資料１－２

Ａ　研究報告（詳細版）

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医薬品 研究報告 調査報告書

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. 5. 20	該当なし	
一般的名称	新鮮凍結人血漿		研究報告の公表状況	星友二, 山岸尚仁; 長谷川隆, 安藤菜穂子, 遠田静穂, 五反田裕子, 内田茂治, 永井正, 佐竹正博(日本赤十字社血液事業本部中央血液研究所) 第64回日本輸血・細胞治療学会総会、2016年4月28日(木)～30日(土)、国立京都国際会館	公表国 日本	
販売名(企業名)	新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)					

研究報告の概要

○輸血用血液のスクリーニングにおけるHBs抗体基準200mIU/mLについての検証。
【はじめに】日本赤十字社で製造する血液製剤の基準では、HBc抗体が陽性であってもHBs抗体が200mIU/mL以上あれば製品試験合格となり、輸血用血液として供給される。これまで、これら血液による輸血HBV感染例の報告はないが、感染性および安全性については不明である。前回の本学会にてヒト肝臓置換マウス(PXBマウス)を用い、HBs抗体によるHBVの感染阻止試験結果を報告した。今回、異なるGenotypeのHBV感染により産生されたHBs抗体による感染阻止効果(交差試験)について検討した。
【材料・方法】HBV Genotype A又はCに各々感染した献血者から、HBV感染初期のHBV陽性血漿と回復期のHBs抗体陽性血漿を採取して、PXBマウスへの感染阻止試験を行った。HBV濃度を1.0E+05copies/mLに調製し、HBs抗体陽性血漿を0, 50, 100, 200mIU/mLの濃度で室温にて1時間反応後、その反応液100μLをPXBマウスに接種した(HBV量1.0E+04copies/匹)。1週間ごとに13週まで採血を行い、PCRにてHBV-DNA量を測定した。交差試験については200mIU/mLの濃度での試験を実施した。
【結果】HBV Genotype C感染により得られたHBs抗体は200mIU/mLでHBV Genotype C感染を阻止することが確認され100mIU/mLでは検出限界域の検出、50mIU/mLでは定量限界域の検出が認められたが、それ以上の増殖は見られなかった。また、交差試験ではHBV Genotype A感染により得られたHBs抗体がHBV Genotype Cの感染を抑えることが判明した。
【考察】Genotypeが異なる HBV感染であっても、200mIU/mL のHBs抗体により感染を阻止する結果が得られた。個別NATの検出感度を大幅に上回る1.0E+05copies/mLのHBV感染を防御できることが判明した。従って現行の輸血用血液スクリーニング基準(HBs抗体200mIU/mL)は、HBV感染リスクを低減するうえで非常に有効であり、その妥当性が示された。

報告企業の意見	今後の対応
ヒト肝臓置換マウス(PXBマウス)を用い、200mIU/mL のHBs抗体によるHBVの感染阻止を確認した。この感染阻止は、Genotypeが異なるHBV感染でも確認されたという報告である。	引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

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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

O-52　輸血用血液のスクリーニングにおける HBs 抗体基準 200mIU/mL についての検証

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【はじめに】日本赤十字社で製造する血液製剤の基準では，HBc 抗体が陽性であっても HBs 抗体が 200 mIU/mL 以上あれば製品試験合格となり，輸血用血液として供給される．しかし，HBs 抗体が 200mIU/mL 以上の血液でも NAT スクリーニングで HBV-DNA が稀に検出されることがある．HBc 抗体陽性で HBs 抗体が 200mIU/mL 以上の血液製剤による HBV 感染例の報告はない．それら血液の感染性については不明である．そこでヒト肝臓置換マウス（PXB マウス）を用いた HBV 感染における HBs 抗体による感染阻止試験を前回の本学会（第 63 回）で報告した．今回 Genotype C および異なる Genotype で産生された HBs 抗体による感染阻止効果についても検討したので報告する．【材料・方法】同一献血者における HBV 感染初期の HBV-DNA 陽性と回復期の HBs 抗体陽性のペア血漿を材料とした．PXB マウスに HBV 感染初期血漿を接種し，感染・増殖を確認したマウス血漿を感染材料に用いた．HBV 濃度を 1.0E+05copies/mL に調製し，HBs 抗体陽性血漿を 0，50，100，200mIU/mL の濃度で室温 1 時間反応したのち，100μL を PXB マウスに接種した．1 週間毎に 13 週まで採血し，PCR にて HBV-DNA 量を測定した．交差試験については 200mIU/mL の抗体での試験を実施した．【結果】Genotype C の HBV に対して産生された HBs 抗体は 200mIU/mL で感染を阻止することが確認できた．100mIU/mL では検出限界域の検出，50mIU/mL では定量限界域の検出が認められたが，増殖は見られなかった．また，交差試験においても 200mIU/mL の抗体で HBV の感染を抑えることが判明した．【考察】今回 Genotype C においても HBs 抗体 200mIU/mL は感染阻止に十分な濃度であることが示唆された．HBs 抗体が 200mIU/mL 以上であれば，異なる Genotype であっても，個別 NAT の検出感度を大幅に上回る 1.0E+05copies/mL の HBV 感染を防御できることが判明した．現行の輸血用血液スクリーニングの HBs 抗体の基準（200mIU/mL）は，HBV 感染リスクを低減するうえで非常に有効であり，その妥当性が示された．

別紙様式第2-1

No. 10

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. 7. 1	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名(企業名)	新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)		Tosti ME, Alfonsi V, Lacorte E, et al. Clin Infect Dis. 2016 Jun 1;62(11):1412-8.	イタリア

研究報告の概要

○イタリアにおける全小児を対象としたワクチン定期接種導入後の急性B型肝炎:22年にわたる調査の結果(1993～2014年)。
背景:B型肝炎ワクチンの接種は非常に安全であり、効果が高いことが証明されている。本研究では、急性B型肝炎患者におけるワクチン接種を受けた者の割合、推奨に従ってワクチン接種を受けていれば予防できた可能性があった者の割合、並びにワクチン接種を受けなかった理由について評価を行った。
方法:我々は1993年から2014年までの期間にイタリアの急性ウイルス性肝炎監視システムに報告されたデータを分析した。
結果:本研究の対象とした急性B型肝炎患者11,311例のうち、計362例(3.2%)がワクチン接種を受けていた。ワクチン接種のデータが入手可能であった277例のうちの50例(18%)は、B型肝炎ウイルス(HBV)に曝露される前に、正確なスケジュールに基づいて一連のワクチン接種を完了していた。このうちの17例について遺伝子的特徴付けを行った結果、6例がS遺伝子変異株に感染していたことが判明した。ワクチン接種を受けていなかった10,949例のうち、213例(1.9%)は義務付けられていたワクチン接種を忌避しており、2,821例(25.8%)は感染リスクが高い状態にあったにもかかわらずワクチン接種を受けていなかった。後者に多く認められたリスク因子は、HBVキャリアとの共同生活、静注薬物の使用並びに同性愛・両性愛行為であった。HBVキャリアがいる世帯で、ワクチン接種を受けていなかった者の37%は、自らが有するリスクを認識していた。ワクチン接種を躊躇する理由として挙げられる頻度が高かったものは、ワクチン接種に対する信頼の欠如、消極的な姿勢、誤った信念とこれに続くコミュニケーションの欠如もしくは不足、並びに当該疾患の重大性に対する認識の低さであった。
結論:B型肝炎ワクチン接種を受けた者における急性肝炎の発症は稀である。高リスク者におけるワクチン接種率を高めるための更なる努力が必要である。

報告企業の意見	今後の対応
イタリアではB型肝炎ワクチン定期接種を導入しており、急性B型肝炎発症者の大半は未接種であったという報告である。	HBV感染に関する新たな知見等について、今後も情報の収集に努める。

使用上の注意記載状況・その他参考事項等

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血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

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Clinical Infectious Diseases

MAJOR ARTICLE

Acute Hepatitis B After the Implementation of Universal Vaccination in Italy: Results From 22 Years of Surveillance (1993–2014)

Maria Elena Tosti,[1] Valeria Alfonsi,[1] Eleonora Lacorte,[1] Alfonso Mele,[1] Cristina Galli,[2] Alessandro Remo Zanetti,[2] and Luisa Romanò[2]; for the SEIEVA Collaborating Group[a]

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Background. Hepatitis B vaccination has proven to be very safe and highly effective. This study assessed the proportion of successfully vaccinated individuals among cases with acute hepatitis B, the proportion of preventable cases if individuals were vaccinated as recommended, and the reasons for failures.

Methods. We analyzed data reported to the Italian Surveillance System for Acute Viral Hepatitis from 1993 to 2014.

Results. A total of 362 of 11 311 (3.2%) cases with acute hepatitis B were vaccinated. Of the 277 cases for whom immunization data were available, 50 (18%) received a complete vaccination course according to the correct schedule and before exposure to hepatitis B virus. Molecular characterization of 17 of these cases showed that 6 were infected with S-gene mutants. Among the 10 949 unvaccinated cases, 213 (1.9%) escaped mandatory vaccination and 2821 (25.8%) were not vaccinated despite being at increased risk of infection. Among the latter, the most common risk factors were cohabitation with hepatitis B surface antigen (HBsAg) carriers, intravenous drug use, and homosexual/bisexual practices. Thirty-seven percent of the unvaccinated households with HBsAg carriers were aware of their risk. Lack of trust in the vaccination, negative attitude, and inaccurate beliefs followed by lack of or poor communication and low perceived severity of the disease were the most frequent reasons for vaccine hesitancy.

Conclusions. Development of acute disease in successfully vaccinated individuals is a rare event. Further efforts are needed to enhance the vaccine coverage rate in individuals at increased risk of infection.

Keywords. HBV; surveillance; hepatitis B vaccination; breakthrough infections; vaccination failure.

Hepatitis B virus (HBV) infection and HBV-related diseases are a major public health issue worldwide. Currently about 240 million people are estimated to be chronically infected with HBV, and more than 780 000 individuals die each year due to hepatitis B complications, including cirrhosis and liver cancer [1].

Vaccination is the most effective and economically favorable measure to control and prevent hepatitis B on a global scale [2–4]. Italy is one of the first countries in the world to implement a vaccination policy, starting in 1983 with a program targeted to individuals at increased risk of infection [5–6]. Vaccination became mandatory in 1991 for all infants and all 12-year-olds. This program also includes the mandatory screening of pregnant women for hepatitis B surface antigen (HBsAg) in order to identify babies in need of treatment with hepatitis B immune globulin and vaccine at birth. The program also includes recommendations for vaccination of groups at higher risk of infection. At the end of 2003, those in the first infant cohort vaccinated in 1991 were age 12 years. Thus, the vaccination of 12-year-olds was stopped, as all children at that age were covered, while the vaccination of infants was maintained. Because of the Italian vaccination delivery system's effectiveness and public awareness of the disease, the take-up of vaccination was rapid and reached a coverage rate of >95% within a few years [7]. To maintain the highest possible coverage rate and, in turn, guarantee an effective protection at a social level through herd immunity, parents/legal guardians who miss vaccination are required to have their children vaccinated. Opponents to vaccination are invited to discuss their objections with the staff of vaccination centers (medical doctors, trained nurses) who encourage vaccination for the benefit of both individuals and the community at large. Noneligible children are exonerated. Parents/legal guardians who choose to opt out despite having been repeatedly invited are asked to sign a waiver. As a consequence of this policy, approximately 20 million people (34 birth cohorts) have been vaccinated against hepatitis B with an outstanding record of safety and efficacy. This resulted in a substantial decrease in the burden of disease, carrier rate,

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[a]Members of the SEIEVA Collaborating Group are listed in the Acknowledgments.

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and HBV-related morbidity and mortality [8, 9]. Despite this success, there are still cases of acute hepatitis B that are reported to the national surveillance system for acute viral hepatitis (SEIEVA [Sistema Epidemiologico Integrato dell'Epatite Virale Acuta], Istituto Superiore di Sanità, Rome), even among individuals who have been vaccinated or who should have been vaccinated as they were eligible for vaccination.

Our aim in this study was to assess the proportion of successfully vaccinated individuals in cases with acute hepatitis B, the proportion of cases that could have been avoided if fully and timely vaccinated, and reasons of failures.

METHODS

National Surveillance System for Acute Viral Hepatitis

SEIEVA is a national passive surveillance system that is a supplement to the Italian Official Surveillance System for Infectious Diseases. It was implemented in 1984 [10]; over 3 decades, SEIEVA has included a network of Italian local health units (LHUs), covering a mean of 60% of the Italian population (approximately 36 million people). As described by Tosti et al [11] and according to the ongoing protocol, clinical acute cases are reported by physicians in charge of the diagnosis to the pertaining LHU. Participation is voluntary. Demographic, clinical, epidemiological, and laboratory data as well as information on risk factors and hepatitis B vaccination status are collected and maintained in a dedicated database. Information about vaccination schedule (timing, number of doses, and type of vaccine administered) is further confirmed for each case by reviewing the vaccination registries of the LHUs. All SEIEVA information can be found on their website (www.iss.it/seieva/).

Study Design and Population

This was a retrospective descriptive study of acute hepatitis B cases reported to SEIEVA between 1993 and 2014. Within the SEIEVA surveillance system, the definition of an acute hepatitis B case is based on clinical and serological criteria including acute symptoms consistent with viral hepatitis, significant (more than 10-fold) increase in serum alanine amino transferase (ALT), immunoglobulin M anti-HBc antibody positivity.

Cases with a previous hepatitis B vaccination were analyzed to assess whether they were properly vaccinated. For the purposes of this study, a properly vaccinated case was defined as a diagnosis of acute hepatitis B (clinical breakthrough) in an individual who had been previously immunized according to our nationally recommended schedule of vaccination (3 doses given at 3, 5, and 11 months of age in infants and at 0, 3, and 6 months in children and adults) and had symptom onset at least 6 months (maximum incubation period) after completing a full vaccination course. This definition of HBV breakthrough does not take into account whether the vaccine recipient developed a post-vaccination immune response (anti-HBs antibody ≥10 mIU/mL as measured 1–3 months after administration of the last dose of the initial series), since post-vaccination testing is not routinely performed in Italy.

All cases with missing information on hepatitis B vaccination status were excluded from the analysis. Moreover, unvaccinated cases were studied to determine whether they belonged to high-risk groups or to groups for which vaccination is mandatory. To gather information about missing vaccinations, an ad hoc questionnaire was administered to households with HBsAg chronic carriers who were aware of the chronic infectious status of their relatives and acquired acute disease.

All data were anonymously analyzed in accordance with Italian privacy legislation.

Virological Testing

Molecular characterization of the specific viral strain causing hepatitis was possible in only a few cases. In particular, the amplification of the S gene of HBV DNA was performed in sera collected during the course of acute hepatitis B. Then, the HBV genotypes and the presence of mutations in the "a" determinant, that is, the neutralizing epitope within the major hydrophilic region of the HBsAg, were determined by direct sequence analysis [12].

Statistical Analyses

Pearson χ^2 test or Fisher exact test, when necessary, were used to assess differences in discrete characteristics between groups,

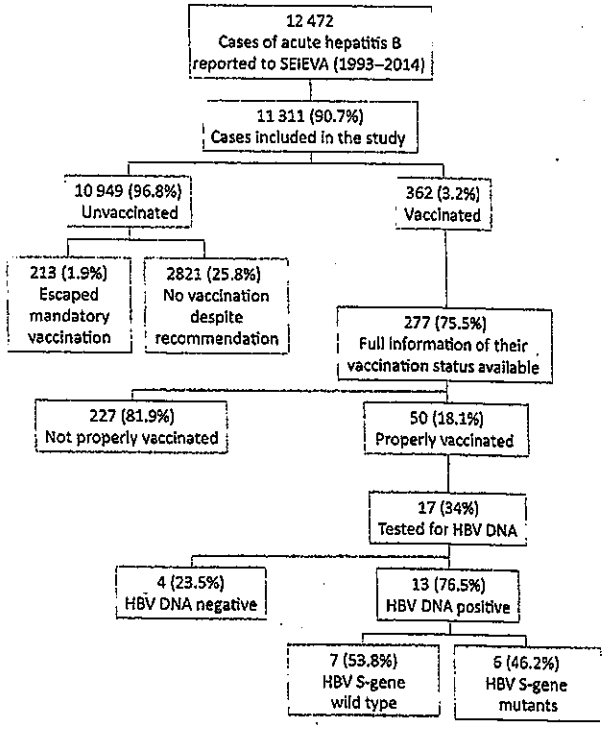

Figure 1. Study design. Abbreviations: HBV, hepatitis B virus; SEIEVA, Sistema Epidemiologico Integrato dell'Epatite Virale Acuta.

such as vaccinated/unvaccinated cases, geographical areas, and year of diagnosis. The Kruskal–Wallis test was used to investigate the significance of by-group differences in continuous variables.

P values <.05 were considered statistically significant. All statistical procedures were performed using the STATA statistical package, version 13.1.

RESULTS

As shown in Figure 1, 12 472 cases of acute hepatitis B were reported to SEIEVA between 1993 and 2014. Information on hepatitis B vaccination status was available for 11 311 (90.7%) cases, which were included in the study population. The median age was 36 years (range, 0–100) and 74.9% were males. During this period, the median age of individuals with hepatitis B significantly rose from 26 years in 1993 to 46 years in 2014 ($P < .001$).

Within the study population, 362 cases (3.2%) occurred in individuals who had received at least 1 dose of vaccine. The yearly percentage of cases in vaccinated individuals did not significantly change during the study period. Complete information on vaccination history (ie, number of doses of vaccine administered, time between doses) was available for 277 (76.5%) vaccinees. In most of these (n = 227; 81.9%), the vaccination schedule was not completed properly. Vaccine was administered postexposure in 151 cases, with a median time lapse between vaccination and onset of disease of 31 days (range, 5–175). Moreover, 40 individuals were administered an incomplete schedule (24 received 1 dose, 16 received 2 doses), and 36 individuals received 3 doses but with an incorrect interval between them (1 individual received the second dose 25 months after the first dose and 35 individuals had a median span of 19 months [range, 16–41] between the second and the third doses).

Table 1. Comparison Between Properly Vaccinated Cases and Unvaccinated Cases, by Demographic Characteristics, Risk Factors, Hospitalization Rate, and Clinical and Biochemical Parameters

	Properly Vaccinated Cases		Unvaccinated Cases		
	N = 50		N = 10 949		
Characteristic	N	%	N	%	*P* Value[a]
Gender					
Male	37	74.0	8176	75.2	ns
Female	13	26.0	2703	24.8	
Age (y)					
0–14	5	10.0	109	1.0	<.001
15–24	13	26.0	1552	14.3	
25–34	13	26.0	3377	31.0	
35–54	17	34.0	4217	38.7	
≥55	2	4.0	1632	15.0	
Risk factor[b]					
Intravenous drug use	10	20.4	1139	10.6	.035
Nosocomial exposure[c]	8	16.0	1863	17.1	ns
Parenteral exposure[d]	15	30.6	3488	32.1	ns
Dental therapy	16	32.7	3312	30.8	ns
Household Hepatitis B surface antigen +	8	21.6	871	10.4	.026
>2 sexual partner	7	22.6	2190	27.7	ns
Unsafe sexual practices[e]	7	17.1	2076	20.9	ns
Clinical characteristics and liver function tests					
Hospitalization	43	86.0	10 176	93.5	.042
Jaundice	42	84.0	9239	85.2	ns
Aspartate amino transferase: mean (SD)	1060 (812)		1453 (1059)		.039
Alanine amino transferase: mean (SD)	1730 (1213)		2357 (1375)		.011
Total bilirubin: mean (SD)	8.3 (6.7)		11.9 (11.4)		.057
Direct bilirubin: mean (SD)	10.0 (20.0)		9.0 (10.1)		ns
International Normalized Ratio: mean (SD)	1.2 (0.1)		1.5 (1.0)		ns

Data from SEIEVA (Sistema Epidemiologico Integrato dell'Epatite Virale Acuta) 1993–2014.

Abbreviations: ns, not statistically significant; SD, standard deviation.

[a] Obtained using Mann–Whitney test for continuous variables and χ^2 test (or Fisher exact test) for discrete characteristics.

[b] Each case may report more than 1 risk factor.

[c] Hospitalization, hemodialysis, surgical intervention, endoscopy, blood transfusion.

[d] Piercing, tattooing, acupuncture, manicurist/chiropodist attendance, barber-shop shaving.

[e] Condom use (occasional/never vs always) during occasional sexual intercourses.

Table 2. Molecular Characteristics of 6 Cases of Acute Hepatitis B in Individuals Properly Vaccinated and Infected With Mutant Hepatitis B Viruses

Case Number	HBV Genotype	Mutation	Anti-HBs
1	D	G145R	≥10 mIU/mL
2	F	T118K	≥10 mIU/mL
3	F	T126A	≥10 mIU/mL
4	D	Q129H	Negative
5	D	D144E	Negative
6	B	T143M	Negative

Abbreviation: HBV, hepatitis B virus.

The remaining 50 (18.1%) individuals (74% males; median age 30 years, range, 2–79 years) developed acute hepatitis B after a median of 7.5 years (range, 223 days–21.5 years), despite having been given a full course of vaccination according to the correct schedule and before exposure to HBV. These cases were uniformly distributed throughout the observation period. Table 1 shows a comparison of demographic characteristics, risk factors, clinical characteristics, and liver function tests between correctly vaccinated and unvaccinated cases. Vaccinated individuals were significantly younger, less frequently hospitalized, and had lower levels of ALT and aspartate amino transferase compared with unvaccinated cases. The percentage of intravenous drug users (IVDUs) and households with HBsAg chronic carriers was significantly higher among vaccinated than among unvaccinated individuals.

Molecular characterization was performed on sera collected from 17 of the 50 (34%) individuals who were successfully vaccinated and showed that 13 of them were HBV-DNA positive. Genotype F was found in 5 cases, genotype D in 4 cases, and genotype A in 2 cases, while genotypes B and E were found in 1 case each. Sequence analysis of the viral "a" determinant showed that 7 of these cases were infected with wild-type HBV, while 6 cases carried S-gene mutations (Table 2). Three of the 6 cases infected with mutant viruses had cocirculating seroprotective concentrations of anti-HBs antibody (≥10 mIU/mL).

During the study period, 10 949 cases of acute hepatitis B in unvaccinated individuals were reported to SEIEVA (Figure 1). Of these, 213 (1.9%) escaped mandatory vaccination. Their distribution showed a significant geographical gradient, with 1.2% and 1.7% in northern and central Italy, respectively, compared with 4.4% in southern Italy ($P < .001$). A total of 187 (87.8%) of 213 cases escaped mandatory vaccination as adolescents and 26 (12.2%) escaped as infants.

Acute hepatitis B was diagnosed in 2821 (25.8%) cases who were at increased risk of exposure to HBV and for whom hepatitis B vaccination was strongly recommended and offered free of charge. The trend of these cases showed a significant yearly decrease (150–250 cases/year in 1993–2001 vs 40–60 cases/year in 2010–2014; $P < .001$). As shown in Figure 2, most of the 2821 cases that were in high-risk groups were IVDUs (n = 1047; 37.1%), including 135 (12.9%) who referred to centers for the treatment of addictions, 843 (29.9%) households with HBsAg chronic carriers, and 800 (28.4%) homosexual/bisexual men. Among the households with HBsAg chronic carriers, 310 (36.8%) were aware of his/her relative's infectious status. SEIEVA collected information about the reasons for missed vaccination from 285 of these 310 (91.9%) cases. As shown in Table 3, the main reasons were lack of trust in the vaccination, negative attitude, and inaccurate beliefs (67.7%) followed by lack of or poor communication (10.5%) and low perceived severity of the disease.

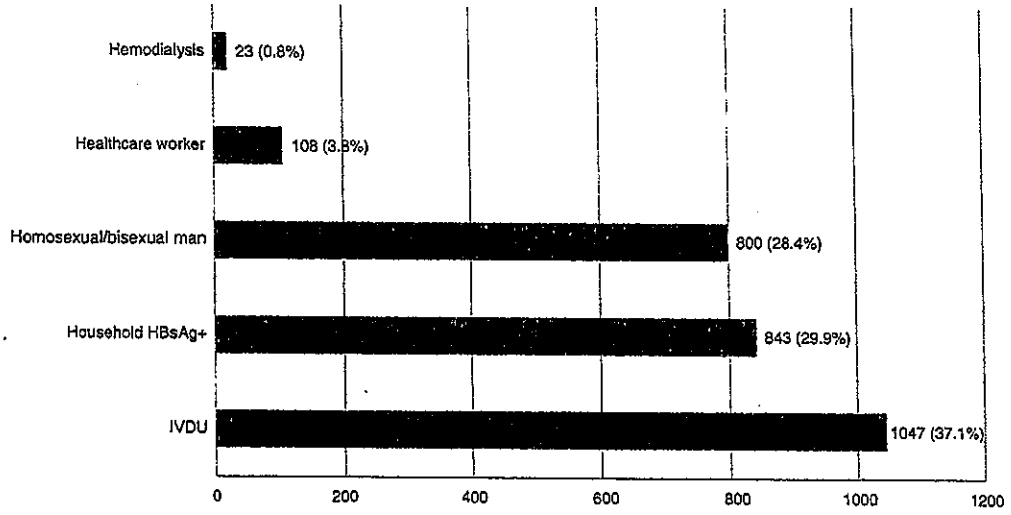

Figure 2. Indication for vaccination in 2821 unvaccinated individuals with acute hepatitis B belonging to high-risk group. Abbreviations: HBsAg, hepatitis B surface antigen; IVDU, intravenous drug users.

Table 3. Reasons for Nonvaccination Among Households With Hepatitis B Surface Antigen Chronic Carriers Who Were Aware of Their Relative's Infective Status

Reason for Nonvaccination	Number of Cases	%
Lack of trust in the vaccination, negative attitude, and inaccurate beliefs	193	67.7
Lack of or poor communication	30	10.5
Low perceived severity of the disease	25	8.8
Doubts about the vaccine's efficacy	24	8.4
Fear of side effects	13	4.6
Total	285	100.0

Data from SEIEVA (Sistema Epidemiologico Integrato dell'Epatite Virale Acuta) 1993–2014.

DISCUSSION

Viral hepatitis B is a vaccine-preventable disease. According to World Health Organization recommendations, more than 183 countries have implemented hepatitis B vaccination into their national childhood immunization programs [1]. Evidence shows that after completing a routine 3-dose schedule of vaccination, seroconversion to protective concentrations (≥10 mIU/mL) of anti-HBs is reached in more than 95% of healthy vaccine recipients, and that protection lasts more than 15–20 years after vaccination, with no need for booster doses [13–17]. The introduction of hepatitis B vaccine led to a global decrease in the incidence of disease, HBV carrier rate, and HBV-related mortality due to cirrhosis or hepatocellular carcinoma (HCC). In Taiwan, after the implementation of universal hepatitis B vaccination in 1984, the HBsAg seropositive rate in children decreased from 10% to 0.9% in 2012 [18]. Concurrent with this decrease, a significant reduction was observed in the incidence of HCC among children and teenagers, indicating that the hepatitis B vaccine was the first vaccine used for a major human cancer [19, 20]. Similar results have been achieved in other previously hyperendemic locations such as Alaska, Gambia, China, and South Africa [21–24]. In Italy, where the vaccination of individuals at increased risk of HBV exposure started in 1983 and universal vaccination started in 1991, data from SEIEVA show a substantial decline in the overall incidence of acute hepatitis B from 5 per 100 000 inhabitants in 1990 to <0.8 in 2014 [25]. The decrease was even more evident in individuals aged 15–24 years and in those aged 0–14 years in whom morbidity rates (per 100 000) decreased, in the same period, from 17 to 0.3 (98% decrease) and from 1 to 0.03 (97% decrease), respectively. Moreover, the prevalence of HBV markers also substantially decreased in children and young adults after vaccination [8, 9]. A further benefit, due to the biological dependence of hepatitis delta virus from HBV, was that hepatitis delta also decreased significantly in Italy as a consequence of the implementation of hepatitis B vaccination [26–28].

Some long-term studies have documented HBV breakthrough, proven by seroconversion to anti-HBc antibody, while virtually no clinical disease or carriage has been reported to date [29, 30].

Between 1993 and 2014, 96.8% of the 11 311 cases reported to SEIEVA and included in this study occurred in unvaccinated individuals; in the remaining 362 (3.2%) cases, acute disease occurred in those who were administered at least 1 dose of vaccine. Complete information on vaccination status was collected for 277 of the 362 (75%) vaccinated individuals, showing that only 50 cases out of 227 (18.1%) had received a full primary course of vaccination according to the proper schedule and before exposure to HBV. Recalculation of the 18.1% of the total 362 cases in vaccinated individuals results in an estimated 66 breakthrough cases (correctly vaccinated before exposure) reported over a 22-year period to SEIEVA. Considering that during the period of surveillance, SEIEVA covered a mean of 60% of the entire Italian population (approximately 60 million), we estimate that 5 vaccination failures could occur in Italy each year. This clearly indicates that breakthrough infections are rare in Italy, where approximately 20 million individuals, or one third of the entire population, have been vaccinated. In this context, the herd immunity that is secondary to the large number of vaccinated individuals may have played a crucial role in the control and prevention of hepatitis B.

Correctly vaccinated cases were younger, hospitalized less frequently, and had lower ALT values than those who were not vaccinated. This could suggest some effect of vaccination in slowing the natural course of the disease, but this interpretation needs further confirmation. The most common risk factors identified among fully vaccinated individuals included intravenous drug use and a household contact with an HBsAg carrier; however, the reason for this association needs to be further investigated. The molecular characterization of the samples collected from 13 of the vaccinated individuals with breakthrough infection who were HBV-DNA positive showed that 7 of them were infected with HBV wild-type and 6 with S-gene mutants potentially able to evade the vaccine-induced immunity. Three of these cases developed acute hepatitis B despite the presence of protective levels of anti-HBs (≥ 10 mIU/mL). It has been reported that vaccination based on recombinant HBsAg from HBV genotypes A and D (the vaccines currently used worldwide) may be less effective toward genotypes E and F since they display marked differences from the other genotypes in the "a" determinant domain toward which neutralizing (protective) antibodies are largely targeted [31]. The fact that 6 (46.2%) cases of breakthrough infection were caused by genotype F (n = 5) or genotype E (n = 1), which are both quite rare in Italy, is intriguing even though is important to say that in Gambia, where genotype E is predominant [32], and in Alaska, where genotype F is highly diffused [33], vaccination had great success.

Despite the high coverage rate achieved over time in Italy, cases of acute hepatitis B continue to be reported among individuals who should have been vaccinated but were not, including a number of cases (213; 1.9%) who escaped mandatory

vaccination. The majority of these (87.8%) were adolescents, most of whom lived in southern Italy.

In 25.8% of unvaccinated cases, acute hepatitis B was diagnosed in individuals at increased risk of exposure to HBV for whom hepatitis B vaccination is strongly recommended and offered free of charge. Grouping those who escaped mandatory vaccination with those who were not vaccinated despite being at high risk of infection, the disease could have been prevented in 27.7% (3034 of 10 949) of cases if duly vaccinated. However, the trend of such cases showed a significant yearly decrease (150–250 cases/year in 1993–2001 vs 40–60 cases/year in 2010–2014; $P < .001$), attributable to an increase in the proportion of vaccinated individuals and possibly to an increasing herd immunity effect.

Cohabitation with chronic HBsAg carriers, IVDU, and homosexual/bisexual practices were the major risk factors associated with acute hepatitis B. Here, we report an alarming number of missed opportunities for immunization in 13% of IVDUs who were being seen at treatment centers before the onset of acute hepatitis and in at least 37% of households with HBsAg carriers who were not vaccinated despite being aware of the condition of their cohabitant(s). For this latter group, data collected using an ad hoc questionnaire showed that lack of trust in the vaccination, negative attitude, and inaccurate beliefs followed by lack of or poor communication and low perceived severity of the disease were the most frequent reasons for vaccine hesitancy.

In conclusion, data from this study show that the Italian program of vaccination resulted in substantial progress toward the prevention and control of hepatitis B infection. Cases of acute hepatitis B reported in successfully vaccinated individuals are currently infrequent and rarely caused by vaccine-escape S gene viral mutants. Further efforts to achieve and maintain a high level of public confidence in the safety and efficacy of hepatitis B vaccination are essential to reaching high coverage rates, especially among individuals at increased risk of HBV infection.

A limitation of this study is that SEIEVA does not collect data on the follow-up of vaccinated individuals with hepatitis B, thus missing the opportunity to determine whether, in case of infection, vaccination can protect against the development of a chronic carrier state. However, since the risk of becoming a chronic carrier is known to be largely age dependent, with a higher frequency in younger individuals compared with adults [34], the fact that the median age of our cases increased from 26 years in 1993 to 46 years in 2014 is reassuring. The vaccine-induced shifting of infection to those who are older, in fact, favors a reduction in the rate of infected individuals who can develop a chronic carrier state. Further studies are needed to better clarify this issue.

Notes

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別紙様式第2-1

No. 11

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2016. 5. 20	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名(企業名)	新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)		高橋勉, 古居保美, 平力造(日本赤十字社血液事業本部) 第64回日本輸血・細胞治療学会総会、2016年4月28日(木)～30日(土)、国立京都国際会館	日本

研究報告の概要

○輸血との因果関係が極めて低いとされたHCV感染疑い症例の解析。
【目的】輸血によるHCV感染症例は、2010年以降に製造された輸血用血液製剤による報告は1例もない。しかし、医療機関からのHCV感染疑い情報は毎年30～40例が日本赤十字社へ報告されている。HCV感染防止対策の一助とするため、輸血以外の感染要因の可能性について調査したので報告する。
【対象】2012年～2014年の3年間に、医療機関から報告のあった99例のうち、受血者の輸血前後でHCV-RNAが陽転した症例で、感染を疑った全ての血液の保管検体等の検査結果が全て「陰性」で、そのHCV感染と輸血との因果関係が極めて低いと評価した39例を対象とした。
【方法】医療機関で記載頂いた「副作用・感染症記録」の原疾患及び併用療法等について調査し、原疾患については東京都の平成25年輸血状況調査結果と、併用療法については2006年～2008年の内部調査と比較した。
【結果】原疾患は、悪性新生物が10例、骨折等の損傷が8例、消化器系疾患が5例、循環器系疾患が4例、血液・造血器疾患・免疫障害、筋骨格系・結合組織疾患、尿路性器系疾患、その他がそれぞれ3例であり、悪性新生物(26%)、血液・造血器疾患・免疫障害(8%)は東京都の報告に比べ低く、骨折等の損傷(21%)は高かった。
併用療法(重複回答)に記載のあった33例では、手術等の侵襲的処置が26例(79%)、内視鏡、カテーテルがそれぞれ6例(18%)、透析、化学療法がそれぞれ4例(12%)、放射線療法が2例(6%)であり、実施率は過去の調査に比べ、カテーテル、内視鏡で低下し、手術等の侵襲的処置で上昇していた。
【考察】医原性の感染経路は、主に汚染器具の使用、外科手術、内視鏡、透析と報告されている。今回の調査では、手術等の侵襲的処置は骨折等の損傷などで、高率で実施されていた。HCV感染との直接的関係は不明であるが、侵襲的処置実施時にはHCV感染防止対策が必要と思われた。医療安全の観点からも、これらの結果を医療機関と共有して、HCV感染防止対策に役立てたい。

報告企業の意見	今後の対応
医療機関から報告のあった輸血によるHCV感染疑い症例は、保管検体等の検査結果が全て「陰性」で、その大半において、手術等の侵襲的処置が行われてい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、陰性の検体について20プールNATを実施していたが、更なる安全性の向上を目的に、2014年8月1日よりNATシステムを変更し、全検体に対し個別検体によるNAT(個別NAT)スクリーニングを開始し、陽性血液を排除している。HCV感染に関する新たな知見等について、今後も情報の収集に努める。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

P-16　輸血との因果関係が極めて低いとされたHCV感染疑い症例の解析

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【目的】輸血によるHCV感染症例は，2010年以降に製造された輸血用血液製剤による報告は1例もない．しかし，医療機関からのHCV感染疑い情報は毎年30～40例が日本赤十字社へ報告されており，HCV感染防止対策の一助とするため，輸血以外の感染要因の可能性について調査したので報告する．【対象】2012年～2014年の3年間に，医療機関から報告のあった99例のうち，受血者の輸血前後でHCV-RNAが陽転した症例で，感染を疑った全ての血液の保管検体等の検査結果が全て「陰性」で，そのHCV感染と輸血との因果関係が極めて低いと評価した39例を対象とした．【方法】医療機関で記載頂いた「副作用・感染症記録」の原疾患及び併用療法等について調査し，原疾患については東京都の平成25年輸血状況調査結果と，併用療法については2006年～2008年の内部調査と比較した．【結果】原疾患は，悪性新生物が10例，骨折等の損傷が8例，消化器系疾患が5例，循環器系疾患が4例，血液・造血器疾患・免疫障害，筋骨格系・結合組織疾患，尿路性器系疾患，その他がそれぞれ3例であり，悪性新生物（26%），血液・造血器疾患・免疫障害（8%）は都に比べ低く，骨折等の損傷（21%）は高かった．併用療法（重複回答）に記載のあった33例では，手術等の侵襲的処置が26例（79%），内視鏡，カテーテルがそれぞれ6例（18%），透析，化学療法がそれぞれ4例（12%），放射線療法が2例（6%）であり，実施率は過去の調査に比べ，カテーテル，内視鏡で低下し，手術等の侵襲的処置で上昇していた．【考察】医原性の感染経路は，主に汚染器具の使用，外科手術，内視鏡，透析と報告されている．今回の調査では，手術等の侵襲的処置は骨折等の損傷などで，高率で実施されていた．HCV感染との直接的関係は不明であるが，侵襲的処置実施時にはHCV感染防止対策が必要と思われた．医療安全の観点からも，これらの結果を医療機関と共有して，HCV感染防止対策に役立てたい．

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識別番号・報告回数			報告日	第一報入手日 2016年07月22日	新医薬品等の区分	厚生労働省処理欄

一般的名称	①②③⑨⑩⑪　pH4処理酸性人免疫グロブリン ④⑤⑥⑦⑧ポリエチレングリコール処理人免疫グロブリン ⑫⑬　人免疫グロブリン	研究報告の公表状況	http://www.cdc.gov/mmwr/volumes/65/wr/mm6528a2.htm?s_cid=mm6528a2_w/2019/07/22	公表国 アメリカ
販売名 （企業名）	①献血ポリグロビンN5%静注0.5g/10mL　（日本血液製剤機構） ②献血ポリグロビンN5%静注2.5g/50mL　（日本血液製剤機構） ③献血ポリグロビンN5%静注5g/100mL　（日本血液製剤機構） ④献血ヴェノグロブリンIH5%静注0.5g/10mL　（日本血液製剤機構） ⑤献血ヴェノグロブリンIH5%静注1g/20mL　（日本血液製剤機構） ⑥献血ヴェノグロブリンIH5%静注2.5g/50mL　（日本血液製剤機構） ⑦献血ヴェノグロブリンIH5%静注5g/100mL　（日本血液製剤機構） ⑧献血ヴェノグロブリンIH5%静注10g/200mL　（日本血液製剤機構） ⑨献血ポリグロビンN10%静注2.5g/25mL　（日本血液製剤機構） ⑩献血ポリグロビンN10%静注5g/50mL　（日本血液製剤機構） ⑪献血ポリグロビンN10%静注10g/100mL　（日本血液製剤機構） ⑫グロブリン筋注450mg/3mL「JB」　（日本血液製剤機構） ⑬グロブリン筋注1500mg/10mL「JB」　（日本血液製剤機構）			

研究報告の概要

妊娠可能年齢の女性におけるC型肝炎ウイルス（HCV）の検出の増加および垂直感染の潜在的リスク－米国および米ケンタッキー州，2011年～2014年；

2011年～2014年の間に，妊娠可能年齢の女性におけるC型肝炎ウイルス（HCV）の検出率（抗体またはRNA陽性）および2歳以下の小児におけるHCV検査（抗体またはRNA陽性）の増加が，全国（22%［妊娠可能年齢の女性］；14%［小児］）ならびにケンタッキー州（>200%［妊娠可能年齢の女性］；151%［小児］）においてみとめられた。同期間における出生証明書のデータでは，HCVに感染している母親から生まれた乳児の割合は，全国で68%およびケンタッキー州で124%の増加を示した。妊婦へのHCV検査の増加、HCV感染女性からの出生児のための調和された検査ガイドライン、標準化された周産期のHCV症例の定義の採用が、HCV感染女性からの出生児の早期特定を改善させ、その後のHCV関連後遺症を予防するためのケアや治療に対する母児のつながりを改善する可能性がある。

使用上の注意記載状況・その他参考事項等

代表として献血ヴェノグロブリンIH5%静注0.5g/10mLの記載を示す。
2．重要な基本的注意
(1)本剤の原材料となる献血者の血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体、抗HTLV-1抗体陰性で、かつALT（GPT）値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohnの低温エタノール分画で

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報告企業の意見	今後の対応	
C型肝炎ウイルス（Hepatitis C virus：HCV）はフラビウイルス科（Flaviviridae）ヘパシウイルス属（Hepacivirus）に属する直径50～60nmの球状のウイルスで、エンベロープとコア蛋白の二重構造を有する一本鎖RNAウイルスである。万一、原料血漿にHCV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。	得た画分からポリエチレングリコール4000処理、DEAEセファデックス処理等により人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理、ウイルス除去膜によるろ過処理及びpH3.9～4.4の条件下での液状インキュベーション処理を施しているが、投与に際しては、次の点に十分注意すること。

Centers for Disease Control and Prevention
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Increased Hepatitis C Virus (HCV) Detection in Women of Childbearing Age and Potential Risk for Vertical Transmission — United States and Kentucky, 2011–2014

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Summary

What is already known about this topic?

Illicit injection drug use is a risk factor for hepatitis C virus (HCV) infection; recent increases in injection drug use and increases in incidence of HCV infection among young persons have been observed in the United States.

What is added by this report?

During 2011–2014, increased rates of HCV detection (antibody or RNA positivity) among women of childbearing age and HCV testing (antibody or RNA) among children aged ≤2 years were observed both nationally and for Kentucky (22% and 14%, nationally; >200% and 151%, Kentucky). During the same period, birth certificate data showed the proportion of infants born to HCV-infected mothers increased 68% nationally and 124% in Kentucky.

What are the implications for public health practice?

Increased HCV testing of pregnant women, harmonized testing guidelines for children born to HCV-infected women, and adoption of a standardized perinatal HCV case definition might improve early identification of infants born to HCV-infected women and subsequent linkage of the mother and infant to care and treatment to prevent HCV-related sequelae.

Hepatitis C virus (HCV) infection is a leading cause of liver-related morbidity and mortality (*1*). Transmission of HCV is primarily via parenteral blood exposure, and HCV can be transmitted vertically from mother to child. Vertical transmission occurs in 5.8% (95% confidence interval = 4.2%–7.8%) of infants born to women who are infected only with HCV and in up to twice as many

infants born to women who are also infected with human immunodeficiency virus (HIV) (*2*) or who have high HCV viral loads (*3,4*); there is currently no recommended intervention to prevent transmission of infection from mother to child (*3*). Increased reported incidence of HCV infection among persons aged ≤30 years (*5,6*) with similar increases among women and men in this age group (*6*), raises concern about increases in the number of pregnant women with HCV infection, and in the number of infants who could be exposed to HCV at birth. Data from one large commercial laboratory and birth certificate data were used to investigate trends in HCV detection among women of childbearing age,* HCV testing among children aged ≤2 years, and the proportions of infants born to HCV-infected women nationally and in Kentucky, the state with the highest incidence of acute HCV infection during 2011–2014 (*6*). During 2011–2014, commercial laboratory data indicated that national rates of HCV detection (antibody or RNA positivity†) among women of childbearing age increased 22%, and HCV testing (antibody or RNA) among children aged ≤2 years increased 14%; birth certificate data indicated that the proportion of infants born to HCV-infected mothers increased 68%, from 0.19% to 0.32%. During the same time in Kentucky, the HCV detection rate among women of childbearing age increased >200%, HCV testing among children aged ≤2 years increased 151%, and the proportion of infants born to HCV-infected women increased 124%, from 0.71% to 1.59%. Increases in the rate of HCV detection among women of childbearing age suggest a potential risk for vertical transmission of HCV. These findings highlight the importance of following current CDC recommendations to identify, counsel, and test persons at risk for HCV infection (*1,7*), including pregnant women, as well as consider developing public health policies for routine HCV testing of pregnant women, and expanding current policies for testing and monitoring children born to HCV-infected women. Expansion of HCV reporting and surveillance requirements will enhance case identification and prevention strategies.

In the United States, incidence of HCV infection has been increasing in young persons, including women of childbearing age, particularly in rural areas such as Appalachia (*5,6*). Although acute HCV infection, as defined by the Council of State and Territorial Epidemiologists,§ is a notifiable condition and reportable to the health department in almost all states,¶ persons with acute HCV infection account for a small fraction of persons with newly diagnosed HCV infection; most new diagnoses are among persons with HCV infection of unknown duration. Because reporting of all cases of HCV infection is not mandated in many states, a substantial proportion of HCV-infected women of childbearing age, including pregnant women, are likely not reported in routine state-based surveillance systems. Commercial laboratory data and birth certificate data provide additional sources of information to supplement HCV surveillance data.

To evaluate HCV infection among women of childbearing age and the potential for mother-to-child transmission of HCV, trends in HCV detection (defined as HCV antibody or RNA positivity) in women of childbearing age and HCV testing (antibody or RNA) among children aged ≤2 years from 2011–2014 were assessed nationally and for Kentucky using commercial laboratory data from Quest Diagnostics (Quest). Detection of HCV infection among infants was not evaluated

because 1) the exact infant dates of birth to allow discrimination between maternal and infant HCV antibody were not available, and 2) very few infants had RNA testing to detect current HCV infection. Trends in proportions of infants born to HCV-infected women were assessed using birth certificate data from the National Center for Health Statistics. Maternal HCV infection status on birth certificates is obtained from the prenatal record, labor and delivery admission form, admission history and physical examination, or delivery record; maternal HCV diagnosis is recorded on the birth certificate if HCV infection is present at pregnancy diagnosis or if HCV infection is confirmed during pregnancy with a positive test for HCV (*8*). Demographic characteristics of HCV antibody-positive pregnant women reported to the Kentucky Department for Public Health (KDPH) during 2011–2014 were also examined. These data were collected as part of routine acute HCV surveillance, and during December 2013–December 2014, were enhanced by a KDPH request for voluntary reporting of all cases of HCV infection identified among pregnant women and infants.

The annual HCV detection rate among women of childbearing age tested by Quest was calculated as cases of HCV detection per 100,000 women of childbearing age served by the laboratory (i.e., women of childbearing age who received a laboratory test for any reason). Quest data were also used to calculate the annual HCV testing rate per 100,000 children aged ≤2 years served by Quest. The proportion of infants born to HCV-infected mothers was calculated using birth certificate data.

During 2011–2014, the national rate of HCV detection among women of childbearing age served by Quest increased 22%, from 139 to 169 per 100,000, and the rate of HCV testing among children aged ≤2 years served by Quest increased 14%, from 310 to 353 per 100,000 (Figure 1). During this time, the proportion of infants born to HCV-infected women nationally increased 68%, from one in 536 (0.19%) to one in 308 (0.32%) (Figure 2). During the same time, the rate of HCV detection among women of childbearing age in Kentucky increased 213%, from 275 to 862 per 100,000, and the rate of HCV testing among children aged ≤2 years increased 151%, from 403 to 1,011 per 100,000 (Figure 1). In addition, the proportion of infants born to HCV-infected women increased 124%, from one in 142 (0.71%) to one in 63 (1.59%) (Figure 2). During 2011–2014, HCV case reporting to KDPH identified 777 pregnant women with HCV antibody positivity; 527 (68%) were aged 20–29 years, 218 (28%) were aged 30–39 years, 653 (84%) were non-Hispanic white, and 293 (38%) reported past or current injection drug use.

Discussion

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The national increases in HCV detection among women of childbearing age, HCV testing among infants, and the proportion of infants born to HCV-infected mothers suggest increased risk for mother-to-child transmission of HCV. This risk might be higher in certain areas of the United States, as illustrated by the findings in this report for Kentucky, which might be related to increasing illicit injection drug use (*5*). KDPH surveillance data for pregnant women are also consistent with demographic patterns of HCV incidence overall in Kentucky and nationally (*6*).

Many opportunities to improve identification and monitoring of HCV infection among women of childbearing age and infants exist. CDC recommends HCV testing for persons with a history of injection drug use and others at risk, including persons infected with HIV and persons with recognized exposures (e.g., health care workers after needle sticks or mucosal exposure to HCV-positive blood) (*1,7*). It is important that providers assess women of childbearing age, particularly pregnant women, for HCV risk and test accordingly. CDC also recommends HCV testing of children born to HCV-infected women (*1,7*). Several organizations have published guidelines on HCV testing of children,** but harmonization is needed to ensure that all women who are pregnant or planning pregnancy and all infants born to HCV-infected women are appropriately tested and linked to care if they are infected.

The potential for mother-to-child transmission of HCV has prompted some jurisdictions to consider changes in HCV case identification strategies and reporting policies. For example, the Philadelphia Department of Public Health recently demonstrated improved identification of infants born to HCV-infected mothers by cross-matching maternal information (including mother's name and date of birth) on birth certificates to women in HCV surveillance registries (*9*). In 2015, Kentucky mandated reporting of all HCV-infected pregnant women and children through age 60 months, as well as all infants born to all HCV-infected women.†† Development of national reporting criteria to include a case definition for perinatal HCV infection could standardize reporting across states. Reporting pregnancy status as part of HCV laboratory-based surveillance would also facilitate case identification. Improved surveillance can inform HCV screening and testing recommendations for pregnant women. Furthermore, there is an opportunity to detect HCV infection through routine HCV testing of infants identified as having perinatal exposure to illicit drugs, or neonatal abstinence syndrome, and their mothers; this could enhance HCV case identification as suggested by the large proportion of HCV antibody-positive pregnant women in Kentucky who report injecting illicit drugs.

The findings in this report are subject to at least four limitations. First, incomplete information on pregnancy status on case report forms used for surveillance in Kentucky and maternal HCV infection status on birth certificates might underestimate rates of infants born to HCV-infected mothers. Second, identifying cases of HCV-infected persons, including pregnant women, relies on completeness of reporting; therefore, the data from KDPH are likely underestimates. Third, laboratory data were limited to a single commercial laboratory and thus might not represent the United States and Kentucky populations. Finally, HCV-infected mothers cannot be linked to their children using laboratory data, and information on children's age in the laboratory data are limited, making it difficult to determine whether children are appropriately tested and have current infection; thus, HCV detection rates among children aged ≤2 years were not included in this report.

These findings underscore the importance of providing primary prevention services (*7*) and following current recommendations to identify persons at risk for HCV infection and test accordingly; doing so among pregnant women would improve early identification of HCV-

infected infants and linkage of the mother and infant to care and treatment. Furthermore, identifying HCV-infected women of childbearing age before pregnancy, with linkage to care, treatment, and cure, would avoid HCV infection during pregnancy and prevent mother-to-child transmission. Expanding current and developing new public health policies to increase HCV detection among women of childbearing age (especially pregnant women) and infants should be considered; however, additional data are needed to better assess HCV prevalence among pregnant women and their infants and investigate options for perinatal prevention, care, and treatment.

Acknowledgments

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* Childbearing age among women is defined as 15–44 years. ^ Top

† Quest Diagnostics detects antibody to HCV by an immunoassay, HCV RNA quantitatively by real-time polymerase chain reaction, and HCV RNA qualitatively by transcription mediated amplification.

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†† Kentucky Reportable Disease Regulations: 902 Ky. Admin. Regs. 2:020.

FIGURE 1. Hepatitis C virus (HCV) detection rate among females aged 15–44 years and HCV testing rate among children aged ≤2 years —United States and Kentucky, 2011–2014*

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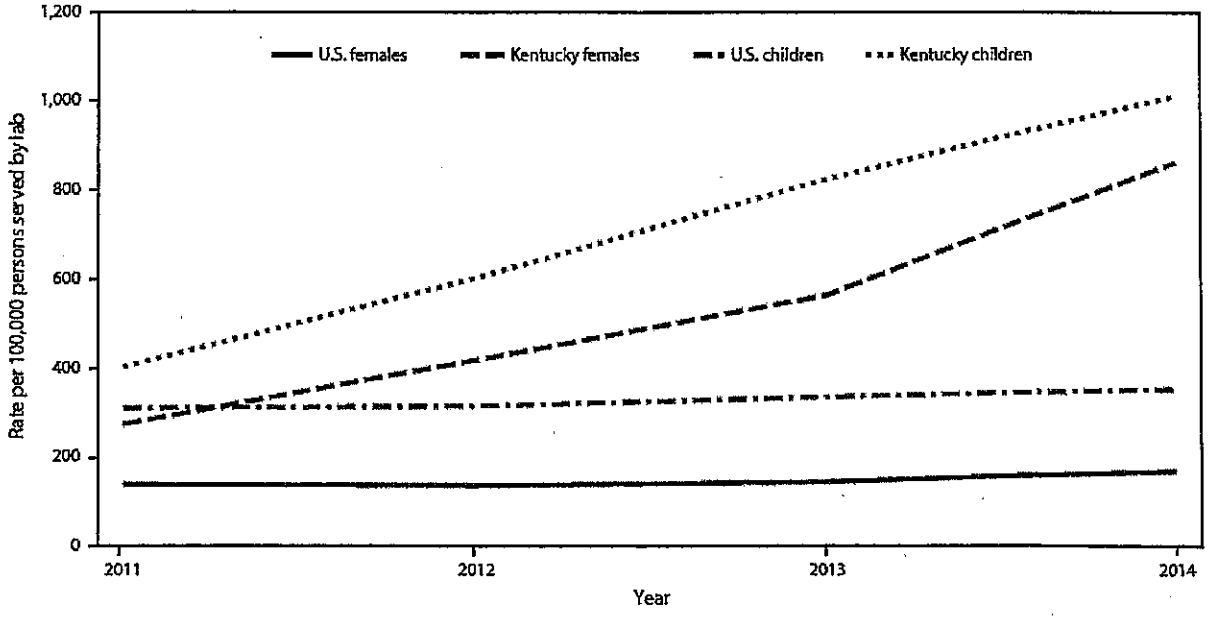

* HCV detection rates were calculated as number of females aged 15–44 years who received a positive HCV antibody and/or RNA result per 100,000 females aged 15–44 years served by Quest Diagnostics (i.e., received a laboratory test for any reason) by area of residence. HCV testing rates among children were calculated as number of children aged ≤2 years who received a test for HCV antibody and/or RNA per 100,000 children aged ≤2 years served by Quest Diagnostics by area of residence.

Source: Quest Diagnostics laboratory data.

FIGURE 2. Proportion* of infants born to hepatitis C virus (HCV)-infected women† — United States and Kentucky, 2011–2014

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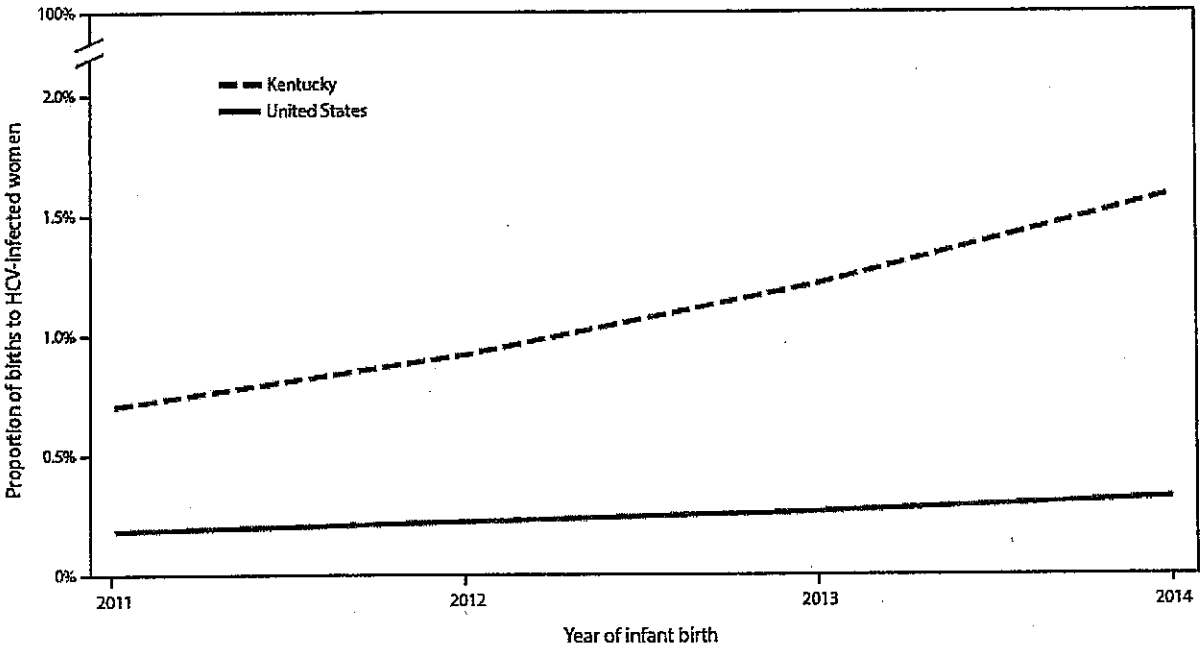

* Proportion calculated annually as infants born to HCV-infected women divided by total infants born.

† HCV infection status of mother is determined by notation on infant's birth certificate. Birth categorization is based on mother's place of residence.

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No. 12

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一般的名称	新鮮凍結人血漿	研究報告の公表状況	Rivero-Juarez A, Frias M, Rodriguez-Cano D, Cuenca-López F, Rivero A. Clin Infect Dis. 2016 Jun 1;62(11):1464.	公表国 スペイン
販売名(企業名)	新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)			

研究報告の概要

○急性感染期における母乳からのE型肝炎ウイルス(HEV)の分離。
潜在的感染経路としての母乳におけるHEVの存在が検討されたことはない。34歳の女性が2週間にわたって持続していた軽度の発熱、疲労及び食欲不振のため当院を受診した。HBs抗原、HBc抗体、HCV抗体、HEV IgM/IgG抗体、HBV DNA、HCV RNA等の検査は全て陰性であったが、HEV RNAが検出され、ウイルス量は31,324 IU/mLで急性HEV感染の診断を受けた。2週間後にはHEV IgG抗体が陽性、無症候状態となり生化学的検査は次第に改善し、4週間で正常化した。
当該患者は生後18ヶ月の男児の母親であり、授乳中であったが急性HEV感染の診断後に中断された。母乳を遠心分離して得た乳清よりサンプルを2つ作成し、PCR法によるHEV RNA検査を2回実施した。同時に採取したサンプルの解析により、HEV RNAが血清と同様の力価で母乳に存在することが判明した。血清中並びに母乳中のHEV RNAの観察を行った結果、経過観察期間におけるRNA力価並びにウイルスクリアランスの達成時点は、血清と母乳で同様であることが明らかになった。
本研究は、急性感染期に母乳からHEVを分離した初めての研究である。我々のデータは、母親から子供へのHEV感染において、授乳が感染経路となり得ることを示唆している。この感染経路における感染力価並びに感染効率の評価を行う研究を実施すべきである。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
急性E型肝炎ウイルス(HEV)感染期の母乳からHEVが検出されたことから、授乳は感染経路となり得るという報告である。	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。

IDSA Infectious Diseases Society of America
hivma hiv medicine association
OXFORD

Isolation of Hepatitis E Virus From Breast Milk During Acute Infection

To the Editor—Hepatitis E virus (HEV) is the world's major cause of acute viral hepatitis [1]. The main routes of transmission are the consumption of water contaminated by enterically excreted HEV (genotypes 1 and 2) and of the food of infected animals (genotypes 3 and 4) [2, 3]. During pregnancy, HEV transmission can be transplacental, increasing the risk of abortions and stillbirths, and of liver necrosis and deaths in newborns [4, 5]. Nevertheless, the presence of the virus in breast milk as a potential route of transmission has not been evaluated.

A 34-year-old woman, with no history of alcoholism or liver disease, presented in our hospital with low-grade fever, fatigue, and loss of appetite over 2 weeks. She had a serum aspartate aminotransferase (AST) value of 126 U/L and alanine AST value of 164 U/L. Immunoglobulin (Ig) M anti–hepatitis A virus, hepatitis B surface antigen, IgM anti–hepatitis B core, anti–hepatitis C virus, IgM anti–Epstein-Barr virus, IgM/IgG anti-cytomegalovirus, IgM/IgG anti-HEV, hepatitis B virus DNA, and hepatitis C virus RNA tested negative, whereas HEV RNA was positive with a quantitative viral load of 31 324 IU/mL. A diagnosis of acute HEV infection was made and the patient was managed conservatively. Two weeks later, IgG anti-HEV was positive. The patient was asymptomatic and the metabolic parameters gradually improved, returning to normal over the following 4 weeks.

The patient was a breastfeeding mother of a boy of 18 months. After acute HEV infection was diagnosed, breastfeeding was interrupted. Breast milk was collected in disposable plastic labware, centrifuged to obtain milk plasma, and tested in duplicate for HEV RNA using 2 PCR assays. Analysis of samples collected simultaneously revealed the presence of HEV RNA in breast milk, showing a similar titer to the serum (Table 1). HEV RNA was monitored in serum and breast milk. During follow-up, milk and serum showed similar HEV RNA titers and time-points for viral clearance (Table 1).

Table 1. Hepatitis E Virus Concentrations in Blood and Breast Milk at Different Time-points

Sample	22 October 2015	9 November 2015	30 November 2015	18 December 2015
Blood	31 324 IU/mL	2130 IU/mL	670 IU/mL	Negative
Milk	. . .	1930 IU/mL	500 IU/mL	Negative

For viral quantification, total viral RNA was isolated from 200 µL of serum and 200 µL of milk plasma using the QIAamp Mini Elute Virus Spin Kit (Qiagen, Hilden, Germany). Presence of the virus was determined by reverse transcription polymerase chain reaction using the LightCycler 480 instrument (Roche Diagnostics, France). The World Health Organization standard strain for hepatitis E virus RNA (Paul-Ehrlich-Institut code 6329/10) was used to quantify the virus.

Our study constitutes the first isolation of HEV in breast milk during the acute phase of infection. Our data suggest that breastfeeding could be a potential route of HEV transmission from mother to child. Studies should be performed evaluating the viral infectious dose and infectious efficiency of this way of transmission. In the absence of such data, however, the interruption of breastfeeding should be considered in women infected with HEV.

Notes

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Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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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 08 月 15 日	新医薬品等の区分 該当なし	厚生労働省処理欄

一般的名称	①②抗 HBs 人免疫グロブリン ③④乾燥抗 HBs 人免疫グロブリン ⑤ポリエチレングリコール処理抗 HBs 人免疫グロブリン	研究報告の公表状況	Transfusion 2016; 56(3): 722-728	公表国 オランダ
販売名 (企業名)	①抗 HBs 人免疫グロブリン筋注 200 単位/1mL「JB」（日本血液製剤機構） ②抗 HBs 人免疫グロブリン筋注 1000 単位/5mL「JB」（日本血液製剤機構） ③ヘブスブリン筋注用 200 単位（日本血液製剤機構） ④ヘブスブリン筋注用 1000 単位（日本血液製剤機構） ⑤ヘブスブリン IH 静注 1000 単位（日本血液製剤機構）			

研究報告の概要

オランダ人の献血者の E 型肝炎ウイルス感染の感染率と持続期間：

背景：オランダにおける E 型肝炎ウイルス（HEV）感染の感染率は高い。献血者は、定期的な HEV 感染のスクリーニングを受けていないが、2013 年 1 月以降、S/D 処理血漿の製造に用いられる献血に対し HEV RNA スクリーニングを実施している。
研究デザインと方法：献血は、96 のプールと 192 の献血で HEV RNA についてスクリーニングを実施した。 加えて、HEV 陽性の前後 60 日間に献血された血液は、個別に HEV RNA および抗 HEV 免疫グロブリン G の試験を実施した。
結果：2013 年 1 月から 2014 年 12 月の間に 59,474 の献血のスクリーニングした結果、41 名のドナーから 45 の HEV RNA 陽性献血（0.076%）が同定された。HEV RNA 量は、80 から 2.3×10（6）IU/mL の範囲であった。陽性献血は時間の経過と共に明らかに増加した（P = 0.03）。HEV RNA が陽性となった前後 60 日間の献血において、90 の献血のうち 32 の献血は、プール検査では陽性とならなかったものの、個別検査で陽性であった。健康な献血者におけるおける HEV のウイルス血症の平均持続期間は 68 日であると推定された。
結論：オランダでは、HEV の感染率が高く、研究期間中に増加した。 2013 年と 2014 年に、S/D 処理血漿の製造のための献血において、762 の献血あたり 1 つの陽性が検出された。

使用上の注意記載状況・その他参考事項等

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

報告企業の意見

E型肝炎ウイルス（Hepatitis E virus：HEV）は直径27～38nmの球状粒子で、エンベロープはなく、長さ約7,300塩基対の一本鎖RNAを内包している。万一、原料血漿にHEVが混入したとしても、各種モデルウイルスのウイルスクリアランス試験成績から、本剤の製造工程において不活化・除去されると考える。

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。

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Incidence and duration of hepatitis E virus infection in Dutch blood donors

Boris M. Hogema,[1,2] Michel Molier,[1] Margret Sjerps,[2] Mirjam de Waal,[2] Peter van Swieten,[2] Thijs van de Laar,[1,2] Marijke Molenaar-de Backer,[1,2] and Hans L. Zaaijer[1,3]

BACKGROUND: The incidence of hepatitis E virus (HEV) infection in the Netherlands is high. Blood donors are not routinely screened for HEV infection, but since January 2013, donations used for the production of solvent/detergent (S/D)-treated plasma have been screened for HEV RNA.

STUDY DESIGN AND METHODS: Donations were screened for HEV RNA in pools of 96 and 192 donations. In addition, all donations made between 60 days before and after each HEV RNA-positive donation were tested individually for HEV RNA and anti-HEV immunoglobulin G.

RESULTS: The screening of 59,474 donations between January 2013 and December 2014 resulted in identification of 45 HEV RNA-positive donations (0.076%) from 41 donors. HEV RNA loads ranged from 80 to 2.3×10^6 IU/mL. The number of positive donations increased significantly over time ($p = 0.03$). Thirty-three of 90 donations made up to 60 days before or after HEV RNA-positive donations were positive when tested individually, while they had not been detected in the pool screening. The mean duration of HEV viremia in the healthy blood donor is estimated to be 68 days.

CONCLUSION: The incidence of HEV infection in the Netherlands is high and increased during the study period. In 2013 and 2014, HEV RNA was detected in 1 per 762 donations intended for production of S/D plasma.

In the Western world, hepatitis E was traditionally seen as a traveler's disease.[1] More recently it became clear that autochthonous infection with hepatitis E virus (HEV) Genotype 3 occurs frequently. Most HEV Genotype 3 infections are asymptomatic or cause minor acute illness. In immunosuppressed patients infection may become chronic resulting in rapidly progressing liver fibrosis and cirrhosis.[1] In the Netherlands, the HEV seroprevalence among blood donors is 27% and strongly increases with age.[2] Testing of 40,173 recent donations in pools of 48 and 480 resulted in the identification of 13 HEV RNA-positive donations.[2] The incidence of HEV infection was estimated to be 1.1% per year, based on the number of seroconversions among blood donors between 2009 and 2011.[2] HEV is highly resistant to inactivation and transfusion-transmitted HEV infection has been observed in various countries.[3,4] A recent study showed that in Southeast England, transmission of HEV via transfusion occurs regularly. HEV RNA was detected in 0.04% of the donations tested, and 42% of the HEV RNA-positive blood products used for transfusion caused HEV transmission to the recipient. Overall, approximately 1 in 6800 components transfused caused transmission. Clinical symptoms in infected recipients were rare.[5] Given the high incidence

ABBREVIATION: LLD(s) = lower limit(s) of detection.

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of HEV infection in donors, testing of blood and plasma donations is currently being considered. One of the products for which testing seems the most urgent is solvent/detergent (S/D)-treated plasma. Due to the small production pools and the limited inactivation of HEV by S/D treatment, one highly viremic donor could potentially infect hundreds of recipients. A recent retrospective study from Canada showed probable transmission by pooled S/D-treated plasma between 2001 and 2003.[6] S/D-treated plasma was not screened for HEV, and two of 17 thrombotic thrombocytopenic purpura patients who received 20 to 40 L of plasma seroconverted for anti-HEV immunoglobulin (Ig)G within 6 months after "transfusion," and both were HEV RNA positive within 1 month of treatment. In contrast, none of the 19 patients from the control group (treated with cryosupernatant plasma from individual donors) showed evidence of HEV infection.[6]

As of January 2015, testing of S/D production pools for HEV RNA is a requirement of the European Pharmacopoeia. In the Netherlands, fresh-frozen quarantine plasma has recently been replaced by S/D-treated plasma produced by Octapharma using plasma from Dutch donors. In anticipation of the change in the European Pharmacopoeia and to increase the safety of the final product, screening of donations used for production of S/D plasma was implemented in January 2013. We here report the results of the first 2 years of screening.

MATERIALS AND METHODS

Screening of minipools for HEV RNA

All donations used for production of S/D plasma were screened for HAV RNA, parvovirus B19 DNA, and HEV RNA in plasma minipools of 96 donations using in-house real-time polymerase chain reaction (PCR) tests. Plasma units were frozen and released for production only once test results were known. Pools of 48 donations were prepared from the nucleic acid test tubes and frozen at −70°C until testing. If fewer than 48 donations were present the volume was adjusted using negative human plasma. Testing for HAV RNA, parvovirus B19 DNA, and HEV RNA was performed once every 4 weeks. Pools of 48 donations were thawed and combined to pools of 96 for testing. Nucleic acid was extracted using automated nucleic acid extraction robots (NucliSens EasyMag, bioMérieux, Boxtel, the Netherlands). When the HEV test was introduced, the most efficient method to implement this test was to use the remaining extract available from the HAV and parvovirus B19 screening. Previously, an HEV RNA PCR had been developed using the virus spin kit extraction method (QIAamp MinElute, Qiagen, Chatsworth, CA).[2] Initial experiments showed a higher sensitivity and less inhibition of the internal control signal with the Qiagen extraction method compared to EasyMag extraction (data not shown). At the moment Octapharma requested to start screening for HEV RNA, the EasyMag extraction method had not yet been validated and had an unknown sensitivity. It was decided to screen all minipools using both extraction methods until the validation and risk assessment were finished. To this end, the remainder of the plasma from the pools of 96 was combined into pools of 192 and screened using the Qiagen extraction method. If pools of 96 or 192 tested HEV RNA positive, resolution testing was performed to identify the HEV RNA-positive donation(s) by first testing all constituent pools of 48 donations using Qiagen extraction. Positive pools of 48 were deconstructed by first testing six pools of eight donations and subsequently the individual donations from positive pools of eight donations. For the last two steps, donations were pipetted from 96-well archive plates using a robotic workstation (Freedom EVO, Tecan, Männedorf, Switzerland) followed by EasyMag extraction.

Follow-up of HEV RNA-positive results

To reduce the risk of using HEV RNA-positive donations that were not detected during screening in production pools and to obtain an improved estimate of the duration of viremia, donations made between 60 days before and 60 days after an HEV RNA-positive donation were deferred and submitted for individual HEV RNA testing using Qiagen and/or Chemagic (Perkin Elmer, Waltham, MA) extraction (see below). This was possible because of the delay between donation and production of S/D plasma. Subsequently, an estimate of the viremic period was made as described in the legend to Fig. 1. The mean duration of viremia was calculated using the combined data of the current and the previous study on HEV RNA screening in plasma pools.[2] The mean proven duration of viremia was calculated from all blood donors with more than one HEV RNA-positive donation. Added to the calculated interval was 0.5 times the mean interval between the last negative and first positive donation and 0.5 times the mean duration between last positive and first negative donation. Arbitrarily, only series of donations with interdonation intervals less than 35 days were included to avoid inclusion of noninformative data and overestimation of the viremic period.

Anti-HEV IgG was measured in all HEV RNA-positive donations using an anti-HEV IgG enzyme immunoassay (Wantai Biological Pharmacy Enterprise Co., Beijing, China). If anti-HEV IgG was detected, anti-HEV IgG was determined in previous donations to confirm seroconversion. If no HEV IgG was detected, anti-HEV IgG was determined in later donations.

Detection and genotyping of HEV RNA

The real-time PCR method for detection of HEV RNA using Qiagen extraction has been described previously.[2]

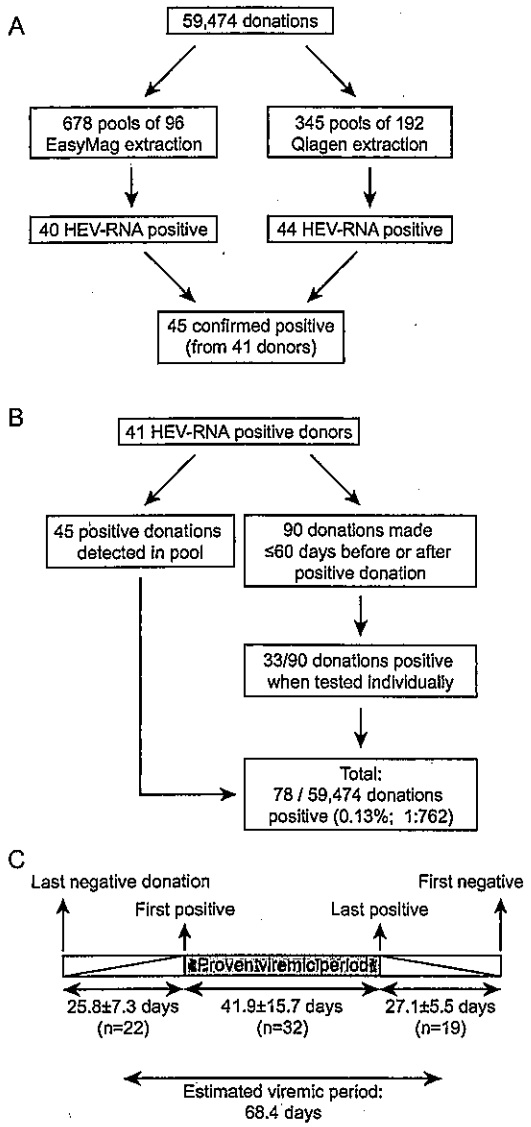

Fig. 1. Screening of donations for HEV RNA. (A) Flow diagram of the screening methods. (B) Follow-up of HEV RNA-positive donations and estimate of the incidence of HEV infection. (C) Estimated duration of viremia based on the testing of serial donations.

Briefly, 0.4 mL of plasma was extracted after addition of MS2 phage as internal control (IC) using the virus spin kit (QIAamp MinElute, Qiagen) and an elution volume of 50 µL. Amplification of 20 µL of eluate was performed in a total volume of 50 µL using TaqMan Fast Virus one-step master mix (Applied Biosystems, Foster City, CA). The HEV PCR was performed twice; to obtain maximum sensitivity one of the reactions was performed without internal control primers and probe (the HEV signal is somewhat inhibited by the internal control). Samples were considered to contain HEV RNA if at least one of the two reactions was positive. If no internal control signal was detected the sample was retested. For EasyMag extraction, 1 mL of pooled plasma was extracted using the NucliSens EasyMag extractor with an elution volume of 45 µL. MS2 phage, used as an internal control for both the HAV and the HEV PCR procedures, was added before extraction. Ten microliters of extract was used for the amplification of each of the three targets (HAV, HEV, and B19). The HEV PCR was performed in a total volume of 40 µL using exactly the same conditions as for the Qiagen extracts. The 95 and 50% lower limit of detection (LLD) of the HEV RNA PCR was determined for both extraction methods by Probit analysis of the frequency of HEV RNA detection at various concentrations of the first international HEV RNA WHO standard (6329/10). The 95 and 50% LLDs were 38.4 and 5.2 IU/mL with the EasyMag extraction method and 10.3 and 1.6 IU/mL when using Qiagen extracts. If a plasma bag was available, testing at maximum sensitivity was performed using a MSM1 extractor with a viral DNA and RNA kit (Chemagic) with an input of 9.6 mL of plasma and an elution volume of 100 µL. Testing 20 µL of eluate in duplicate resulted in a 95% LLD of 3 to 4 IU/mL. Genotyping of HEV by amplification and sequencing of a 304-bp fragment of ORF2 was performed as previously described.[2]

Statistical analysis

The chance that a HEV-positive donation is detected in pools of various sizes and using tests with different sensitivities was calculated using a one-hit model ($p = 1 - e^{(-\text{viral load in pool/slope})}$) with the slope values determined from test validation data. Linear regression and chi-square tests were performed using computer software (Graphpad Prism 6, Graphpad Software, San Diego, CA). Probit analysis was performed using statistical software (SPSS Statistics, Version 23, IBM, Armonk, NY).

RESULTS

Between January 2013 and December 2014, a total of 59,474 donations were screened for HEV RNA in pools of 96 and 192, using two different extraction methods (Fig. 1A). Positive pools were deconstructed to the level of individual donations, resulting in identification of 45 confirmed HEV RNA-positive donations (0.069%; 1:1440) from 41 donors (Fig. 1A, Table 1). The number of HEV RNA-positive donations significantly increased over time (Fig. 2; $p = 0.03$) and no seasonal effects were observed. Eleven of 45 (24%) of the HEV RNA-positive donations were anti-HEV IgG positive. Thirty-nine of 45 (87%) of the HEV RNA-positive donations were identified using both extraction methods. Five donations were only detected with the Qiagen extraction method in pools of 192, whereas NucliSense extraction in pools of 96 donations

TABLE 1. Overview of findings in HEV RNA-positive donors

Donor	Viral loads (IU/mL) of donations positive in screening	HEV genotype	Time of other donations (days, relative to index)	Viral load of other donations (IU/mL)
1	6,230	3c	−59, −28	Neg, <20
2	6,080	3c		
3	28,000	ND	−42, 48	<20, neg
4	14,700	3c	−42, −28, −14	Neg, 50, 82
5	7,890	3c	56	Neg
6	440	3f	−56, 56	Neg, <20
7	2,550	3c	−42, 28, 56	Neg, neg, neg
8	270	3c	−49, −21, 49	Neg, neg, 36
9	330	ND	−56, 49	Neg, <20
10	187,000	3c	-	
11	580	3f	−28, 29	Neg, 47
12	7,210	3c	−56, −28	Neg, <20
13	2,320,000	3c	−56, 28	Neg, 160
14	40,800	3c	28	Neg
15	350	ND	-	
16	420	ND	−26, 58	<20, neg
17	610	ND	−56, −28, 42	neg, 750, neg
18	3,240	3c	−41, −20, 50	<20, 190, neg
19	135,000	ND	−56	Neg
20	300	ND	28	Neg
21	3,510	ND	28, 56	Neg, neg
22	80,200	ND	−49	Neg
23	19,200	ND	−35, 49	<20, neg
24	1,440	ND	−42	Neg
25	770	ND	−42, 36	Neg, <20
26	4,640	ND	−42, 21	Neg, 25
27	2,270 and 620	ND	−56, −28, (37)*, 70	Neg, neg, (620), <20
28	80,600	3c	−35, 55	Neg, 27
29	5,910	ND	−49, −17, 21, 39, 60	Neg, 36, 182, <20, neg
30	10,300	ND	−56, −42, −21, 22, 42	Neg, neg, 22, <20, neg
31	5,350 and 80	ND	−56, −42, −28, −14, (14), 42, 56	Neg, neg, 36, 58, (80), neg, neg
32	51,700	3c	−36, 27, 55	Neg, <20, neg
33	339,000 and 1,690	3c	(20), 34, 48	(1690), 38, 31
34	720	3c	−43, −29, 42	Neg, neg, neg
35	12,900	ND	−55, 32, 52	Neg, <20, neg
36	200,000	ND	42	Neg
37	2,060	ND	−35, 28, 56	neg, neg, neg
38	10,800	ND	−49, 48	Neg, neg
39	2,560	ND	42	Neg
40	400 and 1,620	ND	−56, −28, (21), 42	Neg, <20, (1620), 156
41	549	ND	−56, −28, 35	Neg, <20, neg

* Numbers in parentheses refer to the second donation positive in the screening.
ND = not done (no sample was available for genotyping); neg = negative.

resulted in one donation not detected with the Qiagen extraction method. All six discordant donations had low viral loads, varying between 138 and 620 IU/mL in the individual donation. The 38 donations that were detected using both methods had viral loads between 330×10^6 and 2.3×10^6 IU/mL (Table 1). Many of the donations had relatively low viral loads; 23 of 39 (59%) had a load below the 95% limit of detection in the pool of 96 with EasyMag extraction, and eight donations contained so little HEV RNA that the chance of detection was less than 50%. Weakly HEV RNA-positive donations from two donors who did not seroconvert were interpreted as false positive and are not included in the analysis.

While being more cost-effective, screening in pools has the obvious disadvantage that donations with low viral loads are missed. By testing all donations made up to 60 days before or after the index donations, we were able to estimate how many donations remained undetected in the pool. Presence of HEV RNA was confirmed in 33 of 90 nonindex donations (37%; Table 1). Viral loads among the individual donations that were not detected during mini-pool ranged from less than 20 to 750 IU/mL, which illustrates that detection is not fully reliable at low viral loads. Fifteen plasma bags from donations that tested HEV RNA negative were available from donations made during the 60-day period. Two of 15 of these donations were positive using the more sensitive Chemagic extraction method with an input of 9.6 mL plasma, showing that the total number of HEV RNA positive donations is still slightly underestimated.

Because HEV RNA-positive donors were followed over time, an improved estimate of the duration of HEV

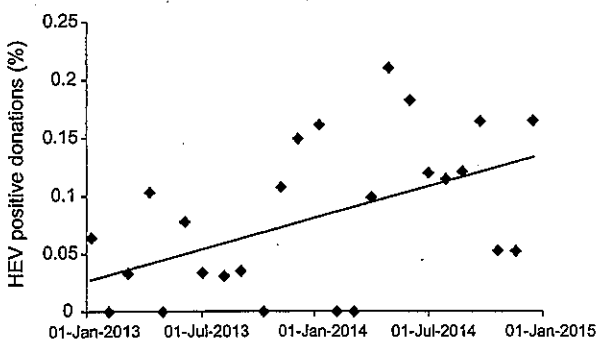

Fig. 2. Percentage of HEV RNA-positive donations in 2013 and 2014. Points show the percentage of positive donations detected each time the screening was performed, approximately once every 4 weeks. Linear regression is indicated by the line.

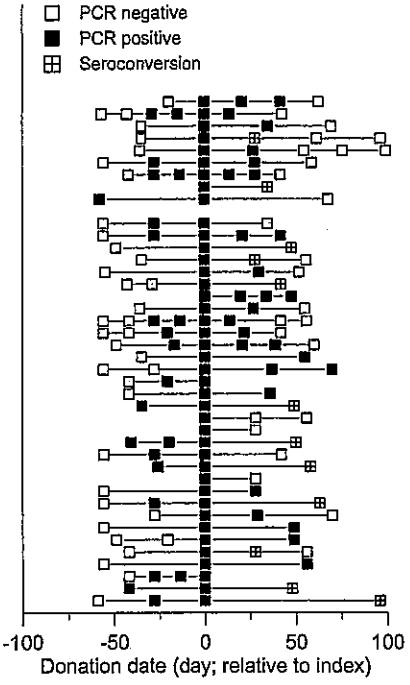

Fig. 3. Detection of HEV RNA in 167 sequentially sampled specimens from 41 blood HEV RNA-positive blood donors. The red and green symbols represent positive and negative results for the HEV-RNA test, respectively. + = the first anti-HEV IgG-positive donation.

viremia could be made. Figure 3 shows PCR results and donation intervals from all donors included in the calculation. The mean duration of proven viremia was 41.9 days in 32 blood donors with more than one HEV RNA-positive donation (Fig. 1C, Fig. 3). Added to this interval was 0.5 times the difference between the last HEV RNA-negative donation and the first positive donation and 0.5 times the

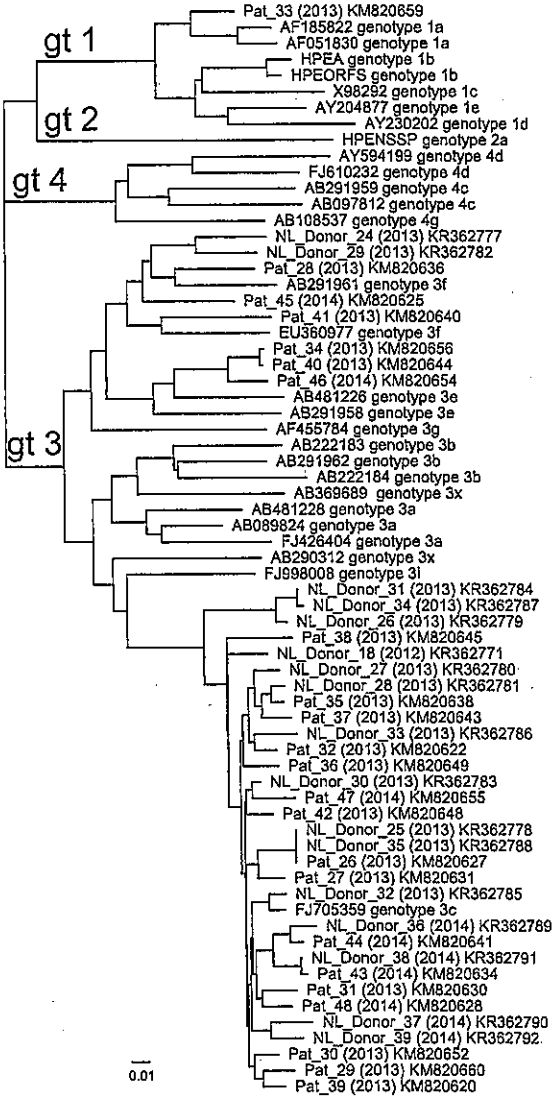

Fig. 4. Phylogenetic tree of HEV ORF2 sequences from 17 plasma donors (blue) and 23 HEV RNA-positive patient samples (green) analyzed by Sanquin Diagnostic Services in 2013 and 2014.[7]

interval between the last HEV RNA-positive and first negative donation, using a maximum interval of 35 days as a cutoff. The resulting estimate of the mean viremic period is 68.4 days. Genotyping of HEV succeeded in 17 cases. All HEV isolates were Genotype 3 and showed high homology to the other HEV sequences from Dutch donors and patients (Fig. 4).[7]

DISCUSSION

The screening of blood donations used for production of S/D plasma in 2013 and 2014 demonstrates a high incidence of HEV infection in the Netherlands. Screening

59,474 donations in pools of 96 and 192 for HEV RNA resulted in the identification of 45 HEV RNA-positive donations from 41 donors (0.076%; Fig. 1). This proportion is much higher than reported in Austria (0.012%, 2013-2014),[8] France (0.045%, 2012-2014),[9] Southeast England (0.035%, 2012-2013),[5] and Catalonia (0.03%, 2013).[10] Only in the western part of Germany was the detected number of positive donations higher (0.081%, 2011; Vollmer et al.[11]). However, screening results cannot be compared directly because different pool sizes and tests are used. Differences between methods can be taken into account if viral loads and sensitivity of the screening methods are known. For example, we estimate that application of our slightly less sensitive screening method in Germany would have resulted in detection of HEV RNA in nine of the 13 HEV RNA-positive donations identified in that study. The resulting HEV RNA detection rate of 0.056% is slightly lower than the number of HEV RNA-positive donations we observed in the Netherlands. The screening in Catalonia was performed by individual testing of samples using a highly sensitive test. The relatively low number of positive donations detected in this study (three in 9998 donations) demonstrates that the incidence of HEV infection is highly variable in different regions in Europe.[10]

Our results indicate that the incidence of HEV infection in the Netherlands has increased during the study period ($p = 0.03$, Fig. 2). This increase is not related to changes in the sensitivity of the HEV RNA test. The sensitivity of all five reagent batches used was checked before use and found to be equivalent. The number of HEV RNA-positive donations during the study period exceeds the incidence of HEV RNA-positive donations observed in our previous study[2] in which 13 of 40,176 (0.032%) HEV RNA-positive donations were identified in 2011 and 2012. We estimate that use of the slightly less sensitive test algorithm used in 2011 to 2012 would have resulted in detection of 15 of 18 and 23 of 27 HEV RNA-positive donations in 2013 and 2014, respectively. The corresponding adjusted number of HEV RNA-positive donations is 15 of 35,214 (0.040%) in 2013 and 23 of 24,260 (0.095%) in 2014, suggesting that the HEV incidence was relatively stable between 2011 and 2013 and then abruptly increased in 2014.

Our study is the first to provide a realistic estimation of the number of HEV RNA positive donations that remain undetected due to screening of donations in pools. By testing serial donations for HEV RNA, 33 additional positive donations were identified from the 41 HEV RNA donors. This high number could only be achieved because of the short interdonation interval of plasma donors, but we assume that the proportion of HEV RNA-positive donations that are not detected in the minipool screening is similar for all types of donations.

The source(s) of HEV remain enigmatic. Although there is little doubt that pigs serve as a major HEV reservoir, the cause of the increasing HEV incidence in the Netherlands is unknown. The number of pigs per capita in the Netherlands is among the highest in the world, but it has not increased in recent years.[12] Already in 2005, pooled stool samples from 53% of Dutch pig farms tested positive for HEV RNA, and HEV-specific antibodies were detected in approximately 70% of slaughtered pigs.[13,14] Hence, presence of HEV in the Dutch swine herds is not a recent phenomenon and it remains unclear what mechanism lies behind the recent increase in the HEV attack rate. Possibly, a new transmission route arose (e.g., via contaminated water, fruit, or vegetables), or the dynamics of HEV infection in pig farms changed, resulting in more pigs being viremic at the moment of slaughter.

Previously we showed that the anti-HEV seroprevalence in the Netherlands decreased strongly among all age groups between 1988 and 2000, which is indicative of an age cohort effect resulting from reduced exposure.[15] After 2000, the decline in HEV IgG seroprevalence stopped and an increase was observed among younger donors. The age-cohort effect of the HEV IgG prevalence has been observed in various European countries including the UK, Denmark, and Germany.[16,17] The high incidence of HEV infection among blood donors in some countries, including the ones where an age cohort effect was shown, suggests that the increased incidence of HEV infection is not restricted to the Netherlands. In the United States an age cohort effect was observed, but without indications of an increasing incidence of HEV infection among blood donors.[18]

HEV RNA screening in the Netherlands is performed for donations used for production of S/D plasma only. Because most recipients clear HEV infection without significant clinical symptoms and because of the high risk of HEV infection from food or other sources, general screening of donations for HEV RNA is unlikely to be cost-effective. Furthermore, even if donor screening is implemented, immune-compromised recipients of blood products should still be monitored for HEV infection because of the high incidence of foodborne HEV infection. To protect immunocompromised patients from HEV infection via blood components, screening of selected donations should be considered. The increasing HEV incidence requires investigation of the source(s) of infection, and measures to abolish circulation of HEV in commercial pig farms must be considered.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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医薬品　研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日 2016 年 6 月 6 日	新医薬品等の区分	総合機構処理欄
一般的名称	－		研究報告の公表状況	Transfusion medicine and Hemotherapy 2016;43:137-141	公表国 ブラジル	
販売名（企業名）	－					

研究報告の概要

この文献は、血液疾患を持つ患者に対する輸血による A 型肝炎の伝播を報告している。
全血の献血を行なった 31 歳の男性ドナーが、献血の 20 日後黄疸の症状で血液バンクに申し出た。
HBV、HCV に対して血清学的検査及び NAT 検査は、陰性であった。
HAV　IgM と IgG の血清検査では、献血サンプルでは陰性であったが追跡のサンプルでは陽性であり、ドナーの HAV の急性感染を確認している。
同じ疑いのある献血からの赤血球と濃厚血小板のレシピエントは両方とも、HAV　IgM は陰性で IgG は陽性であった。
定性的な PCR では、3 人の個人すべてからのサンプルで陽性であり、ウイルスの系統学的分析で 2 人の血液生成物のレシピエントにウイルスの感染が判明した。
ドナーの追跡調査のサンプルと血小板のレシピエントの HAV のウイルス量は、それぞれ 1.3×10^{5}IU/ml、1.5×10^{5}IU/ml であった。
血小板のレシピエントは AML で化学療法を受けている患者で、血小板輸血から退院までの観察期間で肝酵素の高値及び肝炎の臨床症状は見られなかった。
赤血球のレシピエントは HCV にも感染していたが、骨髄移植が行なわれ、HAV 感染の疑いの 26 日後、劇症肝炎にて死亡した。
血液ドナーは、黄疸発症後、血液バンクに自発的に申し出た。このことは、献血後に感染性疾患に関連するサイン、症状が発現したことをすぐに血液バンクに連絡するというドナーの教育の重要性を強調している。
HAV に汚染された血小板輸血を受けた後劇症感染で死亡した HCV をもつイムノコンプロマイズドホストの事象が、これらの患者への HAV ワクチンの重要性をあらわしている。

使用上の注意記載状況・その他参考事項等

重要な基本的注意
［患者への説明］
本剤の投与又は処方にあたっては、疾病の治療における本剤の必要性とともに、本剤の製造に際し感染症の伝播を防止するための安全対策が講じられているが、ヒト血液を原料としていることに由来する感染症伝播のリスクを完全に排除することができないことを、患者に対して説明し、理解を得るよう努めること。

報告企業の意見	今後の対応
HAV は経口伝播が主であるが、輸血による感染例の報告である。 当社血漿分画製剤の製造工程には、各種ウイルスの不活化・除去を目的とした工程が設けられており、さらに最終製品において核酸増幅検査により HAV RNA 陰性であることを確認している。	今後とも輸血による HAV 感染に関する情報に留意し、情報を収集していく。

Transfusion Medicine and Hemotherapy

Case Report

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A Rare Case of Transfusion Transmission of Hepatitis A Virus to Two Patients with Haematological Disease

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Keywords
Hepatitis A · Immunosuppression · Window period · Blood donor · Blood transfusion

Summary
Background: This paper describes the transmission of hepatitis A virus (HAV) to two blood recipients from a healthy donor that later presented to the blood bank with jaundice. **Methods:** The RNA of HAV was detected by qualitative nested reverse transcription polymerase chain reaction (nested RT-PCR) and quantified by real-time RT-PCR. HAV RNA samples were genotyped by direct sequencing of PCR products. A sequence from a fragment of 168 bp from the VP1/2A HAV region was used to construct a phylogenetic tree. **Case Report:** A 31-year-old male donor accepted for donation of a whole blood unit returned to the blood bank with clinical jaundice 20 days after donation. His serological and NAT tests were negative for HBV and HCV. Serological tests for HAV IgM and IgG were negative on donation sample but positive on follow-up sample, confirming donor's HAV acute infection. Both recipients of red blood cells (R1) and platelet concentrate (R2) from the same implicated donation were HAV IgM-negative and IgG-positive. Qualitative PCR was positive on samples from all three individuals and phylogenetic analysis of viruses proved HAV transmission to the two recipients of blood products. HAV viral load on donor follow-up sample and the platelet recipient was 1.3 and 1.5 × 10^3 IU/ml, respectively. The RBC recipient, also infected by HCV, was undergoing bone marrow transplantation and died from fulminant hepatitis, 26 days after the implicated HAV transfusion. **Conclusion:** The blood donor, a garbage collector, spontaneously returned to the blood bank when developing jaundice. This highlights the importance of donor education to immediately report to blood banks of any signs and symptoms related to infectious disease developed after blood donation. The fact that one immunocompromised patient with HCV infection died from fulminant hepatitis after receiving a HAV-contaminated platelet transfusion underpins the importance of a HAV vaccination program for these group of patients.

Introduction

Hepatitis A virus (HAV) is the most common agent causing acute liver disease, with approximately 1.4 million of new cases occurring every year worldwide [1]. In Brazil, two epidemiological scenarios with low and intermediate HAV endemicity are shown. The anti-HAV IgG seroprevalence varies from 33.7% to 68.8%, for the intermediate and low endemic areas [2]. HAV is a non-enveloped single-stranded RNA picornavirus of the genus *Hepatovirus* whose most common genotypes found in human infections are the genotypes I and III [1–4]. Most cases of hepatitis A are self-limited and last 2–3 weeks after onset of symptoms [5]. Hepatitis A carries no risk for development of chronic hepatitis.

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The HAV incubation period usually lasts 2–7 weeks (average 28 days) prior to the onset of signs and symptoms. Viremia and fecal shedding often peak 2 weeks before symptoms appear. Laboratory findings are typical of hepatocellular injury with a marked elevated alanine aminotransferase (ALT) that precedes the elevation of serum bilirubin levels by a few days. When symptoms develop, IgM is frequently detected and confirms cases of hepatitis A [1, 5].

Usually the main route of transmission is fecal-oral, through contaminated food and water [1, 5]. Because viremia develops before symptoms onset transfusion transmission by whole blood, fresh-frozen plasma, platelet concentrates, and red blood cells (RBCs) has been sporadically described since 1974 [6–9]. However, in these reports it was difficult to demonstrate direct virological links between blood components and HAV infection.

Using molecular methods, Gowland et al. [10] documented one case of HAV transmission by RBCs to a recipient that was anti-HAV IgG-positive. The recipient did not develop hepatitis A but viremia ensued 2.5 months later along with elevation of IgG titer. Sequencing of HAV RNA from both the donor's and recipient's samples confirmed the transfusion link between individuals.

This article describes two cases of HAV transmission by blood transfusion from one infected donor. The two recipients were anti-HAV IgG-positive; nevertheless, both developed viremia, and one had elevated liver enzymes and symptoms characteristic of HAV infection. Molecular sequencing of the isolated HAV RNA from the three individuals confirmed the transfusion transmissions of HAV.

Material and Methods

Ethics

This investigation was approved by the Ethics Committee of the National Cancer Institute, Rio de Janeiro, Brazil, under the registration number 100/13.

Blood Collection and Manufacturing

The donor's whole blood was collected on CPDA-1 bag. RBCs and platelet-rich plasma were obtained after centrifugation of whole blood within 4 h of completion of the phlebotomy. The platelet concentrate was prepared by additional heavy-spin centrifugation, followed by removal of supernatant plasma. The platelet concentrate was stored under continuous agitation at a temperature between 20 and 24 °C, and the RBCs were stored at a temperature between 4 and 8 °C. The platelet concentrate was integrated into a pool of platelets that was transfused into a patient (blood recipient R1). The RBC concentrate was filtered and irradiated before transfused into another patient (blood recipient R2). The remaining plasma unit was discarded.

Biochemical Tests

Testing for alanine aminotransferase (ALT) is not mandatory for blood donor screening in Brazil; thus samples from the implicated donor and blood recipients were referred to the clinical laboratory where serum levels of ALT and total bilirubin were measured on a Roche/Hitachi Cobas 501 analyzer (Roche Diagnostics GmbH, Grenzach-Wyhlen, Germany). The values obtained are within the set limits, according to the manufacturer's instructions.

Serological Assays and Nucleic Acid Test (NAT)

The presence of anti-HAV IgM and IgG antibodies was investigated in serum samples using a qualitative microparticle chemiluminescence immunoassay on the Architect system Module i2000 (Abbott, North Chicago, IL, USA). In addition to HAV serological assays, blood donor samples were screened for *Trypanosoma cruzi*, syphilis, HTLV-1/2, HIV1/2, HCV, and HBV (HBsAg and anti-HBc) by serological assays on the Architect platform and for HIV, HCV, and HBV by NAT performed on minipools of six samples using Cobas TaqScreen MPX test, v2.0 on the Cobas s 201 System (Roche Molecular Diagnostics, Pleasanton, CA, USA). Except for HBV NAT, all assays were mandatory by Brazilian government regulations.

Qualitative Nested RT-PCR

The viral RNA was extracted from 140 µl of serum using the QIAmp Viral RNA Kit (Qiagen, Valencia, CA, USA). Reverse transcription was performed at 55 °C for 1 h using 10 µl of RNA, RNasin (30 U/µl) (Invitrogen, Carlsbad, CA, USA), random primer (20 pmol/µl) (Invitrogen, Rockville, MD, USA), dNTP (1.25 mmol/l) (Invitrogen, Carlsbad, CA, USA) and Superscript III transcriptase (200 U/µl) (Invitrogen, Carlsbad, CA, USA). The VP1/2A (247 bp) of the HAV genome was amplified using a nested RT-PCR as previously described [11]. The PCR products were loaded onto a 1.5% agarose gel and stained with ethidium bromide. Fragments of the expected size (247 bp) were purified using the QIAquick PCR purification kit (Qiagen) according to the procedure described by the manufacturer.

Real-Time RT-PCR

The HAV RNA was quantified from 5 µl of the cDNA with the TaqMan real-time PCR assay using an Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). To quantify HAV RNA, a recombinant clone of the 5' non-coding region (5' NCR) of the HAV (strain HAF-203) was used as standard curve, and primers specific for the 5' NCR of HAV and one HAV probe labeled with 5-carboxyfluorescein were used as described previously [12]. The detection limit reported for this assay was 14 copies/ml (3.3 IU/ml) [12]. The viral load results were described as IU/ml, after applying a conversion factor of 4.2 [13].

HAV Sequencing

HAV RNA samples were genotyped by direct sequencing of PCR products by using internal primers and the ABI PRISM Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) in a Gene Amp PCR System 9700 (Applied Biosystems) [14]. The sequence of 168 bp VP1/2A was used to construct a phylogenetic tree [15]. The *HAV* genotype is determined by the inclusion of previously published HAV sequences of different genotypes, including genotype strain IA-15, IB strains HM-175 (Australia), and HAF-203 (Brazil), strain IIA SLF88 (Sierra Leone) strain IIB CF-53 (France) , and IIIA strain GA76.

Case Report

The implicated donor is a 31-year-old male accepted for donation of a whole blood unit on December 10, 2011. All his serological screening tests were non-reactive as were the HIV, HCV and HBV NAT. 20 days after donation (on December 30, 2011), he returned to the blood bank service with clinical evidence of jaundice. An investigation of possible transmission of a hepatitis virus was initiated and new sample collected. All donor regulatory screening tests, including HCV and HBV NAT testing, were again non-reactive. As part of the protocol for lookback investigation of transfusion transmission of hepatitis, the serum levels of ALT and total bilirubin were evaluated, and both were elevated (ALT = 680 U/ml and total bilirubin = 7.75 mg/dl). Because the donor is a garbage collector a suspicion of HAV infection was raised, and anti-HAV IgM and IgG antibody tests were carried out and both found reactive.

A sample from the donation day was recovered from the serum repository and tested for anti-HAV IgM and IgG antibodies being non-reactive for both tests. These results characterize an anti-HAV antibody seroconversion confirming the diagnosis of HAV acute infection.

The two recipients of blood components from the same implicated donation have been identified. The first blood component recipient (R1) was a 27-year-old man under chemotherapy for acute myeloid leukemia (AML). He

Table 1. Laboratory results of samples collected from the implicated donor and blood component recipients (R1 and R2)

Individual	Tx date	Sample date	Anti-HAV		HAV NAT	Anti-HCV
			IgM (s/c)	IgG (s/c)		
Donor	–	December 10, 2011	NR (0,23)	NR (0,54)	ND	NR
	–	December 30, 2011	R (12,90)	R (3,95)	positive	NR
Recipient 1 (PLT)	December 12, 2011	January 8, 2012	NR (0,10)	R (14,71)	positive	NR
Recipient 2 (RBC)	December 14, 2011	January 5, 2012	NR (0,12)	R (14,34)	positive	R

*Tx = Transfusion; s/c = sample/cut-off ratio (values above 1,0 is considered reactive); NR = non-reactive; ND = not done; R = reactive; PLT = platelet concentrate.

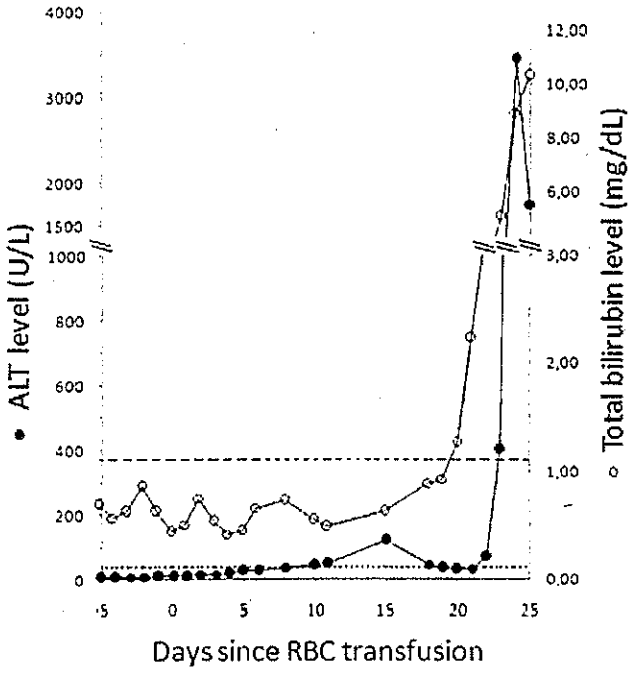

Fig. 1. Liver enzymes level of the R2 patient. ALT level (closed circles); ALT reverence level (41 U/l; dotted line); bilirubin levels (open circles); bilirubin reference level (1.1 mg/dl, dashed line).

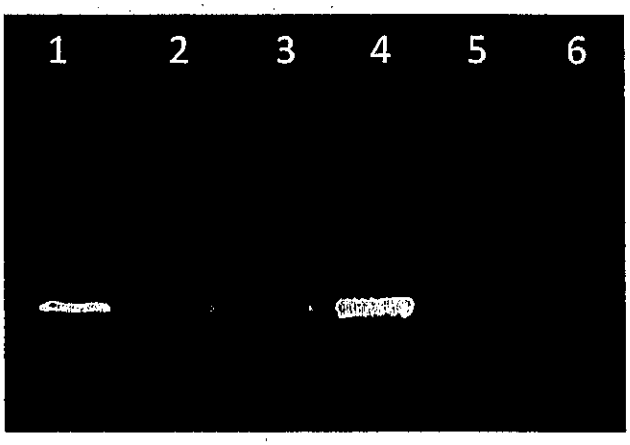

Fig. 2. Detection of HAV RNA (VP1/2A genome). Nested RT-PCR products (247 bp) were visualized by 1.5% agarose gel and ethidium bromide staining. Lanes: (1) blood donor; (2) platelet recipient (R1); (3) RBC recipient (R2); (4) HAV-positive control; (5) negative control; (6) ØX 174 DNA ladder as molecular size standard.

received a transfusion of platelets from the implicated donor on December 12, 2011. His liver enzymes and clinical symptoms were monitored during the 49 days since the platelet transfusion and hospital discharge. During this period he did not show elevated levels of liver enzymes or clinical symptoms of hepatitis. He died on July 2012 of causes related to his underlying disease.

The second blood component recipient (R2) was a 39-year-old man diagnosed with myelodysplastic syndrome, undergoing post-bone marrow transplant immunosuppression and being a chronic carrier of HCV. He received transfusion of RBCs on December 14, 2011, and on January 5, 2012 (22 days after transfusion) serum levels of both ALT and total bilirubin were elevated (fig. 1). The patient died from fulminant hepatitis on January 9, 2012, 26 days after the RBC transfusion. Of note, on the day of his death the HCV viral load was 2.57×10^6 IU/ml, a value almost 19 times greater than the HCV viral load of 1.36×10^5 IU/ml measured on August 2011.

Serum samples from the two patients collected on January 5, 2012 (R2) and January 8, 2012 (R1) were tested for anti-HAV IgM and IgG (table 1). Sera from both recipients were reactive for anti-HAV IgG but non-reactive for anti-HAV IgM.

These two samples, along with donor's second sample were evaluated by qualitative nested RT-PCR directed to the VP1/2A region (247 bp). All three samples were positive, as shown on figure 2. Furthermore, quantitative real-time RT-PCR was performed on samples from donor (sample from December 30, 2011) and from platelet recipient with viral load of 1.3×10^3 and 1.5×10^3 IU/ml, respectively. There was not enough sample volume from R2 patient to perform real-time RT-PCR.

A phylogenetic tree based on 168 nucleotide sequences in the HAV VP1/2A region was constructed (fig. 3). All three viruses sequenced were genotyped as HAV IA. Relatedness between the donor's and recipients' virus sequence was evidenced in this analysis further confirming the HAV transmission by transfusion.

Discussion

We reported two cases of transmission of HAV via blood transfusion with blood products originated from one blood donor. He was asymptomatic and anti-HAV IgM- and IgG-negative at the time of donation, but 20 days later, when he returned to the blood bank, he had jaundice as well as elevated levels of ALT and total bilirubin. Moreover, anti-HAV IgM became positive, and viremia (1.3×10^3 IU/ml) could be detected by PCR. It is likely therefore that blood donation took place during the HAV incubation period.

In adults, HAV infection has an average period of 28 days before symptom onset. In general, viremia precedes peak of liver enzymes by 2 weeks and may last for several months after symptoms disappear [17]. The donor case fitted well with this data.

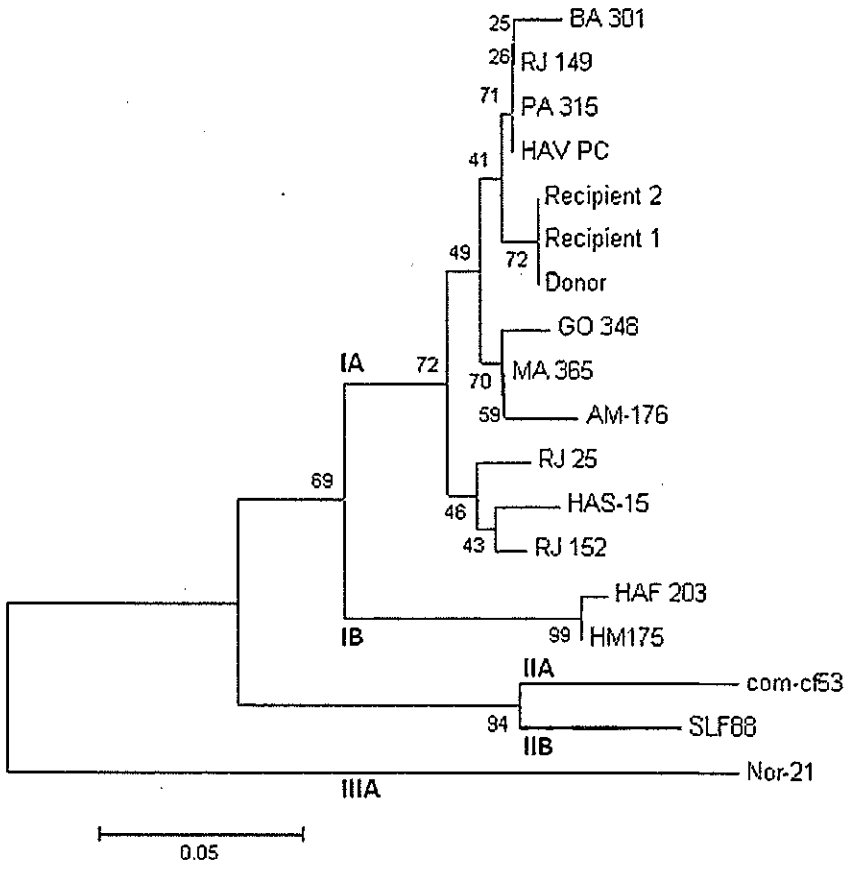

Fig. 3. Phylogenetic tree of the three HAV isolates were constructed by neighbor-joining method and based on HAV 168 nucleotide (positions 3,024 to 3,191) in the VP1/2A region. Roman numerals designate the respective genotype groupings, whereas (A) and (B) designate subgenotypes. Bootstrap percentages were calculated from 2,000 replicates. The horizontal bar at the bottom represents an evolutionary distance of 0.05. Receptor 1 indicates platelet recipient (R1) and receptor 2 indicates RBC recipient (R2); HAV PC indicates positive control for HAV-nested RT-PCR. References sequences from GenBank included subgenotypes IA: HAS-15 (X15464), IB: HM-175 (M14707, Australia), and HAF-203 (AF26896, Brazil), IIA: CF-53 (L07693, France), IIB: (SLF88), and IIIA: Nor-21 (L07668). The sequences from Brazilian states: RJ (Rio de Janeiro), GO-348 (Goias) (AY322994), PA-315 (Pará) (AY322923), BA-301 (Bahia) (AY322898), has been reported by De Paula et al. [16].

The donor is a garbage collector and may have acquired HAV infection through occupational exposure possibly during collection of contaminated water and/or food. Due to the high risk of occupational exposure to HAV infection, health authorities should reevaluate the standards and encourage the practice of vaccination against hepatitis A for this category of workers.

Although viremia was detected in the two blood transfusion recipients, their clinical courses were very distinctive. R1 died 7 months after blood transfusion of causes unrelated to HAV infection but did not show elevated levels of liver enzymes until 49 days after blood transfusion, when he was discharged from the hospital. It is possible that pre-existing IgG were activated by the infection and partially neutralized the virus, which as a consequence, led to a sub-clinical infection. Corroborating this hypothesis are studies with passive immunization with immunoglobulin that can completely suppress or modify the infection in HAV-susceptible persons depending on the concentration of anti-HAV administered [18]. This case is similar to the one described by Gowland et al. [10] and adds platelets concentrate as source of HAV transmission by transfusion.

R2, however, died 26 days after blood transfusion from fulminant hepatitis, and the level of ALT peaked 2 days before death. Importantly, R2 was under immunosuppressive drugs since before the blood transfusion which may have impaired a normal immune response including IgG burst induced by infection. In this scenario, pre-existing anti-HAV IgG may not have been sufficient to completely neutralize the virus. Moreover, R2 had a chronic HCV infection, a condition that when superimposed by HAV infection is associated with a high risk of developing fulminant hepatitis [19, 20], as was the case in this patient.

Timely recognition of post donation symptoms and notification to blood banks is important to limit transmission of infectious agents by transfusion [9]. However, post donation report of donor infection is a procedure which is not clearly defined by blood banks, and as a consequence its communication to the donor at time of donation is often neglected. In this case, and although the blood donor had not been adequately instructed, he voluntarily returned to the blood bank with jaundice. If he had not returned to the blood bank, this lookback investigation would not have been initiated, and a case of HAV transmission by transfusion would have gone unnoticed.

Antibody screening tests for HAV is not recommended for serological screening of blood donors in any country, including Brazil [21, 22]. In contrast, after a series of reports definitely proving the transmission of HAV by solvent-detergent-treated factor VIII, the plasma industry now tests all source plasma by HAV NAT [23, 24]. However, due to the sporadic nature of the HAV acute infection among blood donors and the lack of HAV chronic carriers the current recommendation for not screening blood donors for HAV seems adequate.

In USA, the Advisory Committee on Immunization Practices of the Centers for Disease Control and Prevention recommends vaccination of all chronic liver disease patients against HAV [25]. Specific groups of immunocompromised patients, especially those going for bone marrow or solid organ transplantation, may benefit from a HAV vaccination program as well. These are also patients that may need continuous blood transfusion support. The R2 case, therefore, underpins the importance of HAV vaccination in immunocompromised as well as chronically infected HCV and HBV patients.

Disclosure Statement

The authors declare no conflict of interest.

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医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2016. 6. 3	該当なし	
一般的名称	新鮮凍結人血漿		研究報告の公表状況	D'Ortenzio E, Matheron S, Yazdanpanah Y, et al. N Engl J Med. 2016 Jun 2;374(22):2195-8.	公表国 フランス	
販売名(企業名)	新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)					

研究報告の概要

○ジカウイルス(ZIKV)の性感染のエビデンス。
ZIKVは主にヤブカを介してヒトに伝播するが、性感染のエビデンスが複数存在している。パリ在住の24歳の女性(患者1)におけるZIKV性感染例を報告する。
患者1:ブラジルに滞在していた患者2と性的接触があった。尿及び唾液が、発症の3日後に採取された。RT-PCRによるZIKV RNA検査では、尿が陽性となり、ウイルス量は$3.5x10^3$コピー/mLであった。唾液も陽性反応を示し、ウイルス量は$2.1x10^4$コピー/mLとなった。同検査において血漿は陰性となったが、ZIKV IgM抗体が検出された。発症の10日後に採取された膣スワブは、同検査において陰性と判定された。
患者2:46歳の男性で、ブラジルに滞在していた際に、発熱、無力症、筋肉痛及び悪寒を呈したが、フランスに到着した際には症状は既に消失していた。発症から16日後に採取した尿から$4x10^3$コピー/mLのウイルス量が検出されたが、血漿並びに唾液は陰性であった。一方、採取した精液中のウイルス量は18日目で$2.9x10^8$コピー/mL、24日目で$3.5x10^7$コピー/mLと高値であった。
ZIKVは培養により18日目及び24日目の精液から分離された。
患者1の唾液、並びに患者2の精液から抽出されたZIKVの全ゲノム配列決定結果は系統発生分析にてクラスターを形成した。これは、性交渉を通じて患者2から患者1へZIKVが伝播した(経口または経膣)という仮説を支持している。ディープキスという行為によって、唾液など体液を介して伝播した可能性を除外することはできない。患者2の唾液は、発症後10日目の検査において陰性と判定されたが、これ以前に唾液検査は実施されていなかった。ZIKVが唾液から検出された例はあるが、唾液を介して伝播した例は記録されていない。ZIKVの伝播を阻止するため、より効果的に勧告を定める必要がある。

報告企業の意見	今後の対応
ジカウイルス(ZIKV)に感染した男性との性交渉により女性がZIKVに感染したという報告である。	今後も引き続き、新たなウイルス等による感染症の発生状況等に関する情報の収集に努める。

使用上の注意記載状況・その他参考事項等

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血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

only one cerebellar hemisphere in 3 infants. In 2 infants, the brain stem was globally hypoplastic. In all the infants, there was abnormal hypodensity of the white matter, and in 87% of the patients it diffusely involved all the cerebral lobes. In 1 infant, chronic encephalomalacic changes from ischemic stroke in the vascular territory of the left middle cerebral artery were seen.

Intrauterine ZIKV infection appears to be associated with severe brain anomalies, including calcifications, cortical hypogyration, ventriculomegaly, and white-matter abnormalities, although we cannot determine with certainty when ZIKV infection may have occurred during fetal development in these 23 infants. Our findings are nonspecific and may be seen in other congenital viral infections. The global presence of cortical hypogyration and white-matter hypomyelination or dysmyelination in all the infants and cerebellar hypoplasia in the majority of them suggest that ZIKV is associated with a disruption in brain development rather than destruction of brain. The neuronal and glial proliferation as well as neuronal migration appear to be affected. The mothers of the microcephalic infants in our study population had symptoms (e.g., low-grade fever and cutaneous rash) that were compatible with ZIKV infection during the first or second trimester of pregnancy, similar to the findings in other studies.[4] Tang et al. found that ZIKV directly infects human cortical neural progenitor cells with high efficiency, resulting in stunted growth of this cell population and transcriptional dysregulation.[5] This observation supports the type of disruptive, anomalous brain development that we found in these infants.

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A complete list of authors is available with the full text of this letter at NEJM.org.

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

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Evidence of Sexual Transmission of Zika Virus

TO THE EDITOR: Zika virus (ZIKV), an emerging flavivirus, generally causes mild infection in humans but is associated with severe neurologic complications and adverse fetal outcomes. ZIKV is transmitted to humans primarily by aedes mosquitoes. However, there is some evidence of sexual transmission.[1,2] Two studies have shown the presence of infectious ZIKV in semen.[3] A recent article described detection of ZIKV RNA in semen 62 days after the onset of illness, but infectious virus was not cultured.[4]

We report a case of ZIKV infection in a previously healthy 24-year-old woman (Patient 1) who was living in Paris and in whom acute fever, myalgia, arthralgia, and pruritic rash developed on February 20, 2016. She was not receiving any medication, had not received any blood transfusions, and had never traveled to a region where

Zika was epidemic or to tropical or subtropical areas. Her last trip outside France was to Okinawa, Japan, from December 21, 2015, to January 1, 2016. A clinical examination on February 23 showed a maculopapular rash on the patient's abdomen, arms, and legs and a temperature of 36.6°C. The illness lasted approximately 7 days.

Patient 1 reported sexual contact between February 11 and February 20, 2016, with a man (Patient 2, the index patient) who had stayed in Brazil from December 11, 2015, through February 9, 2016. The sexual contact involved seven episodes of both vaginal sexual intercourse, without ejaculation and without the use of a condom, and oral sex with ejaculation.

Patient 2, a 46-year-old man, reported fever, asthenia, myalgia, chills, and a cutaneous rash that began on February 7, while he was in Rio de Janeiro. The symptoms had resolved on the day he arrived in France on February 10. The clinical examination of Patient 2 was normal on February 23 (details are provided in the Supplementary Appendix, available with the full text of this letter at NEJM.org).

Populations of *Aedes aegypti* and *A. albopictus* mosquitoes are not established in the city of Paris. Moreover, in France, the diapause period of aedes species extends from December to May.

Three days after the onset of her symptoms, on February 23, samples of urine and saliva were obtained from Patient 1. The urine sample tested positive for ZIKV RNA by reverse-transcriptase polymerase chain reaction (RT-PCR) at a viral count of 3.5×10^3 copies per milliliter, and the saliva tested positive at a viral count of 2.1×10^4 copies per milliliter. A plasma sample tested negative for ZIKV RNA by RT-PCR, but serum IgM antibodies to ZIKV were detected (see the Supplementary Appendix). A vaginal swab obtained on March 1 was negative for ZIKV RNA by RT-PCR.

In Patient 2, a urine sample obtained 16 days after the onset of symptoms tested positive for ZIKV RNA by RT-PCR with a viral count of 4×10^3 copies per milliliter, but plasma and saliva samples tested negative. The first and second urine stream samples obtained on day 24 were positive for ZIKV RNA with a viral count of 2.1×10^4 copies per milliliter. Semen samples tested positive for ZIKV RNA by RT-PCR with a high viral load of 2.9×10^8 copies per milliliter in the sample obtained on day 18 and 3.5×10^7 copies per milliliter in the sample obtained on day 24. ZIKV was isolated by means of culture from semen samples on days 18 and 24. Timelines are shown in Fig. 1A.

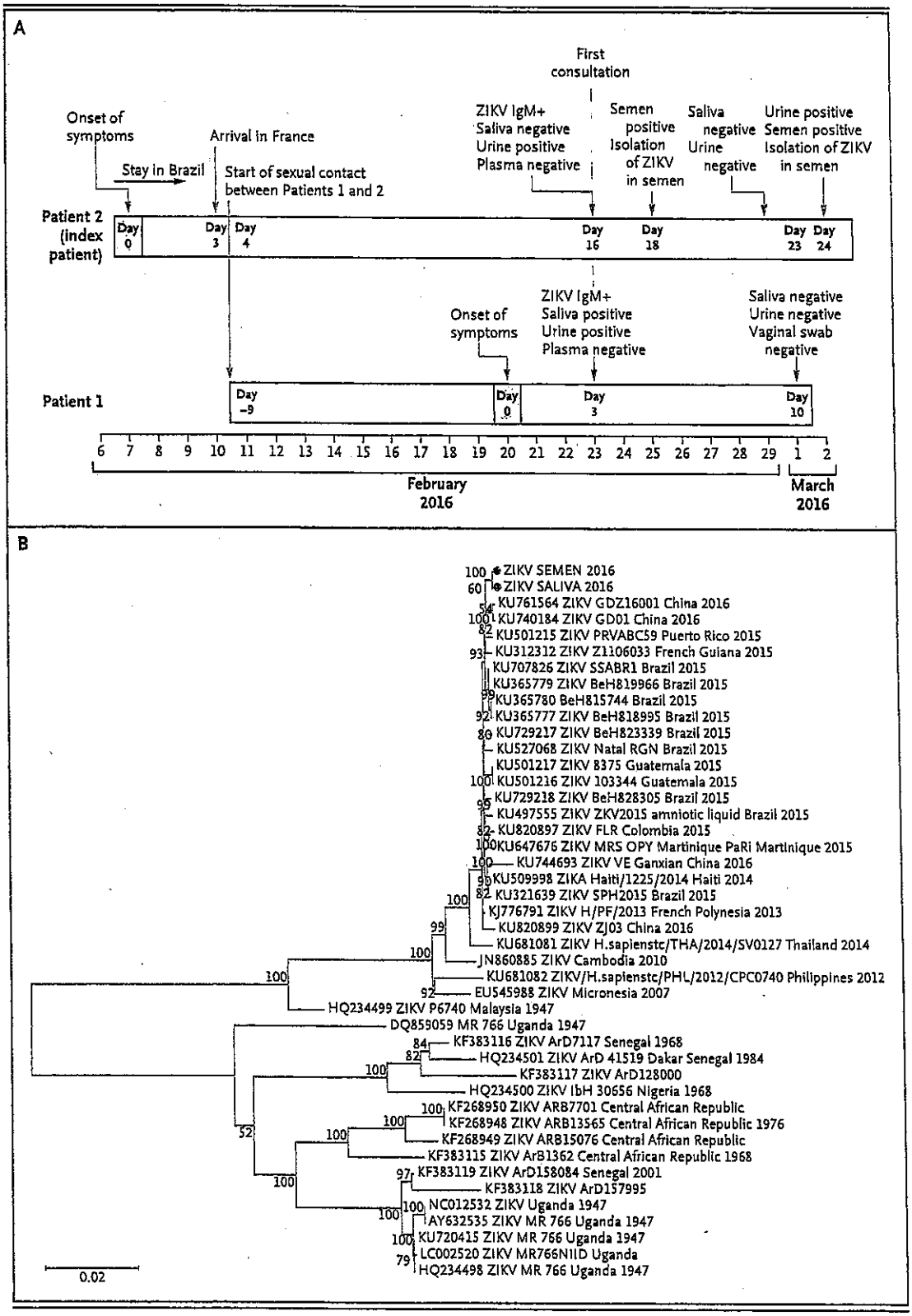
Figure 1 (facing page). Clinical Events and Phylogenetic Analysis of Zika Virus in the Patients.

Panel A shows clinical timelines with the key dates of exposure to Zika virus (ZIKV), the onset of symptoms, the results of reverse-transcriptase–polymerase-chain-reaction tests of plasma, urine, saliva, semen, and vaginal swab samples, and the results of ZIKV IgM from serum in Patient 1 and the index patient (Patient 2). Panel B shows the ZIKV strains (red dots) that were characterized directly from the semen (obtained from Patient 2) and saliva (obtained from Patient 1) at the top of a phylogenetic tree. Available sequence information regarding the virus GenBank number, strain name, country of isolation, and date of isolation is shown. Sequences of the Zika viruses identified in the semen and saliva samples are labeled beside the taxon names. The scale bar shows the nucleotide sequence divergence.

A complete ZIKV genome was sequenced from saliva samples obtained from Patient 1 and semen samples obtained from Patient 2 (see the Supplementary Appendix). Only four mutations, all of them synonymous, differentiate the sequences of the two patients. The complete nucleotide coding sequences of ZIKV identified in these semen and saliva samples cluster together within the phylogenetic tree (Fig. 1B).

These data support the hypothesis of sexual transmission (either oral or vaginal) of ZIKV from Patient 2 to Patient 1. We cannot rule out the possibility that transmission occurred not through semen but through other biologic fluids, such as pre-ejaculate secretions or saliva exchanged through deep kissing. The saliva of Patient 2 tested negative on day 10 after the onset of his symptoms, but it was not tested earlier. ZIKV has been detected in saliva,[5] but, to our knowledge, no cases of transmission through saliva have been documented.

The current outbreaks of ZIKV infection should be an opportunity to conduct studies to understand the natural history of ZIKV. We need to better define recommendations to prevent transmission of the virus. In particular, guidelines regarding how long men who are returning from



an area where active ZIKV transmission is occurring should continue to use condoms during sexual contact with pregnant women and those of child-bearing age are lacking. In addition, recommendations regarding the possibility of oral transmission of the virus through semen are needed.

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- A letter can have no more than five references and one figure or table.
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NOTICES

Notices submitted for publication should contain a mailing address and telephone number of a contact person or department. We regret that we are unable to publish all notices received.

13TH WORLD CONGRESS OF PEDIATRIC DERMATOLOGY (WCPD 2017)

The congress will be held in Chicago, July 6–9. It is presented by the Society for Pediatric Dermatology and cosponsored by the American Academy of Dermatology.

Contact Stephanie Garwood, Society for Pediatric Dermatology, 8365 Keystone Crossing, Suite 107, Indianapolis, IN 46240; or e-mail sgarwood@hp-assoc.com; or see http://pedsderm.net/meetings/world-congress-of-pediatric-dermatology.

38TH ANNUAL ASPEN CONFERENCE ON PEDIATRIC GASTROINTESTINAL DISEASE

The conference, entitled "Pediatric Liver Disease and Liver Transplantation," will be held in Aspen/Snowmass, CO, July 11–15.

Contact Sarah Delaat, Cincinnati Children's Hospital Medical Center, CME, ML 3003, 3244 Burnet Ave., Cincinnati, OH 45229-3039; or call (513) 636-6732; or fax (513) 636-7574; or e-mail cme@cchmc.org.

MAYO CLINIC SCOTTSDALE

The following courses will be offered: "Mayo Clinic Diagnostic Imaging Update and Self-Assessment with Advanced Radiology Life Support™ Workshop" (La Jolla, CA, July 31–Aug. 5) and "19th Annual Mayo Clinic Internal Medicine Update: Sedona 2016" (Sedona, AZ, Oct. 6–9, Oct. 20–23).

Contact Mayo School of Continuous Professional Development, Mayo Clinic, 13400 E. Shea Blvd., Scottsdale, AZ 85259; or call (480) 301-4580; or fax (480) 301-8323; or e-mail mca.cme@mayo.edu; or see http://www.mayo.edu/cme.

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No. 7

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2016. 7. 19	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名(企業名)	新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)		Davidson A, Slavinski S, Komoto K, Rakeman J, Weiss D. MMWR Morb Mortal Wkly Rep. 2016 Jul 22;65(28):716-7.	米国

研究報告の概要

○女性から男性へのジカウイルス(ZIKV)の性感染の疑い ― ニューヨーク市、2016年。
ニューヨーク市の保健精神衛生局は、20代の女性がZIKV流行地域から帰国した日(day0)に男性パートナーと避妊具なしで性交渉を行い、翌日(day1)に発熱、疲労感、丘疹、筋肉痛、関節痛、腰痛等を発症した。女性は帰国の際に空港での待ち時間に頭痛と疝痛の症状を呈していた。さらに、day1より月経が始まり、その症状がいつもより重かったためday3に家庭医を受診したところ、血液及び尿検体からリアルタイムRT-PCRにてZIKV RNAが検出されたが、酵素結合免疫吸着法(ELISA法)による抗体検査ではZIKV IgM抗体は検出されなかった。day6には、20代の男性パートナーも発熱、丘疹、関節痛及び結膜炎を発症した。男性はday9に女性と同じ家庭医を受診したところ、尿検体からZIKV RNAが検出されたが、血清検体からZIKV RNA及びIgM抗体は検出されなかった。day3に女性から採取した血清中からZIKV RNAが検出されたことから、性交渉時にウイルス血症であったと推察された。
本症例は女性から男性へのZIKVの性感染の初報告例であると考えられ、米国疾病予防管理センター(CDC)はガイドラインにてZIKVの伝播する地域へ直近に渡航したパートナーとの性交渉は避けるか、避妊具を適切に装着することを推奨している。

報告企業の意見	今後の対応
ジカウイルス(ZIKV)感染者の女性との性交渉により、相手男性にZIKVが感染した疑い症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、発熱などの体調不良者を献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

使用上の注意記載状況・その他参考事項等

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vCJD等の伝播のリスク

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Suspected Female-to-Male Sexual Transmission of Zika Virus — New York City, 2016

Alexander Davidson, MPH[1]; Sally Slavinski, DVM[1]; Kendra Komoto[1]; Jennifer Rakeman, PhD[1]; Don Weiss, MD[1]

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

A routine investigation by the New York City (NYC) Department of Health and Mental Hygiene (DOHMH) identified a nonpregnant woman in her twenties who reported she had engaged in a single event of condomless vaginal intercourse with a male partner the day she returned to NYC (day 0) from travel to an area with ongoing Zika virus transmission. She had headache and abdominal cramping while in the airport awaiting return to NYC. The following day (day 1) she developed fever, fatigue, a maculopapular rash, myalgia, arthralgia, back pain, swelling of the extremities, and numbness and tingling in her hands and feet. In addition, on day 1, the woman began menses that she described as heavier than usual. On day 3 she visited her primary care provider who obtained blood and urine specimens. Zika virus RNA was detected in both serum and urine by real-time reverse transcription–polymerase chain reaction (rRT-PCR) performed at the DOHMH Public Health Laboratory using a test based on an assay developed at CDC (*1*). The results of serum testing for anti-Zika virus immunoglobulin M (IgM) antibody performed by the New York State Department of Health Wadsworth Center laboratory was negative using the CDC Zika IgM antibody capture enzyme-linked immunosorbent assay (Zika MAC-ELISA) (*2*).

Seven days after sexual intercourse (day 6), the woman's male partner, also in his twenties, developed fever, a maculopapular rash, joint pain, and conjunctivitis. On day 9, three days after the onset of his symptoms, the man sought care from the same primary care provider who had diagnosed Zika virus infection in his female partner. The provider suspected sexual transmission of Zika virus and contacted DOHMH to seek testing for the male partner. That same day, day 9, urine and serum specimens were collected from the man. Zika virus RNA was detected in urine but not serum by rRT-PCR testing at the DOHMH Public Health Laboratory. Zika virus IgM antibodies were not detectable by the CDC Zika MAC-ELISA assay performed at the New York State Department of Health Wadsworth Center. The CDC Arbovirus Disease Branch confirmed all rRT-PCR results for urine and serum specimens from both partners.

During an interview with DOHMH on day 17, the man confirmed that he had not traveled outside the United States during the year before his illness. He also confirmed a single encounter of condomless vaginal intercourse with his female partner (the patient) after her return to NYC and reported that he did not engage in oral or anal intercourse with her. The man reported that he noticed no blood on his uncircumcised penis immediately after intercourse that could have been associated either with vaginal bleeding or with any open lesions on his genitals. He also reported that he did not have any other recent sexual partners or receive a mosquito bite within the week preceding his illness.

Independent follow-up interviews with the woman and man corroborated the exposure and illness history. The patients were consistent in describing illness onset, symptoms, sexual history, and the woman's travel. This information also was consistent with the initial report from the primary care provider.

The timing and sequence of events support female-to-male Zika virus transmission through condomless vaginal intercourse. The woman likely was viremic at the time of sexual intercourse because her serum, collected 3 days later, had evidence of Zika virus RNA by rRT-PCR. Virus present in either vaginal fluids or menstrual blood might have been transmitted during exposure to her male partner's urethral mucosa or undetected abrasions on his penis. Recent reports document detection of Zika virus in the female genital tract, including vaginal fluid. A study on nonhuman primates found Zika virus RNA detected in the vaginal fluid of three nonpregnant females up to 7 days after subcutaneous inoculation (*3*), and Zika virus RNA was detected in specimens from a woman's cervical mucous, genital swab, and endocervical swab collected 3 days after illness onset, using an unspecified RT-PCR test (*4*). Further studies are needed to determine the characteristics of Zika virus shedding in the genital tract and vaginal fluid of humans.

This case represents the first reported occurrence of female-to-male sexual transmission of Zika virus. Current guidance to prevent sexual transmission of Zika virus is based on the assumption that transmission occurs from a male partner to a receptive partner (*5,6*). Ongoing surveillance is needed to determine the risk for transmission of Zika virus infection from a female to her sexual partners. Providers should report to their local or state health department any patients with illnesses compatible with Zika virus disease who do not have a history

of travel to an area with ongoing Zika virus transmission, but who had a sexual exposure to a partner who did travel.

Persons who want to reduce the risk for sexual transmission of Zika virus should abstain from sex or correctly and consistently use condoms for vaginal, anal, and oral sex, as recommended in the current CDC guidance (5). Guidance on prevention of sexual transmission of Zika virus, including other methods of barrier protection, will be updated as additional information becomes available (http://www.cdc.gov/zika).

[1]New York City Department of Health and Mental Hygiene, New York.

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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
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一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名(企業名)	新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)		Kuehnert MJ, Basavaraju SV, Moseley RR, et al. MMWR Morb Mortal Wkly Rep. 2016 Jun 24;65(24):627-8.	米国

研究報告の概要

○ジカウイルス(ZIKV)感染症に対する供血スクリーニング ― プエルトリコ、2016年4月3日～6月11日。
ZIKVは、フランス領ポリネシアにおける2013～2014年の大流行が報告された後に、血液の安全性への脅威となる可能性として認識されている。ブラジルでは少なくとも1例のZIKV輸血感染症例が記録されている。
2016年2月、FDAはアメリカ国内及び同国領土における輸血によるZIKV感染リスクを軽減する目的で、供血者スクリーニング、供血延期及び製品管理に関する勧告を発表した。FDAは、全エリアで供血者に対する行動及び健康リスクに関する問診を追加して、最近まで蚊が媒介するZIKV感染を認める地域に住んでいた、又は旅行した非感染地域の居住者に対し供血延期を勧告する。1)流行地域で採取された供血血液へのZIKVスクリーニング、2)病原体低減化技術による処理(FDAが承認した対象製剤は血小板製剤及び新鮮凍結血漿製剤)、のいずれかが実施されるまでは、プエルトリコのような流行地の血液採取施設に対しては、採血の中止、米国大陸の非感染地域からの製剤の搬送を勧告する。
プエルトリコでは4月3日から、地域で供血された血液に対して新しく開発された核酸検査を用いてスクリーニングを実施した。6月11日までに12,777検体が検査され、68人(0.5%)がウイルス血症と推定された。陽性率がもっとも高い週は6月5～11日の週(1.1%)で、プエルトリコでのZIKV感染率の増加を反映するものと考えられた。ZIKV疾患のために市販されているワクチン又は薬は現時点ではないため、感染の予防には蚊の刺咬防止、蚊の繁殖場の排除、性感染を防ぐなどの措置が取られる。供血延期、供血者スクリーニング、病原体低減化技術を含めた血液供給におけるZIKVに対する防御措置は、輸血伝播のリスク低減が期待される。

使用上の注意記載状況・その他参考事項等

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血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
ジカウイルス(ZIKV)流行地域であるプエルトリコにおいて、2016年4月3日から6月11日までの間に12,777検体についてZIKV検査を行い、ウイルス血症供血者が68人(0.5%)検出された。流行ピーク時に陽性率は1.1%に達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、発熱などの体調不良者を献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

Screening of Blood Donations for Zika Virus Infection — Puerto Rico, April 3–June 11, 2016

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On June 17, 2016, this report was posted as an MMWR *Early Release on the* MMWR *website (http://www.cdc.gov/mmwr).*

Transfusion-transmitted infections have been documented for several arboviruses, including West Nile and dengue viruses (*1*). Zika virus, a flavivirus transmitted primarily by *Aedes aegypti* mosquitoes that has been identified as a cause of congenital microcephaly and other serious brain defects (*2*), became recognized as a potential threat to blood safety after reports from a 2013–2014 outbreak in French Polynesia. Blood safety concerns were based on very high infection incidence in the population at large during epidemics, the high percentage of persons with asymptomatic infection, the high proportion of blood donations with evidence of Zika virus nucleic acid upon retrospective testing, and an estimated 7–10-day period of viremia (*3*). At least one instance of transfusion transmission of Zika virus has been documented in Brazil after the virus emerged there, likely in 2014 (*4*). Rapid epidemic spread spread has followed to other areas of the Americas, including Puerto Rico.

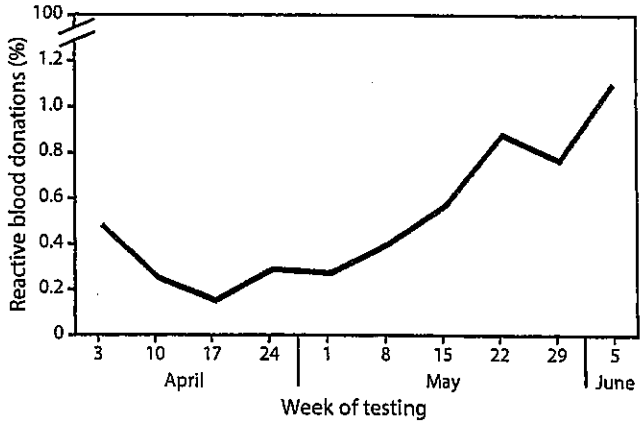
In February 2016, the Food and Drug Administration (FDA) issued recommendations for donor screening, donor deferral, and product management to reduce the risk for transfusion-transmitted Zika virus in the United States and its territories (*5*). In addition to behavioral- and health-risk questionnaires for blood donors in all areas, FDA recommends deferrals for donors in unaffected areas who recently lived in or visited an area with active mosquito-borne transmission of Zika virus. For establishments collecting blood in areas with active, local mosquito-borne transmission, such as Puerto Rico and other U.S. territories, the recommendations include discontinuing local blood collections and importing blood units from unaffected areas of the continental United States unless one of the following is implemented: 1) Zika virus screening of locally collected blood donations or 2) treatment of locally collected units with pathogen-reduction technology (FDA-approved only for plasma and apheresis platelets). In Puerto Rico, interventions initially were limited to importation of blood units from unaffected U.S. areas and to treatment of plasma and apheresis platelets with pathogen-reduction technology; no Zika virus screening test was available. On April 3, 2016, Zika virus screening of locally collected blood donations was implemented using a newly developed nucleic acid test (NAT) (cobas Zika, Roche Molecular Systems, Inc., Pleasanton, California) authorized by FDA under an investigational new drug application (IND) (*6*). As part of the IND, plasma samples from blood donors are screened individually, and specimens with reactive results are subjected to additional testing including an alternate NAT and immunoglobulin M serology. A blood donation with an initial reactive result by NAT is regarded as a presumptive viremic donor, indicating an infected donor, and is interdicted and removed from the blood supply.

During April 3–June 11, 2016, a total of 68 (0.5%) presumptive viremic donors were identified from 12,777 donations tested. The highest weekly incidence was 1.1% for the latest week of reporting, June 5–June 11, and incidence has been increasing over time (Figure).

Although the blood donor population of Puerto Rico is not intended to be statistically representative of the general population, the increasing prevalence of Zika virus nucleic acid among blood donors likely reflects an overall increase in infection incidence in the population at large. Based on data from previous outbreaks caused by arboviruses transmitted by *Aedes aegypti*, the high incidence often associated with these outbreaks can result in a substantial proportion of the population becoming infected. For example, chikungunya virus was introduced into Puerto Rico in 2014. Retrospective screening for chikungunya virus nucleic acid was performed on blood donations collected during June–December 2014, and the estimated detectable viremia was 0.65%, with a peak of 2.1% in October. Testing for chikungunya virus immunoglobulin M antibody of retained individual blood donation samples obtained during March 1–9, 2015, suggested that nearly 25% of the Puerto Rico population became infected during the previous year's epidemic (*7*). Because viremia is only present days after acute infection, immunoglobulin M antibody can provide a more precise estimate of the burden of recent infection. The 2014–2015 chikungunya virus data suggest that detection of viremia in a relatively small proportion of blood donors each week can reflect a substantial proportion of the general population becoming infected during the course of an epidemic season.

Currently, no medication or vaccine is available to treat or prevent Zika virus disease. Prevention relies on avoidance of mosquito bites, elimination of mosquito breeding sites, community mosquito control, and taking measures to prevent sexual transmission. Screening of the U.S. blood supply using

FIGURE. Proportion of screened blood donations reactive for Zika virus infection, by week of testing — Puerto Rico, April 3–June 11, 2016



nucleic acid tests has markedly reduced the risk for transfusion transmission for multiple pathogens, including for West Nile virus after it was associated with arboviral epidemics in the United States. Measures to protect the blood supply from Zika virus, including donor deferrals, laboratory screening, and pathogen reduction technology, are expected to similarly reduce the risk for transfusion transmission.

[1]Zika virus response blood safety team, CDC; [2]Roche Molecular Systems, Inc., Pleasanton, California; [3]Creative Testing Solutions, Tempe, Arizona; [4]Blood Systems Research Institute, San Francisco, California; [5]Banco de Sangre de Servicios Mutuos, San Juan, Puerto Rico; [6]Banco de Sangre de Puerto Rico; [7]Food and Drug Administration, Silver Spring, Maryland; [8]Puerto Rico Department of Health.

Corresponding author: Matthew J. Kuehnert, eocevent281@cdc.gov, 800-232-4636.

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番号 16

医薬品
医薬部外品　　研究報告　調査報告書
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識別番号・報告回数			報告日	第一報入手日 2016 年 07 月 29 日	新医薬品等の区分	厚生労働省処理欄

一般的名称	①②③⑨⑩⑪ pH4 処理酸性人免疫グロブリン ④⑤⑥⑦⑧ポリエチレングリコール処理人免疫グロブリン ⑫⑬ 人免疫グロブリン	研究報告の公表状況	http://www.fda.gov/BiologicsBloodVaccines/SafetyAvailability/ucm513583.htm/2016/07/28	公表国 アメリカ
販売名 (企業名)	①献血ポリグロビン N5%静注 0.5g/10mL (日本血液製剤機構) ②献血ポリグロビン N5%静注 2.5g/50mL (日本血液製剤機構) ③献血ポリグロビン N5%静注 5g/100mL (日本血液製剤機構) ④献血ヴェノグロブリン IH5%静注 0.5g/10mL (日本血液製剤機構) ⑤献血ヴェノグロブリン IH5%静注 1g/20mL (日本血液製剤機構) ⑥献血ヴェノグロブリン IH5%静注 2.5g/50mL (日本血液製剤機構) ⑦献血ヴェノグロブリン IH5%静注 5g/100mL (日本血液製剤機構) ⑧献血ヴェノグロブリン IH5%静注 10g/200mL (日本血液製剤機構) ⑨献血ポリグロビン N10%静注 2.5g/25mL (日本血液製剤機構) ⑩献血ポリグロビン N10%静注 5g/50mL (日本血液製剤機構) ⑪献血ポリグロビン N10%静注 10g/100mL (日本血液製剤機構) ⑫グロブリン筋注 450mg/3mL「JB」 (日本血液製剤機構) ⑬グロブリン筋注 1500mg/10mL「JB」 (日本血液製剤機構)			

研究報告の概要

米フロリダ州におけるジカウイルスの非渡航関連症例に関して，採血を行う施設へアドバイス；

Florida Department of Health State Surgeon General は，マイアミ・デイド郡およびブロワード郡における複数のジカウイルスの非渡航関連症例について疫学調査を行っていることを発表した。これらの症例は米国本土での蚊を媒介したジカウイルス地域内感染の最初の症例と考えられている。 ジカウイルス地域内感染の可能性を考慮し，血液および血液製剤の安全性を確保するための慎重な措置として，米 FDA はマイアミ・デイド郡およびブロワード郡内の採血を行う全施設に対し，採血した個々の血液にジカウイルス RNA に対する治験中のドナースクリーニング検査を実施できるまで，または承認されている／治験中の病原体不活化技術の使用を実施できるまで，採血を中止するよう要求している。

使用上の注意記載状況・その他参考事項等

代表として献血ヴェノグロブリン IH5%静注 0.5g/10mL の記載を示す。
2. 重要な基本的注意
(1)本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-1 抗体陰性で、かつ ALT (GPT) 値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査 (NAT) を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分からポ

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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。	リエチレングリコール4000処理、DEAEセファデックス処理等により人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理、ウイルス除去膜によるろ過処理及びpH3.9～4.4の条件下での液状インキュベーション処理を施しているが、投与に際しては、次の点に十分注意すること。

グロブリン

U.S. Food and Drug Administration
Protecting and Promoting *Your* Health

Advice to Blood Collection Establishments on Non-Travel Related Cases of Zika Virus in Florida

July 27, 2016

The Office of the Florida Department of Health State Surgeon General has announced that it is conducting an epidemiological investigation into a number of non-travel related cases of Zika virus in Miami-Dade and Broward Counties. These may be the first cases of local Zika virus transmission by mosquitoes in the continental United States. Miami-Dade County and Broward County are adjacent counties in South Florida.

In consideration of the possibility of an emerging local outbreak of Zika virus, and as a prudent measure to help assure the safety of blood and blood products, FDA is requesting that all blood establishments in Miami-Dade County and Broward County cease collecting blood immediately until the blood establishments implement testing of each individual unit of blood collected in the two counties with an available investigational donor screening test for Zika virus RNA or until the blood establishments implement the use of an approved or investigational pathogen inactivation technology.

Additionally, FDA recommends that adjacent and nearby counties implement the precautions above to help maintain the safety of the blood supply as soon as possible.

For blood collection establishments outside of this region, FDA suggests that donors who have traveled to Miami-Dade and Broward Counties during the previous 4 weeks be deferred.

FDA will continue to monitor this potential outbreak in cooperation with the Centers for Disease Control and Prevention (CDC) and Florida State public health authorities and provide updates as additional information becomes available.

More in Safety & Availability (Biologics) (/BiologicsBloodVaccines/SafetyAvailability/default.htm)

Biologics Product Shortages Q&A (/BiologicsBloodVaccines/SafetyAvailability/ucm303569.htm)

Recalls (Biologics) (/BiologicsBloodVaccines/SafetyAvailability/Recalls/default.htm)

CBER-Regulated Products: Shortages and Discontinuations (/BiologicsBloodVaccines/SafetyAvailability/Shortages/default.htm)

Report a Problem to the Center for Biologics Evaluation & Research (/BiologicsBloodVaccines/SafetyAvailability/ReportaProblem/default.htm)

Biologic Product Security (/BiologicsBloodVaccines/SafetyAvailability/ProductSecurity/default.htm)

Pandemics (/BiologicsBloodVaccines/SafetyAvailability/Pandemics/default.htm)

Blood Safety & Availability (/BiologicsBloodVaccines/SafetyAvailability/BloodSafety/default.htm)

Tissue Safety & Availability (/BiologicsBloodVaccines/SafetyAvailability/TissueSafety/default.htm)

Vaccine Safety & Availability (/BiologicsBloodVaccines/SafetyAvailability/VaccineSafety/default.htm)

HIV Home Test Kits (/BiologicsBloodVaccines/SafetyAvailability/HIVHomeTestKits/default.htm)

医薬品　研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日 2016 年 8 月 29 日	新医薬品等の区分	総合機構処理欄
一般的名称	—		研究報告の公表状況	The New England Journal of Medicine Downloaded from nejm.org on August 29, 2016	公表国 ブラジル	
販売名(企業名)	—					

研究報告の概要

このレポートは、アフェレーシスにより血小板を献血した発症前の感染者から、輸血によりジカウイルスが伝播した可能性のある2例について報告している。
白血球が除去された二つの血小板ユニットは放射線を照射され、異なる患者に輸血された。
献血後、ドナーは皮膚の発疹、眼窩後方の痛み、両膝の痛みで血液バンクに連絡した。
献血前後のドナーの血液サンプルは、チクングニヤウイルス、デングウイルス、ジカウイルスは陰性であった（RP-PCR）が、14日後の血漿と尿のサンプルではジカウイルスは陽性であった。血清学的検査で、ドナーの急性ジカウイルスの感染を確認した。
レシピエントの二人は、血小板輸血前のサンプル（routine pretransfusion sample）では、PCR にてチクングニヤウイルス、デングウイルス、ジカウイルスは陰性であった。
血小板輸血後、一人目の患者は輸血後 6 日で、二人目の患者は輸血後 23 日、51 日のサンプルで PCR にてジカウイルス陽性であった。
患者は調査の期間中、ジカウイルスの症状を示さなかったが、これらのデータは血小板輸血によるジカウイルスの伝播のエビデンスを表している。

使用上の注意記載状況・その他参考事項等

重要な基本的注意
［患者への説明］
本剤の投与又は処方にあたっては、疾病の治療における本剤の必要性とともに、本剤の製造に際し感染症の伝播を防止するための安全対策が講じられているが、ヒト血液を原料としていることに由来する感染症伝播のリスクを完全に排除することができないことを、患者に対して説明し、理解を得るよう努めること。

報告企業の意見	今後の対応
血小板輸血によるジカウイルスの感染の可能性の高い症例の報告である。 現時点まで血漿分画製剤から伝播したとの報告はないが、今後もジカウイルスに関連する情報に留意していく。	今後ともジカウイルスに関する情報に留意していく。

CORRESPONDENCE

Evidence for Transmission of Zika Virus by Platelet Transfusion

TO THE EDITOR: Zika virus (ZIKV) is a mosquito-borne virus that has important secondary means of transmission that include perinatal and sexual modes.[1-3] The potential for transmission in transfused donated blood components has been a concern owing to the detection of ZIKV viremia in healthy blood donors.[4]

This report from Brazil describes two cases of likely ZIKV transmission by blood transfusion from one presymptomatic infected person who donated platelets by apheresis on January 16, 2016. The two leukodepleted platelet units were irradiated with 25 Gy delivered by an IBL-437C gamma irradiator (Cis Bio International) and were transfused in different patients on January 19 (day 0). On January 21, the donor called the blood bank to report a cutaneous rash, retro-orbital pain, and pain in both knees that had begun on January 18. An investigation was initiated under the hospital's clinical protocol for transfusion-associated adverse events, with the donor and both patients providing written informed consent.

Two samples that were obtained from the donor before and after donation were negative for chikungunya virus (CHIKV) and dengue virus (DENV) on reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay, but the index plasma and urine samples 14 days later were positive for ZIKV (Table 1). (Details of the methods that were used and results are provided in the Supplementary Appendix, available with the full text of this letter at NEJM.org.) Serologic analysis by means of point-of-care testing, in-house indirect immunofluorescence assay (IFA), and plaque-reduction neutralization testing (PRNT) confirmed the presence of acute ZIKV infection in the donor.

The first recipient (Patient 1) was a 54-year-old woman with the primary myelofibrosis syndrome. The second recipient (Patient 2) was a 14-year-old girl with acute myeloid leukemia who had undergone haploidentical bone marrow transplantation on January 6, after which she had been receiving continuous immunosuppressive therapy. Routine pretransfusion samples obtained from the two patients were negative on PCR assay for CHIKV, DENV, and ZIKV, but samples collected 6 days after platelet transfusion in Patient 1 and 23 to 51 days after platelet transfusion in Patient 2 were positive for ZIKV on PCR assay.

Molecular sequencing and phylogenetic analysis of ZIKV RNA isolated from the donor and from the two patients confirmed the identity of their ZIKV isolates, with nucleotide changes in the envelope gene (codons 11 and 186) shared only by the donor and platelet recipients among available isolates from Brazil (GenBank accession numbers, KX173840, KX173841, KX173842, and KX173844) (Table S1 in the Supplementary Appendix). Against a backdrop of a high degree of conservation (>99% nucleotide identity) of ZIKV isolates in the Western Hemisphere,[5] the possibility of a single spatiotemporal cluster of mosquito-acquired cases was further undermined by the fact that Patient 2 lived 200 km away from Rio de Janeiro. Although neither patient was hospitalized in the period immediately preceding viral detection and thus could have been exposed to aedes mosquitoes contemporaneously with the platelet transfusions, the temporal coincidence of the infection (shortly after ZIKV diagnosis in the donor) and the phylogenetic identity of ZIKV samples that were recovered strongly favor transfusion as the source of the infection.

Serologic data supported the findings from the molecular analysis. All the samples obtained from Patient 1 showed antibody reactivity to DENV-2 on both IFA and IgG-capture enzyme-linked immunosorbent assay, findings that were consistent with her report of a history of dengue fever. Seroconversion to ZIKV was evident in both IFA IgG and point-of-care results; her PRNT titer on day 31 was 1:2560. For Patient 2, reactivity on IFA developed between 23 and 51 days after transfusion, as did a modest neutralizing antibody titer. The limited cross-reactivity to DENV-2 suggests ZIKV as the primary flavivirus infection. The limited antibody response in Patient 2 was

Table 1. Results of Molecular and Serologic Testing of Samples Obtained from the Platelet Donor and the Two Recipients.*

Donor or Patient†	Molecular Testing				Serologic Testing						
	ZIKV (Ct)‡		CHIKV	DENV	PRNT§	IFA IgG¶		ZIKV POC‖		DENV-Capture ELISA**	
	Plasma	Urine	Plasma	Plasma	ZIKV	ZIKV	DENV	IgM	IgG	IgM	IgG
Donor											
Day −3	Pos (23)		Neg	Neg							
Day 11	Neg	Pos (33)	Neg	Neg	1:1280	++	+/−	Pos (143)	Pos (239)	Pos (1.4)	Neg (0.5)
Patient 1											
Day −4	Neg		Neg	Neg		−	+++	Neg (7)	Pos (57)	Neg (0.6)	Pos (5.0)
Day 6	Pos (33)		Neg	Neg		+	++++	Neg (9)	Sus (32)	Neg (0.7)	Pos (4.9)
Day 31	Neg				1:2560	++++	++++	Sus (33)	Pos (335)	Pos (2.3)	Pos (5.4)
Patient 2											
Day −1	Neg		Neg	Neg							
Day 1	Neg		Neg	Neg							
Day 23	Pos (36)	Neg	Neg	Neg	1:40	−	−	Neg (7)	Sus (20)	Neg (0.1)	Neg (0.3)
Day 51	Neg/Pos††				1:20	++	+/−	Neg (4)	Neg (17)	Neg (0.2)	Neg (0.3)
Day 71	Neg							Neg (12)	Neg (5)		

* CHIKV denotes chikungunya virus, DENV dengue virus, ELISA enzyme-linked immunosorbent assay, IFA indirect immunofluorescence assay, Neg negative, POC point of care, Pos positive, PRNT plaque-reduction neutralization test, Sus suspected infection, and ZIKV Zika virus.
† Day 0 was January 19, 2016, the date of transfusion for both recipients.
‡ Ct denotes the threshold cycle (indicated by the values in parentheses) at which the result on reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay was positive.
§ PRNT values represent the serum dilution causing plaque reductions of 90%.
¶ IFA intensity ranges from low (+) to high (++++).
‖ The values in parentheses are measures of test-band intensity in arbitrary units, with results classified as negative (<20 units), suspected infection (20 to 39 units), and positive (≥40 units).
** The values in parentheses are the sample optical density divided by the assay cutoff.
†† The positive result on day 51 was obtained in a sample that had four times the starting volume on RT-PCR.

presumably due to her ongoing immunosuppressive therapy. Although neither patient reported symptoms associated with ZIKV infection during the investigation, these data show evidence for ZIKV transmission by means of platelet transfusion.

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Dr. Motta and Mr. Spencer contributed equally to this letter.

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

This letter was published on August 17, 2016, at NEJM.org.

1. Foy BD, Kobylinski KC, Chilson Foy JL, et al. Probable non-vector-borne transmission of Zika virus, Colorado, USA. Emerg Infect Dis 2011;17:880-2.
2. Musso D, Roche C, Robin E, Nhan T, Teissier A, Cao-Lormeau VM. Potential sexual transmission of Zika virus. Emerg Infect Dis 2015;21:359-61.
3. Besnard M, Lastere S, Teissier A, Cao-Lormeau V, Musso D. Evidence of perinatal transmission of Zika virus, French Polynesia, December 2013 and February 2014. Euro Surveill 2014;19(13):pii:20751.
4. Musso D, Nhan T, Robin E, et al. Potential for Zika virus transmission through blood transfusion demonstrated during an outbreak in French Polynesia, November 2013 to February 2014. Euro Surveill 2014;19(14):pii:20761.
5. Lanciotti RS, Lambert AJ, Holodniy M, Saavedra S, Signor Ldel C. Phylogeny of Zika virus in Western Hemisphere, 2015. Emerg Infect Dis 2016;22:933-5.

DOI: 10.1056/NEJMc1607262

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識別番号・報告回数			報告日	第一報入手日 2016 年 08 月 01 日	新医薬品等の区分 該当なし		厚生労働省処理欄
一般的名称	①②抗 HBs 人免疫グロブリン ③④乾燥抗 HBs 人免疫グロブリン ⑤ポリエチレングリコール処理抗 HBs 人免疫グロブリン			研究報告の公表状況	http://www.cdc.gov/media/releases/2016/p0729-florida-zika-cases.html/2016/07/29	公表国 アメリカ	
販売名 （企業名）	①抗 HBs 人免疫グロブリン筋注 200 単位/1mL「JB」（日本血液製剤機構） ②抗 HBs 人免疫グロブリン筋注 1000 単位/5mL「JB」（日本血液製剤機構） ③ヘブスブリン筋注用 200 単位（日本血液製剤機構） ④ヘブスブリン筋注用 1000 単位（日本血液製剤機構） ⑤ヘブスブリン IH 静注 1000 単位（日本血液製剤機構）						

研究報告の概要

米国 CDC は，ヒト 4 例のジカウイルス感染は地元の蚊による咬傷が原因である可能性が高いことについて，フロリダ州より通知を受けた。これらの症例は米国本土で確認されたおそらく初めての地元での蚊媒介性ジカウイルス伝播である。「確認したエビデンス全てが，マイアミのいくつかの地域内で数週間前に発生した蚊媒介性伝播であることを示している」と CDC の Tom Frieden が述べている。 2016 年 7 月 27 日時点，米国本土およびハワイにおけるジカの症例 1658 例が CDC に報告されている。これらの症例には性感染によると考えられる 15 例，および研究所での曝露による 1 例が含まれる。なお，この総数には本報告の地元伝播の可能性の高いフロリダの 4 例は含まれていない。

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

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

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

Florida investigation links four recent Zika cases to local mosquito-borne virus transmission

Press Release

For immediate release: Friday, July 29, 2016
Contact: Media Relations (http://www.cdc.gov/media)
(404) 639-3286

The Centers for Disease Control and Prevention (CDC) has been informed by the State of Florida that Zika virus infections in four people were likely caused by bites of local *Aedes aegypti* mosquitoes. The cases are likely the first known occurrence of local mosquito-borne Zika virus transmission in the continental United States. CDC is closely coordinating with Florida officials who are leading the ongoing investigations, and at the state's request, sent a CDC medical epidemiologist to provide additional assistance.

State officials have responded rapidly with mosquito control measures and a community-wide search for additional Zika cases. Under the current situation, there are no plans for limiting travel to the area.

"All the evidence we have seen indicates that this is mosquito-borne transmission that occurred several weeks ago in several blocks in Miami," said Tom Frieden, M.D., M.P.H., director of the CDC. "We continue to recommend that everyone in areas where *Aedes aegypti* mosquitoes are present—and especially pregnant women—take steps to avoid mosquito bites. We will continue to support Florida's efforts to investigate and respond to Zika and will reassess the situation and our recommendations on a daily basis."

Zika virus spreads to people primarily through the bite of an infected *Aedes* species mosquito (*Ae. aegypti* and *Ae. albopictus*), but can also be spread during sex by a person infected with Zika to their partner. Most people infected with Zika won't have symptoms, but for those who do, the illness is usually mild. However, Zika infection during pregnancy can cause a serious birth defect of the brain called microcephaly and other severe fetal birth defects.

"We have been working with state and local governments to prepare for the likelihood of local mosquito-borne Zika virus transmission in the continental United States and Hawaii," said Lyle Petersen, M.D., M.P.H., incident manager for CDC's Zika virus response. "We anticipate that there may be additional cases of 'homegrown' Zika in the coming weeks. Our top priority is to protect pregnant women from the potentially devastating harm caused by Zika."

CDC has been working with state, local, and territorial health officials to prepare for locally transmitted Zika infection in the United States. Officials from Florida participated in all these activities, and their experience in responding to mosquito-borne diseases similar to Zika, including dengue and chikungunya, has helped guide their current investigations. To date, CDC has provided Florida more than $8 million in Zika-specific funding and about $27 million in emergency preparedness funding that can be used for Zika response efforts.

Because we are in mosquito season, CDC continues to encourage everyone, especially pregnant women and women planning to become pregnant, to protect themselves from mosquito bites. Remember to use an insect repellent registered by the Environmental Protection Agency (EPA), wear long-sleeved shirts and long pants, use or repair screens on windows and doors, use air conditioning when available, and remove standing water where mosquitoes can lay eggs.

We continue to learn about Zika virus, and we are working hard to find out more about these cases. Here is what we do know:

- Zika is spread to people primarily through the bite of an infected *Aedes* species mosquito (*Ae. aegypti* and *Ae. albopictus*).
- A pregnant woman can pass Zika virus to her fetus during pregnancy or during birth.
- Zika virus infection can cause microcephaly and other severe fetal brain defects, and is associated with other adverse pregnancy outcomes.
- A person who is infected with Zika virus can pass it to sex partners.
- Many people infected with Zika virus won't have symptoms or will only have mild symptoms.
- No vaccines or treatments are currently available to treat or prevent Zika infections.

As of July 27, 2016, 1,658 cases of Zika have been reported to CDC in the continental United States and Hawaii; none of these were the result of local spread by mosquitoes. These cases include 15 believed to be the result of sexual transmission and one that was the result of a laboratory exposure. This number does not include the four Florida cases likely caused by local transmission.

For more information about Zika: http://www.cdc.gov/zika/ (http://www.cdc.gov/zika/#_blank).

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

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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08月30日	新医薬品等の区分 該当なし	厚生労働省処理欄

一般的名称	①②抗HBs人免疫グロブリン ③④乾燥抗HBs人免疫グロブリン ⑤ポリエチレングリコール処理抗HBs人免疫グロブリン	研究報告の公表状況	https://my.jbpo.or.jp/cgi-bin/cbgrn/grn.cgi/mail/view?mid=189887/2016/08/29	公表国 アメリカ
販売名 （企業名）	①抗HBs人免疫グロブリン筋注200単位/1mL「JB」（日本血液製剤機構） ②抗HBs人免疫グロブリン筋注1000単位/5mL「JB」（日本血液製剤機構） ③ヘブスブリン筋注用200単位（日本血液製剤機構） ④ヘブスブリン筋注用1000単位（日本血液製剤機構） ⑤ヘブスブリンIH静注1000単位（日本血液製剤機構）			

研究報告の概要

業業界向けガイダンス「血液および血液成分によるジカウイルス伝播のリスクを削減するための勧告の改訂」；

米国食品医薬品局（FDA）は、ジカウイルスに対するさらなる安全対策として、米国とその統治領で献血した全血および血液成分のジカウイルスに対するスクリーニングを推奨する業界向けガイダンスを改訂し発行した。本ガイダンスは米国由来の全血及び輸血用血液成分にZikaウイルスに対する個別ＮＡＴの導入を行い、陽性供血者の場合は120日前まで遡り当該供血者由来の血液製剤の隔離と回収を行うこと、また使われていた場合は、受血者をモニタリングすると共にジカウイルス感染の可能性を情報提供するように求めた勧告で、血漿分画製剤用の原料血漿は対象外としている。

報告企業の意見

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

今後の対応

本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

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

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抗HBs

Revised Recommendations for Reducing the Risk of Zika Virus Transmission by Blood and Blood Components

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Guidance for Industry

This guidance is for immediate implementation.

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

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

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

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

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

Contains Nonbinding Recommendations

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Contains Nonbinding Recommendations

Revised Recommendations for Reducing the Risk of Zika Virus Transmission by Blood and Blood Components

Guidance for Industry

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

I. INTRODUCTION

We, FDA, are notifying you, blood establishments that collect Whole Blood and blood components, that we have determined Zika virus (ZIKV) to be a relevant transfusion-transmitted infection (RTTI) under Title 21 of the Code of Federal Regulations (CFR) 630.3(h)(2) and we are providing you with FDA's assessment. We are also providing you with recommendations to reduce the risk of transmission of ZIKV by Whole Blood and blood components. The recommendations contained in this guidance apply to the collection of Whole Blood and blood components. This guidance does not apply to the collection of Source Plasma.[1]

This guidance document supersedes the guidance document entitled, "Recommendations for Donor Screening, Deferral, and Product Management to Reduce the Risk of Transfusion-Transmission of Zika Virus; Guidance for Industry," (dated February 2016) and the guidance document entitled, "Questions and Answers Regarding "Recommendations for Donor Screening, Deferral, and Product Management to Reduce the Risk of Transfusion-Transmission of Zika Virus; Guidance for Industry," (dated March 2016) no later than 12 weeks after the date of the issuance of this guidance. Implementation of the guidance will be immediate for blood establishments that collect Whole Blood and blood components in states and territories with local transmission of Zika virus by mosquitos, and will be phased in over 4 to12 weeks in other states and territories using a tiered, risk-based approach. Blood establishments should follow the recommendations in the February 2016 guidance until the recommendations in this guidance document have been fully implemented.[2] See section V. of this guidance for further recommendations on implementation.

[1] Source Plasma is used for further manufacture of plasma-derived products. Viral inactivation and removal methods that are currently used to clear viruses in the manufacturing process for plasma-derived products are sufficient to reduce the risk of the transmission of ZIKV.

[2] Except that blood establishments that collect Whole Blood and blood components in states and territories with locally acquired mosquito-borne cases of ZIKV should implement the recommendations in this guidance immediately or cease blood collection until they implement the recommendations in this guidance.

Contains Nonbinding Recommendations

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

II. BACKGROUND

ZIKV is an arbovirus in the *Flaviviridae* family, genus *Flavivirus*. It is transmitted to humans primarily by the *Aedes aegypti* mosquito, but it may also be transmitted by the *Aedes albopictus* mosquito (Ref. 1). In addition, cases of intrauterine, perinatal, sexual, laboratory-acquired and transfusion-associated transmission of ZIKV have been reported (Refs. 2, 3, 4, 5, 6, 7).

The virus was first isolated in 1947 from a sentinel rhesus monkey in the Zika Forest of Uganda (Ref. 2). Human illness due to ZIKV infection was first confirmed in Nigeria in 1953, and epidemiological studies conducted between 1951 and 1981 showed that the virus has circulated in humans in African and Asian countries (Ref. 8). ZIKV illness was first recognized outside of Africa and Asia in 2007 during an outbreak on Yap Island, Micronesia (Refs. 9, 10). An outbreak of ZIKV was next reported in French Polynesia from October 2013 to February 2014, when about 11% of the population had symptomatic infection (Refs. 11, 12).

The global ZIKV epidemic expanded in the region of the Americas by early 2015 when the first local transmission was reported in Brazil (Refs. 13, 14, 15). Local transmission of ZIKV has also been reported in areas outside of the Americas, including the Pacific Islands of Samoa, American Samoa, Marshall Islands and Tonga, and Cape Verde in Africa, and there are now at least 50 countries and territories worldwide with active local transmission of the virus (Refs. 16, 17).

The first local transmission of ZIKV in the United States (U.S.) was reported from Puerto Rico in December 2015, and soon thereafter local transmission was also reported in American Samoa and the U.S. Virgin Islands (Refs. 16, 17). In July 2016, the first cases of local transmission of ZIKV occurring in the continental United States were reported from Miami-Dade County in Florida (Ref. 18). The possibility of further geographic spread of ZIKV exists in regions where the *Aedes aegypti*, and possibly the *Aedes albopictus*, mosquito is present. In January 2016, Zika virus disease was added to the list of nationally notifiable conditions in the U.S. as a subtype of Arboviral diseases (Ref. 18).

ZIKV disease symptoms include fever, arthralgia, maculopapular rash, and conjunctivitis. Less frequently observed symptoms include digestive problems (abdominal pain, diarrhea and constipation), mucous membrane ulcerations (aphthae), and pruritus (Refs. 19, 20). In addition, neurological manifestations have been temporally and spatially associated with ZIKV disease outbreaks (Refs. 1, 2, 20). ZIKV infection has been associated with an increased incidence of Guillain-Barré syndrome (Ref. 21). Zika virus infection during pregnancy is a cause of microcephaly and other serious fetal brain anomalies. Other problems have been detected in pregnancies and among fetuses and infants infected with ZIKV before birth, such as miscarriage,

stillbirth, absent or poorly developed brain structures, defects of the eye, hearing deficits, and impaired growth; however, the full clinical spectrum of the effects of Zika virus infection during pregnancy is not yet known (Refs. 22, 23, 24, 25 26, 27).

Sexual transmission of ZIKV has been reported, including through male to female, male to male, and female to male routes (Refs. 4, 5). ZIKV RNA has been detected for up to 6 months in semen, although the maximum duration of transmissibility remains unknown at this time (Refs. 28, 29, 30). ZIKV has also been detected for up to 11 days in vaginal fluid; however data are currently unavailable on the maximum duration of its persistence in such fluid (Ref. 31). Prolonged circulation of ZIKV RNA in serum has also been demonstrated in pregnant women (Ref. 32).

ZIKV may be spread through blood transfusion. In French Polynesia, 2.8% of samples from asymptomatic blood donors contained detectable ZIKV RNA during the 2013-2014 outbreak, indicating the potential for transmission by blood transfusion (Refs. 11, 33). Confirmation of this finding came in 2016, as close to 1% of blood collected from asymptomatic donors in Puerto Rico tested positive when screened for ZIKV (Ref. 34). Probable transfusion-transmission of ZIKV also has been reported from Brazil (Refs. 6, 7).

Regarding measures to help prevent ZIKV transmission through the transfusion of blood products, ZIKV is likely cleared by the existing viral inactivation and removal methods that are currently used to clear viruses in the manufacturing processes for plasma-derived products. For example, viral clearance steps for various products may include heat, solvent/detergent (S/D) treatment and incubation at low pH (Refs. 35, 36, 37). These methods are highly effective in clearing lipid-enveloped viruses in plasma-derived products, but are not generally applicable for use in blood and blood components intended for transfusion. However, an S/D treated pooled plasma product has been FDA-licensed and is commercially available.

A pathogen reduction device (amotosalen combined with UV illumination) for plasma and platelets has recently been approved by the FDA (Ref. 38) and demonstrated effective reduction of a panel of viruses, including flaviviruses, such as dengue and West Nile virus. The same pathogen reduction technology (PRT) can effectively reduce ZIKV in plasma (Ref. 39). These devices have been used to reduce the risk of ZIKV infection by plasma or apheresis platelet components that are collected in areas experiencing ZIKV outbreaks (Ref. 10).

Risk of ZIKV Transmission by Blood Transfusion

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

1. ZIKV infection is asymptomatic in approximately 80% of individuals, and may be transmitted sexually for an unknown duration of time, potentially for up to 6 months (Refs. 1, 2, 28, 29, 30).
2. When symptoms of ZIKV do develop in individuals, the pre-symptomatic period for ZIKV infection varies from 3 to 12 days, during which viremia may occur (Refs. 1, 2, 40).

3. The viremia with ZIKV infection in non-pregnant individuals may produce up to 8.1 million copies per milliliter in serum, which typically lasts about 1-2 weeks, though duration of viremia may be longer. Whole Blood appears to have longer periods of detectable viremia when compared to serum. ZIKV RNA has been detected in Whole Blood as late as 58 days after symptom onset (Refs. 1, 2, 32, 40, 41).
4. ZIKV RNA has been found in asymptomatic blood donors during the French Polynesia outbreak in 2013-2014 and has been detected in 2016 in asymptompatic blood donors in Puerto Rico (Refs. 11, 33, 34).
5. There has been documented transfusion-transmissions of other flaviviruses such as West Nile virus, dengue virus and yellow fever vaccine virus, all of which have been shown to produce detectable viremia (the presence of virus in the blood) during asymptomatic and symptomatic infections (Refs. 42, 43, 44).
6. Probable transmission of ZIKV by blood transfusion has been reported (Refs. 6, 7).

The totality of the evidence presented above indicates that ZIKV can be transmitted through blood transfusion.

III. DISCUSSION

FDA has identified ZIKV as a transfusion-transmitted infection (TTI) under 21 CFR 630.3(l) and a relevant transfusion-transmitted infection (RTTI) under 21 CFR 630.3(h)(2). This determination is based on the severity of the disease, risk of transfusion-transmission by blood and blood components, the availability of appropriate screening measures and significant incidence and prevalence affecting the potential donor population.[3]

Transfusion-Transmitted Infection

A transfusion-transmitted infection (21 CFR 630.3(l)) means a disease or disease agent:

> (1) That could be fatal or life-threatening, could result in permanent impairment of a body function or permanent damage to a body structure, or could necessitate medical or surgical intervention to preclude permanent impairment of body function or permanent damage to a body structure; and
>
> (2) For which there may be a risk of transmission by blood or blood components, or by a blood derivative product manufactured from blood or blood components, because the disease or disease agent is potentially transmissible by that blood, blood component, or blood derivative product.

[3] Although a licensed screening test is not currently available, investigational screening tests are available at this time for use under investigational new drug applications, and these tests may be approved in the future. The Director of the Center for Biologics Evaluation and Research at FDA, taking into account the available scientific evidence, has issued a variance under 21 CFR 640.120(b), to provide for appropriate blood testing for ZIKV using investigational screening tests. This determination is based upon: 1) the potential severity of outcomes related to ZIKV, 2) the widespread nature of the global spread of ZIKV, 3) the risk of transmission of ZIKV by blood and blood components, and 4) the availability of investigational testing under IND to help reduce the risk of transmission of ZIKV through the blood supply.

Severity of Disease: ZIKV disease symptoms typically include fever, arthralgia, maculopapular rash, conjunctivitis and other less frequent symptoms include digestive problems, mucous membrane ulcerations, and pruritus. Zika virus infection during pregnancy is a cause of microcephaly and other serious fetal brain anomalies. Other problems have been detected in pregnancies and among fetuses and infants infected with ZIKV before birth, such as miscarriage, stillbirth, absent or poorly developed brain structures, defects of the eye, hearing deficits, and impaired growth; however, the full clinical spectrum of the effects of Zika virus infection during pregnancy is not yet known. ZIKV infection has also been associated with Guillain-Barré syndrome. Therefore, infection with ZIKV could be fatal or life threatening, could result in permanent impairment of a body function or permanent damage to a body structure, or could necessitate medical or surgical intervention to preclude permanent impairment of body function or permanent damage to a body structure.

Risk of Transmission: The risk of transmission of ZIKV is considered likely based on demonstrated viremia in symptomatic and asymptomatic infections, documented perinatal transmission of ZIKV, the identification of ZIKV RNA in blood donations, documented transfusion-associated transmission of other flaviviruses; and reports of probable transfusion-transmission in Brazil.

Therefore, FDA has established that ZIKV is a TTI because it is a disease agent that can be fatal or life threatening and can cause permanent damage to a body structure and it is potentially transmissible by blood or blood components.

Relevant Transfusion-Transmitted Infection

Having determined that ZIKV is a TTI, below we provide FDA's assessment that ZIKV meets the conditions for an RTTI as described in 21 CFR 630.3(h)(2).

Under 21 CFR 630.3(h)(2), relevant transfusion-transmitted infection means: a transfusion-transmitted infection not listed in 21 CFR 630.3(h)(1) when the following conditions are met:

> (i) Appropriate screening measures for the transfusion-transmitted infection have been developed and/or an appropriate screening test has been licensed, approved, or cleared for such use by the FDA and is available; and
>
> (ii) The disease or disease agent: (A) May have significant incidence and/or prevalence to affect the potential donor population; or (B) May have been released accidentally or intentionally in a manner that could place potential donors at risk of infection.

Availability of Appropriate Screening Measures or Screening Test: Although an FDA-licensed donor screening test for ZIKV is not currently available, investigational nucleic acid testing under IND has been developed and testing under IND has been implemented in Puerto Rico and in regions of the United States at risk for local mosquito-acquired transmission of ZIKV.[4]

Sufficient incidence and prevalence: The recent outbreak of ZIKV represents a rapid expansion of the virus in the Americas and the Pacific Islands. Currently 50 countries and territories are experiencing active transmission of ZIKV. As of August 17, 2016, 8,000 locally-acquired cases of ZIKV have been reported in U.S. territories; 2,245 travel-associated cases have been reported in U.S. states; and, 14 locally acquired mosquito-borne cases have been reported in Florida (Ref. 18). Epidemiological investigations of additional non-travel related cases of ZIKV are ongoing in Florida. We conclude that there is sufficient incidence and prevalence of ZIKV to affect the potential donor population.

Therefore, FDA has determined that ZIKV meets the criteria in 21 CFR 630.3(h)(2) for an RTTI because of the sufficient incidence and prevalence of ZIKV to affect the potential donor population in the United States and because of the availability of appropriate screening tests for ZIKV.

Donor Eligibility

Consistent with existing regulations, under 21 CFR 630.10(a), a blood establishment must not collect blood from a donor before determining that the donor is eligible to donate, or before determining that an exception to 21 CFR 630.10 applies. Under 21 CFR 630.10(a), "to be eligible, the donor must be in good health and free from transfusion-transmitted infections as can be determined by the processes in this subchapter. A donor is not eligible if the donor is not in good health or if you identify any factor(s) that may cause the donation to adversely affect: . . . (2) the safety, purity, or potency of the blood or blood component." The provision at 21 CFR 630.10(e) requires blood collection establishments to assess a donor's medical history to identify risk factors closely associated with exposure to, or clinical evidence of, an RTTI. Under 21 CFR 630.10(e)(1), "a donor is ineligible to donate when information provided by the donor or other reliable evidence indicates possible exposure to a relevant transfusion-transmitted infection if that risk of exposure is still applicable at the time of donation." Furthermore, under 21 CFR 630.10(e)(2), a donor is ineligible to donate when donating could adversely affect the safety, purity, or potency of the blood or blood component. Under this provision, a donor must be assessed for "travel to, or residence in, an area endemic for a transfusion-transmitted infection, when such screening is necessary to assure the safety, purity, and potency of blood and blood components due to the risks presented by donor travel and the risk of transmission of that transfusion-transmitted infection by such donors." (21 CFR 630.10(e)(2)(iii)).

[4] The Director of the Center for Biologics Evaluation and Research at FDA, taking into account the available scientific evidence, has issued alternative procedures under 21 CFR 640.120(b), to provide for appropriate blood testing for ZIKV using investigational screening tests. This determination is based upon: 1) the potential severity of outcomes related to ZIKV, 2) the widespread nature of the global spread of ZIKV, 3) the risk of transmission of ZIKV by blood and blood components, and 4) the availability of investigational testing under IND to help reduce the risk of transmission of ZIKV through the blood supply.

Pursuant to these provisions, FDA considered appropriate measures to assess a donor for risk factors closely associated with ZIKV. Screening donors residing in an area with local mosquito-borne transmission of ZIKV based on a medical history suggestive of ZIKV is inherently inadequate to protect the blood supply because ZIKV infection is asymptomatic in approximately 80% of individuals. Consequently, the majority of infected persons and their sexual partners in such areas could be unaware of their risk. Although history of prior residence in or travel to an area with local ZIKV transmission, or recent sexual exposure to a person who resided in or traveled to an areas with local ZIKV transmission (with or without a diagnosis of ZIKV infection or suggestive symptoms) conveys increased risk of ZIKV, use of these risk factors to select safe donors becomes ineffective as new areas of local transmission emerge.

As we learn more about the changing epidemiology of ZIKV, we recognize that there may be a significant delay between the time that the first case of locally acquired mosquito-borne transmission is first reported in an area and when the risk of transmission is present and could have been more widespread. Therefore, as the affected areas and number of cases of locally acquired mosquito-borne transmission of ZIKV in the continental U.S. increases, screening donors for risk factors will become increasingly logistically complex and decreasingly effective while also resulting in significant donor deferrals potentially compromising adequacy of the blood supply.

Additionally, the possibility exists that sexual transmission of ZIKV may evolve as a significant mode of spread of ZIKV independent of mosquito borne transmissions, rendering determination of locally affected areas by mosquito-borne transmission an inadequate safeguard. In contrast, testing blood donations for evidence of ZIKV or pathogen reduction by an approved device represents a more effective safeguard against ZIKV, rendering donor screening of de minimus value because general use of these alternate measures would: a) obviate determination of affected geographic areas; b) provide highly sensitive detection of virus-contaminated blood donations independent of a donor's medical or travel history; c) provide a safeguard against transfusion risk related to sexual transmissions; and d) assure continued availability of an adequate blood supply. Based on these considerations, FDA has concluded that it is necessary for blood establishments to implement nucleic acid testing of all donations or pathogen reduction technology using an FDA-approved device to reduce the risk of ZIKV transmission by blood and blood components.

Therefore, to provide for appropriate donor screening and testing, the Director of the Center for Biologics Evaluation and Research is providing an alternative procedure under 21 CFR 640.120(b) to the provisions in 21 CFR 630.10 to assess donors for specific risk factors for ZIKV before collecting blood or blood components. The Director of the Center of Biologics Evaluation and Research at FDA, taking into account the available scientific evidence, has issued alternative procedures to this provision under 21 CFR 640.120(b), to provide for appropriate donor testing for ZIKV with an investigational screening test available for use under investigational new drug applications. Alternatively, you may implement pathogen reduction technology using an FDA-approved device as specified in the instructions for use of the device to reduce the risk of ZIKV transmission.

IV. RECOMMENDATIONS

The following recommendations are intended to reduce the risk of ZIKV transmission by blood and blood components. The recommendations apply to the collection of all Whole Blood and blood components[5] in the United States and its territories. If, based upon the available scientific evidence, the risk of ZIKV transmission by blood and blood components significantly changes, FDA may update these recommendations as warranted. In making this determination, FDA will consider available epidemiologic and other scientific evidence.

A. Testing and Pathogen Reduction

We recommend the following:

1. Test all donations collected in the U.S. and its territories with an investigational individual donor nucleic acid test (ID-NAT) for ZIKV under an investigational new drug application (IND), or when available, a licensed test, *or*

2. Implement pathogen reduction technology for platelets and plasma using an FDA-approved pathogen reduction device as specified in the Instructions for Use of the device. If an FDA-approved pathogen reduction device becomes available for Whole Blood or red blood cells, you may implement pathogen reduction technology for such products rather than testing the donations as described in section IV.A.1.

   Note: Use of investigational pathogen reduction under an investigational device exemption (IDE) may be permitted in situations where approved technologies are unavailable.

Because all donations will be tested using an investigational ID-NAT for ZIKV under an IND or when available, a licensed test, or pathogen-reduced using an FDA-approved pathogen reduction device, you may discontinue providing donor educational material with respect to ZIKV and screening donors for ZIKV risk factors, such as travel history, and deferring them as previously recommended in the February 2016 guidance. Under 21 CFR.630.10(a), if a donor volunteers a recent history of ZIKV infection, you must not collect blood or blood components from that individual. We recommend that you defer such a donor for 120 days after a positive viral test or the resolution of symptoms, whichever timeframe is longer.

[5] The recommendations do not apply to the collection of Source Plasma. Viral inactivation and removal methods that are currently used to clear viruses in the manufacturing process for plasma-derived products are sufficient to reduce the risk of the transmission of ZIKV.

B. Donor and Product Management

1. You may release ID-NAT non-reactive donations provided all other donation suitability requirements are met (21 CFR 630.30).

2. If a donation tests ID-NAT reactive for ZIKV, you must not distribute or use the donation unless an exception exists (21 CFR 610.40(h))[6].

3. You must defer a donor who tests ID-NAT reactive for ZIKV and notify the donor of the deferral (21 CFR 610.41 and 630.30). We recommend that you defer the donor for 120 days[7] from the date of the reactive test or after the resolution of ZIKV symptoms, whichever timeframe is longer. We recommend you counsel the donor regarding a possible ZIKV infection.

4. We recommend that you quarantine and retrieve in-date blood and blood components collected from a donor in the 120 days prior to the donation that is ID-NAT reactive. Additionally, if such blood components were transfused, we recommend that you advise the transfusion service to inform the transfusion recipient's physician of record regarding the potential need for monitoring and counseling the recipient for a possible ZIKV infection.

C. Labeling of Whole Blood and Blood Components Intended for Transfusion

Under 21 CFR 606.122(h), the circular of information must include the names and results of all tests performed when necessary for safe and effective use. When testing is performed, we recommend that you update your circular of information to include the non-reactive ID-NAT results for ZIKV. You should indicate whether the testing has been performed using an investigational or licensed test.

V. IMPLEMENTATION

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

1. Blood establishments that collect Whole Blood and blood components in U.S. states and territories with one or more reported locally acquired mosquito-borne cases of ZIKV should implement the recommendations immediately. You should cease blood collection until testing or the use of pathogen reduction technology is implemented, consistent with the recommendations in this guidance.

[6] This requirement applies to all donations that test ID-NAT reactive for ZIKV, including those that have been pathogen-reduced.

[7] We recommend a deferral period of 120 days until more data become available on the duration of the viremic period and virus transmissibility.

As of the date of issuance of this guidance, this recommendation applies to blood establishments that collect Whole Blood and blood components in Florida and Puerto Rico.[8]

2. Because of their proximity to areas with locally acquired mosquito-borne cases of ZIKV or because of other epidemiological linkage to ZIKV, such as the number of travel-associated cases reported in a state, blood establishments that collect Whole Blood and blood components in Alabama, Arizona, California, Georgia, Hawaii, Louisiana, Mississippi, New Mexico, New York, South Carolina and Texas should implement the recommendations as soon as feasible, but not later than 4 weeks after the guidance issue date.

3. Blood establishments that collect Whole Blood and blood components in all other states and territories should implement the recommendations as soon as feasible, but not later than 12 weeks after the guidance issue date.

Consistent with 21 CFR 601.12, licensed establishments implementing these recommendations should update their annual reports indicating the date that the establishment revised and implemented their standard operating procedures consistent with these recommendations. See 21 CFR 601.12(a)(3). These changes do not require our prior approval.

[8] At this time blood is not being collected on a routine basis in American Samoa and the U.S. Virgin Islands.

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医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2016. 6. 21	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名(企業名)	新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)		Simmons G, Brès V, Lu K, et al. Emerg Infect Dis. 2016 Jul;22(7):1221-8.	米国

研究報告の概要

○米国プエルトリコのチクングニヤウイルス(CHIKV)流行期間における高感染率並びにウイルス血症者による供血(2014年)。
CHIKV は、2014年にカリブ海地域全域において大規模な流行を引き起こした。我々は2014年にプエルトリコにおいて流行が発生していた期間並びに流行後に採取・保管されていた供血者検体を用いて、CHIKV RNA(n = 29,695;16人ミニプール検査26,688検体+個別検査3,007検体)の核酸増幅検査(NAT)並びにCHIKV IgG抗体(n = 1,232;流行前201検体+流行後1,031検体)の検出を目的とする血清学的検査を実施した。
16人ミニプールNAT結果は、感染率が全体で0.6%(161プール陽性 /26,688検体(1,668プール))であった。一方、個別NATの陽性率は全体で1.9%(56検体陽性/3,007検体)、10月が最も高く、供血血液の2.1%がCHIKV RNA陽性となった。個別NATで検出された56検体中、8検体(14.3%)は、RNAコピー数が10^4-10^9 RNA copies/mLと高値を示し、また血清学的検査は陰性であることから感染初期のウイルス血症ピーク時の供血者からの採血であり、輸血によるウイルス感染リスクの高さが提起された。個別NAT陽性検体56検体を16倍に希釈し16人ミニプールNATを想定した評価を行ったところ、僅か21検体(37.5%)しか検出されないことから、残る35検体(62.5%)は検査をすり抜けると考えられる。また、流行前後の血清学的調査により、プエルトリコの供血者のほぼ25%がCHIKVに感染しており、流行期間中にセロコンバージョンを起こしていたことが証明された。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
プエルトリコの供血者におけるチクングニヤウイルス(CHIKV)流行期間の個別核酸増幅検査陽性率は10月が最も高く2.1%であり、流行後のCHIKV血清陽性率はほぼ25%であ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ている。国内でチクングニヤ熱が確認されたため、渡航歴確認の徹底を図っている。また、チクングニヤ熱の既往歴がある場合、治癒後6ヵ月間は献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

High Incidence of Chikungunya Virus and Frequency of Viremic Blood Donations during Epidemic, Puerto Rico, USA, 2014

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Chikungunya virus (CHIKV) caused large epidemics throughout the Caribbean in 2014. We conducted nucleic acid amplification testing (NAAT) for CHIKV RNA (n = 29,695) and serologic testing for IgG against CHIKV (n = 1,232) in archived blood donor samples collected during and after an epidemic in Puerto Rico in 2014. NAAT yields peaked in October with 2.1% of donations positive for CHIKV RNA. A total of 14% of NAAT-reactive donations posed a high risk for virus transmission by transfusion because of high virus RNA copy numbers (10^4–10^9 RNA copies/mL) and a lack of specific IgM and IgG responses. Testing of minipools of 16 donations would not have detected 62.5% of RNA-positive donations detectable by individual donor testing, including individual donations without IgM and IgG. Serosurveys before and after the epidemic demonstrated that nearly 25% of blood donors in Puerto Rico acquired CHIKV infections and seroconverted during the epidemic.

Chikungunya virus (CHIKV), a mosquitoborne, positive-sense RNA virus of the family *Togaviridae*, causes an acute febrile illness and severe polyarthralgia that can persist for months or years in some patients (*1–3*). Serious outcomes and deaths are rarely observed. However, newborns and other vulnerable populations are at risk for severe complications (*4*).

In late 2013, cases of CHIKV infection were reported in the French Collectivity of Saint Martin, which is part of the French Antilles (*5*), constituting the first instance of autochthonous transmissions of CHIKV in the Americas in the past century (*6*). In an immunologically naive population, CHIKV spread rapidly throughout the Caribbean region and beyond to most countries in the Western Hemisphere (*7*), including 11 autochthonous cases reported in Florida, USA, in September 2014 (*8*).

CHIKV has yet to be demonstrated to be transmissible by blood transfusion (*9*). However, this finding might result from difficulties in discriminating transfusion transmission from locally acquired mosquitoborne infection. Transfusion transmission is probable, given previous instances of laboratory-acquired infections and infection of healthcare workers by blood exposures (*10*). Asymptomatically infected persons can have viral loads >10^5 PFU/mL (*11,12*) and are a substantial risk for transfusion transmission.

Estimates of asymptomatic CHIKV infection vary widely. A recent study in Puerto Rico (*13*) confirmed previous estimates that 10%–25% of total infections are subclinical (*14–16*). However, other studies with the Asian genotype suggest that a greater proportion of cases might be asymptomatic or have only mild and transient symptoms (*17,18*). CHIKV infection can result in viral loads >10^8 PFU/mL (*19*). Thus, relatively high viral loads likely present in some presymptomatic donors might be a threat for transfusion transmission. Recently, a case of transfusion transmission of the related alphavirus Ross River virus, has been reported (*20*), stemming from transfusion of the erythrocyte component from a blood donor who reported symptoms of Ross River virus infection 2 days after donating blood.

To mitigate the theoretical risk for transmission, some blood collection organizations in regions with large CHIKV epidemics have suspended local blood collection, implemented nucleic acid amplification testing (NAAT) of erythrocyte and plasma donations for CHIKV RNA, and introduced pathogen-reduction technology for platelet components (*21,22*). To directly assess the threat that CHIKV poses to the blood supply, and given the absence of

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licensed NAAT for donor screening, we conducted NAAT surveys of blood donors in Puerto Rico during the 2014 epidemic and complementary serosurveys before and after the epidemic.

Materials and Methods

Human Subjects Research Approval

We performed retrospective testing of anonymous blood donor samples and minipools. The study was approved by the University of California, San Francisco Committee for Human Research.

Specimens

Creative Testing Solutions (Tempe, AZ, USA) retained, aliquoted, and archived at −70°C residual plasma from EDTA-anticoagulated blood collected in Puerto Rico and supplied for routine blood donor screening during the second half of 2014 and for a brief period during March 2015. Current molecular testing procedures at Creative Testing Solutions require that plasma samples be pooled into a minipool of 16 donor samples. Minipools prepared from blood donations in Puerto Rico were frozen during June 20–December 31, 2014. The sample set consisted of 1,667 minipools representing 26,672 individual donation samples from donors in Puerto Rico. Minipools were irreversibly stripped of their original labels and given a unique bar code that was linked only to month of collection.

In addition, 3,007 individual donor samples (IDS) were collected during the epidemic (September–November 2014), and ≈1,000 samples were saved per month. IDS were irreversibly stripped of all identifying information and given a unique bar code. Only basic demographic data (donor's age, race, sex, county of residence, and week of collection) were retained in a secure database. Anonymous minipools and individual donor samples were retained, aliquoted, frozen, and stored at −70°C.

Finally, we retained 1,031 individual donation samples obtained during March 1–9, 2015, for a postepidemic serosurvey. Demographic data, including the donor's age, sex, and zip code of residence, but not individual donor identifiers, were retained for these samples to enable analysis of serologic test results by using demographic strata.

Viral RNA Testing

We performed viral RNA testing by using a prototype real-time CHIKV/dengue virus (DENV) target-capture, transcription-mediated amplification (TC-TMA) assay (*12*) (Hologic, Inc., San Diego, CA, USA). Plasma samples (0.5 mL) were tested by using the fully automated Panther System (Hologic, Inc.), which performs target capture, amplification, and real-time detection in the presence of an internal control. We achieved detection by using single-stranded, fluorescent-labeled nucleic acid probes that were present during amplification of the target. The time for the fluorescent signal to reach a specified threshold was proportional to the starting CHIKV and DENV RNA concentrations. Target capture oligonucleotides, TMA primers, and detection probes hybridize with highly conserved regions of CHIKV or DENV RNA genomes and were designed to detect all 3 major CHIKV lineages and all 4 DENV types. We set the cutoff value for reactive specimens at 1,000 relative fluorescent units.

Estimated viral loads for CHIKV were calculated relative to the emergence time of the emitted fluorescence of a calibration curve generated by testing logarithmic dilutions of a CHIKV in vitro–synthesized transcript. ID-NAAT–reactive specimens were diluted 1:16 in defibrinated, delipidated, pooled plasma (SeraCare, Gaithersburg, MD, USA) to mimic minipool testing and tested by TC-TMA assay to assess whether donation samples detected by ID-NAAT would have been detectable by minipool NAAT (MP-NAAT).

We determined limits of detection (LODs) by using an in vitro transcript corresponding to each analyte and calculation by using Enterprise Guide 5.1 Probit analysis and the Normal model (SAS Institute, Cary, NC, USA). For DENV-1–4, the 50% LOD was 1.7–2.1 copies/mL, and the 95% LOD was 7.1–13.0 copies/mL in the IDS format. For CHIKV, the 50% LOD was 4.6 copies/mL, and the 95% LOD was 19.7 copies/mL in the IDS format. In 16-member minipools for DENV-1–4, the 50% LOD was from 27.2–33.6 copies/mL, and the 95% LOD was 116.8–208.0 copies/mL. For CHIKV, the 50% LOD was 73.6 copies/mL, and the 95% LOD was 315.2 copies/mL in the MP format.

Serologic Analysis

Plasma samples were tested for CHIKV IgM or IgG by using 2 ELISAs (Euroimmun US, LLC, Morris Plains, NJ, USA). These CHIKV ELISAs had specificities of 82% and 95% and sensitivities of 85% and 88% for IgM and IgG, respectively, when compared with those for 2 established in-house assays (*23*). Samples were diluted 1:100 and tested in duplicate according to the manufacturer's instructions. Sample-to-calibrator ratios were calculated. In validating the assay, we found that preepidemic samples (n = 201) yielded no strongly positive samples when the manufacturer's cutoff value >1.1 sample-to-calibrator ratio was used. However, 5 samples showed borderline reactivity (sample-to-calibrator ratios 1.13–1.37).

These 5 samples did not show positive results by reflex IgM testing, plaque-reduction neutralization testing (PRNT), or Western blot analysis when cell culture–propagated virus (strain 99659) was used as antigen. Testing of randomly chosen highly and moderately IgG-reactive samples from March 2015 by PRNT showed strong neutralization in all instances. Thus, the assay

does not appear to yield strongly reactive false-positive results, but might yield a small frequency (5/201, 2.5%) of low-level reactive false-positive results. Therefore, a new cutoff value was established by using mean sample-to-calibrator ratios of preepidemic samples plus 5 SDs (1.42). Testing of multiple IgG-negative samples from both sample sets by IgM ELISA (20 samples), PRNT (20 samples), and Western blot analysis (10 samples) did not yield any suspected false-negative results, which suggested that false-negative results were also not common.

Estimation of Detection Periods for MP-NAAT and IDS-NAAT

On the basis of the estimate for incidence of infection during the 2014 epidemic derived from serosurveys and MP-NAAT–positive results for the study period, we derived an estimate for duration of viremia detectable by the CHIKV TMA NAAT applied to minipools by using the approach of Busch et al. (*24*). We estimated the number of NAAT-positive donations in each minipool from minipool-testing results by using a program developed at the Centers for Disease Control and Prevention (Atlanta, GA, USA) (*25*). If T_i is the proportion of NAAT-positive donations in month i and P is seroprevalence of CHIKV at the end of the epidemic, then the TMA detection interval of CHIKV virus RNA (W) is estimated as

$$W = \frac{30 \sum_i T_i}{P}$$

Confidence limits for W were estimated by using a delta method estimate of the variance of W. Estimates for length of the individual donor sample-positive detection periods preceding and following the MP-NAAT–detectable period were derived from results of screening 3,007 individual donor samples by using ratios of samples detectable only by ID-NAAT that lacked IgG or contained IgG relative to the number of samples detectable at a dilution of 1:16. Confidence limits for these detection periods were derived by bootstrapping the assay results ratios (2/21) and (33/21) to obtain their variances, and then combining those with the variance associated with the estimate for the minipool detection period to obtain the variance of each of the 2 window estimates.

Results

Of 1,668 minipools tested, 1 was positive for DENV RNA, and 161 (9.7%) were positive for CHIKV RNA (Table 1). This finding indicates a minimum MP-NAAT–detectable infection rate of 0.6% (161 positive donations of 26,688 total donations), assuming only 1 of the 16 donations in each positive minipool was viremic. However, because the reactive minipool proportion peaked at 19.5% in September 2014 (Table 1), some pools would probably contain >1 viremic donation.

Individual donations comprising reactive minipools were not archived for further testing. Thus, we could not directly determine numbers of reactive IDS per reactive minipool. Therefore, we used a published algorithm (*25*) to estimate the proportion of donations that would contain CHIKV RNA at levels detectable by MP-NAAT (Table 1). This modification yielded an estimate for MP-NAAT detectable viremia of 0.65% for the overall season and an upper limit of 0.93%. The highest estimated proportion of MP-NAAT–detectable CHIKV RNA-positive donations was during September and October (1.34% and 1.31% of donations reactive for CHIKV RNA by MP-NAAT, respectively) (Table 1). This estimation represented a slightly delayed peak when compared with suspected and confirmed clinical cases reported in Puerto Rico (Figure 1).

Although not optimized to be quantitative, the TC-TMA assay provided approximate viral RNA copy numbers (Figure 2, panel A). Several minipools, particularly from early in the epidemic, had $>10^7$ copies/mL, although they were tested as a minipool, and thus effectively diluted 1:16. Of 161 reactive minipools, 125 had quantifiable viral loads. Remaining minipools had viral loads less than an estimated value of 0.5 log copies/mL (according to the calibration curve). The median viral load of 161 reactive minipools was 550 copies/mL (range <3.16 copies/mL–2.3×10^7 copies/mL). Donations from November and December had lower viral loads than donations from preceding months.

We also performed testing of archived IDS for CHIKV RNA for 3,007 donations collected in Puerto Rico during September–November 2014. We identified 56 confirmed positive donations, and ID-NAAT yields were 1.7%–2.1% for the 3 months tested (Table 2). When samples were

Table 1. Nucleic acid amplification testing for chikungunya virus in minipools of blood donations during a chikungunya epidemic, Puerto Rico, USA, 2014

Month	No. reactive minipools/no. tested (%)	Infection rate* (upper limit), %
June	0/106 (0.0)	0.0 (0.00)
July	8/193 (4.1)	0.26 (0.50)
August	26/293 (8.9)	0.58 (0.83)
September	51/262 (19.5)	1.34 (1.75)
October	57/299 (19.1)	1.31 (1.69)
November	12/243 (4.9)	0.32 (0.54)
December	7/272 (2.6)	0.16 (0.32)
Total	161/1,668 (9.7)	0.65 (0.93)

*In individual donors on the basis of minipools of 16 samples.

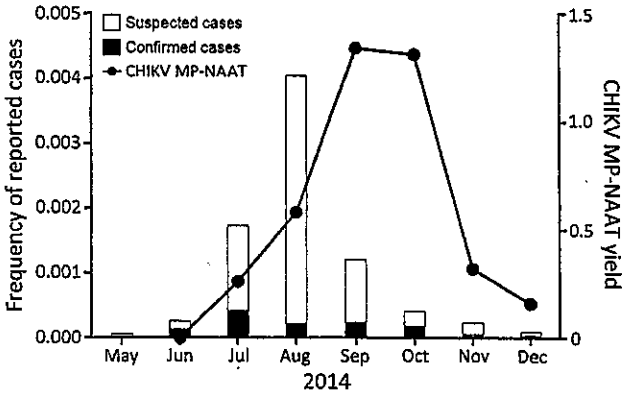

Figure 1. Estimated percentage of blood donations positive for chikungunya virus (CHIKV) RNA during a chikungunya epidemic, Puerto Rico, USA, 2014. CHIKV RNA-positive minipools of 16 donors were used to estimate the percentage of positive donations for the last 7 months of 2014. Estimates were made by using an algorithm for calculating infection rates from pooled data. Data from the Puerto Rico Department of Health for reported (suspected) and confirmed chikungunya case reports was used to transform data into estimated frequency of reported cases in a population in Puerto Rico of ≈3,548,400. MP-NAAT, minipool nucleic acid amplification testing.

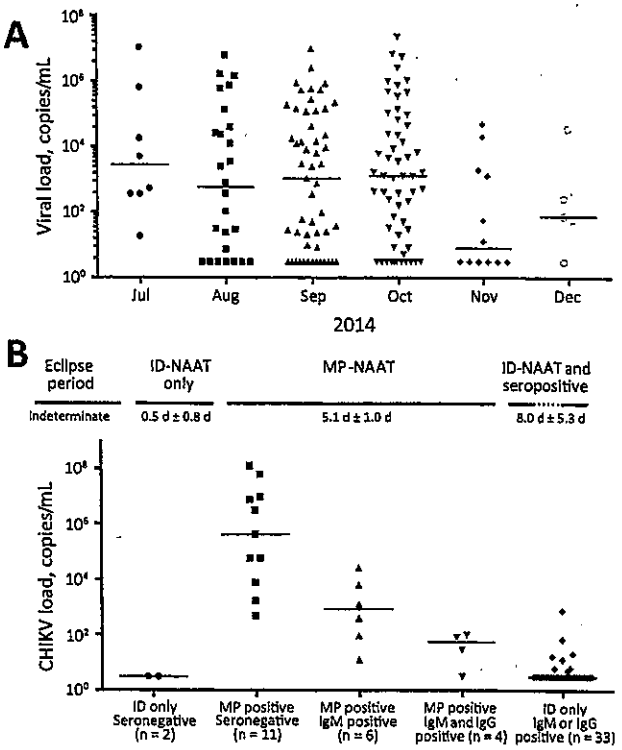

Figure 2. Viral loads for chikungunya virus (CHIKV) in blood donations during a chikungunya epidemic, Puerto Rico, USA, 2014. A) Positive minipool (MP) viral loads. Estimated viral loads (RNA copies/mL) were calculated for each reactive MP identified by using target capture transcription-mediated amplification (TC-TMA) during the epidemic. June 2014 (n = 106) is not plotted because of a lack of positive samples. Positive samples with unquantifiable viral loads are plotted as being at the limit of quantification (3.16 copies/mL) and were included in calculation of medians (horizontal bars). B) Individual donor (ID) viral loads for CHIKV. Estimated viral loads were calculated for each positive specimen identified by using TC-TMA during the 3 peak months of the epidemic. Positive samples with unquantifiable viral loads are plotted as being at the limit of quantification (3.16 copies/mL) and were included in calculation of medians (horizontal bars). Samples are arranged in order of projected time postinfection on the basis of predicted time course of acute infection (shown as estimated mean ±SD time intervals in days). ID only, samples positive by nucleic acid amplification testing (NAAT) but not positive for a 1:16 dilution mimicking minipooling. MP positive, samples positive by ID-NAAT and at a 1:16 dilution. Dynamics of acute infection with CHIKV (26) from the eclipse period (negative for virus RNA and IgM and IgG against CHIKV) to the end of infection (positive or negative for virus RNA and positive for IgM and IgG against CHIKV) is based on similar staging of dynamics of acute infection for other arboviruses (27) and approximate detection periods as described in the text.

diluted 1:16 to mimic minipools, proportions of RNA-positive samples detectable by MP-NAAT for September–November decreased to 0.4%–0.9%. Only 21 (37.5%) of 56 ID-NAAT–reactive specimens were reactive when tested for CHIKV RNA at a dilution of 1:16. Thus, 35 (62.5%) of 56 specimens would probably have been missed by routine MP-NAAT (Table 2). As expected, viral loads were low in donations reactive only by ID-NAAT. Only 8 of the ID-NAAT only–reactive samples had quantifiable viral loads (range 5.2–760 copies/mL) (Figure 2, panel B).

We performed assays to detect IgM and IgG in the 56 ID-NAAT–reactive specimens to characterize the relationship between development of IgG and IgM, viral load, and the ability of minipool testing to detect viremic donations (Table 2). Thirteen (23.2%) of 56 samples were seronegative; 2 were detectable only by ID-NAAT. These 2 samples are presumed to represent donors detected in the earliest stages of acute infection. The remaining 11 seronegative viremic donations had detectable viral loads (range 5 $\times 10^2$–1.3×10^8 copies/mL) (Figure 2, panel B), including 8 (14.3%) of 56 with viral loads $>10^4$ copies/mL. These samples were probably from donors who were near the peak of viremia, but still collected before seroconversion occurred.

Most CHIKV RNA-reactive samples were IgM positive (75%) and IgG positive (64%); 1 sample was IgM negative and IgG positive. Development of IgG titers is an inverse correlate of CHIKV RNA detection (*28*); of the IgG-reactive samples, only 4 (11.1%) of 36 were detectable by the less sensitive MP-NAAT. Viral loads of samples sorted on the basis of NAAT results (ID only vs MP-NAAT detectable) and serologic data demonstrate a typical profile of acute viral infection (Figure 2, panel B). The 43 viremic IgM-positive or IgG-positive donations had significantly lower viral loads (median <3.16 copies/mL) than 13 viremic seronegative donations (60,000 copies/mL; $p<0.0001$ by 2-tailed Mann-Whitney test). Although

similar proportions of ID-NAAT–positive samples were detected in November (1.7%) and September (1.8%), only 2 (11.8%) of 17 were seronegative in November compared with 6 (33.3%) of 18 in September, which suggested waning of the epidemic and a higher proportion of donations at the end of acute infection.

To estimate the incidence of CHIKV infection during the 2014 epidemic, we performed IgG serologic studies on blood donor specimens collected at the beginning of the epidemic (June 2014; preepidemic) and after the epidemic had subsided (March 2015; postepidemic). Collection was delayed until March to maximize detection of IgG seroconversion and to enable the maximum period for potential donors to recover from symptomatic infection, which would result in self-deferral, or deferral by the blood collection organization.

On the basis of IgG testing, we found that there were no unequivocally seroreactive samples in preepidemic samples (n = 201). In contrast, 241 (n = 1,031) postepidemic samples were strongly reactive (sample-to-calibrator ratio >2.5) (Figure 3). An additional indeterminate sample was positive by confirmatory testing with IgM ELISA, PRNT, and Western blot analysis. Thus, 242 (23.5%) of 1,031 samples were conservatively characterized as reactive (Figure 3).

Before we relabeled samples so that CHIKV testing was anonymous, basic demographic data were extracted for many of the specimens from March 2015 tested for seroreactivity (Table 3). No differences were observed in seropositivity rates between men and women. Persons 16–19 years of age had the highest rate of CHIKV recent infection; 40 (43.0%) of 93 of these persons were seropositive. In contrast, only 30 (18.3%) of 164 persons 40–49 years of age were seropositive.

We combined results from MP-NAAT and ID-NAAT screening and the serosurvey to estimate lengths of time that CHIKV RNA is detectable in serial stages of viremia in asymptomatic donors by MP-NAAT and ID-NAAT used in this study (Figure 2, panel B). We estimated that the length of the MP-NAAT–detectable phase for acute CHIKV infection in asymptomatic persons who donated blood was 5.1 days (confidence limit 4.1–6.0 days). By applying the ratios of seronegative ID-NAAT–only donations (2/56), MP-NAAT–detectable donations (21/56), and ID-NAAT–only seropositive donations (33/56), we estimated that there is a transient stage of low viral load infection preceding viremia detectable by MP-NAAT (0.5 days; confidence limit 0–1.3 days), whereas there is a relatively long stage of persistent viremia after seroconversion (8 days; confidence limit 2.7–13.3 days).

Discussion

Large epidemics of CHIKV infection occurred in the Caribbean Islands and in Central and South America over the past 2 years. Although >1.5 million confirmed and suspected cases have been reported (*29*), continued monitoring of CHIKV in these immunologically naive populations is needed for understanding population immunity and predicting dynamics of future epidemics. Using MP-NAAT, we estimated that 0.58% of individual blood donations were positive for CHIKV RNA during August 2014, a finding that is consistent with reported rates for Puerto Rico (*12*) and other Caribbean Islands (*22*).

As the 2014 epidemic in Puerto Rico continued, proportions of CHIKV viremia peaked in blood donors during September and October; >2% of donors were viremic, as indicated by individual donor NAAT results. During September and October, 1,440 chikungunya cases confirmed by real-time reverse transcription PCR were reported to the Puerto Rico Department of Health, which indicated sustained levels of CHIKV in the general population. However, reports of suspected chikungunya cases by month of illness onset received by the Puerto Rico Department of Health through passive surveillance peaked in August 2014 (Figure 1), which resulted in ≈14,000 suspected chikungunya cases in August, including 741 chikungunya cases confirmed by real-time reverse transcription PCR (Puerto Rico Department of Health, 2015, unpub. data).

Several factors probably affect the relative frequency of viremia and seroincidence of CHIKV in blood donors compared with clinical cases documented in the general population, including the focal nature of the epidemic in Puerto Rico during 2014 in relation to blood donor center

Table 2. Individual blood donations tested for chikungunya virus by nucleic acid amplification testing and serologic analysis during a chikungunya epidemic, Puerto Rico, USA, 2014*

Month	No. samples	No. ID-NAAT reactive samples	ID-NAAT yield, %	No. reactive at 1:16 dilution (MP-NAAT)	IgM reactive		IgG reactive	
					Total	IgM+/ID-only reactive	Total	IgG+/ID-only reactive
September	987	18	1.8	8	11	7†	8	7‡
October	1,010	21	2.1	9	15	10	14	10
November	1,010	17	1.7	4	16	12	14	12
Total	3,007	56	1.9	21	42	32†	36	32‡

*ID, individual donor; NAAT, nucleic acid amplification testing; MP, minipool.
†Includes 1 IgM-positive/IgG-negative ID-only positive specimen.
‡Includes one IgM-negative/IgG-positive ID-only positive specimen.

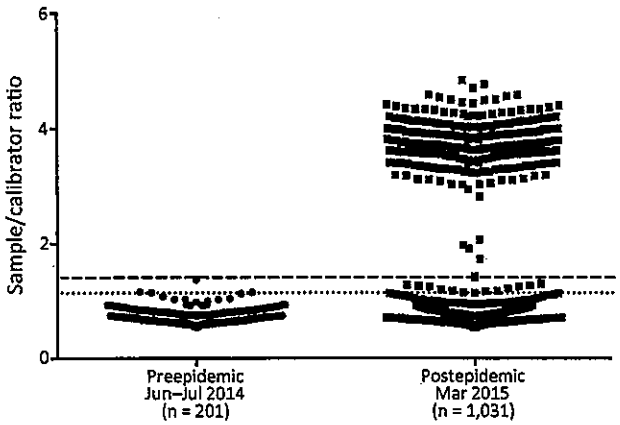

Figure 3. Serosurvey for chikungunya virus IgG in blood donations during a chikungunya epidemic, Puerto Rico, USA, 2014. Preepidemic samples collected in June and July 2014 were tested by using an IgG ELISA. A stringent cutoff value of mean + 5 SD (dashed line) was calculated from preepidemic samples. A less stringent cutoff value of mean + 3 SD (dotted line) was also calculated. These cutoff values were then applied to postepidemic samples collected in March 2015.

locations. It is also likely that many cases went unreported and that as the epidemic progressed many infected persons might not have sought medical care (*13*). Our finding that ≈25% of blood donors had serologic evidence of CHIKV infection after the 2014 epidemic supports these suggestions. Given a population of >3.5 million, and assuming that blood donors are representative of the total population of Puerto Rico with respect to risk for arbovirus transmission, a seroincidence of 23.5% would suggest that >800,000 persons were infected in Puerto Rico during the 2014 epidemic.

Blood safety protocols in place during the study included a Puerto Rico Departmen t of Health requirement for questioning of donors concerning symptoms in the month preceding donation and passive reporting of postdonation febrile illness. Thus, in the absence of specific NAAT screening, asymptomatic donors are likely to result in most viremic donations (*30*).

It is not clear whether asymptomatic infection is correlated with lower viremia levels, and thus would decrease the likelihood of transfusion transmission. However, similar to previous findings (*12*), many presumably asymptomatic donors in our study had viral loads comparable with those for symptomatic patients (*11*,*19*), including some viral loads >10^8 copies/mL. Most donations with low viral loads were IgM positive, which indicates recent acute infections. The proportion of these viremic specimens increased as the epidemic waned, and the percentage of ID-NAAT–only samples increased from 56% in September to 77% in November. Lower average viral copy numbers were also observed in November and December by testing of minipools. Furthermore, all RNA-positive donors in November were seropositive compared with only 78% of NAAT-reactive donors in September.

We estimate that the RNA-detectable window for MP-NAAT was 5.1 days. This value matches viremic periods observed for experimentally infected nonhuman primates (*31*) but is somewhat shorter than estimates for symptomatic patients of 1–2 days before disease onset and 8 days postonset (*9*,*11*,*32*,*33*). This finding is probably caused by a loss in the ability to detect viremia at the 1:16 dilution inherent in creating minipools, but might be a reflection that this study was limited to asymptomatic persons who donated blood. In addition, we calculated a relatively short ramp-up period before MP-NAAT–detectable viremia (0.5 days) and a longer low-level (MP-NAAT negative) viremia at the end of acute infection after seroconversion (8 days). Nevertheless, the 5-day MP-NAAT–detectable period for high-titer viremia is probably the most infectious period in terms of transfusion transmission and transmission to mosquitoes.

The overall threat CHIKV poses to the blood supply remains an open question that requires urgent attention, including in the continental United States, given the risk for travel-acquired and autochthonous transmission. In the absence of routine NAAT for CHIKV, and in regions where pathogen-reduction technology is not implemented, the largest threat is probably from donors with high viral

Table 3. Demographic characteristics of blood donors tested for chikungunya virus during a chikungunya epidemic, Puerto Rico, USA, 2014

Characteristic	No. (%) nonreactive for IgG, n = 786*	No. (%) reactive for IgG, n = 242*	Total, n = 1,031*	Odds ratio (95% CI)
Sex				
F	235 (75.81)	75 (24.19)	310	1.00
M	348 (74.95)	117 (25.05)	567	1.05 (0.75–1.47)
Age, y				
16–19	53 (56.99)	40 (43.01)	93	1.00
20–29	139 (81.29)	32 (18.71)	171	0.31 (0.17–0.55)
30–39	119 (79.33)	31 (20.67)	150	0.35 (0.19–0.62)
40–49	134 (81.71)	30 (18.29)	164	0.30 (0.16–0.54)
50–59	90 (70.54))	38 (29.46)	129	0.55 (0.31–0.97)
60–78	49 (70.00)	21 (30.00)	69	0.57 (0.29–1.10)

*Some specimens did not have complete demographic data.

loads who have not fully seroconverted because it can be assumed that donors with neutralizing IgG responses have a lower probability of transmitting an infectious dose to a recipient.

Although convalescent-phase serum is protective in animal studies (*34*), the ability of IgM and IgG in viremic donors to mitigate CHIKV transfusion transmission requires further study. Likewise, if viral RNA screening is introduced, studies will be needed to evaluate the relative usefulness of ID versus MP-NAAT. In screening of 3,007 individual donations, we identified 7 viremic donors with only IgM responses. However, only 1 of these donors had viremia detected only by ID-NAAT. We also identified 2 seronegative donors who showed reactivity by ID-NAAT, but not minipool testing. Whether blood components from these donations, together with specimens in the so-called eclipse phase between acquisition of infection and detectable ID-NAAT reactivity, are infectious remains unanswered.

In summary, our results indicated a sizable proportion of blood donors had detectable CHIKV RNA during the chikungunya epidemic in Puerto Rico in 2014. Several donations with high viremias were negative for IgM and IgG, which suggested that donors were in the peak phase of acute infection and highlights the risk for transfusion transmission. However, most viremic donations had low levels of viral RNA and were seropositive, which suggests recent subclinical infection and low risk for infectivity. However, these donors were healthy enough to donate blood. Finally, serosurveys before and after peak epidemic months showed that ≈25% of blood donors in Puerto Rico acquired CHIKV during the 2014 epidemic. On the basis of findings of this study, we are now conducting further investigations to determine the risk for transfusion transmission of CHIKV by virus RNA–positive transfusions and outcomes of infection in recipients.

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No. 17

医薬品 研究報告 調査報告書

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一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
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研究報告の概要

○血小板製剤及び赤血球製剤の保管期間におけるデングウイルス(DENV)の存続と複製。
背景:供血者由来の血小板(PLT)製剤並びに赤血球(RBC)製剤の有効期間におけるDENVの生存能力を調査した。
研究デザイン及び方法:精製した10^5～10^6 感染単位/mLのDENV(無症候供血者におけるウイルス力価を再現)を、血液バンクの作業手順に従って製造・保管したPLT製剤及びRBC製剤に添加した。DENV感染力価の測定にはプラーク形成法並びにRT-PCRを用いた。
結果:感染性DENVは、対数的減少が見られたものの保管期間を通じてPLT製剤中(7日間、20～24℃)及びRBC製剤中(42日間、1～6℃)で存続した。一方、緩衝液中では、20～24℃で1日目、1～6℃で14日後には感染力が低下した。翻訳阻害剤を用いて保管PLT製剤中の感染性ウイルス産生を確認し、ウイルスゲノムの複製による裏付けを行った。驚いたことに、DENVはRBC製剤においても産生され、赤血球以外の網状赤血球等の関与が示唆される結果となった。
結論:DENVは本来不安定で、保管期間を通じて急速に減少するが、ウイルス増殖と細胞の機能に依存しない効果の両者によってその減少は緩和される。しかしながら保管期間における全体的かつ急速な減少は、長く保管したPLT製剤及びRBC製剤の方がより安全である可能性を示唆している。今回のデータは、他のより安定であると考えられるRNAウイルスが、血液細胞製剤の保管期間中に存続する可能性についての認識を促している。

報告企業の意見	今後の対応
デングウイルスは、保存前白血球除去処理後の血小板製剤及び赤血球製剤にて、各々の製剤保管温度で有効期間内は複製したという報告である。	今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

Dengue virus persists and replicates during storage of platelet and red blood cell units

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BACKGROUND: Dengue virus (DENV) is a transfusion-transmissible arbovirus that threatens blood donor systems with approximately 200 million high-titer asymptomatic infections occurring annually. Here we investigated the viability of DENV during storage of donor-derived platelet (PLT) and red blood cell (RBC) units. While purified PLTs have been shown to generate viable DENV, RBCs are replication incompetent. Combined with different storage criteria, distinct virus persistence profiles were anticipated in PLT and RBC units.

STUDY DESIGN AND METHODS: Mimicking the virus titer of asymptomatic donors, purified DENV was spiked (10^5-10^6 infectious units/mL) into PLT or RBC units produced and stored according to blood bank operating procedures. DENV was measured by infectious plaque-forming assays and by quantitative reverse transcription–polymerase chain reaction.

RESULTS: In both PLT (7 days, 20-24°C) and RBC (42 days, 1-6°C) units, infectious DENV persisted throughout storage despite logarithmic decay. In buffer alone, DENV infectivity was insignificant by Day 1 at 20 to 24°C or 14 days at 1 to 6°C. Infectious virus production was identified in stored PLT units using a translation inhibitor and supported by virus genome replication. Surprisingly, DENV was also produced in RBC units, implying the involvement of cells other than RBCs.

CONCLUSION: Both virus propagation and effects independent of cell function mitigate the intrinsic lability of DENV. Nevertheless, the overall rapid storage decay suggests that aged PLT and RBC units may be safer. These data raise awareness to the possible persistence of other conceivably more robust RNA viruses during the storage of cellular blood products.

Dengue virus (DENV) has been predicted to infect almost 400 million people annually,[1] making it the most prevalent arbovirus on the globe.[2] Approximately one-quarter of infected people experience painful self-limiting symptoms, known as dengue "breakbone" fever. The World Health Organization estimates that approximately 500,000 of these escalate to life-threatening dengue hemorrhagic fever or dengue shock syndrome, which require hospitalization and cause approximately 20,000 deaths each year. The DENV mosquito vector (primarily *Aedes* sp.) is endemic to the tropics and subtropics. However, its geographic

ABBREVIATIONS: DENV = dengue virus; MOI = multiplicity of infection; PFU = plaque-forming units; qRT-PCR = quantitative reverse transcription–polymerase chain reaction; SFM/BSA = serum-free Medium 199 containing bovine serum albumin; (+)ssRNA = single strand of positive-sense RNA.

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distribution, and that of the virus, is broadening due to global warming and other factors. DENV incidence has increased 30-fold over the past 50 years, placing approximately 40% of the world's population at direct risk.[3] Locally acquired North American reports now reach as far north as Florida, New Mexico, and Texas.[4-6]

Although dengue illness is of paramount concern, more than 200 million asymptomatic infections per annum are a threat to blood donation systems because these too have a typically high virus titer of up to 10^9 copies/mL.[7] Approximately 2% of illness in travelers returning from endemic regions is caused by DENV.[8,9] Therefore, blood donations collected around the world have the potential to conceal DENV due to the lengthy asymptomatic period.[10] DENV infections involve a 3- to 14-day window period before signs of infection may develop. Combined with those who never become symptomatic, there is high probability for contaminated blood donation. Indeed, incidents of transfusion-transmitted DENV have been confirmed.[7,11,12] Understanding the persistence of DENV viability in donor-derived blood components is fundamental to predicting the risk that DENV poses to the blood supply.

DENV is a member of the *Flavivirus* genus and has four genetically related but antigenically distinct serotypes (DENV1-4). These complicate pathology and vaccine development due to antibody-dependent enhanced infection.[13] Typical of *Flaviviruses*, the DENV genome consists of a single strand of positive-sense RNA ((+)ssRNA). Virus-encoded proteins are translated by host cellular cytosolic organelles, leading to amplification of the DENV genome and progeny assembly. Containing ribosomes, endoplasmic reticulum, mitochondria, and Golgi apparatus, platelets (PLTs) are known to be translation competent,[14] although anucleate. We have recently shown that purified PLTs are permissive to DENV, translate the (+)ssRNA genetic material, and thereby allow the production of new infectious virus.[15] This adds to the mechanism of DENV-mediated modulation of PLT function reported by other laboratories[16,17] and helps to explain why thrombocytopenia is common to both mild and severe forms of DENV pathology.[18]

The direct binding and replication of DENV by purified PLTs[15] not only has pathologic implications, but also suggests that DENV virus may reproduce in PLTs that are prepared for transfusion as a biopharmaceutical. Therefore, in the current study DENV persistence was quantified after inoculation of PLT units produced and stored under industry standard conditions. Even less is known about DENV persistence within red blood cell (RBC) units, which was also evaluated here. Our results demonstrate infectious DENV throughout the storage of both PLT and RBC units. Of note, infectious virus progeny were produced within these stored cellular blood products, extending virus persistence. These results highlight the risk of DENV if penetration into the blood system is enabled via asymptomatic donors.

MATERIALS AND METHODS

Reagents and proteins

Chloroform, 2-propanol, and NaCl (Fisher Scientific, Ottawa, ON, Canada); polyethylene glycol 8000 (PEG), methylcellulose, bovine serum albumin (BSA), cycloheximide, methylcellulose (Sigma, Oakville, ON, Canada); leukocount reagent (CD41 antigen, BD Bioscience, Mississauga, ON, Canada); phycoerythrin (PE)-labeled anti CD62P and IgG_1-PE (Beckman-Coulter, Mississauga, ON, Canada); TRIzol reagent, Medium 199, and Turbo DNase (Life Technologies, Burlington, ON, Canada); RNeasy micro kit (Qiagen, Toronto, ON, Canada); iScript advanced cDNA synthesis kit and iTaq universal SYBR green supermix (BioRad, Mississauga, ON, Canada) were from commercial suppliers, as indicated.

PLT and RBC unit production

This study was approved by the research ethics boards of Canadian Blood Services and the University of British Columbia. Informed consent was obtained before whole blood donation at the Canadian Blood Services Network Center for Applied Development laboratory (www.blood.ca/en/blood/blood-research) where subsequent component production was carried out using standard operating procedures. The buffy coat (top/bottom) method, as previously described,[19,20] was used to produce PLT and RBC units prepared from 500 mL of whole blood into collection bags (MacoPharma, Tourcoing, France; LQT7291LX, Leucoflex LCR-Diamond quadruple bottom-and-top system, 500 mL of CPD/SAGM). The buffy coat fractions from four ABO-matched individual donors and plasma from one of the male donors were pooled and leukoreduced (Optipure PLTs, Fenwal, Lake Zurich, IL) to produce the PLT units. Each leukoreduced RBC unit was derived from a single donor. All units were stored under standard blood bank conditions. PLT units were stored at 20 to 24°C with agitation on a PLT incubator (Thermo Forma, Fisher Scientific). RBC units were stored at 1 to 6°C without agitation. After cell component production, 3 ABO-matched PLT or 2 ABO-matched RBC units were transferred into a large 1-L component bag. Once combined, the pooled units were gently mixed and separated (approx. 125 mL) into five conventional product-specific storage bags (MacoPharma; PLT bags were gas permeable with no bis(2-ethylhexyl) phthalate [DEHP] plasticizer; RBC bags had DEHP) fitted with a sampling site.

The cell types contained in each PLT and RBC unit were quantified by flow cytometry using a hematology analyzer (ADVIA 120, Siemens, Deerfield, IL). On average, PLT units contained $8.9 \times 10^{11} \pm 1.3 \times 10^{11}$/L PLTs, less

than 2×10^7/L white blood cells (WBCs), and $9.5 \times 10^{10} \pm 2.4 \times 10^{10}$/L RBCs (n = 19; ± SD). RBC units contained on average $7.0 \times 10^{12} \pm 0.5 \times 10^{12}$/L RBCs, $4.5 \times 10^9 \pm 3.3 \times 10^9$/L PLTs and $2.9 \times 10^7 \pm 2.1 \times 10^7$/L WBCs (n = 15; ± SD). Cellular profiles did not change throughout the experimental storage period. For some experiments, the requirement for cellular integrity was evaluated by subjecting PLT and RBC units to three cycles of freezing at −80°C followed by a rapid thaw in a 37°C water bath before inoculation with DENV at conventional storage temperature and conditions.

DENV purification

DENV Serotype 2 (DENV2; New Guinea C strain) was the primary focus of the study. When virus was available for certain experiments this was compared to DENV1 (Hawaii strain), DENV3 (H87 strain), and DENV4 (H241 strain) and data are presented as supplementary material. The initial inoculum for each virus serotype was provided by Dr M. Drebot (Viral Zoonoses Laboratory, Public Health Agency of Canada, Winnipeg, MB, Canada). To generate purified DENV, confluent African green monkey kidney cell (Vero, CCL-81: ATCC, Manassas, VA) monolayers were inoculated with individual DENV serotypes in serum-free Medium 199 containing BSA (1 mg/mL; SFM/BSA). All subsequent preparative steps were performed at 4°C to preserve DENV viability. The infected cell supernatants were collected daily and clarified at 180 × *g* for 10 minutes. Virus-containing supernatants were then incubated with PEG (10% wt/vol) and NaCl (1.5 mol/L) overnight with gentle mixing. After centrifugation at 6000 × *g* for 20 minutes, the virus-containing pellets were resuspended in phosphate-buffered saline (PBS), layered onto a stepwise sucrose (10%-30%-60%) gradient and centrifuged at 25,000 × *g* for 3.5 hours. The virus band at the 30%/60% sucrose interface was removed, suspended in PBS, and centrifuged at 25,000 × *g* for 1.5 hours. The resulting purified DENV pellet was frozen in PBS at −80°C. Virus preparations were evaluated for purity and quantified by electron microscopy, as we previously described for herpes viruses.[21] The amount of infectious DENV in each virus preparation was measured in Vero cells using standard plaque assays.[15] Newly thawed virus aliquots were used for each experiment.

Infectious DENV quantification

The viability of purified DENV was determined by plaque assays using Vero cells grown in six-well plates. Before inoculation, the cells were washed once with PBS and SFM/BSA. Samples were then serially diluted in SFM/BSA and incubated with the cell monolayer for 90 minutes at 37°C (1 mL/well). After the inoculum was removed, the cells were washed and replaced with reduced serum (2%) medium in a methylcellulose (1%) overlay. The infection was allowed to proceed for 5 days for DENV2 or 7 days for DENV1, 3, and 4, before staining with crystal violet to visualize and count the number of productive infectious events (i.e., plaque-forming units [PFU]) using a low-power dissecting microscope.

DENV RNA quantification

Two-step quantitative reverse transcription–polymerase chain reaction (qRT-PCR) was conducted as previously described[15] using TRIzol reagent to extract DENV RNA from PLT and RBC unit samples followed by purification with the RNeasy micro kit. DENV RNA samples (1 μg) were converted into first-strand cDNAs at 42°C for 30 minutes and then 85°C for 5 minutes using iScript advanced cDNA synthesis kit. qRT-PCR was performed using iTaq universal SYBR green supermix with denaturation at 95°C for 30 seconds, followed by 40 cycles of amplification at 95°C for 15 seconds and melting curve analysis at 60°C, 1 minute conducted on a real-time PCR system (Model 7900HT, Applied Biosystems, Foster City, CA) in quadruplicate for each experimental time point. The number of RNA genome copies was derived from a standard curve of serial dilutions of in vitro RNA transcripts for each DENV serotype ranging from 10 to 10^9 known copies of RNA quantified by electron microscopy. The standard curve samples were run in triplicate as well as a no-template background control. The limit of detection for qRT-PCR was 10 copies of DENV RNA. The serotype-specific primers used were as follows: DENV1 (NC_001477, forward CCCGGCACATCTGGATCTCCTATC and reverse GTCCTCAATCTCTGGTAGAGGCCC); DENV2 (NC_001474, forward CTCTCAGTGAACTGCCGGAGACC and reverse CGTACCATAGGAGGATGCTAGCCG); DENV3 (NC_001475, forward CCTACCTCTACCATGGGCATCAGG and reverse GTAGCTCCTGTCAGTGCGGTATGC); and DENV4 (NC_002640, forward GGCGGCTTACGTGATGAGT GGTAG and reverse CCTCGACGTCCCGTATGGAGAAAG). To assess PLT and RBC RNA stability throughout the storage period in the presence of DENV, PLT β-actin (*ACTB;* forward CCAGGCTGTGCTATCCCTGTACG and reverse CATGAGG TAGTCAGTCAGGTCCCG) or RBC glyceraldehyde-3-phosphate dehydrogenase (*GAPDH;* forward GGCTCCC ACCTTTCTCATCC and reverse GGGCCATCCACAGTCTTC TG) reporter gene levels were also monitored. Data were analyzed using computer software (SDS 2.3, Applied Biosystems).

Inoculation of blood cell units with DENV

DENV in autoclaved PBS (1 mL final volume) was added to PLT and RBC units giving a final concentration of 10^5 to 10^6 PFU/mL. PBS without virus was added to a control unit. After inoculation, the PLT and RBC numbers were confirmed by flow cytometry (ADVIA, Siemens) and all units were stored under standard blood bank conditions. At the end of the storage period (PLTs, 7 days; RBCs, 42

days), each blood cell unit was determined to be free of bacterial contamination as assessed by a bacterial detection system (BacT/ALERT, bioMérieux, St Laurent, QC, Canada) as well as growth in tissue culture. Each PLT or RBC experiment was repeated at least three times over several months using products generated from several unique blood donors.

Statistical analysis

Where appropriate, statistical comparisons were performed using t test between two groups. Differences were considered significant at p values of not more than 0.05 and indicated with asterisks in the figures with specific values given in the corresponding legend. All values are expressed as either mean ± standard error of the mean (SEM) or mean ± standard deviation (SD) as noted.

RESULTS

Infectious DENV persists and genome replicates during PLT unit storage

PLT units were inoculated with purified DENV2 at a primary cell ratio of 0.0001 (i.e., multiplicity of infection [MOI]), which is intermediate in the reported broad range for asymptomatic viremia.[7,22-24] During the course of storage under blood bank conditions, quantification of infectious virus demonstrated a logarithmic decrease of infectivity (Fig. 1A). DENV1, 3, and 4 (Fig. S1A, available as supporting information in the online version of this paper) had similar profiles. Of note, all serotypes remained infectious for the duration of the current 5-day standard for PLT storage (approx. 300-8000 PFU/mL) and even up to 7 days postinoculation ranging from approximately 150 to 5000 PFU/mL depending on the serotype.

Our recent report[15] showed that at a relatively high MOI of 5, purified PLTs were capable of replicating the (+)ssRNA DENV genome. In this study, stored PLT units were evaluated for similar activity, but at a much lower MOI typical of asymptomatic viremia (0.0001). Consistent with the earlier work, qRT-PCR showed that the DENV2 genome increased by approximately 3-fold during the 7-day storage period (Fig. 1B). This reached a maximum by Day 4 and declined. DENV1, 3, and 4 (Fig. S1B) had similar qRT-PCR time courses as that of DENV2. To demonstrate the specificity of the DENV genome profiles, the samples were also assayed for PLT β-actin RNA (i.e., *ACTB*). As shown in Fig. 1C, unlike the DENV genome, *ACTB* did not vary over the 7-day storage period.

Infectious DENV persists and genome replicates during RBC unit storage

The persistence of DENV viability in cellular blood products was further studied in RBC units (approx. 7×10^{12} RBCs/L). These were inoculated with the same virus concentration as that in the PLT unit experiment (Fig. 1), which is typical of a donation from an asymptomatic donation. The resulting MOI in RBC units was respectively lower (0.000014). Figure 2A shows that DENV2 decayed much more slowly in RBC units than PLT units with more than 90% infectivity remaining by 7 days postinfection. All four serotypes persisted over the duration of storage (Fig. S2A, available as supporting information in the online version of this paper). After 28 days, 84 to 435 PFU/mL were measured.

Unlike PLTs, RBCs do not have the required intracellular organelles to copy DENV (+)ssRNA. Regardless, we

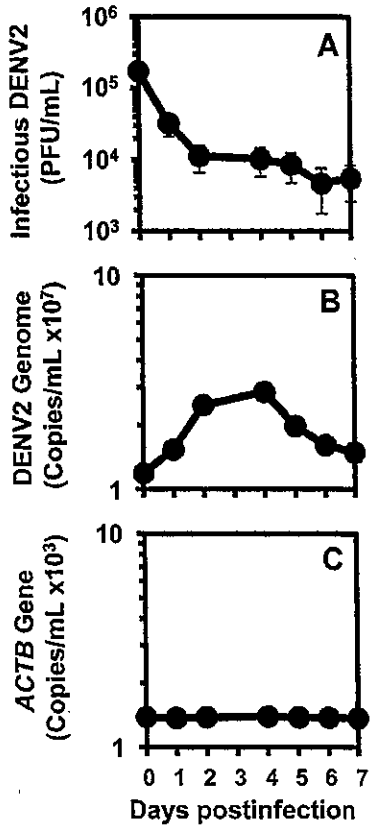

Fig. 1. DENV2 survival and genome replication in stored pooled PLT units. PLT units (approx. 8.9×10^{11} PLTs/L) were inoculated with purified DENV2 (approx. 10^5 PFU/mL) and stored under standard blood bank conditions (20-24°C, constant agitation). (A) Samples were removed daily and assayed for infectious virus by following plaque formation 5 days postinfection of confluent Vero cells. (B) The inoculated PLT unit samples were assayed for DENV2 genome by qRT-PCR using a standard derived from quantified virus. (C) At the same time, the DENV2-inoculated PLT unit samples were assayed for the β-actin gene (*ACTB*). (DENV2 infectious virus and genome, n = 4 ± SEM; *ACTB*, n = 2 ± SD. Error bars may be smaller than the size of symbols.)

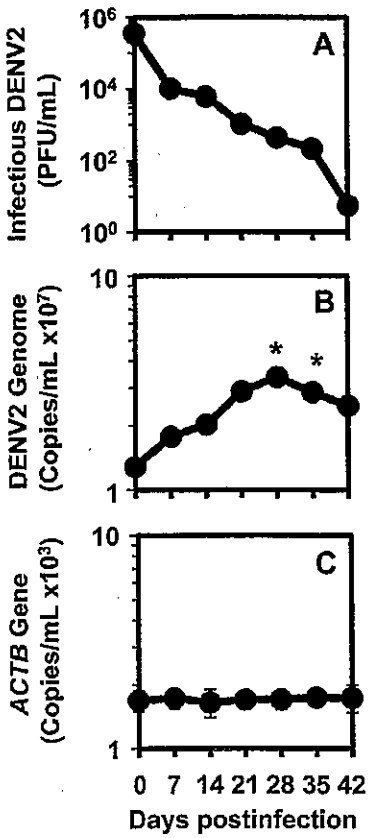

Fig. 2. DENV2 survival and genome replication in stored RBC units. RBC units (approx. 7×10^{12} RBCs/L) were inoculated with purified DENV2 (approx. 10^5 PFU/mL) and stored under standard blood bank conditions (1-6°C, stationary). (A) Samples were removed weekly and assayed for infectious virus using standard plaque assays in Vero cells as in Fig. 1. (B) The inoculated RBC unit samples were assayed for DENV2 genome by qRT-PCR using a standard derived from quantified virus. (C) At the same time, the DENV2-inoculated PLT unit samples were assayed for the glyceraldehyde-3-phosphate dehydrogenase gene (*GAPDH*). (DENV2 infectious virus n = 6 ± SEM, and genome n = 4 ± SEM; *ACTB*, n = 2 ± SD. Error bars may be smaller than the size of symbols. *p = 0.0003 when compared to Day 0.)

evaluated the effect of storage on DENV2 in RBC units at the genetic level. Surprisingly, viral (+)ssRNA increased similar to PLT units and showed an approximately threefold peak increase in genome production by Day 28 (Fig. 2B, p = 0.05). DENV1, 3, and 4 had comparable qRT-PCR profiles (Fig. S2B). To confirm the specificity of the observed DENV genome increase, the RBC glyceraldehyde-3-phosphate dehydrogenase gene (i.e., *GAPDH*) was evaluated in samples that were simultaneously obtained in the DENV2 inoculation experiment. Figure 2C shows that unlike the DENV genetic material, *GAPDH* was invariant, indicating selective enhancement of the virus genome.

DENV persistence is dependent on intact and disrupted cell properties

To investigate the contribution of PLTs and RBC cells to DENV2 persistence in stored units, infectivity was compared under three conditions (Fig. 3). The first condition was intact cells (Fig. 3, closed circles), where purified DENV2 was added to either PLT units or RBC units as in Figs. 1A and 2A, respectively. The second condition disrupted the PLTs and RBCs by subjecting the units to three freeze-thaw cycles (Fig. 3, open circles), which resulted in approximately 2-log reduction of infectious virus over the storage duration compared to the untreated cells (Fig. 3, closed circles). Since the loss of DENV viability was even more pronounced when stored in PBS alone (Fig. 3, open squares), effects conferred by both intact and disrupted PLTs and RBC units were suggested to prolong virus persistence. These results clearly demonstrate the intrinsic lability of DENV2.

The infectivity results (Figs. 3A and 3C) were further evaluated using qRT-PCR, which showed very similar trends when comparing stored PLT and RBC units due to the freeze-thaw process (Fig. 3, open circles) or complete lack of cells (Fig. 3, open squares). These concordant data suggest that the instability of viral (+)ssRNA under these conditions is partly the basis for lost viability. Interestingly, DENV2 genome copies/mL was observed to increase in both intact PLT (Fig. 3B, closed circles; p = 0.035) and RBC units (Fig. 3D, closed circles; p = 0.0003) indicating that the overall infectivity decay may be attenuated by new virus replication.

Infectious DENV is produced in PLT and RBC units

The finding that intact PLT and RBC units produce DENV RNA and freeze-thawing the cells attenuates this effect with concomitantly reduced virus infectivity may suggest that viable DENV is being produced. An alternative explanation is that the freeze-thaw cycle could release cellular nucleases that may contribute to viral RNA damage but this is unlikely due to protection of the genetic material by the viral envelope and nucleocapsid. Nevertheless, to avoid this ambiguity and to further investigate the possibility that new virus is produced during storage of cellular blood products, the translation elongation inhibitor, cycloheximide, was added to intact cells. When samples were withdrawn from PLT or RBC units over the storage period and assayed for DENV2 (Fig. 4, top panels), cycloheximide was shown to reduce infectious virus detection by plaque formation assays. For both PLTs and RBCs, viable cellular translational machinery enhances productive DENV2 replication by at least several fold (Fig. 4, bottom panels). Thus, generation of infectious virus contributes to prolonging the overall decay of DENV2 in both cellular blood products that were evaluated.

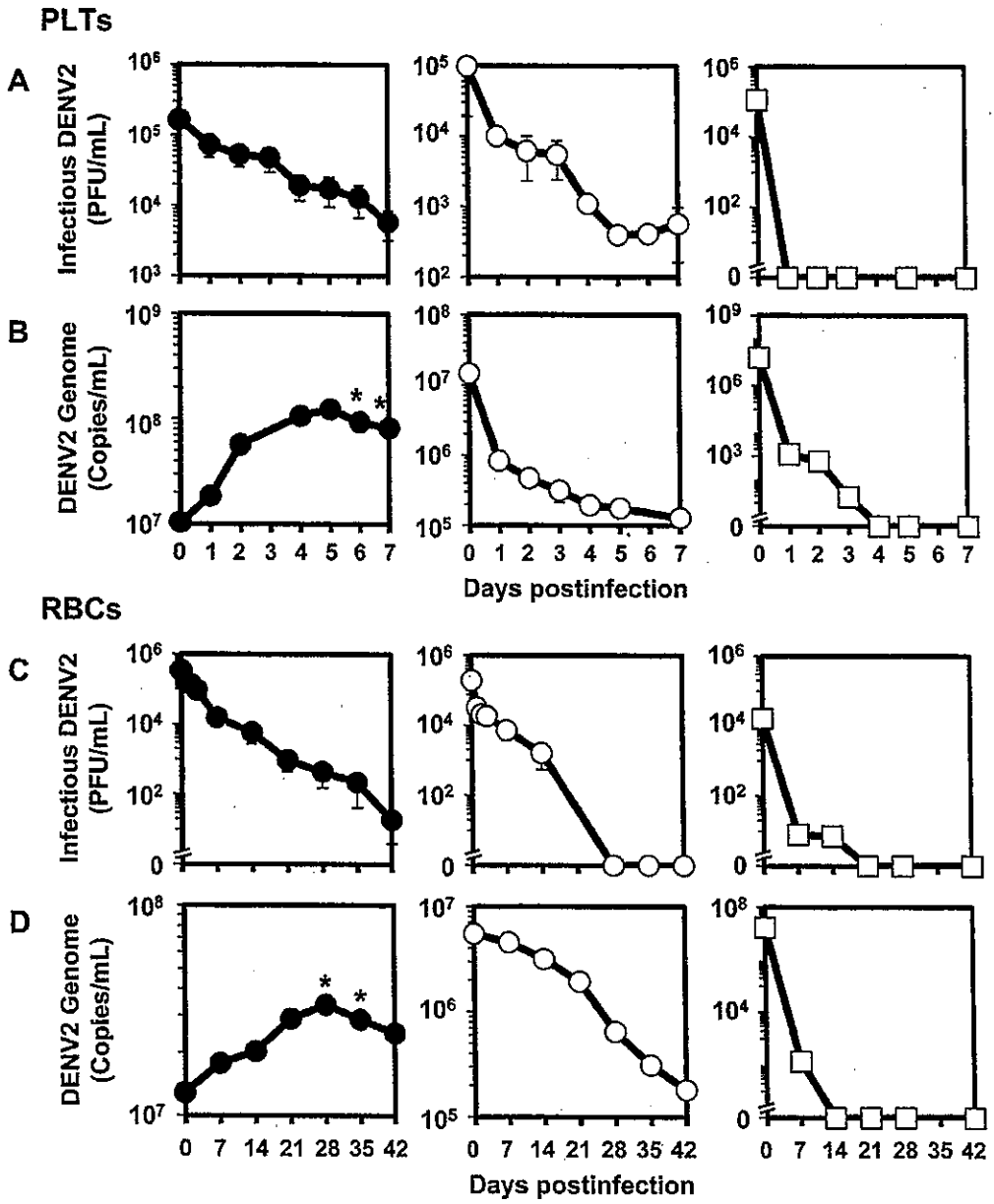

Fig. 3. DENV2 persistence is dependent on biologic and carrier effects. (Top panels) Intact PLT units (●; 2×10^{11} PLTs/L) were subjected to three cycles of freeze (−80°C)/thaw (37°C; ○) or PBS (□;) were inoculated with purified DENV2 (approx. 10^5 PFU/mL) and assayed for infectious DENV2 by plaque formation (infectious DENV2) or viral RNA by qRT-PCR (DENV2 genome). (Bottom panels) Inoculation experiments were conducted as in A and B except on RBC units (approx. 7×10^{12} RBC/L). (PLTs plus DENV2 [infectious virus and genome n = 4 ± SEM] or RBCs plus DENV2 [infectious virus n = 6 ± SEM and genome n = 4 ± SEM]; frozen PLTs or RBC plus DENV2 [infectious virus and genome], n = 3 ± SEM; PBS plus DENV2 [infectious virus and genome], n = 2 ± SD. Error bars may be smaller than the size of symbols. [A] *p = 0.035 when compared to Day 0. [C] *p = 0.0003 when compared to Day 0.)

DISCUSSION

Blood donor systems worldwide are vulnerable to DENV because of the high number of asymptomatic carriers (approx. 200 million/year). Testing has confirmed many cases of viremic blood donors in Puerto Rico (approx. 0.19%), Honduras (approx. 0.15%), and Brazil (0.06%).[7,23] A similar frequency (0.1%) was extrapolated by mathematical modeling of a local outbreak in Australia.[25]

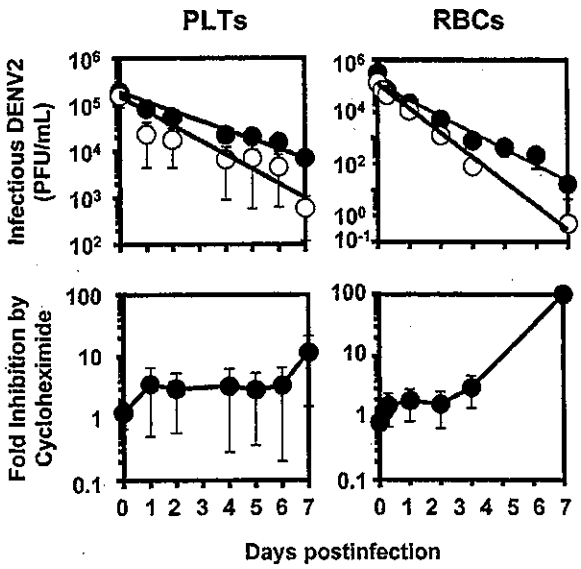

Fig. 4. Generation of DENV2 progeny in stored blood products. (Top panels) PLT units (2×10^{11} PLTs/L) or RBC units (7×10^{12} RBCs/L) were inoculated with purified DENV2 (approx. 10^5 PFU/mL) in the presence (○) or absence (●) of cycloheximide (1 mmol/L final) and assayed for infectious virus by plaque formation. (Bottom panels) PFU without/PFU with cycloheximide expressed as fold infectious virus inhibition due to treatment with the translation inhibitor. (PLTs plus DENV2 [n = 5 ± SEM] or plus DENV2 and cycloheximide [n = 3 ± SEM]; RBCs plus DENV2 [n = 6 ± SEM] or plus DENV2 and cycloheximide [n = 2 ± SD]. Error bars may be smaller than the size of symbols.)

Encompassing the entire country in a model still suggested that DENV-positive donors would be captured in the blood pool, but with approximately 10-fold lower incidence than local outbreaks.[26] The reported virus load of asymptomatic donors ranges broadly from 10^3 to 10^9 copies/mL.[7,24] Although penetrance of DENV into blood systems has been established, it is unknown whether this pathogen remains infectious throughout the storage of respective blood products. In the current study the persistence of DENV1 to 4 infectivity within leukoreduced PLT and RBC units stored under blood center criteria was investigated. Highly purified virus was used to exclude contributions of host cellular debris or plasma found in semipurified virus or patient samples. Purified DENV was added to PLT and RBC units at 10^5 to 10^6 copies/mL, which is intermediate within the reported range of infected donor blood.[7] For both types of blood cells, all four DENV serotypes remained infectious over the duration of storage, which is consistent with reported cases of transfusion-transmitted disease.[7,11,12] For the first time we also show that DENV viability in both PLT and RBC units is not stable, but decays exponentially over the shelf life duration. Thus, the time between sampling and pathogen quantification is a variable not previously considered, to our knowledge. The rapid decay of DENV in PLT and RBC units may furthermore explain why the incidents of transfusion transmission are relatively rare compared to DENV prevalence. More importantly, these data suggest that the age of the blood product may attenuate the transmission of DENV by transfusion.

Compared to buffer alone, the infectivity of DENV was greatly prolonged in stored PLT or RBC units. The instability of DENV in the absence of cells has been reported previously.[27] Our data show that components within PLT and RBC units promote the survival of DENV. When either type of donor cell unit was inoculated, infectious virus was detectable for the entire storage duration, whereas in buffer alone there was complete loss of infectivity before 24 hours at the PLT storage temperature (20-24°C) or after 2 weeks at the RBC storage temperature (1-6°C). This difference in viral decay rate coincided with a prolonged rise in DENV genome copy number for the latter. To demonstrate that the stabilizing effects of PLT and RBC units on DENV involved viral protein synthesis, we compared DENV infectivity when a translation elongation inhibitor (cycloheximide) was added during storage. The finding that this treatment enhanced viral decay demonstrated that infectious virus was produced during storage. However, the amount produced was insufficient to reverse the dramatic logarithmic overall decay profile. Freeze-thaw treatment of cells also attenuated DENV survival and prevented RNA replication. Flow cytometry forward and side scatter measurements and hemolysis assays (not shown) confirmed the disruption of cells after freeze-thaw treatment of PLT and RBC units (respectively). These data further supported the generation of viral progeny during storage because DENV genome amplification was observed only when cells and presumably the subcellular organelles were intact. Loss of DENV infectivity was far greater during storage in buffer alone compared to either cycloheximide or freeze-thaw treatment of cells, suggesting that PLT or RBC units prolong the infectivity of DENV by a combination of new virus production and effects not requiring intact cellular function.

While overwhelming literature supports a direct DENV-PLT link,[15,28,29] there is little to presume a similar pathophysiologic involvement for RBCs, except perhaps the inference from an altered RBC sedimentation rate during DENV disease.[30] Moreover, RBCs contrast PLTs by lacking the requisite translational organelles and, therefore, cannot directly generate protein encoded by the viral RNA genome. Therefore, it was somewhat surprising to observe an increase in the DENV genome copy number and infectious virus production during storage of RBC units. These data imply the involvement of other cells inherent to RBC units. WBCs are considered to be a primary target for DENV replication[31] and PLTs are also

known to be permissive.[15] Although in relatively low abundance within the RBC units used here (0.0004 and 0.06% vs. RBCs/mL, respectively), these may account for some of the observed DENV genome amplification once the virus-encoded polymerase is translated. Not previously considered as mediators of RNA virus replication is the RBC precursor, reticulocytes. These constitute a comparatively high proportion of cells in RBC units at approximately 1% (approx. 7×10^{10} cells/L)[32,33] and are long known to have translational function.[34] Data from studies involving RBC units has shown that reticulocytes diminish over the 42-day storage period, but nevertheless persist[33] despite maturation and loss of translational capacity. Therefore, the observation of increased DENV genome production suggests reticulocytes may also be a reservoir for DENV production during RBC unit storage. Regardless, these findings insinuate that other permissive RNA viruses may also be propagated during storage of cellular blood products.

Cumulatively, the results presented here demonstrate that the viability of DENV1 to 4 persists in PLT and RBC units over the respective storage duration. The bases of this persistence are complex and consist of the novel production of virus during storage, effects not requiring intact cells or protein synthesis (also conferred by plasma; data not shown), and low storage temperature. These factors diminish the intrinsic decay of DENV, thereby prolonging infectivity. Virus generation in stored units implies that cellular biomarkers could be used as a surrogate to detect DENV contamination. As examples, PLT activation or RBC permeability may be affected. However, at least at the relatively low virus-to-cell ratio evaluated in this study, insignificant effects were observed on CD62P expression or hemolysis in PLT or RBC units, respectively (data not shown). At least these typical cell quality markers would not be applicable surrogates for current DENV screening methods. Direct DENV screening protocols have recently been implemented in some endemic regions, but at significant cost. The advent of universal inactivation technologies promises to reduce the risk of pathogen transmission for some blood products, although certain toxicity concerns and incompatibility with cellular components continue to delay its general implementation.[35] Nevertheless, for as-yet-unknown reasons DENV inactivation is relatively resistant to these technologies in the presence of cells.[36] The infectivity decay profiles presented in this study, combined with screening, pathogen inactivation, and vigilant travel deferral methods, may help to further reduce the risk of DENV transmission.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's Web site:

Fig. S1. DENV serotype survival and genome replication in stored pooled platelet units. Platelet units (~8.9×10^8 platelet/mL) were inoculated with purified DENV1, 2, 3, or 4 (~10^5–10^6 PFU/mL), and stored under standard blood bank conditions (20-24°C, constant rocking). A. Samples were removed daily and assayed for infectious virus by following plaque formation 5 days (DENV2) or 7 days (DENV1, 3, and 4) post-infection of confluent Vero cells. B. The inoculated platelet unit samples were assayed for DENV1-4 genome by qRT-PCR using a standard derived from quantified virus. DENV1, 3, and 4 infectious virus and genome $n = 3 \pm$ SEM. DENV2 infectious virus and genome $n = 4 \pm$ SEM. Error bars may be smaller than the size of symbols.

Fig. S2. DENV serotype survival and genome replication in stored red cell units. RBC units (~7×10^9 RBC/mL) were inoculated with purified DENV1, 2, 3, or 4 (~10^5-10^6 PFU/mL), and stored under standard blood bank conditions (1-6°C, stationary). A. Samples were removed daily and assayed for infectious virus using standard plaque assays in Vero cells as in Figure 1. B. The inoculated RBC unit samples were assayed for DENV1-4 genome by qRT-PCR using a standard derived from quantified virus. DENV1, 3, and 4, infectious virus and genome, $n = 3 \pm$ SEM. DENV2 infectious virus $n = 6 \pm$ SEM and genome $n = 4 \pm$ SEM. Error bars may be smaller than the size of symbols. * indicates $p < 0.05$ when compared to Day 0.

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識別番号・報告回数			報告日	第一報入手日 2016 年 07 月 11 日	新医薬品等の区分		厚生労働省処理欄
一般的名称	①②③⑨⑩⑪ pH4 処理酸性人免疫グロブリン ④⑤⑥⑦⑧ポリエチレングリコール処理人免疫グロブリン ⑫⑬ 人免疫グロブリン			-		公表国 オーストラリア	
販売名 (企業名)	①献血ポリグロビン N5%静注 0.5g/10mL (日本血液製剤機構) ②献血ポリグロビン N5%静注 2.5g/50mL (日本血液製剤機構) ③献血ポリグロビン N5%静注 5g/100mL (日本血液製剤機構) ④献血ヴェノグロブリン IH5%静注 0.5g/10mL (日本血液製剤機構) ⑤献血ヴェノグロブリン IH5%静注 1g/20mL (日本血液製剤機構) ⑥献血ヴェノグロブリン IH5%静注 2.5g/50mL (日本血液製剤機構) ⑦献血ヴェノグロブリン IH5%静注 5g/100mL (日本血液製剤機構) ⑧献血ヴェノグロブリン IH5%静注 10g/200mL (日本血液製剤機構) ⑨献血ポリグロビン N10%静注 2.5g/25mL (日本血液製剤機構) ⑩献血ポリグロビン N10%静注 5g/50mL (日本血液製剤機構) ⑪献血ポリグロビン N10%静注 10g/100mL (日本血液製剤機構) ⑫グロブリン筋注 450mg/3mL「JB」 (日本血液製剤機構) ⑬グロブリン筋注 1500mg/10mL「JB」 (日本血液製剤機構)			研究報告の公表状況	EcoHealth 2015; 12(2): 347-353		
研究報告の概要	ロスリバーウイルスとバルマフォレストウイルスに対する抗体の血清陽性率： 極端な気象現象後の輸血安全性への関連性の可能性； 気候変動は、血液供給の安全性に脅威を増し、ベクター媒介性病原体の感染率を増加させると予測される。2011 年初めに、オーストラリアは壊滅的な降雨と洪水を経験し、アルボウイルス感染の増加につながった。この降雨が増加した期間後のオーストラリアの血液供給にもたらされた可能性のあるリスクを調査するため、我々はロスリバーウイルス (RRV) とバルマフォレストウイルス (BFV) をテストケースとして用いた。我々は、RRV は 2,500-58,000 例に 1 例、BFV は 2,000-28,000 例に 1 例の輸血のリスクを推定した。気候変動は、血液の安全性に対するアルボウイルスの脅威を増大させる可能性がある。						使用上の注意記載状況・その他参考事項等 代表として献血ヴェノグロブリン IH5%静注 0.5g/10mL の記載を示す。 2. 重要な基本的注意 (1)本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-1 抗体陰性で、かつ ALT(GPT) 値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査 (NAT) を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分からポリエチレングリコール 4000 処理、DEAE セファデックス処理等に

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報告企業の意見	今後の対応	
アルボウイルス（arbovirus）は、節足動物内で増殖し、それらの吸血活動によって脊椎動物に伝播されるウイルスの総称。節足動物体内での増殖を行わず、機械的伝播のみを起こすウイルスはアルボウイルスに含まれない。およそ 10 のウイルス科が含まれ、ヒトに病原性を示すものは 100 種を超える。アルボウイルスのうちフラビウイルス科、ブニヤウイルス科に属するものには重篤な症状を引き起こすものが多い。万一、原料血漿にアルボ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。	より人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理、ウイルス除去膜によるろ過処理及び pH3.9～4.4 の条件下での液状インキュベーション処理を施しているが、投与に際しては、次の点に十分注意すること。

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EcoHealth

Short Communication

Seroprevalence of Antibodies to Ross River and Barmah Forest Viruses: Possible Implications for Blood Transfusion Safety After Extreme Weather Events

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Abstract: Climate change is predicted to increase the transmission of many vector-borne pathogens, representing an increasing threat to a safe blood supply. In early 2011, Australia experienced catastrophic rainfall and flooding, coupled with increased arbovirus transmission. We used Ross River (RRV) and Barmah Forest (BFV) viruses as test cases to investigate the potential risk posed to Australia's blood supply after this period of increased rainfall. We estimated the risk of collecting an infected donation as one in 2,500–58,000 for RRV and one in 2,000–28,000 for BFV. Climate change may incrementally increase the arbovirus threat to blood safety.

Keywords: arbovirus, climate, rainfall, blood donor, risk, safety, transfusion

Emerging transfusion-transmissible pathogens, including arboviruses such as West Nile (WNV), chikungunya (CHIKV) and dengue viruses (DENV), are a threat to transfusion safety (Petersen and Busch 2010; Dodd 2012; Faddy et al. 2013). Increased rainfall generally favours mosquito reproduction, resulting in increased arboviral transmission (Gould and Higgs 2009; Knope et al. 2013). Climate change is predicted to increase the transmission of many vector-borne pathogens, including arboviruses, representing a significant threat for the maintenance of a safe future blood supply (Semenza and Domanovic 2013).

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Ross River virus (RRV) and Barmah Forest virus (BFV) have the highest incidence among human arbovirus diseases in Australia (Knope et al. 2013). Both belong to the genus *Alphavirus*, have a transmission cycle with kangaroos and wallabies as the main reservoir hosts and share similar mosquito vectors (Chapman et al. 1999; Robertson et al. 2004; Hu et al. 2006; Kay et al. 2007; Jansen et al. 2009). Rainfall, temperature, tidal levels and humidity correlate with changes in the incidence of RRV and BFV infections in humans (Tong et al. 2004; Gatton et al. 2005; Jacups et al. 2008, 2011). Increased rainfall, however, is the primary climatic factor influencing RRV disease transmission in eastern Australia, due to its effect on the vector population

(Tong and Hu 2002; Hu et al. 2004). In the southern hemisphere summer of 2010–2011, eastern Australia experienced extreme rainfall (Coumou and Rahmstorf 2012). Rates of RRV infection were elevated nationally during the 2010–2011 summer compared to historical averages, and in some regions, the number of BFV cases also increased (Knope et al. 2013).

Both RRV and BFV can cause debilitating, rheumatic symptoms in humans (Mackenzie and Smith 1996). Subclinical infection and viraemia are common, so transmission through blood transfusion is possible; however, no cases have been reported in the literature (Harley et al. 2001; Shang et al. 2012). Transfusion-transmission has, however, been documented for other arboviruses, including WNV and DENV, and is suspected for CHIKV (Petersen and Busch 2010). We therefore aimed to determine the proportion of blood donors that had recently been exposed to RRV or BFV, assess the proportion of the donor population with evidence of previous infection and quantify the risk of collecting an infected blood donation following a period of increased rainfall as an indicator of theoretical transfusion-transmission risk.

Plasma samples were collected in the first half of 2011 from 'higher-risk' regions (defined as those that experienced increased rainfall in late 2010 to early 2011, coupled with increased arbovirus transmission: Bundaberg, Cairns, Mackay, Rockhampton, Toowoomba, Townsville and the Murray Valley). A 1–3 month lag between peak rainfall and elevated rates of RRV or BFV notifications is predicted (Gatton et al. 2004). Australian notification data confirmed samples were acquired during the peak viral transmission period in 2011 (Knope et al. 2013). Samples were tested for RRV and BFV IgM antibodies using an indirect enzyme linked immunosorbent assay (ELISA; Panbio, Brisbane, Australia), with positive samples confirmed by an in-house indirect immunofluorescence antibody test (IFA) (Harnett et al. 1985), modified for RRV or BFV. We detected RRV IgM antibodies, indicating recent infection, in 0.9% of donors from 'higher-risk' areas (Table 1). Region ($P = 0.017$) had an influence on the proportion of donors with RRV IgM. A similar proportion (1.2%) of donors from 'higher-risk' areas had evidence of a recent BFV infection (Table 1), also influenced by region ($P < 0.001$). Only one donor, from the Murray Valley area, had RRV and BFV IgM antibodies. Donors did not report diagnosed RRV or BFV infections pre- or post-donation, indicating either asymptomatic infection or, less likely, symptomatic infection without informing the Australian Red Cross Blood Service. Asymptomatic, viraemic individuals may donate with consequent risk for transfusion-transmission.

Additional samples were obtained from capital cities (Adelaide, Brisbane, Darwin, Hobart, Melbourne, Perth and Sydney). These samples, as well as those from Townsville, were tested for RRV and BFV IgG antibodies using an indirect ELISA (Panbio), with confirmatory testing performed using an in-house haemagglutination inhibition (HI) (Clarke and Casals 1958). We detected RRV IgG antibodies, indicating past infection, in 8.4% of donors, with region ($P < 0.001$), age ($P < 0.001$) and sex ($P < 0.001$) associated with previous infection (Tables 2, 3). Previous RRV exposure was observed in donors from all states; however, the highest IgG seroprevalence was in the tropics and sub-tropics, from where the majority of clinical cases are reported (Knope et al. 2013). Prevalence increased with age, probably due to increased lifetime exposure. We detected BFV IgG in a smaller proportion (1.7%) of donors, and this proportion was influenced by region only ($P = 0.007$; Tables 2, 3). No age dependency was observed for previous BFV infection, and while historical exposure did vary regionally, no clear latitudinal trend was observed. Previous BFV exposure was relatively low in all regions, while serological evidence of recent exposure was similar to that for RRV in some regions. After first being isolated in 1974, BFV infection in humans has been nationally notifiable since 1995 (Marshall et al. 1982; Herceg et al. 1996; Knope et al. 2013). BFV notification rates have increased in recent years (Knope et al. 2013), demonstrating that BFV infection may be increasing in endemic and epidemic regions.

The risk of collecting a RRV or BFV infected donation was estimated using modified published models (Weinstein et al. 1995; Shang et al. 2012; Faddy et al. 2013). The probability of collecting an infected donation (for each virus) was estimated over 6 months for each 'higher-risk' region. We assumed that the potential for collecting an infected donation directly correlated with the RRV or BFV IgM seroprevalence among the blood donor populations studied. The risk of collecting a donation containing RRV from 'higher-risk' regions was estimated at one in 7,333 (range 2,497–58,284) and for BFV was estimated at one in 5,616 (range 2,074–27,774; Figure 1). These risks are of a similar magnitude to previous estimates for RRV and DENV during outbreaks (Shang et al. 2012; Faddy et al. 2013). During DENV outbreaks, supplementary measures, such as donation restrictions, are implemented to mitigate the risk of transfusion-transmitted DENV (Faddy et al. 2013).

Table 1. Serological Evidence of Recent RRV or BFV Exposure (by RRV or BFV IgM) in Australian Blood Donors and Donations.

	Donors				Donations			
	Number tested	Reactive			Number tested	Reactive		
		Number	%	95% CI		Number	%	95% CI
RRV IgM								
Overall	5,114	46	0.90	0.64–1.16	5,935	46	0.78	0.55–1.00
Region								
Bundaberg	843	5	0.59	0.07–1.11	959	5	0.52	0.07–0.98
Cairns	701	2	0.29	0.00–0.68	969	2	0.21	0.00–0.49
Mackay	891	4	0.45	0.01–0.89	1,007	4	0.40	0.01–0.79
Rockhampton	903	15	1.66	0.83–2.49	1,030	15	1.46	0.72–2.19
Townsville	830	11	1.33	0.55–2.10	973	11	1.13	0.47–1.79
Murray Valley	946	9	0.95	0.33–1.57	997	9	0.90	0.25–1.36
Age group								
≤24	747	4	0.54	0.01–1.06	786	4	0.51	0.01–1.01
25–34	704	5	0.71	0.09–1.33	801	5	0.62	0.08–1.17
35–44	755	4	0.53	0.01–1.05	892	4	0.45	0.01–0.89
45–54	1,332	19	1.43	0.79–2.06	1,615	19	1.18	0.65–1.70
55–64	1,184	10	0.84	0.32–1.37	1,422	10	0.70	0.27–1.14
≥65	392	4	1.02	0.03–2.02	419	4	0.95	0.02–1.89
Sex								
Male	2,680	25	0.93	0.57–1.30	3,205	25	0.78	0.48–1.08
Female	2,434	21	0.86	0.50–1.23	2,730	21	0.77	0.44–1.10
BFV IgM								
Overall	5,033	61	1.21	0.91–1.51	5,791	68	1.17	0.90–1.45
Region								
Bundaberg	843	7	0.83	0.22–1.44	959	8	0.83	0.26–1.41
Cairns	701	8	1.14	0.35–1.93	969	12	1.24	0.54–1.93
Rockhampton	903	26	2.88	1.79–3.97	1,030	28	2.72	1.73–3.71
Toowoomba	810	0	0.00	–	863	0	0.00	–
Townsville	830	2	0.24	0.0–0.57	973	2	0.21	0.00–0.49
Murray Valley	946	18	1.90	1.03–2.77	997	18	1.81	0.98–2.63
Age group								
≤24	699	11	1.57	0.65–2.50	736	11	1.49	0.62–2.37
25–34	683	11	1.61	0.67–2.55	769	11	1.43	0.59–2.27
35–44	744	5	0.67	0.08–1.26	863	7	0.81	0.21–1.41
45–54	1,281	18	1.41	0.76–2.05	1,538	22	1.43	0.84–2.02
55–64	1,201	8	0.67	0.21–1.13	1,431	9	0.63	0.22–1.04
≥65	425	8	1.88	0.59–3.17	454	8	1.76	0.55–2.97
Sex								
Male	2,637	34	1.29	0.86–1.72	3,116	37	1.19	0.81–1.57
Female	2,396	27	1.13	0.70–1.55	2,675	31	1.16	0.75–1.56

Currently in Australia, individuals diagnosed with an arboviral infection, such as RRV or BFV, are not permitted to donate blood until 4 weeks after they have recovered. In addition, blood components are quarantined or recalled from donors reporting any significant illness within 7 days of donation. RRV and BFV notifications occur in all Australian states and territories, although spatio-temporal as well as seasonal variation exists; RRV is endemic to

Table 2. Prevalence of Previous RRV or BFV Infection (by RRV or BFV IgG) in the Australian Blood Donor Population.

Variable	Number tested	IgG reactive		
		Number	%	95% CI
RRV				
Overall	2,839	238	8.38	7.36–9.40
Region				
Hobart	355	19	5.35	3.01–7.69
Adelaide	359	13	3.62	1.69–5.55
Brisbane	355	49	13.80	10.21–17.39
Darwin	354	54	15.25	11.51–19.00
Melbourne	359	3	0.84	0.00–1.78
Perth	358	10	2.79	1.09–4.50
Sydney	360	16	4.44	2.32–6.57
Townsville	339	74	21.83	17.43–26.23
Age group				
≤24	375	18	4.80	2.61–6.89
25–34	460	17	3.70	1.97–5.42
35–44	446	35	7.85	5.35–10.34
45–54	678	77	11.36	8.97–13.75
55–64	657	65	9.89	7.61–12.18
≥65	223	26	11.66	7.45–15.87
Sex				
Male	1,527	159	10.41	8.88–11.94
Female	1,312	79	6.02	4.73–7.31
BFV				
Overall	2,839	47	1.66	1.19–2.12
Region				
Hobart	355	3	0.85	0.00–1.80
Adelaide	359	5	1.39	0.18–2.61
Brisbane	355	4	1.13	0.03–2.22
Darwin	354	13	3.67	1.71–5.63
Melbourne	359	1	0.28	0.00–0.82
Perth	358	5	1.40	0.18–2.61
Sydney	360	11	3.06	1.28–4.83
Townsville	339	5	1.47	0.19–2.76
Age group				
≤24	375	5	1.33	0.17–2.49
25–34	460	7	1.52	0.40–2.64
35–44	446	6	1.35	0.28–2.41
45–54	678	15	2.21	1.11–3.32
55–64	657	10	1.52	0.59–2.46
≥65	223	4	1.79	0.05–3.54
Sex				
Male	1,528	27	1.77	1.11–2.43
Female	1,311	20	1.53	0.86–2.19

coastal areas of northern and central Australia and is epidemic in the rest of the country, while the majority of BFV cases are in coastal New South Wales (Cashman et al. 2008; Knope et al. 2013; Yu et al. 2014). There are blood donor centres in areas where RRV and BFV transmission occurs, including some 'higher-risk' regions.

Table 3. Multivariate Logistic Regression Analysis: Influence of Factors on RRV or BFV Seropositivity in Australian Blood Donors.

Variable	Multivariate logistic regression analysis		
	Odds ratio	95% CI	*P* value
RRV			
Region (reference group: Hobart)			
Adelaide	0.66	0.32–1.36	0.254
Brisbane	2.80	1.61–4.88	<0.001
Darwin	3.37	1.94–5.85	<0.001
Melbourne	0.16	0.05–0.55	0.004
Perth	0.51	0.23–1.11	0.088
Sydney	0.89	0.45–1.77	0.746
Townsville	5.04	2.95–8.60	<0.001
Age group (reference group: ≤24)			
25–34	0.69	0.34–1.38	0.292
35–44	1.39	0.76–2.55	0.286
45–54	2.07	1.19–3.58	0.010
55–64	1.71	0.98–2.98	0.059
≥65	2.43	1.27–4.67	0.008
Sex (reference group: male)			
Female	0.57	0.42–0.76	<0.001
BFV			
Region (reference group: Hobart)			
Adelaide	1.68	0.40–7.12	0.480
Brisbane	1.33	0.30–6.00	0.709
Darwin	4.52	1.27–16.01	0.020
Melbourne	0.33	0.03–3.22	0.342
Perth	1.65	0.39–6.97	0.495
Sydney	3.81	1.05–13.82	0.042
Townsville	1.75	0.41–7.37	0.449
Age group (reference group: ≤24)			
25–34	1.01	0.31–3.22	0.994
35–44	0.93	0.28–3.11	0.909
45–54	1.52	0.54–4.27	0.431
55–64	1.08	0.36–3.23	0.892
≥65	1.13	0.30–4.31	0.86
Sex (reference group: male)			
Female	0.82	0.45–1.48	0.504

Given that the estimated transmission risk for RRV and BFV (albeit 'theoretical' at present) is similar to that for DENV outbreaks, which trigger further donation restrictions (Faddy et al. 2013), additional precautionary measures may be indicated to mitigate the potential increased risk of RRV or BFV transmission through blood components after extreme rainfall in 'higher-risk' areas. Such approaches could include additional donor questioning to identify donors residing in or travelling to risk areas, coupled with either donation restriction or the implementation of licenced donation screening assays (if available). An alternative approach could be the implementation of pathogen reduction technology (a process designed to inactivate a broad range of pathogens in blood components), which has the added advantage of concurrently safeguarding the blood supply against a large number of pathogens, including as yet unknown agents (Faddy et al. 2014). However, any approaches need to take into account

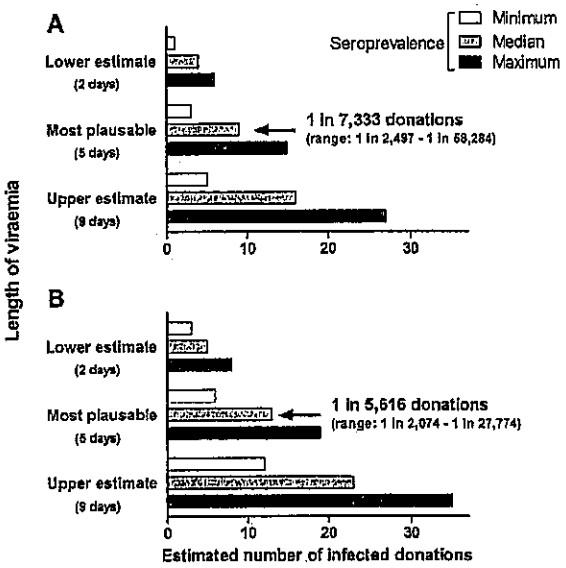

Figure 1. Risk of collecting an infectious donation in high-risk areas after increased rainfall. Estimated risk for RRV (a) and BFV (b).

the severity of the illness in recipients, with both RRV and BFV predominantly resulting in asymptomatic infections or self-limiting illness. The occurrence of RRV or BFV transfusion-transmission would remain a minimal mode of transmission compared to mosquito-borne transmission, with the major risk for transfusion-transmission being increased mosquito-borne transmission resulting in incident infections in blood donors. This risk may be better managed through vector control programs.

Transfusion-transmissible pathogens threaten blood safety globally. Risk reduction strategies must, therefore, incorporate consideration of changes in epidemiology, virus biology and population dynamics (i.e. of humans, animal hosts and disease vectors), as well as climatic and environmental conditions. Many disease vectors are projected to expand their population range to higher latitudes with increasing temperatures under climate change (Semenza and Domanovic 2013). Such range shifts may bring vectors and viruses into contact with larger human populations, increasing risk to blood safety. Moreover, vector-borne disease abundance may also be affected by other factors, such as population movements and manmade environmental changes. Our study highlights the need for continuous re-evaluation of risk mitigation strategies to ensure optimal management of the risk posed by arboviruses and other emerging pathogens. We suggest that risk models must better incorporate projected climate change scenarios in order to maintain the highest level of blood safety.

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別紙様式第2-1

No. 14

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. 7. 1	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名(企業名)	新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)		Yamagishi N, Furui Y, Koshinami S, et al. Transfusion. 2016 Jun;56(6):1305-1310.	日本

研究報告の概要

○ヒトサイトメガロウイルス(CMV)感染極低出生体重児への感染経路として輸血と母乳伝播を評価するCMV- DNA可変領域の塩基配列分析。

極低出生体重児におけるCMV感染は深刻な臨床症状を来す。輸血後にCMV関連症状を呈した時には、母乳よりも輸血用血液製剤に起因するものと考えられがちである。しかしながら、輸血感染と母乳感染を鑑別するのは困難であることが多い。患児は早産児(27週)で超低出生体重児(689g)で出生し、後にCMV-DNAが検出された照射白血球除去赤血球製剤を輸血された。授乳された母乳からもまたCMV-DNAが検出された。その後、血小板減少、黄疸、CRP陽性が認められ、CMV抗原陽性が確認された。出生時には臍帯血にてCMV-DNA陰性が確認されており、感染経路を決定するために、患児全血、当該製剤の保管検体血餅、母乳について、それぞれのCMV-DNA可変領域であるUL139、UL146領域の塩基配列をPCR Direct Sequenceで決定し、各々比較した。塩基配列の一致率は、患児と献血者においてUL139領域が64.6%、UL146領域が68.6%であった。その一方で、患児と母乳の一致率は両領域で100%であった。さらに、Deep Sequencingの結果、献血者から患児のCMV株は検出されなかった。よって、本症例は輸血による感染ではなく、経母乳感染であることが示唆され、白血球除去製剤の輸血によるCMV感染は稀であるとする主張に一致する結果である。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
ヒトサイトメガロウイルス(CMV)に感染した極低出生体重児の感染経路を特定するために、CMV-DNA可変領域の塩基配列分析を行ったところ母乳による伝播が確認され、輸血した照射白血球除去赤血球製剤による伝播は否定されたという報告である。	日本赤十字社では、CMVの感染防止に有効とされる、保存前白血球除去した輸血用血液製剤のみを供給している。さらに、必要に応じてCMV抗体が陰性であることを確認した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。

Sequence analysis of two variable cytomegalovirus genes for distinction between transfusion- and breast milk–transmitted infections in a very-low-birthweight infant

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BACKGROUND: Cytomegalovirus (CMV) infections in very-low-birthweight infants can lead to serious clinical consequences. When CMV-related symptoms occur after transfusion, CMV transmission is often attributed to the transfusion products rather than to breast milk. However, it is sometimes difficult to distinguish between transfusion-transmitted and breast milk–transmitted CMV infections.

PATIENT AND METHODS: A patient was born at 27 gestational weeks with a weight of 689 g. He was transfused with leukoreduced red blood cells (LR-RBCs), which were later found to be CMV seropositive and CMV DNA positive. He was also fed with CMV DNA–positive breast milk. Thereafter, he developed CMV disease with thrombocytopenia and jaundice. To determine the route of transmission, we analyzed the sequences of two variable CMV genes, UL139 and UL146, by direct sequence analysis. We also performed deep sequence analysis to determine whether there were polyclonal CMV strains in the LR-RBCs transfused.

RESULTS: CMV DNA sequence-matching rates for the LR-RBCs and the patient's blood were 64.6% for the UL139 gene and 68.6% for the UL146 gene. In contrast, the sequences of these genes in the patient's blood were 100% matched with those in the breast milk. Furthermore, by deep sequence analysis, the CMV strain found in the patient's blood was not detected in the LR-RBCs transfused.

CONCLUSION: The results indicate that the pathogenic CMV strain was transmitted through breast milk, which is consistent with the claims that transfusion-transmitted CMV infection due to leukoreduced blood products is uncommon.

Cytomegalovirus (CMV) might be transmitted from mothers to their children during the postnatal period. While most full-term infants rarely develop severe CMV diseases, CMV infections in very-low-birthweight (VLBW) infants or premature infants sometimes lead to serious clinical consequences due to insufficiency of transferred maternal antibodies. There are several possible sources for CMV transmission to seronegative VLBW or premature infants, including breast milk, other CMV-infected individuals, and blood transfusion products.

Transfusion-transmitted CMV (TT-CMV) is often suspected when VLBW infants develop a symptomatic CMV infection after transfusion of blood products. In such cases, proving the presence of CMV in transfused components and matching the sequence identity of CMV between the transfused and patient's blood would provide a definitive diagnosis of TT-CMV. However, such sequence

ABBREVIATIONS: BM-CMV = breast milk–transmitted cytomegalovirus; LR-RBCs = leukoreduced red blood cells; VLBW = very low birthweight; TT-CMV = transfusion-transmitted cytomegalovirus.

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[†]NY and YF contributed equally to this study, and the order in which they are listed should be considered arbitrary.

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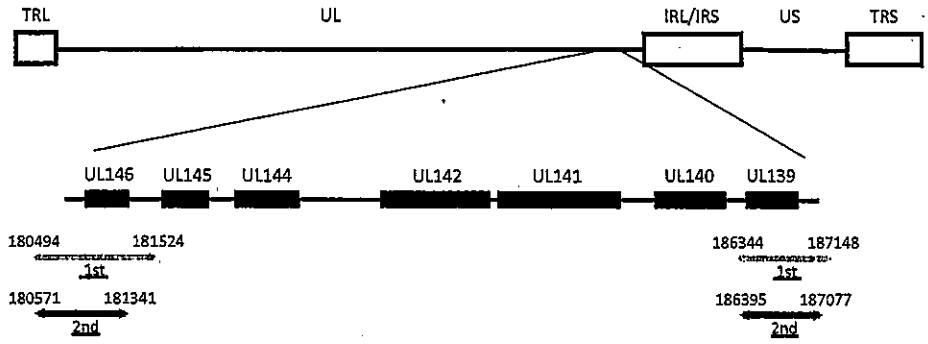

Fig. 1. Schematic map of the CMV strain. A map of the CMV strain Merlin (GenBank Accession Number AY446894.2) is shown. The terminal repeat long (TRL), unique long (UL), internal repeat long (IRL), internal repeat short (IRS), unique short (US), and terminal repeat short (TRS) areas are indicated with white boxes. The gray and black arrows represent the first and second PCR products, respectively. Numbers shown with each arrow indicate the corresponding genome location of the Merlin CMV strain.

analysis has not been performed in most cases. Interestingly, some recent studies showed that the frequency of TT-CMV infection is lower than was generally believed when leukoreduced blood products are used,[1] suggesting that accurate diagnosis of TT-CMV by sequence analysis is of significant clinical importance.

For prevention of TT-CMV, use of either CMV-seronegative or leukoreduced blood products has been shown to be effective,[2-4] although selection policies regarding these two types of blood products do not always coincide among institutions.[5] The risk of TT-CMV is believed to still remain even when leukoreduced blood is transfused, particularly when CMV-seropositive blood products are used.[6] Furthermore, it is possible that CMV-seronegative blood products contain CMV if they are collected during a "window period." Based on these facts, currently transfusion with CMV-seronegative leukoreduced products is actually recommended for VLBW infants.[7,8]

It is also well known that CMV DNA can be found at high rates, of more than 90%, in the breast milk of CMV-seropositive mothers.[9] At present, effective prevention strategies for breast milk–transmitted CMV (BM-CMV) have not yet been established. As a result, 2% to 37% of infants who are fed with CMV DNA–positive breast milk could be infected with CMV.[8,10,11]

Here we report a case of BM-CMV in a VLBW infant, in whom TT-CMV was first suspected. We performed sequence analysis of two variable CMV genes from samples of the patient's blood, the transfused blood products, and the patient's mother's breast milk.

MATERIALS AND METHODS

Polymerase chain reaction for CMV DNA

The Japanese Red Cross Society stores and retains repository blood samples, which have been separated into a blood clot and a serum fraction, from all blood donors for 11 years. For this study, DNA samples from serum fractions of the pertinent repository blood samples were prepared using the virus/pathogen midi kit (QIAsymphony DSP, Qiagen Inc.). The blood clot of the repository blood sample was pulverized using a homogenizer (Bio-musher V, Nippi Inc.). Subsequently, DNA from nucleated cells was isolated using the DNA mini kit (QIAsymphony, Qiagen Inc.). We found in our previous study that the efficiency of DNA isolation from the blood clot of the repository blood sample is almost equal to that from the buffy coat.[12] DNA from the whole blood of the patient and his mother, as well as from her breast milk, was also isolated using the DNA mini kit (QIAsymphony, Qiagen Inc.). After DNA samples were prepared, polymerase chain reaction (PCR) tests to detect CMV DNA were performed using an CMV TM PCR kit (artus, Qiagen Inc.), as described previously.[12]

Sequence analysis of the UL139 and UL146 genes of CMV

Using DNA samples from the breast milk, the patient's blood, and the repository blood sample corresponding to the transfused leukoreduced red blood cells (LR-RBC), in which CMV DNA was detected, nested PCR for the CMV UL139 gene, which encodes a membrane glycoprotein, and the UL146 gene, which encodes a CXC chemokine, was performed (Fig. 1). The primers used for nested PCR, which were designed and modified based on previous reports,[13,14] are shown in Table 1. Sequences of the UL139 and UL146 genes were then determined by direct sequence analysis. Using computer software (Mega 6.0),[15] the phylogenetic trees for CMV genotypes were created based on the predicted amino acid sequences, which were determined by computer software (GENETYX, Version 12, GENETYX Corp.).

For genetic sequencing by deep sequence analysis, we created libraries from PCR amplicons, which had been prepared from DNA samples, using the DNA sample preparation kit (Nextera XT, Illumina). Nucleotide sequences were then determined using a reagent kit and sequencer

TABLE 1. Primers used for nested PCR

Genes	Forward	Reverse
UL139 (first)	5′-aagccttagcctctacggtgt-3′	5′-atctactgtaaaccctctgctctg-3′
UL139 (second)	5′-gtgaaagtgacgtctcagg-3′	5′-gtggaaatttttacgtcatt-3′
UL146 (first)	5′-tagacactacgtcgtaaatg-3′	5′-tgtagaattagtctagattcctga-3′
UL146 (second)	5′-gcttgcgcgttaggattgagacac-3′	5′-ataccggatattacgaatt-3′

(Miseq v2, 500 cycles, and Miseq, respectively, Illumina). Previous reports demonstrated that there are eight genotypes for the UL139 gene and 14 genotypes for the UL146 gene;[13,16] therefore, we used these 22 variable sequences as a reference. The resulting data were analyzed by computer software (Sequencher 5.3, Gene Codes Corp.), in which the BWA-MEM algorithm was used for the alignment of paired-end reads to the reference sequences. To reduce sequence artifacts, reads with a low-quality score (<35) or shorter than 150 bp were excluded.

Detection of CMV antigen and CMV antibody

CMV antigen was examined by the pp65 antigenemia method using an enzyme-labeled antibody kit (LSIM kit, LSI Medience Co.) in accordance with the manufacturer's instructions. Briefly, the patient's white blood cells (WBCs) were fixed on glass slides, and then the slides were incubated with the C10/C11 anti-CMVpp65 mouse monoclonal antibody to detect the presence of CMVpp65 antigen in the WBCs. Anti-CMV IgM (Enzygnost, Siemens Healthcare Diagnostics Products GmbH) was used to detect anti-CMV IgM in the serum fraction of the transfused LR-RBCs and the plasma of the patient. Presence of anti-CMV IgG was also examined using the anti-viral antibody enzyme immunoassay Cytomegalo IgG (Denka Seiken Co., Ltd. Tokyo, Japan), in accordance with the manufacturer's instructions. In our hospital, we use CMV-seronegative blood products for VLBW infants, except for during urgent situations when we cannot afford to find such products.

CASE REPORT

The patient was born at a gestational age of 27 weeks and had a birthweight of 689 g. Analysis of his mother's blood performed 1 month before delivery revealed positivity for anti-CMV IgG, but anti-CMV IgM testing was negative. At the time of delivery, the patient was assessed to be CMV negative because his dry umbilical cord was negative for CMV DNA. On Day 3, the patient's mother began to feed him breast milk not treated by freeze-thawing. On Day 7, the patient was treated with a transfusion of 20 mL of LR-RBCs to improve his hemodynamics. Because it was an urgent situation, CMV-undetermined LR-RBCs, which had been stored at the hospital, were used instead of ordering a CMV-seronegative supply. Thereafter, he received transfusions four times, up to a total of 120 mL of CMV-seronegative LR-RBCs. Later, by testing the repository blood samples, we found that the first transfused LR-RBC unit was CMV seropositive and CMV DNA positive.

On Day 9, laboratory examinations revealed extremely high levels of C-reactive protein (10.3 mg/dL), as well as decreased platelets (10.3×10^{10}/L). In addition, the patient developed jaundice. On Day 23, the CMV antigen test was positive (3/15 cells/50,000 peripheral blood WBCs), indicating that CMV infection had been established in the patient. After antiviral treatment with ganciclovir, the clinical symptoms disappeared with improvement of laboratory examinations. The results of CMV-related testing on Day 87 were still all positive for anti-CMV IgM, anti-CMV IgG, and qualitative CMV DNA. CMV DNA positivity in the patient's whole blood was also confirmed by a qualitative analysis performed in an external institution. However, there was no further recurrence of clinical symptoms or thrombocytopenia. On Day 188, we found that the mother's breast milk was positive (1640 IU/mL), whereas her whole blood was negative, for CMV DNA.

RESULTS

A previous study showed that there is much diversity in the sequences of the UL139 and UL146 genes of CMV.[13] When comparing CMV strains found in the transfused LR-RBCs and the patient's blood by direct sequence analysis, DNA sequence-matching rates were 64.6 and 68.6% for the UL139 and UL146 genes, respectively (Figs. 2A and 2B). The UL139 and UL146 genes from the LR-RBCs were assigned to Genotypes G5 and G3, whereas those from the patient's blood were assigned to G4 and G12, respectively, using the predicted amino acid sequences method (Figs. 3A and 3B).[13-16] In contrast, when comparing CMV found in the breast milk and the patient's blood, DNA sequence-matching rates were 100% for both genes (Figs. 2A and 2B). Thus, it is highly likely that the CMV in the patient's blood and that in the breast milk were identical.

However, it still remained possible that the CMV in the LR-RBCs was polyclonal and that a minor strain, which was accidentally identical to that in the breast milk, had proliferated advantageously in the patient. To address this question, we next performed deep sequence analysis of the repository blood sample of LR-RBCs, to determine whether there were CMV strains other than those found by direct sequence analysis. In this analysis, we could not find additional CMV strains, including the one found in the

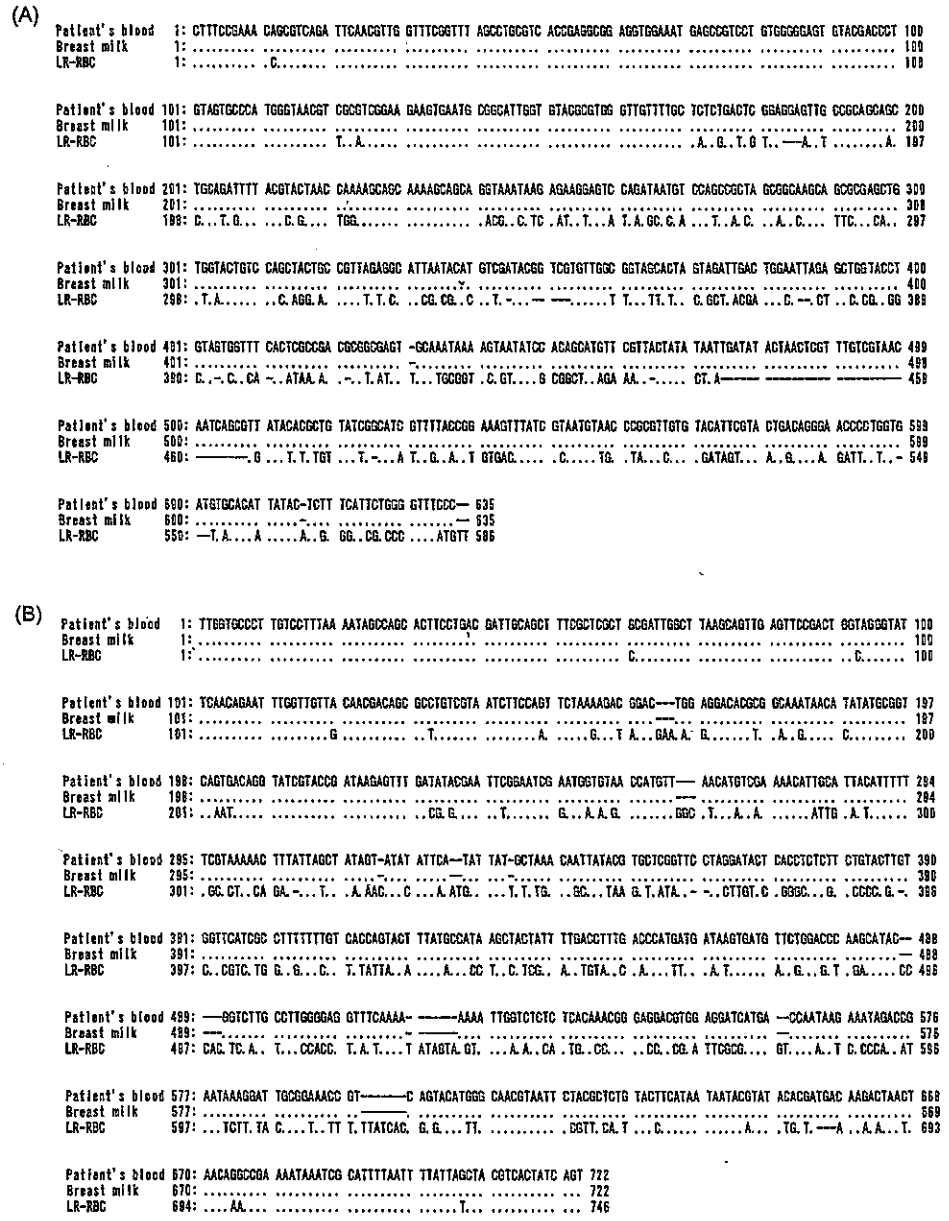

Fig. 2. Nucleotide sequence alignment of the UL139 and UL146 genes of CMV. Sequences of the UL139 (A) and UL146 (B) genes of CMV detected from patient's blood, breast milk, and LR-RBCs were determined and compared. Sequences of the two genes from the breast milk and the patient's blood matched completely. Dots indicate identical nucleotides and dashes indicate deletions. Sequences shown in the figure correspond to the regions with genome locations of 186395 to 187077 (A) and 180571 to 181341 (B) of the Merlin CMV strain.

patient's blood, supporting the results of direct sequence analysis indicating that the CMV strains found in the transfused LR-RBCs were not transmitted to the patient.

DISCUSSION

In this case, TT-CMV, rather than BM-CMV, was first suspected, because CMV-seropositive LR-RBCs were transfused before development of CMV infection–related clinical symptoms. Furthermore, CMV DNA was positive in the blood clot of a repository blood sample for the transfused LR-RBCs. It is possible that the transfused LR-RBCs contained excess amounts of WBCs as a result of filtration failure. Moreover, although there is no definitive evidence to support this, potential CMV in (cell-free) plasma, which could remain even after leukoreduction of

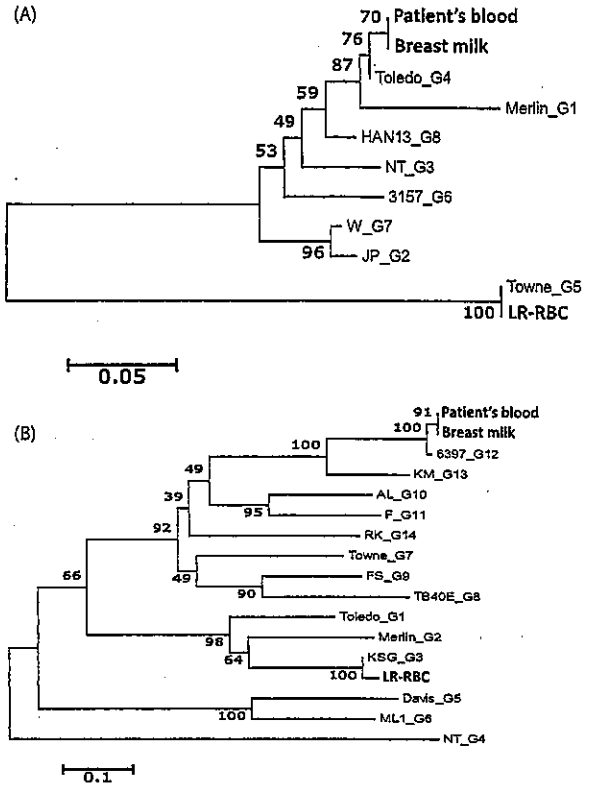

Fig. 3. Genotypes of CMV found in patient's blood, breast milk, and LR-RBCs. Unrooted neighbor-joining trees for the UL139 (A) and UL146 (B) genes are shown.[13-15] Scale bar indicates the number of amino acid substitutions per site. Bootstrap values (out of 100) are shown.

CMV-infected blood products, could be regarded as a possible source of transmission. CMV DNA was, however, not detected in the serum fragment of the repository blood sample of this LR-RBC unit (data not shown).

Most previously reported TT-CMV cases never underwent CMV sequence analysis; instead they were diagnosed based only on the clinical course—the fact that CMV diseases developed after a transfusion. In other words, the possibility of BM-CMV cannot be completely ruled out in previously reported TT-CMV cases. This fact raises the possibility that the frequency of TT-CMV is much lower than what was previously believed. Indeed, Seed and colleagues[1] recently reported that the risk of TT-CMV with use of leukoreduced blood products is extremely low. Therefore, we felt a need to distinguish TT-CMV from BM-CMV in this case.

For this purpose, we analyzed the sequences of the two CMV genes, UL139 and UL146, which were previously reported to have sequence variation among different strains.[13] As a result, we clearly showed that the sequences of these genes in the patient's blood completely matched those in the fed breast milk. Furthermore, these sequences could not be detected in the repository blood sample of LR-RBCs using sensitive deep sequence analysis. We also demonstrated that this analysis could detect a specific genotype of UL139 or UL146 that comprised only 0.3% (1.4 copies/PCR procedure) of a mixture of two CMV clones having different genotypes of these genes, indicating the high sensitivity of sequencing for CMV (data not shown). These results strongly support the conclusion that the CMV strain in the patient's blood was identical to that in the fed breast milk and that the patient acquired a BM-CMV infection.

Josephson and coworkers[8] showed that risk factors for BM-CMV are the number of breast milk feeding days, viral load in breast milk, and premature rupture of membranes. In this case, premature rupture of membranes was not observed. However, the number of breast milk feeding days was relatively high (24 days). Although the CMV viral load in breast milk was relatively low (1640 IU/mL), it might have been much higher when the patient was fed. A previous study showed that the CMV viral load in the breast milk of a transmitter increases significantly after delivery, peaking 4 to 6 weeks later.[17] We performed CMV PCR test of the breast milk of the mother on Day 188, which was much later than the reported peak period. Considered together, all these findings suggest that the CMV in breast milk in this case was highly transmittable.

Since VLBW infants with CMV infection not only often receive a blood transfusion, but are also sometimes fed breast milk, it is of importance to definitively determine the source of the transmitted CMV. To the best of our knowledge, this is the first report of sequence analysis of the UL139 and UL146 genes performed to distinguish TT-CMV from BM-CMV. Our results are consistent with those of recent reports showing that TT-CMV associated with leukoreduced blood products is uncommon. Since most previous studies comparing the roles of leukoreduced and CMV-seronegative blood (performed for the prevention of TT-CMV), and studies regarding the application of CMV-safe blood to VLBW infants, were performed without confirmation of CMV sources, it might be desirable to reexamine those issues after determination of the definitive route of CMV transmission. Results of the current case report also suggest that development of an effective way to prevent BM-CMV may be an even more critical issue remaining to be solved than has been previously recognized.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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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06月08日	新医薬品等の区分		厚生労働省処理欄
一般的名称	①②③⑨⑩⑪ pH4処理酸性人免疫グロブリン ④⑤⑥⑦⑧ポリエチレングリコール処理人免疫グロブリン ⑫⑬ 人免疫グロブリン			研究報告の公表状況	Vox Sanguinis 2016; 110( ): 310-316	公表国 イギリス	
販売名（企業名）	①献血ポリグロビンN5%静注0.5g/10mL（日本血液製剤機構） ②献血ポリグロビンN5%静注2.5g/50mL（日本血液製剤機構） ③献血ポリグロビンN5%静注5g/100mL（日本血液製剤機構） ④献血ヴェノグロブリンIH5%静注0.5g/10mL（日本血液製剤機構） ⑤献血ヴェノグロブリンIH5%静注1g/20mL（日本血液製剤機構） ⑥献血ヴェノグロブリンIH5%静注2.5g/50mL（日本血液製剤機構） ⑦献血ヴェノグロブリンIH5%静注5g/100mL（日本血液製剤機構） ⑧献血ヴェノグロブリンIH5%静注10g/200mL（日本血液製剤機構） ⑨献血ポリグロビンN10%静注2.5g/25mL（日本血液製剤機構） ⑩献血ポリグロビンN10%静注5g/50mL（日本血液製剤機構） ⑪献血ポリグロビンN10%静注10g/100mL（日本血液製剤機構） ⑫グロブリン筋注450mg/3mL「JB」（日本血液製剤機構） ⑬グロブリン筋注1500mg/10mL「JB」（日本血液製剤機構）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
背景と目的：本報告は、英国における輸血によるvCJD伝播の新規症例を調査する研究で2015年3月31日までの結果を報告する。また、他のタイプのCJDが輸血により感染した可能性を示す証拠を調査した結果も報告する。 材料と方法：適切な年齢におけるvCJD全症例と孤発性CJD（sCJD）または家族性CJD（fCJD）症例の患者の献血歴及び輸血歴は、UKBSに報告された。報告された献血記録を確認し、全ての献血の使用記録をルックバックにより確定した。死亡診断書には、CJD患者への全ての献血者と死亡したCJD患者からの成分輸血の受血者情報が含まれる。 結果：本研究では、370の報告から、29名のsCJD献血者から211名への輸血を確認した。これらの受血者のうち5症例で痴呆を伴う死亡が報告された。しかしCJD症例と考えられる症例は無かった。vCJDの供血は、177名の英国vCJD症例のうち24名の献血者を特定し、医療機関で使用された献血血液は18名のvCJD献血者のものであることが判明した。 現在までに、この受血者グループの特定された67名の受血者で3症例のvCJDが発生し、1名の受血者は、死亡確認に脾臓に異常プリオンの沈着があった（全て報告済み）。 結論：現在進行中のTMER研究の結果は、2007年以降、輸血に関連したvCJDの新しい症例とsCJDの輸血伝播の証拠はなかったことを示す。	代表として献血ヴェノグロブリンIH5%静注0.5g/10mLの記載を示す。 2. 重要な基本的注意 (1)略 1)略 2)現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

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

JB2016-014

VoxSanguinis

ISBT International Society of Blood Transfusion

Vox Sanguinis (2016) 110, 310–316

ORIGINAL PAPER

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Creutzfeldt–Jakob disease and blood transfusion: updated results of the UK Transfusion Medicine Epidemiology Review Study

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Vox Sanguinis

Background and Objectives This paper reports the results to 31 May 2015 of an ongoing UK study to look for additional cases of variant Creutzfeldt–Jakob disease (vCJD) transmission by blood transfusion, and to seek evidence whether other subtypes of Creutzfeldt–Jakob disease (CJD) may be transmissible via blood components.

Materials and Methods All vCJD cases of appropriate age and any sporadic CJD (sCJD) or familial CJD (fCJD) cases with a history of blood donation or transfusion are notified to the UKBS. Donation records are sought and the usage of all donations is determined by look back. Death certificates are obtained for all donors to patients with CJD and recipients of transfused components from patients with CJD who are deceased.

Results The study identified 29 sCJD blood donors, of 370 reported, with transfusion to 211 recipients. Five of these recipients were reported to have died with or of dementia, but were not believed to be cases of CJD. The vCJD arm found 18 vCJD blood donors who had donated blood which was issued for clinical usage, of 24 traced donors from 177 UK vCJD cases. To date, 3 cases of vCJD have occurred in 67 recipients identified in this recipient group, and one recipient had post-mortem confirmation of abnormal prion protein deposition in the spleen (all previously reported).

Conclusion The results of the ongoing TMER study show no new cases of transfusion-associated vCJD since 2007 and no evidence of transfusion transmission of sCJD.

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Key words: blood transfusion, epidemiology, prion disease.

Introduction

Creutzfeldt–Jakob disease (CJD) is an untreatable and invariably fatal member of a group of neurodegenerative conditions known as prion diseases or transmissible spongiform encephalopathies (TSEs). Prion diseases are recognized in both humans and other mammals and have a number of aetiologies including sporadic, acquired or familial forms. Despite this apparent heterogeneity, there is a unifying hypothesis linking all prion diseases: the 'protein hypothesis' described by Prusiner [1], which proposes that a post-translational change occurs in the normal prion protein (PrP^{C} – cellular) forming the infective form of the prion protein (PrP^{Sc} – Scrapie). PrP^{Sc} essentially replicates by catalysing further transformation of PrP^{C} into PrP^{Sc}.

The variant form of Creutzfeldt–Jakob disease (vCJD) is the zoonotic form of bovine spongiform encephalopathy, a prion disease in cattle, which entered the human food

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chain in the UK between 1980 and 1996. vCJD has been transmitted by blood transfusion on three occasions [2], as well as one non-symptomatic transmission [3]. The most recent UK primary case of vCJD had symptom onset in 2012 and died in 2013, although surveillance to look for further cases continues. In contrast, sporadic Creutzfeldt–Jakob disease (sCJD), the most commonly occurring human subtype, is believed to be a spontaneous illness with no identified causative event or exposure. There has been one epidemiological study, which has suggested blood transfusion may be a risk factor for the development of sCJD [4], but this has not been supported by a similar study in the UK [5] or through look-back studies [2, 6, 7]. The familial form of CJD (fCJD) is caused by a mutant copy of the PRNP gene, encoding a form of endogenous PrP^{c} prone to spontaneous conversion to PrP^{Sc}. fCJD is inherited in an autosomal dominant pattern, but family history may not be present in some cases due to loss of contact, non-paternity, variable penetrance, etc.

This study updates the 2006 Transfusion Medicine Epidemiology Review (TMER) paper [2] and describes the results of the UK study on blood transfusion and the development of CJD, for all CJD subtypes.

Study design and methods

CJD surveillance

The National Creutzfeldt-Jakob Disease Research & Surveillance Unit (NCJDRSU) was founded in Edinburgh, UK, in 1990 to identify all cases of CJD in the UK and to look for evidence of a link between BSE in cattle and CJD in humans. The methodology of the surveillance process has been described previously [8] and includes referral of suspected cases to the Unit from clinicians from a number of professional backgrounds, including neurologists, psychiatrists, other physicians and neuropathologists. The referred cases are seen, when possible, by a neurologist from the Unit, who carries out a detailed interview with the family of the patient and reviews the specialist investigations. The interview includes details about past medical history, blood transfusion and donation. Cases are categorized according to WHO diagnostic criteria [9]. The Transfusion Medicine Epidemiology Review (TMER) was created in 1997 as a collaboration between the NCJDRSU and the UKBS to identify any evidence that CJD was transmissible via blood transfusion.

Notification of CJD cases with a history of donation

sCJD and fCJD cases with a reported history of blood donation, including cases where a family may be uncertain, are notified to UKBS retrospectively, following the visit by the NCJDRSU clinician. All vCJD cases old enough to be a blood donor are notified to UKBS at diagnosis irrespective of whether they have a reported history of blood donation. Following this notification, all computer and any archived paper records are searched at blood centres for evidence of the documented donation – using name, date of birth and address at time of donation as identifiers. If available, information about dates and places of donation is used to target the search. If donor records are identified, a list is generated of all components issued for clinical usage. The outcome for each component is determined from hospital transfusion laboratory records, with the names of recipients of these components cross-checked against the NCJDRSU database of known CJD cases and flagged with the Health and Social Care Information Centre (HSCIC, formerly Office of National Statistics) to collect data from death certificates regarding cause and date of death.

Notification of CJD cases with a history of transfusion

Where relatives have indicated any patient with fCJD, sCJD or vCJD is suspected to have received blood or blood components, the information collected is passed to the relevant blood service, which contacts the hospital transfusion laboratories to confirm, if possible, details of the transfusion. The transfused components are identified from records, and details passed back to the blood centre for attempted identification of the donors. As above, the donor details are checked against the NCJDRSU database and flagged with the HSCIC.

Further information

UKBS and hospital transfusion records prior to 1980 are extremely limited, making such historical searches frequently unrewarding. For cases where HSCIC data list potentially relevant diagnoses on the death certificate (e.g. dementia, Alzheimer's disease) but the individual concerned has not been seen by the NCJDRSU clinician, we have sought further information where possible regarding the nature of this illness, from either general practitioner or hospital notes.

Results

sCJD blood donors

A total of 370 sCJD cases were reported to be blood donors, with 204 of these believed to have donated after 1980. In only 29 of 204 cases were these individuals

traced as blood donors; blood components from these donors were transfused to 211 recipients.

Fate of recipients from sCJD blood donors

To date, 143 individuals (67·8%) of the 211 recipients identified in this study have died, 44 (20·9%) were alive, and 24 individuals (11·4%) were of unknown status due to insufficient information to identify the individual, or relocation of that individual abroad. Of the 143 who had died, death certificates are available. The underlying causes of death for all cases are listed in Table 1. Five of 143 had dementia (including Alzheimer's disease) listed on their death certificates but are not thought to represent cases of CJD. These five cases had mean age at death of 88 years, and in each case, dementia was not listed as the primary cause of death. In one of these five cases, dementia was considered a relevant comorbidity, rather than the underlying cause of death on the certificate; hence, only four dementia deaths are listed in Table 1. The first case received whole blood donation about 21 years before becoming symptomatic of dementia; and had a 6 year, slowly progressive illness, dying 26 years after receiving the transfusion. The donor became symptomatic of CJD nearly 21 years after the donation. The second case received red cells (non-leucocyte depleted) 10 months prior to death, while the donor became symptomatic of sCJD almost 4 years after donation (more than 3 years after the recipient died). The third case received red cells (non-leucocyte depleted) 8 years before death; the donation occurred 4 ½ years before the donor became symptomatic of sCJD. The fourth case received fresh frozen plasma 28 months before death; the donor became symptomatic 5 ½ years after donation. The fifth case received red cells (non-leucocyte depleted) 18 years before death; the donor became symptomatic 15 ¾ years after donation.

Table 1 Underlying causes of death in recipients from sCJD donor

Cause of death	Number
Cancer (non-haematological)	39
Leukaemia, myeloma, myelodysplasia, myelofibrosis, sideroblastic anaemia	34
Ischaemic heart disease, other cardiac disease	25
Pneumonia	10[a]
Stroke	7
Liver disease	5
Dementia (including Alzheimer's)	4
Abdominal aortic aneurysm	3
Chronic obstructive pulmonary disease	2
Old age	2
Renal failure	2
Atherosclerosis (not otherwise specified)	1
Diverticulosis and bowel angiodysplasia	1
Haemorrhage	1
Multiple sclerosis	1
Peritonitis	1
Polytrauma	1
Pulmonary embolism	1
Septicaemia	1
Small bowel obstruction	1
Vasculitis	1

[a]In one case, Alzheimer's disease was recorded as a co-morbidity.

Eighty-eight of 143 (61·5%) sCJD recipients died less than 1 year after transfusion, 25 (17·5%) between one and 5 years after transfusion and 28 (19·6%) more than 5 years after transfusion (range in this group 5·77–26·10 years, median 8·97); for two recipients, the transfusion date was unknown.

Of the 44 recipients still alive as of May 2015, all have survived more than 9 years from the date of transfusion. Twenty-two (50%) of these recipients received donations from eight donors who donated less than 5 years before they became symptomatic of sCJD (range 0·23–4·92 years, median 2·21). None of these living recipients have developed sCJD and been referred to the NCJDRSU.

vCJD blood donors

Of the 177 UK cases of vCJD, 167 were old enough to have been blood donors. In 32 of the 167 cases, family reported to the NCJDRSU clinician a possible history of blood donation. A further four cases not reported by the families as donors appeared on UK databases, but only one of these four had donated. In total, 24 had records with the UKBS, but only 18 of these 24 had donations which were subsequently used clinically. Sixty-seven blood components from these 18 donors were traced to identified recipients; a further six components known to have been issued could not be traced.

Fate of recipients from vCJD blood donors

Thirty-four (50·7%) of the 67 successfully traced recipients died within 5 years of their transfusion – none were thought to have died from CJD, but none of these cases had post-mortem examination to look for PrP^{Sc} deposition. Three cases (4·5%) of vCJD have already been reported from this cohort of 67 [2]; these three developed vCJD between 6 ½ and 8 years 4 months after their transfusion. Five (7·5%) of the 67 died more than 5 years after transfusion and had post-mortem examination including examination for PrP^{Sc} – only the single case already reported tested positive [3], with PrP^{Sc} deposition in the spleen. A further 11 recipients who died more than

5 years after transfusion did not have post-mortem examination to look for PrP^{Sc} deposition. To date, 14 of the 67 recipients remain alive. One recipient has moved abroad, and their fate is currently unknown while the remaining 13 have now survived more than 10 years after receiving transfusion from vCJD donors. There have been no new cases of vCJD identified by the NCJDRSU among the recipients of blood from vCJD donors.

fCJD blood donors

Of the 17 familial/genetic cases reported to have been donors, four were traced by the UKBS, one with a D178N mutation, one with an E200k mutation and two with octapeptide repeat insertion mutations. Fifteen recipients were traced, and of these eight have died, all of a non-neurological disorder, other than one with a history of stroke. Four are alive and three could not be identified. Blood transfusions took place between 1977 and 2002, and four recipients are alive more than 13 years after the transfusion. None of the recipients appear on the NCJDRSU database as CJD cases.

Transfusion history in sCJD recipients

A total of 199 sCJD cases were reported to have received blood or blood component transfusion, 111 of these after 1980. The records were traced in 23 (20·7%) of these 111 cases, with 214 donors identified. These 23 cases received their first blood or blood components between 0·3 and 14·2 years before becoming symptomatic of sCJD (mean 3·89, median 2·61).

Fate of donors to sCJD transfusion recipients

To date, 205 (95·8%) of the 214 donors are still alive, four (1·9%) have died, and five (2·3%) were of unknown status due to insufficient data (four) or relocation abroad (one). The surviving donors ranged from 25 to 82 years of age (median 56). Three of the four deceased donors died of causes other than dementia (intracerebral tumour, liver disease and suicide), but for one individual, dementia was listed on the death certificate; this donor died almost 12 years after the donation, aged 76, and was thought likely to have vascular dementia, rather than CJD. The other three donors died aged 54, 59 and 63 years, respectively 4, 10 and 12 ½ years after their donations.

Transfusion history in vCJD recipients

Fifteen of the 177 UK vCJD cases were reported to have received blood or blood components. Transfusion laboratory records were traced in 10 of these 15 cases, which include the three cases of transfusion-associated vCJD previously published and listed earlier in this study [2]. One of the 10 recipients had onset of symptoms less than 1 year after transfusion and is unlikely to represent possible transfusion-associated vCJD given the timings of the three known cases. Four of the 10 received blood components from 112 donors; the remaining two recipients received a total of six blood components, but it was not possible to identify the donors in these cases.

Fate of donors to vCJD transfusion recipients

Six of these donors have died of causes unrelated to CJD (Table 2), 104 are currently alive, and the fate of two is not known (one having moved abroad).

Transfusion history in fCJD recipients

None of the familial/genetic human prion disease cases who were reported to have had a history of having received a blood transfusion were traced by UKBS.

Discussion

This study has not identified any new cases of transmission of vCJD by blood transfusion, with only four documented infections to date, as described in an earlier TMER publication in 2006 [2]. The possibility that there are significant numbers of missed transfusion cases is judged to be unlikely, not least because the great majority of vCJD cases have no history of blood transfusion [2, 10]. It is surprising that there have been no further transfusion-transmitted cases in view of the estimated prevalence of abnormal PrP positivity of 1/2000 in the general UK population, derived from an anonymized survey of routine appendix tissue [11]. All clinical cases of vCJD with data on genotype have been methionine homozygotes at codon 129 of the PRNP gene. Analysis of the codon 129 distribution in the UK population indicates that 44% are MM homozygotes, with 45% MV heterozy-

Table 2 Cause of death in six donors

Donor	Cause of death
1	Haemorrhage due to abdominal aortic aneurysm
2	Hypertensive heart disease
3	Pulmonary Embolus/deep vein thrombosis/ischaemic heart disease
4	Bronchopneumonia/disseminated sigmoid colon carcinoma
5	Complication of heart valve surgery
6	Bronchopneumonia/stroke/atrial fibrillation/ischaemic heart disease

gotes and 11% VV (valine homozygotes) [12]. It is possible that individuals who are either heterozygotes or valine homozygotes may experience a longer presymptomatic phase before developing clinically evident vCJD and all codon 129 genotypes were represented in the positive appendix samples in the recent prevalence study. However, it is 20 years since the onset of symptoms in the first case of vCJD and no definite or probable case of vCJD with a non-MM homozygous genotype has yet been identified in the UK or internationally.

The codon 129 genotype is known in 19 of the 67 recipients, including the three vCJD cases (MM homozygotes) and the preclinical infection (MV heterozygote). Four deceased recipients with no evidence of abnormal PrP in brain or peripheral tissues have been genotyped. These four individuals had survived for 6·3–15·9 years post-transfusion, and the interval from the donation to the onset of clinical symptoms in the donor was between 2 months and 6·8 years. Two were MV heterozygotes and two were MM homozygotes. Two further cases without post-mortem examination were MM homozygotes. Nine recipients who are currently alive have been genotyped. Five are MV heterozygotes and four are MM homozygotes. One of the MV heterozygote recipients had a tonsil biopsy, which showed no evidence of abnormal PrP deposition. This recipient received a transfusion from an individual from whom earlier donations were implicated in two of the three known transfusion-transmitted cases, who were, as previously stated, MM homozygotes. It is of interest that in three surviving asymptomatic MM homozygotes, red cells had been leucodepleted, a policy introduced in the UK as a vCJD risk-reduction measure in 1999. In addition, the fourth surviving asymptomatic MM homozygote had received cryo-depleted plasma.

The high proportion (50·7%) of blood transfusion recipients who died within 5 years of transfusion reflects the comorbidities which led to blood transfusion. Extrapolating from other acquired prion diseases, kuru and iatrogenic CJD, the minimum incubation periods are at least 4·5 years [13, 14], and it is unlikely that this group would have manifest symptoms of vCJD prior to death, even if infected. The observed interval from transfusion to symptom onset in the identified transfusion cases was 6 ½–8 years, 4 months. It is also likely that there may be variable levels of infectivity in blood from vCJD donors relating to the proximity of the time of the donation to symptom onset in that individual and early donations might have a lower level of infectivity, which could be associated with a longer presymptomatic phase in recipients.

Recipients of any blood transfusion are now deferred from themselves donating blood, which prevents the potential propagation of a transfusion vCJD epidemic, which is important if some donors have a subclinical infection. It is of note that laboratory transmission studies using splenic tissue from the subclinically infected vCJD case have confirmed the presence of infectivity and this case was a codon 129 heterozygote [15].

In contrast to the vCJD group, as yet there have been no cases of sCJD with definite epidemiological evidence to support a transfusion link. Evidence of an increased risk through blood transfusion in sCJD with a lag period of more than 10 years in an Italian study [4] was not replicated by a similar analysis of UK data [5]. In our study of sCJD, there has been a total of 1194 patient-years survival following transfusion from a sCJD donor with no evidence of transmission via blood transfusion. The absence of any observed cases supports the hypothesis that blood infectivity, should it be present at all, is lower in sCJD than vCJD. This would be compatible with the extensive PrP^{Sc} deposition in lymphoreticular tissues in vCJD, which contrasts with sCJD where there is comparatively much less peripheral PrP^{Sc}. Nevertheless, animal studies using transgenic mice overexpressing human PrP^{C} have suggested there can be infectivity in sCJD blood [16] and work is ongoing to attempt to use amplification techniques, such as real-time quaking induced conversion (RT-QuIC), to identify a positive signal in blood in sCJD. The identification of positive findings in sCJD blood using highly sensitive techniques may be difficult to interpret in relation to actual risk, and epidemiological data remain important in assessing risks for public health.

As in the recipients of blood from the vCJD donor group, early mortality among recipients of blood transfusion is high in the sCJD study and it is possible that some of these recipients could be in the presymptomatic phase of sCJD infection at time of death. Post-mortem uptake is low in the UK population, and, unless explicitly looked for, changes of early sCJD may be missed. The combination of these factors raises the possibility of as yet undetected transmission of sCJD by blood transfusion, although this is unlikely given the negative data in this study and similar findings from other studies [2, 6, 7]. The cumulative data from look-back studies in sCJD suggest that transfusion transmission of sCJD is a rare event, should it occur at all.

The data on fCJD show no evidence of transfusion transmission and, although the data are very limited, there is some evidence of restricted peripheral pathogenesis in hereditary forms of human prion disease similar to sCJD.

The study has some limitations. Both the sCJD and fCJD arms depend on relatives reporting blood transfusion or donation at the time of the NCJDRSU clinician interview; if the relative was uncertain, or believed a

patient may have possibly donated blood or received a transfusion (e.g. intraoperatively), this was still flagged to the UKBS. Despite this it is likely that some patients with sCJD and fCJD who had either donated blood or received transfusion were not identified by the current methodology. By comparison, all vCJD cases of donation age are flagged to UKBS, reducing potential under-reporting. Investigation of all sCJD and fCJD cases, whether or not they have been reported to be blood donors or recipients, has been considered, but follow-up of cases is labour intensive, and investigating these cases regardless of the transfusion history is unlikely to provide much additional information.

A further limitation is that no transfusion or donation records have been identified for some cases. In some instances, this may be because the possible donors or recipients as reported by the family had never donated or received blood, but these cases are in the minority. The lack of centralized computer records, particularly prior to 1980, and the tendency of many hospital trusts to destroy old, unused, medical records means a large pool of potentially useful data has been lost. This problem is compounded in the non-vCJD group, as these patients tend to be older and are therefore more likely to have had their contact with the UKBS prior to the improvement in record keeping.

The reliance on data derived from death certificates is also a limitation as the final diagnostic classification of identified cases relies on the conditions listed on the death certificate. There have been widely variable estimates of inaccuracy on death certificates. An Office for National Statistics survey suggested inaccuracy in 22% of certificates, with under-reporting of common underlying conditions including heart disease and cancer [17]. It is likely that dementia of any cause is also under-reported, and CJD may not be diagnosed in life, with symptoms attributed to an alternative cause of dementia. Review of death certificate data on CJD since 1990 at the NCJDRSU suggests that the sensitivity and specificity of a correct diagnosis on death certificates is about 80%. It is also likely that not all UK cases of CJD are referred in life or identified through death certificates or post-mortem findings.

Despite these caveats, the data presented in this paper has provided no evidence that cases of sCJD have developed the condition as a result of prior blood transfusion. The unexplained mismatch between the observed data in vCJD and prevalence estimates in the general population and the recent experimental evidence of infectivity in sCJD blood underline the importance of continuing the epidemiological studies of blood transfusion in all forms of human prion disease.

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Author contributions

RGW, PEH, CAL and JMM all contributed to the research design, acquisition and analysis of data, PU contributed to data acquisition and data analysis and wrote the first draft of the paper. All authors contributed to drafting the paper and all approved the final version.

Conflict of interest

The authors declare no conflict of interests.

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一般的名称	①②抗 HBs 人免疫グロブリン ③④乾燥抗 HBs 人免疫グロブリン ⑤ポリエチレングリコール処理抗 HBs 人免疫グロブリン	研究報告の公表状況	Transfusion 2016; 56(3): 712-721	公表国 アメリカ
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研究報告の概要

リスザルにおけるプリオンの血液感染に関する研究：バクスター研究：

背景：変異型クロイツフェルト・ヤコブ病（vCJD）感染の 4 例の 2 次感染は、疾患の無症候期間に vCJD 患者から採血した白血球除去を行っていない赤血球製剤の輸血に関連していた。輸血を介して、vCJD の感染性の感染リスクを評価するための有効な実験モデルを確立することは、英国において有病率が 2,000 分の 1 と推定される無症候性の vCJD 患者の虫垂サンプルを保管することと共に極めて重要なことである。本研究では、実験的な挑戦としてリスザルを用いて血液を介したヒトのプリオン病である vCJD と sCJD の感染性の研究を行った。
研究デザインと方法：vCJD と sCJD に感染リスザルから採取した全血を、リスザルに複数回輸血した。輸血を受けたリスザルは、最初の輸血から 7 年後に安楽死させた。
結果：ｖCJD およびｓCJD の輸血を受けたリスザルにおいてプリオン病の臨床症状は観察されなかった。免疫組織化学的および生化学的検査において、中枢神経系またはリンパ組織に PrP（TSE）は認められなかった。同様に、vCJD と sCJD 患者のバフィーコートまたは血漿のいずれかを脳内接種（IC）および静脈内接種（IV）されたリスザルは、疾患を発症しなかった。しかしながら、ヒトゲルストマン-シュトロイスラー-シャインカー（GSS）病を継代したチンパンジーからの白血球の脳内接種を受けた 2 匹のサルは、34 と 39 ヶ月の観察期間後に剖検により感染を認めた。
結論：血液を介して、GSS は伝播するが、7 年間の観察期間内に脳内接種または静注投与のリスザルに vCJD および sCJD は感染しなかった。

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

使用上の注意記載状況・その他参考事項等

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

Blood transmission studies of prion infectivity in the squirrel monkey (*Saimiri sciureus*): the Baxter study

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BACKGROUND: Four secondary transmissions of variant Creutzfeldt-Jakob disease (vCJD) infectivity have been associated with the transfusion of nonleukoreduced red blood cells collected from vCJD patients during the asymptomatic phase of the disease. Establishing efficient experimental models for assessing the risk of future transmissions of vCJD infectivity via blood transfusion is of paramount importance in view of a study of archived appendix samples in which the prevalence of asymptomatic vCJD infection in the United Kingdom was estimated at approximately 1 in 2000 of the population. In this study, we investigated transmission of vCJD and sporadic CJD (sCJD) infectivity from blood using the squirrel monkey, which is highly susceptible to experimental challenge with human prion disease.

STUDY DESIGN AND METHODS: Whole blood collected from vCJD- and sCJD-infected squirrel monkeys was transfused at multiple time points into recipient squirrel monkeys. Blood recipients were euthanized approximately 7 years after their first blood transfusion.

RESULTS: No clinical or pathologic signs of a prion disease were observed in either the sCJD- or the vCJD-transfused monkeys, and immunohistochemistry and biochemical investigations showed no PrP^{TSE} in central nervous system or lymphoreticular tissues. Similarly, monkeys inoculated intracerebrally (IC) and intravenously (IV) with either buffy coat or plasma from vCJD and sCJD patients failed to develop disease. However, white blood cells from a chimpanzee-passaged strain of human Gerstmann-Sträussler-Scheinker (GSS) disease transmitted autopsy-proven disease to two IC-inoculated monkeys after incubation periods of 34 and 39 months.

CONCLUSION: Blood transmits GSS but not sCJD or vCJD infectivity to IC- or IV-inoculated squirrel monkeys within a 7-year observation period.

Prion diseases are a group of rare degenerative and invariably fatal disorders affecting the central nervous system (CNS). Although the infectious agent in prion diseases remains to be fully elucidated, it is generally accepted that they result from the posttranslational misfolding of a normal host-encoded cellular isoform of the prion protein (PrP^{C}) into an infectious and partially protease-resistant, disease-specific isoform (PrP^{TSE}). Indeed, the presence of PrP^{TSE}

ABBREVIATIONS: CNS = central nervous system; GSS = Gerstmann-Sträussler-Scheinker; IC = intracerebrally; NaPTA = sodium phosphotungstic acid; NCJDRSU = UK National CJD Research & Surveillance Unit; PET = paraffin-embedded tissue; PK = proteinase K; sCJD = sporadic Creutzfeldt-Jakob disease; vCJD = variant Creutzfeldt-Jakob disease.

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within CNS tissues is generally regarded as a surrogate marker for infectivity.

Enormous public and scientific attention has focused on prion diseases, not only because of their unique biologic properties, but more recently because of their impact on public health, particularly after the appearance of variant Creutzfeldt-Jakob disease (vCJD) in the United Kingdom.[1] In contrast to other human prion diseases, PrP^{TSE} readily accumulates within lymphoreticular tissues in vCJD patients, indicating a widespread involvement of the peripheral lymphoid system.[2] PrP^{TSE} has also been demonstrated to a lesser extent in peripheral nervous tissues,[2,3] in peripheral motor nerve fibers in skeletal muscle,[4,5] and in a number of other extraneuronal tissues.[4,6,7] The detection of PrP^{TSE} is primarily based on autopsy tissues from patients with clinically apparent disease; however, PrP^{TSE} has also been detected in appendix samples surgically removed from two individuals who went on to develop clinical vCJD 8 months and 2 years after their appendectomies.[8,9]

PrP^{TSE} accumulation in tissues before the onset of clinical symptoms heightened concerns over possible iatrogenic spread of vCJD by secondary human transmission through surgical procedures or surgical instruments and of greatest concern the possibility of secondary human-to-human spread of vCJD via blood transfusion from asymptomatic carriers. These concerns were confirmed by the probable transmission of vCJD infectivity to four individuals transfused with red blood cells (RBCs) from vCJD patients during their asymptomatic phase of the disease.[10-13] Subsequently, PrP^{TSE} was detected in the spleen of a hemophiliac patient who had received coagulation factor concentrate derived from UK-sourced plasma pools prepared from vCJD-implicated batches.[14] Although only one new case of vCJD has been identified within the United Kingdom during the past 3 years, a recent study of 32,000 archived appendix samples removed between the years of 2000 and 2012 estimated the prevalence of subclinical vCJD infection at 1 in 2000 of the UK population (95% confidence interval, 282–801 per million),[15] more than double that estimated by an earlier, smaller study of archived appendix and tonsil.[16] With such high levels of estimated subclinical infection, assessing the potential risk from further transmission of vCJD infectivity through contaminated blood donations remains a priority for public health.

Experimental transmission in animal models remains the definitive tool for demonstrating and estimating levels of infectious titers in prion-affected tissues. Initial public health policies regarding blood safety in the United Kingdom, developed to limit the transmission risk from blood and blood products, were formulated based on evidence from rodent models, applying infectious titers found per milliliters of blood in experimentally infected animals to human beings. More recently, experimental transmission by sheep-to-sheep transfusion has provided a model more comparable to that carried out in clinical practice and predicts that infective doses may be much lower than those estimated by rodent models.[17,18] These widely differing estimates encourage the continued development of experimental models that can provide an accurate assessment of the actual risk associated with human blood-borne prion transmission.

In this study we have used a primate model (squirrel monkey) to assess the potential risk from the transmission of vCJD, sporadic CJD (sCJD), and Gerstmann-Sträussler-Scheinker (GSS) infectivity via blood and blood components. The advantages of using squirrel monkeys are its high susceptibility to prion disease (93% compared to 97% in the chimpanzee, which is no longer used as an experimental animal) and comparatively short incubation period averaging 2 years.[19] Neuropathologic and biochemical analyses of CNS tissue from vCJD- and sCJD-infected squirrel monkeys have shown that the two forms of disease can be successfully distinguished in this animal model and that many of the characteristics that define these disease subtypes are conserved on transmission.[20] The squirrel monkey is therefore a highly appropriate model for assessing the transmission risk via blood transfusion in humans.

MATERIALS AND METHODS

Ethical statement

Human tissue samples used in this study originated from the United Kingdom and the United States. All human tissue samples originating from the United Kingdom were collected at autopsy by the UK National CJD Research & Surveillance Unit (NCJDRSU) with consent and ethical approval for the retention and research use (Lothian Research Ethics Committee reference number LREC/2000/4/157). All animal experiments were carried out in compliance in strict accordance with the recommendations in the "Guide for the Care and Use of Laboratory Animals" of the National Institutes of Health. The protocol was approved by the IACUC of the University of South Alabama (Number 020365).

Human tissue and inoculation protocols

Frozen brain samples from eight clinically typical and neuropathologically confirmed cases of prion disease were used for transmission. Brain tissue was obtained from three vCJD and two sCJD patients of UK origin and two sCJD and one GSS patient (192-bp insertion) originating from the United States. In addition, samples of plasma and buffy coat collected from the UK vCJD and sCJD patients were analyzed in this transmission series.

TABLE 1. UK cases of vCJD and sCJD inoculated into squirrel monkeys followed by monkey-to-monkey blood transfusions (Studies 1 and 2)*

Donor	Inoculum	Route of inoculation	Dilution	Incubation period (months)	Survival (months)	Disease incidence
Study 1						
vCJD (three-case pool)	Brain	IC	10^{-1}	24, 26, 29, 34		4/4
			10^{-2}	33, 34, 39, 48		4/4
			10^{-5}	38	62, 53†	1/3
			10^{-7}		61, 61, 61,	0/3
sCJD (two-case pool)	Brain	IC	10^{-1}	20, 22, 24, 25		4/4
			10^{-3}	24, 24, 28, 29		4/4
vCJD-infected squirrel monkey	Whole blood	IV	Undiluted every 3 months‡		43,† 85, 90, 90	0/4
sCJD-infected squirrel monkey	Whole blood	IV	Undiluted every 3 months‡		81, 81, 90, 90	0/4
Study 2						
vCJD						
Case 1	Plasma	IV and IC	Undiluted		37,† 62, 62	0/3
	Buffy coat	IV	Undiluted		62, 62	0/2
Case 2	Plasma	IV and IC	Undiluted		62, 62, 62	0/3
	Buffy coat	IV	Undiluted		62, 63	0/2
Case 3	Plasma	IV and IC	Undiluted		63, 63, 63	0/3
	Buffy coat	IV	Undiluted		63, 63	0/2
sCJD (two-case pool)	Plasma	IV and IC	Undiluted		60, 61, 61	0/3
	Buffy coat	IV and IC	Undiluted		62, 62, 62	0/3

* Disease incidence is expressed as the number of squirrel monkeys showing clinical signs of a prion disease and/or neuropathologic evidence of a prion disease.
† Animals euthanized before schedule due to non–prion-related causes.
‡ See Materials and Methods for details.

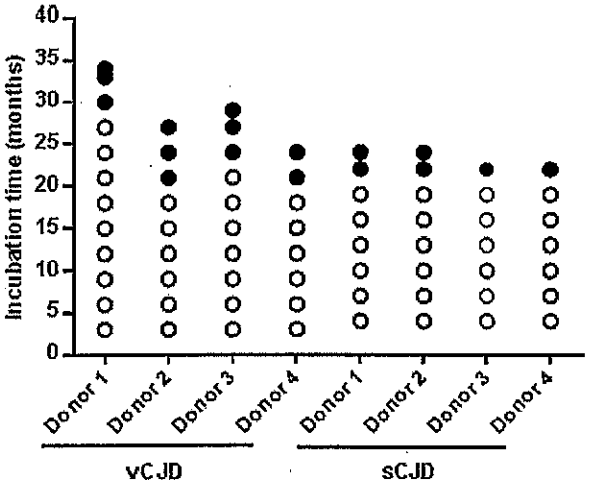

Fig. 1. Incubation period in blood donors after IC inoculation with variant and sCJD brain homogenate (10^{-1} dilution). All sCJD- and vCJD-infected squirrel monkeys were euthanized with clinical signs of a prion disease. Each circle represents the time of blood collection and subsequent transfusion to recipients. The uppermost circle shows the last blood collected at autopsy. (●) Blood collected from donors showing clinical signs of disease. (○) Blood collected at which time the donor was asymptomatic.

Inoculation protocols varied among three study subsets, depending on tissue availability and goals of the studies.

Study 1: transfusion of squirrel monkeys with whole blood from either vCJD- or sCJD-infected squirrel monkeys (the "buddy study")

Brain tissue pools of three UK vCJD and two sCJD patients were separately homogenized as 10% (10^{-1}) suspensions in saline from which 0.1 mL of undiluted and 100-fold serially diluted samples were inoculated intracerebrally (IC) into groups of three to four squirrel monkeys (Table 1). Animals were kept under continuous clinical surveillance and subjected to detailed behavioral testing at regular intervals throughout the preclinical and clinical stages of disease until the animals met the ethical criteria for euthanasia.[20]

Squirrel monkeys inoculated with 10^{-1} dilution of brain homogenate were used as blood donors in this study. At approximately 3-month intervals throughout their incubation period and clinical course, 1.5 mL of blood was drawn from each donor into tubes containing EDTA and immediately transfused into the femoral veins of individual ("buddy") monkeys. The number of individual transfusions depended on the length of the donor incubation and clinical phases of disease in each monkey and varied from 7 to 8 months in sCJD-transfused animals to 8 to 12 months in vCJD-transfused animals (Fig. 1). The total volume of transfused blood thus amounted to 10 to 12 mL in the sCJD recipients and 12 to 18 mL in the vCJD recipients. Animals were held under observation for periods of approximately 7 years after their first transfusion,

TABLE 2. US cases of chimpanzee-passaged sCJD and GSS before inoculation of purified blood components into squirrel monkeys (Study 3)*

Apheresis	Donor	Inoculum	Route of inoculation	Dilution	Incubation period (months)	Survival (months)	Disease incidence
6 months postinoculation (preclinical)	sCJD-infected chimpanzee (Case W)	Plasma	IC and IV	Undiluted		60, 60	0/2
		WBCs	IC and IV	Undiluted		60, 60	0/2
	sCJD-infected chimpanzee (Case R)	Plasma	IC and IV	Undiluted		60, 60	0/2
		WBCs	IC and IV	Undiluted		60, 60	0/2
	sCJD-infected chimpanzees Cases W and R (pooled)	RBCs	IC and IV	1:1 in saline		50, 50	0/2
	GSS-infected chimpanzee (Case C)	Plasma	IC and IV	Undiluted		60, 60	0/2
		WBCs	IC and IV	Undiluted	35, 38		2/2
		RBCs	IC and IV	1:1 in saline		50, 50	0/2
8 months postinoculation (symptomatic)	sCJD/GSS-infected chimpanzees Cases W, R, and C (pooled)	Plasma	IC	Undiluted		61,61	0/2
			IV	Undiluted		61,61	0/2
		WBCs	IC	Undiluted		61,61	0/2
			IV	Undiluted		61,61	0/2
		RBCs	IC and IV	1:1 in saline		50, 50	0/2
		PLTs	IC and IV	Undiluted		50, 50	0/2

* Disease incidence is expressed as the number of squirrel monkeys showing clinical signs of a prion disease and/or neuropathologic evidence of a prion disease.

at which time they were euthanized, terminating the experiment (Table 1).

Study 2: inoculation of squirrel monkeys with plasma or buffy coat from UK vCJD or sCJD patients

Undiluted plasma from individual UK vCJD cases was inoculated IC (0.1 mL) and intravenously (IV; 0.5 mL) into groups of three monkeys. Undiluted buffy coat was inoculated IC (0.1 mL) into groups of two monkeys. Undiluted plasma from UK sCJD cases was pooled and inoculated IC (0.1 mL) and IV (0.8 mL) into three monkeys. Undiluted buffy coat from sCJD cases was pooled and inoculated IC (0.1 mL) and IV (0.6-0.8 mL) into three monkeys. Animals were held under observation for periods of 5 to 6 years after their first transfusion, at which time they were euthanized, terminating the experiment (Table 1).

Study 3: inoculation of squirrel monkeys with purified blood components from chimpanzees infected with brain tissue from US cases of either sCJD or GSS

Brain specimens sampled from two US sCJD patients and a single GSS patient were homogenized as 10% saline suspensions and inoculated IC into single chimpanzees. Inoculated chimpanzees were held under observation and euthanized when symptomatic (approx. 26 weeks postinoculation). Brain tissue from each prion-infected chimpanzee was inoculated IC into a pair of chimpanzees. Six and a half months after inoculation, while still asymptomatic, each of the six inoculated chimpanzees were placed under general anesthesia and subjected to a 2- to 3-hour apheresis procedure (Apheresis Model No. LN 9000/MCS+, Haemonetics Corporation, Braintree, MA). Plasma and cellular blood components were separated and purified using a combination of multiple centrifugation–wash cycles and filtrations, using filters for plasma (Pall LPS1, Pall Corporation, Port Washington, NY) and filters for separation of RBCs and platelets (PLTs) from white blood cells (WBCs; Pall Neo 1 and LRFXL filters, respectively, Pall Corporation). All purified samples were frozen at −70°C. Six to 8 weeks later, after the onset of symptomatic disease, the animals were again anesthetized and subjected to a terminal apheresis and plasma and blood components separated and purified as described. Samples were subsequently thawed and plasma, WBCs, and RBCs from the first apheresis procedure, and plasma, WBCs, RBCs, and PLTs from the terminal apheresis procedure, were each inoculated into groups of two to four young adult squirrel monkeys via either a single IC (0.1 mL) or IV (0.5 mL) inoculation or a combination of IV and IC routes (Table 2).

Harvesting of CNS and peripheral tissues at postmortem

All experimentally challenged squirrel monkeys from Study 1 were subjected to a full autopsy, from which CNS tissues and a selection of peripheral tissues were examined. Brains were removed with half the brain sliced and frozen at −80°C for biochemical analysis with the remaining half immersed in 15% formalin for histologic analysis. In addition to brain tissue, samples from the spinal cord (cervical, thoracic, and lumbar regions), spleen, tonsil,

and cervical lymph node were fixed in 15% formalin and separate samples frozen at −80°C. In addition, the two squirrel monkeys transfused with WBCs purified from chimpanzee-passaged GSS were subject to a full autopsy.

Neuropathology

After formalin fixation, tissues were immersed in 96% formic acid for 1 hour before processing to reduce titers of infectivity. Serial sections (5 μm) were cut for hematoxylin and eosin staining, for immunohistochemistry, and for paraffin-embedded tissue (PET) blotting. Immunohistochemistry was performed as previously described.[16] PrP was detected using three monoclonal anti-PrP recognizing different residues of the prion protein (3F4, Cambridge Bioscience, Cambridge, UK; 12F10, Bioquote Ltd, York, UK; 6H4, Prionics, Schlieren, Switzerland). Briefly, tissue sections were autoclaved in distilled water at 121°C for 10 minutes followed by immersion in 96% formic acid for 5 minutes. Sections were immersed in proteinase K (PK) solution (5 μg/mL) for 5 minutes before blocking in normal rabbit serum. Sections were incubated for 1 hour with the anti-PrP, and immunolabeling was completed using the catalyzed signal amplification system (DAKO, Ely, UK).[21] Staining was visualized with 3,3'-diaminobenzidine chromogen. PET blot analysis for PrP^{TSE} was carried out on CNS and peripheral tissues as described previously.[22] PrP^{TSE} was detected with the anti-PrP 12F10 and 6H4 and immunolabeling was completed using the a detection system (Vectastain ABC-AmP, Vector Laboratories, Peterborough, UK). Staining was visualized using 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium.

Western blot analysis of PrP^{TSE} tissue deposition

Detection of PrP^{TSE} in frozen tissue samples was investigated using the standard diagnostic Western blot protocol carried out at the NCJDRSU for the detection and classification of PrP^{TSE} in human CNS tissue.[23] An additional step of centrifugal concentration of tissue homogenates was included for increased sensitivity.

Briefly, samples were homogenized in a nondenaturing detergent buffer at 10% tissue weight/volume (wt/vol) of buffer. Aliquots of the cleared 10% brain homogenates were subjected to digestion with PK used at 50 μg/mL for 1 hour at 37°C. One-hundred microliters of the PK-treated sample was given a brief mixing before centrifugation at 20,000 × *g* for 1 hour at 4°C. PrP^{TSE} was analyzed by Western blotting using 10% NuPage Bis-Tris gels, buffers, and the manufacturer's instructions (Life Technologies, Paisley, UK).[23] Western blots were probed with the monoclonal antibody 3F4, used at final concentrations of 75 ng/mL for 1 hour. The secondary antibody was ECL anti-mouse IgG, peroxidase-linked species-specific F(ab')2 fragment, used at a concentration of 1/25,000 for 1 hour, and signal was captured by Western blotting detection reagent (ECL Prime, Amersham, Buckinghamshire, UK) using x ray film. In all Western blot runs, a 5-μL protease-treated sample of a 10% brain homogenate from a vCJD- and sCJD-infected squirrel monkey and from a sCJD MM1 and vCJD (MM2B) patient were included serving as an appropriate reference control for both fragment mobility and glycoform ratio.

The presence of PrP^{TSE} in frozen tissue samples was also investigated using the high-sensitivity Western blot protocol described by Peden and colleagues.[5] Briefly, tissue samples were homogenized to a 10% (wt/vol) in 2% sarkosyl/phosphate-buffered saline by using a benchtop tissue homogenizer system (FastPrep, Fisher Scientific, Loughborough, UK). Samples were precipitated with sodium phosphotungstic acid (NaPTA) and subject to PK digestion; immunoblotting was performed as described above.[5]

RESULTS

All squirrel monkeys inoculated IC with 10^{-1} and 10^{-3} dilutions of vCJD and sCJD brain homogenates (Study 1) developed clinical signs of disease with incubation periods ranging from 20 to 48 months, and one of four animals inoculated with a 10^{-5} dilution of vCJD became symptomatic after an incubation period of 38 months (Table 1). Neuropathologic, immunohistochemical, and biochemical investigations on CNS tissue from squirrel monkeys inoculated with a 10^{-1} brain homogenate (blood donors) confirmed the diagnosis of prion disease. The distinctive pattern of spongiform change and PrP^{TSE} accumulation in the brain (Figs. 2A-2D) as well as PrP^{TSE} type observed in the brains of the infected squirrel monkeys (Fig. 3) demonstrated that vCJD and sCJD could be successfully distinguished in this animal model and that many of the neuropathologic and biochemical features that define these disease subtypes are conserved on transmission.[20]

Western blot analysis of spleen samples from the vCJD- and sCJD-infected monkeys detected PrP^{TSE} in one of the four animals in each group. The PrP^{TSE} isoform resembled that found in the brains of the same donor monkeys and in the human brain samples used to infect them, in terms of molecular mass, mobility, and glycosylation (Fig. 3).

No evidence of transmission occurred in squirrel monkeys serially transfused with whole blood collected from the 10^{-1} brain-inoculated monkeys during the entire span of their observational period (approx. 7 years). Postmortem examination of hematoxylin and eosin–stained brain sections showed no evidence of vacuolar pathology in recipients of whole blood from either vCJD- or sCJD-infected monkeys. Immunohistochemical and PET blot analysis of fixed brain tissue using a panel of anti-PrP showed no evidence of PrP^{TSE} accumulation in any of the transfused animals (Figs. 2E and 2F). Western blot analysis of 500-mg frozen samples of frontal cortex tissue following

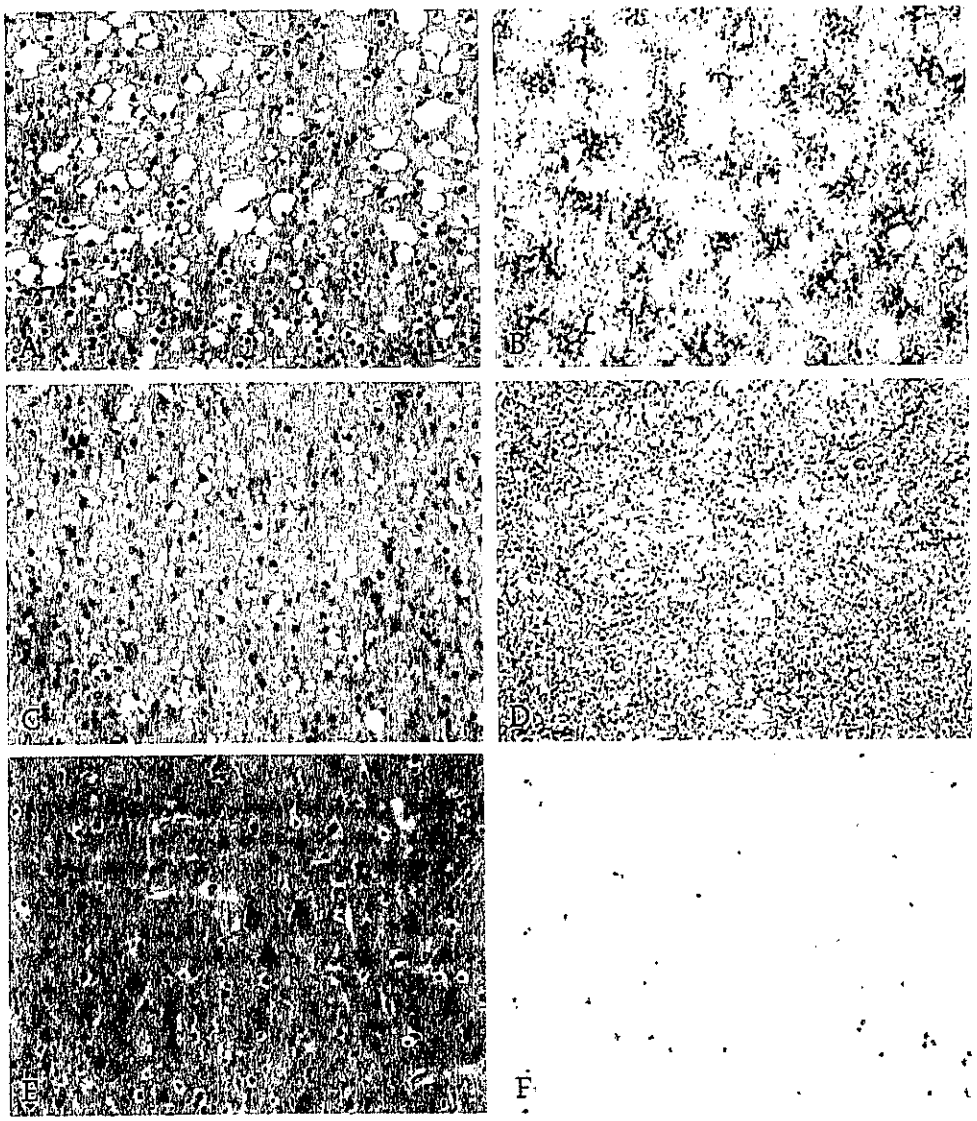

Fig. 2. Pathologic features in squirrel monkeys challenged with human prion disease. (A) Widespread spongiform change and (B) PrP accumulation in a predominantly pericellular pattern in the cerebral cortex in a squirrel monkey inoculated with vCJD brain homogenate. (C) Widespread microvacuolar change, with a (D) predominantly synaptic pattern of PrP accumulation in the in the cerebral cortex of a squirrel monkey inoculated with sCJD brain homogenates. (E, F) No evidence of spongiform vacuolation or PrP accumulation was detected in squirrel monkeys serially transfused with whole blood collected from vCJD-infected squirrel monkeys. Original magnifications ×400.

NaPTA precipitation failed to detect any disease-specific banding pattern consistent with the presence of PrP^{TSE} in the recipient animals (data not shown). Further analysis of samples of spleen, tonsil, cervical lymph node, spinal cord, and vagus nerve using immunohistochemistry, PET blot analysis, and NaPTA precipitation also failed to show evidence of PrP^{TSE} accumulation in the recipient animals (Figs. 3C and 3D). Therefore, we have been unable to detect evidence of transmission of vCJD or sCJD infectivity via blood transfusion in this primate model.

All squirrel monkeys inoculated with plasma and buffy coat from vCJD and sCJD patients (Study 2) were asymptomatic after an observation period of approximately 6 years (Table 1). Autopsies were not performed on these animals.

In the experiment using US cases of sCJD and GSS (Study 3), squirrel monkeys inoculated with purified blood components from chimpanzee-passaged sCJD or with purified plasma from chimpanzee-passaged GSS remained asymptomatic more than 6 years after inoculation (Table

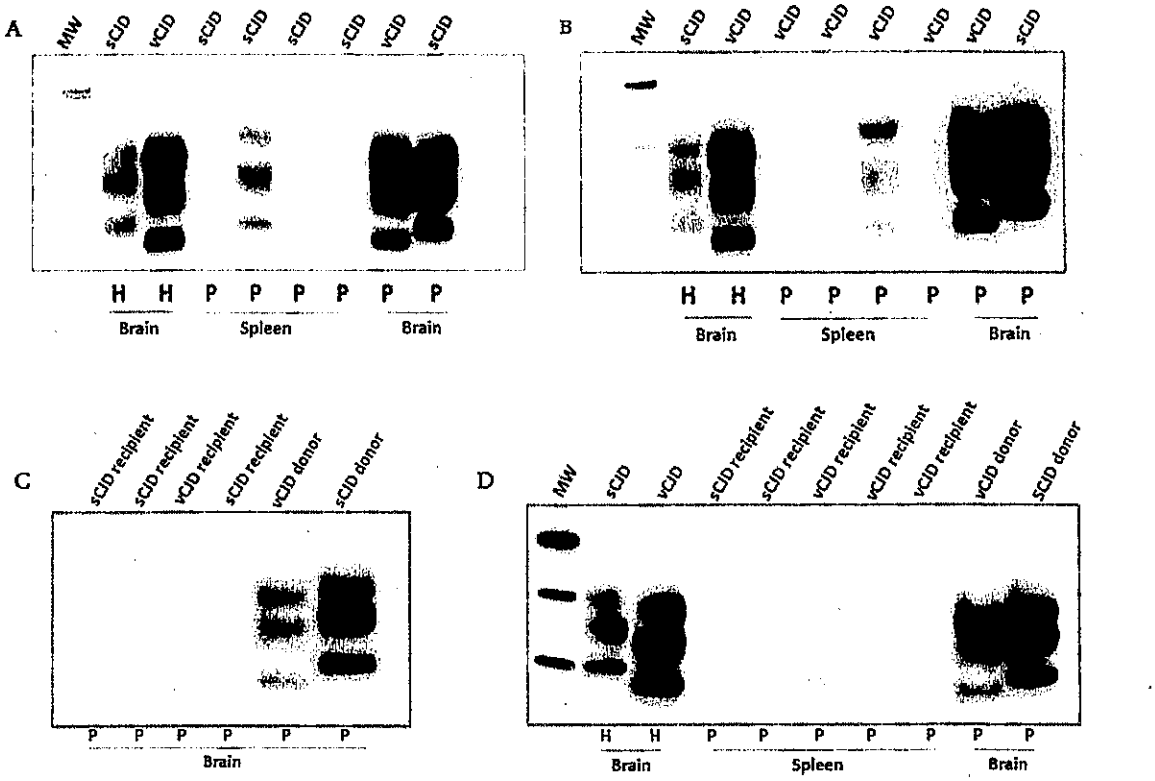

Fig. 3. Western blot analysis of PrP^{TSE} in spleen and brain samples from squirrel monkeys. (A, B) Squirrel monkeys (blood donors) challenged with vCJD or sCJD brain homogenate. (C, D) Squirrel monkeys (recipients) transfused with blood collected from vCJD- or sCJD-infected donors. Tissues were analyzed after digestion with PK. Squirrel monkey samples (P) were run alongside a human (H) sCJD and a vCJD diagnostic standard.

2). However, the two squirrel monkeys inoculated with purified WBCs from chimpanzee-passaged GSS did transmit disease 35 and 38 months postinoculation (Table 2). Both animals were symptomatic and had spongiform vacuolation (Fig. 4A) with PrP^{TSE} detectable by immunohistochemistry (Fig. 4B) and Western blot analysis (Fig. 4C).

DISCUSSION

This study investigated infectivity in the blood and blood components of squirrel monkeys inoculated with different forms of human prion disease and examined whether blood transfusions from these monkeys could transmit disease to other monkeys. Ongoing surveillance of blood recipients from donors who subsequently died of sCJD in the United States and United Kingdom have so far failed to identify any recipient dying of CJD. In the United States, there are currently 645 such recipients from 54 CJD donors, of whom 187 have survived 5 to 20 years after transfusion.[24] In the United Kingdom, current data are being analyzed for publication, but as of 2006, a total of 20 recipients from three sCJD donors had been identified, none of whom had developed CJD.[25]

In contrast to these negative surveillance studies of blood-borne sCJD, four instances of human vCJD infection have been identified among a total of 67 recipients of blood or blood components from individuals who later died of vCJD, of whom 34 survived 5 or more years.[10-14] In an as-yet-unpublished study conducted in cynomolgus monkeys, transfusions of whole blood from vCJD-infected monkeys (but not directly from human vCJD cases) transmitted disease (J.P. Deslys and E. Comoy, personal communication, 2015).

Blood is regularly infectious in rodent models of CJD, and in a recent study conducted in transgenic mice overexpressing the PrP gene, infectivity was detected in plasma, WBCs, and RBCs from a single vCJD case and in plasma from two of four cases of sCJD.[26] Comparison of this study to the primate studies is hindered by our continued ignorance of how well the results in highly susceptible engineered mice correspond to the results in parallel studies of the same specimens in primates.

In our study, plasma and buffy coat from vCJD patients inoculated both IC and IV into squirrel monkeys

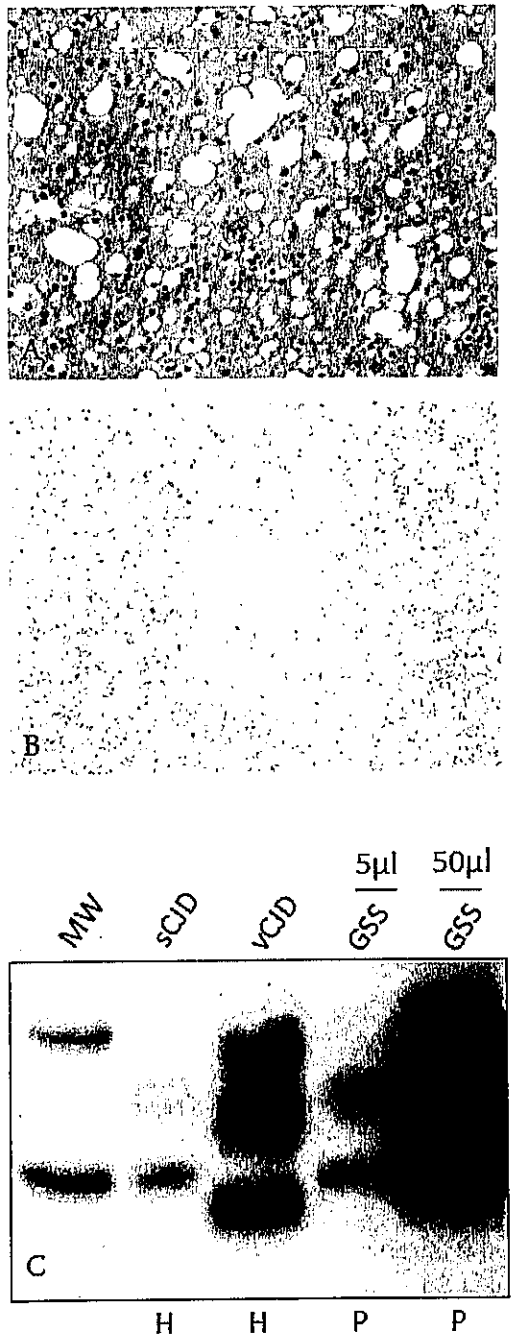

Fig. 4. Pathologic and biochemical features in a squirrel monkey challenged with WBCs from a GSS-infected chimpanzee. (A) Widespread, confluent spongiform change and (B) PrP accumulation in a predominantly synaptic pattern of PrP accumulation in the cerebral cortex. Original magnifications ×400. (C) PrP^{TSE} in extracts of frontal cortex from a squirrel monkey inoculated with WBCs from GSS-infected chimpanzee. Tissues were analyzed after digestion with PK. Squirrel monkey samples (P) were run alongside a human (H) sCJD and a vCJD diagnostic standard.

failed to transmit disease, as did multiple small transfusions of whole blood collected throughout the incubation and symptomatic phases of disease from vCJD-infected squirrel monkeys. Blood recipients showed no clinical signs of disease and no PrP^{TSE} was detected in the brain, spinal cord, spleen, tonsil, or lymph nodes after periods of 6 to 7 years after receiving the first of multiple transfusions.

What factors might be responsible for these apparently discrepant results? One possibility is that the squirrel monkey is less sensitive than the cynomolgus monkey to vCJD infections. Large numbers of IC-inoculated squirrel and cynomolgus monkeys at the NIH showed that for sCJD, the squirrel monkey was a more sensitive assay animal than the cynomolgus monkey (vCJD had not yet appeared when these studies were done).[19] In this study, parallel brain infectivity titrations of sCJD and vCJD were similar. Therefore, for IC infections with either sCJD or vCJD, the squirrel monkey is at least as sensitive as the cynomolgus monkey, and although it could still be argued that this equivalence may not extend to IV infections, we know of no precedent in conventional viral or prion disease to validate such a species-dependent IC versus IV discrepancy. However, in a recent independent study comparing vCJD and sCJD in cynomolgus monkey, survival times were significantly shorter after vCJD than sCJD infections.[27] Thus, despite our results in sCJD infections, we cannot altogether discount the possibility that the cynomolgus monkey could be more sensitive to vCJD.

A second possibility is that we administered a subinfectious amount of blood. Our IC and IV inoculations of plasma and buffy coat from chimpanzee-passaged cases of sCJD and GSS used far lower IV volumes than were used in the cynomolgus study, in which monkeys were transfused with up to 40 mL of whole blood (equivalent to 1-1.5 transfusion units in humans). In our multiple transfusion study of monkey-to-monkey–passaged sCJD or vCJD, each 1.5 mL of transfused blood represented approximately 2% of the squirrel monkey circulating blood volume; the combined transfused volumes amounted to approximately 7 mL in sCJD recipients and 10 mL in vCJD recipients or approximately 1 to 1.3 units of blood in terms of transfusions in humans. Although this total transfusion volume was comparable to that of the cynomolgus study, its administration in a series of multiple small transfusions may have been insufficient to infect the recipients. Moreover, the fact that preclinical blood from the pair of GSS-infected chimpanzees transmitted disease, but clinical stage blood pooled with the two sCJD cases did not transmit disease, may have resulted from dilution of GSS blood infectivity by blood from sCJD-infected animals to levels below the threshold of disease transmissibility.

Currently, the most relevant experimental model for studying transfusion-associated, blood-borne vCJD comes from sheep orally infected with bovine spongiform

encephalopathy.[17] Statistical models based on the results of these sheep transfusion studies estimated the infectious doses per unit of prion-infected blood (approx. 400 mL) at approximately 0.8 ID per unit of nonleukoreduced RBCs, which is equivalent to approximately 0.002 ID/mL.[18] Applying estimated infectious titers from the sheep model directly to this study would indicate that recipient animals were transfused at each time point with 0.003 ID. No blood infectivity titrations were conducted in this series of experiments. However, in view of the irregular transmission results in both humans and experimental animals, depending on the exact levels of infectivity within blood of the donors, it is likely that the levels of infectivity must be very close to or below the threshold for disease transmission.

A third possible explanation of our failure to detect infectivity is whether longer observation periods might have been needed for clinically manifest disease to appear. There is abundant experimental evidence from studies of various prion strains across a range of species that PrP^{TSE} can be detected by Western blots or immunohistochemistry in brain and lymphoid tissues well before infectivity can be demonstrated, sometimes as early as midway through the incubation period.[28-32] The absence of PrP^{TSE} in the brains of our assay monkeys therefore suggests that they would not have become ill within at least a year or two after termination of the experiment, but does not exclude the possibility that incubation periods might have extended many years beyond our observation endpoint. A recently concluded study in which scrapie-infected sheep brain was inoculated IC into a cynomolgus monkey yielded an incubation period of 10 years.[33] Previous studies of hundreds of patients whose brain tissue was inoculated IC into squirrel monkeys yielded a bell-shaped incubation curve that peaked at 2 years, with a maximum duration of 3 years,[19] exactly the same as the incubation period in our vCJD assay animal inoculated at the limiting brain dilution of 10^{-5} and in the assay animal inoculated with WBCs from chimpanzee-passaged GSS.

Our study of apheresed blood from sCJD- and GSS-infected chimpanzees was undertaken to obtain sufficient blood volumes to examine purified components. No component-transmitted disease from either case of sCJD, corroborating similarly negative results of earlier IC inoculations of WBCs or whole blood into squirrel monkeys from 7 cases of sCJD, and transfusions of units of whole blood from three additional cases into chimpanzees held under observation for 10 to 15 years.[19] However, WBCs from chimpanzee-passaged GSS case did transmit infectivity similar to an earlier study conducted in wild-type mice in which WBC-transmitted disease from a different (mouse-adapted) case of GSS.[28]

An unexpected finding was that all six chimpanzees used as blood donor animals infected with either sCJD or GSS became symptomatic within 4 to 6 weeks of having been apheresed 6 months after inoculation, that is, having an incubation period of approximately 7 months. Because this finding was not anticipated, no additional nonapheresed animals inoculated with the same samples were included in the study. However, earlier human sCJD transmission experiments in chimpanzees infected IC with sCJD brain tissue from 28 different sCJD cases yielded a mean incubation period of 17 months (minimum of 11 months), suggesting that the stress of a 3- to 4-hour-long apheresis procedure under general anesthesia may have triggered the onset of symptomatic disease in these animals.

In conclusion, we suggest that the variation in transmission results obtained in different experimental studies is mostly due to sampling issues inherent in studies in which small numbers of animals are used to evaluate comparatively uncommon events, and thus always subject to erratic or even contradictory results. Therefore, in this study, the fact that infectivity was present in WBCs from chimpanzee-passaged GSS during the incubation period, but not the clinical stage of disease or from chimpanzee-passaged sCJD at any period of disease, should not be overinterpreted to mean that GSS blood is only infectious during the incubation period, or is more infectious than sCJD blood, which may or not be the case. Nor do the negative results in our monkey-to-monkey transfusion study mean that vCJD blood is not infectious (we know from human epidemiologic studies that it can be). Considering the ensemble of human epidemiologic studies together with the limited experimental blood transmission studies, the most reasonable conclusion is that vCJD (and possibly GSS)-infected donors pose a greater risk than sCJD donors and that even if a small proportion of sCJD blood donations were to contain low levels of infectivity, disease transmission must be a very rare event, if it occurs at all.

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CONFLICT OF INTEREST

TRK is an employee of Baxalta, formerly Baxter BioScience, a company that produces plasma protein therapies. The other authors have disclosed no conflicts of interest.

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研究報告の概要

○*Trypanosoma cruzi (T. cruzi)*:食物が媒介する原虫として国際的に認識されるべき時。

*T. cruzi*の食物媒介感染は、食物、もしくはより高い頻度で飲料がトリポマスチゴート型に汚染し、ヒトがこれを飲食することで発生する。トリポマスチゴート型は加熱によって不活化されると考えられているが、冷蔵及び冷凍に対しては非常に高い耐性を示すことが複数の実験により証明されている。

食物媒介によるシャーガス病については、2010年以降に4ヶ国以上から16を超えるアウトブレイクが報告されているが、感染者数は約170名となり、多数の死者も出ている。過去10年間に寄せられた73件の報告には、959例の急性シャーガス病症例が含まれており、経口感染例は638例(67%)、先天性感染例は258例であり、ベクター媒介による直接的な皮膚感染例はわずか23例であったことが示されている。

食物媒介感染の初期段階における罹患率と死亡率は比較的高い。これは高濃度の原虫に感染することや、胃ムチンや上皮細胞に結合する*T. cruzi*の糖たんぱく質gp82の発現による、胃粘膜を介した効率的な感染メカニズムに関連している。感染の初期段階の発症については、経口感染者の80～100%は高熱が遷延するが、これに対して、ベクター媒介感染は軽症であることが多く、症状が認められないことさえもある。ベクター媒介感染では、原虫の摂取はサシガメの糞を介しており、3,000～4,000トリポマスチゴート型/μLが含まれるが、皮膚からの侵入に成功するのはこの一部である。しかし、一匹のブラジルサシガメには経口により何百人も感染させることができる684,000トリポマスチゴート型が潜伏する。

食物媒介感染という問題が認識されることによって、初めてコントロールの検討が可能となる。我々は、食物媒介感染が会議等の場においてテーマとして取り上げられる機会を増やすことを目的として、本稿を作成した。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
Trypanosoma cruzi の感染経路は、67%が経口感染によるもので、高濃度の原虫に感染するため80～100%が高熱などの急性期症状を呈するという報告である。	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。かについて問診を行い、該当する献血者については、中南米諸国を離れて6か月未満の人は献血延期とする。6か月以上経過している人については、献血の都度*T.cruzi*抗体検査を行う。 *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

VIEWPOINTS

Trypanosoma cruzi: Time for International Recognition as a Foodborne Parasite

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A Neglected Transmission Route of a Neglected Tropical Disease

Chagas disease is one of the "neglected tropical diseases" (NTDs) listed by the World Health Organization (WHO). Depending on the study, the global prevalence has been estimated to be between 9 and 10 million people, with a disease burden from 300,000 to over 800,000 disability adjusted life years (DALYs) [1–4]. At least 10,000 people die from Chagas disease annually. Chagas disease accounts for over 11% of heart failure in Brazil [3], and over 7% of deaths due to heart failure during 2006 were due to Chagas disease [5]. The global costs of Chagas disease have been estimated at US$7.19 billion per year.

Transmission of *Trypanosoma cruzi*, the protozoan etiological agent of Chagas disease, was traditionally considered almost exclusively vectorborne, transmitted by bugs (Hemiptera) in the family Reduviidae, sub-family Triatominae. However, other transmission routes exist, including intrauterine, by blood transfusions and organ transplantation, laboratory accidents, and foodborne transmission. In foodborne infections, food is, or more commonly, drinks are, contaminated with metacyclic trypomastigotes, which are then ingested by susceptible humans. These contaminant metacyclic trypomastigotes may be derived from either the feces of triatomines or from the whole insect. This indicates that although such transmission is not classical vector-borne transmission, the vector is still essential in this foodborne modality. Alternatively, contaminant metacyclic trypomastigotes have the potential to be derived from the secretions of reservoir hosts such as opossums that may also contaminate food [6]; for this transmission route the vector is not involved. It should be noted that as metacyclic trypomastigotes are inactivated by the action of drying or by low moisture content [7], drinks such as fruit juices are the most common transmission vehicles. Other food items may also be relatively unsuitable as transmission vehicles due to toxic effects, and some food preparation treatments may also inactivate the parasites. Whereas heating seems to inactivate trypomastigotes [8], experiments have indicated that they are quite resistant to refrigeration and freezing [9].

Whereas direct vectorborne transmission has gradually been controlled, particularly due to initiatives in housing organized by international health institutions such as WHO and PAHO [10], foodborne infection continues to be relatively neglected. Although several researchers

have drawn attention to this mode of transmission (e.g. [11,12]), and foodborne Chagas disease was mentioned by the WHO Foodborne Disease Burden Epidemiology Reference Group (FERG), it was not considered of sufficient interest to merit evaluation with the resources available [13]. Similarly, a "call for action" article for Chagas disease [14] does not mention foodborne transmission.

Why Is Foodborne *T. cruzi* Infection Becoming More Common?

Outbreaks of foodborne Chagas disease are documented from 1966 [15] and oral transmission may always have been the usual infection route between wild and domestic fauna; stercorarian transmission is relatively inefficient, and fur and thick skin create a barrier for cutaneous penetration [10,16]. Much of the experimental work on oral transmission using animal models was conducted between 1960 and 1980, and human outbreaks occur regularly. Furthermore, earlier clusters of cases previously considered vectorborne may have been foodborne [15]. However, although we can better recognize outbreaks of foodborne Chagas disease, there seems to be little doubt that foodborne transmission is increasingly reported. Since 2010, over 16 outbreaks of foodborne Chagas disease have been reported from at least four countries, involving around 170 individuals and a dozen deaths [15]. A summary of 73 reports from the past 10 years that includes 959 cases of acute Chagas disease suggests that 638 (>66%) were due to oral transmission, 258 congenital, and only 23 due to direct cutaneous vectorborne transmission [17]. The relative increase in foodborne transmission may not only reflect decreases in cutaneous vectorborne transmission due to efficient control of the vector *Triatoma infestans* by domiciliary spraying [18] but also result from alterations in aspects of ecology and host behavior [19]. Progressive invasion and domestication of wild triatomines has occurred in rural and urban localities. The species *Triatoma dimidiata*, *Panstrongylus rufotuberculatus*, *Rhodnius stali*, *Eratyrus mucronatus* and *Panstrongylus geniculatus* have all been reported as being domiciled in urban areas [18,20,21]. This could have exacerbated possibilities for foodborne transmission. As well as "urbanization" of wild triatomines and reservoir hosts, human encroachment into areas where wild reservoirs live may also exacerbate the potential for transmission, both orally and by direct vectorborne transmission, particularly as environmental imbalance caused by man through the invasion and deforestation of woodlands, results in reduction of the biodiversity of mammals available as food sources for triatomines [19]. In the Brazilian Amazon around 70% of cases of acute Chagas disease recorded between 2000 and 2010 were associated with food consumption [15]. Another important factor is that many more species of triatomines are suitable as vectors for oral transmission than cutaneous vectorborne transmission, as a rapid defecation reflex after feeding is unnecessary in foodborne transmission [15]. The rapidity of defecation following feeding has long been used as a measure of the suitability of different triatomine species for vectorial transmission, and a defecation index was even proposed as a measure of rapidity and frequency of defecations, and hence importance as a transmission agent following biting [22,23]. This means that triatomines such as *P. geniculatus*, the most widely distributed species in the Americas, are suitable for foodborne transmission, but not for cutaneous vectorborne transmission. In cities such as Caracas, the high prevalence of infection of *P. geniculatus* with *T. cruzi* [24,25], the wide distribution of this vector in neighborhoods, and its presence in homes during its immature stages (five nymph stages) are factors that lead to the direct contamination of food and beverages. Nymphs may easily contaminate food unnoticed due to their small size (1st nymphal instar is around 2 mm).

Why Is Foodborne Transmission Potentially More Serious Than Vectorborne Transmission?

The route of infection influences the success of *T. cruzi* in its vertebrate host and also the severity of clinical outcome. Relevant factors include the number of metacyclic trypomastigotes, the biodeme involved, and the host's immune response. The relatively high percentage of morbidity and mortality in the early stages of infection in foodborne transmission [26] have been related to higher parasite load and the efficient mechanism of infection through the stomach mucosa, as first demonstrated by Hoft et al. [27]. This is based on expression of gp82, a stage-specific surface glycoprotein that binds to gastric mucin and epithelial cells, triggering the signaling cascades leading to intracellular Ca^{2+} mobilization and promoting parasite entry [28]. During the early stages of infection, prolonged high fever occurs in 80–100% of cases with oral transmission, whereas with vectorborne transmission, symptoms are often mild or even absent [15]. Furthermore, cardiac pathology occurs relatively frequently, and is potentially severe. In the Chacao outbreak severe clinical signs occurred in 34.4% of patients, compared with 5–10% by cutaneous vectorborne transmission [29].

A range of experimental studies in mice has also demonstrated significantly greater infectivity through oral challenge [30,31]. As with many other unrelated foodborne parasites, the potential for heavy contamination of the infection vehicle is critical for transmission success. In vectorborne transmission, the parasite inoculum is through triatomine feces, perhaps containing 3,000–4,000 metacyclic trypomastigotes per μl, of which only a proportion succeed in penetrating the epidermis. However, *T. infestans* can harbor 684,000 infective trypomastigotes, able to infect hundreds by the oral route [15].

Timely diagnosis and effective treatment are important for decreasing disease progression and the likelihood of congenital transmission. However, the absence of the classical signs (a skin chagoma or Romaña's sign) and ignorance regarding the presence of triatomines may delay diagnosis in foodborne infections.

The importance of other routes of infection with *T. cruzi* should not be underestimated. These include the fact that transmission via blood transfusion and/or tissue donation can deliver high infectious inoculum and tends to result in the most virulent acute disease especially in immunosuppressed persons. Thus the greater likelihood of infecting higher numbers of people, combined with the potential for delayed diagnosis and more severe symptoms, means that foodborne Chagas disease is likely to have a greater impact than vectorborne transmission at both individual and community level. Nevertheless, it is important to remember that domestic vectors may still colonize houses across wide areas, and vector elimination efforts should be sustained [32].

Estimating the Burden of Foodborne Chagas Disease

While the evidence of foodborne transmission of *T. cruzi* is convincing, to date there have been no systematic reviews or other published work that might indicate the proportion of Chagas disease transmitted via food. However, as an indication, data can be extracted from a narrative review of acute Chagas disease [17]. On a crude level, 638 of 959 cases (67%) were orally transmitted [17], and, in consideration of the large foodborne outbreaks that have been reported, we suggest that the majority of these would be through contaminated food, including beverages. Furthermore, 21 were through reactivation and 258 through congenital transmission. The former would have a primary transmission of similar proportions to the total, and likewise for women who transmit the parasite to their children congenitally. The only cases definitively not linked to oral transmission are the cutaneous vectorborne cases [12], and the transfusion and transplantation cases [5]. Adding in the unknown to this group, and making an adjustment

(some reactivation and congenital cases would result from vectorborne transmission) would result in approximately 910 cases that are acquired through oral transmission (95%). Even if only around half of these are actually foodborne, this would result in 273,000 DALYs per annum attributable to contaminated food, assuming the Chagas disease burden reported in GBD 2010 [2]. This would rank foodborne Chagas disease as approximately the 8th most important foodborne parasitic disease on a global basis [13], despite much of the world being free from this parasite. This may give some pointers as to the burden of foodborne Chagas disease, but it should be noted that both foodborne and vectorborne disease may also be sporadic and hence using outbreak data might underestimate the burden from vectorborne transmission. It should be noted that oral infection does not necessarily indicate contaminated food—*per os* infection can also occur from dirty hands contaminated with triatomine feces. Such an infection route is less likely to result in a large-scale outbreak. Furthermore, chronic disease, with no acute stage symptomatology, provides a substantial burden and the contribution of different transmission pathways leading to that clinical picture have yet to be addressed.

Clearly, a systematic review of the evidence rather than the superficial overview reported here might better untangle the contributions to the burden of Chagas disease by the different transmission pathways.

Message to Stakeholders and Policy-Makers

WHO launched FERG in order to provide data and tools to support policy-makers and other stakeholders when setting appropriate, evidence-informed priorities of food safety at country level. However, by focusing on global impact, important pathogens that have a restricted distribution may be overlooked. It seems as though *T. cruzi* could be one of these. In calling to the relevant governments from Latin America and elsewhere for sustained support for prevention, control, and treatment of Chagas disease, Schmuñis [14] provided an overview of the disease, including transmission and mitigation initiatives; however, foodborne infection was not mentioned. Likewise, in the CODEX Alimentarius draft guidelines on the application of the general principles of food hygiene to the control of foodborne parasites, *T. cruzi* is not mentioned.

By concerted efforts, relevant authorities have managed to reduce vectorborne transmission of Chagas disease considerably. Foodborne transmission may be a more complex situation, with multiple and changing factors that mean transmission reduction may be more difficult to achieve. However, before we can think about control, the problem must be first acknowledged. The intention of this article is to bring foodborne transmission further onto the table.

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医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2016. 6. 3	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名(企業名)	新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)		Nolen LD, Osadebe L, Katomba J, et al. Emerg Infect Dis. 2016 Jun;22(6):1014-21.	米国

研究報告の概要

○コンゴ民主共和国で発生したサル痘のアウトブレイクにおけるヒトーヒト感染の拡大。
コンゴ民主共和国のボクング保健ゾーンでは、2013年の後半に計104例のサル痘疑い例が報告され、例年の6倍*(2011年17症例、2012年13症例)を超える症例数の増加がみられた。オルソポックスウイルス特異的リアルタイムPCR検査が行われた60例のうち、50例(48.1%)はサル痘ウイルス(MPXV)感染が確認され、10例(9.6%)は陰性と判定された。家族内発症率(MPXV感染者と同居し、感染症状を発症した者の割合)は、これまで報告された3～11%から50%となった。9世帯において、感染事象の件数が1件以上となった。また、ボクング保健ゾーン内では6件以上の感染事象が発生していた。平均潜伏期間は8日であった(範囲:4日～14日)。本研究で確認された高い発症率と感染は、サル痘症例の調査並びに迅速に症例を特定することの重要性を強調している。アウトブレイク発生時には、MPXV感染の伝播を阻止するため、地域社会の教育並びに訓練を実施する必要がある。

*訳者注:
抄録には600倍と記載されているが、計算(前2年の平均15症例との比較)すると約6倍(5.9倍)の誤り。

報告企業の意見	今後の対応
サル痘症例数が、コンゴ民主共和国にて2013年の後半に例年の6倍を超え、家族内発症率は既報告3～11%が50%まで増加したという報告である。	今後も引き続き、新たなウイルス等による感染症の発生状況等に関する情報の収集に努める。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

Extended Human-to-Human Transmission during a Monkeypox Outbreak in the Democratic Republic of the Congo

Leisha Diane Nolen, Lynda Osadebe, Jacques Katomba, Jacques Likofata, Daniel Mukadi, Benjamin Monroe, Jeffrey Doty, Christine Marie Hughes, Joelle Kabamba, Jean Malekani, Pierre Lokwa Bomponda, Jules Inonga Lokota, Marcel Pie Balilo, Toutou Likafi, Robert Shongo Lushima, Benoit Kebela Ilunga, Frida Nkawa, Elisabeth Pukuta, Stomy Karhemere, Jean-Jacques Muyembe Tamfum, Beatrice Nguete, Emile Okitolonda Wemakoy, Andrea M. McCollum, Mary G. Reynolds

A 600-fold increase in monkeypox cases occurred in the Bokungu Health Zone of the Democratic Republic of the Congo during the second half of 2013; this increase prompted an outbreak investigation. A total of 104 possible cases were reported from this health zone; among 60 suspected cases that were tested, 50 (48.1%) cases were confirmed by laboratory testing, and 10 (9.6%) tested negative for monkeypox virus (MPXV) infection. The household attack rate (i.e., rate of persons living with an infected person that develop symptoms of MPXV infection) was 50%. Nine families showed ≥1 transmission event, and ≥6 transmission events occurred within this health zone. Mean incubation period was 8 days (range 4–14 days). The high attack rate and transmission observed in this study reinforce the importance of surveillance and rapid identification of monkeypox cases. Community education and training are needed to prevent transmission of MPXV infection during outbreaks.

Monkeypox virus (MPXV), which belongs to the genus *Orthopoxvirus*, is zoonotic and endemic to western and central Africa. MPXV is a close relative of the variola virus, and monkeypox illness resembles a smallpox-like infection but is less severe than smallpox. Most patients initially develop a fever, followed by rash a few days later. Lymphadenopathy is a common sign and occurs just before or concomitant with the rash (*1,2*). Up to 11% of unvaccinated affected persons die (*3*). No targeted medications are licensed to treat this infection. Although smallpox vaccination can provide some protection against infection, this vaccination is not used in MPXV-endemic areas because of cost considerations and safety concerns about using a vaccine that contains live vaccinia virus.

MPXV transmission among close contacts within households is well documented; previous reports have shown up to 6 intrafamily transmission events (*4*). Transmission is thought to occur by means of salivary or respiratory droplets or contact with lesion exudate (*5,6*); however, evidence suggests that infection can occur by direct inoculation (*7*). Previous household attack rates (i.e., rates of persons living with an infected person and developing symptoms of MPXV infection) of 3%–11% have been reported (*6,8*). Although some reports show a high incidence of households with single isolated cases (*8*), other reports have documented frequent transmission events within households (*6,9*). Attack rates have been found to be much higher among persons living in households with an MPXV patient and among persons with no evidence of prior smallpox vaccination (*6,8*).

Monkeypox is a reportable disease in the Democratic Republic of the Congo (DRC), and cases are reported from 26 health districts (containing 512 health zones). During 2013, a substantial increase in the number of suspected human monkeypox cases was noted in the Bokungu Health Zone within Tshuapa District of DRC's Equateur province (Figure 1). In December 2013, we conducted an

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investigation of monkeypox for this health zone and focused on cases reported during July 1–December 8, 2013.

Methods

DRC has a regional surveillance system that collects reports of all suspected monkeypox cases. When possible, cases are investigated, a monkeypox-specific case report form is completed, and replicate diagnostic specimens (derived from lesions) are collected. During this investigation, additional retrospective cases within affected villages and households were identified on the basis of physical symptoms that were reported by patients and family members but that had not previously reported to the surveillance system.

Case Definitions

Cases in our investigation must have occurred during July 1–December 8, 2013. We used the following case definitions as part of the enhanced surveillance system in Tshuapa District. A confirmed case occurred in a person with a history of high fever, a vesicular-pustular rash, and ≥1 of the following 3 characteristics: 1) rash on the palms and soles, 2) lymphadenopathy, 3) fever preceding rash. In addition, this person has a PCR-tested diagnostic specimen that yielded a positive result for *Orthopoxvirus* or had MPXV DNA signatures. A probable case occurred in a person with a history of high fever, a vesicular-pustular rash, and ≥1 of the following 3 characteristics: 1) rash on the

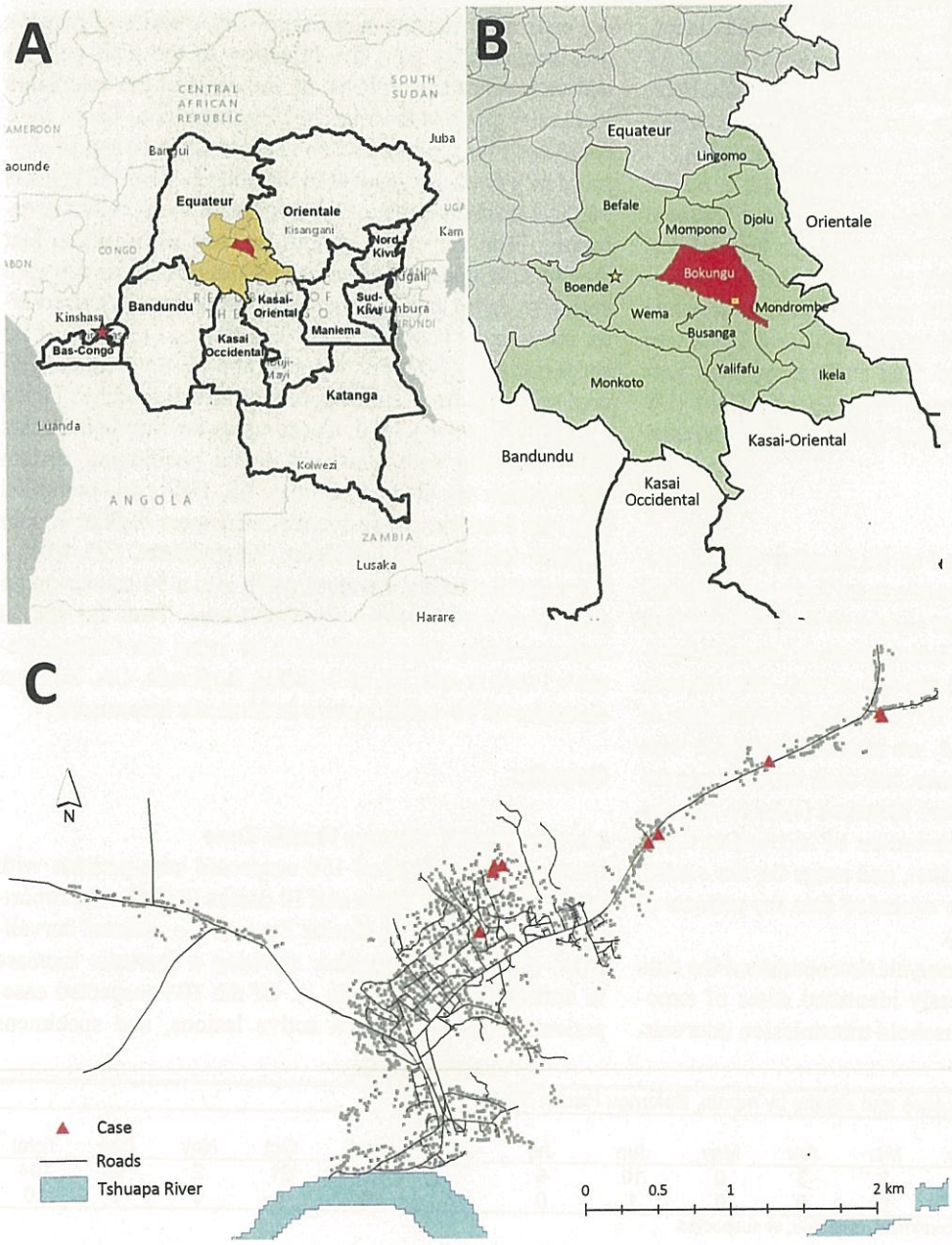

Figure 1. Region affected by monkeypox illness. A) The Democratic Republic of the Congo is outlined; Tshuapa District is highlighted in yellow and Bokungu Health Zone in red. B) Health zones within Tshuapa District; Bokungu Health Zone is highlighted in red. The village with the largest cluster of cases is indicated by a yellow square. C) Distribution of cases (shown by red triangles) in the village with the most cases during this outbreak.

palms and soles, 2) lymphadenopathy, 3) fever preceding rash. In addition, this person has a history of contact with a person or animal with confirmed monkeypox during the 14 days before illness onset. A suspected case occurred in a person with a history of high fever, a vesicular-pustular rash, and ≥1 of the following three characteristics: 1) rash on the palms and soles, 2) lymphadenopathy, 3) fever preceding rash.

Clinical symptoms were documented by a trained healthcare provider or investigator who used a standardized case-reporting form. Smallpox vaccination status was documented on the basis of patient recall and presence of a vaccination scar on the left upper arm. Analysis of vaccination status was performed with χ^2 test by using STATA 14.0 (https://www.stata.com/). Age was considered dichotomous because vaccination was available only to those >33 years of age, as a result of the discontinuation of smallpox vaccination in DRC (and other countries) 33 years earlier (1980) after the announcement of smallpox eradication.

Diagnostic specimens (crusts, vesicular fluid, or ocular fluid) were collected and shipped to the Institut National de Recherche Biomedicale in Kinshasa, DRC, for analysis. DNA was extracted from each specimen, and an *Orthopoxvirus*-specific real-time PCR assay (*10*) was performed for diagnostic confirmation. If no *Orthopoxvirus* DNA was amplified, then a second real-time PCR assay was performed for varicella zoster virus (VZV)–specific DNA (US Army Medical Research Institute of Infectious Diseases, unpub. data).

Symptom Intervals

Incubation period was defined as the number of days between contact with a symptomatic monkeypox patient and development of rash. Rash was chosen as the benchmark of infection for estimating incubation periods because families were better able to recall the day of rash onset than to recall the day of fever onset. To obtain the best estimate of the MPXV incubation period, we identified patients who reported clear dates of exposure and rash onset in our investigation and in the published literature (*4,9,11*). We determined a mathematical distribution of incubation times and calculated the mean, median, and range for the central 75% of the cases (that is, we excluded data for patients at either end of the distribution).

We conducted a larger analysis that combined the data from those persons with clearly identified dates of exposure with data containing household transmission intervals. The household data was calculated by determining the time between onset of rash for the first and second cases in a household. Cases were eliminated from this analysis if the first 2 cases in a household were separated by ≤3 days because we assumed that these case-patients were infected by the same source. We determined a mathematical distribution for the incubation times of this larger group. A secondary analysis of the dataset containing only persons with clearly defined dates of exposure and the dataset which included household transmission was performed by using an alternative formula that was developed to model serial case intervals for respiratory infections (*11*).

Transmission Chains

We estimated transmission chains (i.e., a series of persons who sequentially pass the infection to the next person) within families and villages on the basis of the calculated incubation periods for household transmission. Cases were considered independent when the interval between the onset of rash for a case-patient in the household or village was >8 days from the onset of symptoms for the previous case-patient. This value was chosen because we assumed that the first case occurred after the shortest possible incubation period (5 days) and that the last possible case occurred after the longest possible incubation period (13 days). Any cases occurring after this window of 5–13 days are considered to result from an independent infection either inside or outside the household. Coordinates for case households were recorded with handheld global positioning system units (eTrex 10; Garmin, Olathe, KS, USA) and compiled with the locations of residential structures digitized from satellite images (DigitalGlobe, Westminster, CO, USA). Household counts were aggregated into a 50-square-meter grid covering the entire populated area. Tests for spatial autocorrelation were performed by using the Global Moran's I tool in ArcGIS 10.2 (ESRI, Redlands, CA, USA) at distances of 50–1,000 meters in 50-meter increments.

Results

Monkeypox in Bokungu Health Zone

During 2013, a total of 104 suspected case-patients with human MPXV infection and 10 deaths (9.6%) were reported from the Bokungu Health Zone to the national surveillance system, with October showing a dramatic increase in number of cases (Table 1). Of the 104 suspected case-patients, 60 (57.7%) had active lesions, and specimens

Table 1. Reported monkeypox cases and deaths by month, Bokungu Health Zone, 2013*

Cases and deaths	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Cases, no.	3	0	2	3	0	10	6	0	1	61	2	16	104
Deaths, no.	0	0	0	0	0	1	0	0	0	8	0	1	10

*All cases, not yet characterized as confirmed, probable, or suspected.

Table 2. Characteristics of patients with monkeypox infections, Bokungu Health Zone, July–December 2013

Characteristic	Total cases, N = 63	Confirmed cases, n = 20	Probable cases, n = 19	Suspected cases, n = 24	Unaffected household members,* n = 53
Median age, y (mean)	10 (15.5)	14 (20.4)	7 (6.7)	10 (16.4)	20 (23)
Age range	4 mo–68 y	8 mo–68 y	4 mo–21 y	6 mo–65 y	2 mo–72 y
Male sex, no. (%)†	36 (57)	12 (60)	9/18 (50)	15/22 (68)	19/50 (38)
Vaccinated, no. (%)†	9/59 (15)	5/18 (28)	0/18 (0)	4/23 (17)	14/53 (26)

*Persons in households without symptoms and not tested.
†Denominators indicate no. patients with data available in that category.

were collected from these persons for testing. Of tested specimens, 50 (83.3%) were confirmed MPXV infections. Because MPXV infection and VZV infection have clinical similarities, testing for VZV was also performed. Five (8.3%) of the 60 patients had specimens that tested positive for VZV, and specimens for 5 (8.3%) failed to yield a positive result for either virus.

During the focused investigation period (July–December 2013), we identified and interviewed 63 case-patients in 16 households (Table 2). Of these case-patients, 26 had previously been identified, investigated, and reported by local health authorities; our investigation identified an additional 37 case-patients, including 4 with acute illness. Of the total 63 case-patients, 20 were confirmed, 19 were probable, and 24 were suspected cases. Median age of case-patients was 10 years (range 4 months–68 years); 17.7% were <5 years of age (Table 2). Of the 63 case-patients, 36 (57.1%) were male. Most cases occurred within a 74-day period between the first week of September and the last week of October (Figure 2). All 63 cases included in the 6-month investigation occurred within a 144-day window.

In the 16 investigated households, 9 (15%) of affected household members had evidence of a prior smallpox vaccination, compared with 30% of unaffected household members; χ^2 analysis showed that that this difference was not significant ($p>0.05$). However, vaccination status and age >33 years were nearly perfectly correlated ($p< 0.001$).

The median number of persons affected within each household was 3 (mean 3.9; range 1–8). The median attack rate within households was 50%; mean was 52.1% (range 50%–100%). For 1 of the 16 families investigated, all 6 household members were affected. For all households, the median interval between the time that rash developed in the first person in the household to time that rash developed in the last person was 10 days (range 2–41 days).

Incubation Period

Four case-patients were able to identify a specific date of monkeypox exposure and rash onset. These persons reported that rash developed 5–8 days after contact with an earlier case-patient. A PubMed (http://www.ncbi.nlm.nih.gov/pubmed) search identified 12 additional persons who had confirmed or probable infection and well-defined incubations periods; these case-patients had an incubation period of 9–14 days (*4,9,11*). When the 4 case-patients in our investigation and the 12 historical case-patients were considered together, mean incubation period for all was 9.6 days and median was 9 days. The central 75% of these case-patients had an incubation period of 6–13 days (Figure 3).

A second analysis was conducted with additional incubation periods that were calculated by using the difference in time of onset between the first and second cases within 12 households. These additional data were added to the 16 data points from the first analysis. For the total 28 data points available for this second analysis, the mean incubation period was 8.3 days and median was 8 days. From the second analysis, the central 75% of case-patients had an incubation period of 5–12 days (Figure 3). For subsequent

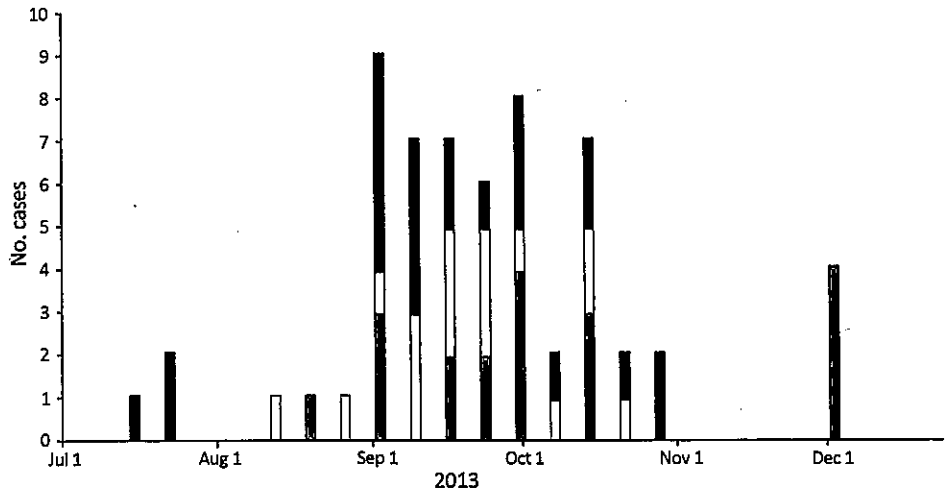

Figure 2. Epicurve of cases included in investigation and monkeypox cases during investigation period (July 1–December 8, 2013). Black represents suspected cases, white represents probable cases, and gray represents confirmed cases.

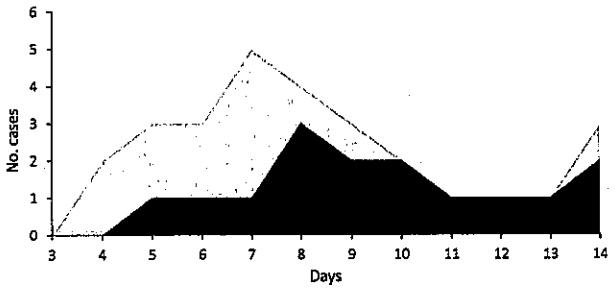

Figure 3. Distribution of incubation periods from 2 separate analyses. Dark gray shows the distribution of incubation periods on the basis of case-patients with well-defined dates of exposure identified in our investigation and in the published literature (n = 16). Light gray shows the distribution of incubation periods from the literature and incubation periods calculated by using the first 2 case-patients in each family (n = 28).

calculations in this investigation, we used the extremes in the 2 analyses as the incubation range (5–13 days, a range indicating the least and most number of days between exposure and onset of rash).

A third analysis of this data was performed by using the model described by Jezek et al. (*11*). In this analysis, which generates a model of serial intervals from the observed data, the transmission interval of the 16 well-described cases was 9.7 (95% CI 8.35–10.95) days; the interval for all 28 cases was 7.4 (95% CI 6.76–7.99) days.

Transmission Chains

Using the range of 5–13 days as the incubation period, we reconstructed transmission events within families and villages. When the longest incubation period (13 days) was used, 9 of 16 households showed ≥1 transmission event. When the shortest time of incubation was used (5 days), an additional 4 households showed patterns consistent with transmission within the household (Figure 4, panel A). Two households had cases separated by a considerable period, suggesting the occurrence of either an unknown transmission event within the household or an exposure outside of the household (Figure 4, panel B). When community-wide transmission was considered within the health zone, longer transmission chains were observed, with the longest being in the village of Bokungu, where ≥7 suspected transmission events resulted in 42 apparent cases (Figure 4, panel C). Tests for spatial autocorrelation showed that case households for were more spatially clustered (z-scores >2.0) than would be expected randomly at all distances of 50–1,000 meters.

Discussion

Human MPXV infection is endemic to DRC, with cases occurring throughout the Congo Basin. Many of these cases occur in isolation or in small clusters, but large outbreaks occasionally occur that involve many persons over a large geographic area. During 2013, a total of 104 cases of human monkeypox illness were reported in the Bokungu Health Zone. In contrast, only 17 cases were reported in 2011 and 13 in 2012. The surveillance system did not change substantively during this period; consequently, the rate in 2013 represents an increase of >600%, compared with rates for previous years. Our investigation focused on cases that occurred during the height of the 2013 outbreak.

Within the investigation period, 57% of affected persons were male, and median age was 10 years; 18% were <5 years of age. According to the United Nations World Population Prospects (http://esa.un.org/unpd/wpp/), 17.8% of the population in DRC is <5 years of age. Consequently, the age demographics of patients during the monkeypox outbreak correspond to those of the general population, suggesting that young children are not more prone to MPXV infection than others. A parallel study performed during this outbreak found no association between monkeypox illness and hunting or consumption of specific animals (*12*).

Previous publications have reported attack rates of 3%–11% (*6*,*8*); our investigation found a median attack rate of 50%, and 1 family had 100% of persons affected. The previously published attack rates are considerably lower than those for other viruses with similar routes of transmission; for example, smallpox has attack rates of 35%–88% (*13*–*15*), and variola virus had an attack rate of 90% (*16*). The difference between the findings reported here and those reported previously may result from several different causes. First, the high attack rate reported here possibly results from changing individual- and population-level immunity caused by elimination of routine childhood smallpox vaccination (*17*). Earlier investigations may have found lower attack rates because more persons had vaccine-derived immunity; however, some persons in the outbreak that we investigated had MPXV infection and prior smallpox vaccination, which suggest possible waning immunity over time, a factor that should be considered in future investigations. Second, a viral strain different from that found in previous investigations could have circulated in this outbreak and resulted in the high attack rate. However, we have no evidence for accepting or rejecting this possibility. Third, the high attack rate possibly reflects a high rate of case-patient identification in this investigation. We found that many persons are often affected in a household but that only 1 household member usually seeks medical attention, causing only 1 case to be recorded or investigated for surveillance. Case reporting on the basis of persons seeking healthcare may have caused the surveillance system to underestimate of the number of human monkeypox cases. Because we used in-home interviews, many previously unidentified cases were uncovered, enabling the calculation of a more accurate attack rate.

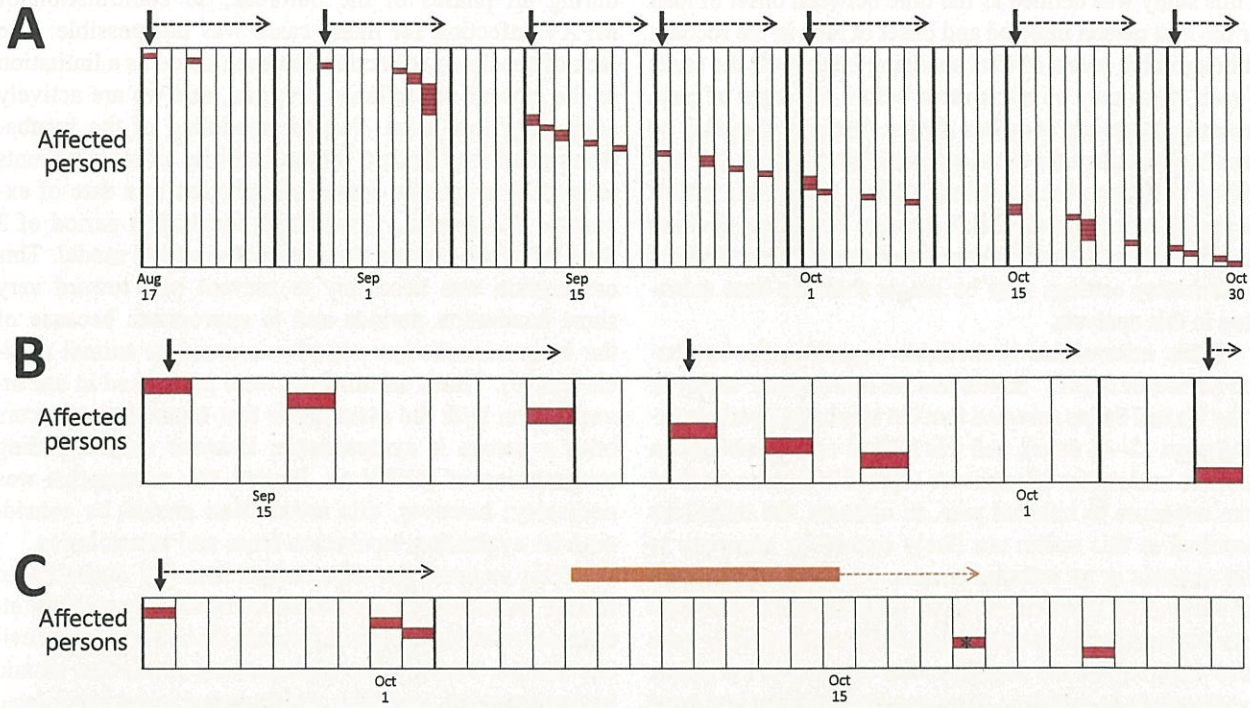

Figure 4. Reconstruction of monkeypox virus transmission events in the Democratic Republic of the Congo by using an estimated incubation period. Each column represents a calendar day. Red boxes represent a single case of monkeypox infection. A cluster is defined as a set of case-patients that could have resulted from a single exposure and are delimitated with dark vertical lines. Dark arrows indicate the first case within a cluster, and the dotted arrow indicates the time during which a potential single exposure could have produced symptoms in the first person in that group to the last (i.e., 5–13 days). A) Transmission events in the village of Bokungu in the Democratic Republic of the Congo. B) A household with evidence of 3 known transmission events. C) A household with evidence of 1 known and 1 unknown transmission event. The orange bar represents the days when the case-patient, represented by an asterisk (*), would be expected to have been exposed.

Previous investigations have shown a limited transmission capacity of MPXV within the human population. The highest number of suspected serial transmission events previously recorded is 6 (*4,9*). The ability to identify transmission events is limited by our lack of knowledge of the dynamics of infection. Often, whether 2 persons were coinfected by the same person and have different incubation periods or whether the persons were sequentially infected is difficult to determine. Understanding the incubation period of MPXV is vital for creating accurate transmission chains and determining if multiple introductions (human or zoonotic) occurred.

Sixteen patients with well-defined incubation periods were identified in our investigation and in the literature. Although these defined incubation periods are the best information available, they are also limited in number. Consequently, we also included apparent incubation periods within households. The time between onset of rash in 1 case and onset of rash in a subsequent case within a household provide an approximate incubation period. The longer that MPXV is present in a household, the more difficult identifying a clear infection chain is; therefore, only the transmission between the first and second case was used for the analysis. However, these 2 persons could have been infected by an outside source instead of by human-to-human transmission. The 3 analyses that we developed and presented here yielded similar results; 75% of the incubation periods were 5–13 days. Analysis of the same data by using the model proposed by Jezek et al. (*11*) yielded transmission intervals that matched data from our mathematical distribution model when we analyzed the 16 well-described cases. When all 28 cases were analyzed, the Vink model produced an interval 1 day shorter than that for the mathematical distribution. The difference between these numbers likely results from the weighting that is included in the Vink model. Further work is needed to evaluate which model best fits the biology of MPXV.

Our investigation suggests a shorter incubation period for MPXV than that observed in many animal models (*7,18,19*). Differences in organism and exposure may account for this difference. Experimental animals are often exposed to a virus for a brief time, and the interval between that exposure and development of symptoms is recorded as the incubation period. In contrast, the incubation period

in this study was defined as the time between onset of rash for the first person infected and onset of rash in the second. Although high levels of viral shedding begin with the onset of rash, virus may be transmitted before the onset of rash. Research in prairie dogs has shown that oral shedding of virus begins before the development of dermal rash (*5*); this finding indicates that spread of MPXV is possible before the appearance of external skin lesions. Therefore, the time from first exposure to development of symptoms calculated in laboratory settings may be longer than the time calculated in this analysis.

Little information is available regarding the incubation period of MPXV in humans. A monkeypox outbreak in the United States revealed that the incubation period varied (range 12–14 days), and this period was dependent on the route and nature of exposure (*7*). All US cases resulted from exposure to infected pets. In contrast, the infections described in this article are likely caused by exposure to wild animals or an infected human. The type of exposure and route of virus transmission may result in incubation periods during the US outbreak that differ substantially from those observed in the outbreak that was the focus of our investigation. In addition, previous outbreaks were caused by viruses from a different genetic clade than that which caused the outbreak reported here. Transmission times may differ because of the specific virus involved.

Altogether, the Bokungu Health Zone had 42 cases in ≥7 infection clusters (i.e., a group of cases that could have resulted from a single infectious exposure). These clusters could have been created in 3 different ways. First, the clusters could be linked sequentially, whereby the infection could be externally introduced into a cluster and then passed by 1 person from that group to cluster 2 and so on. Second, transmission may not have occurred in a clear linear fashion, but persons may have had multiple human exposures. Third, MPXV could have been reintroduced into the community from an external source (zoonotic or human) during the course of the outbreak. Although we cannot determine which of these possibilities is most likely, we favor the second model because community interactions would make a strictly linear pattern of spread unlikely to occur. Further, the limited number of cases in the population as a whole makes it less likely that an external source was causing frequent reintroductions. We can conclude that ≥6 transmissions or introductions occurred in this health zone after the initial infection.

This report has limitations that should be considered. First, MPXV infection was laboratory confirmed in 48% of the cases by using PCR; the remaining cases were identified by patients' symptoms. Laboratory confirmation was not possible for many cases because patients were interviewed after symptoms had resolved. Local resources for performing specimen collection were unavailable during all phases of the outbreak, so confirmation of MPXV infection for many cases was not possible. The lack of specimen collection has been noted as a limitation of the current surveillance program, and we are actively addressing this issue. Second, modeling of the incubation period was limited by the inability of most patients to identify a specific source of infection or a date of exposure. We assumed a minimum incubation period of 3 days when we created the incubation period model. This assumption was necessary to prevent bias toward very short incubation periods and is appropriate because of the longer incubation periods observed in animal models (*18,20*). Third, calculations were performed in our investigation with the assumption that transmission occurs once a person is symptomatic. Because data regarding transmission of MPXV are limited, this assumption was necessary; however, this assumption should be considered for evaluating incubation times and transmission.

This analysis provides insight into the dynamics of MPXV infection. We observed an average household attack rate of 50%, a much higher rate than reported in previous studies. Measures to decrease this attack rate should be implemented, including family-based education related to hygiene and isolation of patients. The transmission patterns observed in this outbreak also suggest transmission at the community level; therefore, community-wide education should begin as soon as the first monkeypox case is identified in an area. The calculated incubation period of 5–13 days further refines our understanding of the longest period of MPXV transmission risk after exposure in a natural setting. Knowledge of transmission risk is helpful for considering the appropriate monitoring period for exposed persons. This investigation and future work will improve our understanding of MPXV infection and our ability to limit its spread.

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感染症定期報告に関する今後の対応について

平成１６年度第５回
運営委員会確認事項
（平成１６年９月１７日）

１ 基本的な方針

運営委員会に報告する資料においては、

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

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

２ 具体的な方法

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

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

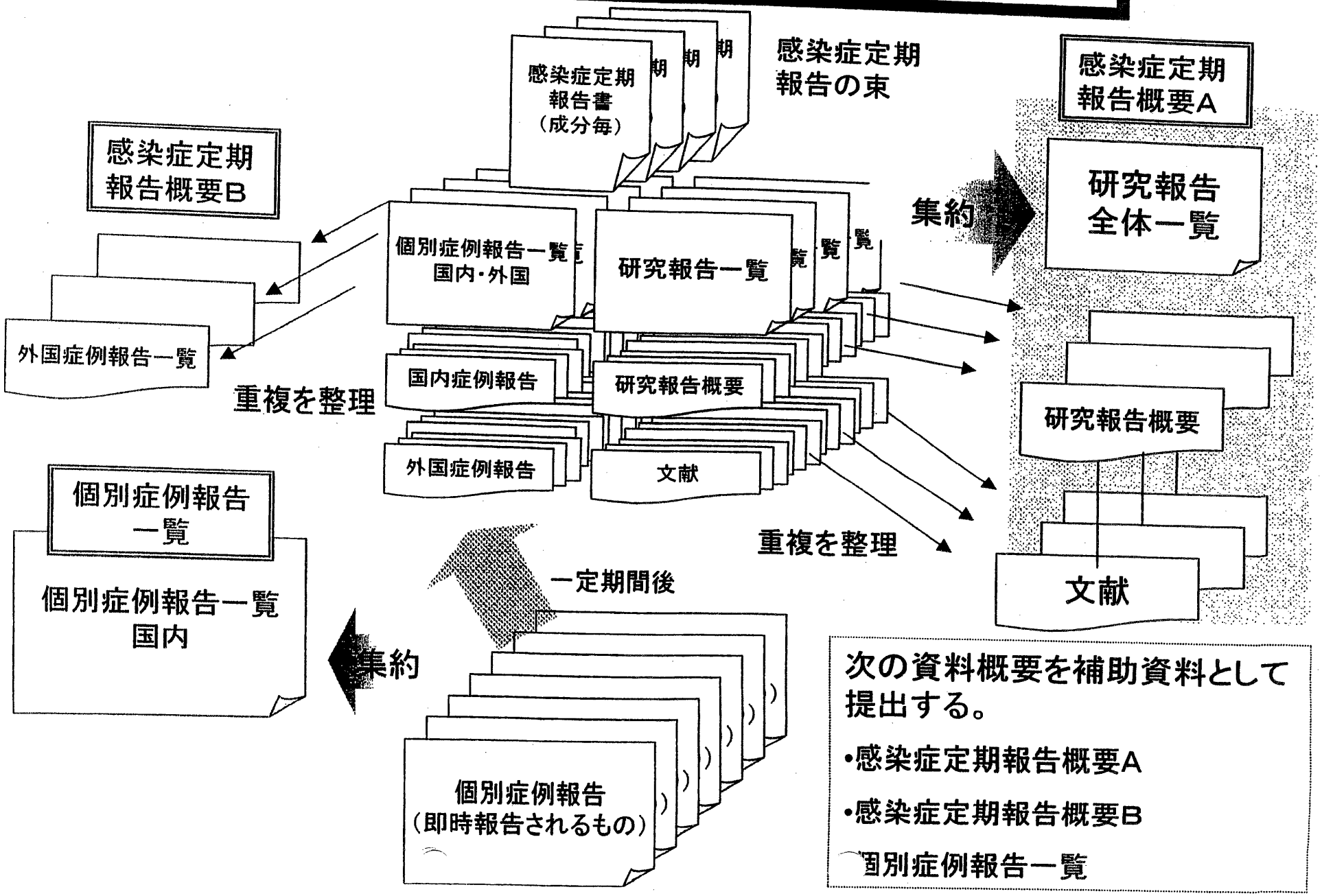
② 感染症発生症例報告のうち、発現国が「外国」の血漿分画製剤の使用による症例は、同一製品毎に報告期間を代表する感染症発生症例一覧（別紙様式第4）をまとめた**「資料概要B」**を事務局が作成し、送付する。

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

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

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

感染症定期報告・感染症個別症例報告の取り扱い



次の資料概要を補助資料として提出する。

- 感染症定期報告概要A
- 感染症定期報告概要B
- 個別症例報告一覧