCV RNA was tested in 250 μL of serum, in 2 mL of ultracentrifuged serum, and in PBMNCs from the 42 anti-HCV core–reactive donors and from 100 anti-HCV core–negative donors. RNA testing was negative in all sera (250 μL) and PBMNCs, but was positive (Ct value, 37.11; 10-100 IU/mL) in the 2 mL of ultracentrifuged serum from one donor who tested indeterminate for anti-HCV by immunoblot (Donor 26, Table 2). Viral RNA was also then tested by a different laboratory worker, who was unaware of the previous results, in 2-mL ultracentrifuged plasma samples from the 142 donors mentioned above. Donor 26 tested also positive in plasma (Ct value, 36.84: 10-100 IU/mL), as well as two additional donors (Donors 14 and 19; Ct values, 37.48 and 37.73, respectively) who had tested negative in ultracentrifuged serum. Positive results were obtained in different runs on different days, excluding intersample cross-contamination. Donor 14 did not meet the tentative criteria for anti-HCV positivity, but Donor 19 met them and tested indeterminate (single E2 line) in immunoblot. The 5′ NC region of these three donors was again amplified from the remaining FEHV-concentrated plasma in CNM, by nested RT-PCR, with positive results.

Overall, three of 42 (7.1%) anti-HCV core–reactive donors displayed low amounts of circulating viral RNA, while none of the 100 anti-HCV core–negative donors tested positive ($p = 0.025$). HCV RNA was also tested in ultracentrifuged serum, plasma, and PBMNC samples from the two donors yielding reactivity for anti-HCV in the screening tests at the blood banks, and all samples tested negative.

Sequencing and sequence analysis

Analysis of the sequences of the PCR products identified all isolates as HCV Genotype 1b (BLAST analysis, NCBI). Donors 14 and 19 shared an identical nucleotide sequence for the genome fragments amplified independently in two laboratories. Identity reached 100% in the BLAST analysis performed with 94 sequences of the NCBI database, including the sequence used as reference in the alignment (Fig. 1). In contrast, the sequence from Donor 26 displayed six nucleotide changes (C114G, A116C, G253C, G256C, G271C, and A276G) in comparison with the former. Substitutions C114G and G256C were detected in fragments amplified from serum but not from plasma, and substitutions A116C and G253C were found only in the product amplified at one of the two laboratories (CNM).

Liver function tests

Abnormal liver function tests were recorded in 306 of 2007 (15.3%) donors. Altered transaminase levels were detected in one of five (20%) anti-HCV core–positive donors and in five of 37 (13.5%) among the remaining anti-HCV core–reactive donors (four males and two females). The frequency of anti-HCV core reactivity did not display significant differences in regard to this feature (6/306 with altered values vs. 36/1701 with normal values).

DISCUSSION

The prevalence of anti-HCV among blood donors declined dramatically in Spain during the past decade and was

TABLE 2. Results obtained on individual samples from anti-HCV core–reactive donors

Donor	Sex	AST/ALT/ GGTP (IU/L)†	Anti-HCV core test and inhibition assay			Supplemental anti-HCV core ELISA*				Supplemental anti-HCV immune blot	Interpretation of anti-HCV testing	HCV RNA in plasma (2 mL), IU/mL
						21-40 test		101-120 test				
			Abs.	AI	PI (%)	Abs.	AI	Abs.	AI			
1	Female	8/9/18	0.206	1.67	9.8	0.394	**1.76‡**	0.299	**1.77‡**	Negative	Negative	0
2‡	Male	10/17/35	0.310	2.53	**75.7‡**	0.060	0.44	0.073	0.43	**C1 (1+)‡**	**Positive‡**	0
3	Female	11/18/21	0.766	6.20	**90.5‡**	0.106	0.77	0.112	0.66	Negative	Negative	0
4	Female	6/6/14	0.348	2.63	**79.0‡**	0.075	0.55	0.100	0.59	Negative	Negative	0
5	Male	11/14/22	0.476	3.59	**80.1‡**	0.068	0.50	0.092	0.54	Negative	Negative	0
6	Male	25/54/29	0.176	1.39	20.1	0.134	0.60	0.120	0.71	Negative	Negative	0
7	Female	18/20/25	0.142	1.21	<5	0.073	0.53	0.146	0.82	Negative	Negative	0
8	Male	14/27/65	0.167	1.42	<5	0.262	1.17	0.244	**1.44‡**	Negative	Negative	0
9	Female	14/11/11	0.229	1.78	12.5	0.105	0.77	0.126	0.74	Negative	Negative	0
10	Male	24/22/16	0.202	1.79	<5	0.075	0.55	0.101	0.59	Negative	Negative	0
11	Female	17/13/27	0.150	1.33	<5	0.304	**1.67‡**	0.424	**2.39‡**	Negative	Negative	0
12	**Male‡**	36§/36/24	0.175	1.53	11.3	0.109	0.48	0.102	0.60	Negative	Negative	0
13	Male	12/14/18	0.250	2.18	**70.0‡**	0.064	0.47	0.080	0.47	Negative	Negative	0
14‡	Male	16/19/20	0.139	1.21	<5	0.085	0.62	0.164	0.92	Negative	Negative	**Positive (<100)‡**
15	Male	19/17/18	0.192	1.49	**52.2‡**	0.087	0.47	0.157	0.88	Negative	Negative	0
16	Female	14/15/13	0.155	1.20	<5	0.082	0.60	0.240	**1.36‡**	Negative	Negative	0
17	Female	29/26/14	0.157	1.21	10.4	0.199	1.09	0.306	**1.73‡**	Negative	Negative	0
18	Male	18/28/24	0.171	1.23	11.2	0.076	0.41	0.130	0.73	Negative	Negative	0
19‡	Male	17/19/23	0.220	1.81	**67.5‡**	0.069	0.51	0.096	0.57	**E2 (1+)‡**	Positive	**Positive (<100)‡**
20	Female	16/15/23	0.225	1.86	32.3	0.068	0.50	0.092	0.54	Negative	Negative	0
21‡	Female	30/22/21	0.202	1.66	**66.9‡**	0.082	0.36	0.082	0.49	C1 (1+)	Positive	0
22	Female	14/18/16	0.218	1.82	<5	0.273	**1.22‡**	0.210	**1.24‡**	Negative	Negative	0
23	Female	20/17/18	0.181	1.51	10.1	0.268	**1.20‡**	0.286	**1.69‡**	Negative	Negative	0
24	Male	26/39/31	0.147	1.22	<5	0.061	0.33	0.160	0.90	Negative	Negative	0
25	Male	32/33/40	0.166	1.38	<5	0.105	0.57	0.195	1.10	Negative	Negative	0
26‡	Female	17/24/12	0.643	5.35	<5	0.059	0.43	0.075	0.44	NS4 (1+)	Negative	**Positive (<100)**
27	Female	18/21/18	0.189	1.40	<5	0.287	**1.57‡**	0.419	**2.37‡**	Negative	Negative	0
28	Female	19/28/**40‡**	0.224	1.66	38.1	0.206	0.92	0.129	0.76	Negative	Negative	0
29	Male	29/53§/21	0.171	1.44	**60.5‡**	0.131	0.59	0.127	0.75	Negative	Negative	0
30‡	Male	32/28/20	0.202	1.70	**51.0‡**	0.099	0.44	0.092	0.54	**E2 (1+)‡**	**Positive‡**	0
31	Male	30/40/39	0.167	1.46	7.8	0.122	0.67	0.175	0.99	Negative	Negative	0
32	Male	25/28/26	0.190	1.60	<5	0.533	**2.92‡**	0.675	**3.81‡**	Negative	Negative	0
33‡	Female	17/15/20	0.238	1.99	**67.3‡**	0.072	0.39	0.142	0.80	**C1 (1+)‡**	Positive‡	0
34	Male	21/22/18	0.154	1.25	8.0	0.446	**2.43‡**	0.343	**1.82‡**	Negative	Negative	0
35	Female	25/28/**118‡**	0.165	1.40	6.2	0.250	**1.36‡**	0.271	**1.44‡**	Negative	Negative	0
36	Female	23/18/24	0.165	1.40	<5	0.524	**2.86‡**	0.551	**2.92‡**	Negative	Negative	0
37	Male	13/16/20	0.224	1.88	**67.6‡**	0.089	0.49	0.101	0.54	Negative	Negative	0
38	Male	24/16/19	0.188	1.56	6.7	0.320	**1.75‡**	0.326	1.72‡	Negative	Negative	0
39	Male	19/19/30	0.262	2.02	<5	0.166	0.90	0.161	0.85	Negative	Negative	0
40‡	Female	17/16/16	0.176	1.51	<5	0.416	2.27‡	0.370	**1.96‡**	**C1 (1+), C2 (1+)‡**	**Positive‡**	0
41	Male	22/28/47	0.261	2.10	<5	0.620	**3.38‡**	0.674	**3.57‡**	Negative	Negative	0
42	Female	12/13/23	0.293	2.52	<5	0.075	0.41	0.078	0.41	Negative	Negative	0

* Additional HCV core peptide antibody reactivity against epitopes spanning Amino Acids 21-40 and/or 101-120 of the HCV core sequence (European Patent EP2258714B1).
† Normal values for AST, ≤34 IU/L; ALT, males ≤40 IU/L, females ≤32 IU/L; normal values for GGTP, males ≤50 IU/L, females ≤30 IU/L).
‡ Values above the normal level.
Bold highlight means significant reactivity in the test.

recently reported 0.013% from 1.7 million first-time donors.[15] Among the general population of adults, the overall prevalence is more than 100-fold higher (1.8%).[16] The small size of the sample studied might account for the lack of anti-VHC–positive donors resulting in this study from the screening performed at the transfusion centers.

In contrast, anti-HCV core testing selected 42 donors to investigate, and supplemental studies could identify, according to the criteria explained, five donors displaying prior contact with HCV but not actual infection and three donors displaying OCI (Table 3). The yield of this alternative testing was 0.39% (8/2007). Most of the donors (34/42, 81%) lacked, therefore, any evidence of prior contact with

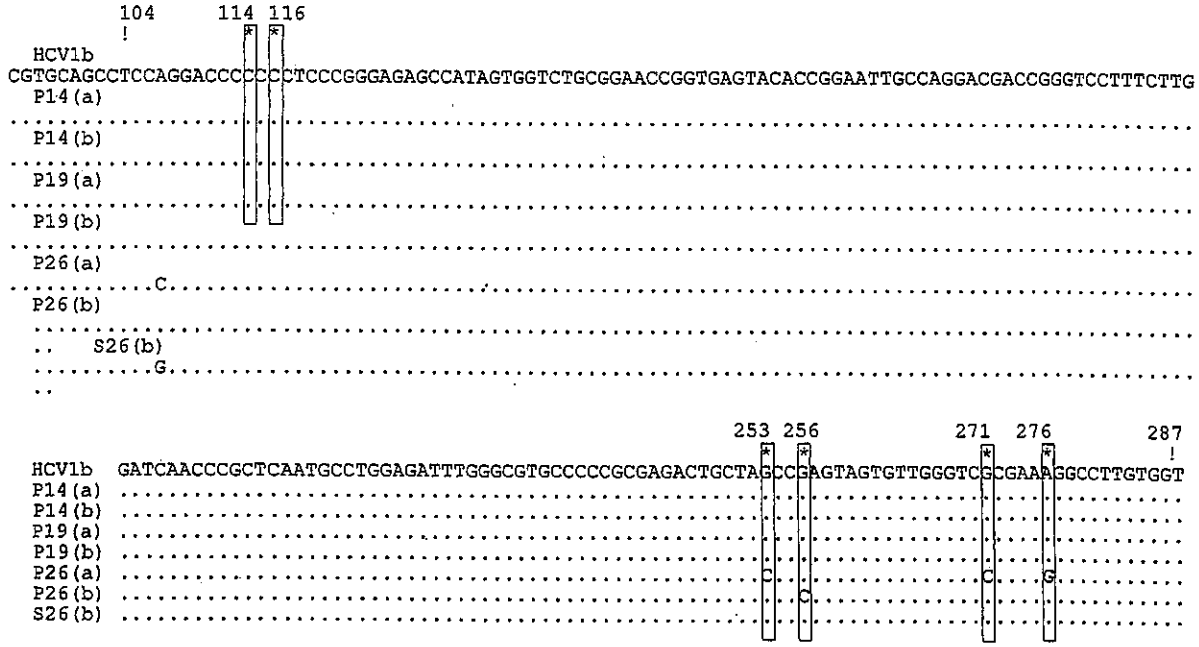

Fig. 1. Sequence comparison of PCR products amplified from plasma and serum of the three positive donors. Nucleotide positions and sequence of HCV 1b were taken from AB249644. (A) PCR products obtained at the CNM; (B) PCR products obtained at the FEHV. P = plasma sample; S = serum sample.

TABLE 3. Interpretation of the results shown in Table 2

Anti-HCV present	Conventional HCV infection	OCI	Number of donors (donor number)
No	No	No	34
Yes	No	No	5 (2, 21, 30, 33, 40)
Yes	No	Yes	1 (19)
No	No	Yes	2 (14, 26)

HCV, and these false-positive cases represented 1.7% of the sample studied.

The novel assay could actually detect anti-HCV where some current assays did not, including some assays using a sample dilution lower than 1:10. These findings would suggest that minute amounts of antibody directed toward an epitope present in the single peptide used could be more prone to be detected when the specific activity of the target is not diluted in the multiantigen design of the solid phase of a conventional immunoassay (European Patent EP2258714B1).[10] The results obtained by immunoblot supported the interpretation for seven samples, and recording of isolated reactivity to core antigens by INNOLIA among four of them was of interest.[17] This advantage was, however, jeopardized by the finding of a large number of false-positive results, which might be elicited by some human protein displaying cross-reactivity with epitopes of this region of the HCV core protein.[18-20] Alternatively, presence of maturation-altered or low-avidity anti-HCV[21-23] could explain the results, as suggested previously for OCI.[10]

Although sample concentration by ultracentrifugation does not account among the procedures standardized for HCV diagnosis by PCR, it offers a valuable research tool for improving the knowledge of the natural history of the HCV infection. Sample contamination, either from external sources before testing or from sample to sample during testing, must be carefully considered to discuss the results obtained with this approach. Three facts would support the results of this study in regard to this important issue: 1) positive results were obtained in different runs on different days; 2) negative controls, the rest of the samples included in each PCR run, and 100 anti-HCV–negative donors tested negative; and 3) results were reproduced from the RNA extracts by a second laboratory using a different set of primers and a totally independent aliquot of sample, which is of special significance for supporting the results obtained on Sample 14 (presence of HCV RNA in absence of anti-HCV). Sequencing confirmed the identity of the products as corresponding to the expected region of HCV Genotype 1b, a viral genotype common in Spain.[24] The fragments displayed, in addition, sequence diversity, which excludes contamination from a common source. Given that the 5′ NC region of the HCV genome is highly conserved,[25] the sequence identity found for two of the three isolates would fall within the expected and should not be necessarily interpreted as reflecting sample-to-sample contamination. In regard to the interlaboratory discrepancy found in the sequencing of the third isolate, it

might represent an artifact of the amplification procedures or could alternatively reflect differential amplification of viral quasispecies present in the sample.

In conclusion, these findings reinforce the notion of OCI as part of the natural history of the HCV infection,[26-30] although a couple of points remain obscure. First, the discrepancy between the results obtained by PCR on samples of serum and plasma taken at once from the same donor, which perhaps reflects unknown technical issues that could be critical when the viral load is extremely low. Second, the differences found in the sequence of the fragments amplified from samples from Donor 26 at the two participating laboratories, which can respond either to technical or biologic reasons. Third, the weak antibody response and the very low viral load found at once in these donors, which is a feature characteristic of OCI that awaits satisfactory explanation in terms of the putative pathogenic mechanisms leading to it. Although not conclusive yet, the findings of this report suggest that the safety of the blood supply might be improved by the anti-HCV core test used in the study and would justify future studies with larger samples of traceable donors who can be fully characterized and submitted to follow-up studies after selection by the novel test. In regard to such future investigations, collecting and storing PBMNCs for performing cellular recall HCV antigen studies would be of interest, and the evaluation of the outcome of anti HCV core–positive units among their recipients must be regarded in these studies as an ethical commitment.

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CONFLICT OF INTEREST

Fundación para el Estudio de las Hepatitis Virales holds patent grants related to occult HCV detection. RP is scientific founder and stockholder of DIATER S.A., and JA is an employee of the company. The remaining authors have disclosed no conflicts of interest.

AUTHOR CONTRIBUTIONS

JAQ, AA, JB, IC, and JA performed experiments, analyzed results, and prepared tables and figures; MAA, EE, MIG, RG, SP, and LAR performed mandatory testing at transfusion centers and managed the sample delivery logistics; and RP, VC, and JME designed the study and wrote the manuscript.

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別紙様式第2-1

No. 16

医薬品 研究報告 調査報告書

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				2016. 8. 5	該当なし	

一般的名称	人血小板濃厚液	研究報告の公表状況		公表国
販売名(企業名)	濃厚血小板-LR「日赤」(日本赤十字社) 照射濃厚血小板-LR「日赤」(日本赤十字社) 濃厚血小板HLA-LR「日赤」(日本赤十字社) 照射濃厚血小板HLA-LR「日赤」(日本赤十字社) 照射洗浄血小板-LR「日赤」(日本赤十字社) 照射洗浄血小板HLA-LR「日赤」(日本赤十字社)		Huang F, Li Y, Yu W, et al. Hepatology. 2016 Aug;64(2):350-9.	中国

研究報告の概要

○雌牛の乳汁への感染性E型肝炎ウイルス(HEV)の排出は、高い人獣共通感染症のリスクをもたらす。
HEVは世界中において急性肝炎の主要原因となっている。HEVは、豚が主要な保有宿主であると考えられている。しかしながらこの考えは、開発途上国と西洋諸国の双方における著しく高いHEVの抗体陽性率を説明するには不十分である。したがって本研究では、ヒトにおけるHEV感染の原因となる危険性が高い、新たな人獣共通の感染源を特定することを目的とした。我々は、家畜の混合飼育が一般的となっている中国南西部・雲南省の農村地域の雌牛140頭から、糞、血液及び乳汁を検体として採取した。定量リアルタイムPCR法を用いてHEV RNAを定量し、全ゲノムの配列を決定した。HEVの感染力は、アカゲザルを用いて評価した。糞検体から52頭のHEV感染牛を確認し、雌牛における感染性のHEV感染の罹患率が高いことを確認した。驚くべきことに、感染した雌牛の100%(52頭/52頭)において乳汁にHEVが排出されることが判明し、系統発生解析により、全ての株がGenotype 4(subtype 4h)に属することが明らかになった。アカゲザルにHEVに汚染された生乳または低温殺菌乳を経口投与した結果、感染性が確認された。重要なことには、HEVの完全な不活化は、短時間の煮沸(100℃で3分間)によって可能となった。
結論:HEVに感染した雌牛の乳汁が、ヒトへのHEV感染の原因となる危険性が高く、新たな人獣共通の感染源であることが確認された。これらの結果は、特に家畜の混合飼育が行われている環境を対象として、HEVによる人獣共通感染を適切に評価・コントロールする方法を理解し、これを確立することへの注意を喚起している。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
家畜を混合飼育する中国の農村において、E型肝炎ウイルス(HEV)感染が確認された雌牛全頭の乳汁、及びこれを低温殺菌した市販牛乳からHEVが検出され、アカゲザルへの感染性が確認されたという報告である。	日本赤十字社では、AMED「経口感染によるウイルス性肝炎(A型及びE型)の感染防止、病態解明、治療等に関する研究」の一環として、重症化が懸念されるHEV Genotype 4の輸血感染報告があった北海道で献血血液について試行的個別NATを実施している。今後もHEV感染の実態に関する情報の収集及び安全対策に努める。

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Excretion of Infectious Hepatitis E Virus Into Milk in Cows Imposes High Risks of Zoonosis

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Hepatitis E virus (HEV) represents the main cause of acute hepatitis worldwide. HEV infection in immunocompromised patients involves a high risk for the development of chronic hepatitis. Because HEV is recognized as a zoonotic pathogen, it is currently believed that swine is the primary reservoir. However, this is not sufficient to justify the strikingly high seroprevalence of HEV in both developing and Western countries. Thus, this study aimed to identify new zoonotic sources that bear a high risk of transmission to humans. We collected fecal, blood, and milk samples of cows in a typical rural region of Yunnan Province in southwest China, where mixed farming of domestic animals is a common practice. HEV RNA was quantified by quantitative real-time polymerase chain reaction, and the whole genome was sequenced. HEV infectivity was assessed in rhesus macaques. We found a high prevalence of active HEV infection in cows as determined by viral RNA positivity in fecal samples. Surprisingly, we discovered that HEV is excreted into milk that is produced by infected cows. Phylogenetic analysis revealed that all HEV isolates from cow/milk belong to genotype 4 and subtype 4h. Gavage with HEV-contaminated raw and even pasteurized milk resulted in active infection in rhesus macaques. Importantly, a short period of boiling, but not pasteurization, could completely inactivate HEV. *Conclusion:* Infectious HEV-contaminated cow milk is recognized as a new zoonotic source that bears a high risk of transmission to humans; these results call attention to understanding and establishing proper measurement and control of HEV zoonotic transmission, particularly in the setting of mixed farming of domestic animals. (HEPATOLOGY 2016;64:350-359)

Hepatitis E virus (HEV) is a positive single-stranded RNA virus with four defined genotypes and other newly discovered strains that have not been assigned to these known genotypes.[1] It is the most common cause of acute hepatitis globally.[2] In the Western world, chronic hepatitis has been frequently described in immunocompromised patients.[3] Thus, HEV infection has emerged as a global public health issue with a particularly high mortality rate in pregnant women.[4] Seroprevalence is rather high in the developing world, ranging 30%-80%. Strikingly, it is also very high in Western countries. In the United States, population-based surveys have indicated a seroprevalence of 21% from 1988 to 1994 and 6% from 2009 to 2010.[5] An overall seroprevalence of 22.4% and 27% was found in French[6] and Dutch[7] blood donors, respectively.

In the developing world, epidemics of hepatitis E occur periodically and are mainly attributed to genotypes 1 and 2. They account for annually 20 million infections, over 3 million cases with symptomatic diseases, and 70,000 deaths.[8] Fecal contamination of drinking water is a major route of transmission of these two genotypes.[8]

In contrast, in developed countries, HEV genotype 3 is predominant and spread by zoonotic transmission.

Abbreviations: cDNA, complementary DNA; HEV, hepatitis E virus; IgG/IgM, immunoglobulins G and M; ORF, open reading frame; PCR, polymerase chain reaction; qRT-PCR, quantitative real-time PCR.

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Potential conflict of interest: Nothing to report.

It is believed to be mainly transmitted through consumption of uncooked or undercooked pig meat.[9] Genotype 4 is also a zoonotic strain, mainly found in China[10-12] and Japan[13,14] as well as some sporadic cases reported in Europe.[15-17] Thus, zoonotic strains are circulating in both developing and developed countries. The initial concept of zoonotic transmission was based on the identification of HEV stains in various animals, including swine, boars, deer, and rabbits.[18-21] Recent studies have confirmed the genetic similarity between strains circulating in pigs and in indigenous human cases as well as cross-species infection,[18,22] indicating that swine is the most important reservoir of HEV zoonotic transmission.

Given the factor of cross-species infection, traditional mixed farming of different types of domestic animals may potentially expand the zoonotic sources that mediate the transmission of HEV to humans. Thus, besides direct contact with infected pigs or consumption of uncooked pork contaminated with HEV, other unidentified zoonotic sources may also contribute to the high prevalence of HEV. In this study, we unexpectedly found a high prevalence of HEV infection in cows in a rural area of Yunnan Province, southwest China. We discovered that infected cows excreted HEV into milk. Furthermore, we demonstrated that HEV from raw and even pasteurized fresh milk are infectious in rhesus macaques yet can be inactivated by boiling.

Materials and Methods

SAMPLE COLLECTION

Fresh stool, serum, and milk samples of Holstein cows were collected from Dali, Yunnan, China, during September to December 2015. The region where samples were collected has traditional mixed farming of different types of domestic animals. Typically, each household owns one to three cows, whereas a few families own larger numbers of cows. The samples were stored at -80°C until use. Details are described in Supporting Table S1.

DETECTION OF HEV RNA

Stool specimens were suspended at 10% w/v in phosphate-buffered saline (pH 7.4), containing 0.01% diethyl pyrocarbonate, and centrifuged at 12,000*g* for 10 minutes. Total RNA was extracted from the supernatant of stool, milk, or serum using the QIAamp Viral RNA mini-kit (Qiagen, Germany) according to the manufacturer's instructions. Reverse-transcription was performed using a reverse transcriptase kit (AMV XL for real-time polymerase chain reaction [RT-PCR]; Takara, Japan) according to the manufacturer's directions. A 348-nucleotide amplicon from HEV open reading frame 2 (ORF2) was amplified by nested RT-PCR as described (Supporting Table S2).[23]

GENOME SEQUENCING

Three full-length nucleotide sequences of HEV were obtained by amplification of five overlapping fragments covering the complete genome (Supporting Table S2). The extreme 5′ and 3′ ends of the viral genome were amplified using the rapid amplification of complementary DNA (cDNA) ends technique as in our previous study.[24] The complete nucleotide sequence was assembled and analyzed using the MegAlign software. All sequences have been submitted to the GenBank database.

VIRAL TITER QUANTIFICATION BY QUANTITATIVE RT-PCR

The viral titer of HEV in serum, feces, or milk was quantified using SYBR green-based quantitative RT-PCR (qRT-PCR) with HEV-specific primers as described (Supporting Table S2).[23] In brief, 200 μL of milk or serum or 0.4 g of fecal samples was subjected

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to RNA isolation. Isolated RNA was used to synthesize the first-strand cDNA, and cDNA was added as a template for qRT-PCR. qRT-PCR was performed under the following conditions: 95°C for 30 seconds, followed by 39 cycles of 95°C for 5 seconds and 60°C for 31 seconds. It was performed using an ABI PRISM 7300 Real-Time PCR System.

VIRAL TITER CALCULATION

A standard plasmid was constructed by cloning the RT-PCR-amplified ORF2 partial gene (348 bp) into the pMD 18-T clone vector. The copy number of the recombinant plasmid was calculated using the following formula: (DNA concentration [ng/μL] $\times$ 10^{-9} $\times$ 6.0233 $\times$ 10^{23} copies/mol)/(DNA size [bp] $\times$ 660). The standard curve was drawn based on the copy number and the cycle threshold value of qRT-PCR. The accuracy of HEV RNA quantification by qRT-PCR was validated with a 10-fold serial dilution of this standard plasmid. We first performed dilutions from 1×10^1 copies/mL to 1×10^7 copies/mL to generate a linear range. However, there was no clear distinction of cycle threshold values between 1×10^1 copies/mL and 1×10^2 copies/mL, suggesting that this assay is not sensitive to quantify at a low copy number of 1×10^1 copies/mL. Therefore, a linear range was generated from 1×10^2 copies/mL to 1×10^7 copies/mL with $R^2 = 0.9966$ (Supporting Fig. S1).

PHYLOGENETIC ANALYSIS

The nucleotide sequences of the amplified PCR products and of prototypes of different genotypes of HEV strains were aligned using MEGA 4.1 software (version 4.10, http://www.megasoftware.net). The reference sequences of prototype HEV strains were obtained from the GenBank database. The standard classification of HEV genotypes and subtypes was according to previous studies.[25,26]

Phylogenetic trees were generated by the minimum evolution and interior branch methods. Bootstrapping with 1,000 resamplings of the data was performed to calculate branch percentages. The identity between nucleotide sequences was calculated using the MegAlign program (DNAstar package, version 5.03).

HEV INFECTIVITY IN RHESUS MACAQUES

Healthy male rhesus macaques (n = 7), 2-3 years old, negative for HEV RNA and anti-HEV immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies, were used. The protocol of animal experimentation was approved by the committee of Laboratory Animal Welfare and Ethics of the Kunming University of Science and Technology. Monkeys were numbered randomly and housed in individual cages. HEV-contaminated raw or pasteurized milk and a swine HEV strain (KM01, 2×10^4 copies/mL) that we have demonstrated has robust infectivity in rhesus macaques[27] were used. The first monkey was inoculated with gavage of 5 mL supernatant of KM01 (viral titer 2×10^4) pretreated at 62°C for 30 minutes. The second monkey was inoculated with gavage of 5 mL supernatant of KM01 pretreated at 72°C for 30 seconds. The third monkey was inoculated with gavage of 5 mL supernatant of KM01 pretreated at 100°C for 3 minutes. The fourth monkey was gavaged with 5 mL of pasteurized (the exact pasteurization condition was unclear) commercial milk positive for HEV RNA (shelf life 24 hours, viral titer 3×10^4 copies/mL). The fifth monkey was gavaged with 5 mL of raw milk from the cow that was HEV-positive in both feces and milk (viral titer in milk 2×10^4 copies/mL). The sixth monkey was gavaged with 5 mL of phosphate-buffered saline. The seventh monkey was gavaged with 5 mL of milk negative for HEV RNA. Heating treatment of the indicated samples was performed using a PCR machine. The effectiveness of our pasteurization protocols was validated on *Escherichia coli* (Supporting Fig. S2). Fecal samples were collected from each monkey twice per week for HEV RNA detection/quantification. Sera were collected every week for anti-HEV IgG and IgM testing, HEV RNA quantification, or liver enzymes analysis. All samples were tested immediately or stored at -80°C until use.

DETECTION OF ANTI-HEV IgG AND IgM ANTIBODIES

Serum samples were tested for the presence of anti-HEV IgG and IgM antibodies using commercial enzyme-linked immunosorbent assay kits (KHB, China) containing recombinant ORF2 peptides from the HEV genome as well as both positive and negative controls. Samples were tested in duplicate according to the manufacturer's instructions, with cutoff values for IgG and IgM assays set at 0.22 and 0.26, respectively, which were determined based on the mean optical density 450 values from the negative controls ($\pm$ standard deviation).

SERUM LIVER CHEMISTRY PROFILE

The activities of alanine aminotransferase and aspartate aminotransferase in serum were measured with an automated biochemistry analyzer (Olympus 2700, Japan).

Results

HIGH PREVALENCE OF ACTIVE HEV INFECTION IN COWS

To investigate the prevalence of active HEV infection in cows, we collected stool samples from 140 individual Holstein cows from Dali, Yunnan Province, southwest China, from September to December 2015. Livestock are important components of the agricultural economy in that region, and many of the households typically own one to three cows, as well as other types of domestic animals (Supporting Table S1). The presence of HEV genomic RNA (a hallmark of active infection) in stool samples was examined by nested RT-PCR as described.[23] We found that 52 out of 140 samples were positive (37.14%), in line with previous reports of HEV RNA positivity in cows (8.79%, 8/91) on dairy farms in Xinjiang Province (northwest China)[28] and in swine (7.8%, 20/256) in Kunming, the capital of Yunnan Province.[29]

Complete genomic nucleotides of three strains of cow HEV were sequenced by amplification of five overlapping fragments covering the complete genome from fecal samples (Supporting Table S2). The extreme 5′ and 3′ ends of the viral genome were amplified using the rapid amplification of cDNA ends technique, similar to our previous study.[24] The complete genomic sequence was assembled and analyzed using the MegAlign software. Sequences were submitted to the GenBank database (KU356187, KU356188, and KU356189 for HEV isolated from fecal samples of cows and KU974927, KU974928, KU974934, KU974936, KU974938, KU974946, KU974947, KU974948, KU974949, KU974950, and KU974952 for partial HEV ORF2 sequences isolated from raw milk in Dali, Yunnan Province, China) (Supporting Table S3).

EXCRETION OF HEV INTO COW MILK

A recent case report showed the presence of HEV in breast milk of a woman with acute hepatitis E.[30] We thus investigated whether HEV is excreted in the milk of infected cows. Paired serum, feces, and milk samples were collected from 6 out of the 52 HEV-infected cows that we have identified. As shown by qRT-PCR analysis, all samples were positive, and the titers in milk were considerably high (Fig. 1A; Supporting Table S4).

We further extended the collection of milk samples from all of the identified HEV-positive cows. The presence of HEV RNA (100%, 52/52) was first confirmed by RT-PCR, and the genomic copy number was subsequently quantified by qRT-PCR (Fig. 1B; Supporting Table S4). With respect to the milk samples derived from cows for which the complete HEV genome was sequenced, the amplified ORF1 and ORF2 (partial) sequences were also determined. As expected, these ORF1 and ORF2 sequences were perfectly matched with their parental genome (Supporting Fig. S3), providing further confirmation that HEV in milk was excreted by the infected cows.

HIGH HOMOLOGY OF COW/MILK HEV TO HUMAN AND SWINE STRAINS

To find the homology relationship, phylogenetic analysis was performed based on the complete genomic sequences of our identified cow HEV strains and ORF2 (partial) sequences from milk. A phylogenetic tree clearly illustrated that all HEV isolates from cow/milk belong to genotype 4 (Fig. 2A), subtype 4h (Fig. 2B). More importantly, these HEV strains were closely clustered to human and swine HEV isolated in Kunming City, the capital of Yunnan Province.

Homology analysis based on the complete sequence of HEV indicated that these cow HEVs shared 99.2%-99.4% similarity to human HEV isolated from a pregnant woman in Kunming City, in early 2015,[31] and shared 99.5%-99.8% identity with swine HEV isolated in Kunming City in 2010.[32] These results strongly suggest that these HEV isolates prevalent in human, swine, and cows in Yunnan Province probably originated from the same source.

HEV FROM RAW OR PASTEURIZED MILK IS INFECTIOUS IN RHESUS MACAQUES

Cow milk produced in this area is mainly for household consumption and local market supplies. To address the key issue of whether HEV-contaminated milk can be a mediator of zoonosis, we next evaluated

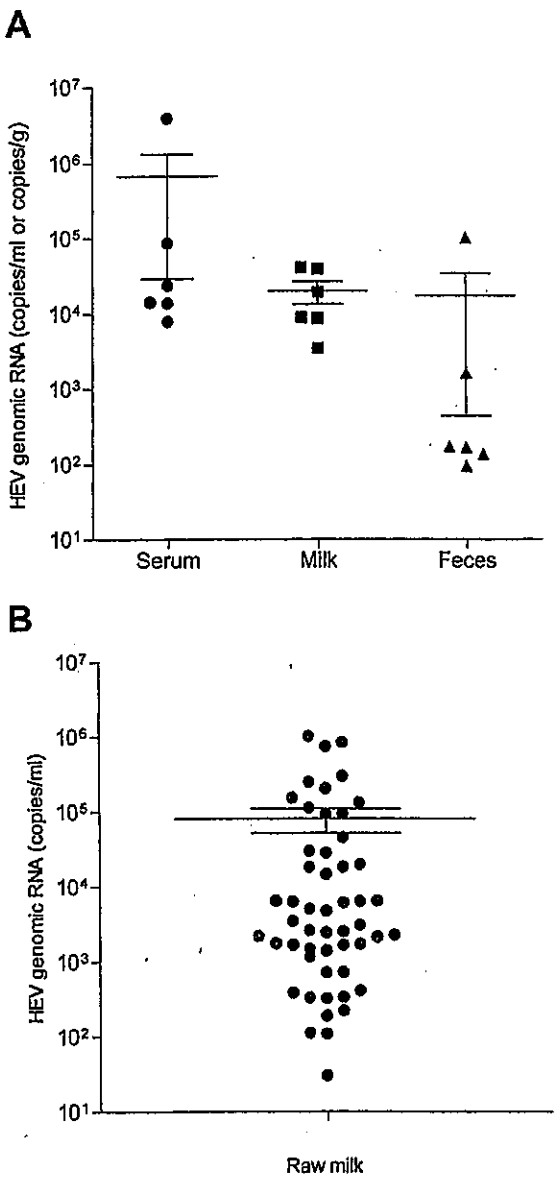

FIG. 1. Quantification of HEV genomic RNA from stool, serum, and milk samples of cows. (A) HEV genomic RNA copy numbers of stool, serum, and milk samples collected from six HEV-infected cows (mean ± standard error of the mean). (B) HEV genomic RNA copy numbers of 52 milk samples from HEV-infected individual cows (mean ± standard error of the mean). Units for fecal samples were copies per gram, and units for serum and milk samples were copies per milliliter.

whether it is infectious in rhesus macaques. We employed this sophisticated animal model for HEV infection that had been previously used by our group[27] and others.[33,34] We selected a representative milk sample with intermediate HEV viral titers (approximately 2×10^4 copies/mL of genomic RNA) (Fig. 1B).

Experimental rhesus macaques were prescreened and confirmed as being HEV-negative. Oral gavage of raw milk containing HEV resulted in active infection in a monkey showing an elevated viral titer from 7-10 days postinoculation in feces (Fig. 3A) and a high titer at 4 weeks postinoculation in serum (Fig. 3B). In contrast, HEV was not detectable in the monkey gavaged with HEV-negative raw milk.

Fresh pasteurized milk is readily available at local markets supplied by the farmers. As expected, we identified HEV-contaminated packages but were curious to know whether they remained infectious. Surprisingly, oral gavage of pasteurized milk in a rhesus macaque resulted in a substantially high level of HEV viral load (viral genomic RNA approximately 1.1×10^5 copies/mL) in serum at 4 weeks postinoculation (Fig. 3B), although viral RNA was hardly quantifiable in feces (Fig. 3A). Thus, pasteurization seems insufficient to completely inactivate HEV.

COMPLETE INACTIVATION OF HEV BY BOILING

To more clearly demonstrate the effects of pasteurization on HEV infectivity, we mimicked the commonly used protocols of pasteurizing raw milk by preheating at 62°C for 30 minutes or at 72°C for 30 seconds using a PCR machine. We used a swine genotype 4 strain that we have previously demonstrated to produce robust infection in monkeys.[27] We found that pretreatment with our pasteurization protocols is not sufficient to completely inactivate the HEV infectivity in monkeys as shown by quantified viral load in both feces and serum (Fig. 4).

We next explored whether boiling of HEV at 100°C for 3 minutes is sufficient for complete inactivation. Indeed, similar to the phosphate-buffered saline-treated negative control monkey, HEV viral RNA was not detectable in both feces and serum of the monkey inoculated with the boiled HEV sample (Fig. 4). Thus, these results alert us to the potential zoonotic risk of both raw and pasteurized cow milk. Fortunately, a short period of boiling is already sufficient to completely inactivate the virus, providing a cost-effective approach that can be easily implemented even in resource-limited regions.

Discussion

Shortly after the discovery of the HEV genome, which was attributed to the cause of non-A, non-B

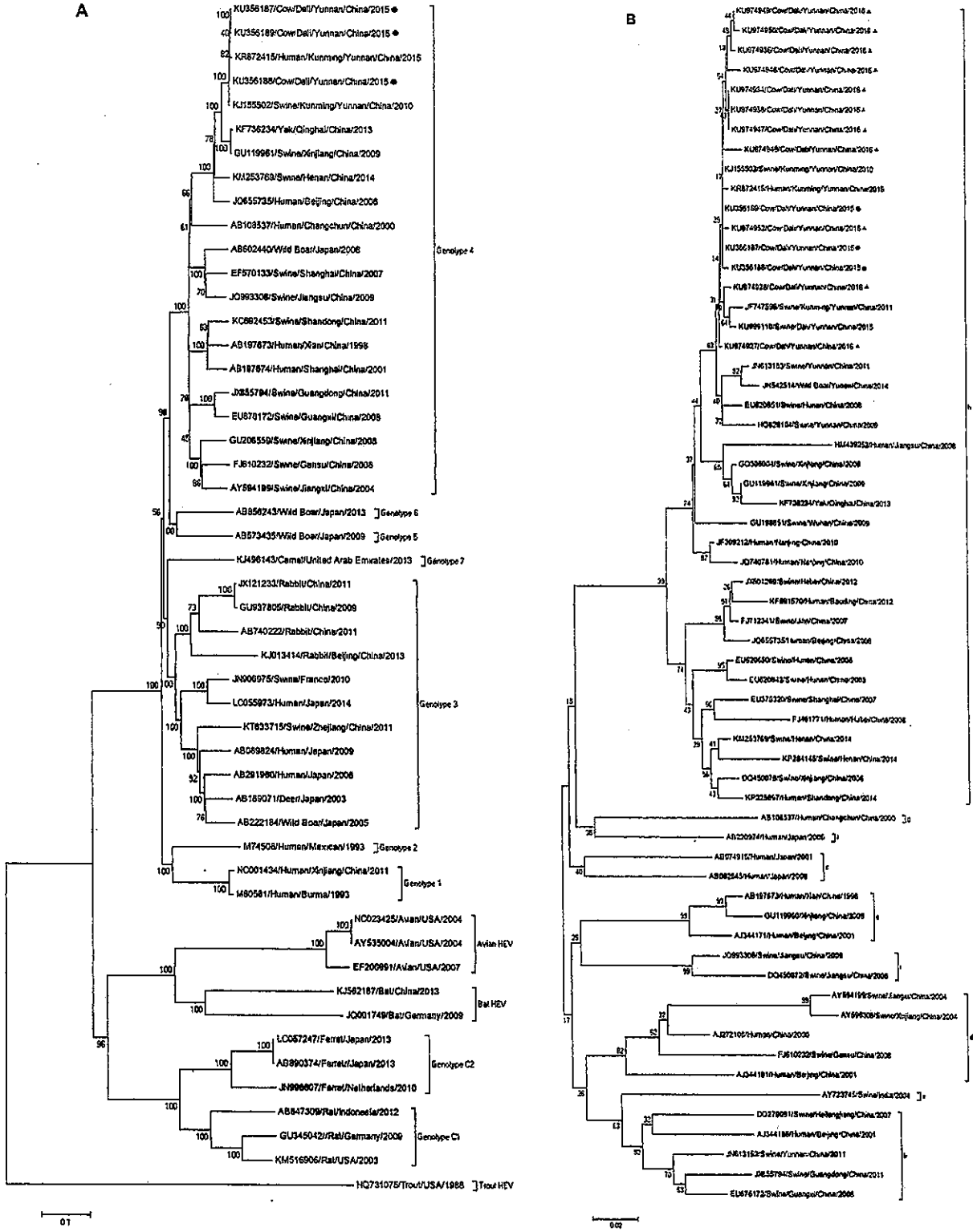

FIG. 2. Phylogenetic analysis of HEV strains identified from cow and milk. (A) Three complete genome sequences (KU356187, KU356188, and KU356189; highlighted by dark dots) amplified from stool samples of infected cows were assigned to genotype 4. (B) Further analysis of the three complete genomes of cow HEV (highlighted by dark dots) and 11 ORF2 partial sequences (348 bp; highlighted by gray triangles) amplified from HEV-contaminated milk samples revealed that all these strains belong to subtype 4h.

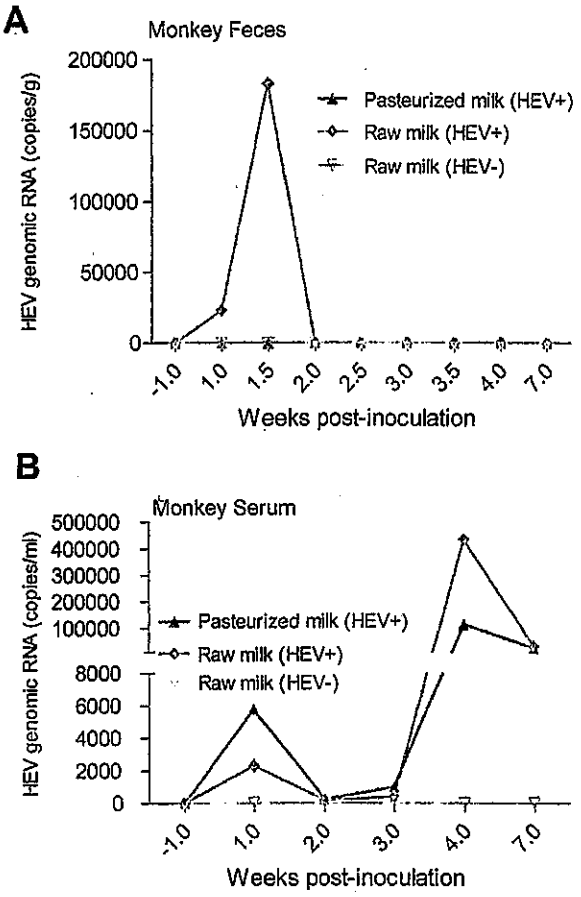

FIG. 3. Infection of rhesus macaques by HEV-contaminated raw or pasteurized milk. (A) Quantification of HEV genomic RNA in fecal samples collected preinoculation and postinoculation. (B) Quantification of HEV genomic RNA in serum samples collected preinoculation and postinoculation.

hepatitis in patients,[35] the identification of HEV in pigs[36] has raised the assumption of a potential zoonotic feature of this virus. The cross-species infectivity of swine HEV demonstrated in nonhuman primates[37] and of human HEV in pigs,[22] as well as the strong association of the consumption of undercooked animal meat with HEV infection in humans,[38] have now firmly established its zoonotic nature. The evidence of HEV infection in animals is rapidly expanding.[1,39] Besides in swine, genotype 3 and 4 strains have also been identified in other hosts, including deer, wild boar, mongoose, macaque, sheep, yak, and cattle. Many new strains that have not been assigned to any genotype were recently discovered from camel, birds, rabbits, bats, rats, ferrets, and fish.[1,39]

Because many of these newly identified animal hosts are not closely associated with human life, we currently only recognize swine as the primary source of zoonotic transmission. However, the recent discovery of HEV in other common domestic animals may revise our classical concept. In cattle, a seroprevalence of 15% was described in the United States[40] and an HEV RNA positivity of 8% was reported in cows on dairy farms in Xinjiang Province, China.[28] Seroprevalence of HEV in cows has been described in various regions in China.[41-43] In this study, we described a 37% positivity of HEV RNA in cows surveyed in a rural area of Yunnan Province, China. In this area, cows are regarded as an important component of the agricultural economy and each household typically owns one to three cows. Mixed farming in this region is a common practice. Family-based, small-size farms host diverse domestic animals including buffalo, cows, goats, sheep, pigs, chicken, and ducks. In fact, a mixed farming system is one of the oldest and most traditional farming methods practiced all over the world and remains the

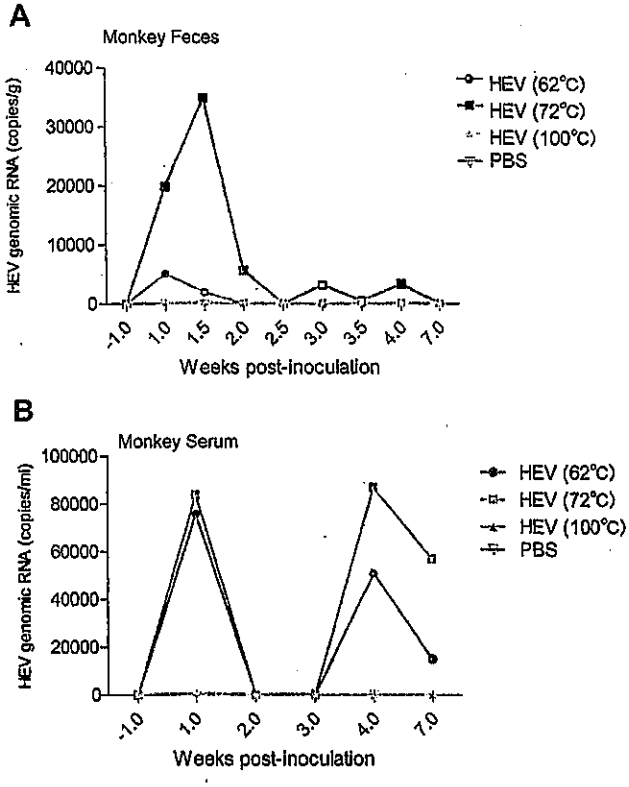

FIG. 4. Boiling, but not pasteurization, completely inactivated the infectivity of HEV in rhesus macaques. (A) Quantification of HEV genomic RNA in fecal samples collected preinoculation and postinoculation of HEV. (B) Quantification of HEV genomic RNA in serum samples collected preinoculation and postinoculation. Abbreviation: PBS, phosphate-buffered saline.

backbone of agriculture in Asia and many developing countries.[44] However, an increased risk of zoonosis has been raised as an important concern in this system.[45] Domestic animals are reared primarily on grass and naturally grown crops, while composted animal wastes are often used to fertilize the soil for growing crops. When livestock are reared outdoors, this increases the potential for contact with disease vectors including rodents, wild birds, and insects, as well as interaction among different types of domestic animals. Given the ability of cross-species infection, we were not astonished by a high rate of HEV infection in cows found in our study with a high homology to human and swine HEV strains as a high prevalence of HEV has been reported in both swine and patients in Yunnan Province.[11,46] Importantly, especially with respect to mixed farming systems, we call attention to the proper measurement and control of HEV circulating among different types of domestic animals, to eventually prevent transmission to humans.

We were indeed concerned by the discovery of HEV in milk that was produced by the infected cows. The most common virus contaminating cow milk is bovine leukemia virus. Bovine leukemia virus-infected lymphocytes circulate through the blood of infected cattle and can also infect the mammary epithelial cells of cows. Infected cells could be disseminated into milk. Exposure to bovine leukemia virus through consumption of cow milk has been associated with breast cancer in women.[47] Although there is no direct evidence yet to illustrate milk-mediated transmission of HEV, a recent study has demonstrated isolation of the virus from breast milk of an acute hepatitis E patient, suggesting that breast-feeding could be a potential route of HEV transmission from mother to child.[30] Another case of liver transplantation with chronic hepatitis E infected with a camel strain was related to regular consumption of camel meat and milk.[48] Because camel meat is often well cooked but the milk is often consumed fresh without processing, this might indicate that camel milk is a possible source of transmission.

To concretely prove whether milk represents a valid source for HEV transmission, we assessed the infectivity of HEV-contaminated cow milk in rhesus macaques in this study. The rhesus macaque is one of the most robust animal models for HEV infection,[27,34,37] although it is not commonly accessible for most researchers. We successfully demonstrated that gavage of raw milk resulted in active infection, as shown by the quantification of HEV RNA in feces and blood. Surprisingly, inoculation of pasteurized milk also led to active infection, although it appeared less robust. Consistently, mimicking the commonly used protocols of pasteurization by preheating at 62°C for 30 minutes or at 72°C for 30 seconds still resulted in active infection in monkeys. In contrast, boiling of HEV at 100°C for 3 minutes is sufficient for complete inactivation. Rhesus macaques are in general tolerant of HEV infection, often with weak or no clear humoral immune response, as we have described.[27] Except for one monkey that was inoculated with HEV preheated at 62°C for 30 minutes and showed a dramatic elevation of alanine transaminase 2 weeks postinoculation (Supporting Fig. S4), a strong indication of liver injury, all of the others had normal alanine aminotransferase and aspartate aminotransferase levels (Supporting Figs. S4 and S5). Most of these monkeys had no clear humoral immune responses (Supporting Figs. S6 and S7), except one that was inoculated with HEV preheated at 72°C for 30 seconds and had a high level of anti-HEV IgM at 3 weeks postinoculation (Supporting Fig. S7).

In fact, several factors have been proposed to affect the thermal stability of HEV. An early study reported that an HEV isolate from Guangzhou, China, could be inactivated by heating at 56°C for 30 minutes.[49] There are probably also genotype-dependent differences and variations between different isolates. The genotype 1 strain (Akluj) could be completely inactivated at 56°C for 1 hour. Another genotype 1 strain (Sar55) was more heat-resistant and was not inactivated at 56°C, but ~80% of the viruses were inactivated at 60°C for 1 hour. Approximately 50% of the viruses of a genotype 2 strain (Mex 14) could be inactivated at 56°C, and 96% of the viruses were inactivated by incubation at 60°C for 1 hour.[50] Heat treatment for 1 minute up to 75°C was not able to completely inactivate a genotype 3 strain (47832c). Complete inactivation was achieved at 80°C or higher temperatures. These data have been used to calculate predictive heat inactivation models for HEV.[51] Thus, we speculate that the tradition of consuming raw pork and raw milk in regions where HEV is highly prevalent could be the key route of transmitting HEV to humans.

In summary, we found a high prevalence of active HEV infection in cows in a typical rural region of Yunnan Province in China, where mixed farming of domestic animals is a common practice. Furthermore, we discovered that infectious HEV is excreted into the milk that is produced by the infected cows, although the mechanism of action remains to be elucidated. Importantly, we demonstrated that a short period of

boiling, but not pasteurization, could completely inactivate HEV. This provided a cost-effective approach that can be easily implemented even in resource-limited regions to eliminate the risk of milk-mediated HEV transmission to humans. Finally, we call attention to understanding and establishing proper measurement and control of HEV zoonotic transmission, in particular in the setting of mixed farming of domestic animals.

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Supporting Information

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販売名(企業名)	赤血球液-LR「日赤」(日本赤十字社) 照射赤血球液-LR「日赤」(日本赤十字社)			

研究報告の概要

○日本において献血者から採取された血漿中に存在するE型肝炎ウイルス(HEV)。
背景及び目的:ヒトHEVは世界中に広まっている。近頃では、輸血感染症例の報告に基づいて、医原性HEV感染が確認されている。日本ではHEV-RNAの検出率並びにHEV IgG/IgM抗体の血清陽性率が地域的に評価されている。現在は北海道において献血者から採取された血漿を対象として、核酸増幅検査によるスクリーニングが実施されているが、日本の北海道以外の地域の献血者におけるHEV-RNA検出率は報告されていない。
材料及び方法:2004年から2014年までの期間に、北海道を除く日本各地において献血適格と判定された献血者から採血された計620,140本の血漿検体を対象として、HEV-RNA検査(50プールまたは500プール)を実施した。HEV-RNA陽性血漿バッグを特定し、ウイルス量、遺伝子型並びにHEV IgG/IgM抗体について評価を行った。
結果:HEV-RNAの検出率(50プール)は1/15,075であり、東日本(東京都を含む関東甲信越ブロック;1/9,135)における検出率が西日本(大阪府を含む近畿ブロック;1/75,930)と比較して高かった。HEV-RNA陽性となった36検体の全てのHEVの遺伝子型は3型であった。ウイルス量の範囲は、1.69 log10copies/ml未満～7.22 log10 copies /mlであった。
結論:日本(北海道を除く)の献血者集団におけるHEV-RNA検出率(1/15,075)は、欧州で報告されている検出率と比較して全体的に低く、またこれまでに報告されている北海道の検出率(1/8,173)よりも低かった。手法が異なっていたため、これらの差異がRNA検出限界値の違いを反映している可能性を除外することはできない。献血血液からHEV遺伝子型4型が報告された北海道とは対照的に、我々が陽性を確認した全ての献血血液における遺伝子型は、病原性がより低い3型であった。

報告企業の意見	今後の対応
北海道を除く日本各地で献血された血液のE型肝炎ウイルスRNAの検出率(50プール)は1/15,075であり、北海道(1/8,173)よりも低く、東日本(1/9,135)が西日本(1/75,930)より高い検出率であったという報告である。	日本赤十字社では、AMED「経口感染によるウイルス性肝炎(A型及びE型)の感染防止、病態解明、治療等に関する研究」の一環として、重症化が懸念されるHEV Genotype 4の輸血感染報告があった北海道赤十字血液センターで輸血用血液について試行的個別NATを実施している。今後もHEV感染の実態に関する情報の収集及び安全対策に努める。

使用上の注意記載状況・その他参考事項等

赤血球液-LR「日赤」
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血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

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ORIGINAL PAPER

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Hepatitis E virus in donor plasma collected in Japan

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Background and objectives Human hepatitis E virus (HEV) is prevalent worldwide. Iatrogenic HEV has recently been identified based on the reports of transfusion-transmitted cases. The detection rate of HEV-RNA and seroprevalence of HEV-IgG/IgM have been regionally evaluated in Japan, and donor plasma collected in Hokkaido is currently screened by nucleic acid amplification testing. However, the detection rate of HEV-RNA in blood donors in Japan outside of Hokkaido has not been reported.

Materials and Methods A total of 620 140 qualified donor plasma samples from Japanese regions excluding Hokkaido were tested for HEV-RNA (pools of 50 or 500) between 2004 and 2014. HEV-RNA-positive plasma bags were identified, and the HEV viral load, genotype and anti-HEV immunoglobulin (Ig)G/IgM were evaluated.

Results The detection rate of HEV-RNA (pools of 50) was 1/15 075 and higher in eastern than in western Japan. All 36 HEV-RNA-positive samples were genotype 3 with viral load ranging from <1·69 to 7·22 log10 copies/ml.

Conclusions Our detection rate of HEV-RNA in donor populations in Japan outside Hokkaido (1/15 075 donations) is generally lower than reported in Europe and lower than previously reported for Hokkaido (1/8173 donations). As methods varied, we cannot exclude that these differences are reflective of differing RNA detection limits. In contrast to Hokkaido where genotype 4 has been reported among blood donations, all our positive donations were genotype 3, which is less pathogenic.

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Key words: blood donors, HEV, NAT testing, serological testing.

Introduction

Human hepatitis E virus (HEV) is the causative agent of viral hepatitis E [1]. HEV, which belongs to the genus *Hepevirus* of the family Hepeviridae, has a diameter of approximately 27–34 nm. The viral capsid is nonenveloped, and the nucleocapsid contains positive-sense single-stranded RNA of approximately 7·3 kb. HEV has one serotype, with genotypes 1–4 (G1–G4) and recently identified genotypes 5 and 6 (G5, G6) [2–4]. Genotypes 3 and 4 are zoonotic, with transmission occurring mainly through ingestion of uncooked or undercooked meat in developed countries [5, 6]. In addition, HEV infections transmitted by transfusion have been reported in Japan [7, 8]. HEV can also be transmitted through solvent-/detergent-treated pooled plasma [9]. On the other hand, HEV-RNA is not detected in plasma derivatives, which have not been implicated in any reported cases of transmission [10]. Previous studies have described the detection rate of HEV-RNA in donor plasma in several countries [11–13], and the results suggest regional variations. The seroprevalence of HEV has also been found to vary by region [14–17]. In Hokkaido, Japan, an HEV nucleic acid amplification testing (NAT) screening trial for donated blood was implemented by Japanese Red Cross (JRC) in 2005 [18], since most HEV patients in this region are infected with genotype 4, which has a higher

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pathogenicity than genotype 3 [19]. Plasma derivatives have been manufactured from donor plasma (source plasma) collected in all regions of Japan. However, the detection rate of HEV-RNA in the donor population in areas other than Hokkaido and the relationship between HEV-RNA detection and seroprevalence of HEV-IgG/IgM in these areas are unclear. This study addressed this issue by evaluating the detection rate of HEV-RNA in source plasma donated between 2004 and 2014.

Materials and methods

A total of 620 140 source donor plasma samples donated between 2004 and 2014 in six Japanese regions (excluding Hokkaido, see figure 1). NAT was used to detect HEV genomes in donor plasma pools of 50 or 500, as briefly described below. Each donor sample was collected from the segment tube of donor bag. A mini-pool of 10 donors was a mixture of 10 donor samples. On the other hand, a mini-pool of 50 or 500 donors was a mixture of 5 or 50 mini-pools of 10 donor samples. A mini-pool sample of 0·2 ml was tested. When HEV-RNA was detected in the mini-pool sample of 50 or 500 donors, the positive donor bag was identified by testing individual donor samples following the mini-pool of 10 donors. Nucleic acids were extracted from test samples using the MagNA Pure 96 System with the MagNA Pure 96 DNA and Viral NA Small Volume kit (Roche Diagnostics, Mannheim, Germany). Viral RNA amplification was carried out on a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using the QuantiTect Probe RT-PCR kit (Qiagen GmbH, Hilden, Germany) and the JVHEVF/JVHEVR and JVHEVP primer and probe set [20]. The detection limit was 152 IU/ml (95% detection limit) for undiluted samples. HEV-RNA-positive donor plasma bags were identified and removed by retesting individual plasma samples when the mini-pool yielded positive results. HEV-RNA-positive plasma samples were evaluated for HEV-RNA viral load, genotype and the presence of anti-HEV immunoglobulin (Ig)G/IgM antibodies. HEV viral load was quantified with the JVHEVF/JVHEVR and JVHEVP primer and probe set as described above. To identify HEV genotype, sequences amplified with primers HE044 and HE041 covering open reading frame 2 were analysed using a 3500 Genetic Analyzer and the BigDye Terminator Cycle Sequencing kit (Applied Biosystems). Sequences were aligned with Clustal W from the DNA Data Bank of Japan based on a 412-bp sequence corresponding to nucleotides 5969–6380 (GenBank accession no. AB073912) [21]. An IgG/IgM anti-HEV enzyme-linked immunosorbent assay kit (Institute of Immunology Co., Tokyo, Japan) was used to detect anti-HEV-IgG/IgM antibodies in samples.

Table 1 (a) Positivity of HEV-RNA of donor plasma (500 pool). (b) Positivity of HEV-RNA of donor plasma (50 pool)

Donated year	Tohoku	Kanto & Koshinetsu	Tokai & Hokuriku	Kinki	Chugoku & Shikoku	Kyushu & Okinawa	Total
(a)							
2004	NT	NT	NT	NT	NT	0/61	0/61
2005	0/34	0/26	0/65	0/82	0/359	0/47	0/613
2006	0/1763	0/3710	0/2255	0/5575	0/7512	0/6152	0/26 967
2007	0/3186	0/11 452	0/5864	0/15 269	0/7547	0/11 713	0/55 031
2008	0/3769	2/16 095 (1/8 048)	0/3740	0/970	0/330	NT	2/24 904 (1/12 452)
Total	0/8752	2/31 283 (1/15 642)	0/11 924	0/21 896	0/15 748	0/17 973	2/107 576 (1/53 788)
(b)							
2007	NT	NT	0/42	0/396	0/372	NT	0/810
2008	0/774	1/4 254	0/3853	0/2769	0/1778	0/1832	1/15 260
2009	0/6041	5/21 367 (1/4 273)	0/7737	1/18 774	0/12 187	0/16 126	6/82 232 (1/13 705)
2010	1/6 561	4/20 168 (1/5042)	0/11 230	0/11 132	2/7526	0/3550	7/60 167 (1/8 595)
2011	1/8 149	5/35 191 (1/7 038)	1/14 273	0/10 798	0/2422	0/3	7/70 836 (1/10 119)
2012	0/25 646	4/52 978 (1/13 245)	0/13 007	0/16 152	0/9 309	NT	4/117 092 (1/29 273)
2013	3/25 872 (1/8 624)	3/62 698 (1/20 899)	1/16 683	0/3956	NT	NT	7/109 209 (1/15 601)
2014	0/8426	2/22 573 (1/11 287)	0/4771	0/11 953	0/9235	NT	2/56 958 (1/28 478)
Total	5/81 469 (1/16 294)	24/219 229 (1/9135)	2/71 596 (1/35 798)	1/75 930	2/42 829 (1/21 415)	0/21 511	34/512 564 (1/15 075)

NT: Not tested.

Results

Donor plasma samples were evaluated; 36 of these tested positive for HEV-RNA, corresponding to an HEV-RNA detection rate of 1/53 788 (500 pool) and 1/15 075 (50 pool) (Table 1a,b). HEV genotype 3, but not genotype 4, was detected among the samples (<1·69–7·22 log_{10} copies/ml). Of the 36 HEV-RNA-positive samples, seven were positive for anti-HEV IgM or IgG (Table 2). Detection rates of 50-pool NAT in each area were as follows: Kyushu and Okinawa (including Fukuoka), 0/21 511; Kinki (including Osaka), 1/75 930 (0·001%); Tokai and Hokuriku (including Aichi), 2/71 596 (1/35 798, 0·003%); Chugoku and Shikoku (including Okayama), 2/42 829 (1/21 415, 0·005%); Tohoku (including Miyagi), 5/81 469 (1/16 294, 0·006%); and Kanto and Koshinetsu (including Tokyo), 24/219 229 (1/9135, 0·011%) (Fig. 1).

Discussion

We determined the rate of HEV-RNA in source plasma collected in Japan between 2004 and 2014. We detected HEV-RNA in 34/512 564 50 donor pools (1/15 075, 0·007%) from donations outside of Hokkaido, whereas the reported detection rate in donors from Hokkaido between 2005 and April 2015 was 348/2 844 182 (1/8 173,

Table 2 Properties of HEV-RNA-positive donor plasma

Collected year	Genotype	Genome amount (log_{10} copies/ml)	Anti-HEV antibody		Collected area
			IgG	IgM	
2008	3	7·22	–	–	Kanto & Koshinetsu
	3	4·79	+	+	Kanto & Koshinetsu
	3	4·64	–	–	Kanto & Koshinetsu
2009	3	3·60	+	–	Kinki
	3	4·14	–	–	Kanto & Koshinetsu
	3	2·34	–	–	Kanto & Koshinetsu
	3	3·34	–	–	Kanto & Koshinetsu
	Unclassified	<1·69	+	–	Kanto & Koshinetsu
	3	3·46	–	–	Kanto & Koshinetsu
2010	3	4·99	–	–	Kanto & Koshinetsu
	3	3·38	–	–	Kanto & Koshinetsu
	3	3·48	–	–	Chugoku & Shikoku
	3	<1·69	+	–	Kanto & Koshinetsu
	3	4·57	–	–	Tohoku
	3	3·56	+	–	Kanto & Koshinetsu
	3	3·93	–	–	Chugoku & Shikoku
2011	3	2·53	–	–	Kanto & Koshinetsu
	3	2·80	+	+	Kanto & Koshinetsu
	3	3·63	–	–	Tohoku
	3	3·56	–	–	Kanto & Koshinetsu
	3	4·06	–	–	Tokai & Hokuriku
	3	3·89	–	–	Kanto & Koshinetsu
	3	3·89	+	–	Kanto & Koshinetsu
2012	3	3·67	–	–	Kanto & Koshinetsu
	3	5·73	–	–	Kanto & Koshinetsu
	3	<1·69	–	–	Kanto & Koshinetsu
	3	4·65	–	–	Kanto & Koshinetsu
2013	3	4·81	–	–	Tohoku
	3	3·12	–	–	Kanto & Koshinetsu
	3	3·86	–	–	Tohoku
	3	<1·69	–	–	Tohoku
	3	4·89	–	–	Tokai & Hokuriku
	3	4·01	–	–	Kanto & Koshinetsu
	3	4·62	–	–	Kanto & Koshinetsu
2014	3	4·75	–	–	Kanto & Koshinetsu
	3	3·75	–	–	Kanto & Koshinetsu

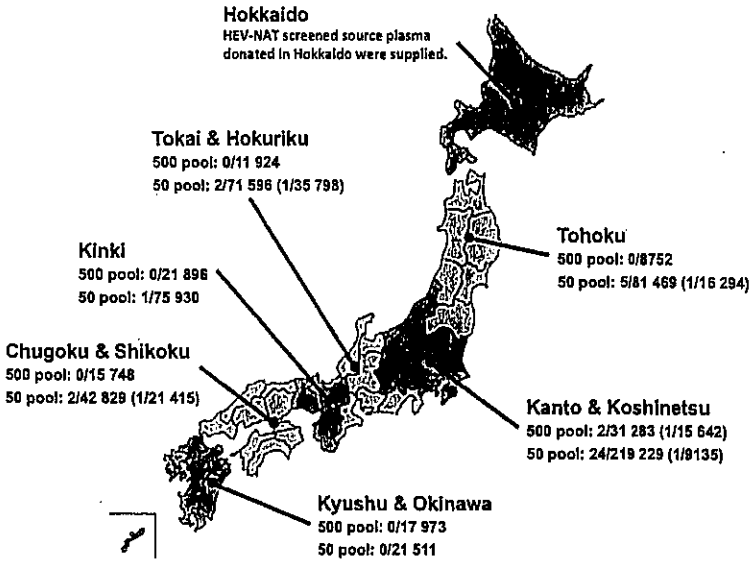

Fig. 1 Positivity rate of HEV-RNA in donor plasma samples derived from seven geographical areas in Japan.

0·012%) [13]. While our rate for donations outside Hokkaido is lower, it is not appropriate to compare the rates directly, since HEV-RNA detection limits differed. Seropositive rates in western Japan – namely, Fukuoka, Okayama, Osaka and Aichi – were 1·7%, 1·0%, 1·1% and 3·2%, respectively; in eastern Japan – namely, Tokyo, Miyagi, and Hokkaido – the rates were 8·6%, 4·4% and 3·9%, respectively [14]. HEV has been reported in the general population as well as in animals in Japan [19]. Reported HEV transmission routes in a nationwide Japanese study were unknown (58%), zoonotic food-borne (31%), travel and import (7·9%), blood transfusion (2·3%) and contact with animal (0·5%) [22]. Also, prevalence seems to be affected by consumption of pork [14]. These findings suggest that rate of HEV-RNA carriage and seroprevalence of HEV-IgG/IgM correlates across regions. In our study, the detection rates of HEV-RNA in Japan were generally lower than in European countries and higher than in the U.S.A [11–13]. However, as the detection limit for the tests applied in these studies vary, it is not appropriate to compare rates directly. The impact of the test detection limit can be seen in our own data where the HEV-RNA detection rate in pools of 50 was almost fourfold higher than in pools of 500. Detection of only genotype 3 in our study, along with previous data from the JRC, demonstrates genotype 4 has to date only been detected in samples donated in Hokkaido [13]. Interestingly, detection rate of HEV genotype 4 in Hokkaido is higher than other areas in the general population and the pathogenicity of genotype 4 is more severe than genotype 3 [22]. It remains unclear why genotype 4 has only been detected in donor samples from Hokkaido. Hence, the publication on HEV issued by the European Medicines Agency is useful for the risk assessment of plasma derivatives of donor plasma samples collected in Japan [23]. More discussion may be needed for the risk of genotype 4 since the genotype 4 has not been detected in the donors in European countries and has more severe pathogenicity than genotype 3. Further studies on solid transplantation and vertical modes of transmission as well as chronic infection are needed to clarify the risk of genotype 4. In addition, the risk posed by animal reservoirs must also be evaluated.

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Conflicts of interest

The authors declare no conflict of interests.

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医薬品
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識別番号・報告回数			報告日	第一報入手日 2016年09月05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	Transfusion 2016; 56(4): 831-836	公表国 スペイン
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

メチレンブルー光処理新鮮凍結血漿によるHIVの伝播：

背景：輸血感染症（TTI）のリスクは、核酸増幅検査（NAT）、および病原体不活性化（PI）を導入することによって最小化されている。この症例報告は、これらの対策にもかかわらず、2人のレシピエントへのHIV-1の感染症例について記述する。
研究デザインと方法：2005年11月にプールされたバフィーコート濃厚血小板（BC-PLT）を輸血された患者において、HIV-1によるTTIの可能性症例が2009年3月に確認された。その後の遡及調査により、同じドナーからメチレンブルー（MB）処理された新鮮凍結血漿（FFP）と赤血球製剤（RBCs）を輸血されていた他の2人の患者を特定した。2005年11月にそのドナーは、44ミニプール（44MP）NATによるHIV-1 RNAと抗HIV抗体の両方とも陰性であった。この献血の保管サンプルとレシピエントのサンプルのウイルス量の測定と配列の解析を行った。
結果:2005年のウインドウピリオドの献血の保管サンプルで個別NATによりHIV-1 RNAが検出され、ウイルス量は135 copy/mLであった。HIV-1感染は、BC-PLT（血漿65 mL）とMB-FFP（血漿261 mL）の両方のレシピエントで確認されたが、4週間前のRBCs（血漿20 mL）のレシピエントにおいて感染は確認されなかった。配列解析は、TTIと相同性のあるドナーとレシピエントから分離されたウイルス株の間に近い系統学的関係を明確にした。
結論：MB-FFPとBC-PLT中の約17600と4400のビリオンは、感染を引き起こしたが、赤血球中の1350のビリオンでは感染しなかった。個別NATは、この感染を阻止したが、MP-NATとMB-PIの組み合わせでは阻止しなかった。

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、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
ヒト免疫不全ウイルス（Human Immunodeficiency Virus：HIV）は、レトロウイルス科（retrovirus）レンチウイルス属（Lentivirus）に属し、直径約110nmのエンベロープを持つ一本鎖RNAウイルスである。血清学的にHIV-1とHIV-2に分類され、HIV-1は塩基配列により4群に分類され、グループM(Major)、グループO（Outlier）、グループN（non-M/non-O）、グループP（pending）に分けられるが、世界的に分布しているウイルスの多くがグループMに属している。本剤の原料となる血液は抗HIV-1抗体，抗HIV-2抗体陰性であることを確認し、更にNATスクリーニングを実施し、適合した血漿を使用している。万一、原料血漿にHIVが混入したとしても、モデルウイルスのHIV-1のウイルスクリアランス試験成績から、本剤の製造工程において不活化・除去されると考える。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

Transmission of human immunodeficiency virus Type-1 by fresh-frozen plasma treated with methylene blue and light

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BACKGROUND: The risk of transfusion-transmitted infection (TTI) has been minimized by introduction of nucleic acid testing (NAT) and pathogen inactivation (PI). This case report describes transmission of human immunodeficiency virus Type 1 (HIV-1) to two recipients despite these measures.

STUDY DESIGN AND METHODS: In March 2009 a possible TTI of HIV-1 was identified in a patient that had received pooled buffy coat platelet concentrate (BC-PLT) in November 2005. The subsequent lookback study found two more patients who had received methylene blue (MB)-treated fresh-frozen plasma (FFP) and red blood cells (RBCs) from the same donation. In November 2005 the donor had tested negative for both HIV antibodies and HIV-1 RNA by 44 minipool (44 MP) NAT. Repository samples of this donation and samples from the recipients were used for viral load (VL) and sequence analysis.

RESULTS: HIV-1 RNA was detectable by individual donation (ID)-NAT in the repository sample from the 2005 window period donation and a VL of 135 copies/mL was measured. HIV-1 infection was confirmed in both recipients of both BC-PLT (65 mL of plasma) and MB-FFP (261 mL of plasma), but not in the patient that had received 4-week-old RBCs (20 mL of plasma). The sequence analysis revealed a close phylogenetic relationship between the virus strains isolated from the donor and recipients, compatible with TTI.

CONCLUSIONS: Approximately 17,600 and 4400 virions in the MB-FFP and BC-PLT were infectious, but 1350 virions in the RBCs were not. ID-NAT would have prevented this transmission, but the combination of MP-NAT and MB-PI did not.

The main cause of transfusion-transmitted infections (TTIs) are incident window period (WP) infections in donors that are not detected by the screening tests.[1] It is estimated that a validated donor selection policy can eliminate 86% to 90% of this risk.[2] The length of the WP for human immunodeficiency virus Type-1 (HIV-1) has decreased from 56 days, with first-generation serologic tests,[1] to approximately 19 and 15 days with the third- and fourth-generation serologic assays, respectively.[3] The introduction of nucleic acid amplification technology (NAT) has further reduced the diagnostic WP. The length of the infectious WP depends on the sensitivity of the NAT method, the minipool (MP) size, and the transfusion plasma volume.[4] In addition, the first-generation polymerase chain reaction (PCR) assays

ABBREVIATIONS: BC-PLT = buffy coat platelet concentrate; ID = individual donation; LOD(s) = limit(s) of detection; MB = methylene blue; MID50 = 50% minimum infectious dose; MP = minipool; MSM = man practicing sex with men; PI = pathogen inactivation; TMA = transcription-mediated amplification; TTI(s) = transfusion-transmitted infection(s); VL(s) = viral load(s); WP = window period.

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are known to have caused detection failures due to mismatches in primers or probes.[5-7]

In July 1999, hepatitis C virus (HCV) NAT was introduced in the Valencian Regional Blood Transfusion Center (Valencia, Spain) in minipools of 44 donations (44 MP-NAT).[8] A few years later, in February 2004, HIV-1 RNA screening was incorporated in this 44 MP-NAT procedure. Later, in July 2006, individual-donation (ID) NAT was introduced in our center, which reduced the infectious WP to 1 to 4 days depending on the estimates of the 50% minimum infectious dose (MID50).[3,4] In addition, since November 1997 pathogen inactivation (PI) has been performed for fresh-frozen plasma (FFP) by adding methylene blue (MB) and subjecting the plasma bags to a visible light source. Validation studies claimed more than 6 logs reduction of infectivity of HIV model virus in tissue culture by this PI procedure.[9] Hence, MP-NAT screened MB-FFP units are believed to be safe with regard to HIV-1 transmission. This report calls this in question.

CASE REPORT

In March 2009, a Valencian hospital identified HIV-1 seroconversion in a 24-year-old patient who was diagnosed with T-cell acute lymphoblastic leukemia. He had received blood components from 77 donors from September 2005 to April 2006. During the traceback process, 63 donations were identified from returning donors who had negative serologic results. Hence, they were excluded from being the source of transmission. The remaining 14 donors were called back for a follow-up sample. Twelve donors returned, of whom 11 tested HIV-1 negative. The remaining donor tested positive for HIV-1 antibodies and RNA by ID-NAT in April 2009. The donor acknowledged being an HIV carrier since May 2006. His last blood donation was on November 23, 2005, from which red blood cells (RBCs), MB-FFP, and buffy coat platelet concentrate (BC-PLT) had been prepared. The buffy-coat had been included in a platelet (PLT) pool transfused to the recipient who subsequently had seroconverted to anti-HIV, as confirmed in a sample taken in 2009. At the time of donation, all mandatory screening assays were negative, including HIV-1 RNA by reverse transcription–PCR (RT-PCR) in 44 MP format. The cellular components from this donation had been leukoreduced. The lookback identified another HIV-1–infected recipient who had received MB-FFP in the context of a liver transplant. The other patient, who had received the RBC unit, was not infected, according to anti-HIV test, 23 months after transfusion. This could not be confirmed in 2009, because she had died earlier by a cause not related to transfusion.

MATERIALS AND METHODS

Routine tests

All blood products transfused were screened using a chemiluminescent immunoassay for anti-HIV-1 and HIV-2 antibodies (Abbott PRISM anti-HIV-1/2 assay, Abbott Laboratories, North Chicago, IL) and HIV-1 RNA in 44 MP-NAT using PCR (COBAS AmpliScreen HIV-1 test, Version 1.5, Roche Molecular System, Branchburg, NJ). For each donor, plasma containing EDTA was centrifuged at 1750 × *g* and held at 4°C until processed. An application for the Hamilton dispenser designed in the Galicia Blood Transfusion Center was used for pooling. The final volume of each 44-member MP was 1 mL. To minimize dilution effects, each MP was centrifuged at 23,600 × *g* for 60 minutes at 2 to 8°C. Then 900 µL of supernatant was discarded, and the 100 µL of RNA pellet was extracted.[8] The 95 and 50% lower limit of detection (LOD) of HIV-1 RNA in the multiprep Ampliscreen method was 78 (60-137) and 22 (13-29) IU/mL, respectively, according to analytical sensitivity studies on the WHO 97/656 International Standard reported by the manufacturer in the package insert. Recalled donors in April 2009 were also tested by ID-NAT by transcription-mediated amplification (TMA; Procleix Ultrio Assay on Tigris System, Chiron/Novartis, Emeryville, CA). The 95 and 50% LOD of HIV-1 RNA on the WHO International Standard 97/650 in the Ultrio assay was reported to be 20.3 (18.1-23.1) and 4.5 (3.5-5.9) IU/mL.[10] The serum sample from the HIV-1–infected donor obtained in 2009 tested anti-HIV positive repeat reactive and confirmed by immunoblot assay (INNOLIA HIV I/II Score, INNOGENETICS N.V., Gent, Belgium). The donor sample was also ID-NAT reactive and was identified as HIV-1 RNA positive by TMA discriminatory testing. Viral loads (VLs) were determined using the Roche COBAS Amplicor HIV-1 Monitor Test Version 1.5 (Roche Molecular Systems). Recipient plasma samples were obtained from the hospitals where they were receiving treatment; each one was frozen and sent to the reference center for amplification, sequencing, and phylogenetic analysis.

Phylogenetic analysis

Two plasma samples of the donor were available for sequence analysis, one from the donors' repository sample of 2005 and the other obtained in 2009. Plasma samples from the infected recipients were obtained in 2009. Plasma from 37 newly HIV-1 diagnosed persons obtained in the same city between 2004 and 2009 were used as local control samples. Specimens from the donor, the infected recipients, and the local controls underwent HIV RNA purification followed by RT-PCR and direct sequencing using procedures described elsewhere with minor modifications.[11] Sequences were obtained for a 728-nucleotide-long region from the *pol* gene, comprising partial protease (Codons 10 to 99) and reverse transcriptase (Codons 1 to

TABLE 1. First HIV-1–infected patient results: traceback study

Subject	Diagnosis for transfusion	Time of storage (days)	Day of transfusion	Date of blood drawing	Viral testing results				
					NAT (TMA)	HBsAg	Anti-HCV	Anti-HIV	HIV Imblot
BC-PLT recipient	T-cell acute lymphoblastic leukemia	2	Nov 25, 2005	Oct 14, 2005	ND	Neg	Neg	Neg	NA
		NA	NA	Feb 06, 2009	ND	Neg	Neg	Pos	ND

Imblot = immunoblot; NA = not applicable; ND = not done or not reported; Neg = negative; Pos = positive.

TABLE 2. Subsequent lookback study

Subject	Diagnosis for transfusion	Time of storage (days)	Day of transfusion	Date of blood drawing	Viral testing results				
					NAT (TMA)	HBsAg	Anti-HCV	Anti-HIV	HIV Imblot
Donor	NA	NA	NA	Nov 23, 2005	Pos*	Neg	Neg	Neg	NA
	NA	NA	NA	Apr 28, 2009	Pos	Neg	Neg	Pos	Pos
MB-FFP recipient	In the context of liver transplant	15	Dec 8, 2005	May 24, 2009	ND	Neg	Neg	Pos	ND
RBC recipient	Anemia of chronic disease	27	Dec 20, 2005	Nov 15, 2007	ND	Neg	Neg	Neg	ND

* In 2005 NAT tested negative in 44 MP-NAT using PCR. This same sample, from the repository, tested positive in 2009 by ID-NAT using TMA.
Imblot = immunoblot; NA = not applicable; Neg = negative; ND = not done or not reported; Pos = positive.

152) genes. To establish the relationship between viruses obtained from the donor and the infected recipients, a phylogenetic analysis was carried out as described before.[12]

Pathogen inactivation

Plasma inactivation was performed using MB-Plasma Theraflex (MacoPharma, Tourcoing, France), following the manufacturer's instructions.

RESULTS

On November 23, 2005, a donation that transmitted HIV to two recipients tested negative for anti-HIV and HIV-1 RNA by NAT in 44-member MP and all other required screening tests. In March 2009, HIV seroconversion was detected in the BC-PLT and MB-FFP recipients. The RBC recipient remained healthy and anti-HIV negative 23 months after transfusion. In April 2009, a repository sample collected in 2005 from the donor tested HIV-1 RNA positive, with a VL of 135 copies/mL. The infectious blood was donated by a man practicing sex with men (MSM) aged 42 who knew of his HIV infection in May 2006, although he did not report this information to the blood center. A more recent blood sample from this donor, collected in April 2009, tested positive for anti-HIV by chemiluminescent immunoassay, immunoblot assay, and HIV-1 RNA by TMA (Tables 1 and 2).

Viral RNA was isolated from two donors' samples (the 2005 repository and 2009 blood drawing) and from each recipient's samples. HIV-1 RNA could not be isolated by the RT-PCR procedure in the reference laboratory from the 2005 repository sample probably due to its low VL and therefore could not be sequenced. The HIV RNA from the second donor sample and the samples from the MB-FFP recipient and the BC-PLT recipient, all collected in 2009, was successfully amplified and sequenced. Using different amplification strategies, two closely related nucleotide sequences were obtained from the sample corresponding to the MB-FFP's recipient. The four sequences corresponded to HIV-1 subtype B and they grouped in a well-supported monophyletic clade when compared with local reference sequences for this subtype (Fig. 1). The mean nucleotide identity for these sequences (excluding one redundant sequence from the MB-FFP's recipient) was 99.3% (range, 99.0%-99.7%) whereas the mean nucleotide identity to unrelated control sequences was 94.2% (range, 91.9%-96.8%). This type of phylogenetic grouping and high genetic identity are indicative of samples sharing a relatively recent common origin, compatible with a TTI from blood components from a single donor.

DISCUSSION

To our knowledge this is the first case of HIV-1 transmission related to a blood component undergoing a PI process. It is difficult to imagine how MB-treated plasma that

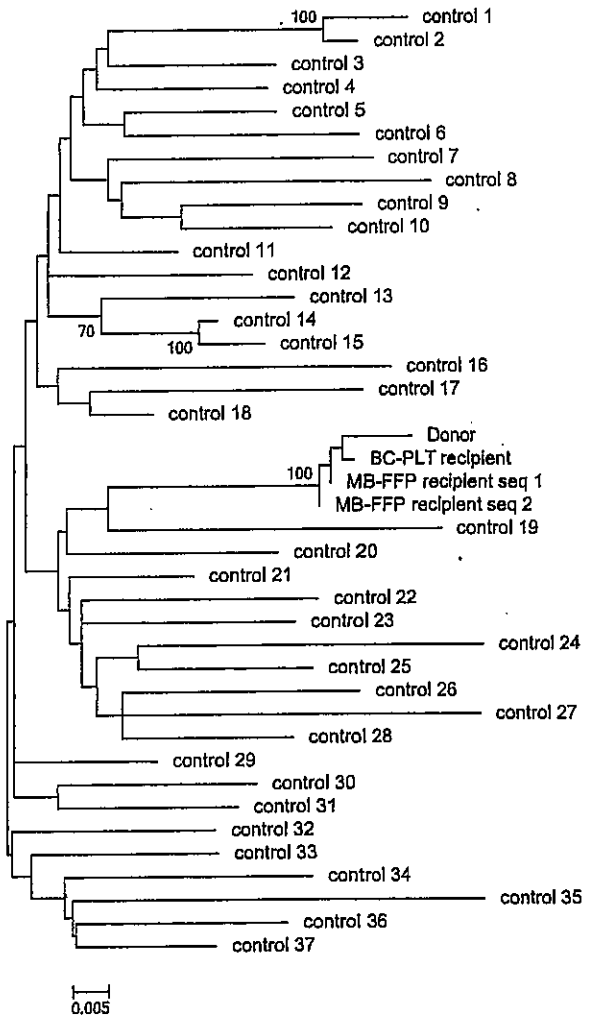

Fig. 1. Maximum likelihood phylogenetic tree for pol sequences (728 nucleotides) from blood donor, BC-PLT recipient, and MB-FFP recipient (two sequences) along with 37 local control sequences. The phylogenetic tree was built with RAxML with bootstrap support for branches after 1000 replicates. Only bootstrap values higher than 70% are shown. The scale bar indicates number of substitutions per nucleotide.

tested nonreactive in 44 MP-NAT (Ampliscreen) method used in 2005 might have transmitted HIV-1. One explanation is that the PI failure could be due to manufacturing or process errors, such as: 1) the Plasmaflex PLAS4 filter was defective, allowing the passage of residual HIV-infected white blood cells into the illumination bag. The inactivation of intracellular virus by MB is known to be inefficient;[9] 2) there was no MB pill in the bag system; 3) the MB tablet was not completely dissolved or homogeneously mixed over all compartments of the bag; 4) something had gone wrong with the illumination of the plasma bag (the inactivation is known to be limited without visible light); 5) standard operating procedures related to good manufacturing practices in the blood center were violated. Another explanation may be that viruses associated with human cell membranes or lipids in plasma are partially protected from inactivation by MB. The manufacturer found more than 6-log reduction of HIV-1 by MB treatment in tissue culture experiments but these may not be representative for the infectivity of HIV in the WP of plasma transfused to humans. Finally, it is theoretically possible that the viremia level (135 copies/mL) of the HIV-1 strain detected in the infectious donation was underquantified by the Amplicor Monitor PCR assay as a consequence of oligonucleotide mismatches. This, however, seems unlikely since the RBC unit did not transmit HIV infection and other differential transmission cases in which RBCs were not infectious were associated with even lower VL.[13]

The observed transmission of HIV-1 by MB-FFP and BC-PLT but not by the RBC unit is not surprising because:

1. RBCs contain approximately 20 mL plasma;[14] therefore, fewer virions than the BC-PLT and MB-FFP unit in which 65 and 261 mL of plasma was present.
2. The MB-FFP and BC-PLT recipients were receiving immunosuppressive therapy, whereas the RBC recipient was not.
3. The storage period of the RBCs before transfusion was 27 days (in contrast to only 2 days of the infectious BC-PLT) during which the infectivity of the viral particles has likely been more than 10-fold reduced as has been observed in tissue culture experiments.[15] Weusten and coauthors[4] constructed a probabilistic infectivity risk model for NAT-screened units donated within the WP. A major driver of the residual risk in this model is the MID50 that may lie between 1 and 10 virions in FFP units or BC-PLT but is likely much higher (between 100 and 1000 virions) in longer-stored RBC units as estimated from MP-NAT breakthrough transmission cases.[14]

Since plasma volume was 261 mL, the amount of virions in MB-FFP was calculated to be 17,618 particles (since one virion contains two RNA copies); likewise, 65 mL of BC-PLT contained 4388 virions and RBC carried 1350 virions (20 mL of plasma). One can imagine that the amount of infectious virus in the BC-PLT stored for 2 days was logarithmically higher than in the RBC unit, in which the infectivity of the virus had likely reduced during the long storage period of 4 weeks. For example, with the formulas of Weusten and colleagues,[4] it can be estimated that the probability of infectivity of 1350 virions in the RBC unit would be reduced from 100% to 25% if the MID50 had reduced 1000-fold (from 1-10 to 1000-10,000 copies). Finally, it must be emphasized that the RBC recipient was immunocompetent, while the others were under immunosuppressive therapy. If the MB-FFP unit (like the RBC

unit) had also been borderline infectious the immune status of the liver transplant patient could very well have contributed to the outcome of the infection. It is important to note that these transmission cases would not have occurred if ID-NAT had been in place in 2005. The 95 and 50% LODs of the Ultrio assay are estimated at 13.8 and 2.4 copies/mL, whereas for the multiprep Ampliscreen assay these were 135 and 38 copies/mL, respectively (calculated from the LODs reported in IU in methods with a conversion factor of 0.58).[10] With a MID50 of one virion and a doubling time of 0.85 days it can be calculated with the formulas given by Weusten and coworkers[4] that the lengths of the infectious WPs for RBC, BC-PLT, and FFP transfusion were 4.2, 5.7, and 7.4 days, respectively, and for the previously used 44 MP-NAT system, 12.2, 13.6, and 15.3 days. Hence the introduction of ID-NAT reduced the infectious WP by 8 days.[16]

Nevertheless, in Spain, HIV RNA blood donation screening is not mandatory nowadays. However, there is no doubt of the added value of NAT or additional sensitivity of ID-NAT compared to MP-NAT.[5,17] It should be recognized that all blood safety programs have limitations and that absolute safety, in terms of absence of infectious risk, cannot be guaranteed.[18] Careful donor selection remains critical, even in the era of application of both NAT and PI.[19] This is the first step to avoid individuals at risk of being in an early infection stage from donating. In our case, the donor did not admit MSM practices and risk for HIV infection when he donated in 2005. In Spain, 74% of HIV-positive blood donations are given by noncompliant MSM donors.[20]

In conclusion, the following lessons can be learned from these HIV-1 transmission cases: 1) PI methods may not always be sufficiently efficacious (as was recently also observed by transmission of hepatitis E virus by FFP treated with the Intercept method).[21] 2) Zero risk is not attainable even if a combination of MP-NAT and PI is used, but the residual risk may become negligible when ID-NAT is combined with PI. 3) Efficient quality control checks on proper performance of NAT and PI may need to be developed to guarantee consistent efficacy of these interventions. 4) Further improvements in the sensitivity of NAT screening tests and the effectiveness of PI are desirable, considering that, according to conservative estimates, one infectious virion in a blood component is enough to cause infection in a recipient. 5) Continuing attention should be given to the selection of safe blood donors[22] and finally 6) with limited resources currently available the cost-effectiveness of the applied blood screening methods and PI needs to be seriously monitored.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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No. 7

医薬品 研究報告 調査報告書

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販売名(企業名)	濃厚血小板-LR「日赤」(日本赤十字社) 照射濃厚血小板-LR「日赤」(日本赤十字社) 濃厚血小板HLA-LR「日赤」(日本赤十字社) 照射濃厚血小板HLA-LR「日赤」(日本赤十字社) 照射洗浄血小板-LR「日赤」(日本赤十字社) 照射洗浄血小板HLA-LR「日赤」(日本赤十字社)			

研究報告の概要

○ハイチからイタリアに帰国した渡航者にて発症後6ヶ月間にわたり精液から持続的にジカウイルス(ZIKV)RNAを検出(2016年2月)。
2016年1月後半にローマのイタリア国立感染症研究所に、30代前半の男性が、2016年1月中旬から2月初めまでのハイチ滞在時に発熱、脱力感及び紅斑性発疹を呈し、その4日後にZIKV IgM抗体によりZIKV感染と診断されたことが報告された。
患者は発症後14日目にイタリアに帰国し、17日目に採取した唾液を用いたリアルタイムRT-PCRによりZIKV RNAが検出されたが、血清と尿検体は共に陰性であった。91日目の尿、唾液及び精液からは検出された。134日目では精液のみから検出され、続く188日目(発症の6ヶ月後)の時点においても再び精液検体からZIKV RNAが検出され、当該患者は未だ観察下にあった。当該患者は、いかなる慢性疾患及び免疫学的疾患にも罹患していなかった。
回復期に得られた体液検体によりVero-E6細胞を用いた感染実験を行ったが、感染は確認できなかったことから、ベクターを介することなく唾液、尿及び精液がZIKV感染源となる可能性を決定的に提示することはできない。
我々が得た知見は、ZIKVの性感染の可能性を支持するものであり、ベクターを介さないZIKV感染について調査を継続することの重要性を強調している。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
ジカウイルス(ZIKV)感染症の発症後91日目の尿、唾液及び精液、並びに188日目の精液からZIKV RNA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、平成28年2月3日付厚生労働省医薬・生活衛生局血液対策課長事務連絡「ジカウイルスによることが疑われる小頭症等の増加に関するWHO緊急委員会宣言について(注意喚起)」の発出を受け、同月4日付で各血液センターに対し問診時の帰国(入国)後経過日数の確認を徹底するよう指示した。さらに同年7月1日から、ジカウイルス感染症と診断され、治癒後1カ月間経過していない場合は献血不適としている。今後もジカウイルス感染症に関する新たな知見等について情報の収集に努める。

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RAPID COMMUNICATIONS

Persistent detection of Zika virus RNA in semen for six months after symptom onset in a traveller returning from Haiti to Italy, February 2016

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A man in his early 30s reported in January 2016 a history of fever, asthenia and erythematous rash during a stay in Haiti. On his return to Italy, ZIKV RNA was detected in his urine and saliva 91 days after symptom onset, and in his semen on day 188, six months after symptom onset. Our findings support the possibility of sexual transmission of ZIKV and highlight the importance of continuing to investigate non-vector-borne ZIKV infection.

Case description and laboratory investigations

In the second half of January 2016, a previously healthy man in his early 30s reported to the National Institute of Infectious Diseases in Rome, Italy, a history of five-day self-limiting febrile syndrome (<38 °C) associated with asthenia and an erythematous rash during a stay in Haiti from mid-January to early February 2016. Zika virus (ZIKV) infection was diagnosed in Haiti by ZIKV-specific IgM serology four days after symptom onset (Figure). He returned to Italy 14 days after symptom onset.

Dengue virus and chikungunya virus infections were ruled out following testing of serum and urine samples taken 17 days after symptom onset by both qualitative real-time reverse transcription (RT)-PCR (RealStar Dengue RT-PCR Kit and RealStar Chikungunya RT-PCR Kit, altona Diagnostics, Germany) and serology (indirect immunofluorescence assay (IFA), Arbovirus Fever Mosaic 2, IgM and IgG, Euroimmun, Germany). ZIKV serology (IFA, Arbovirus Fever Mosaic 2, Euroimmun) was positive: ZIKV IgM and IgG antibody titres were 1:160 and 1:640, respectively. Serum ZIKV-specific neutralising antibodies were confirmed by microneutralisation test [1]. ZIKV real-time RT-PCR (RealStar Zika Virus RT-PCR Kit, altona Diagnostics) in saliva was positive with a threshold cycle (CT) value of 36.4; serum and urine samples were both negative.

Testing of convalescent sera taken 91 and 134 days after symptom onset were ZIKV real-time RT-PCR negative. On day 91, the test was positive for urine, saliva and semen samples, with CT values of 36.1, 35.4, and 29.6, respectively. On day 134, only a semen sample was positive (CT: 32.5). At the subsequent follow-up, on day 188, a semen sample was again positive (CT: 30.2); the patient is still under evaluation. The patient was not affected by any chronic disease or immunological impairment.

All samples were tested also using a pan-flavivirus NS5 nested RT-PCR (modified from [2]), followed by sequencing of the amplicons (data not shown) to exclude any sample mismatch.

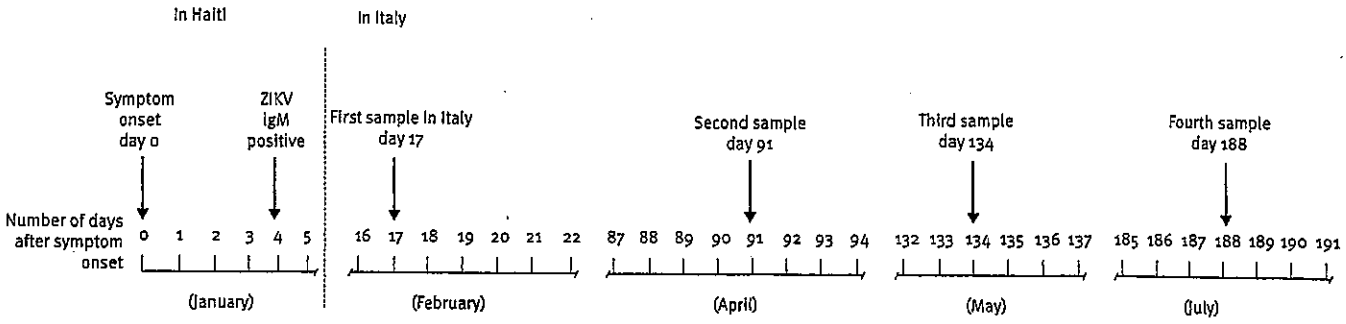
On day 91, ZIKV IgM and IgG titres were 1:40 and 1:1,280, and on day 134, 1:20 and 1:2,560, respectively.

ZIKV isolation on Vero-E6 cells was attempted with all the collected samples. Briefly, bodily samples were diluted 1:5 in serum-free Dulbecco's-modified Eagle's medium (D-MEM) with antibiotics, inoculated into Vero-E6 cells that were 24 hours-old and then incubated for 60 minutes at 37 °C. After incubation, D-MEM with 2% heat-inactivated fetal bovine serum was added. The cells were followed daily for the appearance of cytopathic effects. After seven days, the cells were subcultured by scraping them and adding fresh cells. Each blind subpassage (three times) was checked for the presence of ZIKV RNA by real-time RT-PCR. No ZIKV isolates were obtained from samples collected during the convalescent phase.

Throughout the course of the ZIKV infection, the patient always had protected sexual intercourse with his spouse, using condoms. His spouse did not report ZIKV-related symptoms, and as at 18 July 2016, her ZIKV serology was still negative.

FIGURE

Laboratory findings related to Zika virus infection in a traveller returning from Haiti to Italy, February–July 2016



Type of test and sample	Results			
	Day 17*	Day 91*	Day 134*	Day 188*
ZIKV real-time RT-PCR serum	Neg	Neg	Neg	NT
ZIKV real-time RT-PCR urine	Neg	Pos (CT: 36.1)	Neg	NT
ZIKV real-time RT-PCR saliva	Pos (CT: 36.4)	Pos (CT: 35.4)	Neg	NT
ZIKV real-time RT-PCR semen	NT	Pos (CT: 29.6)	Pos (CT: 32.5)	Pos (CT: 30.2)
IFA ZIKV IgM titre	1:160	1:40	1:20	1:20
IFA ZIKV IgG titre	1:640	1:1,280	1:2,560	1:640
MNT antibody titre	1:160	≥1:320	≥1:320	NT

*Number of days after symptom onset.

CT: threshold cycle; IFA: indirect immunofluorescence assay; NT: not tested; Neg: negative; Pos: positive; RT-PCR: reverse transcription-PCR; ZIKV: Zika virus; MNT: microneutralisation test.

Background

Zika virus is a single-stranded RNA virus (genus *Flavivirus*) mainly transmitted by the *Aedes* mosquito, as well as through sexual contact with symptomatic and, possibly, asymptomatic individuals [3,4]. This non-vector-related mode of transmission was first described in 2008 in the United States [5] and was then reported in several other countries [3,4,6,7].

ZIKV RNA can be detected in different bodily fluids with a wide range of viral loads, depending on the sampling time since acute infection [8,9]. ZIKV from human semen samples has been isolated in African green monkey Vero cells [10] and higher viral loads have been detected in sperm compared with other bodily samples during the convalescent phase [11]. Previous reports have shown that ZIKV RNA has been detected in semen up to day 62 after symptom onset [12-14]. Taken together, these data suggest that virus could replicate specifically in the male genital tract and may persist in semen, with implications for potential male-to-female sexual transmission, even in the absence of haematospermia.

Discussion

In previous reports, convalescent phase saliva and urine samples were positive by ZIKV real-time RT-PCR in 39 days after symptom onset [3,14].

For the case described here, detection of ZIKV RNA in urine and saliva 91 days after symptom onset and in semen up to day 134 might indicate a possible role played by other non-vector modes of transmission during kissing or vaginal, oral and anal sex. Because of the lack of virus isolation from all the collected samples, we cannot definitively state that saliva, urine and semen represent a potential source of ZIKV that could be transmitted without a vector. During the outbreak in French Polynesia, ZIKV was more frequently detected in saliva than in blood after the first week from symptom onset [13] and it was isolated on day 6 from the saliva of a patient during acute ZIKV infection [14]. No cases involving ZIKV transmission through biological fluids other than semen have been reported, but potential transmission of ZIKV through saliva warrants investigation [15].

The detection of ZIKV RNA in semen up to day 134 might indicate a prolonged potential risk for sexual transmission, for a period longer than previously reported [12]. In reports of Ebola virus disease, suspected sexual transmission of Ebola virus occurred 179 days after onset of the disease [16] and Ebola virus RNA has been detected in semen for 4–6 months after disease onset in 43% of survivors [17].

The lack of isolation of ZIKV from the various biological samples of our patient, during the convalescent phase, is not unexpected. The high CT values found are consistent with a low Zika viral load during the convalescent phase of infection, making it difficult to obtain viral cultures and thus sequence data.

Because of prolonged detection of ZIKV RNA and isolation of replication-competent virus in semen [11,13], the testes are considered an immunoprivileged replication site for ZIKV [18]. Seminal shedding of ZIKV seems to coincide with the duration of spermatogenesis (69–80 days), suggesting a hypothesis of infection of sperm progenitors and viral shedding during the differentiation process [18]. Our results showed the persistence of ZIKV RNA for 188 days after symptom onset, but this is not sufficient to support a hypothesis of ZIKV RNA being present in sperm progenitors until spermatozoa are fully differentiated and eliminated. Further studies are needed in order to understand persistence of ZIKV in semen and the potential risk of ZIKV sexual transmission.

Public health impact

The European Centre for Disease Prevention and Control and the World Health Organization recommend that all travellers returning from areas with ongoing ZIKV transmission should adopt safer sex practices or consider abstinence for at least eight weeks after their return [4,19]; if men have ZIKV-related symptoms, they should adopt safer sex practices or consider abstinence for at least six months.

Considering the 80% incidence rate of asymptomatic ZIKV infection [20], further studies are needed to assess viral persistence in asymptomatic men and the potential risk for sexual transmission and fetal abnormalities following infection during pregnancy. The prolonged genital shedding reported here may have implications for screening measures to detect ZIKV RNA for semen cryopreservation in sperm banks [21].

Acknowledgements

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Conflict of interest

None declared.

Authors' contributions

Emanuele Nicastri was the physician in charge of the patient, Giuseppina Liuzzi was the physician in charge of the spouse of the patient; Concetta Castilletti was the virologist in charge of the virological assay for Zika virus diagnosis, Marco Iannetta wrote the manuscript, Maria R. Capobianchi, who is the person responsible for the virology laboratory unit, and Giuseppe Ippolito, who supervises all the clinical and translational research on emerging and re-emerging pathogens, contributed to the discussion and reviewed the manuscript.

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別紙様式第2-1

No. 8

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2016. 9. 12	該当なし	

一般的名称	人血小板濃厚液	研究報告の公表状況		公表国
販売名(企業名)	濃厚血小板-LR「日赤」(日本赤十字社) 照射濃厚血小板-LR「日赤」(日本赤十字社) 濃厚血小板HLA-LR「日赤」(日本赤十字社) 照射濃厚血小板HLA-LR「日赤」(日本赤十字社) 照射洗浄血小板-LR「日赤」(日本赤十字社) 照射洗浄血小板HLA-LR「日赤」(日本赤十字社)		Septfons A, Leparc-Goffart I, Couturier E, et al. Euro Surveill. 2016 Aug 11;21(32).	フランス

研究報告の概要

○フランス本土における渡航関連及び地域内のジカウイルス(ZIKV)感染(2016年1月1日～7月15日)

フランスの本土では、2016年の夏に、蚊媒介によるZIKVの地域内感染の条件(ウイルスを伝播する能力を有するベクターであるヒトスジシマカの存在、ZIKV感染地域から帰国した多数の渡航者の存在、ZIKVに対する未感染集団であること)が全て満たされた。フランス本土では、2016年1月1日から7月15日までの期間に、ZIKV感染検査により625名の感染者が報告された。617名(99%)はZIKV流行地への最近の渡航が報告され、8名(1%)は感染した渡航者との性交渉により感染しており、現在まで欧州におけるZIKVのベクター伝播は報告されていない。2016年7月15日の時点で156名(25%)の感染者は、ヒトスジシマカが存在する地域の活動期(5月～11月)にウイルス血症であったことが確認されている。これらの症例は適切なベクターコントロール対策が実施されていない状況下では、地域のベクター伝播の引き金となる可能性がある。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
フランス本土で2016年1月1日から7月15日までの期間に、625名のジカウイルス(ZIKV)感染者が報告された。617名(99%)はZIKV流行地への渡航により、残る8名(1%)は感染した渡航者との性交渉により感染しており、現在まで欧州におけるZIKVのベクター伝播は報告されていないという報告である。	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、平成28年2月3日付厚生労働省医薬・生活衛生局血液対策課長事務連絡「ジカウイルスによることが疑われる小頭症等の増加に関するWHO緊急委員会宣言について(注意喚起)」の発出を受け、同月4日付で各血液センターに対し問診時の帰国(入国)後経過日数の確認を徹底するよう指示した。さらに同年7月1日から、ジカウイルス感染症と診断され、治癒後1カ月間経過していない場合は献血不適としている。今後もジカウイルス感染症に関する新たな知見等について情報の収集に努める。

Travel-associated and autochthonous Zika virus infection in mainland France, 1 January to 15 July 2016

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During summer 2016, all the conditions for local mosquito-borne transmission of Zika virus (ZIKV) are met in mainland France: a competent vector, *Aedes albopictus*, a large number of travellers returning from ZIKV-affected areas, and an immunologically naive population. From 1 January to 15 July 2016, 625 persons with evidence of recent ZIKV infection were reported in mainland France. We describe the surveillance system in place and control measures implemented to reduce the risk of infection.

From 1 January to 15 July 2016, 625 persons with evidence of recent Zika virus (ZIKV) infection were reported in mainland France. This large influx of ZIKV-infected travellers reflects the current epidemic of ZIKV infection in the French departments and collectivities of the Americas – Martinique, Guadeloupe, Saint Martin, Saint Barthélemy and French Guiana [1] – and coincides with the activity period (May to November) of the vector *Aedes albopictus* in mainland France. Because of an increase in the number of travellers from the French departments and collectivities of the Americas during the summer holidays, the risk of introduction and transmission of ZIKV in mainland France is at its height in the summer months of 2016. We describe the surveillance system and control measures implemented in mainland France to reduce this risk, as well as some preliminary results.

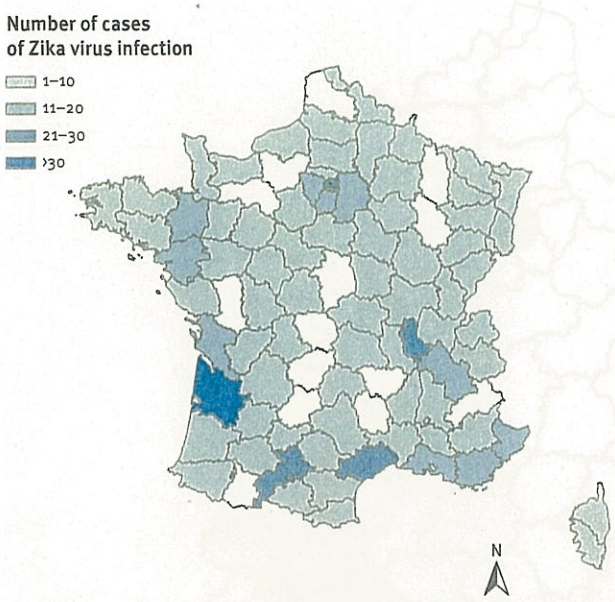
Surveillance of Zika virus infection in mainland France

Surveillance of ZIKV infections has been integrated into the system implemented for chikungunya and dengue in mainland France, which has been in place since 2006 [2]. The objectives of the surveillance are to detect imported or autochthonous cases early and to prevent local transmission by the early implementation of vector control measures. An additional specific objective for ZIKV surveillance is to identify ZIKV-infected pregnant women, in order to ensure enhanced follow-up of their pregnancies in specialised centres, and describe their pregnancy outcomes.

The surveillance system comprises several components related to ZIKV infection:

FIGURE 1

Cases of Zika virus infection by administrative department, mainland France, 1 January–15 July 2016 (n = 625)



Source: Santé publique France, French national public health agency, France, 2016.

• nationwide year-round notification of probable and confirmed cases of ZIKV infection (in place since 1 January 2016, mandatory since 5 June 2016);

• seasonal enhanced surveillance in administrative departments where the vector is established. From 1 May to 30 November, when the vector is active, all suspected imported cases must be immediately reported to the regional health authorities. Without waiting for laboratory confirmation, an entomological investigation is immediately carried out around the places visited by the patient during their likely viraemic period (defined as two days before until seven days after the onset of symptoms). According to the findings, appropriate vector control measures, comprising the elimination of larval breeding sites and spraying of larvicides (*Bacillus thuringiensis israelensis*) and adulticides (pyrethroids) [2,3], are implemented in an area of 200 m around these places;

• daily reporting from a network of laboratories of the results of Zika serological or RT-PCR tests to the French national public health agency. This allows catching up on confirmed cases which have not been reported through the notification system and the seasonal enhanced surveillance;

• notification of pregnancy outcomes for pregnant women infected by Zika virus, or possibly exposed to the virus through sexual or mosquito-borne transmission.

A suspected case of ZIKV infection is defined as a person presenting with rash, with or without fever and at least two of the following: arthralgia, myalgia or conjunctivitis/conjunctival hyperaemia, not explained by another medical condition.

A probable case is a suspected case with anti-ZIKV IgM antibodies in serum sample(s).

Cases are confirmed by serology (anti-ZIKV IgG antibodies confirmed by plaque-reduction neutralisation test, or fourfold increase in IgG titre or seroconversion) or by detection of viral nucleic acids in body fluids (blood, cerebrospinal fluid, urine, semen, saliva, etc.) by reverse transcription (RT)-PCR.

To characterise ZIKV infection, information on patients' demographics, recent travel history and exposure, clinical presentation and symptoms are collected for each confirmed case.

Since January 2016, the National Reference Centre for Arboviruses in Marseille has contributed to diagnostic capacities for ZIKV in hospital and private medical laboratories by making available reference material, operating procedures and testing/diagnosis algorithms. The Ministry of Health has ensured the reimbursement of serology and RT-PCR tests for ZIKV, under certain conditions, through the National Health Insurance Scheme.

Cases of Zika virus infection in mainland France

From 1 January 2016 to 15 July 2016, 625 cases of ZIKV infection, 537 confirmed (86%) and 88 probable (14%), were reported (Figure 1).

Among the 625 cases, 617 (99%) reported recent travel to an area with active ZIKV transmission and 8 (1%) were infected after sexual intercourse with an infected traveller [4-6].

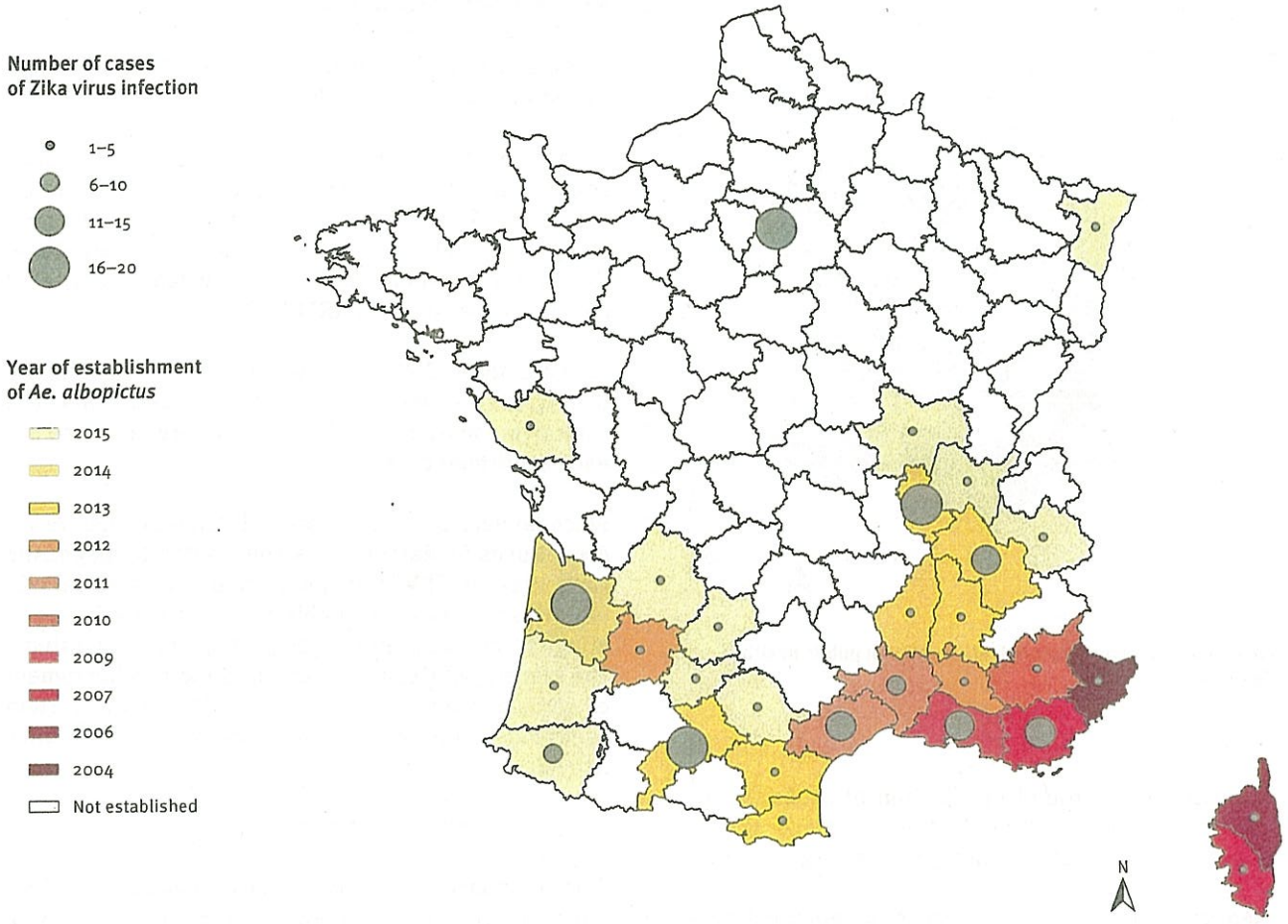
A total of 357 cases (57%) were female. The median age of the cases was 45 years (range: 2–84) (Table).

ZIKV infection was confirmed by detection of viral nucleic acids by RT-PCR in blood or urine for 487 (78%) cases, RT-PCR in blood or urine and serum IgM positivity for 36 cases (6%), seroconversion for two (0.3%) cases, detection of ZIKV RNA by RT-PCR in semen for 6 cases (1%) and in cerebrospinal fluid for 1 case (0.2%) with meningoencephalitis, by detection of neutralising antibodies against ZIKV for 5 cases (0.8%). For 88 (14%) cases, only a positive serological test (IgM) was available.

Clinical illness was reported in 570 cases (91%), 46 (7%) are still under investigation to obtain clinical information and 7 (1%) were asymptomatic.

FIGURE 2

Establishment of *Aedes albopictus* in mainland France, by administrative department and year (2004–15), and number of cases of Zika virus infection since the start of the vector activity season, 1 May–15 July 2016 (n = 185)



Source: Santé publique France, French national public health agency, France, 2016.

Among the seven asymptomatic cases, three were tested because of a planned medically assisted procreation intervention (one woman, two men). One woman was tested because she had been in a ZIKV-epidemic area and wanted to get pregnant, one woman was tested during the investigation of an instance of likely sexual transmission of the virus and two women were tested because they had been exposed in an epidemic area and were pregnant. All asymptomatic cases were confirmed by detection of viral nucleic acids by RT-PCR (four in urine and three in blood).

Among the 570 cases with clinical illness, the most commonly reported signs or symptoms were rash (84%, n = 480), fever (64%, n = 367), arthralgia (64%, n = 367), myalgia (57%, n = 325) and headache (52%, n = 295). Only 20% (n = 112) reported conjunctivitis. Three cases had neurological complications: two had Guillain–Barré syndrome, one had meningoencephalitis [7].

Nine patients reported other neurosensitive symptoms including paraesthesia of the hands, arms or around the mouth (n = 4), hypoesthesia of the hands (n = 3), cutaneous hyperesthaesia (2/9).

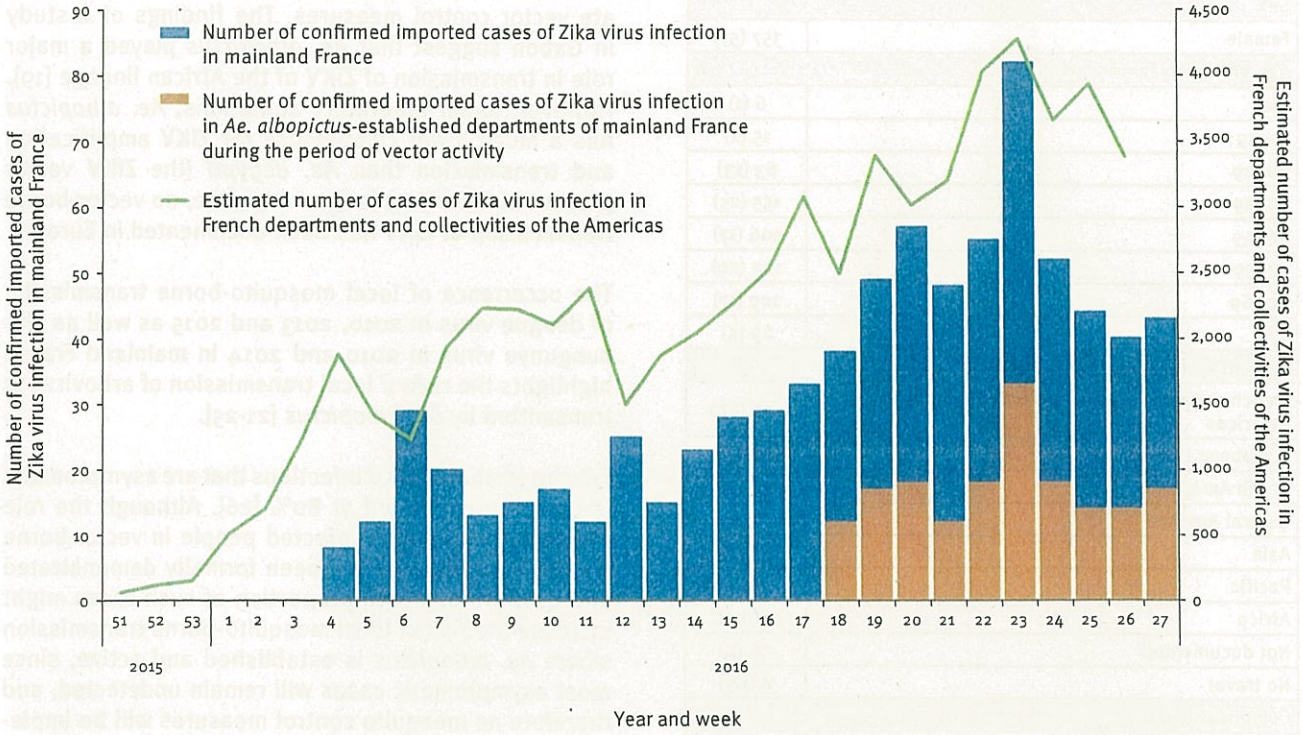
Hospitalisation was required for 29 (5%) patients and there were no deaths. There were 16 pregnant women among the cases.

A majority (85%, n = 527) of confirmed imported cases of ZIKV infection were travellers returning from the French departments and collectivities of the Americas (327 from Martinique, 160 from Guadeloupe, 21 from French Guiana, 16 from Saint Martin and 3 from unspecified locations in the French departments and collectivities of the Americas). The remaining cases had returned from other Caribbean islands and Central or South American countries (Table).

On their return to mainland France, 185 (30%) had stayed in an *Ae. albopictus*-established area during the

FIGURE 3

Imported cases of Zika virus infection in mainland France (weeks 4–27 2016[a], n = 617), imported cases staying in an *Aedes albopictus*-established area in mainland France during the period of vector activity (weeks 18–27 2016[b], n = 183) and estimated number of cases in the French departments and collectivities of the Americas (week 51 2015–week 26 2016[c], n = 62,825)[d]



[a] 25 January–10 July 2016.

[b] 2 May–10 July 2016

[c] 14 December–3 July 2016.

[d] The numbers are based on cases reported by a sentinel network of general practitioners and are then extrapolated [1,8].

Source: Santé publique France, French national public health agency and Regional unit Antilles Guyane, France, 2016.

vector activity period (Figure 2), 84% (n = 156) of them were viraemic. The median delay between the onset of symptoms and date of return in an area with active vectors was two days (range: –7 to 10) with 82% (n = 128) of cases staying in those areas during the entire period of viraemia. Entomological investigations led to the implementation of vector control measures for 21% (32/156) of the cases. The median delay between onset of symptoms and implementation of vector control measures was 13 days (range: 4–58) and between notification and intervention 5 days (range: 2–38).

Before 2016, few imported cases of ZIKV infection were reported by the National Reference Centre in mainland France, with the majority returning from French Polynesia. The number of imported cases steadily increased in 2016, reflecting the epidemic in the French departments of the Americas [1,8] (Figure 3), as observed during the chikungunya virus outbreak in 2014 [9].

Background

Zika virus is an emerging mosquito-borne flavivirus which typically causes mild disease. Since 2015, ZIKV has spread rapidly throughout the Americas, including the French departments and collectivities [8], and revealed new ways of transmission and severe complications [10-12], including sexual transmission, congenital malformations [13,14] and neurological syndromes [15]. By 5 August 2016, 43 countries and territories had confirmed local, vector-borne transmission of ZIKV in South and Central America since 2015 [16,17].

TABLE

Characteristics of cases of Zika virus infection, mainland France, 1 January–15 July 2016 (n=625)

Characteristic	Number (%)
Sex	
Female	357 (57)
Age group in years	
<10	6 (1)
10–19	15 (2)
20–29	83 (13)
30–39	155 (25)
40–49	106 (17)
50–59	122 (20)
60–69	109 (17)
≥70	29 (5)
Regions visited during the incubation period[a]	
French departments and collectivities of the Americas	527 (84)
Caribbean islands	28 (4)
South America	25 (4)
Central America	8 (1)
Asia	1 (0.2)
Pacific	1 (0.2)
Africa	1 (0.2)
Not documented	26 (4)
No travel	8 (1.3)
Complications	
Guillain–Barré syndrome	2 (0.3)
Meningoencephalitis	1 (0.2)
Hospitalisation	29 (5)
Viraemic cases[b]	156 (25)
Month of notification	
January	8 (1)
February	76 (12)
March	74 (12)
April	121 (19)
May	144 (23)
June	158 (25)
July[c]	44 (7)

[a] During the two weeks before symptom onset.

[b] In an area in which the vector *Aedes albopictus* is established and active.

[c] Until 15 July 2016.

Discussion

Although no local mosquito-borne transmission of ZIKV has been documented in mainland France to date, criteria for local mosquito-borne transmission of ZIKV are met: a population that is immunologically naive to the virus; a high probability of introduction of the virus by travellers returning from ZIKV-affected areas; and an established competent vector. The number of returning travellers is expected to further increase over the summer months (there are approximatively 2.5 million passengers travelling by air between mainland France and Martinique, Guadeloupe and French Guiana annually [18]). In mainland France, as at 15 July 2016, 156 (25%) cases were viraemic in an area where *Ae. albopictus* is established, during the period of vector activity. These cases have the potential to trigger local vector-borne transmission in the absence of appropriate vector control measures. The findings of a study in Gabon suggest that *Ae. albopictus* played a major role in transmission of ZIKV of the African lineage [19]. However, under laboratory conditions, *Ae. albopictus* has a much lower competence for ZIKV amplification and transmission than *Ae. aegypti* (the ZIKV vector present in Americas) [20], and to date, no vector-borne transmission of ZIKV has been documented in Europe.

The occurrence of local mosquito-borne transmission of dengue virus in 2010, 2013 and 2015 as well as chikungunya virus in 2010 and 2014 in mainland France highlights the risk of local transmission of arboviruses transmitted by *Ae. albopictus* [21-25].

The proportion of ZIKV infections that are asymptomatic is currently estimated at 80% [26]. Although the role of asymptomatic ZIKV-infected people in vector-borne transmission has not yet been formally demonstrated and quantified, a high proportion of such cases might increase the risk of local mosquito-borne transmission where *Ae. albopictus* is established and active, since most asymptomatic cases will remain undetected, and therefore no mosquito control measures will be implemented around these cases.

Eight cases of sexual transmission of ZIKV have been reported in mainland France as at 15 July 2016, including transmission by an asymptomatic man [5]. Some authors have suggested that sexual transmission may play a significant role in transmission of ZIKV and has contributed to the higher proportion of female cases observed in Brazil [27]. Case finding should therefore not only focus on travellers returning from areas with ZIKV transmission but also on their sexual partners, even in the absence of symptoms in the traveller. Cases infected by sexual transmission can initiate further vector-borne transmission, emphasising the importance of the implementation of vector control measures around all cases. The lack of knowledge on the persistence of ZIKV and the dynamics of RNA viral load in semen still pose a considerable challenge to guidance on prevention of sexual transmission of ZIKV.

Other questions remain regarding the aetiological link between ZIKV infection and neurological presentations and their spectrum [28]. Since January 2016, two cases of Guillain–Barré syndrome and one case of meningoencephalitis were reported (0.5% of all cases) in mainland France. Paraesthesia, hypoaesthesia or hyperaesthesia were reported for nine additional cases (1.5% of all cases): the frequency and relevance of these milder symptoms deserves further attention.

The expected high number of imported cases of ZIKV infection in areas where *Ae. albopictus* is established and severe ZIKV-related adverse outcomes trigger the need to monitor closely cases of ZIKV infection. Vector control measures are essential during the vector's active period.

Furthermore, it is essential to maintain a high level of commitment of healthcare professionals, especially family practitioners, to continue their participation in surveillance and in health education. They are a major source of information for patients on the risk of ZIKV infection and for the general population on measures to prevent infection by ZIKV and other arboviruses.

Zika Surveillance Working Group in French departments and collectivities of the Americas

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Conflict of interest

None declared.

Authors' contributions

Alexandra Septfons analysed the data. Alexandra Septfons and Elisabeth Couturier drafted the manuscript. Isabelle Leparc Goffart contributed to the validation of laboratories techniques and the virological tests and the extension of the laboratories' access to diagnosis capacities in France. Florian Franke, Anne Guinard, Guillaume Heuzé, Anne Hélène Liebert, Jean Rodrigue Ndong, Isabelle Poujol, Sophie Raguet, Cyril Rousseau, Asma Saidouni-Oulebsir, Caroline Six, Véronique Servas, Elodie Terrien, Hélène Tillaut, Marguerite Watrin, Anita Balestier, Marion Subiros, Delphine Viriot, K. Wyndels, Alexandra Mailles, Alexandra Septfons, Elisabeth Couturier, Harold Noël, Marie Claire Paty contributed to the surveillance and epidemiological investigations in mainland France. Joel Deniau and Florian Franke managed the national database. The Zika Surveillance Working Group took part in alert and surveillance systems of Zika in the French departments and collectivities of the Americas and sent their data. Marie Claire Paty and Harold Noël are in charge of the coordination of the arboviruses surveillance system at Santé publique France and contributed to data analysis and writing of the manuscript. Henriette De Valk coordinated and supervised the writing of the manuscript.

All authors contributed to the review of the manuscript and approved the final version.

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別紙様式第2-1

No. 9

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2016. 9. 12	該当なし	

一般的名称	人血小板濃厚液	研究報告の公表状況		公表国
販売名(企業名)	濃厚血小板-LR「日赤」(日本赤十字社) 照射濃厚血小板-LR「日赤」(日本赤十字社) 濃厚血小板HLA-LR「日赤」(日本赤十字社) 照射濃厚血小板HLA-LR「日赤」(日本赤十字社) 照射洗浄血小板-LR「日赤」(日本赤十字社) 照射洗浄血小板HLA-LR「日赤」(日本赤十字社)		Richard B. Brooks, Maria Paz Carlos, Robert A. Myers, et al. MMWR Morb Mortal Wkly Rep. 2016, 65(34)915-6	米国

研究報告の概要

○感染症の症状がない男性からのジカウイルス(ZIKV)の性感染が疑われる症例 — メリーランド州、2016年。
2016年6月、メリーランド州の健康・精神衛生部(DHMH)は発熱とかゆみを伴う発疹(担当医の診断は丘疹性発疹)を呈して受診した女性の報告を受けた。DHMHの検査によりZIKV感染が確認され、症例調査からこの女性にはZIKVが流行する地域への渡航歴がないこと、最近までドミニカ共和国に渡航していた男性パートナーと性交渉があったことが明らかとなった。この男性は渡航中、蚊がいる環境下にいたが、アメリカへの帰国前後にZIKV感染症の症状はなかった。また、女性は自分が発症するまでの14日間にこの男性以外との性交渉はなく、輸血又は臓器移植を受けていなかった。
男性の帰国後10日(day10)そして14日(day14)に避妊具を未使用で2回の性交渉があり、day16に女性はZIKV感染症を発症、day19に受診、RT-PCR法により尿からZIKV RNAを検出、血清によるデング熱ウイルスおよびチクングニヤ熱ウイルスに対するRT-PCR法は陰性であった。ZIKV、デング熱ウイルスのIgM抗体はいずれも陽性であった。
男性はday26の問診では、発熱、発疹、結膜炎、関節痛などのZIKV感染症の症状はなく、day29の血漿、血清及び尿を用いたRT-PCR法は陰性、ZIKV及びデング熱ウイルスのIgM抗体検査は共に陽性、尿は判定不能であり症状もなく、day31に精液が採取されたがZIKV RNAは検出されていない。
現時点では、症状のない男性が女性パートナーへ性感染によりZIKVを伝播させたかもしれない例が他に1例報告されているが、男女ともにZIKV感染流行地域へ渡航しており、渡航中における女性への蚊の刺咬があったことを否定できない。女性に渡航歴のないケースは初めてであることから、流行国から帰国した場合、少なくとも8週間は性交渉を控えるべきであると考えられる。

報告企業の意見	今後の対応
ジカウイルス(ZIKV)流行地域から帰国した後、感染症状が無い男性との性交渉にて相手女性がZIKVに感染した疑い事例という報告である。	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、平成28年2月3日付厚生労働省医薬・生活衛生局血液対策課長事務連絡「ジカウイルスによることが疑われる小頭症等の増加に関するWHO緊急委員会宣言について(注意喚起)」の発出を受け、同月4日付で各血液センターに対し問診時の帰国(入国)後経過日数の確認を徹底するよう指示した。さらに同年7月1日から、ジカウイルス感染症と診断され、治癒後1カ月間経過していない場合は献血不適としている。今後もジカウイルス感染症に関する新たな知見等について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

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

JRC2016T-032

Likely Sexual Transmission of Zika Virus from a Man with No Symptoms of Infection — Maryland, 2016

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In June 2016, the Maryland Department of Health and Mental Hygiene (DHMH) was notified of a nonpregnant woman who sought treatment for a subjective fever and an itchy rash, which was described as maculopapular by her provider. Laboratory testing at the Maryland DHMH Laboratories Administration confirmed Zika virus infection. Case investigation revealed that the woman had not traveled to a region with ongoing transmission of Zika virus, but did have sexual contact with a male partner who had recently traveled to the Dominican Republic. The male partner reported exposure to mosquitoes while traveling, but no symptoms consistent with Zika virus infection either before or after returning to the United States. The woman reported no other sex partners during the 14 days before onset of her symptoms and no receipt of blood products or organ transplants.

The couple reported having had condomless vaginal intercourse twice after the man's return from the Dominican Republic and before the woman's symptom onset, approximately 10 days (day 10) and 14 days (day 14) after the man's return. The man also reported that he received fellatio from the woman during their sexual encounter on day 14. On day 16 (2 and 6 days after the episodes of condomless vaginal intercourse) the woman developed symptoms of Zika virus infection, including fever and rash. On day 19 (3 days after symptom onset) she sought medical care; the provider suspected Zika virus infection, and serum and urine specimens were collected. Flavivirus and chikungunya virus tests were performed at the Maryland DHMH Laboratories Administration. Zika virus RNA was detected in urine, but not in serum, by real-time reverse transcription–polymerase chain reaction (rRT-PCR) using a test based on an assay developed at CDC (*1*). Serum rRT-PCR testing for dengue virus and chikungunya virus was negative. Serologic testing was negative for Zika virus immunoglobulin M (IgM) antibodies using the CDC Zika IgM antibody capture enzyme-linked immunosorbent assay (Zika MAC-ELISA) and negative for dengue virus and chikungunya virus IgM antibodies using InBios ELISA kits (InBios International, Inc., Seattle, Washington). Confirmatory serologic testing at the CDC Arbovirus Diagnostic Laboratory was equivocal for Zika virus IgM antibodies using the Zika MAC-ELISA. Plaque-reduction neutralization tests (PRNTs) performed at the CDC Arbovirus Diagnostic Laboratory confirmed a recent Zika virus infection. Convalescent serologic testing performed at the Maryland DHMH Laboratories Administration on day 56 (40 days after symptom onset) was equivocal for Zika virus IgM antibodies using the CDC Zika MAC-ELISA and negative for dengue virus and chikungunya virus IgM antibodies using InBios ELISA kits. PRNTs performed at the CDC Arbovirus Diagnostic Laboratory confirmed a recent, unspecified flavivirus infection.

The woman's male sex partner was interviewed on day 26 after his return to the United States. He reported that he had no symptoms consistent with Zika virus infection (i.e., fever, rash, conjunctivitis, or arthralgias) either during his travel or since his return, and he did not have any of the following other symptoms: myalgias, chills, eye pain, oral ulcers, genital ulcers, anal ulcers, hematospermia, hematuria, dysuria, and prostate pain. He reported feeling tired, which he attributed to having recently traveled. Serum, plasma, and urine specimens were collected from him on day 29, at which time he reported no new symptoms. Zika virus rRT-PCR testing performed at the Maryland DHMH Laboratories Administration was negative on serum and plasma and equivocal on urine. Serologic testing was positive for Zika virus IgM antibodies using the CDC Zika MAC-ELISA and positive for dengue virus IgM antibodies using an InBios ELISA kit. PRNTs performed at the CDC Arbovirus Diagnostic Laboratory confirmed a recent, unspecified flavivirus infection. Semen collected on day 31 had no detectable Zika virus RNA by rRT-PCR testing performed at the Maryland DHMH Laboratories Administration.

To date, only one other case has been reported in which a man without symptoms might have sexually transmitted Zika virus to his female partner (*2*). However, in that reported case, both the man and the woman had traveled to a country with ongoing Zika virus transmission where they were likely exposed to mosquitoes. In that case, although the detection of Zika virus RNA in the woman's serum and urine by rRT-PCR 39 days after return from travel suggested sexual transmission from her male partner, it could not be ruled out that she had been infected from a mosquito bite during travel and had a longer than average incubation period or a prolonged period of viremia. No cases of sexual transmission of Zika virus from an asymptomatic man returning from travel to an area with active Zika transmission to his female sex partner who did

not travel have been reported. Absence of Zika virus symptoms in persons returning from areas with ongoing Zika virus transmission might not preclude sexual transmission of Zika virus to their sex partners. Ongoing surveillance is needed to determine the risk for sexual transmission of Zika virus infection from asymptomatic persons. The findings in this report indicate that it might be appropriate to consider persons who have condomless sex with partners returning from areas with ongoing Zika virus transmission as exposed to Zika virus, regardless of whether the returning traveler reports symptoms of Zika virus infection. Providers should request Zika virus testing for any patients with illness compatible with Zika virus disease who have had sexual exposure without barrier devices to prevent infections to a partner who traveled to an area with active Zika virus transmission (*3*). Such patients should also be reported to local or state health departments (*4,5*).

Current recommendations for the prevention of sexual transmission of Zika virus in returning travelers differ depending on whether the returning traveler is symptomatic and on whether the couple is planning to become pregnant (*3,6*). Couples in areas without active Zika transmission with circumstances in which one partner traveled to an area with active Zika virus transmission but did not develop symptoms of Zika virus disease should wait at least 8 weeks after the partner who traveled returned from the Zika-affected area before attempting conception, regardless of the sex of the traveler. Men with a diagnosis of Zika virus infection should wait at least 6 months before attempting conception, and women with a diagnosis of Zika virus infection should wait at least 8 weeks before attempting conception. Health care providers should counsel couples that correct and consistent use of condoms reduces the risk for sexually transmitted diseases and discuss the use of the most effective contraceptive methods that can be used correctly and consistently (*6*). Couples who do not desire pregnancy should consider abstaining from sex or using the most effective contraceptive methods that can be used correctly and consistently in addition to barrier methods, such as condoms, which reduce the risk for sexual transmission of Zika virus and other sexually transmitted infections (*3*). As more is learned about the incidence and duration of seminal shedding of Zika virus in infected men, recommendations to prevent sexual transmission of Zika virus will be updated if needed.

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医薬品　研究報告　調査報告書

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販売名（企業名）	濃厚血小板-LR「日赤」（日本赤十字社） 照射濃厚血小板-LR「日赤」（日本赤十字社） 濃厚血小板HLA-LR「日赤」（日本赤十字社） 照射濃厚血小板HLA-LR「日赤」（日本赤十字社） 照射洗浄血小板-LR「日赤」（日本赤十字社） 照射洗浄血小板HLA-LR「日赤」（日本赤十字社）			米国

研究報告の概要

○ジカウイルス（ZIKV）：世界的影響を与える血液供給の安全性に対する新たな脅威。
ZIKVはヒト集団に影響を及ぼす最も新しい新興ウイルスとして認められており、血液の安全性及び安定供給にも重大な影響を与えている。フランス領ポリネシアでアウトブレイクが発生していた期間に行われた供血者スクリーニングに関する研究では、ウイルス血症の罹患率は最高で2.8%が記録されている。ブラジルでは輸血感染疑い例が数例報告されており、ZIKV感染のウイルス血症期（最長で14日間持続する可能性がある）には高いウイルス量（最高で8.1×10^6 copies/mL）が検出されているため、輸血感染のリスクに対する懸念が高まっている。
米国食品医薬品局（FDA）、AABB（米国血液バンク協会）、WHO並びに欧州疾病予防管理センターは、ZIKVが伝播していない地域における輸血感染のリスクを低減するため、ZIKV感染のアウトブレイクを経験した国へ渡航した後、もしくは感染リスク地域へ渡航した男性と性交渉を持った後に28日間の供血延期を基礎としたガイダンスを発出している。更に、FDAは伝播している地域に関するガイダンスを発出し、全血製剤及び血液成分製剤を米国のZIKVが伝播していない地域から入手することを義務付けているが、病原体の不活化を実施した血漿製剤並びにアフェレーシス血小板製剤は例外となり、流行地における採血が許可されている。全ての血液成分製剤の輸血を認めるための選択肢には、FDA認可済みの供血者スクリーニングNATもしくは、短期的には、FDAの承認を得る前の、治験薬申請免除に基づく検査によるZIKV RNAの供血者スクリーニングがある。WHOは供血の延期、病原体の不活化並びにNATに加えて、供血者による供血後情報（14日間以内に症状が現れたら連絡する）、並びに血液成分製剤を供血後3日間～14日間クアランティンとすることを推奨している。しかしながら、ZIKV感染は無症候性であることを考えた場合、実施の有用性は限られていると言える。

使用上の注意記載状況・その他参考事項等

濃厚血小板-LR「日赤」
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血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
フランス領ポリネシアでのジカウイルス（ZIKV）の流行時に、ウイルス血症の罹患率は最高で2.8%が記録され、高いウイルス量（最高で8.1×10^6 copies/mL）が検出された。FDAのガイダンスにおいては、ZIKV流行地域では、病原体不活化を実施した製剤に限り採血を許可しているという報告である。	日本赤十字社では、輸血感染症対策として献血時に海外渡航歴の有無を確認し、帰国（入国）後4週間は献血不適としている。また、平成28年2月3日付厚生労働省医薬・生活衛生局血液対策課長事務連絡「ジカウイルスによることが疑われる小頭症等の増加に関するWHO緊急委員会宣言について（注意喚起）」の発出を受け、同月4日付で各血液センターに対し問診時の帰国（入国）後経過日数の確認を徹底するよう指示した。さらに同年7月1日から、ジカウイルス感染症と診断され、治癒後1カ月間経過していない場合は献血不適としている。今後もジカウイルス感染症に関する新たな知見等について情報の収集に努める。

Zika virus: a new threat to the safety of the blood supply with worldwide impact and implications

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An emerging pathogen is an infectious agent that was previously unknown or whose geographic range or virulence has expanded.[1-3] Zika virus (ZIKV) qualifies as the latest emerging virus impacting the human population,[4-6] with major implications for blood safety and availability.[2,3,7-9] Here we review historical and recent outbreaks of ZIKV and consider the response of transfusion medicine experts to the current explosive ZIKV epidemic, in light of recent efforts to define processes and criteria for emerging infectious disease risk assessment and response measures related to blood safety.[10-12]

HISTORICAL CONTEXT

ZIKV is an emerging arthropod-borne virus (arbovirus) of the *Flavivirus* genus in the *Flaviviridae* family.[13] ZIKV was first isolated in 1947 from a sentinel Rhesus macaque in the Zika forest in Uganda and then from humans during its westward spread throughout Central Africa.[14-16] The first human infection was reported in 1954 in Nigeria.[17] Serologic and molecular evidence of viral circulation was generated over the next two decades within several African countries including Tanzania, Egypt, Central African Republic, Sierra Leone, and Gabon (Fig. 1).[14,18,19] The virus then migrated to Asia where its circulation was reported in India, Pakistan, and southeast Asian countries including Thailand, Cambodia, Vietnam, Malaysia, Indonesia, and the Philippines.[19] Approximately 80% of infections are asymptomatic; of those that are symptomatic, most result in mild illness[19-21] with mild fever, maculopapular rash, conjunctivitis, and arthralgia or myalgia.[22] As only 14 clinical cases of human infection had been reported before 2007,[23] ZIKV did not attract sustained interest from the scientific and medical communities.

In 2007, a ZIKV outbreak on the Micronesian island of Yap caused about 5000 infections (75% of the population) over a short period of 3 months,[20,24] suggesting that ZIKV could be responsible for explosive outbreaks (Fig. 1). The subsequent French Polynesian ZIKV outbreak in 2013 and 2014 attracted international attention after the virus was implicated in about 30,000 symptomatic cases and 42 cases of Guillain-Barré syndrome (GBS; a 20-fold increase of GBS incidence), over a period of 5 months.[25-27] This was the first report of severe complications associated with ZIKV infection. A case-control study conducted in French Polynesia that was recently published confirmed the link between ZIKV infection and GBS.[26] Subsequently ZIKV has spread throughout the Pacific.[25]

RECENT ZIKV EPIDEMICS AND EXPANDED DISEASE ASSOCIATIONS IN THE AMERICAS

The first confirmed cases of ZIKV infection in the Americas were reported in north-central Brazil in May 2015,[28,29] although there is genetic evidence that the Asian strain of

ABBREVIATIONS: CHIKV = chikungunya virus; DENV(s) = dengue virus(-es); GBS = Guillain-Barré syndrome; IND = investigational new drug; ZIKV = Zika virus.

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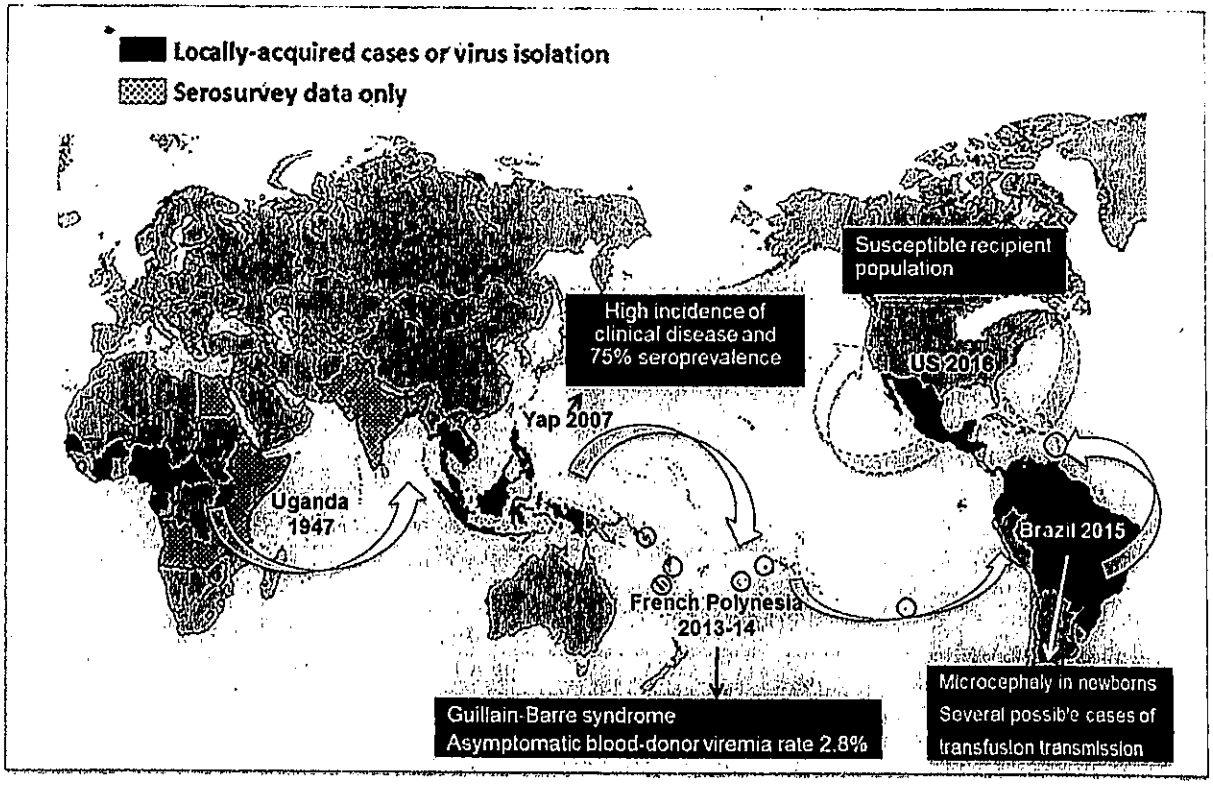

Fig. 1. ZIKV spread, expanded clinical associations, and evidence for potential transfusion transmissions.

the virus was probably carried by a viremic traveler to Brazil in 2014.[30,31] As the virus spread throughout northeastern Brazil, astute pediatricians noted a temporal association between regional ZIKV circulation and severe neurologic malformation in neonates (e.g., microcephaly).[30,32-37] Microcephaly is a rare newborn condition that has been linked to genetic disorders and fetal infections with other viruses, but not previously with arboviruses. While it was difficult to prove retrospectively that ZIKV was responsible for this severe outcome, the temporal and regional associations between ZIKV outbreaks and severe neurologic diseases became increasingly compelling.[38-40] Over the subsequent months ZIKV RNA positivity was documented in mothers (blood, placenta, and amniotic fluid) and fetuses (including replicating virus isolated from brain tissue) suffering from microcephaly, which supported the association between maternal ZIKV infection and newborn congenital disorders.[39,41-45] The increase in GBS cases described in French Polynesia has been reported in nine countries and territories as of March 2016[46] and, similarly, an increase in microcephaly cases has been reported retrospectively in French Polynesia,[47,48] confirming that these complications were not related to local conditions in Latin America (e.g., pesticide exposure).

In February 2016, ZIKV was declared a public health emergency of international concern by the World Health Organization (WHO).[40] By March 2016, according to the Brazilian Ministry of Health, of 6480 suspected cases of microcephaly reported by the media in Brazil, 863 cases of microcephaly and other neurologic syndromes were confirmed including 97 cases that had unequivocal evidence of ZIKV infection by nucleic acid testing (NAT) and serology (many additional cases are pending evaluation). Significantly elevated numbers of cases were diagnosed in several other Latin American countries compared to what had been previously reported. However, the proportion of infections of pregnant women resulting in ZIKV-related microcephaly and other neurologic syndromes is unknown due to reporting bias,[49] and case-control studies are needed to clearly demonstrate the rate of congenital syndromes after maternal ZIKV infection.[50]

While studies are being launched to better understand the incidence and pathogenesis for newborn congenital diseases after maternal ZIKV infection, the virus has rapidly spread to almost all countries in South and Central America as well as in the Caribbean and reemerged in Africa (Cape Verde).[19] There are also increasing numbers of symptomatic ZIKV infections diagnosed in travelers returning from epidemic areas to the United States, Canada, Europe, Asia (Japan and China), and Pacific (Australia and New Zealand) countries, including cases of ZIKV-infected pregnant women with severely affected fetuses and infants.[51] After the confirmation of

five cases of imported ZIKV infection in the United States, including a woman with a history of travel to an epidemic country (Brazil) delivering a baby with microcephaly in Hawaii, the Centers for Disease Control and Prevention (CDC) issued a travel advisory recommending pregnant women avoid travel to countries currently experiencing ZIKV outbreaks.[52]

ROUTES OF TRANSMISSION

ZIKV, like dengue viruses (DENVs), chikungunya virus (CHIKV), and yellow fever virus, can be transmitted in a human-mosquito-human transmission cycle by *Aedes* mosquitoes.[53,54] Given that the mosquito vectors, primarily *Aedes aegypti* and potentially *Aedes albopictus*, are present throughout the Caribbean islands and in the southern part of the United States,[55] emergence of ZIKV in the continental United States is likely[56] (Fig. 1). In addition, other *Aedes* mosquito species may serve as vectors. Indeed, ZIKV was shown to be transmitted by *Aedes hensili* during the Yap island outbreak, even though this mosquito has a very limited distribution in the Pacific area.[57] As of March 2016, the United States and French territories in the Caribbean have already reported hundreds of autochthonous cases of ZIKV infection and such cases have been doubling by the week in Puerto Rico, where large arbovirus outbreaks peak every year in August and September.[58] Noteworthy, approximately half of the world population lives in areas where ZIKV competent vectors are present.[19,54]

Intrauterine,[39,42] perinatal,[59] and sexual[60,61] routes of transmission have also been documented and the potential for transfusion transmission has been recognized.[62] ZIKV RNA and infectious ZIKV have been found in urine,[63] breast milk,[59] saliva,[64] and semen.[65] Considering documented viral persistence in urine for up to 1 month and in semen for up to 62 days after symptom development, persistence in solid organs and tissues and transmission through transplantation, as previously documented for WNV[66] and DENVs[67] can be suspected.

DIAGNOSTIC CHALLENGES

The association between microcephaly and ZIKV maternal infection was difficult to prove in great part due to the fact that ZIKV diagnosis previously relied on serology, which has proven to be challenging in areas endemic for dengue[24,68] (and all endemic areas for ZIKV are also endemic for DENVs);[69] ZIKV is a flavivirus closely related to DENVs and existing DENV antibodies may cross-react as ZIKV antibodies or may blunt ZIKV-specific immunoglobulin (Ig)M and IgG seroconversion after infection.[6] Serology-based assays should be confirmed by neutralization assays that can only be performed by a limited number of laboratories.[19] Consequently, the WHO and CDC have encouraged moving away from serology-based assays and transitioning to NAT assays that are more specific but are only sensitive if performed during the acute phase of infection.[63,64] NAT assay sensitivity could be increased by processing larger volumes of plasma or testing saliva during the acute phase of infection,[64] and the period of detection of ZIKV RNA could be extended by testing urine.[63] Application of sensitive NAT assays may also prove useful for monitoring pregnant women who were infected with ZIKV to detect persistent low level RNA derived from infected fetal and placental tissues as a prognostic marker for congenital disease.[51] Virus isolation is challenging and can only be performed by a limited number of laboratories.[19] Significant effort is now being devoted to development of improved molecular and serologic assays for acute, recent, and past ZIKV infection, tools that are needed for diagnosis; surveillance; and blood, organ, and tissue donor screening.

A NEW THREAT TO THE SAFETY OF THE BLOOD SUPPLY

Blood donor viremia rates of up to 2.8% were documented in a blood donor screening study during the French Polynesia outbreak.[62] With several probable cases of transfusion transmission reported in Brazil[70] and high viral loads (up to 8.1×10^6 copies/mL) detected during the viremic phase of ZIKV infection, which may last up to 14 days,[24,63,71] there are growing concerns about the risk of transfusion transmission.[62,70,72,73] There is particular concern over ZIKV infections and consequent severe outcomes in at-risk recipient populations such as pregnant women and those with sickle cell disease in need of regular blood transfusions.[8]

With endemic transmission of ZIKV confirmed in over 38 countries/territories in the Americas, including Mexico, Puerto Rico, and numerous other Caribbean islands, and travel-related cases diagnosed throughout the continental United States and Europe, the Food and Drug Administration (FDA),[74] AABB,[75,76] WHO,[77] and European Center for Disease Prevention and Control[78] have issued guidance documents to reduce the risk of transfusion transmission of ZIKV in areas without active transmission of ZIKV based on deferral from blood donation for 28 days after travel to countries experiencing ZIKV outbreaks or sexual contact with a male who has traveled to ZIKV risk regions.[28-31,79-81] FDA also issued guidance for areas with active transmission (including Puerto Rico and the US Virgin Islands), which requires that whole blood and blood components be obtained from areas of the United States without active transmission, except for plasma and apheresis platelets (PLTs) that can be collected locally if pathogen inactivated. Cerus Corporation has demonstrated 6-log kill (highest level of infectivity that could be evaluated) of ZIKV in plasma (with a reasonable inference that the

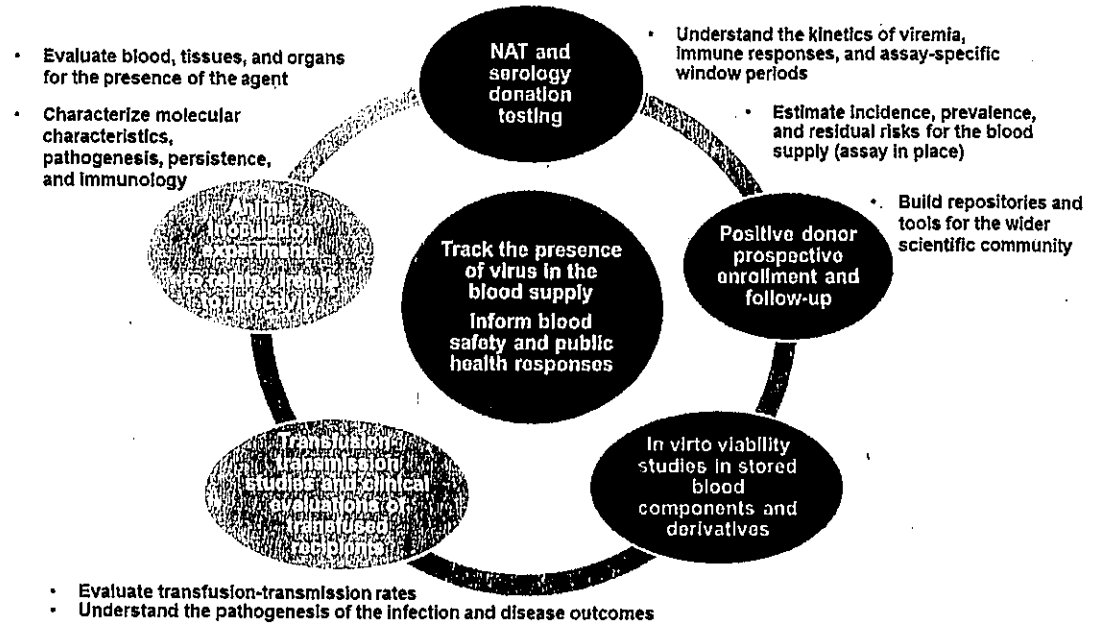

Fig. 2. Assessing the risk of transfusion transmission and studies of donors and recipients to provide insights into epidemiology, natural history, and pathogenesis of ZIKV and other emerging infectious diseases.

same will occur in PLTs)[69,82] and blood collection organizations in Puerto Rico can consider preparing pathogen-inactivated PLTs and plasma. Although not yet available, additional efforts are under way to develop pathogen-inactivated RBC components. The other option to allow for ongoing blood collection and transfusion of all blood components from donations collected in active ZIKV transmission regions is to screen donors for ZIKV RNA using an FDA-licensed blood donor screening NAT or in the near term a test performed under an FDA investigational new drug [IND] exemption before licensure.[8] On March 30, 2016, the first ZIKV NAT assay for blood donor screening, developed by Roche Molecular Systems, became available under an FDA IND. The NAT manufacturing industry is working with regulators and blood testing laboratories to develop and implement additional ZIKV NAT assays for blood screening as well as multiplexed NAT assays for simultaneous detection of ZIKV, DENV, and CHIKV.

In addition, WHO is currently working on the provision of international reference preparations for ZIKV RNA and for ZIKV antibodies to be used for comparative evaluation of both diagnostic and screening assays. Indeed, in its interim guidance document to maintain a safe and adequate blood supply during ZIKV outbreaks,[83] WHO encourages sensitive NATs designed for diagnostic purposes and in house–developed NATs to be used for blood screening as long as they are properly validated. In addition to donor deferral, implementation of pathogen inactivation for plasma and PLTs, and NAT, WHO also recommends that donors self-report the development of any ZIKV-compatible symptoms within a 14-day time frame and encourages quarantine of blood components for a period of 3 to 14 days postdonation or until lack of symptom development; however, this latter recommendation will be challenging to implement (especially for PLTs with a 5-day shelf life) and is acknowledged to be of limited value given that most ZIKV infections are asymptomatic. WHO also acknowledges that the implementation of new transfusion transmission mitigation strategies will represent an economic challenge for some countries.

PLANNED STUDIES IN RESPONSE TO THE ZIKV EMERGENCY AND BLOOD SAFETY CONCERNS

In response to WHO declaring ZIKV a public health emergency of international concern, and because of growing concerns about the risk of transfusion transmission, the National Heart, Lung, and Blood Institute (NHLBI) of the US National Institutes of Health announced in February its interest in supporting research to evaluate the risk of and clinical impact of potential transmission of ZIKV by transfusion (NOT-HL-16-307). NHLBI also announced its participation in an NIH program announcement with review (PAR-16-106) to encourage rapid assessment of ZIKV complications related to blood safety. In addition, NHLBI will exploit its existing Recipient Epidemiology and Donor Evaluation Study-III (REDS-III) blood safety research program to investigate ZIKV in the context of blood donations and transfusion (Fig. 2). One of these REDS-III studies will be launched over the next few months in Puerto Rico and the

continental United States. Blood screening by NAT was implemented under IND to screen blood donations in Puerto Rico in April 2016 and it is anticipated that such screening (under IND) may potentially also be implemented mid-2016 in regions of the United States such as Florida and Texas that are at high risk for local transmission. Blood components from ZIKV RNA–positive donors will be interdicted to prevent ZIKV transfusion transmission. From a research perspective, index donation ZIKV-positive plasma will be made available for further study and acutely infected donors will be asked to participate in follow-up studies. These follow-up studies will investigate the natural history and clinical outcomes of ZIKV infection, including dynamics of viral and immune variables, kinetics of persistence or clearance in blood compartments and other body fluids, and viral and immune mechanisms leading to viral clearance or clinical pathogenesis. The studies will also establish repositories of pedigreed longitudinal samples to advance molecular and serologic test development.

Three other REDS-III studies will be launched in Brazil to evaluate ZIKV or CHIKV transfusion transmission rates and evaluate risk of disease outcome in transfusion recipients, as well as evaluate the rate of viremia among blood donors in several geographical areas. The REDS-III central laboratory and REDS-III Brazil program, both led by Blood Systems Research Institute in San Francisco, California, are working with the Fundação Faculdade de Medicina and Hospital das Clinicas of the Medical School of the University of Sao Paulo in Brazil. This study plans to enroll up to 3500 hospital patients receiving blood transfusions in April and May 2016 and January to June 2017. The study is examining ZIKV, in addition to DENV and CHIKV. A new research NAT assay to simultaneously detect ZIKV, CHIKV, and DENV RNA is being developed in the United States and is expected to be ready for research use by this summer. The study will collect blood samples in recipients before and after transfusions. The posttransfusion blood samples will then be tested for direct evidence of ZIKV, CHIKV, and DENV using the new research test. In cases where recipients are identified with one or more of these viral infections after transfusion, stored samples from the donations transfused to that recipient and pretransfusion samples from the infected and control uninfected recipients will be tested to determine if the infection was probably transfusion-transmitted or acquired from mosquito exposure. Participants will also be evaluated for symptoms that could be caused by these viral infections, both through prospective symptom ascertainment and through chart review, similar to a previous study focused on transfusion transmission of DENV.[84] Another study is also being established to determine the rate of ZIKV RNA detection in six donation minipools of plasma from blood donors at four Hemocenters in Brazil participating in the REDS-III program (located in Recife, Belo Horizonte, Rio, and Sao Paulo). A third study is being planned to evaluate chronically transfused sickle cell disease patients for acquisition of transfusion-transmitted ZIKV at the four REDS-III Brazil Hemocenters and to evaluate clinical symptoms if ZIKV positive (regardless of the route of transmission). These studies performed in a collaborative manner with Brazilian investigators should help Brazil further build its expertise and infrastructure so as to maintain an adequate and safe blood supply.[85]

Additional studies under consideration would investigate the transfusion transmission of ZIKV in macaque and murine models. For example, it would be possible in a relevant nonhuman primate model to 1) rigorously characterize the dynamics of acute ZIKV in blood compartments, 2) determine the minimal infectious dose for ZIKV required for transfusion transmission from donors in serial stages of acute and resolving ZIKV infection, and 3) determine the efficacy of pathogen inactivation on prevention of transmission from blood products with high viral loads.

PROACTIVE BUT EVIDENCE-BASED APPROACH TO ZIKV RESPONSE

It is worth considering the recent response to ZIKV in the context of efforts in the past 5 years to develop tools for systematic risk assessment of emerging infectious diseases and consideration of potential interventions.[7,10,12] Despite the lack of unequivocal evidence of ZIKV transfusion transmission (although likely) or the lack of data as to whether ZIKV transfusion transmission would result in serious ZIKV disease, a preemptive approach to safeguarding the safety of the blood supply including donor deferral, blood importation, pathogen inactivation, and/or NAT seems justified. This rapid and proactive response strategy was motivated by the explosive ZIKV epidemic in the Americas and the global urgency precipitated by previously unrecognized neurologic complications of ZIKV infection, particularly congenital infections causing death and severe neurologic disease in fetuses and newborns. The studies summarized should confirm and help us quantify the risk of ZIKV transfusion transmission and consequent disease, inform additional refinement of donor screening and deferral policies, and allow for the collection of samples from healthy donors, transfused recipients, and animal models with ZIKV infection collected longitudinally throughout the acute and resolution phases of ZIKV infection. Such biospecimen collections will be available for the scientific community to optimize serology-based diagnostics and identify host immune markers of infection and pathogenesis. Such studies would not be possible without strong collaboration between all stakeholders including industry partners, Health and Human Services agencies, Brazilian governmental agencies (Fapesp, Fapemig, and CNPQ), international and US investigators, and the patients and blood donors who agree to participate.[85] These investigations

are performed in a partnership manner and build on the existing collaborative networks established through the REDS-II-III programs by mobilizing multidisciplinary teams of experts in the fields of infectious diseases, virology, immunology, and epidemiology with interest in blood banking, blood screening, hemovigilance, and public health. It is hoped that, as a result, the findings from these studies will inform donor screening strategies, diagnostics, vaccine development, and clinical care nationally and internationally.[85]

CONFLICT OF INTEREST

BSC, MPB, and MCL have received research support from Grifols, Roche, Terumo, and Cerus, which are the manufacturers of NAT assays and pathogen reduction technology systems relevant to prevention of transfusion-transmitted ZIKV. The other authors have disclosed no conflicts of interest.

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研究報告の概要

BWP：ジカウイルスに関する血漿分画製剤と尿由来製剤のウイルス安全性報告：

EMAおよびEU加盟国の規制当局は、血漿由来および尿由来医薬品を使用した患者に、ジカウイルスによる感染リスクの増加がないことを確認した。 血漿分画製剤に使用される製造プロセス（ウイルス不活化のための有機溶媒／界面活性剤法、液状加熱処理による不活化、ウイルス除去膜ろ過など）により、エンベロープウイルスに対する不活化／除去工程の効果は、十分に安全性を担保するものであると結論付けた。そのため、検査または特定の血漿ドナーの除外などの追加の安全対策は必要ないとした。

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-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、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。

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

European Medicines Agency - Science, medicines, health

Zika virus infection: plasma- and urine-derived medicines safe to use

21/09/2016

Zika virus infection: plasma- and urine-derived medicines safe to use

Manufacturing processes for these products successfully inactivate or remove virus

Assessments carried out by the European Medicines Agency (EMA) and competent authorities in the EU Member States have confirmed that there is no increased risk of contamination with the Zika virus for patients who take plasma-derived or urine-derived medicines.

Plasma-derived medicines are manufactured from human blood. They are used to treat and prevent serious diseases and include coagulation factors (treatments which help blood to clot) and immunoglobulins (proteins used in patients who need more antibodies in their blood to help fight infections and other diseases). Urine-derived products are manufactured from pooled human urine and include certain hormone-based treatments and urokinase products (medicines used to break up blood clots).

These medicines are produced from body fluids, which might be sourced in parts of the world where the Zika virus is prevalent. EU regulators sought reassurance that there is no risk of the virus contaminating the final product and thus affecting the patients taking it if the plasma or urine came from donors who had contracted the Zika virus.

EMA's Committee for Medicinal Products for Human Use (CHMP) has addressed the potential risk from Zika virus for plasma-derived medicinal products. The CMDh has coordinated the assessment by EU Member States on the potential risk from Zika virus for urine-derived medicinal products.

The CHMP concluded at its meeting last week that the manufacturing processes used for plasma-derived products, including for example the solvent/detergent method to inactivate viruses, pasteurisation (liquid heat inactivation) and virus filtration, inactivate or remove the Zika virus from the finished product. The CHMP therefore considered that no additional safety measures such as the testing or exclusion of certain plasma donors was necessary.

Concerning urine-derived products, the CMDh, following the assessment of the data, concluded that the manufacturing processes for these products contain complementary steps with inactivation/removal capacity for enveloped viruses, which are considered sufficient for Zika virus safety of these products. Additional safety measures such as the screening of urine donors or donations or the deferral of donors returning from affected areas are not considered necessary.

The findings from these assessments on the viral safety of plasma-derived and urine-derived medicines are available in a report from the CHMP's Biologics Working Party (BWP) published today.

Notes:

- The CMDh is a medicines regulatory body representing the European Union (EU) Member States.
- The Biologics Working Party (BWP) provides recommendations to EMA's scientific committees on all matters relating directly or indirectly to quality and safety aspects relating to biological and biotechnological medicines.
- The BWP recommendation on plasma-derived products is in line with the guidance published in July 2016 by the European Centre for Disease Prevention and Control (ECDC) entitled "Zika virus and safety of substances of human origin - A guide for preparedness activities in Europe".

Name	Language	First published	Last updated
Zika virus infection: plasma- and urine-derived medicines safe to use	(English only)	21/09/2016	

How useful is this page?

Average rating:

別紙様式第2-1

No. 8

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2016. 11. 4	該当なし	

一般的名称	人赤血球液	研究報告の公表状況	Tyler M. Sharp , Aidsa Rivera , Melissa Bello Pagan , et al. The ASTMH 65th Annual Meeting, November13-17, 2016, Atlanta, GA, United States, LB-5149	公表国
販売名(企業名)	赤血球液-LR「日赤」(日本赤十字社) 照射赤血球液-LR「日赤」(日本赤十字社)			米国

研究報告の概要

○ジカウイルス(ZIKV)感染に関連する重篤な血小板減少症の発生率及び転帰－プエルトリコ(2016年)。
南北アメリカ大陸に近年出現したZIKVは、2015年後半にプエルトリコで初めて検出された。ZIKV疾患(発熱、発疹)は一般的に軽度であるが、重篤な血小板減少症を含む致死的な結果との関連が認められている。ZIKV感染患者における重篤な血小板減少症(血小板数 20,000/μL未満)の発生率及び転帰を明らかにすることを試みた。ZIKV感染患者・既往者13,200例のうち、計121例(0.9%)が血小板減少症(血小板数 100,000/μL以下)を報告していた。35例の診療記録について検討が行われ、19例(54%)の血小板減少症が確認されている。7例が重篤な血小板減少症に罹患しており、最低血小板数の範囲は1,000/μL～18,000/μL(中央値 6,000/μL)であった。これらの患者における年齢の中央値は45歳(範囲 30歳～88歳)であり、5例(71%)が男性であった。発症から血小板数が最低となった日までの日数の中央値は5日(範囲 3日～7日)であった。7例全例が入院しており、入院期間の中央値は3日(範囲 1日～7日)であった。6例(86%)に血便または下血(n = 3)、斑状出血または血腫(n = 2)、明らかな血尿(n = 2)、頭蓋内出血(n = 1)等の重篤な出血が認められており、2例(25%)が死亡していた(共に入院後24時間以内に死亡)。この2例に対しては、コルチコステロイドの投与、免疫グロブリンの静脈内投与(IVIG)のいずれも行われていなかった。非致死例5例のうち、4例がコルチコステロイドの投与を受け、免疫性血小板減少性紫斑病の診断を受けた2例がIVIGを受けていた。致死例1例並びに非致死例全例が血小板または赤血球の輸血を受けていた。これまで、重篤な血小板減少症はZIKV感染の稀な合併症であると考えられているが、ZIKVに関連する重篤な血小板減少症の病因並びに生存に関わる治療介入について特徴付けを行うためには、更なる調査が必要である。

報告企業の意見	今後の対応
ジカウイルス感染患者は0.9%(121/13,200)に血小板減少症を伴い、重篤であった7例中の2例の患者は入院後24時間以内に死亡し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、平成28年2月3日付厚生労働省医薬・生活衛生局血液対策課長事務連絡「ジカウイルスによることが疑われる小頭症等の増加に関するWHO緊急委員会宣言について(注意喚起)」の発出を受け、同月4日付で各血液センターに対し問診時の帰国(入国)後経過日数の確認を徹底するよう指示した。さらに同年7月1日から、ジカウイルス感染症と診断され、治癒後1ヶ月間経過していない場合は献血不適としている。今後もジカウイルス感染症に関する新たな知見等について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

赤血球液-LR「日赤」
照射赤血球液-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

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The ASTMH 65th Annual Meeting, November13–17, 2016, Atlanta, GA, United States

Session 27 - Late Breakers in Clinical Tropical Medicine and Global Health

LB-5149 - Incidence and outcome of severe thrombocytopenia associated with Zika virus infection — Puerto Rico, 2016

November 14, 2016, 12:15 - 12:25 PM | Marriott - Imperial A

Authors

Tyler M. Sharp[1], Aidsa Rivera[1], Melissa Bello Pagan[2], Laura Adams[1], Jorge Munoz-Jordan[1], Miriam Garcia[2], Jennifer S. Read[1], Stephen H. Waterman[1], Brenda Rivera-Garcia[2]

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Disclosures

T.M. Sharp: None.

Abstract

Zika virus (ZIKV) recently emerged in the Americas, and was first detected in Puerto Rico in late 2015. ZIKV disease is generally mild (fever, rash); however, ZIKV infection has been associated with life-threatening outcomes including severe thrombocytopenia. We sought to define the incidence and outcome of ZIKV-infected patients with severe thrombocytopenia (platelet count < 20,000/µl). We reviewed passive surveillance data for patients with ZIKV infection (defined by RT-PCR or anti-ZIKV IgM ELISA) and reported thrombocytopenia (platelet count ≤ 100,000/µl). Patients with a condition that would explain thrombocytopenia were excluded. Of 13,200 patients with current or recent ZIKV infection reported through August 24, 2016, a total of 121 (0.9%) had reported thrombocytopenia. Of 35 medical records from patients with reported thrombocytopenia reviewed to date, thrombocytopenia was confirmed in 19 (54%). Seven patients had severe thrombocytopenia, in whom nadir platelet count ranged from 1000-18000/µl (median: 6000). Median age of these patients was 45 years (range: 30-88), and 5 (71%) were male. Six (86%) reported a recent illness, which in all cases included fever and rash. Nadir platelet count occurred a median of 5 days after illness onset (range: 3-7). All 7 were hospitalized, and median duration of hospitalization was 3 days (range: 1-7). Six (86%) patients had major hemorrhage, including hematochezia or melena (n = 3), ecchymoses or hematomas (n = 2), frank hematuria (n = 2), and intracranial hemorrhage (n = 1). Two (25%) patients died, both within 24 hours of hospitalization; neither received corticosteroids or intravenous immunoglobulin (IVIG). Of 5 non-fatal cases, 4 received corticosteroids, and 2 were diagnosed with immune thrombocytopenic purpura and received IVIG. One fatal case and all non-fatal cases received transfusions of platelets or red blood cells. Thus far, severe thrombocytopenia appears to be a rare complication associated with ZIKV infection. Further investigation is needed to characterize the pathogenesis of ZIKV-associated severe thrombocytopenia and interventions associated with survival.

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. 11. 4	該当なし	

一般的名称	人赤血球液	研究報告の公表状況	Torben Schmidt, Denis Kuehnel, Sebastian Mueller, et al. 58th ASH Annual Meeting & Exposition, December 3-6, 2016, San Diego, CA, 2630	公表国
販売名(企業名)	赤血球液-LR「日赤」(日本赤十字社) 照射赤血球液-LR「日赤」(日本赤十字社)			ドイツ

研究報告の概要

○有機溶剤/界面活性剤(S/D)処理または液状低温殺菌法(パスツリゼーション)によるジカウイルス(ZIKV)の不活化。
背景:南北アメリカ大陸では2015年以降、また他の地域においては2015年以前からZIKVの拡大が見られており、2016年にはWHOが「国際的に懸念される公衆衛生上の緊急事態(PHEIC)に該当する」と宣言している。病原体混入防止という側面から血漿製剤の製造においては、確実な病原体除去/低減手段が必要であり、S/D処理並びに液状低温殺菌法の双方がZIKVを有効に不活化することを示す。
研究デザイン及び方法:様々な製剤並びに処理条件における液状低温殺菌法及びS/D処理のZIKV不活化能力を検討した。製造過程にある製剤用原料血漿のバッチからサンプルを採取し、ZIKVを約1:10の割合で添加した。不活化の動態を調べるため、ウイルス量を処理工程前、更に各プロセスの間にも事前に定めた間隔で測定を行った。
結果:ヒト血漿製剤の製造過程では、S/D処理の60分後にウイルス力価が検出限界以下となり、結果的にウイルス低減率(LRF)の平均は6.78 log10以上、免疫グロブリン(IgG)製剤については、240分後のLRFが7.00 log10以上、第VIII因子製剤では、480分後に6.18 log10以上不活化されていた。
ヒトアルブミン製剤の液状低温殺菌過程においては、加熱段階を経た後、ZIKVの感染力が既に検出限界以下となっていることを2時間の処理によって確認した。LRFは7.48 log10以上であることが実証された。
結論:様々なS/D処理手順によりZIKVが検出限界以下まで不活化されることを証明した。更に液状低温殺菌法が非常に有効なZIKV不活化手段であることを証明した。これらの結果は、ヒト血漿製剤並びに血漿分画製剤(IgG製剤、第VIII因子製剤など)の製造過程において、ZIKVのような新興エンベロープウイルスに対してもS/D処理が有効であることを実証している。

報告企業の意見	今後の対応
有機溶剤/界面活性剤(S/D)処理並びに液状低温殺菌法(パスツリゼーション)は、ジカウイルスに対して有効な不活化法であり、ウイルス力価が検出限界以下まで低減化されたことが確認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、平成28年2月3日付厚生労働省医薬・生活衛生局血液対策課長事務連絡「ジカウイルスによることが疑われる小頭症等の増加に関するWHO緊急委員会宣言について(注意喚起)」の発出を受け、同月4日付で各血液センターに対し問診時の帰国(入国)後経過日数の確認を徹底するよう指示した。さらに同年7月1日から、ジカウイルス感染症と診断され、治癒後1ヶ月間経過していない場合は献血不適としている。今後もジカウイルス感染症に関する新たな知見等について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

赤血球液-LR「日赤」
照射赤血球液-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

58th ASH® Annual Meeting & Exposition, December 3-6, 2016, San Diego, CA

2630 Inactivation of Zika Virus By Solvent/Detergent Treatment or Pasteurization

Basic Science and Clinical Practice in Blood Transfusion
Program: Oral and Poster Abstracts
Session: 401. Basic Science and Clinical Practice in Blood Transfusion: Poster II

Sunday, December 4, 2016, 6:00 PM-8:00 PM
Hall GH (San Diego Convention Center)

Torben Schmidt[1]*, Denis Kuehnel[1]*, Sebastian Mueller[1]*, Alexander Pichotta[1]*, Kai Uwe Radomski[1]*, Andreas Volk[1]* and Sigurd Knaub[2]

[1]Octapharma Biopharmaceuticals GmbH, Frankfurt am Main, Germany
[2]Octapharma AG, Lachen, Switzerland

BACKGROUND: Since 2015 Zika virus (ZIKV) has spread within the Americas and even before in other regions. In 2016, the World Health Organisation (WHO) declared the ZIKV as a "Public Health Emergency of International Concern" (PHEIC). Therefore, it is very important to confirm the pathogen safety of blood plasma products. These products generally demand an effective donor selection as well as robust pathogen elimination/reduction steps within the manufacturing processes. Here, we provide experimental evidence that both solvent/detergent (S/D) treatment and pasteurization effectively inactivate ZIKV.

STUDY DESIGN AND METHODS: The ZIKV inactivation capacity of the pasteurization step and the S/D treatment for different products and process conditions were investigated. Therefore, in-process material from commercial batches was collected and spiked with ZIKV in a ratio of approximately 1:10. Viral loads were determined prior to the safety steps and at pre-defined intervals during the individual processes to investigate the inactivation kinetics. Each process was investigated in duplicate runs.

RESULTS: Sixty minutes after S/D treatment the viral titer was below detection limit during manufacturing of human plasma resulting in mean ZIKV reduction factor (LRF) of ≥ 6.78 $\log_{10}$. For immunoglobulin (IgG) after 240 min of S/D treatment the LRF was determined to be ≥ 7.00 $\log_{10}$. ZIKV was inactivated ≥ 6.18 $\log_{10}$ after 480 min by S/D treatment during the manufacture of factor VIII.

During pasteurization of human albumin ZIKV infectivity was already below the detection limit after the heat-up phase. This finding was confirmed by subsequent test samples up to 2 hours. A LRF of ≥ 7.48 $\log_{10}$ was demonstrated.

CONCLUSION: We showed that different S/D treatment procedures inactivate the ZIKV to below the detection limit. This effect was seen independently of various product matrices or the choice of S/D reagent at various concentrations and temperatures, while taking product-specific treatment times into consideration. We also showed that pasteurization is a very efficient inactivation step for the ZIKV. These results demonstrate the effectiveness of S/D treatment against even newly emerging lipid-enveloped viruses like ZIKV in the manufacturing of human plasma and derivatives thereof, such as IgG or factor VIII.

Disclosures: Schmidt: *Octapharma:* Employment. **Kuehnel:** *Octapharma:* Employment. **Mueller:** *Octapharma:* Employment. **Pichotta:** *Octapharma:* Employment. **Radomski:** *Octapharma:* Employment. **Volk:** *Octapharma:* Employment. **Knaub:** *Octapharma:* Employment.

See more of: 401. Basic Science and Clinical Practice in Blood Transfusion: Poster II
See more of: Basic Science and Clinical Practice in Blood Transfusion
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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 2016 年 11 月 28 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	https://www.cdc.gov/media/releases/2016/s1122-microcephaly-onset-after-birth.html/2016/11/22	公表国 アメリカ； ブラジル	
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

米 CDC，米国およびブラジルの研究者がジカに関連した小頭症および出生後のその他の神経学的合併症に関するエビデンスを確認：

米 CDC の研究者は，米国およびブラジルの研究者と共同で，出生後の小頭症発症が記録された先天性ジカウイルス感染の臨床検査値上の証拠を有する乳児の最初のシリーズについて調査を行った。この報告は MMWR において公表され，ブラジルの先天性ジカウイルス感染を伴う乳児 13 例（出生時には小頭症を伴っていなかったが，後に頭部の成長遅延を発症した）について記述している。これらの乳児のうち，11 例が小頭症を発症した。出生時には小頭症を発症していなかったにも関わらず，乳児は先天性ジカ症候群に一致するその他の脳異常を有していた。 この研究により，妊娠中に母親がジカウイルスに曝露した乳児において，出生時に小頭症ではないことによって先天性ジカウイルス感染またはジカ関連の脳異常の存在が除外されるわけではないということを明らかにしたことなどについて報告されている。

報告企業の意見	今後の対応
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、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。

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

CDC, US and Brazilian researchers find evidence of onset of Zika-associated microcephaly and other neurologic complications after birth

Media Statement

For Immediate Release: Tuesday, November 22, 2016
Contact: Media Relations (https://www.cdc.gov/media), (404) 639-3286

CDC researchers in collaboration with researchers from the United States and Brazil investigated the first series of infants with laboratory evidence of congenital Zika virus infection documented to have onset of microcephaly after birth.

The report, published today in CDC's Morbidity and Mortality Weekly Report, describes 13 infants in Brazil with congenital Zika virus infection who did not have microcephaly at birth, but later experienced slowed head growth. Among these infants, 11 later developed microcephaly. Slowed head growth and microcephaly were accompanied by significant neurologic complications. Although microcephaly was not present at birth, the infants had other brain abnormalities consistent with congenital Zika syndrome.

The study reveals that among infants of mothers exposed to Zika virus during pregnancy, the absence of microcephaly at birth does not rule out congenital Zika virus infection or the presence of Zika-related brain abnormalities.

The findings highlight the importance of recent CDC guidance (https://www.cdc.gov/zika/hc-providers/infants-children.html) on initial and continuing medical and developmental evaluations of infants with possible congenital Zika virus infection and the importance of early neuroimaging for infants who were exposed to Zika virus prenatally.

CDC Guidance for Pregnant Women and Women Considering Pregnancy

CDC continues to recommend that pregnant women not travel to areas with Zika. If a pregnant woman travels to or lives in an area with active Zika virus transmission, she should talk with her healthcare provider and strictly follow steps to prevent mosquito bites and sexual transmission of Zika virus. Pregnant women with possible exposure to Zika virus should be tested for Zika infection even if they do not have symptoms. For more information, please visit www.cdc.gov/zika/pregnancy/ (https://www.cdc.gov/zika/pregnancy/).

CDC continues to encourage women considering pregnancy and their partners in areas with active Zika transmission to talk to their healthcare providers about pregnancy planning so that they know the risks and the ways to reduce them. For more information, please visit www.cdc.gov/zika/pregnancy/thinking-about-pregnancy.html (https://www.cdc.gov/zika/pregnancy/thinking-about-pregnancy.html).

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

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医薬品 研究報告 調査報告書

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研究報告の概要

○リベリアにおけるエボラウイルス疾患生存者男性を対象とした国内精液検査・カウンセリングプログラムによるエボラの性感染の予防:エボラウイルスRNA検査の結果及び行動データの解析。
背景:リベリアのMen's Health Screening Program(MHSP)によって、エボラウイルス疾患の生存者男性を対象に精液のエボラウイルス検査が実施されており、中間結果並びに行動変化を報告する。
方法:MHSPはリベリア国内の3ヶ所で実施されており、エボラ治療病棟退院証明書を有する15歳以上の男性を対象としている。リアルタイムRT-PCR法による参加者の精液のエボラウイルスRNA検査が行われ、2回連続で陰性となった後にプログラムからの離脱が認められた。当プログラムは、現在も実施中である。
結果:2016年5月6日現在、466名のエボラウイルス疾患生存者が当該プログラムに登録しており、429名のリアルタイムRT-PCR検査結果が入手可能であった。24名については、エボラウイルス疾患の回復から12ヶ月もしくはこれ以上の期間が経過した後に精液が陽性を示し、最長は565日であった。40歳以上の精液は、40歳までの若者の検体に比較して陽性となる確率がより高くなった(p=0.0004)。登録時にコンドームを使用していないと報告した113名の参加者のうち、84名(74%)が初回の追跡調査来院時にコンドームの使用を報告していた(p<0.0001)。登録時に性的に活発であると報告した385名の参加者のうち、176名(46%)が追跡調査来院時に性交渉を断っていると報告していた(p<0.0001)。
考察:リアルタイムRT-PCR検査によってエボラウイルスRNAが検出される期間の長さは参加者によって異なり、年齢に関連している可能性が考えられる。MHSPは、行動に関するカウンセリングと検査を組み合わせることによって、男性のエボラウイルス疾患生存者が自身のリスクを理解し、性的パートナーを守るために適切な行動をとることを促している。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
リベリアのエボラウイルス疾患の生存者男性466名中24名は回復後12ヶ月間以上、最長では565日間目の精液からウイルスRNAが検出され、40歳以上の陽性率が有意に高かっ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、更にリベリアを含むエボラウイルス流行地域はマラリア流行地域と重なる為、これら地域の滞在者は1年間の採血延期の措置をとっている。また、発熱などの体調不良者を献血不適としている。今後も引き続き情報の収集に努める。

Prevention of sexual transmission of Ebola in Liberia through a national semen testing and counselling programme for survivors: an analysis of Ebola virus RNA results and behavioural data

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Summary

Background Ebola virus has been detected in semen of Ebola virus disease survivors after recovery. Liberia's Men's Health Screening Program (MHSP) offers Ebola virus disease survivors semen testing for Ebola virus. We present preliminary results and behavioural outcomes from the first national semen testing programme for Ebola virus.

Methods The MHSP operates out of three locations in Liberia: Redemption Hospital in Montserrado County, Phebe Hospital in Bong County, and Tellewoyan Hospital in Lofa County. Men aged 15 years and older who had an Ebola treatment unit discharge certificate are eligible for inclusion. Participants' semen samples were tested for Ebola virus RNA by real-time RT-PCR and participants received counselling on safe sexual practices. Participants graduated after receiving two consecutive negative semen tests. Counsellors collected information on sociodemographics and sexual behaviours using questionnaires administered at enrolment, follow up, and graduation visits. Because the programme is ongoing, data analysis was restricted to data obtained from July 7, 2015, to May 6, 2016.

Findings As of May 6, 2016, 466 Ebola virus disease survivors had enrolled in the programme; real-time RT-PCR results were available from 429 participants. 38 participants (9%) produced at least one semen specimen that tested positive for Ebola virus RNA. Of these, 24 (63%) provided semen specimens that tested positive 12 months or longer after Ebola virus disease recovery. The longest interval between discharge from an Ebola treatment unit and collection of a positive semen sample was 565 days. Among participants who enrolled and provided specimens more than 90 days since their Ebola treatment unit discharge, men older than 40 years were more likely to have a semen sample test positive than were men aged 40 years or younger ($p=0\cdot0004$). 84 (74%) of 113 participants who reported not using a condom at enrolment reported using condoms at their first follow-up visit ($p<0\cdot0001$). 176 (46%) of 385 participants who reported being sexually active at enrolment reported abstinence at their follow-up visit ($p<0\cdot0001$).

Interpretation Duration of detection of Ebola virus RNA by real-time RT-PCR varies by individual and might be associated with age. By combining behavioural counselling and laboratory testing, the Men's Health Screening Program helps male Ebola virus disease survivors understand their individual risk and take appropriate measures to protect their sexual partners.

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Introduction

In March, 2015, a 44-year-old female from Monrovia, Liberia, contracted Ebola virus disease and died in an Ebola treatment unit (ETU). An extensive investigation revealed one epidemiological link to Ebola virus exposure: unprotected sexual intercourse with a male Ebola virus disease survivor.[1] A semen specimen collected from the Ebola virus disease survivor tested positive for Ebola virus RNA by real-time RT-PCR (rRT-PCR) 199 days after he first became ill with Ebola virus disease. Although no infectious virus was isolated from the semen, genetic analysis of the Ebola virus collected

Research in context

Evidence before this study

We searched PubMed and MEDLINE for the following search terms: "Ebola" and "sexual transmission", "semen", and "viral persistence". The search was done from Nov 15, 2015, to May 30, 2016. Search results were then limited to research studies using RT-PCR or virus culture, or both, to detect the presence of Ebola virus in semen of survivors of Ebola virus disease. This search yielded seven articles. Four publications reported results for Ebola virus disease survivors of the 2014 west Africa Ebola virus disease outbreak treated in west Africa. Of these, the largest cohort of Ebola virus disease survivors tested was 100. The longest period of time between disease onset and the detection of Ebola virus disease RNA by RT-PCR was 276 days. Viral culture results were not reported in the four articles reporting semen test results of Ebola virus disease survivors cared for in west Africa. One article reported semen test results from five male Ebola virus disease survivors cared for in the USA. The longest period of time between disease onset to the detection of Ebola virus disease RNA by RT-PCR in these men was 290 days. The highest cycle threshold value for the nucleoprotein gene target for semen specimens from which Ebola virus disease was isolated by viral culture was 30.

Added value of this study

We describe the preliminary test results and behavioural outcomes for, to our knowledge, the first national semen testing and counselling public health programme for Ebola virus disease survivors. We present serial RT-PCR semen test results for 429 Ebola virus disease survivors in Liberia, and report a possible association of age and the duration of detection of Ebola virus RNA by RT-PCR. We also report, to our knowledge, the longest interval between discharge from the Ebola treatment unit and the collection of a positive semen sample (565 days). We found that counselling paired with laboratory testing favourably affected reported condom use by men enrolled in the programme, with 74% of participants who reported not using a condom at enrolment subsequently reporting using a condom at their last sexual encounter.

Implications of all the available evidence

We found that the duration for which Ebola virus is detected in the semen of Ebola virus disease survivors varies by individual. As such, semen testing programmes that combine behavioural counselling and laboratory testing can play an important part in educating male survivors of Ebola virus disease of their risk of transmitting Ebola virus through sex and could potentially mitigate future outbreaks associated with sexual transmission. The Men's Health Screening Program can serve as a model for future semen testing programmes for Ebola virus. We also found that the duration in which Ebola virus is detected in the semen of Ebola virus disease survivors might be associated with age. Future studies should be designed to investigate this possible association and to identify other factors that might be associated with prolonged viral persistence in semen.

from the semen of the Ebola virus disease survivor closely matched the Ebola virus recovered from the female patient.[2]

Based on virus-isolation results from previous Ebola virus disease and Marburg virus disease survivors,[3–5] Ebola virus disease survivors were encouraged to practice abstinence or use condoms for 90 days after recovering from the disease. However, the possibility of infectious Ebola virus persisting in the semen of survivors beyond this timeframe prompted WHO to issue new guidance. In May, 2015, WHO released interim guidance for male Ebola virus disease survivors.[6] This interim guidance recommended the following: (1) in addition to receiving condoms and sexual risk reduction counselling at ETU discharge, all male survivors should be offered semen testing for Ebola virus RNA by rRT-PCR until their semen tests negative twice for Ebola virus RNA; (2) male survivors and their sexual partners should be provided with condoms and receive counselling to ensure safe sexual practices until their semen has twice tested negative; and (3) if a survivor's semen has not been tested, he should practise safe sex for at least 6 months after onset of symptoms.

See Online for appendix

At the time WHO released their interim guidance, semen testing for Ebola virus RNA was not widely available in Liberia. To address this gap, on July 7, 2015, the Liberian Ministry of Health, in collaboration with WHO, the Academic Consortium Combating Ebola in Liberia, and the US Centers for Disease Control and Prevention (CDC), launched the Men's Health Screening Program (MHSP). Based on the principles outlined in WHO's interim guidance,[6] the MHSP provides male Ebola virus disease survivors with semen testing for Ebola virus RNA by rRT-PCR and behavioural counselling on safe sex practices. We describe Liberia's national semen testing programme for Ebola virus, present preliminary semen testing results, and report sexual risk behaviours.

Methods

Study design and participants

The MHSP operations manual is provided in the appendix (pp 2–34). The MHSP operates out of three locations in Liberia: Redemption Hospital in Montserrado County, Phebe Hospital in Bong County, and Tellewoyan Hospital in Lofa County (figure 1). Men are eligible to enrol if they are aged 15 years or older and can provide an ETU discharge certificate. Due to insufficient laboratory capacity and challenges in specimen transportation during the height of the Ebola virus disease outbreak in Liberia, laboratory confirmation of Ebola virus infection was not available for all patients with suspected Ebola

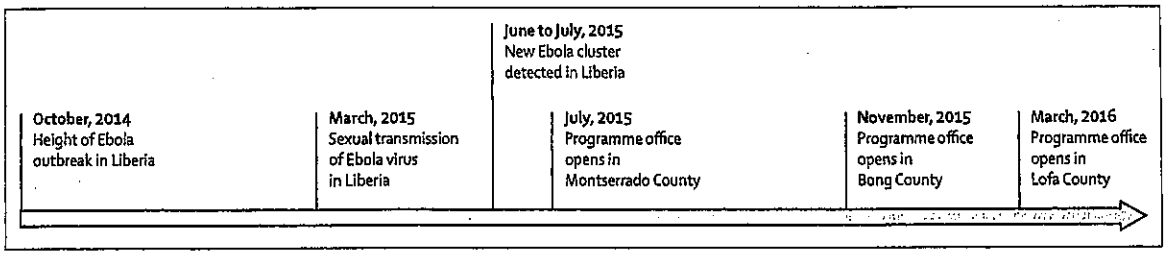

Figure 1: **Programme implementation timeline**

virus disease.[7–9] As such, possession of an ETU discharge certificate was used as proof of survival from the disease.

Potential programme participants are identified through two primary methods: informational events held in conjunction with Ebola virus disease survivor association meetings and through the national Ebola virus disease survivor registry. Maintained by the Liberia Ministry of Health, as of June 17, 2015, the national Ebola virus disease survivor registry listed 1541 laboratory-confirmed Ebola virus disease survivors, 534 of whom were males individuals aged 15 years or older.

Created to implement a WHO-recommended semen testing programme for male Ebola virus disease survivors, the MHSP was granted a non-research determination by the CDC. On June 18, 2015, the Liberian Ministry of Health officially adopted the MHSP as a public health programme of Liberia. Programme participation was voluntary and written informed consent was obtained from participants before programme enrolment.

Procedures

Upon programme enrolment, trained counsellors collect information from enrollees on sociodemographics and sexual behaviours since ETU discharge of the enrollee using a standardised baseline questionnaire (panel; appendix pp 35–47); provide counselling on safe sexual practices (appendix pp 48–69); and provide condoms and instruction on condom use. Date of discharge from an ETU is established from the partitipant's discharge certificate. Additionally, all participants receive a brochure listing health-care facilities that provide clinical care services to Ebola virus disease survivors. Participants presenting with physical or psychological complaints are referred for health-care services as needed or if requested. In keeping with the MHSP's non-research status, data collection was limited to those items that directly affected programme delivery and services. As such, participants were not asked about any pre-existing medical disorders (eg, hypertension, diabetes, or HIV).

Participants were also asked to provide a semen sample for testing by rRT-PCR. After collection of the first semen sample, the frequency of subsequent semen tests was dependent on the test result of the previous sample (figure 2). Participants whose previous semen sample tested positive for the presence of Ebola virus RNA were tested once a month. Participants whose previous semen sample did not detect Ebola virus RNA were tested every 2 weeks, which is the minimum turnaround time for specimen transport and processing. In accordance with WHO guidelines,[6,10] participants graduate from the programme after receiving two consecutive semen test results that do not detect the presence of Ebola virus RNA by rRT-PCR. Participants who are repeatedly unable to produce a specimen (at least three unsuccessful attempts) are referred for health-care services but may remain enrolled in the programme.

During follow-up visits, participants are informed of their individual rRT-PCR results, asked about their sexual

Panel: **Selected questionnaire data fields**

Baseline questionnaire
Whether he (the patient) has resumed sexual activity
Frequency of sexual intercourse
Condom use at last sexual encounter; where he procured the condoms
Signs and symptoms of a sexually transmitted infection*
Age
Highest level of education attained
Marital status
Date of ETU admission or discharge
Did he receive counselling on when they were safe to resume sexual activity; if so, who provided this counselling?
After ETU discharge, did he discuss with his sexual partner when to resume sexual activity?

Follow-up questionnaire
Whether he has had sex since the last visit
Frequency of sexual intercourse
Condom use at last sexual encounter
Signs and symptoms of a sexually transmitted infection*

Graduation questionnaire
Whether he has had sex since the last visit
Frequency of sexual intercourse
Condom use at last sexual encounter
Signs and symptoms of a sexually transmitted infection*
Whether he plans to share his semen test results with his sexual partner or partners
Confidence in correctly putting on and taking off a condom
Participant satisfaction with programme services
Why he choose to receive services from the clinic or mobile team

ETU=Ebola treatment unit. *Dysuria, penile discharge, testicular pain or swelling, or genital sores or blisters.

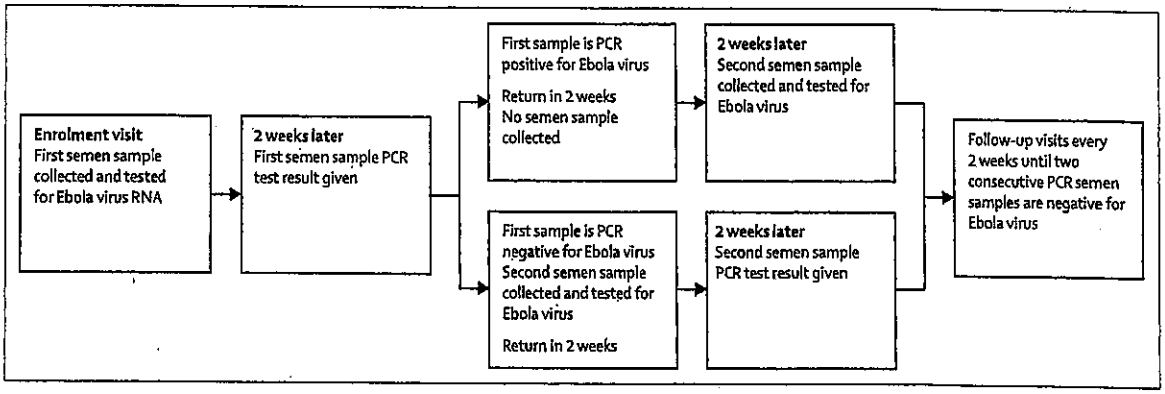

Figure 2: **Semen testing algorithm**
Frequency of semen collection is dependent on the previous semen test result: if positive, the next sample is taken 4 weeks later; if negative, the next sample is taken 2 weeks later.

practices since their last visit using a standardised follow-up questionnaire (panel), counselled on safe sexual practices, and provided with condoms. In addition to questions about their sexual practices, questions were added on Oct 20, 2015, to assess graduating participants' satisfaction with programme services, and their confidence in correctly putting on and taking off a condom (graduation questionnaire; panel). Consistent with other semen testing services for Ebola virus disease survivors in Liberia, MHSP participants were given US$5 to cover transportation costs and $20 for semen samples.

The primary location for service delivery for both the enrolment and follow-up visits was the programme clinic. However, Ebola virus disease survivors who were unable to travel to the programme clinic were offered services at a location of their choosing by a two-person mobile team composed of a counsellor and a semen technician. In addition to the services offered in the clinic setting, participants who receive services from the mobile team were offered the opportunity to include their sexual partners in their counselling sessions.

The risk reduction behavioural counselling used in the MHSP was adapted from the Ebola virus disease Viral Persistence Study in Sierra Leone.[11] The goal of the counselling programme was to (1) encourage abstinence or condom use among programme participants until they had received two consecutive semen test results that did not detect the presence of Ebola virus RNA by rRT-PCR; and (2) refer programme participants to available Ebola virus disease survivor services in the community as needed for other health concerns.

Behavioural counselling to reduce the risk of spreading Ebola virus disease was provided at each visit (appendix pp 48–69). At the first visit, counsellors introduced the participant to the session; provided information about the semen rRT-PCR test; conveyed the importance of abstinence or condom use, or both, to reduce risk of transmitting Ebola virus; engaged the participant in a conversation regarding his sexual behaviour and intimate relationships; negotiated risk reduction steps with the participant; and demonstrated how to safely use and dispose of condoms using a wooden penis model. At each subsequent visit, counsellors delivered the semen test results from the previous visit, explained what the test results mean, and provided education on ways to reduce the risk of sexual transmission of the virus. These processes continued until the participant was discharged from the programme after having two consecutive negative semen test results. At all counselling sessions, counsellors asked participants to report any medical, psychosocial, or other issues they were experiencing. Counsellors referred these participants to appropriate health-care services.

All self-collected semen specimens submitted by the MHSP were stored and transported at –20°C or colder to the Tappita Ebola virus disease laboratory in Nimba County. Upon receipt in the laboratory, specimens were maintained at –20°C or colder until testing with the CDC's Ebola Virus NP and VP40 Real-Time RT-PCR Assays (CDC, Atlanta, GA, USA).[12] Briefly, semen specimens were allowed to thaw for up to 30 min before total nucleic acid isolation using the MagMAX Pathogen RNA/DNA Kit (Applied Biosystems, Foster City, CA, USA). Inactivation of the semen specimen with MagMax lysis buffer (ThermoFisher Scientific, Waltham, MA, USA) was done in a glove box. After proper exterior decontamination of the vial, automated nucleic acid extraction was done using the BeadRetriever platform (Applied Biosystems). rRT-PCR was done on the Bio-Rad CFX96 Touch (Hercules, CA, USA) instrument as per the Emergency Use Authorization protocols.[12] The combined Emergency Use Authorization assays detected specific sequences of the Ebola virus' nucleoprotein and viral matrix protein (VP40) genes and the human RNase P gene that controlled for nucleic extraction and specimen quality. Samples of the extracted nucleic acids were stored at –70°C or colder for potential retesting.

Interpretation of the rRT-PCR results was done in a similar way to that described previously.[11] Semen test

results were reported to the MHSP with target PCR cycle threshold values (ie, the number of cycles needed for the fluorescent signal to exceed the background level) and interpretation. Specimens were deemed positive if both targets (nucleoprotein and VP40) were amplified within 40 cycles of replication. Specimens were deemed negative if neither of the Ebola virus targets was amplified within 40 cycles of replication and the RNase P target yielded amplification in less than 30 cycles of replication. Specimens were considered indeterminate if only one of the Ebola targets showed positive amplification. Specimens without amplification of either Ebola virus target and RNase P amplification of over 30 cycles of replication were judged to be of poor quality and re-collection was requested. Testing of semen samples was limited to rRT-PCR because undertaking virus isolation was not possible in Liberia.

Statistical analysis

Data were collected through the use of questionnaires at baseline and all follow-up visits and were entered into a Microsoft Access database. We used the χ^2 test to test for an association between categorical variables and the Kruskal-Wallis test to test for a difference between the median of non-normally distributed numerical variables across categorical groups. McNemar's test was used to test for differences between paired nominal data between baseline and the first follow-up visit. We used SAS version 9.3 for data analysis. Because the programme is ongoing, data analysis was restricted to data obtained from July 7, 2015, to May 6, 2016.

	Participants (n=466)
Age (years)	33 (15–79)
County of residence	
Montserrado	266 (57%)
Margibi	67 (14%)
Bong	49 (11%)
Lofa	49 (11%)
Other	35 (8%)
Point of service delivery	
Montserrado Program Office	273 (59%)
Bong Program Office	64 (14%)
Lofa Program Office	50 (11%)
Mobile team	79 (17%)
Time from ETU discharge to programme enrolment (days)	384 (7–697)
Sexually active at time of programme enrolment	424 (91%)
Reported sexual frequency at programme enrolment	
Twice a week or more	126/454 (28%)
More than once a month but less than twice a week	195/454 (43%)
Once a month or less	133/454 (29%)
Reported using a condom the last time they had sex	190/422 (45%)
Reported experiencing at least one sign or symptom of a sexually transmitted disease at programme enrolment*	118/463 (25%)

Data are median (range), number (%), or n/N (%). Some percentages do not add up to 100 because of rounding. ETU=Ebola treatment unit. *Dysuria, penile discharge, testicular pain or swelling, or genital sores or blisters.

Table 1: Participant characteristics

Results

As of May 6, 2016, 466 Ebola virus disease survivors had enrolled in the programme. Median time from ETU discharge to programme enrolment was 384 days (range 7–697; table 1); four survivors who were a part of an Ebola virus disease cluster in July, 2015 (figure 1), were enrolled within 2 weeks of ETU discharge. The median age of participants was 33 years (range 15–79), 266 (57%) were residents of Montserrado County, and 79 (17%) chose to receive services from the mobile team.

rRT-PCR results were available for 429 of 466 programme participants. Semen test results for nine participants were not available at the time of data analysis for this manuscript (May 6, 2016); five cited personal or religious objections to masturbation; seven were unable to provide a semen specimen because of erectile dysfunction; two reported being unable to produce a specimen as a result of ongoing medical issues, one because he was recovering from a hernia operation and the other because of testicular pain; eight were lost to follow-up according to the programme protocol after missing several follow-up appointments despite several attempts to re-engage them; and six were unable to produce a specimen at enrolment and had follow-up appointments scheduled after the cutoff date for data analysis for this report (May 6, 2016).

Among the 429 programme participants with rRT-PCR results, 38 (9%) had at least one semen sample test positive for Ebola virus RNA by rRT-PCR. The median age for those with positive Ebola virus results was 40 years (range 18–68) compared with 32 years (15–70) for those who never had a positive test (table 2). The proportion of men reporting at least one sign or symptom of a sexually transmitted disease did not differ between those who had at least one semen sample test positive for Ebola virus RNA (42%) and those who never had a semen sample test positive for Ebola virus RNA (39%; p=0·74).

24 (6%) of 429 participants provided semen specimens that tested positive for Ebola virus RNA at least 12 months after their ETU discharge. The longest interval between ETU discharge and the collection of a positive specimen was 18 months (565 days). One participant whose semen tested rRT-PCR positive more than 12 months after ETU discharge self-disclosed that he was diagnosed with HIV infection in 2009 and was taking antiretroviral therapy.

Ebola virus RNA was detected in the initial semen specimen of all four participants who were enrolled within 90 days of their ETU discharge, eight (22%) of 37 enrolled within 181–270 days, ten (8%) of 123 enrolled within 271–360 days, 11 (8%) of 133 enrolled within

	Participants with at least one positive semen test for EBOV (n=38)	Participants who never had a positive semen test for EBOV (n=391)	p value
Participants with detectable Ebola virus in semen ≥12 months after ETU discharge	24 (63%)	NA	NA
Age (years)	40 (18–68)	32 (15–70)	0·0002*
Reported ≥1 sign or symptom of a sexually transmitted infection	16 (42%)	154 (39%)	0·74†
Reported sexual frequency at baseline			
Twice a week or more	11 (29%)	106/381 (28%)	0·45†
More than once a month but less than twice a week	13 (34%)	167/381 (44%)	..
One a month or less	14 (37%)	108/381 (28%)	..

Data are number (%), median (range). EBOV=Ebola virus RNA. ETU=Ebola treatment unit. NA=not applicable. *Kruskal-Wallis. †χ^2 test.

Table 2: Participant characteristics by semen test result

	MHSP*	Sierra Leone[31]†	Guinea[13]‡	USA[14]§
1–90 days	4/4 (100%)	9/9 (100%)	4/14 (29%)	4/4 (100%)
91–180 days	0	26/40 (65%)	3/18 (17%)	3/4 (75%)
181–270 days	8/37 (22%)	11/43 (26%)	2/31 (6%)	2/5 (40%)
271–360 days	10/123 (8%)	NA	1/29 (3%)	0/1 (0%)
361–450 days	11/133 (8%)	NA	0/6 (0%)	0/1 (0%)
451–540 days	5/72 (7%)	NA	NA	NA
541–630 days	0/1 (0%)	NA	NA	NA

Data are n/N (%). MHSP=Liberia's Men's Health Screening Program. ETU=Ebola treatment unit. NA=not applicable. *Days from acute illness calculated from ETU discharge. †Days from acute illness calculated from symptom onset. ‡Days from acute illness calculated from disease onset. §Days from acute illness calculated from first blood sample negative for Ebola virus on PCR.

Table 3: Proportion of patients with initial samples positive for Ebola virus on qualitative RT-PCR by days from acute illness

361–450 days, and five (7%) of 72 enrolled within 451–540 days (table 3). No participants were enrolled 91–180 days after ETU discharge.

In semen specimens collected within 90 days of ETU discharge, the mean cycle threshold value was 32·28 (range 27·55–36·38) for VP40 and 33·37 (29·17–37·10) for nucleoprotein (table 4). After 90 days, mean cycle threshold values for both gene targets plateaued (VP40 range of means 35·91–36·82, nucleoprotein 36·99–38·70).

Among participants who enrolled and provided specimens more than 90 days since their ETU discharge, men older than 40 years were more likely to have at least one semen sample test positive for Ebola virus RNA by rRT-PCR than were men aged 15–40 years (p=0·0004; table 5). Also, reported sexual frequency did not differ between the two age groups (p=0·45). Participants who were tested within 90 days of ETU discharge were excluded from this analysis because Ebola virus persists in semen during this time period.[3,5]

427 (92%) of 466 participants reported being counselled by ETU staff to either abstain from sex or to use condoms for 90 days after recovering from Ebola virus disease. At enrolment, 424 (91%) participants reported having resumed sexual activity; 42 (9%) reported current abstinence (table 1). Of the 410 participants who reported the date they resumed sexual activity, 363 (89%) waited 90 days or more after their ETU discharge before resuming sex. Frequency of sexual intercourse was not associated with age (p=0·41; appendix p 69).

Of the 424 participants who reported resuming sexual activity on their initial enrolment questionnaire, 190 (45%) reported using a condom the last time they had sexual intercourse. At the first follow-up visit, 84 (74%) of 113 participants who reported not using condoms at enrolment reported use at their last sexual encounter (intrasubject comparison between enrolment and follow-up visit: p<0·0001). At graduation, 80 (76%) of 105 participants who reported not using condoms at enrolment reported use at their last sexual encounter (intrasubject comparison between enrolment and graduation: p<0·0001). Additionally, 176 (46%) of 385 participants for whom follow-up data were available who reported being sexually active at enrolment reported abstinence at the first follow-up visit (intrasubject comparison between enrolment and first follow-up: p<0·0001). At graduation, 109 (36%) of 300 participants who reported being sexually active at enrolment and for whom graduation data were available reported abstinence (intrasubject comparison between enrolment and graduation visit: p<0·0001).

326 participants graduated from the programme after two consecutive semen samples tested negative for Ebola virus RNA. 24 (63%) of 38 participants who had at least one semen sample that tested positive for the presence of Ebola virus RNA have graduated from the programme. The median time from programme enrolment to graduation was 50 days (range 24–226). Of the 299 programme graduates who were asked, 290 (97%) reported that they would refer a family member or friend to participate in the semen testing programme and 257 (86%) shared or planned to share their semen tests results with their sexual partners. Of the 294 programme graduates who provided a response to the graduation questionnaire question about confidence with condom use, 220 (75%) reported feeling "very confident" about knowing how to correctly put on and take off a condom, 52 (18%) reported feeling "somewhat confident", and 22 (7%) reported feeling "not confident" or "not at all confident".

Discussion

Among participants enrolled in the MHSP, 38 (9%) of 429 produced at least one semen sample that tested positive for Ebola virus RNA by rRT-PCR. Of these, 24 provided semen specimens that tested positive for the presence of Ebola virus RNA at least 12 months after ETU discharge. The longest interval between ETU

discharge to the collection of a positive semen sample among MHSP participants was 565 days, which exceeds previously reported time intervals.[11,13,14] However, detection of Ebola virus RNA by rRT-PCR does not necessarily indicate the presence of infectious virus.[3,5,9,11]

Consistent with findings from previous studies,[11,14] the lowest mean rRT-PCR values for both gene targets occurred in MHSP participants who were within 3 months of ETU discharge. Also consistent with findings from previous studies,[11,13,14] the total number of MHSP participants whose semen tested positive for the presence of Ebola virus RNA declined over time. Unlike findings from previous studies,[11,14] the mean cycle threshold values for both gene targets for MHSP participants seemed to plateau. Although some of these differences probably occurred because this study had a larger sample size and longer follow-up period than previous studies, this finding also suggests that heterogeneity exists in Ebola virus persistence in semen among survivors of Ebola virus disease.

Among men for whom more than 90 days had passed since their ETU discharge, age older than 40 years seemed to be a factor in the likelihood of having at least one semen sample test positive for Ebola virus RNA by rRT-PCR. This possible association, to our knowledge, has not been reported previously and the reason for this finding is not clear. Also, for men enrolled in the programme, reported sexual frequency was not associated with age. In view of these findings, differences in the persistence of Ebola virus RNA in semen might be a result of age-related factors such as changes in semen composition[15,16] or age-related changes in immune function. Because participants are not asked about pre-existing medical disorders, sustained viral persistence in semen could also be a result of the presence of other immunocompromising disorders, such as HIV or diabetes.

91% of men reported having resumed sexual activity at the time of programme enrolment. This finding is not surprising because the programme launched 9 months after the peak of the Ebola virus disease outbreak in Liberia. Initial examination of condom use and abstinence showed that counselling, paired with laboratory testing, led to a reduction in reported high-risk sexual behaviours. This reduction was noted at the first follow-up visit and at programme graduation. However, because these measures are all self-reported, the implications of these findings are limited by social desirability bias.

Preliminary data analysis of participants enrolled in the MHSP suggests that duration of detection of Ebola virus RNA by rRT-PCR varies by individual and might be associated with age. In view of this finding, semen testing programmes such as the MHSP, which combine behavioural counselling with laboratory testing, can play an important part in educating male Ebola virus disease survivors of their risk of transmitting Ebola virus through sex and could potentially mitigate future outbreaks associated with sexual transmission. However, our experience shows that implementing and sustaining such a programme is time and resource intensive. Health-care facilities caring for survivors after ETU discharge should offer semen testing and behavioural counselling as part of a standard package of health-care services. In addition to fostering a holistic approach to survivor care, integration would probably reduce the time and costs associated with implementing a semen testing programme and ensure a better surveillance for potential future transmission events.

	Number of semen samples testing positive for Ebola virus	Number of participants with at least one semen sample testing positive for Ebola virus*	VP40 mean (range)	Nucleoprotein mean (range)
1–90 days	8	4	32·28 (27·55–36·38)	33·37 (29·17–37·10)
91–180 days	3	2†	36·82 (36·38–37·62)	38·18 (37·43–39·22)
181–270 days	7	6	36·02 (30·58–38·39)	37·25 (32·00–39·54)
271–360 days	12	11	36·59 (34·31–39·09)	37·72 (34·87–39·58)
361–450 days	31	18	36·22 (31·40–39·50)	37·39 (32·94–39·68)
451–540 days	13	8	35·91 (31·58–39·74)	36·99 (32·14–39·74)
541–630 days	2	2	36·43 (35·20–37·66)	38·70 (38·48–38·91)

*These numbers are cumulative and only include patients who had not yet graduated. †Two patients produced samples that were of poor quality and could not be tested.

Table 4: Mean RT-PCR cycle threshold values by days from Ebola treatment unit discharge to collection of a positive semen sample

	Participants with at least one semen sample testing positive for Ebola virus (n=34)	Participants who never had a semen sample test positive for Ebola virus (n=391)
Age ≤40 years	17 (50%)	302 (77%)
Age >40 years	17 (50%)	89 (23%)

Analysis excluded four participants who enrolled within 90 days of Ebola treatment unit discharge. χ^2 p=0·0004 for the difference between age groups.

Table 5: Semen test results stratified by age

Contributors

MJS and MJC co-wrote the manuscript. AB, NM, MMa, JKo, PKB, AC, VL, SP, MMu, EG, KEV, SMZ, HHWG, BM, TGN, AG, BK, and DW developed the programme and wrote the programme description. SW, ER, CW, SD, OCS, JKp, SN, US, JAB, and PR wrote the laboratory methods section. LJP, SK, and MS did the data analysis and wrote the data analysis section. NA wrote the counselling section.

Declaration of interests

We declare no competing interests.

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No. 18

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. 9. 12	該当なし	

一般的名称	人血小板濃厚液	研究報告の公表状況		公表国
販売名（企業名）	濃厚血小板-LR「日赤」（日本赤十字社） 照射濃厚血小板-LR「日赤」（日本赤十字社） 濃厚血小板HLA-LR「日赤」（日本赤十字社） 照射濃厚血小板HLA-LR「日赤」（日本赤十字社） 照射洗浄血小板-LR「日赤」（日本赤十字社） 照射洗浄血小板HLA-LR「日赤」（日本赤十字社）		Strauss RG. Transfusion. 2016 Aug;56(8):1921-4.	米国

研究報告の概要

○現行の白血球除去法のみによるサイトメガロウイルス輸血感染（TTCMV）の十分な防止。
CMVは、ほぼ例外なく白血球中に存在しており、白血球除去によって、輸血によるCMV感染のリスクが大きく低下することが絶えず証明されている。しかし、白血球除去では血漿中に「浮遊する」ウイルス粒子を取り除くことはできない。感染初期は、このウイルス粒子の数は非常に少なく、この時点においてCMV抗体検査の結果は陰性のままである。感染が進行すると、「浮遊」ウイルス粒子の数は急速に増加するが、抗体量の急速な増加によって「浮遊」ウイルス粒子は消失する。
　現在使用されている白血球除去血液製剤によるTTCMVの正確なリスクは不明だが、比較的小規模の臨床試験並びにコホート研究の結果によると、全リスクは1%をはるかに下回ると考えられている。白血球除去血液製剤の輸血を受けた877名の新生児や骨髄移植患者等の易感染性患者に関する18件の報告によると、CMV感染者は877名中2名のみ（0.2%）であった。
白血球除去の代替としては、CMV抗体陰性の供血者から採取した血液の輸血がある。しかしながら、この方法が成功する確率は100%ではなかったため、CMV抗体陰性の供血者から血液を採取した後に、白血球除去を行う方法を推奨している専門家もいる。CMV抗体陰性血液から製造した血液製剤によるTTCMVのリスクは不明であるが、これまでに実施された研究から2%～3%を下回ると思われる。
現在使用されている白血球除去フィルターを赤血球製剤や血小板製剤といった血液細胞成分の製剤の白血球除去に使用した場合、CMV感染のリスクが著しく低下することは明らかである。全ての臨床的状況下でCMVを伝播する白血球数は不明だが、1製剤あたりの白血球数が5×10^6 個以下である場合、輸血による感染の可能性は極めて低い。

報告企業の意見	今後の対応
現行の白血球除去法によるサイトメガロウイルス輸血感染のリスクは0.2%程度と試算され、1製剤あたりの白血球数は5×10^6 個以下である場合、輸血による感染の可能性は極めて低いという報告である。	日本赤十字社では、保存前白血球除去した輸血用血液製剤のみを供給している。1製剤あたりの白血球数は1×10^6 個以下（適合率が95%以上）。さらに、必要に応じてCMV抗体が陰性であることを確認した輸血用血液製剤を供給している。今後もCMV感染に関する新たな知見等について情報の収集に努める。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

EDITORIAL

Optimal prevention of transfusion-transmitted cytomegalovirus (TTCMV) infection by modern leukocyte reduction alone: CMV sero/antibody-negative donors needed only for leukocyte products

Cytomegalovirus (CMV) is a ubiquitous DNA virus of the human herpesvirus group that infects most individuals at some time during life by a variety of routes, including blood transfusions. Children and young adults are infected most commonly via exposure to respiratory secretions from other individuals with primary infections. When CMV infection occurs during infancy, an important route of primary infection is from mother to infant via breast milk. It is well known by the transfusion community that another route of primary infection can be via transfusion transmission.

Primary infection with CMV begins as an acute upper respiratory infection that, despite the presence of viremia, is often subclinical with few overt signs/symptoms. As with other herpes viruses, primary infection and viremia resolve as antibodies emerge. As is true for all human herpesvirus infections, CMV then progresses to a "latent" phase of chronic infection or carrier state, during which the patient is asymptomatic, but the CMV virus resides within blood leukocytes. If activation of the latent infection occurs—which can happen with many perturbations of the immune system, including normal pregnancy, cancer, chemotherapy/radiotherapy, and solid organ or stem cell transplantation—the virus proliferates and can reappear in body fluids and/or tissues as a reactivation/secondary infection.

In healthy individuals, primary and reactivation CMV infections are of little consequence; however, in patients with immunodeficiency, these infections can produce severe, even fatal, disseminated disease. Patients at risk of severe infections include children with congenital immunodeficiency disorders, patients with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), recipients of organ allografts and/or stem cells, patients with cancer who are undergoing chemotherapy/radiotherapy, fetuses who receive intrauterine transfusions, and preterm neonates. During the latent or carrier stage of CMV infection, previously infected individuals are asymptomatic and fulfill all requirements to donate blood. However, in the bloodstream of individuals who have latent infection, CMV is associated almost exclusively with mononuclear leukocytes, and the transfusion of blood containing these leukocytes can infect the recipient with CMV. Although there has been debate about the subpopulation(s) of leukocytes carrying the virus, it has been consistently demonstrated that effective leukocyte reduction (i.e., that done in the blood center/bank with quality control to document satisfactory leukocyte removal, *not* filtration at the bedside) profoundly reduces—and, in some reports, actually eliminates—the risk of CMV transmission by transfusion.[1-5]

Despite the resounding success/efficacy of modern leukocyte reduction and the fact that this technology has never been found to be inferior to any other method of preventing transfusion-transmitted CMV (TTCMV), a potential problem does exist during the onset of primary infection: namely that, before anti-CMV antibodies form, CMV virions are "free" and are present in plasma. Clearance of these plasma "free" CMV virions shortly follows the emergence of CMV antibodies, and the virus becomes partitioned within leukocytes, where it can be removed along with the carrier leukocytes by effective leukocyte reduction to prevent the transmission of CMV by transfusion. In contrast, leukocyte reduction *cannot* remove plasma "free" virions. Accordingly, leukocyte-reduced blood components that are collected from donors who are in the earliest stage of primary infection and are still both asymptomatic and CMV sero/antibody-negative (i.e., in the "window phase" of primary CMV infection) theoretically could transmit CMV—findings that initially gave rise to the unsettling possibility that neither leukocyte reduction nor the selection of CMV sero/antibody-negative donors could prevent occasional TTCMV infections.

In this regard, it is important to note that later longitudinal studies of seroconverting donors in the early stages of primary CMV infections largely dispelled this concern by several interrelated findings. Those studies found that the number of plasma "free" CMV virions was very low at the time CMV infection began—when CMV antibody test results were still negative. As infection progressed, the number of CMV virions increased

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quickly as antibodies began to emerge, until rapidly increasing amounts of CMV antibody led to the disappearance of plasma "free" CMV virions and their partitioning into leukocytes. The possibility must be vanishingly small that an individual will present for donation during the early stages of primary CMV infection when they are clinically asymptomatic, have a sufficiently large quantity of plasma "free" CMV virions to be infectious, and still be CMV sero/antibody-negative. This pattern seriously challenges the notion that transfusion of plasma "free" CMV virions during the window phase of CMV infections explains[6,7] TTCMV "breakthrough" infections (i.e., those that might occur rarely in susceptible patients despite the use of leukocyte-reduced products). It must be remembered that convincingly documented breakthrough TTCMV infections have not been reported recently[3,5] but only in more historic reports in which leukocyte-reduction methods were less rigorous (e.g., bedside filtration) and donor infectious disease exclusions were less stringent per questioning and testing (i.e., donors who are at risk for hepatitis and/or HIV also pose a risk for CMV, so that deferring them should decrease the risk of TTCMV). Moreover, alternative routes of CMV infection were not thoroughly investigated in alleged cases of TTCMV infections (e.g., breast milk as the source in neonatal patients).

The precise risk of acquiring TTCMV from modern leukocyte-reduced blood products is unknown but is based on results from a growing number of relatively small clinical trials and cohort studies, with the overall risk believed to be well below 1%. In 18 reports involving 877 susceptible patients who received leukocyte-reduced blood products, only 2 of 877 patients (0.2%) became infected with CMV.[1-5,8]

As an alternative technique to leukocyte reduction, the risk of TTCMV can be reduced by transfusing blood collected from CMV sero/antibody-negative donors. However, because this technique has not been 100% successful in preventing TTCMV, some experts advocate first collecting blood from CMV sero/antibody-negative donors and then reducing leukocytes in the units collected. This technique, referred to as the "belt-and-suspenders" approach, as compelling as it appears, has never been shown in properly designed, head-to-head clinical trials to be superior to either transfusing only CMV sero/antibody-negative blood products or to leukocyte reduction alone when effectively performed by the blood center.

The current risk of acquiring TTCMV from sero/antibody-negative blood components is unclear, but it probably is smaller than the 2% to 3% reported in historical studies.[1,2,8] The risk from the combined "belt-and-suspenders" approach must be <1.0%—perhaps much less, because a prospective study involving 310 very-low-birth-weight (VLBW) infants found no CMV infection after 2081 transfusions of blood components that were both leukocyte-reduced and CMV sero/antibody-negative.[9] Unfortunately, that study was not designed to concurrently compare the prevention of TTCMV by leukocyte reduction versus CMV sero/antibody testing alone. However, in this issue of **TRANSFUSION**, investigators from the "belt-and-suspenders" study joined forces with investigators from another institution to compare the results of TTCMV prevention using leukocyte-reduction alone according to a study that was designed to be as comparable as possible at the two institutions.[10] Results from the two studies were identical, and neither detected a single case of TTCMV, whether prevented either by leukocyte-reduction alone[10] or by the combined approach of leukocyte-reduction of CMV sero/antibody-negative blood products.[9]

The report by Delaney and colleagues does not provide a definitive answer to the issue of "belt-and-suspenders" vs. leukocyte-reduction alone as a means of preventing TTCMV infections.[10] Actually, it was a pilot study that involved only a very few infants who were susceptible to acquiring TTCMV via transfusion of CMV sero/antibody-positive blood components that were leukocyte-reduced. If it were not for the link to the earlier "belt-and-suspenders" study, the current report might not have achieved sufficient priority for acceptance. However, it is an important report because it contributes to the mounting evidence that leukocyte reduction alone effectively prevents TTCMV infections. This information is useful for clinical practice, because a three-arm randomized clinical trial comparing efficacy of leukocyte-reduction alone vs. CMV sero/antibody-negative blood products alone vs. the "belt-and-suspenders" combined approach probably will never be done. The large numbers of susceptible recipients that would be required to detect a significant difference among the study arms of a TTCMV endpoint, which is quite rare, along with the enormous expense of such a large clinical trial make it impractical. Moreover, it would probably not be possible to show a statistically significant clinical difference large enough to demonstrate convincing superiority of any one method over the three other approaches to dictate optimal practice. Accordingly, physicians will have to make evidence-based decisions without the benefit of a definitive randomized control trial but on the basis of reported data.

In their report on a randomized observational study in this issue of **TRANSFUSION**, Delaney and colleagues show that the leukocyte-reduction alone approach completely prevented TTCMV in VLBW preterm infants.[10] Three other nonrandomized observational studies indicated that leukocyte reduction alone strikingly reduced[3] or completely prevented[4,5] TTCMV in hematopoietic progenitor cell (HPC) transplant recipients, and all three of those reports recommended against the use of blood

from CMV sero/antibody-negative donors—favoring the selection of leukocyte-reduced blood alone to prevent TTCMV in HPC transplant recipients.[3-5] Although the comparative degree of immunosuppression and susceptibility to symptomatic TTCMV infections in HPC recipients versus VLBW infants is unknown, it is not likely that VLBW infants are more immunosuppressed and at greater risk of TTCMV than HPC recipients; so, it seems appropriate to apply the results from the HPC studies to VLBW infants.

Therefore, in the absence of superior efficacy in preventing TTCMV using the "belt-and-suspenders" approach because of the lack of properly designed clinical trials, coupled with the undesirable and sometimes dangerous delays posed to some patients who require urgent transfusions (delayed by searching for suitable blood units to transfuse; i.e., both CMV sero/antibody-negative and leukocyte-reduced units)—plus the extra expenses involved in applying both preventive measures—the "belt-and-suspenders" approach is difficult to justify. These considerations are particularly relevant when the local blood center does not perform CMV antibody testing or wishes to discontinue testing.

It is logical to conclude that, because multiple reports have demonstrated that the practice of leukocyte reduction alone is an effective way to reduce and, in some studies, to eliminate TTCMV in high-risk patients and that, because no data demonstrate the superiority of other practices, leukocyte reduction alone should be the current standard of practice to prevent TTCMV infections. With apologies for stating the obvious, it is important to emphasize one group of exceptions—namely, when the transfused blood product consists of viable leukocytes (e.g., granulocyte/neutrophil concentrates, peripheral blood stem cells, and donor lymphocytes to boost immunologic function)—for which CMV sero/antibody-negative donors must be selected.

There are two special considerations for neonates as follows: First, precautions should be taken to prevent TTCMV in all neonates/infants for at least the first 4 to 6 months of life, regardless of whether their mothers are CMV sero/antibody-negative or positive. This recommendation is because CMV sero/antibody-positive infants will lose/catabolize CMV antibodies acquired from their mothers via the placenta during their first months of postnatal life. Moreover, infants with passive maternal antibody have not been truly infected with CMV and, accordingly, will neither produce their own antibodies nor have cellular immunity against CMV. Thus, all newborns are at risk for TTCMV whether or not they or their mothers test positive for CMV antibody, and all should receive leukocyte-reduced red blood cell (RBC) and platelet units.

Second, assuming pathogen-reduction technology (PRT) becomes widely used in the future to prevent transfusion-transmitted infections, including TTCMV infections, for which >4.0 log10 viral reduction for CMV has been reported, the use of leukocyte reduction or any other method(s) to prevent TTCMV will be supplanted by PRT. However, as is true for all medical technologies, careful attention must be paid to all precautions/caveats.

As a case-in-point, the INTERCEPT Blood System for Platelets (Cerus Corporation) has been approved for use by the US Food and Drug Administration. Because this PRT system uses Amotosalen S-59 psoralen derivative, which is activated by ultraviolet A (320-400 nm) illumination, platelets processed by INTERCEPT technology should not be transfused to neonates treated with phototherapy devices that emit energy wavelengths <400 nm, as specified in the package insert, because they may cause erythema. Phototherapy devices in use at this time in the United States generally do not emit energy wavelengths in the ranges specified, and INTERCEPT platelets can be transfused safely. However, for neonates being treated with phototherapy, it is prudent to confirm with the manufacturer of the phototherapy device or with the package insert that the problematic energy wavelengths are not emitted by the device in use. Similarly, as other methods of PRT gain approval by the US Food and Drug Administration, particularly for RBC units, it will be prudent to carefully review all cautions and warnings.

In summary, it is clear that the risk of infection with CMV is strikingly reduced if current-generation leukocyte-reduction filters are used to remove leukocytes from cellular blood components such as RBC and platelet units. The precise dose of white blood cells (WBCs) known to transmit CMV in all clinical situations is unknown, but transfusions that contain $<5 \times 10^6$ WBCs/unit are extremely unlikely to be infectious.[2,3] Although some physicians would prefer more definitive data from randomized comparative clinical trials, it is reasonable to conclude that leukocyte reduction of cellular blood components (e.g., RBCs and platelets) by any method capable of consistently achieving a residual WBC count $<5 \times 10^6$/unit optimally reduces the risk of TTCMV. Although it is logical to hypothesize that first collecting blood from CMV sero/antibody-negative donors and then removing the WBCs might improve safety, no data are available from randomized comparative clinical trials to document the superiority of this combined "belt-and-suspenders" approach. Moreover, in the absence of documented benefit, the substantial additional costs of the "belt-and-suspenders" approach and the delay of transfusions—some of which are urgently needed—while searching for CMV sero/antibody-negative units that have been leukocyte-reduced, cannot be justified. Accordingly, the

"belt-and-suspenders" approach to preventing TTCMV cannot be recommended.

NOTE ADDED IN PROOF

Recently, a special AABB Committee issued a report addressing the prevention of transfusion-transmitted cytomegalovirus (TTCMV) in high-risk patients (AABB Committee Report: Reducing transfusion-transmitted cytomegalovirus infections. Transfusion 2016;56:1581-1587). Following a comprehensive search of multiple databases, the Committee concluded that data published to date were inadequate to provide definitive practice guidelines to prevent TTCMV. In particular, the superiority could not be demonstrated of leukocyte-reduction alone, selection of CMV-antibody negative blood components alone, or the combination of both techniques. Accordingly, rather than creating guidelines of questionable utility, the report consisted of a focused, critical analysis of available information. In contrast, for all of the reasons presented in the Strauss Editorial, the conclusion is that leukocyte-reduction alone is the recommended method to prevent TTCMV — despite the lack of definitive randomized clinical trials.

CONFLICT OF INTEREST

The author has disclosed no conflicts of interest.

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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. 8. 3	該当なし	

一般的名称	人血小板濃厚液	研究報告の公表状況		公表国
販売名(企業名)	濃厚血小板-LR「日赤」(日本赤十字社) 照射濃厚血小板-LR「日赤」(日本赤十字社) 濃厚血小板HLA-LR「日赤」(日本赤十字社) 照射濃厚血小板HLA-LR「日赤」(日本赤十字社) 照射洗浄血小板-LR「日赤」(日本赤十字社) 照射洗浄血小板HLA-LR「日赤」(日本赤十字社)		CDC, Salmonella Homepage, 2016 Outbreaks http://www.cdc.gov/salmonella/live-poultry-05-16/index.html	米国

研究報告の概要

○小規模飼育(Backyard Flock)の生きた家禽に関連して、複数の州にわたり8件のヒトサルモネラ菌感染アウトブレイクが発生している。(2016年7月19日付更新)
小規模飼育の生きた家禽を介し、複数の州にわたり発生しているサルモネラ菌感染アウトブレイクは、2016年6月2日の情報更新以降、計8件となり、現在調査中である。2016年7月14日までに、全アウトブレイクにおいて611人のサルモネラ菌感染が45州から報告されている。
情報が得られた中では、患者は2016年1月4日から6月25日の間に発症し、年齢は1歳未満から93歳(中央値;20歳)で、52%は女性である。情報が得られた患者496人中、138人(28%)が入院、1人が死亡したがサルモネラ菌が死亡原因となったとは考えられていない。
疫学、遡及調査及び検査結果により、8件のアウトブレイクが複数の孵化場からのひよこやアヒルのような生きた家禽との接触に関連することが示された。
聞き取り調査の中で、患者は発症の前週における食べ物や動物との接触についての質問に回答した。聞き取りを行った患者493人中434人(88%)が発症の前週に、生きた家禽(ひよこ、鶏、アヒル、コガモ)と接触したと回答した。
患者は、飼料販売店、インターネットサイト、孵化場及び友人を含めた、複数の州における供給業者から生きた家禽のひなを購入していた。また、その購入目的は卵生産のため、農業を学ぶため、趣味として楽しむため、ペットとして飼うため、又はイースターの贈り物とするためであった。生きた家禽に触れる場所としては、自宅、他人の家、職場又は学校と回答した。
公衆衛生当局は複数の州にて、患者の家で飼育していた家禽及びその飼育環境から検体を採取し、4種類のサルモネラ菌のアウトブレイク株を分離した。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
2016年1月から6月の間に、米国にて生きた家禽を介して8件のヒトサルモネラ菌感染アウトブレイクが発生し、45州で611人が感染、1人が死亡したという報告である。	今後も細菌やウイルスの検出や不活化する方策について情報の収集及び安全対策に努める。

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

Eight Multistate Outbreaks of Human *Salmonella* Infections Linked to Live Poultry in Backyard Flocks

Posted July 19, 2016 1:00PM ET

What's New?

- One more outbreak was identified, bringing the total to eight outbreaks under investigation.
- 287 more ill people have been reported.
- 10 more states have reported cases, bringing the total to 45 states with ill people.
- In the eight outbreaks, 611 people infected with the outbreak strains of *Salmonella* were reported from 45 states.

Highlights

- **Read the Advice to Backyard Flock Owners »**
- CDC, multiple states, and the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (USDA-APHIS) are investigating eight separate multistate outbreaks of human *Salmonella* infections linked to contact with live poultry in backyard flocks.
  - In the eight outbreaks, 611 people infected with the outbreak strains of *Salmonella* were reported from 45 states.
  - Illnesses started on dates ranging from January 4, 2016 to June 25, 2016.
  - 138 ill people were hospitalized, and one death was reported. *Salmonella* infection was not considered to be a cause of death.
  - 195 (32%) ill people were children 5 years of age or younger.
- Epidemiologic, traceback, and laboratory findings have linked the eight outbreaks to contact with live poultry such as chicks and ducklings sourced from multiple hatcheries.
- Regardless of where they were purchased, all live poultry can carry *Salmonella* bacteria, even if they look healthy and clean.
- These outbreaks are a reminder to follow steps to enjoy your backyard flock and keep your family healthy.
  - Always wash hands thoroughly with soap and water right after touching live poultry or anything in the area where the birds live and roam.
  - Do not let live poultry inside the house.
  - Do not let children younger than 5 years of age handle or touch chicks, ducklings, or other live poultry without adult supervision.

- These outbreaks are expected to continue for the next several months since flock owners might be unaware of the risk of *Salmonella* infection from live poultry or participate in risky behaviors that can result in infection.

July 19, 2016

Outbreak Summary Update

Since the last update on June 2, 2016, one more outbreak was identified, bringing the total to eight outbreaks under investigation. Another 287 ill people have been reported from 45 states for these eight outbreaks.

As of July 14, 2016, 611 people infected with the outbreak strains of *Salmonella* have been reported from 45 states. A list of states and the number of cases in each can be found on the Case Count Map page.

Among people for whom information is available, illnesses started on dates ranging from January 4, 2016 to June 25, 2016. Ill people range in age from less than 1 year to 93, with a median age of 20. Of ill people, 52% are female. Among 496 ill people with available information, 138 (28%) reported being hospitalized, and one death was reported. *Salmonella* was not considered to be a cause of death.

Illnesses that started after June 16, 2016 might not be reported yet due to the time it takes between when a person becomes ill and when the illness is reported. This takes an average of 2 to 4 weeks.

Investigation Update

Epidemiologic, traceback, and laboratory findings have linked the eight outbreaks to contact with live poultry such as chicks and ducklings from multiple hatcheries.

In interviews, ill people answered questions about contact with animals and foods consumed during the week before becoming ill. Contact with live poultry (chicks, chickens, ducks, ducklings) in the week before becoming ill was reported by 434 of 493 ill people interviewed, or 88%.

Ill people reported purchasing live baby poultry from several suppliers, including feed supply stores, Internet sites, hatcheries, and friends in multiple states. Ill people reported purchasing live poultry to produce eggs, learn about agriculture, have as a hobby, enjoy for fun, keep as pets, or to give as Easter gifts. Some of the places ill people reported contact with live poultry include their home, someone else's home, work, or school settings.

Public health officials collected samples from live poultry and the environments where the poultry live and roam from the homes of ill people in several states. Laboratory testing isolated four of the outbreak strains of *Salmonella*.

More information about each outbreak is available in the outbreak summaries below.

Summaries of the Eight Separate Multistate Outbreak Investigations

- Outbreak 1: *Salmonella* Enteritidis Investigation
- Outbreak 2: *Salmonella* Muenster Investigation
- Outbreak 3: *Salmonella* Hadar Investigation
- Outbreak 4: *Salmonella* Indiana Investigation
- Outbreak 5: *Salmonella* Mbandaka Investigation
- Outbreak 6: *Salmonella* Infantis Investigation
- Outbreak 7: *Salmonella* Braenderup Investigation
- Outbreak 8: *Salmonella* Infantis Investigation

Previous Updates

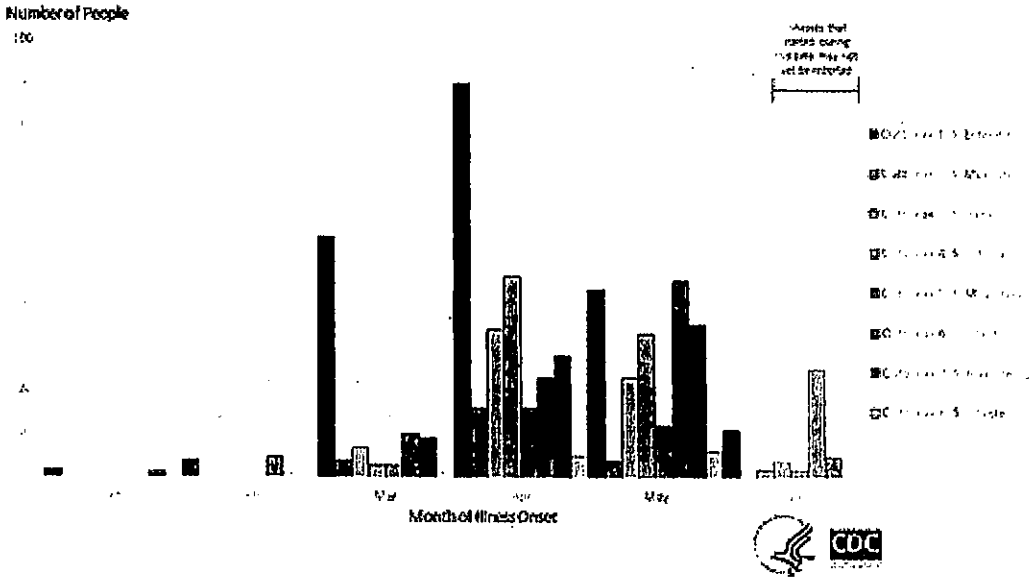
- Initial Announcement

At A Glance

- Case Count: 611
- States: 45
- Deaths: 1
- Hospitalizations: 138

More Information

- Advice to Consumers & Retailers
- Frequently Asked Questions
- Signs & Symptoms
- Key Resources



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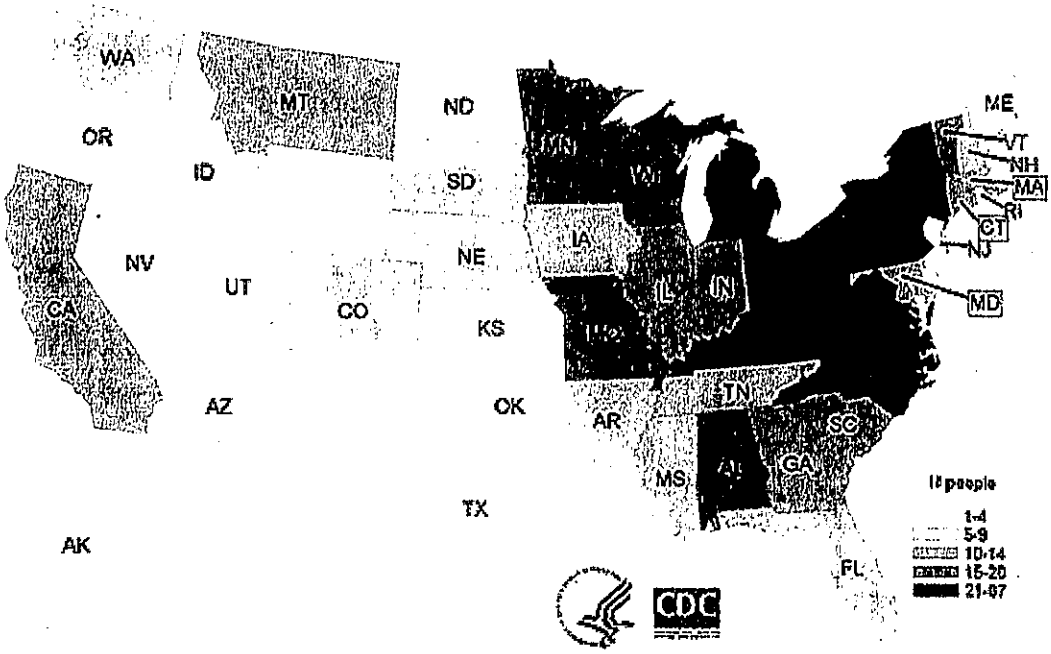


CLICK TO VIEW CASE COUNT MAPS

Page last reviewed: June 2, 2016

Page last updated: July 19, 2016

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

National Center for Emerging and Zoonotic Infectious Diseases (NCEZID) (/ncezid/index.html)

Division of Foodborne, Waterborne, and Environmental Diseases (DFWED) (/ncezid/dfwed/index.html)

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

Eight Multistate Outbreaks of Human *Salmonella* Infections Linked to Live Poultry in Backyard Flocks (Final Update)

- Advice to Consumers and RetailersLive Poultry FAQsCase Count Maps
- Epi CurvesSigns & SymptomsKey Resources

Posted October 6, 2016 2:45PM ET

These outbreak investigations are over. However, people can still get a *Salmonella* infection from live poultry, including those in backyard flocks. Read more information about *Salmonella* from live poultry and how people can reduce the chance they or their children will get an infection.

Highlights

- **Read the Advice to Backyard Flock Owners »**
- Although these outbreak investigations are over, people can still get a *Salmonella* infection from live poultry, including those kept in backyard flocks. Read more information about *Salmonella* from live poultry (http://www.cdc.gov/features/salmonellapoultry/index.html) and how people can reduce their risk of infection. Regardless of where they were purchased, all live poultry can carry *Salmonella* bacteria, even if they look healthy and clean.
- This year saw the largest number of illnesses linked to contact with backyard poultry ever recorded. These outbreaks are a reminder to follow steps (http://www.cdc.gov/features/salmonellapoultry/index.html) to keep your family healthy while enjoying your backyard flock.
  - Always wash hands thoroughly with soap and water right after touching live poultry or anything in the area where the birds live and roam.
  - Do not let live poultry inside the house.
  - Do not let children younger than 5 years handle or touch chicks, ducklings, or other live poultry without adult supervision.
- CDC, multiple states, and the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (USDA-APHIS) investigated eight separate multistate outbreaks of human *Salmonella* infections linked to contact with live poultry in backyard flocks.

- In the eight outbreaks, 895 people infected with the outbreak strains of *Salmonella* were reported from 48 states.
- Illnesses started on dates ranging from January 4, 2016 to September 10, 2016.
- 209 ill people were hospitalized, and three deaths were reported. *Salmonella* infection was considered to be a cause of death for one person in Mississippi. Although the two people who died in Kentucky and New Jersey had a *Salmonella* infection, the infection was not considered to be a cause of death.
- 254 (28%) ill people were children 5 years or younger.

- Epidemiologic, traceback, and laboratory findings linked the eight outbreaks to contact with live poultry, such as chicks and ducklings, sourced from multiple hatcheries.

Outbreak Summary

Introduction

As of September 26, 2016, 895 people infected with the outbreak strains of *Salmonella* were reported from 48 states. A list of states and the number of cases in each can be found on the Case Count Map page (http://www.cdc.gov/salmonella/live-poultry-05-16/map.html).

Among people for whom information is available, illnesses started on dates ranging from January 4, 2016 to September 10, 2016. Ill people ranged in age from less than 1 year to 106, with a median age of 27. Of ill people, 52% were female. Among 761 ill people with available information, 209 (27%) reported being hospitalized, and three deaths were reported. *Salmonella* was considered to be a cause of death for one person in Mississippi. Although the two people who died in Kentucky and New Jersey had a *Salmonella* infection, the infection was not considered to be a cause of death.

Investigation of the Outbreak

Epidemiologic, traceback, and laboratory findings linked the eight outbreaks to contact with live poultry, such as chicks and ducklings, from multiple hatcheries.

In interviews, ill people answered questions about contact with animals and foods consumed during the week before becoming ill. Contact with live poultry (chicks, chickens, ducks, ducklings) in the week before becoming ill was reported by 552 of 745 ill people interviewed, or 74%.

Ill people reported purchasing live baby poultry from several suppliers, including feed supply stores, Internet sites, hatcheries, and friends in multiple states. Ill people reported purchasing live poultry to produce eggs, learn about agriculture, have as a hobby, enjoy for fun, keep as pets, or to give as Easter gifts. Some of the places ill people reported contact with live poultry included their home, someone else's home, work, or school settings.

Public health officials collected samples from live poultry and the environments where the poultry live and roam from the homes of ill people in several states or at locations of purchase in several states. Laboratory testing isolated five of the outbreak strains of *Salmonella*.

More information about each outbreak is available in the outbreak summaries below.

Summaries of the Eight Separate Multistate Outbreak Investigations

- Outbreak 1: *Salmonella* Enteritidis Investigation
- Outbreak 2: *Salmonella* Muenster Investigation
- Outbreak 3: *Salmonella* Hadar Investigation
- Outbreak 4: *Salmonella* Indiana Investigation
- Outbreak 5: *Salmonella* Mbandaka Investigation
- Outbreak 6: *Salmonella* Infantis Investigation
- Outbreak 7: *Salmonella* Braenderup Investigation
- Outbreak 8: *Salmonella* Infantis Investigation

Previous Updates

- July 19, 2016
- Initial Announcement

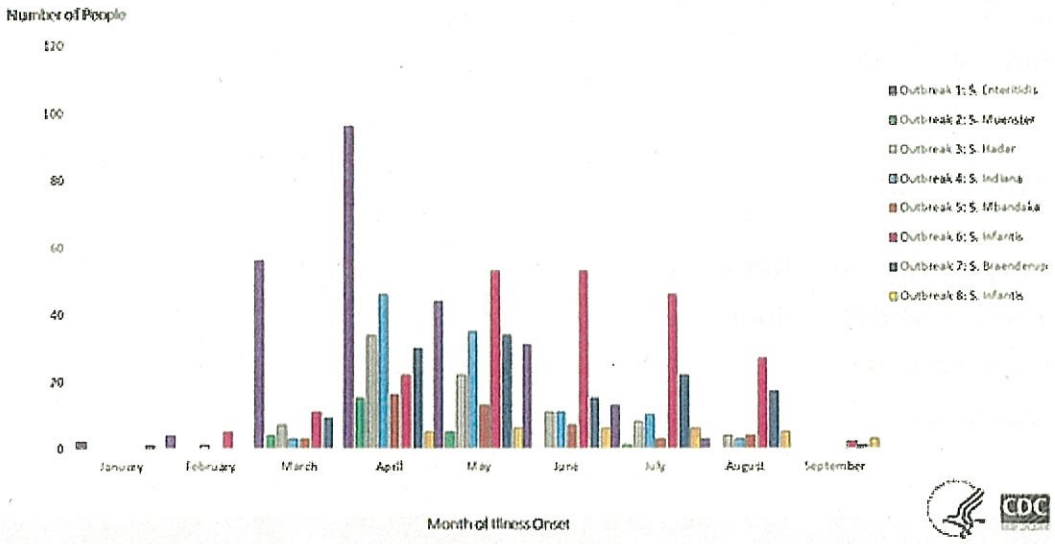
At A Glance

- Case Count: 895
- States: 48

- Deaths: 3
- Hospitalizations: 209

More Information

- Advice to Consumers & Retailers
- Frequently Asked Questions
- Signs & Symptoms
- Key Resources



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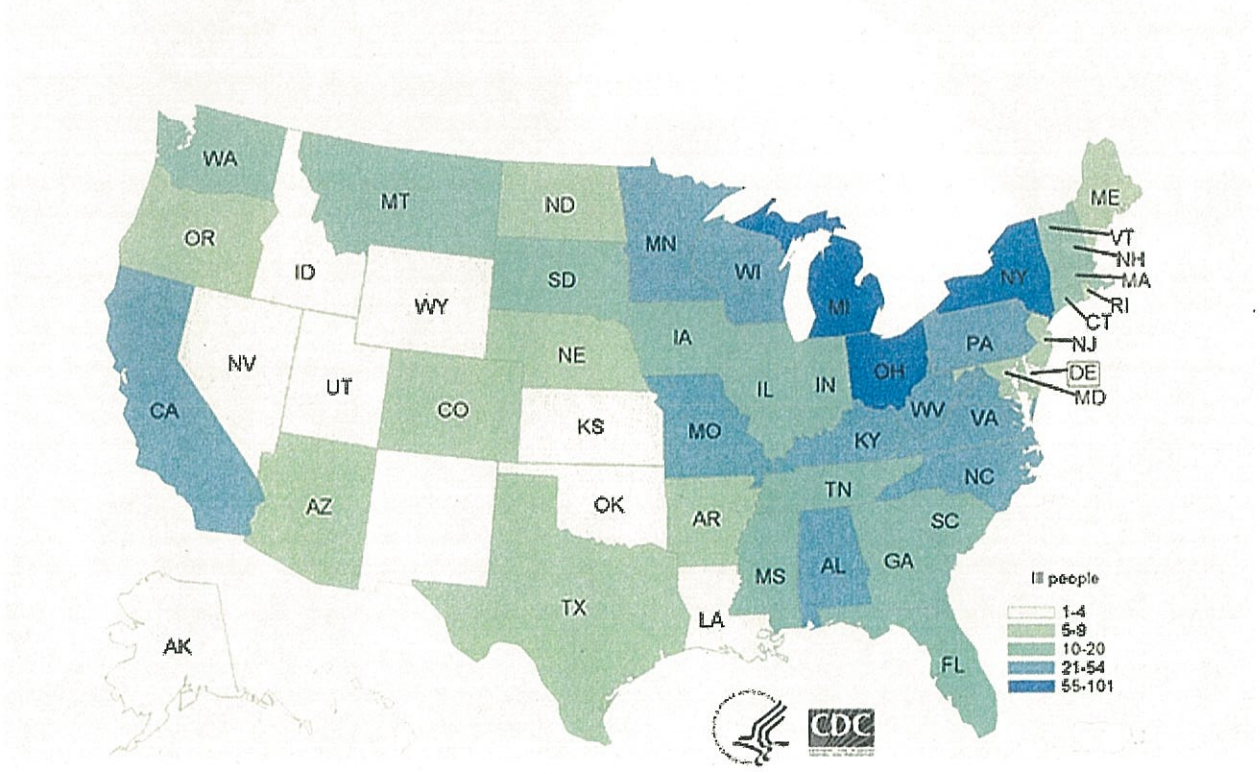

CLICK TO VIEW CASE COUNT MAPS

Page last reviewed: June 2, 2016

Page last updated: October 6, 2016

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

National Center for Emerging and Zoonotic Infectious Diseases (NCEZID) (/ncezid/index.html)

Division of Foodborne, Waterborne, and Environmental Diseases (DFWED) (/ncezid/dfwed/index.html)

別紙様式第2-1

No. 18

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. 11. 4	該当なし	

一般的名称	人赤血球液	研究報告の公表状況	Garcia MN, O'Day S, Fisher-Hoch S, et al. PLoS Negl Trop Dis. 2016 Nov 10;10(11):e0005074. doi: 10.1371	公表国 米国
販売名(企業名)	赤血球液-LR「日赤」(日本赤十字社) 照射赤血球液-LR「日赤」(日本赤十字社)			

研究報告の概要

○シャーガス病のベクターとイヌ科動物宿主とテキサス州のメキシコ国境沿いの住民との間に見られる衛生環境。
背景:中南米ではシャーガス病(*Trypanosoma cruzi*感染)は非虚血性拡張型心筋症の主要原因となっている。テキサス州(特に南部)には*T. cruzi*の伝播に寄与する可能性がある因子が存在しているが、疫学的研究は不十分である。本研究の目的は、森林-家屋周辺-家屋の感染サイクルにおけるシャーガス病の負担を理解するため、特にこの感染サイクルで森林と家屋の橋渡しで重要な役を担うと考えられるイヌ科を含めた3種類の哺乳類(コヨーテ、野良犬、ヒト)並びにベクター(サシガメ種)における*T. cruzi*感染率を明らかにすることであった。
方法/主要結果:感染率を調査するため、コヨーテ、公共の保護施設に収容されていた野良犬、並びに関連する調査研究に参加した住民から採取した血清に対して*T. cruzi*抗体を検査したところ、コヨーテの8%(16/200)、野良犬の3.8%(8/209)、住民の0.36%(3/841)が陽性であることを確認した。PCRを用いて当該地域の家屋周辺から採取したベクターにおける*T. cruzi* DNA保有率を調査した結果、56.5%(65/115)が陽性となり、再び当該地域における伝播のリスクが確認された。
結論/有意性:テキサス州南部における土着シャーガス病感染のエビデンスは増加しており、我々が得た結果もそれを示唆するものである。当該地域の人口が130万人であり、最高で30%の*T. cruzi* 感染者が重篤な心疾患を発症していることを考慮すると、調査及び治療を目的として高リスクグループを特定することは必要不可欠であると言える。

使用上の注意記載状況・その他参考事項等

赤血球液-LR「日赤」
照射赤血球液-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
テキサス州南部における土着シャーガス病の疫学研究として行った*T. cruzi* 抗体検査の陽性率は、コヨーテが8%、野良犬が3.8%、住民が0.36%であった。また、家屋周辺から採取したサシガメ種の*T. cruzi* DNA陽性率は56.5%であり、同地域の同症例増加傾向を示す結果であったという報告である。	日本赤十字社では、献血時の受付にて(1)中南米諸国で生まれた、又は育った。(2)母親又は母方の祖母が中南米諸国で生まれた、又は育った。(3)中南米諸国に連続して4週間以上滞在または居住したことがあるか問診を行い該当献血者については①中南米諸国を離れて6ヶ月未満の人は献血延期②6ヶ月以上経過している人については、献血の都度*T.cruzi*抗体検査を行い③ *T.cruzi*抗体陽性が確認された献血者は、永久に献血不可とする対策を実施している。今後も引き続き情報の収集に努める。

PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

One Health Interactions of Chagas Disease Vectors, Canid Hosts, and Human Residents along the Texas-Mexico Border

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OPEN ACCESS

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

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Abstract

Background

Chagas disease (*Trypanosoma cruzi* infection) is the leading cause of non-ischemic dilated cardiomyopathy in Latin America. Texas, particularly the southern region, has compounding factors that could contribute to *T. cruzi* transmission; however, epidemiologic studies are lacking. The aim of this study was to ascertain the prevalence of *T. cruzi* in three different mammalian species (coyotes, stray domestic dogs, and humans) and vectors (*Triatoma* species) to understand the burden of Chagas disease among sylvatic, peridomestic, and domestic cycles.

Methodology/Principal Findings

To determine prevalence of infection, we tested sera from coyotes, stray domestic dogs housed in public shelters, and residents participating in related research studies and found 8%, 3.8%, and 0.36% positive for *T. cruzi*, respectively. PCR was used to determine the prevalence of *T. cruzi* DNA in vectors collected in peridomestic locations in the region, with 56.5% testing positive for the parasite, further confirming risk of transmission in the region.

Conclusions/Significance

Our findings contribute to the growing body of evidence for autochthonous Chagas disease transmission in south Texas. Considering this region has a population of 1.3 million, and up to 30% of *T. cruzi* infected individuals developing severe cardiac disease, it is imperative that we identify high risk groups for surveillance and treatment purposes.

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Author Summary

In this study, we contribute to the growing body of evidence for autochthonous Chagas disease transmission in south Texas along the US-Mexico border. We found that coyotes, shelter dogs, and vectors in this region demonstrated high infection rates of *T. cruzi*. Random sampling of residents also revealed a higher than expected disease burden that had previously been undiagnosed. With up to 30% of infected individuals developing potentially fatal cardiac disease, it is imperative that we identify and treat patients before irreversible clinical manifestations have occurred. Future prospective studies are necessary to elucidate and validate the disease burden in this area.

Introduction

Chagas disease (*Trypanosoma cruzi* infection) can cause fatal cardiomyopathy in up to 30% of infected people [1]. Transmission to mammals occurs via vector, oral, congenital, and/or transfusion/transplantation routes [2]. The triatomine vector, or "kissing bug," serves as the predominate mode of transmission, particularly in established sylvatic and/or domestic transmission cycles [3]. Over 100 different wildlife mammalian species are competent reservoirs of disease and have been implicated in propagation of sylvatic transmission cycles in nature [4]. Canines, in particular, are important components of peridomestic transmission, resulting in a bridge between sylvatic and domestic transmission cycles [5–7]. Finally, human infections can occur when vectors establish nests inside or near the home, and vectors feed on both humans and domesticated animals [7, 8].

Disease prevalence is highest in impoverished regions of endemic countries due to a plethora of societal factors, including substandard living conditions that result in increased exposure to vectors [9]. While the southern United States is not traditionally considered an endemic area, recent evidence has implicated the establishment of vector transmission cycles, particularly in Texas [10, 11]. Historical evidence of *T. cruzi* infected vectors and mammalian reservoirs date back to the early 1900s [12]. While the first documented locally acquired human case was published in Corpus Christi, Texas in 1955, the south Texas region, including the Rio Grande Valley, has been the subject of investigation by public health authorities dating back to the 1940s [12].

South Texas has compounding factors that could contribute to this area being a high-risk region for transmission. Within the state, sylvatic transmission cycles have been reported with seven different vector species and 27 sylvatic mammalian reservoirs [10]. The potential for sylvatic spillover to humans in this region has been implicated from increased outdoor exposure and interactions in rural environments [13]. In addition, colonias (primarily Hispanic communities) in this region of Texas have unprecedented poverty rates and living conditions that allow for easy access for vectors to enter and colonize homes, which might place residents at an increased risk of domestic transmission [5, 14]. Despite this compounding evidence of increased potential for Chagas disease in the region, epidemiologic assessments are lacking. The aim of our current assessment was to ascertain the prevalence of *T. cruzi* in three different mammalian species (coyotes, stray domestic dogs, and humans) and vectors (*Triatoma* species) to understand the disease burden attributable to Chagas disease among sylvatic, peridomestic, and domestic cycles.

Methods

Ethics Statements

Texas Department of State Health Services in the lower Rio Grande Valley originally collected terminal samples of coyote sera as part of their rabies control programs in 2005–2006, and secondary aliquots from these specimens were shared for *T. cruzi* testing for the purposes of this study. Canine sera collection and Chagas disease testing were approved by the University of Texas Health Science Center Animal Welfare Committee (AWC-07-147 and AWC-03-029). For the human seroprevalence aspects of our study, the original Cameron County Hispanic Cohort study was reviewed and approved by the University of Texas Health Science Center at Houston Committee for the Protection of Human Subjects (HSC-SPH-03-007B), and Chagas disease testing on coded samples was approved under Baylor College of Medicine Institutional Review Board (H-32192).

Study Population

We conducted a retrospective analysis of previously collected sera from coyotes, stray domestic dogs housed in public shelters, and residents participating in related research studies. With regards to the coyote specimens, secondary aliquots from specimens noted above were shared by the Texas Department of State Health Services for *T. cruzi* testing. For domestic dog specimens, sera were collected in 2007 and 2009 from juvenile (less than 6 months of age and over 8 weeks of age based on tooth development) stray dogs housed in public shelters at one of two locations (Brownsville in Cameron County and Edinburg in Hidalgo County). The rationale for collecting samples from dogs under 6 months of age was to identify new, acute cases of infection so that incidence, as opposed to prevalence, could be determined. We purposefully excluded puppies under 8 weeks of age to eliminate issues related to the possible transfer of Chagas-positive maternal antibodies.

Investigators from the University of Texas Health Science Center at Houston, School of Public Health, Brownsville Regional Campus, collected sera from an established cohort living in Cameron County, TX. The participants were recruited from randomly selected households between 2005 and 2008 as a means of assessing the general health of residents along the US-Mexico border. Potential participants were not excluded based on race/ethnicity, with all race/ethnicities eligible for study inclusion. Data from the original health questionnaire and echocardiograms performed by the Cameron County Cohort (CCC) study were available for descriptive analysis [15].

From 2012 to 2013, we received 115 Triatomine insects that were collected in peridomestic areas by citizens across 6 counties in south Texas. Insect specimens were shipped, typically live, to The University of Texas Rio Grande Valley for further processing. PCR testing was performed in collaboration with Baylor College of Medicine Laboratory for Vector-Borne and Zoonotic Diseases.

Trypanosoma cruzi Diagnostics

Serum samples were thawed and analyzed using Chagas Stat-Pak and DPP assays (Chembio Diagnostic Systems, Inc, Medford, NY). These rapid immunochromatographic assays test for antibodies against *T. cruzi*. These highly sensitive and specific assays were designed for feasibility in field-testing of both human and canine blood [6, 16–18]. Tests were examined visually and scored as negative or positive, following manufacturer's directions. A positive sample was defined as being positive on both assays. Negative samples included those that were positive on only one diagnostic but negative on the second diagnostic. Any equivocal samples were retested

for further clarification. Due to the samples being retrospectively tested without potential for prospective clinical intervention and the exploratory nature of the project, additional confirmation testing with alternate diagnostics was not performed.

For *T. cruzi* testing and taxonomic species identification of *Triatoma* insects, the posterior third of the insects' abdomen was homogenized with a 5 mm stainless steel bead in AL buffer (Qiagen, Valencia, CA) in TissueLyser II (Retsch, Haan, Germany) for 3 min at 25 Hz. Following manufacturer's instructions, DNA was then extracted using DNeasy Blood & Tissue kit (Qiagen, Valencia, CA). *T. cruzi* DNA detection and insect-specific mitochondrial 16S DNA for speciation were performed using PCR and sequencing as previously described [8, 19].

Data Analysis

Descriptive statistics were used to identify prevalence infection rates with 95% confidence interval (CI) and stratified by pertinent variables. For domestic dogs, positive infection was translated to incidence since all dogs would have acquired infection in the first 6 months of life. Statistical analysis was performed using STATA v12 (College Station, TX). Spatial analysis was performed using MapInfo Professional v11.5 (Stamford, CT).

Results

Chagas Seroprevalence in Coyotes

Coyote samples collected in the Rio Grande Valley had an overall seroprevalence rate of 8% (16 out of 199; 95% CI = 4.2% to 11.8%) (Table 1). Sampled coyotes were evenly distributed by gender (45% female) and all but one were adults. There was no difference in seropositivity by year of sampling. Interestingly, seroprevalence varied with regards to county of collection, with the highest seroprevalence identified in Zapata County (16%; 10/64), followed by Jim Hogg County (14%; 3/22), Dimmit County (10%; 2/20), and Webb County (1%; 1/83) (Fig 1). No positive coyotes were identified in Cameron, Hidalgo, Starr, or Wallacy counties, although sample sizes from each of these counties were low (range 1 to 4, total tested = 10).

Chagas Seroprevalence in Domestic Canines

Samples collected from juvenile domestic dogs from neighboring Hidalgo and Cameron counties had an overall serologic incidence of 3.8% (8 out of 209 samples; 95% CI = 1.2% to 6.4%). We found a pronounced increase (4.4 fold) in Chagas incidence when comparing sampling in 2007 to 2009 (Fisher's exact test, p-value = 0.04, 95% CI = 1.1 to 18.0), with 2% (3/152) of dogs positive in 2007 versus 9% (5/57) found positive in 2009.

Chagas Seroprevalence and Clinical Data in South Texas Residents

Of 841 human sera samples tested from participants in the CCC, 3 individuals (0.4%; 95% CI = 0% to 0.8%) tested positive on both Stat-Pak and DPP assays. Limited residential history,

Table 1. *Trypanosoma Cruzi* (Chagas Disease) Prevalence In Coyotes, Shelter Dogs, Human Residents, And Vectors Of South Texas.

Samples tested	Number tested	Chagas positive N (%)
Coyote (*Canis latrans*)	200	16 (8.0%)
Shelter dogs (*Canis lupus familiaris*) < 6 months of age	209	8 (3.8%)
Human adult cohort	841	3 (0.36%)
Triatoma species vectors	115	65 (56.5%)

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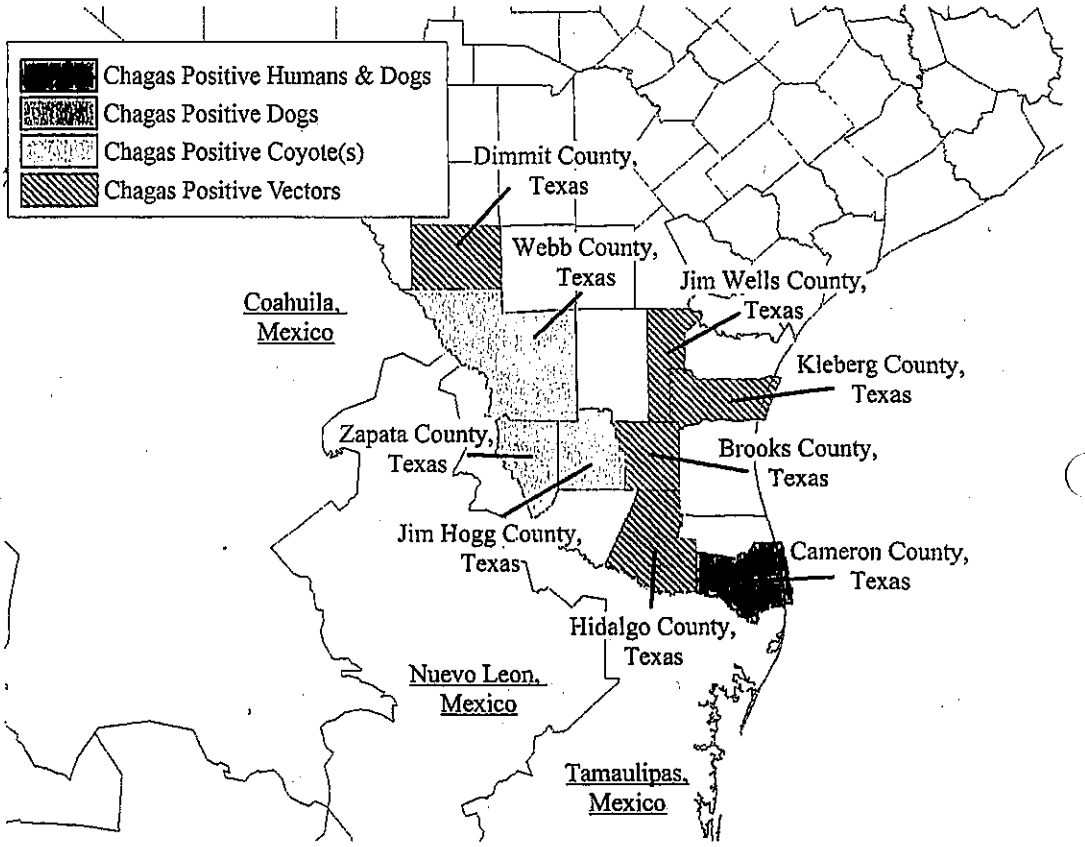

Fig 1. *Trypanosoma Cruzi* (Chagas Disease) Positive Samples By Species And Geographic Origin.

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medical histories and socioeconomic variables were reported as listed below. The precise origin and duration of their infection is unknown.

CCC Participant 1 was a 76-year-old female born in Canary, Texas (now known as Livingston, Texas) with a 52-year residential history in Brownsville, Texas. Case-patient 1 reported no current employment with an annual disability-benefit income of $3,336. Her medical history included diabetes, stroke, and hypertension. Case-patient 1's mother was born in Texas while her father was born in central Mexico (Guanajuato). No data regarding any abnormal cardiac findings were available for this case-patient. On follow-up, participant's husband reported that the participant had died recently with an apparent cause of death reported as leukemia.

CCC Participant 2 was a 45-year-old male born in San Luis Potosi, San Luis Potosi, Mexico with a 6-year residential history in Brownsville, Texas. In addition, he reported a prior 6-year residential history (while attending school) in the Brownsville, Texas border town of Matamoros, Tamaulipas, Mexico. Case-patient 2 was employed at the time of enrollment, reporting an annual income of $12,000. His past medical and social histories included diabetes and smoking. Both parents were born in north-central Mexico (San Luis Potosi). An echocardiogram performed on this participant showed normal left ventricular and right ventricular systolic function, mild concentric left ventricular hypertrophy, grade 1 left ventricular diastolic dysfunction,

and no significant valvular abnormalities. The participant reported no symptoms related to any type of infection, and no additional cardiac evaluations were performed.

CCC Participant 3 was a 63-year-old male born in Matamoros, Tamaulipas, Mexico with a 22-year history of living in Brownsville, Texas. Case-patient 3 was retired with a prior occupational history in agriculture (occupational duration unknown) and a current annual income of $10,248. His medical history was negative for pre-existing conditions or co-morbidities. Case-patient 3's parents were born in northern Mexico (Nuevo León). An echocardiogram performed at the same time as the original blood collection demonstrated normal biventricular systolic function, mild concentric left ventricular hypertrophy, grade 1 left ventricular diastolic dysfunction, and no significant valvular abnormalities. Similarly, the participant reported no symptoms, and no additional cardiac evaluations were performed.

Prevalence of *T. cruzi* in Vectors

Finally, to determine the likelihood of infection in vectors in the region, PCR was performed on 115 insects (*Triatoma* species) collected around homes across 6 counties of south Texas. We found 65 (56.5%) positive for *T. cruzi* DNA, with prevalence ranked by county as follows: Brooks County (84%; 21/25), Hidalgo County (60%; 6/10), Jim Wells County (50%; 12/24), Kleberg County (47%; 22/47), Dimmit County (33%; 2/6), and Cameron County (0%; 0/1); 2 positive insects did not have a georeference provided. The most common insect collected was *Triatoma gerstaeckeri* (96.5% of insects; 62/111 *T. cruzi* positive), followed by *T. lecticularia* (2.6% of insects; 2/3 *T. cruzi* positive) and *T. sanguisuga* (0.9% of insects; 1/1 *T. cruzi* positive).

Discussion

Chagas disease transmission has been identified along the Texas-Mexico border dating back to the 1970s [20, 21]. Our current study is the first to assess the infection status of vectors and seroprevalence among mammalian and human populations all living in the same geographic region of south Texas. Seroprevalence was highest among the sylvatic adult coyote reservoir (8%), moderate among peridomestic juvenile dogs in community shelters (3.8%), and lowest among local residents (0.36%), with one of the three positive CCC participants having a lifelong history of living in Texas. In addition to finding evidence of infection in canines and humans, we found a high percentage (56.5%) of vectors carrying the parasite, further solidifying the risk of Chagas disease transmission in the region. Prior case reports have suggested the potential for domestic transmission along the eastern side of the Texas-Mexico border [5, 20], and now our larger regional assessment confirms this risk. Compounding evidence of poverty, substandard housing, rural residential exposure to sylvatic animals, and high infection prevalence of multiple species all can contribute to an increased risk of Chagas disease transmission to local residents [10, 14, 22].

Coyotes (*Canis latrans*) are den dwelling animals native to North America. Habitat preferences include caves and natural holes, or abandoned domestic structures such as drainage pipes, vacant homesteads and railroad tracks [23]. Similarly, triatomine vectors prefer natural or domestic habitats, living in large numbers within dens that provide constant access to a host meal source [3]. Our finding of 8% seroprevalence among coyote populations in the Rio Grande Valley is slightly lower than a prior study in 1978 which found a 12.8% (20 out of 156) prevalence of infection [20]. A second study published in 1984 found a 14% seroprevalence rate in coyotes from across Texas; however, none of the eastern Rio Grande Valley counties were included in this sampling [24]. Tennessee, Georgia, and Virginia are other southern states with known *T. cruzi* positive coyote populations [25–27]. Comparable to our study, these more

recent studies found seroprevalence rates between 7–10%, suggesting that infection rates might be decreasing with time or current diagnostic tests have better sensitivity-specificity.

Dog (*Canis lupus familiaris*) populations in the United States can be feral or domesticated; however, both groups can serve as bridge hosts for transferring Chagas disease between sylvatic environments and humans. Dogs serve as important sentinel for disease surveillance purposes as their infection rates can be early predictors of transmission risk to humans, especially considering dogs develop clinical cardiac disease quicker than humans [5, 21, 28–30]. Using public health veterinary shelters as a sampling venue is a convenient methodology to capture feral, community-owned, and domesticated dog populations. The shelter dogs in our study of the Rio Grande Valley had a seroprevalence of 3.8%, which is considerably lower than other published infection prevalence estimates among shelter dog populations from across the state. Over 48 different dog breeds in Texas have demonstrated natural infection with *T. cruzi*, with prevalence estimates ranging from 8.8–20.3% [31, 32]. In the greater Brownsville, Texas area, infection prevalence of shelter dogs has ranged from 7.5% in 2003 to 6.7% in 2014 [5, 32]. While our prevalence is slightly lower than other studies, the reason is most likely related to our decision to sample dogs that were under 6 months of age, allowing us to estimate incidence related to recent vector-borne or congenitally-acquired infection. By estimating incidence, we can better understand the annual contribution of disease transmission in this geographic area.

The epidemiology and seroprevalence of human infection in the southern United States is largely unknown. Even in endemic areas, human seroprevalence is typically lower than sylvatic and domestic animals due to multiple factors, including increased mammalian-vector habitat exposure, mammalian predilection for oral ingestion of the triatomine vector, and varying defecation behaviors of different triatomine species [3, 30, 33]. While sylvatic transmission cycles between wildlife and vectors have been established in the southern United States, we are still in our infancy of understanding disease burden and transmission source in infected populations. A prior study conducted in 1977 found a seroprevalence of 2.4% (12 out of 500) among eastern Rio Grande Valley residents [20], which is a sharp contrast to our finding of 0.4% (3 out of 841). Our study sampling included random selection of participants, while their study biased their results by recruiting patients at Texas Chest Hospital in Harlingen. It is likely our sampling methodologies influenced the varying rates, especially as other historical random-selection population studies reported 0.01–0.9% seroprevalence [12]. Despite our selection methodology differences, both Burkholder et al.'s study and ours included long-time residents of the Rio Grande Valley, with one positive participant in our study very likely acquiring the infection in Texas. Based on our findings of a seroprevalence estimate of 0.4%, and considering a population of 1.3 million for the Rio Grande Valley, we can estimate that ~4,600 people in this region are currently infected with Chagas, with ~1,300 at risk for developing Chagas-related cardiomyopathy. If this estimate is accurate, then the burden of Chagas disease in the Rio Grande Valley is 23 times higher than what we had previously estimated based on our findings of 1 out of 6,500 (0.02%) blood donors in Texas testing positive for the disease [34]. Future studies should aim to further clarify the true disease burden and rate of autochthonous transmission in the Rio Grande Valley, an area with documented sylvatic and domestic *T. cruzi* transmission [5].

Our study had a few important limitations notable for discussion. The current World Health Organization guidelines require a minimum of two positive results on different antibody-based assays for diagnostic confirmation [35]. While we used two different assays, neither are currently FDA approved in the United States; however, Stat-Pak rapid immunochromatographic assay has demonstrated efficacy in all three populations of mammals in multiple studies [6, 16–18, 27]. For the purposes of this retrospective study we felt confident in the test results, especially as they were relatively consistent with other published literature. In addition

to our finding of a high rate of infection (56.5%) among local vector species, other studies have also confirmed high rates of infection (51–82%) in Triatomine vectors throughout Texas [7, 8, 10]. Provided the retrospective nature of our study, the obvious lack of travel history in these coyote and dog populations, and the establishment of known *T. cruzi* positive vector populations in our study, we would argue that these are true infections acquired via local vector-sylvatic mammal transmission cycles. Another possible limitation, due to our retrospective sampling of frozen sera collected 8–10 years prior, is the potential for antibody decay resulting in a lower prevalence rate. Handling of the specimens included freezing aliquots to -80°C immediately following collection, constant monitoring of freezer temperature, and adhering to discipline standards during the serum thawing process in an effort to maintain sample preservation. Finally, we cannot rule-out the potential for cross-reaction with leishmaniasis. Rare reports of cutaneous leishmaniasis have been reported in the state [36]; however, none of our three Chagas-positive study participants presented with skin ulcers, lowering the potential for cross-reaction.

In conclusion, we contribute to the growing body of evidence for autochthonous Chagas disease transmission among mammals in south Texas. Coyotes, shelter dogs, and vectors in this region continue to demonstrate high infection rates of *T. cruzi*. Random sampling of residents also revealed a higher than expected disease burden that had previously been undiagnosed, with one human patient suspected of having locally acquired the disease. With up to 30% of infected individuals developing a potentially fatal cardiac disease, it is imperative that we identify and treat patients before irreversible clinical manifestations have occurred. Future prospective studies are necessary to elucidate and validate the disease burden in the Rio Grande Valley.

Supporting Information

S1 Checklist. Strobe Checklist.
(PDF)

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Author Contributions

Conceived and designed the experiments: SFH TPFA JEL KMJ KOM.

Performed the experiments: SOD RG RP AI.

Analyzed the data: MNG SFH TPFA STL KOM.

Contributed reagents/materials/analysis tools: SFH TPFA JEL KMJ KOM.

Wrote the paper: MNG SOD AI KOM.

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医薬品
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化粧品

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研究報告の概要

業界向けガイダンス（案）；輸血用全血および血液成分におけるクルーズ・トリパノソーマ感染リスク低減化を目的とした血清学的検査の使用に関する業界向けガイダンスの改訂について：

このガイダンスは、2010年12月付の「業界向けガイダンス：輸血用全血および血液成分におけるクルーズ・トリパノソーマ感染リスクの低減化を目的とした血清学的検査の使用」の改訂を目的としている。
1）医療機器の成分として用いる、あるいは医療機器の製造に用いることを意図した供血など、製品の製造に使用するための血液又は血液成分の採血を含むよう、ガイダンスの範囲を広げる。
2）提供者にシャーガス病の病歴について尋ねる問診の勧告を削除する。
3）クルーズ・トリパノソーマ抗体のスクリーニング試験結果またはシャーガスの問診の質問に「はい」との回答に基づいて延期していた特定の供血者のための再入アルゴリズムの勧告を提供する。
このガイダンスの勧告は、輸血用あるいは製品の製造用（医療機器の成分としてあるいは製造に用いることを意図した供血を含む）の血液又は血液成分の採血（血漿分画用の原料血漿は除く）に適用される。血液業界は、血漿分画用の原料血漿の献血にクルーズ・トリパノソーマ感染のエビデンスを見つけるための検査は要求されていない。

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、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
クルーズ・トリパノソーマ（Troanosoma cruzi）は、べん毛虫の一種でありキネトプラスト目トリマノソーマ科に属し、サシガメによって媒介され、ヒトのシャーガス病の原因となる。大きさは錐べん毛型で16～20μm、無べん毛型で1.5×40μmである。仮に、血漿にトリパノソーマ・クルジが混入したとしても、凍結により24時間以内に死滅することが分かっており、－20℃以下で貯留保管される血漿分画製剤の原料血漿においては死滅している状態にあると考えられる。さらに、万一、製造工程に混入したとしても除菌フィルター（孔径0.22μm）等の工程で除去できると考える。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

JB2016-026

Amendment to "Guidance for Industry: Use of Serological Tests to Reduce the Risk of Transmission of *Trypanosoma cruzi* Infection in Whole Blood and Blood Components Intended for Transfusion"

Draft Guidance for Industry

This guidance document is for comment purposes only.

Submit one set of either electronic or written comments on this draft guidance by the date provided in the *Federal Register* notice announcing the availability of the draft guidance. Submit electronic comments to http://www.regulations.gov. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

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

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

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

Contains Nonbinding Recommendations

Draft – Not for Implementation

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Contains Nonbinding Recommendations

Draft – Not for Implementation

Amendment to "Guidance for Industry: Use of Serological Tests to Reduce the Risk of Transmission of *Trypanosoma cruzi* Infection in Whole Blood and Blood Components Intended for Transfusion"

Draft Guidance for Industry

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

I. INTRODUCTION

This guidance, when finalized, is intended to amend the document entitled "Guidance for Industry: Use of Serological Tests to Reduce the Risk of Transmission of *Trypanosoma cruzi* Infection in Whole Blood and Blood Components Intended for Transfusion" dated December 2010 (the "2010 Chagas Guidance") (Ref. 1) by 1) expanding the scope of the guidance to include the collection of blood and blood components for use in manufacturing a product, including donations intended as a component of, or used to manufacture, a medical device, 2) removing the recommendation to ask donors about a history of Chagas disease, and 3) providing a recommendation for a reentry algorithm for certain donors deferred on the basis of screening test results for antibodies to *Trypanosoma cruzi* (*T. cruzi)* or on the basis of answering "yes" to the Chagas screening question.

The recommendations in this guidance apply to the collection of blood and blood components, except Source Plasma, for transfusion or for use in manufacturing a product, including donations intended as a component of, or used to manufacture, a medical device. Blood establishments are not required to test donations of Source Plasma for evidence of infection due to *T. cruzi* (21 CFR 610.40(a)(2)(ii)). Within this guidance, "you" refers to establishments that collect blood and blood components.

This guidance notifies you that *T. cruzi* is defined as a relevant transfusion-transmitted infection (RTTI) in 21 CFR 630.3(h)(1) and subject to the testing requirements in 21 CFR 610.40, the donor deferral practices in 21 CFR 610.41, and the donor notification requirements in

21 CFR 630.40. Further, this guidance notifies you that FDA has licensed a supplemental test for antibodies to *T. cruzi* and further testing of donations found repeatedly reactive to a screening test for *T. cruzi* is therefore required under 21 CFR 610.40(e).[1]

When finalized, we will update the 2010 Chagas Guidance by incorporating the new recommendations provided in this guidance into an updated final guidance. All other recommendations in the 2010 Chagas Guidance will remain unchanged.

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

II. BACKGROUND

Chagas disease is caused by the protozoan parasite *T. cruzi*. Natural infections are transmitted by infected blood sucking insects (triatomine bugs). Other primary forms of transmission include oral, congenital (mother to unborn infant), organ transplantation and blood transfusion. The disease is found primarily in Mexico and Central and South America. Several cases of natural transmission also have been reported in the United States (U.S.), which were associated with documented infections in insect vectors and reservoir hosts in the southern U.S. (Refs. 2, 3). The presence of the pathogenic agent in U.S. donors, however, has increased due to immigration of infected individuals from endemic areas. Some experts estimate that there may be as many as 300,000 persons unknowingly infected with *T. cruzi* who reside in the U.S. (Ref. 4). These individuals could serve as a potential source of transfusion-transmitted infection should they become U.S. donors. In the U.S. and Canada, 10 cases of transfusion-transmitted *T. cruzi* and five cases of infection from organ transplantation have been documented through 2013 (Refs. 5, 6).

The voluntary testing of U.S. blood donors for antibodies to *T. cruzi* was initiated in January 2007, subsequent to FDA licensure of the first blood donor screening test. As stated above, in 2015,[2] FDA defined *T. cruzi* as a RTTI and, as of May 23, 2016, blood establishments must test for *T. cruzi* consistent with the requirements in 21 CFR 610.40, subject to the exceptions found in 21 CFR 610.40(c) and (d). Additionally, consistent with 21 CFR 610.40(a)(2)(iii)(A), FDA currently recommends one-time testing of each donor of allogeneic units of blood using a licensed test for antibodies to *T. cruzi* (Ref. 1). This guidance document, when finalized, will extend FDA's recommendations relating to one-time testing of donors to the collection of blood and blood components for use in manufacturing a product, including donations intended as a component of, or used to manufacture, a medical device. An online report of the AABB Chagas'

[1] See Requirements for Blood and Blood Components Intended for Transfusion or for Further Manufacturing Use; Final Rule (80 FR 29842, May 22, 2015), effective May 23, 2016.
[2] See footnote 1.

Biovigilance Network (http://www.aabb.org) dated May 1, 2015,[3] showed that between January 1, 2007 and May 1, 2015, 10,575 donors gave collections that were repeatedly reactive on a licensed screening test for antibodies to *T. cruzi*. Of those collections, 2,046 (19.3%) were reported as confirmed, 8,010 (75.7%) negative, 427 (4.0%) indeterminate, and 92 (0.9%) cases were pending at the time the report was generated. FDA's 2010 Chagas Guidance recommends that all donors whose collections test repeatedly reactive on a licensed test for *T. cruzi* antibodies should be deferred indefinitely and notified of their deferral.

A. Donor Screening for History of Chagas Disease

FDA's 2010 Chagas Guidance recommends asking the question "Have you ever had Chagas disease?" to all donors at each donation, to identify donors with a history of Chagas disease. The 2010 Chagas Guidance also recommends that donors who answer "no" to the question should be tested with a licensed screening test for antibodies to *T. cruzi*, and donors who answer "yes" to this question should be deferred indefinitely and notified of their deferral. In a recent study, Steele, et al., identified 34 donors deferred because of a history of Chagas disease as revealed by the question among approximately 76 million qualified donors screened by the American Red Cross (ARC) between January 2000 and August 2011 (Ref. 7). In comparison, ARC identified 488 donations positive by the unlicensed supplemental Radioimmunoprecipitation Assay (RIPA) among approximately 21 million donations tested between January 2007 and August 2011. The 488 *T. cruzi* RIPA positive donors had not responded in the affirmative to the Chagas history question during the predonation screening process. This report also showed that only one of the six who provided a follow-up sample, among the 34 donors deferred based on the Chagas disease history question, had a repeatedly reactive result with a licensed screening test. This donor was also *T. cruzi* RIPA positive on further testing. The authors concluded that the Chagas question has no added value when all donors are tested at least once.

Based on this study, the clinical sensitivity of the two currently licensed screening tests (Refs. 8, 9), the low (0.8%) risk of transfusion-transmitted *T. cruzi* infection in the blood donor population (Ref. 10), and the observation that *T. cruzi* RIPA positive donors are likely not aware of their infection, we are recommending that one-time testing alone, without donor questioning for history of Chagas disease, is adequate to identify donors at risk for Chagas disease. We explain in section III of this guidance that the recommendation in the 2010 Chagas Guidance to ask the question "Have you ever had Chagas disease?" to all donors at each donation is no longer recommended, and the question can be removed from donor history questionnaires.

[3] Facilities report their data intermittently; consequently, the numbers reported represent what was available in the database at the time.

B. Supplemental Testing of Donors Repeatedly Reactive with a Licensed Screening Test for Antibodies to *T. cruzi*

Consistent with 21 CFR 610.40(e), you must further test each donation found to be reactive by a donor screening test using a licensed, approved or cleared supplemental test, when available.[4] In November 2011, FDA licensed a supplemental test for antibodies to *T. cruzi*. This test is intended for use as an additional, more specific test for human serum or plasma specimens found to be repeatedly reactive using a licensed screening test for antibodies to *T. cruzi*.

A positive test result on the licensed supplemental test indicates that antibodies to *T. cruzi* were detected, providing further confirmation of the repeatedly reactive screening test result. It is FDA's view that donors whose blood samples are found to be repeatedly reactive on a licensed screening test, but negative on a licensed supplemental test, may be considered for reentry as set forth in section III.D of this guidance.

C. Donor Reentry

The reentry of donors deferred on the basis of screening test results for antibodies to *T. cruzi* was discussed at the July 31, 2014 Blood Products Advisory Committee (BPAC or the Committee) meeting (Ref. 11).

FDA presented an analysis of donor follow-up studies used to develop a proposed donor reentry algorithm and four alternative scenarios. In these follow-up studies, donors whose collections were repeatedly reactive on a licensed screening test for antibodies to *T. cruzi* and negative on a licensed supplemental test for antibodies to *T. cruzi* on their initial donation were further evaluated to determine their eligibility for requalification/reentry as donors. Follow-up testing was performed to assess their most likely *T. cruzi* infection status and determine those who could safely be reentered.

Results of the follow-up studies showed that 117/238 (49.2%) of donors in the FDA analysis had follow-up samples that were non-reactive with the two licensed screening tests. Among the 117 donors with negative screening tests on follow-up, 115/117 (98.3%) had non-reactive results with the licensed supplemental test. Conversely, 2/117 (1.7%) of these donors had indeterminate results with the licensed supplemental test.

It is FDA's current thinking that it would not be safe to reenter a donor with any reactivity with a licensed supplemental test given the higher analytical sensitivity of the currently licensed supplemental test compared with the licensed screening tests and the consequent uncertainty regarding the donor's infectious status. FDA currently considers donors whose follow-up samples are tested with all three currently licensed tests and show no reactivity with any of the three tests to be eligible for reentry, provided all other donor eligibility criteria are met. A least burdensome approach to identifying potentially

[4] See footnote 1.

eligible donors would be to perform sequential testing. The donors' follow-up samples would be first tested with the two licensed screening tests, which are run on automated instruments. Only specimens which are non-reactive on both screening tests would be subsequently tested with the manual licensed supplemental test.

It is FDA's current thinking that previously deferred donors who have had positive test results with either the unlicensed *T. cruzi* RIPA test or with an investigational or licensed supplemental test for antibodies to *T. cruzi* are not eligible for reentry and therefore should not be considered for reentry using the recommended algorithm (See section III.D of this guidance). The *T. cruzi* RIPA test has a long history of being used to identify individuals infected with *T. cruzi*. In a study by ARC of *T. cruzi* RIPA positive donors, a high proportion, 74.5% (117/157), were born in a *T. cruzi* endemic country (Ref. 12). Data from the licensed supplemental test clinical trial showed high concordance, 98.7% (151/153), between *T. cruzi* RIPA positivity and licensed supplemental test positivity among screening test repeatedly reactive donors (Ref. 13). Similarly, previously deferred donors who have had an indeterminate test result with either the *T. cruzi* RIPA test or with an investigational or licensed supplemental test are not eligible for reentry and therefore should not be considered for reentry using the recommended algorithm. These donors represent a small percentage of currently deferred donors (4.0%, according to an online report of the AABB Chagas' Biovigilance Network (http://www.aabb.org) dated May 1, 2015, as stated in section II of this guidance) and because their infectious status is unclear due to low level antibody reactivity to *T. cruzi* specific antigens, FDA considers them not eligible for reentry.[5] Only deferred donors with negative test results on the unlicensed *T. cruzi* RIPA (if so tested) and the investigational or licensed supplemental test for Chagas (if so tested), and deferred donors who have never been tested by *T. cruzi* RIPA or an investigational or licensed supplemental test should be considered for reentry using the recommended algorithm. FDA recommends that deferred donors who previously answered "yes" to the predonation screening Chagas question also be considered for reentry using the recommended algorithm provided that they have had no positive or indeterminate test results on the unlicensed *T. cruzi* RIPA or on the investigational or licensed supplemental test for Chagas.[6]

Donors who may be considered for reentry using the recommended algorithm may provide a follow-up blood sample for testing after a minimum of 6 months since the time of their last deferral. Although all *T. cruzi* positive U.S. blood donors identified since testing was initiated in 2007 have shown evidence of a long term rather than recent infection, the six-month time period prior to reentry testing would add a safeguard by allowing time for maturation of an early antibody response in a donor with low level

[5] FDA may reconsider in the future the eligibility of donors with an indeterminate test result using the unlicensed *T. cruzi* RIPA test, or an investigational or licensed supplemental test for antibodies to *T. cruzi* based on newly acquired supporting scientific evidence that these donors are not infected.

[6] If donors participated in follow-up studies, those with a positive or indeterminate test result with an investigational or licensed supplemental test for antibodies to *T. cruzi* or with the unlicensed *T. cruzi* RIPA test should not be considered eligible for reentry.

antibodies at the index donation due to recent infection. Six months would also allow for resolution of potential cross-reacting medical conditions that may have produced the repeatedly reactive screening test result.

While the BPAC did not take a formal vote on the donor reentry algorithm proposed by FDA at its July 31, 2014 meeting, the Committee discussed this approach and did not express concerns about the adequacy of this plan as a reentry algorithm (Ref. 11).

III. RECOMMENDATIONS

The recommendations set forth below are intended to update the recommendations in FDA's 2010 Chagas Guidance at section III.A and section III.C. The recommendations regarding product management in section III.B of the 2010 Chagas Guidance are unchanged.

These recommendations apply to the collection of blood and blood components, except Source Plasma, for transfusion or for use in manufacturing a product, including donations intended as a component of, or used to manufacture, a medical device.[7]

A. Donor Screening for History of Chagas Disease

We no longer recommend that the question "Have you ever had Chagas disease?" should be asked to all donors at each donation. The question may be removed from your donor history questionnaire.

B. Donor Testing

You must test donations for evidence of *T. cruzi* infection using a licensed test for antibodies to *T. cruzi* (21 CFR 610.40(a)), subject to the exceptions found in 21 CFR 610.40(c) and (d). We recommend one-time testing of each donor of blood and blood components (21 CFR 610.40(a)(2)(iii)(A)). We recommend one-time testing of autologous donors of blood and blood components only when the circumstances described in 21 CFR 610.40(d)(1) through (3) are applicable.

Donors who test non-reactive are qualified to return to donate without further testing of subsequent donations for antibodies to *T. cruzi*.

C. Donor Deferral and Counseling

Donors who test repeatedly reactive on a licensed test for *T. cruzi* antibody must be deferred (21 CFR 610.41(a)).

[7] Blood establishments are not required to test donations of Source Plasma for evidence of infection due to *T. cruzi* (21 CFR 610.40(a)(2)(ii)).

You must further test each donation which tests repeatedly reactive using a licensed test for antibodies to *T. cruzi* with a licensed, approved, or cleared supplemental test for antibodies to *T. cruzi* (See 21 CFR 610.40(e)). Further, you must make reasonable attempts to notify any donor that tests repeatedly reactive for antibodies to *T. cruzi* of their deferral and of their test results including the results of further testing required under 21 CFR 610.40(e) within 8 weeks after determining that the donor is deferred (See 21 CFR 630.40).

Donors whose blood tests positive or indeterminate on the licensed supplemental test should be deferred permanently and informed of the likelihood and medical significance of infection with *T. cruzi.* Donors whose blood tests negative on a licensed supplemental test may be considered for reentry using the recommended algorithm and informed of the procedure to follow for reentry.

D. Reentry for Donors Deferred on the Basis of Screening Test Results for Antibodies to *T. cruzi* or Predonation Screening Question

1. FDA recommends that donors with the following Chagas test results are not eligible for reentry:

   a. Positive or indeterminate with an investigational or licensed supplemental test for antibodies to *T. cruzi.*

   OR

   b. Positive or indeterminate with the unlicensed *T. cruzi* RIPA test.

2. Donors deferred on the basis of screening test results for antibodies to *T. cruzi* who had (at the time of the donation that prompted the deferral) the following Chagas test results may be considered for reentry using the recommended algorithm below, provided that they do not meet any of the ineligibility criteria described in item 1 above:[8]

   a. Negative with an investigational or licensed supplemental test for antibodies to *T. cruzi.*

   OR

   b. Negative with the unlicensed *T. cruzi* RIPA test.

[8] Effective May 23, 2016, blood collection establishments must use a licensed supplemental test for *T. cruzi* in accordance with 21 CFR 610.40(e). Accordingly, only donors who were deferred prior to May 23, 2016 should be considered for reentry on the basis of Chagas test results, at the time of the donation that prompted the deferral, with the investigational supplemental test for antibodies to *T. cruzi* or with the unlicensed *T. cruzi* RIPA test.

OR

c. Not tested with an investigational or licensed supplemental test for antibodies to *T. cruzi*, and not tested with the unlicensed *T. cruzi* RIPA test.

3. Donors deferred on the basis of answering "yes" to the predonation screening question "Have you ever had Chagas disease?" may also be considered for reentry using the recommended algorithm, provided that they do not meet any of the ineligibility criteria described in item 1 above.[9]

4. To reenter a donor who meets the criteria described in 2 or 3 above, we recommend that you do the following (also see algorithm in the Appendix):

   a. At least 6 months after the date of deferral, obtain a new blood sample from the donor (no donation is made at this time) and perform follow-up testing as follows:

      i. Test sample using two different licensed screening tests for antibodies to *T. cruzi.*

      If applicable, one of the two screening tests should be the test that was repeatedly reactive on the original donation.

      AND

      ii. If the follow-up sample is non-reactive with the two licensed screening tests, then test the follow-up sample with a licensed supplemental test for antibodies to *T. cruzi.*

      Note: As part of this reentry algorithm, FDA recommends that only follow-up samples that are non-reactive with the two licensed screening tests should be tested with a licensed supplemental test.

   b. Evaluate the results of the follow-up testing on the donor's new sample as follows:

      i. If either one or both screening tests are repeatedly reactive, we recommend that you defer the donor permanently.

[9] If donors participated in follow-up studies, those with a positive or indeterminate test result with an investigational or licensed supplemental test for antibodies to *T. cruzi* or with the unlicensed *T. cruzi* RIPA test should not be considered eligible for reentry.

ii. If the licensed supplemental test is either positive or indeterminate, we recommend that you defer the donor permanently.

iii. If the two licensed screening tests are non-reactive and the licensed supplemental test is negative, you may reenter the donor provided all other donor eligibility criteria are met at the time of donation. Testing for *T. cruzi* is not required on future blood donations from the reentered donor.

IV. IMPLEMENTATION

Note: This guidance is being issued for comment purposes only. Implementation of the recommendations contained herein is not recommended at this time.

A. Donor Screening

If you hold an approved biologics license and you remove the "Have you ever had Chagas disease?" question from your donor history questionnaire (DHQ), you must report this change under 21 CFR 601.12, as follows:[10]

- Revision of your own DHQ and accompanying materials: report in your annual report consistent with 21 CFR 601.12(d), noting the date the question was removed from your DHQ and accompanying materials.

- Revision of a previously FDA accepted DHQ and accompanying materials: report in your annual report consistent with 21 CFR 601.12(d), noting the date the question was removed from the accepted DHQ and accompanying materials.

B. Reentry of Deferred Donors

We consider the recommendations in section III.D for donor reentry in this guidance to be an acceptable requalification method or process, within the meaning of 21 CFR 610.41(b), for reentry of donors deferred due to repeatedly reactive screening tests for antibodies to *T. cruzi* and within the meaning of 21 CFR 630.35(b)[11] for donors deferred for previously answering "yes" to the donor history question, "Have you ever had Chagas disease?"

Licensed establishments implementing the recommendations for donor reentry must report this change to FDA as required under 21 CFR 601.12. Specifically, licensed establishments must submit a statement of this change in an annual report under

[10] See 21 CFR 601.12(a)(3).
[11] See footnote 1.

21 CFR 601.12(d), indicating the date that the revised standard operating procedures were implemented.[12] Unlicensed establishments implementing recommendations for donor reentry in this guidance in their entirety and without modification are not required to report this change.

Sections 610.41(b) and 630.35(b) require that a donor requalification method or process used to requalify a donor be acceptable to FDA. Accordingly, before you implement an alternative requalification method or process from that described in this guidance, FDA must first find the alternative method or process to be acceptable for such purpose. Licensed establishments intending to use an alternative requalification method must submit a supplement for prior approval, as required under 21 CFR 601.12(b). Similarly, FDA must find an alternative requalification method proposed by an unlicensed establishment to be acceptable before it is implemented (21 CFR 610.41(b) and 630.35(b)).

[12] See 21 CFR 601.12(a)(3).

V. REFERENCES

1. Guidance for Industry: Use of Serological Tests to Reduce the Risk of Transmission of *Trypanosoma cruzi* Infection in Whole Blood and Blood Components Intended for Transfusion, December 2010. http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/ucm235855.htm.

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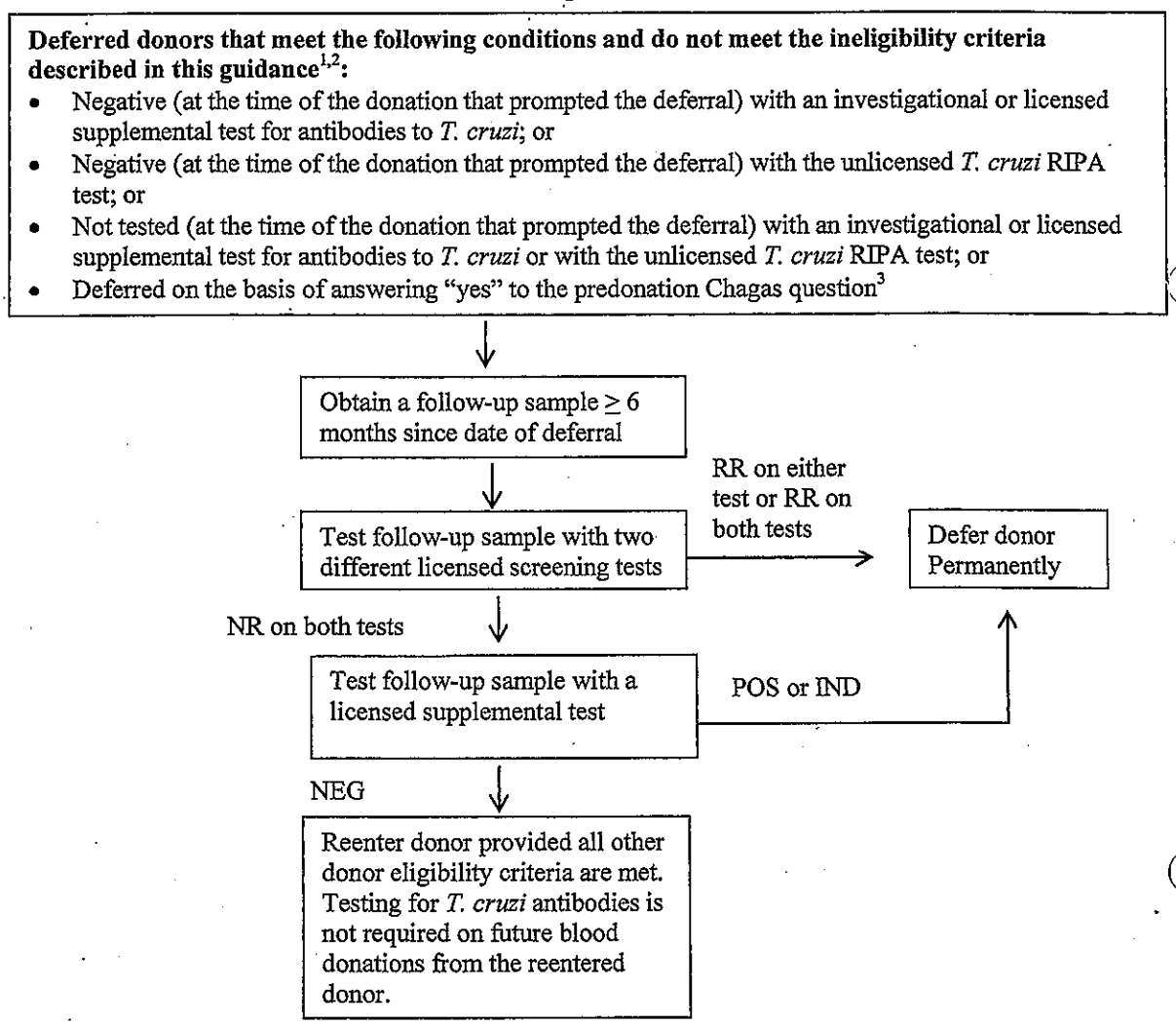
APPENDIX

RECOMMENDED REENTRY ALGORITHM FOR DONORS DEFERRED ON THE BASIS OF SCREENING TEST RESULTS FOR ANTIBODIES TO *T. CRUZI* OR PREDONATION SCREENING QUESTION



RR = repeatedly reactive; NR = non-reactive; POS = positive; NEG = negative; IND = indeterminate

[1] Effective May 23, 2016, blood collection establishments must use a licensed supplemental test for *T. cruzi* in accordance with 21 CFR 610.40(e). Accordingly, only donors who were deferred prior to May 23, 2016 should be considered for reentry on the basis of Chagas test results, at the time of the donation that prompted the deferral, with the investigational supplemental test for antibodies to *T. cruzi* or with the unlicensed *T. cruzi* RIPA test.

[2] FDA recommends that donors with the following Chagas test results are not eligible for reentry: (1) Positive or indeterminate with an investigational or licensed supplemental test for antibodies to *T. cruzi* or (2) Positive or indeterminate with the unlicensed *T. cruzi* RIPA test.

[3] If donors participated in follow-up studies, those with a positive or indeterminate test result with an investigational or licensed supplemental test for antibodies to *T. cruzi* or with the unlicensed *T. cruzi* RIPA test should not be considered eligible for reentry.

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。

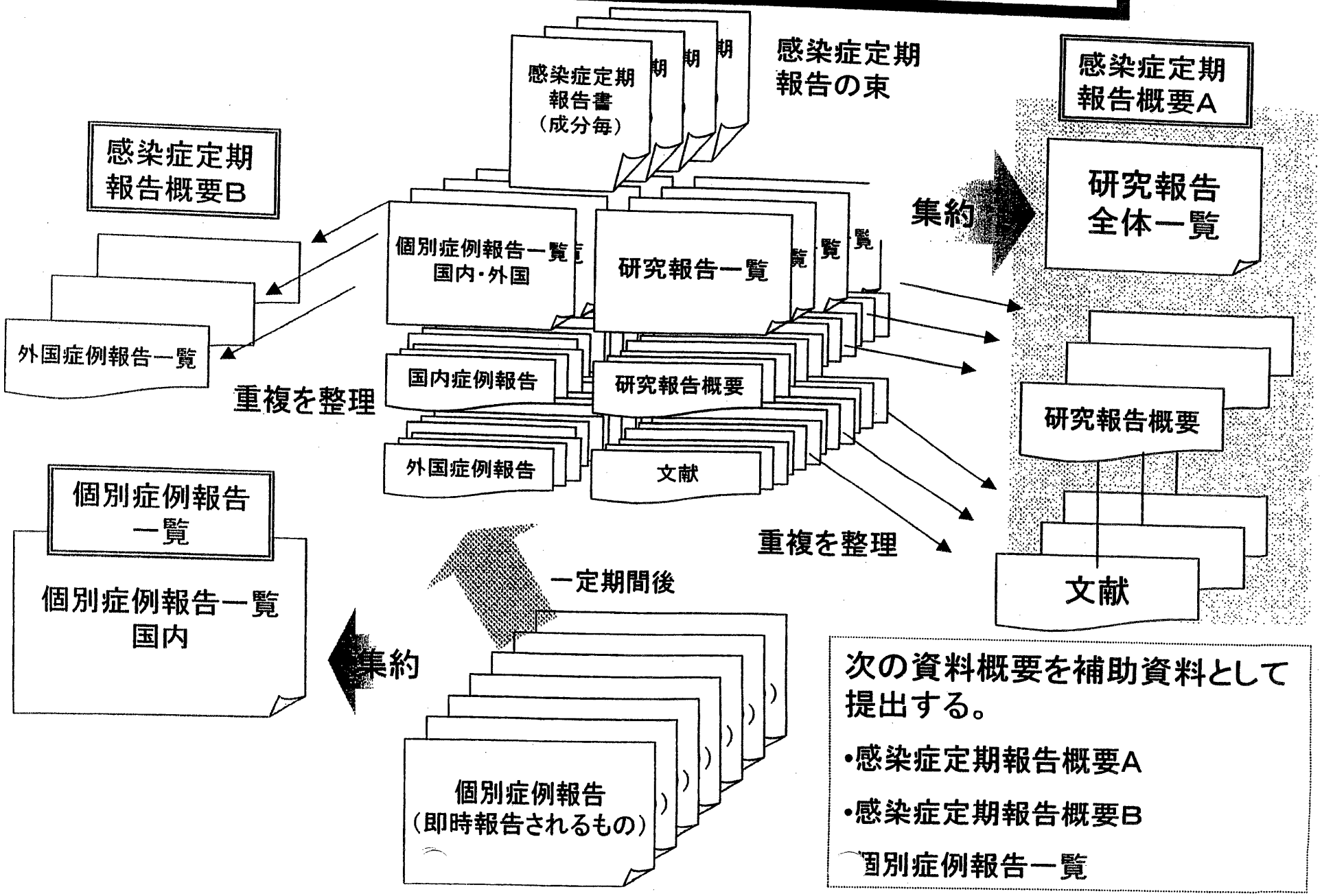
② 感染症発生症例報告のうち、発現国が「外国」の血漿分画製剤の使用による症例は、同一製品毎に報告期間を代表する感染症発生症例一覧（別紙様式第4）をまとめた**「資料概要B」**を事務局が作成し、送付する。

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