資料１－２

Ａ　研究報告（詳細版）

平成２９年９月６日
（平成２９年４月～平成２９年７月受理分）

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
				2017. 3. 24	該当なし	

一般的名称	人全血液	研究報告の公表状況		公表国
販売名(企業名)	人全血液-LR「日赤」(日本赤十字社) 照射人全血液-LR「日赤」(日本赤十字社)		Tedder RS, Ijaz S, Kitchen A. Transfusion. 2017 Feb;57(2):267-272. doi: 10.1111/trf.13976.	英国

研究報告の概要

○E型肝炎のリスク:豚か血液か一それが問題だ

背景:先進国では、E型肝炎ウイルス(HEV)の遺伝子3型による感染症は食物媒介性の人獣共通感染症として認識されており、軽症の急性肝炎を引き起こすことが多い。しかし、免疫不全者においては、肝硬変に移行し得る持続感染を引き起こす可能性がある。英国では、輸血によるHEV感染防止を目的として、HEVスクリーニング検査が陰性となった供血者由来の血液成分製剤のみを有リスク患者に提供するための方策を講じている。この方策は食事による曝露を防止するものではなく、輸血と食事の相対リスクの推定値に対して疑問を投げ掛けるものとなっている。

研究デザイン及び方法:HEVのウイルス血症に関するデータを用いて、成分製剤の輸血、残存血漿量、結果として生じた感染、輸血された製剤に含まれていたウイルス量及び結果的な感染率を判定し、血液による曝露と食事による曝露の関係の推測を可能とするモデルを適用した。

結果:抗体が陽転化した集団における年間発病率より、HEVのウイルス血症の状態にある供血者由来の成分製剤の輸血によるリスクの推定値が得られる。感染を引き起こしたウイルス量の最低値は2×10^4 IUであり、当該量のウイルスが含まれていた成分製剤の55%が感染を伝播させていた。輸血による感染リスクは、単一の供血者に由来する成分製剤の輸血本数が13本を超えた場合に、ようやく食事による感染の年間リスクを上回った。

結論:多くの固形臓器移植患者において、食事による曝露のリスクは、スクリーニング検査を受けていない供血者に由来する製剤の輸血に伴うリスクをはるかに上回る。輸血によるリスクは、多量の成分製剤による輸血を必要とする免疫不全患者においてのみ、食事によるリスクを上回る。この知見は、供血血液のHEV RNAスクリーニング検査に関する方針を決定する上で有益と考えられる。

使用上の注意記載状況・その他参考事項等

人全血液-LR「日赤」
照射人全血液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
輸血と食事によるE型肝炎ウイルス感染リスクを推定し、輸血による感染リスクは、多量の成分製剤の輸血を必要とする免疫不全患者においてのみ、食事によるリスクを上回るという報告である。	日本赤十字社では、献血者には問診等により肝炎履歴を含めた健康状態を確認している。北海道においては、HEVの陽性率が高く、また重症化が懸念されるHEV genotype 4の輸血感染症例があったことから、経口肝炎研究班※と共同して試行的にHEV-NATを実施し、献血者におけるHEVの感染状況、病態、臨床経過等の調査を行っている。今後も情報の収集に努める。 ※日本医療研究開発機構研究 肝炎等克服実用化研究事業「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」

JRC2017T-010

Hepatitis E risks: pigs or blood—that is the question

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BACKGROUND: Infection with hepatitis E virus (HEV) Genotype 3 is recognized as a food-borne zoonosis in developed countries where it usually causes a mild self-limited acute hepatitis. It may cause a persistent infection in the immunosuppressed human that can progress to cirrhosis. To protect the patient from transfusion-acquired HEV infection, steps have been taken in the United Kingdom to provide for at-risk patients only components from donors screened for HEV viremia. This strategy does not protect from dietary exposure and calls into question estimation of relative risk between blood transfusion and diet.

STUDY DESIGN AND METHODS: Using data on HEV viremia, component exposure, residual plasma volume, and resulting transmission, the dose of virus administered and subsequent transmission rates were determined and used to populate a model that can infer the relationship between blood and dietary exposure.

RESULTS: The annual attack rate of a population, defined as seroconversion, provides an estimate of the risk of receiving a component containing HEV from a viremic donor. The lowest viral dose that resulted in infection was 2×10^4 IUs and 55% of components containing this dose transmitted infection. The transfusion risk of infection only exceeds the annual dietary risk when more than 13 individual donor components are transfused.

CONCLUSION: For many solid organ transplant patients dietary exposure far exceeds the risk of transfusion from unscreened donors. It is only in the immunosuppressed patient requiring extensive blood component support that transfusion risk dominates. This understanding should inform policy decisions on HEV RNA screening of blood donations.

Hepatitis E virus (HEV) is a widespread family of related viruses, so closely related that often these agents may cross species causing, in human terms, zoonotic infections. Four major closely related genotypes of HEV infect humans causing disease with different levels of pathogenicity. Genotypes 1 and 2 (G1 and G2)[1] are human pathogens and do not infect other hosts; in contrast Genotypes 3 and 4 (G3 and G4) are enzootic in swine, also infect humans, and are found globally. Persistent HEV infections are believed not to occur in the immunocompetent host and consequently these viruses are maintained in populations through a rolling series of acute infections from primary host to primary host within humans (G1 and G2) and animals (G3 and G4), the latter principally within the genus *Sus*, which includes pigs raised for human consumption. HEV G3 and G4 are transmitted by the oral–fecal enteric route in pigs and cause little or no morbidity among the natural hosts, certainly not enough to invoke consideration on husbandry grounds alone of a vaccine in pigs.[2] As an important animal protein food source, pig meat is consumed in a large number of countries and provides an essential component in the diet of many people.[3] Porcine viruses, principally but not exclusively G3 and G4, are able to infect humans as an end-line infection. Although these

ABBREVIATION: G = genotype (when followed by a number).

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This study was funded by NHS Blood and Transplant.

Received for publication August 25, 2016; revision received November 10, 2016; and accepted November 22, 2016.

doi:10.1111/trf.13976

TRANSFUSION 2017;57;267–272

TABLE 1. Blood components ranked by included plasma volume, showing the minimum donor plasma viral load that could be expected to constitute the observed lowest infectious dose of 20,000 IU

Component	Included plasma volume	Minimum load (IU/mL) in donor for infection to occur in recipient
Pooled granulocytes	10	2000
RBCs in AS	Mean, 12.5	1600
PLT individual preparation	25	800
Apheresis PLTs	180	111
Plasma contributing to a PLT pool	225	89
FFP	275	73

infections are termed zoonotic there is little evidence of human to human transmission although blood transfusion[4] and potentially organ transplantation[5] will facilitate human to human spread. Recent studies in blood donors have indicated that infection with G3 is likely to be widespread in England and the cause of an annual attack rate in the region of 0.2% in the population as a whole.[4] It is accountable for the majority of the 850 annual cases of acute hepatitis in England and Wales.[6]

The significance of G3 infection has changed with the recognition that persistent virus infection occurs in immunosuppressed patients and may be associated with rapid progression to liver fibrosis culminating in cirrhosis.[7] Management of the persistently infected patient is problematic, particularly in light of the complexity of immunosuppression that occurs in an increasingly diverse group of patients under treatment. To mitigate the transfusion risk, provision has been made in the United Kingdom since the early part of 2016 for HEV screened blood for subsets of patients who are particularly at risk from persistent infection.[8] Such policies, however, do not per se address or mitigate the coincidental dietary exposure to HEV of at-risk patients and hereby lies the conundrum. Dietary exposure affects both the population at large and the donors therein and consequently the transplant recipient who receives blood components. At the same time it also affects the transplant recipient directly. Here we present an holistic approach to this paradox, determining the minimum infective dose for HEV transmission by transfusion, defining the relative risks of both routes of infection, and demonstrating the linkage between annual attack rate and transfusion risk. This approach will inform the consequent management of both dietary and transfusion risks in patients undergoing iatrogenic immunosuppression.

MATERIALS AND METHODS

Recipient exposure

Forty-three patients exposed to components from viremic donors were enrolled into a follow-up study. Imputable proof of transmission was confirmed in all viremic recipients by demonstrating phylogenetic identity between donor and recipient viruses. An anti-HEV immunoglobulin (Ig)M response and high IgG levels detectable in the posttransfusion period were considered indicative of transfusion-associated transmission in the absence of detectable HEV RNA in the recipient.[4]

Determination of dose

The outcome in recipients exposed to blood components from donors found to be viremic was determined as previously described.[4] The level of virus was determined for each donor plasma and expressed in international units per mL (IU/mL). Estimates of the plasma volume included in each component and the quantitative viral load in that plasma were used to calculate the dose of virus in IUs contained in components that transmitted infection compared with those components that did not transmit. Assuming that plasma HEV RNA represents potentially infectious virus and that this does not partition with any cellular components the residual plasma volume for each type of blood component (Table 1) was used in the calculation of the infectious dose.

Model to compare transfusion and dietary risk

A range of variables based on previous experience[9] was used to estimate the probability of infection from blood and from diet. Table 2 gives the values used and the formula for the calculation. This is available as a macro on request.

Serology

Antibody to HEV was sought using IgM and IgG assays (Wantai, Fortress Diagnostics) in accordance with the manufacturer's recommendations.

RESULTS

Determination of a minimum infectious dose

The plasma viral load expressed in IU/mL in the index sample from each of the 79 viremic donors ranged from 2.5×10^1 to 2.4×10^6 IU/mL and followed a normal distribution on a log scale (chi-square test for lack-of-fit p value = 0.7) with a geometric mean value of 4.7×10^3 (log mean, 3.67 IU/mL, SD 1.07; Fig. 1). Using the included volume of the plasma in each transfused component

TABLE 2. Variables used for the model to compare donation and dietary risks

Variable	Symbol	Values used	Rationale for values used
Infection risk when receiving a component from a viremic donor	I_v	0.5	As a generalization of any component[4]
Dietary risk per year in England and Wales	μ	0.002	Estimated annual seroconversion rate 0.2%
Period of infectiousness (weeks)	d	8	Duration of viremia[8]
Prevalence of viremia in donor population	F_v	Estimated as $\mu \times d/52$	Governed by each seroconversion providing 8/52 viremia and representative of volunteer donors
Number of donations received	N	1, 5, 10, 60	Covering the range of transfusion exposure
Probability of infection from blood	P_b	$1 - (1 - (I_v \times F_v))^N$	Risk accrues with each exposure
Probability of infection from diet in Y years	P_d	$Y \times \mu$	Risk accrues with life survival

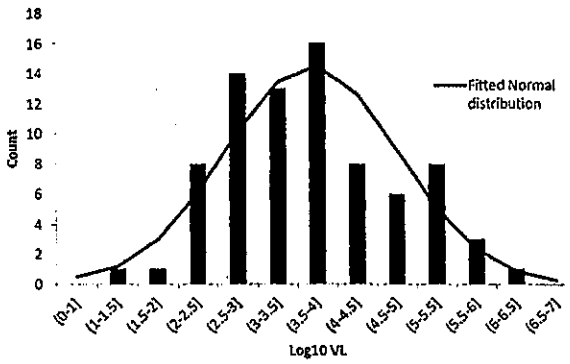

Fig. 1. Log normal distribution of the HEV level (log IU/mL) detected at pickup in 79 donors found to have HEV RNA in their plasma at the time of donation.

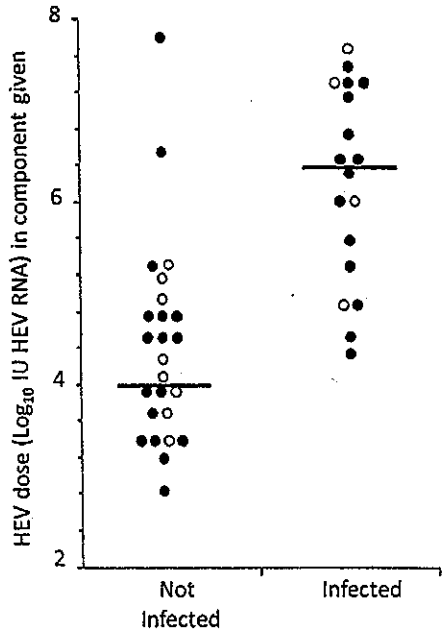

Fig. 2. Spread of the HEV dose (log IU HEV RNA) in those transfused components that gave rise to infection and those that did not. (O) Presence of detectable antibody to HEV in the donation; solid bar indicates the median viral load.

and the viral load of the plasma, it was possible to ascribe a dose of virus to which recipients were exposed. This dose ranged from 6.3×10^3 to 5.6×10^7 IUs per component from a viremic donor. Eighteen recipients were considered to have been infected.

The distribution of virus dosage received by each of the 43 patients available for follow-up differed between the 18 recipients who were infected (mean, 6.16 log IU) and the 25 who were not infected (mean, 4.38 log IU; t test $p < 0.001$; Fig. 2). The lowest dose that led to infection was 1.9×10^4 IUs (4.3 log IU); however, not all recipients exposed to this or a larger dose were infected; 15 of the 25 nontransmitting components contained or exceeded this dosage. Using the lowest dose that led to infection as a minimum infectious dose, 33 transfused components contained a potentially infectious dose and only 18 gave rise to infection, indicating a transmission rate of 55%. The proportion of seropositive donors was similar in the transmitting and nontransmitting groups (4/18 and 8/25, respectively).

Determination of the likelihood of infection through receipt of unscreened blood versus dietary sources

The probability of infection from blood component receipt as well as from dietary risk over a number of years (Y) can be estimated based on the variables in Table 2. The infection risk due to diet is simply calculated as the annual infection risk multiplied by the time period. The risk from transfusion will depend on the number of transfused blood components, the diet-related attack rate in donors, the infectious period, and the risk of infection from each component type with the calculation as shown in Table 2. For the simple situation comparing transfusion risk from a single component to a year's dietary risk the ratio of these two risks is simply

$$(I_v \times \mu \times d/52)/\mu = I_v \times d/52 = 0.5 \times 8/52 = 1/13 \text{ or } 0.077.$$

The transfusion infection risk is less than a typical year's dietary risk until 13 components have been received. If we now consider five, 10, and 60 donations the ratio between

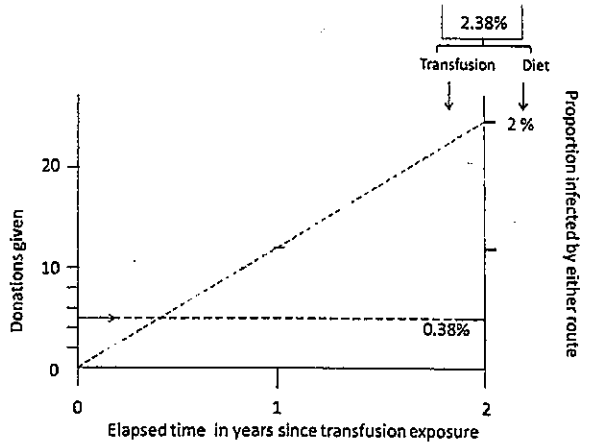

Fig. 3. Outcome 2 years after receiving five components in a country with an annual seroconversion rate of 1%. The combined infection risk is 2.38% comprising both dominant cumulative dietary and smaller transfusion risks.

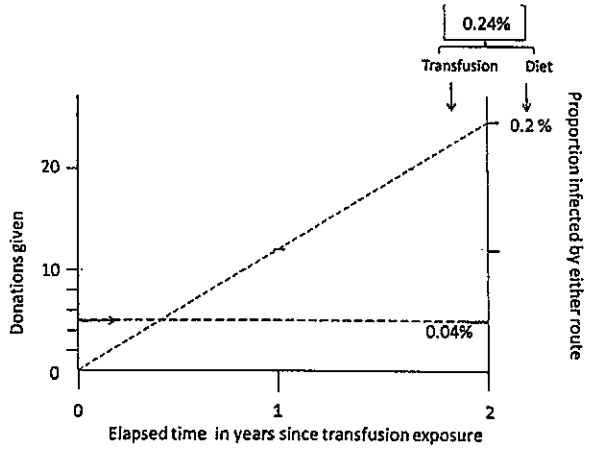

Fig. 4. Outcome at 2 years after receiving five components in a country with an annual seroconversion rate of 0.1%. The combined infection risk is 0.24% comprising both dominant cumulative dietary and smaller transfusion risks.

the transfusion risk and 1 year of contemporary dietary risk is 0.38, 0.77, and 4.6, respectively. This ratio between dietary and transfusion risks will remain fixed in any population of a country as long as the attack rate in donors reflects the attack rate in the population as a whole. Clearly the absolute magnitude of both risks will depend on the attack rate in the population, which in turn defines the prevalence of viremia in blood donors.

That the ratio of dietary to transfusion risk is constant can be demonstrated graphically using the model above by examining the outcome of exposure to five components in two countries, one with an annual seroconversion rate of 1% (Fig. 3) and the other with a rate of 0.1%

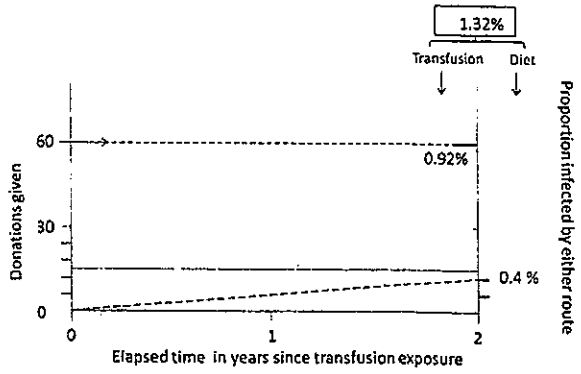

Fig. 5. Outcome at 2 years after receiving 60 components in a country with an annual seroconversion rate of 0.2%. The combined infection risk is 1.32% comprising both dominant transfusion and smaller cumulative dietary risks.

(which would be representative of the United Kingdom; Fig. 4) at 2 years on from the transfusion episode. The topography remains the same though the summated risk is greater in the former country. Of the two major groups of immunosuppressed patients who may be exposed through transfusion—the stem cell recipient and the solid organ recipient—it is the allograft stem cell recipient, who may be exposed to 60 or more components from different donors, who is the more likely to acquire infection, once again depicted in the United Kingdom (Fig. 5). The topography now is very different and the increased donor exposure confers an immediate and dominant transfusion risk of HEV acquisition in the United Kingdom at close to 1%, equivalent to approximately 5 years of dietary exposure.

DISCUSSION

The epidemiology of HEV in England is such that there is no manner in which donors at risk of acquiring HEV can be selectively removed from the donor panel.[9] Consumption of processed pork is widespread and the fraction of the population who are not pork meat eaters is small and would not provide a robust supply of blood and blood components. Similarly, selection of HEV-immune donors would not provide a sufficiently large or robust donor panel. The other remaining option is identification of viremic donors by screening for HEV RNA. This can be achieved but at considerable cost for the blood transfusion services and can only be justified if there is significant health gain as a result and, perhaps, where there is significant reputational risk in not so doing. The first confirmed case of posttransfusion hepatitis E in England was identified in 2005[10] and to this day it remains a rarely reported occurrence. In the recent study from this country there was only a single mild case of posttransfusion hepatitis, which would have remained unreported but for the study.[4]

What, however, was the important sequel to the study was the recognition of the propensity of immunosuppression to reduce or prevent viral clearance.

Persistent HEV is a feature of infection in the immunosuppressed patient, particularly among solid organ transplant recipients where the immunosuppression is unremitting. These infections are frequently immunosilent and can only be detected by screening patients for plasma HEV RNA. Although not all infections will lead to persistence,[11] it is likely in the majority. In this situation HEV can lead to progressive hepatic fibrosis followed by cirrhosis in a matter of a few years.[7] Although treatment with ribavirin often leads to clearance of the infection, this therapy is not without risk of decreasing red blood cell (RBC) survival, is difficult in the face of renal insufficiency, and is beset with problems of late viral recrudescence. Furthermore, a question remains over the proportion of solid organ transplant recipients that are currently infected. Although patients can be expected to display elevated plasma transaminases compatible with chronic hepatitis, it is likely that a significant proportion of patients will maintain plasma transaminases within the normal range in spite of persistent HEV infection. Whether these individuals are also at risk of progression of liver fibrosis and development of cirrhosis remains uncertain. Taking all of these matters together, there are therefore justifications for examining the role of screening for the protection of immunosuppressed patients to reduce the risk of acquiring HEV from blood components.

For a component to be potentially infectious the viral load in the donor plasma must be sufficient to produce an infectious dose of HEV in the volume of plasma contained in the component. Huzly and colleagues[12] report two transmissions, confirmed in one case by phylogeny, after doses of between 7×10^3 and 9×10^3 IU HEV RNA in recipients of apheresis platelets. For a component which contains a small plasma volume, for example, RBCs in additive solution (AS) or granulocyte concentrates, the minimum viral load in the donor plasma that could be expected to lead to transmission is far higher than that required in a large plasma volume component such as fresh-frozen plasma (FFP; Table 1). Interestingly, none of the 13 recipients who received doses less than 2×10^4 IU became infected, suggesting that challenges below this dose (Fig. 2) are less likely to transmit. Conversely, the demonstration that only a proportion, in this study 55%, of patients challenged with components containing more than the observed lower limit of 2×10^4 IU become infected may indicate contemporaneous exposure to components containing neutralizing antibody. Since, however, the virus in human plasma is likely to be lipidated,[13] it may not be susceptible to neutralization, leaving two alternatives to explain refractoriness to infection. First, it might indicate previous exposure, although this is unlikely in the younger patient. Second, it could simply be that some humans may be innately resistant to infection and express a refractory phenotype for infection by this porcine virus.

Removal of the risk for transfusion acquisition is only part of the mitigation of HEV risk in humans. It is, however, something that can be addressed immediately, and on these grounds it was considered essential in the United Kingdom to provide components from HEV-screened blood donors for heavily transfused immunosuppressed patients and in principle for all immunosuppressed transfusion recipients. In contrast to the former group, however, a solid organ transplant patient who might receive two components at the time of transplantation would have a transfusion risk approximately equivalent to 2 months of dietary exposure. In this situation, the health gain to a solid organ transplant recipient of receiving components from HEV-screened donor is more limited, underlining the potential need for considering a different approach to mitigating HEV infection in solid organ transplants and the wider issue of the population as a whole.[14] There is an interesting parallel emerging for the management of the transfusion risk posed by Zika where removal of the transfusion risk will have little impact on the burden of human disease. In patients who have a lifetime stretching in front of them supported by a solid organ transplant, HEV dietary acquisition very quickly becomes the dominant cumulative risk for acquiring HEV persistence. This acquisition can only be addressed in terms of risk prevention by societal changes both in the way food is cooked and in the way that food is produced. It also raises the question of how best to identify those infections that arise through dietary exposure in the large number of people who are not transplant recipients but are currently receiving immunosuppressive therapy.

ACKNOWLEDGMENTS

The authors thank colleagues in PHE and NHSBT for their support in the conduct of this study.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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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
				2017. 3. 24	該当なし	

一般的名称	人全血液	研究報告の公表状況	de Vos AS, Janssen MP, Zaaijer HL. Transfusion. 2017 Feb;57(2):258-266. doi: 10.1111/trf.13978. Epub 2017 Jan 31.	公表国
販売名(企業名)	人全血液-LR「日赤」(日本赤十字社) 照射人全血液-LR「日赤」(日本赤十字社)			オランダ

研究報告の概要

○オランダにおける供血血液のE型肝炎ウイルススクリーニング検査の費用対効果

背景:近年、欧州ではE型肝炎ウイルス(HEV)感染の発生率が大幅に上昇しており、輸血用血液の安全性が脅かされている。オランダにおける供血血液を対象とするHEVスクリーニング検査に係る費用対効果の解析を試みた。

研究デザイン及び方法:供血、供血者集団における感染、供血血液の検査及び受血者への伝播のプロセスについて、シミュレーションモデルを構築した。供血者におけるウイルス量のばらつきは、観察されたウイルス量を用いてモデル化した。臓器移植患者及び幹細胞移植患者における慢性HEV感染(治療抵抗性)症例の数、並びに血液スクリーニング検査の実施により回避されるコストを推定した。

結果:輸血による慢性HEV感染の推定年間症例数は4.94例であり、全数のHEVスクリーニング検査を24本プールで実施することにより、4.52例の発生が防止され、1例当たりのコストは約310,000ユーロになると考えられる。リバビリン治療が無効な症例では、1例当たりのコストが約10倍となる。人員の都合上可能であれば、選択的スクリーニング検査を実施することによりスクリーニング検査コストが85%削減される可能性がある。感度分析により、HEVの感染性及び除去率における不確かさが、推定費用対効果に大きく影響することが判明している。同国内全てのHEV感染症例の700例に1例、また慢性感染症例については3.5例に1例が輸血による感染と推定される。

結論:この評価結果には不確実な部分も含まれるが、供血血液のスクリーニング検査によるHEV感染の防止にかかる費用は、オランダで用いられている他のスクリーニング検査と比較して、過度に高額ではないと思われる。しかし、輸血によるHEV感染症例は全感染症例のごく一部であるため、血液のスクリーニング検査がHEV疾患全体に与える影響は限定的である。

使用上の注意記載状況・その他参考事項等

人全血液-LR「日赤」
照射人全血液-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
オランダにおける供血血液を対象としたHEVスクリーニング検査に係る費用対効果を解析し、供血血液のスクリーニング検査によるHEV感染防止にかかる費用は、オランダで用いられている他のスクリーニング検査と比較し過度に高額ではない。しかし、輸血HEV感染症例は全感染症例のごく一部であるため、血液のスクリーニング検査がHEV疾患全体に与える影響は限定的と考えられるという報告である。	日本赤十字社では、献血者には問診等により肝炎履歴を含めた健康状態を確認している。北海道においては、HEVの陽性率が高く、また重症化が懸念されるHEV genotype 4の輸血感染症例があったことから、経口肝炎研究班※と共同して試行的にHEV-NATを実施し、献血者におけるHEVの感染状況、病態、臨床経過等の調査を行っている。今後も情報の収集に努める。 ※日本医療研究開発機構研究 肝炎等克服実用化研究事業「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」

Cost-effectiveness of the screening of blood donations for hepatitis E virus in the Netherlands

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BACKGROUND: The incidence of hepatitis E virus (HEV) has increased substantially in Europe recently, thereby threatening blood safety. A cost-effectiveness analysis for HEV screening of blood donations in the Netherlands was performed.

STUDY DESIGN AND METHODS: A simulation model was developed to mimic the process of donation, infections in the donor population, donation testing, and transmission to transfusion recipients. The variability of viral loads among donors was modeled using observed loads. The number of (incurable) chronic HEV infections among organ and stem cell transplant patients and the costs avoided by implementing blood screening were estimated.

RESULTS: HEV screening of whole blood donations in pools of 24 would prevent 4.52 of the 4.94 transfusion-associated chronic HEV infections expected annually, at approximately €310,000 per prevented chronic case. Per case not curable by ribavirin prevention, costs are approximately 10 times higher. Selective screening, if logistically feasible, could reduce screening costs by 85%. Sensitivity analyses show that uncertainty in the HEV transmissibility and the frequency of HEV clearing greatly impact the estimated cost-effectiveness. Of all HEV infections nationwide one in 700 is estimated to be due to blood transfusion, while for chronic infections this is one in 3.5.

CONCLUSION: Despite uncertainties in our estimates, preventing HEV transmission by screening of blood donations appears not excessively expensive compared to other blood-screening measures in the Netherlands. However, the impact on HEV disease burden may be relatively small as only a minority of all HEV cases is transmitted by blood transfusion.

The incidence of hepatitis E virus (HEV) Genotype 3 infection has increased in Europe recently.[1,2] As many as 25% of individuals aged 50 to 70 in England in 2012 and 2013 were found to be HEV seropositive.[3] Dutch blood donors showed an even higher seroprevalence of 27%, and the HEV incidence was estimated at 1.07% per year.[2] Meat consumption is thought to be a major route of transmission for HEV.[4] HEV transmission by blood transfusion has been demonstrated in several European and Asian countries.[5-8]

HEV Genotype 3 infections are mostly asymptomatic, or benign and self-limiting, with recovery from any acute illness usually within 4 to 6 weeks.[9] However, in patients with chronic liver disease or immunosuppression HEV Genotype 3 can cause serious acute or chronic liver disease, cirrhosis, and eventually liver failure. For these reasons it has become a pertinent question whether blood and blood products should be tested for HEV and, if so, which testing strategy is best. Recently some countries, including Ireland and the United Kingdom, have decided to implement HEV screening.

In this article we determine the cost-effectiveness of various blood donation screening strategies in the Netherlands. We modeled the process of donation testing as well as the treatment of immunocompromised patients who acquired chronic HEV infection from infected blood

ABBREVIATIONS: LOD = limit of detection; QALY(s) = quality-adjusted life-year(s).

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Received for publication July 22, 2016; revision received November 10, 2016; and accepted November 22, 2016.

doi:10.1111/trf.13978

TRANSFUSION 2017;57;258–266

TABLE 1. Model variable values as well as minimum and maximum credible values as used in sensitivity analysis

Variable	Best estimate	Minimum	Maximum
HEV positivity rate per donation	0.188%[2,11] (also see Materials and Methods)	×1/2	×2
Viral load distribution	Weibull distribution of log viral load: shape parameter = 1.50, scale parameter = 2.54 (see Appendix S1 and Fig. 1)	Shape parameter = 1.34, scale parameter = 2.12 (see Appendix S1)	Shape parameter = 1.68, scale parameter = 2.99 (see Appendix S1)
Detection probability	$1-e^{-\frac{v}{s}}$: v = viral load, s = 6.68 (see Materials and Methods)	s = 3.34	s = 10.01
Transmissibility	Binomial fit to log viral dose: intercept = −7.29, shape = 1.34 (see Appendix S2 and Fig. 1)	95% CI (see Appendix S2)	95% CI (see Appendix S2)
Cost of testing	$M+E*(1-\frac{n}{H+n})$: n = the number of tests performed, M = €30, E = €70, H = 20,000 (H = 2,000 for pool sizes of 6 and 1; see Materials and Methods)		x 2
Annual blood donations	441,503[12]		
Annual transfused blood products	487,761 (see Table 2)		
Annual products transfused to immunocompromised patients	5,337 (see Table 3)		+10% per type
Probability to clear HEV spontaneously when immunocompromised	34%[13]	24%* &‡ 20%*	45%* &‡ 46%*
Probability to clear chronic HEV by decreased immune suppression	32%[9]	&‡ 73%*	&‡ 93%*
Probability to clear chronic HEV by ribavirin monotherapy	85%[9]		
Time on ribavirin	80% × 3, 20% × 6 months[9]		
Costs for monitoring per chronic HEV patient	€2000 (see Materials and Methods)		€5000 &‡
Mean added costs after failed HEV treatment	€1067 (see Materials and Methods)		€3965†

* 95% CIs for binomial proportions (exact method) surrounding original data, that is, 56 of 85 cleared spontaneously,[13] 18 of 56 cleared after reduced immunosuppression, and 50 of 59 responded to ribavirin treatment.[9]
† Cirrhosis probability for chronic HEV patients changed to 26% (upper 95% confidence limit as eight of 56 chronic HEV patients developed cirrhosis)[13] and ignoring lowered survival for blood product recipients.
‡ For our sensitivity analyses these variables are altered simultaneously.

products. We considered universal screening and the selective screening of blood intended for patients at risk of chronic HEV infection. This latter option is currently performed in the United Kingdom.

Although our calculations are specific for the Dutch setting, our analysis may be informative for other countries. Insight in costs and health effects of blood screening strategies supports the decision-making process and increases transparency with respect to consequences of HEV screening.

MATERIALS AND METHODS

Simulation of HEV transmission

The cost-effectiveness of screening was analyzed by simulating the stochastic processes of the occurrence of infected donations, positive test results, and HEV transmission. The model was implemented in the open source software package R for statistical computing (Version 3.1.2).[10] An overview of all model variables and their respective values is given in Table 1.

Incidence and viral load distribution

Based on seroconversions in blood donors between 2009 and 2011, Slot and colleagues[2] found the Dutch incidence of HEV infection to be 1.07% per year. Hogema and coworkers[11] identified 41 HEV-infected donors when testing donations in pools; for these donors, previous and subsequent donations were tested individually. From the resulting individual time series, applying a maximum likelihood method, we estimated current viremic HEV infection to last 64 (37-94) days on average. With this value the likelihood of a donation containing HEV RNA is estimated to be (64/365) × 1.07% = 0.188%. Hogema and colleagues[2,11] also determined the viral load for each HEV-positive donation (see Fig. S1 [available as supporting information in the online version of this paper]). We approximated the cumulative (log) distribution of these viral loads by a

Weibull distribution, compensating for the fact that not all infections were detected when screening in pools (see Appendix S1, available as supporting information in the online version of this paper).

Detection probability

The probability for detection was modeled as $1 - e^{-v/s}$, where v is the viral load in IU/mL blood and s is a scale factor that depends on sensitivity of the test.[11] The 95% limit of detection (95% LOD) is reported for several commercially available tests (i.e., for Grifols at 7.9, Altona at 12.4, Roche at 18.6, and Mikrogen at 36.13 IU/mL), and for the in-house tests used for HEV screening in the Netherlands (10.3 and 38.4 IU/mL). Here we considered a test with an "average" sensitivity of 20 IU/mL (i.e., $s = 6.68$).

Infectivity

The probability of transmission upon transfusion of contaminated blood products depends on viral dose received, that is, the viral load within the product times the volume of plasma in the product transfused. Information on this dependency is difficult to obtain. We fitted the transmissibility as a binomial process based on data from Tedder and colleagues[14] on infected and uninfected cases after transfusion with a contaminated blood product (see Appendix S2, available as supporting information in the online version of this paper).

Pooled testing

Our scenarios ranged from testing individual donations to testing in pools of 192. Resolution testing is performed for all HEV-positive pools; larger pools are subdivided into pools of first 48 and next of six, with individual testing determining which donations to discard. One billion donations were simulated. A fraction equal to the likelihood of infection was randomly assigned an HEV load from the Weibull distribution. Donations were grouped in pools based on their donation number. A test status for each pool was assigned randomly in accordance with the likelihood of detecting an HEV load in a pool. One billion recipients were simulated per type of blood product. The viral load per product was calculated from the original load of the donation and the volume of plasma present in the final product. Doses received from pooled platelet (PLT) concentrates, which are produced from five whole blood donations, were calculated from the individual donation loads and the volume contributed per donor. The mean probability for infection per simulated product was obtained using the infectivity function described above.

Calculation of costs and numbers prevented

Products

Annually in the Netherlands approximately 487,800 blood products that may transmit HEV are produced, from around 441,500 donations (see Table 2).[12]

Selective or universal screening

First, we calculated the overall annual number of HEV infections prevented in case all donations are tested. Next we considered a screening strategy that could reduce costs, namely, the screening of only blood products intended for organ transplant and allogeneic stem cell transplant patients. In 2014 in the Netherlands 1353 organ transplants[18] and 369 stem cell transplantations from unrelated donors were performed.[19] As Dutch data on transfusions to these patients are lacking, English data on the mean number of blood products per patient were used.[20] It was estimated that 4700 red blood cell (RBC) units and 640 PLT units are transfused to these patients annually (Table S1, available as supporting information in the online version of this paper). In the Netherlands quarantine plasma has been replaced by solvent/detergent (S/D)-treated plasma. Since this product is currently already being screened for HEV, the costs and effects of plasma screening are not included in our calculations (these are included in Appendix S3, available as supporting information in the online version of this paper). The number of products intended for high-risk patients comprises 11% of all blood donated.

Cost of testing

The model keeps track of the total number of tests, including those for resolution testing of pools. We estimated the price per test, including costs for logistics (personnel), pooling of samples, disposables, and reagents, but not including hardware and software investments that might be required for implementation. Depending on efficiency the estimated price per test is between €30 and €100. Efficiency increases with the number of tests performed, since at larger scale there is less variation between peak and mean work load, also expert personnel and hardware are more consistently in use, and larger runs require fewer controls and maintenance. The modeled per-test price is

$$M+E \times \left(1-\frac{n}{H+n}\right),$$

in which $M = €30$ is the minimum price paid, $E = €70$ the additional price paid hypothetically at lowest efficiency, and $H = 20{,}000$ scales how quickly efficiency increases with n, the number of tests that are performed. Since at Sanquin nucleic acid testing (NAT) for other infections is performed in pools of six, testing at this pool size is

TABLE 2. Use of blood products and HEV transmission risk per blood product in the Netherlands

Type of product	Number of products*	Number of donors contributing to a single product	Approximate plasma volume included in the product[15-17]	Infection probability per product without screening (%)
RBCs	428,393	1	10 mL	0.03
Buffy coat–derived pooled PLT concentrate in plasma	39,341	5	4 × 2 mL + 1 × 322 mL†	0.13
Buffy coat–derived pooled PLT concentrate in plasma/PASIII	16,861	5	4 × 2 mL + 1 × 124 mL†	0.12
Apheresis PLTs in plasma	2,533	1	400 mL	0.07
Apheresis PLTs in plasma/PASIII	633	1	160 mL	0.05
Quarantine plasma	57,070‡	1	310 mL	0.06
S/D-treated plasma§	10,450‡	1170	1170 × 200 mL/1170	7.3¶

* The total number of blood products supplied to Dutch hospitals in 2014 (unpublished data, provided by Sanquin Blood Bank).
† PLTs, derived from buffy coats of five whole blood donations, are pooled and resuspended in the plasma of one of those donations.
‡ During 2014, quarantine fresh-frozen plasma was phased out as the standard plasma product for transfusion in the Netherlands. From 2015 onward, S/D-treated plasma is the standard plasma product for transfusion.
§ Omniplasma (200 mL), produced by Octapharma AG from apheresis plasma of Dutch donors, that is, the Dutch equivalent of octaplasLG.
¶ Plasma collected for the production of S/D-treated plasma is currently routinely screened for HEV RNA by NAT in pools of 96 donations as well as in pools of 192 (combined test sensitivity $s = 3.625$). Additionally, donations from donors are not used for the production of S/D-treated plasma from 60 days before to 60 days after an HEV-positive index donation. In our main analysis, we therefore assume that transfused plasma has no potential for transmitting HEV in the Netherlands (but see Appendix S2 for an alternative scenario).

rendered efficient much more quickly: for this pool size or when testing individually we set $H = 2000$.

Number of infections advancing to chronic HEV

Only immunocompromised individuals are expected to advance to chronic HEV infection, but as many as 34% of organ transplant patients may clear HEV spontaneously.[4,13] In the Netherlands at-risk patients are screened for infection either annually or when liver enzymes are elevated. For approximately 32% of early-stage chronic cases a temporary decrease of immune suppression will clear HEV.[13] Otherwise ribavirin monotherapy may be given; among 59 chronically HEV-infected organ transplant recipients 85% eventually achieved sustained virologic response after ribavirin treatment.[9]

Costs avoided by preventing chronic HEV infections

Ribavirin treatment costs €523 when the patient receives 600 mg/day for 3 months.[21] Some 20% of patients require a second course of ribavirin.[9] Additional monitoring of chronic HEV patients (e.g., checking HEV load and ribavirin-induced anemia) is estimated to cost €2000 per patient.[22] Approximately 14% of organ recipients who developed chronic HEV infection developed cirrhosis.[8] Since information on further disease progression of HEV-infected patients is not available, we extrapolated from information on hepatitis C patients the following variables: the mean time living with compensated cirrhosis is 10 years[23] (at €300 per year), 39% and 14% progress eventually to decompensated cirrhosis (€27,905) and hepatocellular carcinoma (€21,389) respectively.[22] Conservatively, we reduce these costs by 50% since 50% of individuals receiving blood products do not survive 2 years posttransfusion.[24,25]

Sensitivity analysis

To quantify the impact of uncertain model variables we performed univariate sensitivity analyses. For each model variable we established credible lower and upper limits (see Table 1). By recalculating model outcomes for these alternative values, the separate effect of each variable is shown.

RESULTS

The implementation of HEV testing enables interception of donations containing HEV RNA, but donations with low levels of HEV will not be detected (see Fig. 1). Increasing the pool size will lower the sensitivity for individual donations, resulting in fewer detections and in higher viral loads that go undetected. This increases the probability of recipients becoming infected.

Universal screening

The expected number of HEV transmissions and the associated costs are shown in Table 3 for different test strategies. The model predicts that in the absence of testing 187 HEV transmissions occur annually in the Netherlands via blood products. Screening in smaller pools is more costly, but would prevent a greater fraction of these cases. Additionally, of pools of larger size a considerable proportion would test positive, necessitating frequent resolution testing. When the pool size is increased beyond 96 the costs of testing barely reduce. At a pool size of 96 the cost per transmission prevented is €3679 for universal screening,

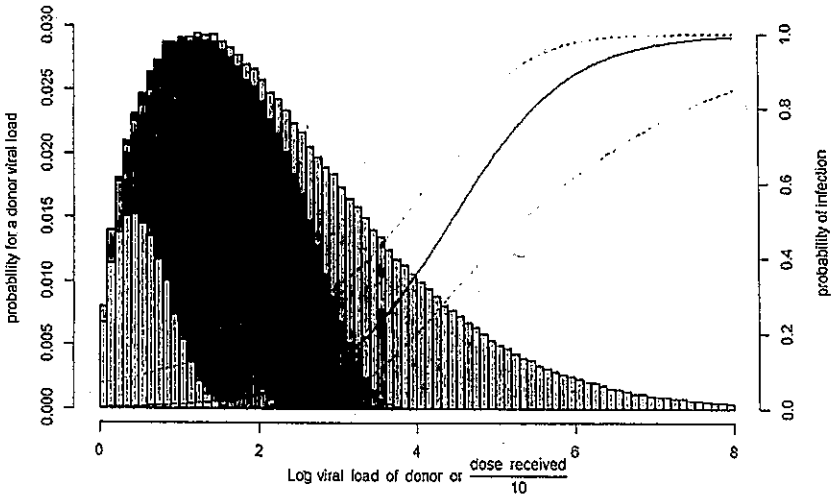

Fig. 1. Distribution of the viral loads of undetected HEV-infected donations for various screening strategies and the transmission probability by dose. Given infection of the donor, the probability for a specific log viral load in IU/mL donated blood is shown (bar plot overall, any color). Superimposed, probability distributions of viral loads for those infected remaining undetected despite testing is shown: bar plots foreground (left) to background (right) for testing in pools of 1, 6, 24, 48, 96, and 192, respectively. (Line curve in front) Probability for infection given a single-RBC-unit transfusion from one infected donor, with the load in the donated blood corresponding to the received dose/10 (as RBCs contains 10 mL of plasma). Assuming a binomial probability distribution by dose, this transmission probability is fit on the 43 cases described by Tedder and coworkers[14] of infection transmission (or lack thereof) after transfusion with HEV-infected blood products. The dashed lines represent the 95% CI surrounding the fit (see Fig. S2 [available as supporting information in the online version of this paper] for this fit to the transmission data, as well as the transmission probabilities for other blood products). [Color figure can be viewed at wileyonlinelibrary.com]

and in this scenario 27 cases of HEV transmission would still remain (14% of the 187 without testing). Testing in pools of 24 instead would approximately halve the number of remaining transmissions while doubling the cost to €8099 per transmission averted.

Following the English SaBTO Advisory committee, we assume that only organ transplant and allogeneic stem cell transplant patients are at risk for chronic HEV.[20] In that case we expect that approximately five of the annual transmissions progress to chronic HEV infection and that one patient every 2 years cannot be cured by either lowered immune suppression or ribavirin treatment. Only these chronic HEV cases are expected to have a substantial health impact. Compared to the cost per transmission prevented the cost per chronic case prevented is approximately 38 times higher, while the cost per incurable chronic case prevented is about 376 times higher.

TABLE 3. Modeled annual costs and cost per HEV transmission prevented

Test strategy	Expected HEV transmissions	Expected chronic HEV cases	Expected chronic HEV cases despite treatment	Pools testing positive (%)	Testing costs (€)	Costs avoided (€)	Cost per transmission averted (€)	Cost per chronic case averted (€)	Cost per incurable case averted (€)
No testing	187	4.94	0.50		0	0			
Testing all whole blood donations in pools of:									
192→48→6→1	32.8	0.98	0.10	10.7	558,711	10,830	3,558	138,426	1,357,121
96→48→6→1	27.0	0.82	0.08	6.50	598,939	11,274	3,679	142,628	1,398,311
48→6→1	18.1	0.56	0.06	3.89	1,008,109	11,991	5,904	227,292	2,228,349
24→6→1	13.4	0.42	0.04	2.29	1,416,739	12,377	8,099	310,458	3,043,707
6→1	5.81	0.19	0.02	0.75	2,443,796	13,017	13,432	510,968	5,009,495
1	1.33	0.04	0.00	0.16	13,384,459	13,406	72,103	2,729,103	26,755,907
Testing only 11% of whole blood donations in pools of:									
192→48→6→1	169.8	0.98	0.10	10.7	72,835	10,830	3,661	15,666	153,589
96→48→6→1	169.2	0.82	0.08	6.50	78,992	11,274	3,854	16,435	161,130
48→6→1	168.2	0.56	0.06	3.89	148,431	11,991	7,352	31,132	305,219
24→6→1	167.7	0.42	0.04	2.29	228,537	12,377	11,332	47,786	468,490
6→1	166.9	0.19	0.02	0.75	367,040	13,017	17,784	74,418	729,592
1	166.4	0.04	0.00	0.16	1,591,423	13,406	77,358	322,082	3,157,662

TABLE 4. Univariate sensitivity analysis

Changed variable*	Expected chronic HEV cases	Expected chronic HEV cases despite treatment	Costs of testing (€)	Costs avoided (€)	Cost per incurable chronic case averted (€)	Cost per incurable case averted compared to baseline scenario (%)
Baseline, screening 11% of donations in pools of 24→6→1	0.42	0.04	228,537	12,377	468,490	100
Higher transmissibility	2.31	0.24	228,537	20,403	273,651	58
Lower transmissibility	0.11	0.01	228,537	7,209	823,593	176
Higher HEV loads per infected	0.38	0.04	236,270	16,645	353,958	76
Lower HEV loads per infected	0.44	0.04	221,291	9,024	631,033	135
Incidence × 2	0.83	0.08	266,555	24,701	262,654	56
Incidence × 0.5	0.21	0.02	209,064	6,182	880,301	188
Less clearing of HEV (spontaneous or induced)	0.48	0.10	228,537	14,886	189,892	41
More clearing of HEV	0.35	0.01	228,537	10,056	1,533,303	327
Screening 40% of all donations	0.42	0.04	704,796	12,377	1,500,696	320
Higher chronic HEV costs	0.42	0.04	228,537	27,285	436,179	93
Higher screening costs	0.42	0.04	457,075	12,377	963,804	206
Low test sensitivity	0.52	0.05	225,113	12,077	473,182	101
High test sensitivity	0.29	0.03	234,594	12,725	467,727	100
High test sensitivity and screening in pools of 48→6→1	0.40	0.04	156,749	12,428	311,505	66

* See Table 1.

Selective screening

Cost-effectiveness could be enhanced by selective screening of the blood products received by organ and allogeneic stem cell transplant patients. Screening only 11% of all whole blood donations in pools of 24 would lower the cost by 85%, to €47,786 per chronic case and €468,490 per incurable chronic case prevented, and reduce the total cost of screening from €1,416,739 to €228,537 per year. Note that the cost per case prevented actually increases by selective screening compared to universal screening, as the per test price increases due to lowered efficiency when performing fewer tests.

Sensitivity analysis

The univariate sensitivity analyses show the effect of alternative plausible values on our model outcomes (Table 4). The uncertainty in the probability of transmission by viral dose (which is based on only 43 observations; see Appendix S2) translates to a substantial credible range in expected transmission numbers, and consequently cost-effectiveness could be up to 42% lower or 76% higher. Another source of uncertainty concerns the distribution of viral loads of infected donors. A higher expected HEV load in a donation increases the transmission potential, but at the same time increases the probability of detection which eliminates transmission risk. As a result, with HEV loads on average higher (or lower) the cost per chronic HEV case prevented is estimated 24% lower (35% higher, respectively).

The number of prevented infections and inversely the cost per prevented case depend almost linearly on the incidence rate (nonlinearity results from additional resolution testing at high incidence). In case both the fraction of immunocompromised individuals who clear the infection spontaneously and the ribavirin effectiveness are actually at the lower (higher) limit of their estimation, the cost per case prevented is estimated 59% lower (227% higher, respectively).

We may have underestimated the number of blood products received by organ recipients or the stock of tested products needed to guarantee administration of typed blood products. If a greater proportion of donations needs to be screened to supply screened blood products to all patients at risk for chronic HEV, this obviously renders selective screening less cost saving compared to universal screening. Using a test with better sensitivity, even if it were more expensive per test, might result in a lower overall cost by allowing testing of larger pools; the model predicts slightly fewer remaining chronic cases when screening in pools of 48 with A 95% LOD at 10 IU/mL compared to testing in pools of 24 with a 95% LOD of 20 IU/mL (=baseline scenario).

DISCUSSION

We investigated the cost-effectiveness of HEV screening of Dutch blood donations. The estimated cost per chronic HEV case averted would be approximately €310,458 when applying universal screening in pools of 24. The cost per incurable case prevented is estimated 10-fold higher at approximately €3,043,707. The total per year cost of testing would be €1,416,739.

Costs could potentially be reduced substantially by screening blood products for immunocompromised patients, who are known to develop chronic infection,[26] only. The cost for selective screening in pools of 24 is

estimated at €47,786 per chronic case prevented or €228,537 in total per year.

However, in case of selective screening additional costs are incurred for keeping a double inventory, additional transports, and possibly for increased product expiration. These factors are difficult to estimate but they may negate the benefits of selective screening.

The model predicts that per year 187 HEV transmissions occur via blood products in the Netherlands. This likely is an overestimate since some recipients already are HEV seropositive and thereby possibly protected from reinfection.[5] Of Dutch blood donors 27% were found seropositive.[2] This proportion could be higher among recipients who are generally older than blood donors.[24] However, as immunocompromised patients remain at risk for HEV infection, our cost-effectiveness estimates are unaffected by acquired immunity for chronic HEV prevention.

We estimated the number of chronically infected patients and took into account costs avoided by preventing such infections. We took variation in HEV viral load into account.[11] Based on current knowledge, the modeled probability of transmission depends on the HEV exposure of recipients.[14] Our model therefore not only addresses the benefit of lowered numbers of infected products, but also accounts for the lower HEV doses received when screening in smaller pools. As shown in Table 4 however, the inconclusiveness in donor viral loads—and especially in the dose response—contributes considerably to the uncertainty in our final estimates.

We may have underestimated the number of individuals at risk for chronic HEV infection. Following the English SaBTO Advisory committee, we assumed only organ transplant and allogeneic stem cell transplant patients to be at risk for chronic HEV, ignoring the risk that for example other oncology patients may run.[20] In general, considering a larger group of blood recipients at risk for chronic HEV will lower the estimated cost per prevented case, but it would also make selective screening a relatively less attractive option.

Since there is much uncertainty about the health impact for different patients affected by HEV, an estimate of quality-adjusted life-years (QALYs) saved by HEV screening was outside the scope of our investigation. Tentatively, if we account for a health utility of 0.72 during ribavirin treatment and of 0.55 during cirrhosis[22] (ignoring rare further complications and the lowered quality of life for transplant patients) we arrive at a loss of 0.12 QALYs per chronic case of HEV. Our rough estimate for the cost per gained QALY would be in the order of €2,600,000 using universal screening in pools of 24, or €400,000 when screening selectively. Thereby, screening for HEV seems relatively inexpensive and effective compared to, for example, multiplex NAT (for HBV, HCV, and HIV) in the Netherlands, estimated to cost €5,200,000 per QALY gained.[27]

Recently in the Netherlands almost all quarantine plasma has been replaced by pooled S/D-treated plasma (Omniplasma). Donations used for Omniplasma and Omniplasma production pools are screened for HEV RNA; therefore, potential transmission by plasma was not considered in our analysis. Because fresh-frozen or quarantine plasma remains in use in most other countries,[20] we considered a scenario in which all plasma used is fresh-frozen quarantine plasma (see Appendix S3). This resulted in 43 additional HEV transmissions (230 rather than 187 overall), of which 0.27 would progress to chronic HEV infection (5.21 instead of 4.94 cases among organ and stem cell recipients) (Table A2, available as supporting information in the online version of this paper).

The most common transmission route for HEV in European countries probably is food related.[4] From the estimated HEV incidence per year and the number of Dutch citizens (including 27% seropositive persons), it follows that in the Netherlands 133,000 cases of HEV infection are expected annually.[2] Our estimate of 187 HEV infections by blood transfusion therefore corresponds to only one in 700 HEV transmissions being due to blood transfusion. As the transfusion risk is proportional to the background risk, we expect the relative importance of the risk of transmission by blood transfusion to be similarly low in other countries. However, especially patients at risk for chronic hepatitis E receive a considerable amount of blood products. Among the annual 1722 organ and bone marrow transplant patients, 18.4 background HEV infections are expected, of which 12.1 would become chronic. Combined with our estimate of 4.94 chronic cases due to infected blood products, it follows that of chronic HEV infections one in 3.5 would be via blood transfusion.

We conclude from our analysis that a small health gain through prevention of a few chronic cases per year is expected by screening blood donations for HEV. We emphasize that it is important to put this risk for HEV transmission by blood transfusion in perspective. If the dietary source(s) of HEV are investigated and eliminated, the entire population will benefit rather than only recipients of blood products. In the long run, the investments necessary to accomplish this are likely to be more cost-effective than the screening of donated blood. Meanwhile, blood establishments must choose between providing unscreened products that may seriously harm some recipients, and expensive screening that provides only limited protection, since most HEV transmissions occur not via blood transfusion.

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.

Appendix S1. The distribution of viral loads of HEV positive donations.
Appendix S2. Transmissibility of HEV by viral dose.
Appendix S3. Including transmission risk by plasma.
Fig. S1. Parametric fit of the cumulative log10 viral load distribution. Cumulative distribution of the log10 viral load in IU per mL blood of identified HEV cases from

Hogema et al.[11] (open circles), the adjusted cumulative distribution, where the viral loads were weighed according to their detection probability (as explained in the Appendix S1 text) (closed circles), with confidence intervals from bootstrapping (dotted lines), and the best fitting Weibull cumulative density function (line). Reliable quantification was not possible for absolute viral loads below 20 (= log10 value of 1.3).

Fig. S2. Parametric fit of the transmission probability. Based on 43 cases described by Tedder *et al.* of infection transmission (or lack thereof) after transfusion with HEV infected blood products.[12] Top: Data (circles, jittered vertically to enhance visibility), the moving average per 10 cases (blue diamonds) and the best fitting binomial probability function (line), $e^{b+m\cdot LVL}/(1+e^{b+m\cdot LVL})$, where *LVL* represents the log10 viral dose received, and b and m scale the function. The best fitting function was found for the parameters $b = -7.29$ and $m = 1.34$, the 95% confidence interval surrounding this fit is shown in dotted lines. Bottom: same as Figure 1 within the main text, but the probability of infection is here shown for receiving different products. From left to right, receiving (included plasma volume in parentheses): apheresis platelets in plasma (400 mL), apheresis platelets in plasma/PASIII (160 mL), red cell concentrate (10 mL), buffy coat-derived pooled platelet concentrate in plasma (2 mL), S/D-treated plasma (0.17 mL) (note that for the latter two products we assume that only one of the donors contributing to the pooled product is infected).

Table A1. Number of blood products transfused to transplant patients in the Netherlands.

Table A2. Modeled annual costs and cost per HEV transmission prevented assuming use of quarantine plasma instead of S/D treated plasma.

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一般的名称	人ハプトグロビン	研究報告の公表状況	Transfusion 2016; 56( ): 2532-2537	公表国 ドイツ
販売名 (企業名)	ハプトグロビン静注 2000 単位「JB」(日本血液製剤機構)			

研究報告の概要

血漿分画製剤のためのプール血漿における E 型肝炎ウイルス量：

背景：献血者は、E 型肝炎ウイルス（HEV）に不顕性感染している可能性があり、血漿由来医薬品の製造に使用されるプール血漿は、検出可能なウイルス RNA を含むことが現在認識されている。HEV に感染した血液および血漿ドナーの発生率は、地域的な疫学によって大幅に異なる可能性がある。
研究計画と方法：HEV RNA の TMA による拡散増幅を用いて、北アメリカ、ヨーロッパ、中東、およびアジアからの製造用プール血漿について、HEV の存在を調べた。HEV の確認のために、リアルタイム逆転写 PCR を行い、配列決定を分析した。
結果：合計 484 プールを試験した。アジア系プールは HEV RNA 陽性頻度が最も高く、300 IU/mL を超えなかったものの、ウイルス負荷が高かった。1 つの明らかな変異を含むジェノタイプ 4 に属するストレインが確認された(n = 5)。北米（n = 5）および欧州（n = 5）プールでは、HEV のジェノタイプ 3 のみが同定された。試験を行った中東からのプール血漿には HEV の証拠はなかった。
結論：HEV は、3 つの異なる大陸からの製造用プール血漿において検出された。プールサイズが大きく個々の献血における HEV 量が中程度であったことと一致して、製造用プール血漿へのウイルス負荷は低かったが、にもかかわらず血漿由来医薬品のリスク評価に有益であった。シーケンスの分析が可能な場合、それぞれの領域における地域的に流行する遺伝子型と一致するウイルスの存在が確認された。中東のプールに HEV が存在しないことは、おそらく豚肉消費が低いことによるこの地域の HEV の低罹患率と一致している。

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

使用上の注意記載状況・その他参考事項等

2. 重要な基本的注意
(1) 本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-1 抗体陰性で、かつ ALT(GPT)値でスクリーニングを実施している。更に、HBV、HCV 及び HIV について核酸増幅検査(NAT)を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分から人ハプトグロビンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及びウイルス除去膜によるろ過処理を施しているが、投与に際しては、次の点に十分注意すること。

C3 ハプトグロビン

Hepatitis E viral loads in plasma pools for fractionation

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BACKGROUND: It is now recognized that blood donors may be silently infected with hepatitis E virus (HEV) and that plasma pools used in the manufacture of plasma-derived medicinal products may also contain detectable virus RNA. The occurrence of HEV-infected blood and plasma donors can vary considerably depending on local epidemiology.

STUDY DESIGN AND METHODS: Manufacturing plasma pools from North America, Europe, the Middle East, and Asia were examined for the presence of HEV using transcription-mediated amplification of HEV RNA; confirmatory testing was performed using real-time reverse transcription polymerase chain reaction and sequencing.

RESULTS: A total of 484 pools were tested. Asian pools were most frequently positive for HEV RNA and had higher viral loads, although none exceeding 300 IU/mL, and the sequenced strains (n = 5) clustered with Genotype 4, including one significantly divergent sequence. Only HEV Genotype 3 was identified in North American (n = 5) and European (n = 5) pools. There was no evidence of HEV in any pools tested from the Middle East.

CONCLUSIONS: HEV was detected in manufacturing plasma pools from three different continents; viral loads were low—consistent with large pool sizes and moderate levels of HEV viremia at the individual donation level—but are nevertheless informative for risk assessment of plasma-derived medicinal products. Where sequencing was possible, analysis confirmed the presence of viruses consistent with locally circulating genotypes in the respective regions. The absence of HEV in Middle Eastern pools is consistent with the low prevalence of HEV in this region, likely due to low pork consumption.

Hepatitis E virus (HEV) belongs to the *Hepeviridae* family of viruses and, as a result of poor sanitation in developing countries, is a major cause of acute hepatitis with the virus transmitted by the fecal-oral route. In developed regions of the world, hepatitis E can occasionally be found in travelers' who have visited areas in Asia and Africa endemic for hepatitis E. In recent years, it has become apparent that autochthonous acquisition of HEV occurs in developed countries and that locally acquired hepatitis E in these regions is the result of zoonotic transmission, particularly after consumption of certain types of meat such as pork. These differences in transmission are related to the different genotypes of HEV. Genotypes 1 and 2 only infect humans and cause large water-borne outbreaks and sporadic cases of hepatitis E in developing countries, whereas Genotypes 3 and 4 usually infect pigs, wild boar, and several other animal species but are also zoonotic infecting people. Although hepatitis E is usually an acute infection, Genotype 3 HEV may cause chronic infection in certain patient groups, such as immunosuppressed transplant.[1-3]

Although not a major route of transmission, HEV can be transmitted by blood transfusion. Transfusion transmission of HEV by blood components has been reported from several countries including, for example,

ABBREVIATIONS: S/CO = signal-to-cutoff ratio; TMA = transcription-mediated amplification.

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Received for publication April 12, 2016; revision received May 24, 2016; and accepted May 24, 2016.

doi:10.1111/trf.13722

TRANSFUSION 2016;56;2532–2537

Japan, England, and France.[4-11] On one occasion, there was evidence of initial infection of a blood donor via food[7] and another example of a plasma donor who worked in a slaughterhouse and developed hepatitis E after donation.[12]

Studies in Japan and China have identified acute HEV infections in blood donors confirmed by the detection of HEV RNA with an incidence of 0.014% to 0.02%.[13-15] Analysis of European blood and plasma donors has also identified HEV viremic donors in Germany, England, Sweden, Scotland, the Netherlands, France, Spain, and Austria.[10,12,16-25] In Europe, the incidence of HEV RNA-positive viremic units ranges from approximately 0.15% to 0.007%.[15]

In North America, fewer studies have been performed and HEV RNA–positive viremic units have not been identified in some groups of donors from Canada and the United States,[15,17,26] suggesting a low incidence. However, a recent study found an incidence of HEV RNA–positive donations of 0.11%[27] and in another study a plasma fractionation pool was found to be positive for HEV RNA.[28]

With respect to fractionated plasma-derived medicinal products, one study indicated increased anti-HEV seroprevalence in recipients of nonvirally inactivated clotting factor concentrates.[29] There are also cases of transmission of HEV to recipients of solvent/detergent (S/D)-treated plasma.[30-32] We previously identified HEV RNA in several plasma fractionation pools from Europe, Asia, and, in one case, North America.[28] Since the improvement in nucleic acid tests for the detection of HEV and the introduction of the World Health Organization (WHO) International Standard for HEV RNA[33] we have expanded our initial study to investigate a larger number of plasma fractionation pools from different parts of the world, including North America, to get an overview about the incidence of viremic donations in the pools used for the manufacture of plasma-derived medicinal products, to investigate the virus genotypes by sequence analysis and assess the levels of HEV RNA present in plasma pools.

MATERIALS AND METHODS

Samples

Samples of manufacturing plasma pools submitted to the Paul-Ehrlich-Institut were stored at −20 to −40°C until analysis. Manufacturing pools were sourced from donations collected in Europe, North America, the Middle East, and Asia between 2001 and 2014 with volumes of the pools ranging from approximately 750 to approximately 4500 L.

Nucleic acid test screening and confirmatory testing

Initial pool screening was performed using transcription-mediated amplification (TMA), while confirmatory testing used another nucleic acid amplification technology (NAT), real-time reverse transcriptase–polymerase chain reaction (RT-PCR). For screening, the automated Procleix Panther system and the Procleix HEV assay was chosen (Hologic, Inc., and Grifols Diagnostic Solutions, Inc.). The Procleix HEV assay is CE-marked and is based on TMA technology. For each plasma pool, 700 µL was analyzed in the initial screening procedure; testing was performed in accordance with the manufacturer's instructions. All pools were tested without dilution. The 95% cutoff of the screening assay was 7.8 IU HEV RNA/mL plasma.[25] Reactive pools, with a signal-to-cutoff ratio (S/CO) of 1.0 or more were subjected to confirmatory testing. It was not possible to retest initially reactive pools using the Procleix HEV assay due to limitations in sample volumes available for retesting. Therefore, confirmatory testing was performed using RT-PCR technology, which also provided some measure of quantification of the HEV loads in the pools.

Extraction of RNA was performed from 200 µL of plasma using the virus spin kit (QIAamp MinElute, Qiagen GmbH). Elution was performed with 70 µL of elution buffer, and 10 µL of the eluate was used for the RT-PCR. Amplification and detection were performed using an HEV RT-PCR kit (RealStar 1.0, Altona Diagnostics GmbH) using a real-time PCR system (LightCycler 480, Roche Applied Science GmbH) in accordance with the manufacturer's instructions. Pools were extracted singly and RT-PCR was performed in duplicate and samples were deemed positive when one or more reaction was found positive.

To determine the levels of HEV RNA in the reactive plasma pools, a standard curve was prepared using the WHO International Standard for HEV RNA (Code 6329/10).[33] The 95% cutoff of the confirmatory assay was 64 IU HEV RNA/mL plasma.

Sequence analysis

For sequence analysis, HEV RNA was extracted using a virus spin kit (MinElute, Qiagen). Amplification of a partial region of the HEV RNA-dependent RNA polymerase (*RdRp*) gene was performed as described previously.[34] Maximum likelihood phylogeny of sequences (283 nucleotide fragment) obtained in this study and reference *Orthohepevirus A* strains, as recently defined,[35] were calculated using a GTR nucleotide substitution model, the complete deletion option, and 1000 bootstrap replicates in MEGA 6.0 (http://www.megasoftware.net/). Sequencing served as a further confirmatory test where repeat testing using RT-PCR was negative.

TABLE 1. Analysis of plasma pools for HEV RNA: results of initial screening by TMA and confirmatory testing by RT-PCR and nucleic acid sequencing

Manufacturer	Plasma origin	Number of plasma pools tested	Number positive by TMA assay (% positive pools)	Number positive by RT-PCR analysis (% positive pools)	Number positive by sequence analysis (after RT-PCR) (% positive pools)
A	Asia	78*	32 (42.7)	17 (22.7)	19 (25.3)
G	North America	99	10 (10.1)	3 (3.0)	3 (3.0)
H	North America	70	9 (12.9)†	4 (5.7)†	4 (5.7)†
C	Europe	77	8 (10.4)	7 (9.1)	8 (10.4)
F	North America	51	7 (13.7)	6 (11.8)	6 (11.8)
E	Europe	49	6 (12.2)	6 (12.2)	6 (12.2)
D	Europe	15	1 (5.6)	0 (0)	0 (0)
D	North America	3	0 (0)	0 (0)	0 (0)
E	Europe/North America	6	0 (0)	0 (0)	0 (0)
G	Europe	3	0 (0)	0 (0)	0 (0)
B	Middle East	33	0 (0)	0 (0)	0 (0)

*Test results for three pools were invalid.
†Only five of the initially TMA-reactive samples from Manufacturer H were followed up.

TABLE 2. Overview of pools with viral loads exceeding 100 IU/mL

Manufacturer	Plasma origin	Number of plasma pools tested	Number positive by RT-PCR analysis (% positive pools)	Number of pools with loads > 100 log IU/mL
A	Asia	78	17 (22.7)	5
C	Europe	77	7 (9.1)	1
E	Europe	49	6 (12.2)	0
F	North America	51	6 (11.8)	0
G	North America	99	3 (3.0)	0
H	North America	70	4 (5.7)	0

RESULTS

A total of 484 pools, from seven different manufacturers (A-G), were screened using the Procleix HEV assay. The results of the initial screening are shown in Table 1. Pools were deemed initially reactive when the S/CO was more than 1.0. Asian pools were most frequently positive for HEV RNA, with 43% being positive after the initial screening. Plasma pools from Europe and North America had similar rates of HEV-positive pools, that is, between 6% to 12% and approximately 10% to 14%, respectively. All pools (n = 33) from the Middle East were negative for HEV RNA. The Asian pools had the highest volumes of all pools tested in the study (i.e., more than 4000 L vs. 700-3500 L). The higher positive rate of the Asian pools in the Procleix assay might be explained by the higher number of pooled donations. A higher number of pooled donations would be expected to result in a higher number of positive pools if tested with a sensitive assay.

Upon confirmatory testing, the number of positive Asian pools fell to 22% based on RT-PCR (Table 1). In some cases it was possible to obtain a sequence for some samples which were negative by RT-PCR using different primers (i.e., using ORF2/3 primers compared to ORF1 primers, respectively). By combining the RT-PCR results and HEV-positive sequence results, 25% of Asian pools were positive after confirmatory testing. In the case of the European pools found initially reactive and the pools from Manufacturer F from North America, the majority of these pools were also reactive in the confirmatory testing, either by RT-PCR or by sequencing. The North American pools from Manufacturer G had an initial reactive rate of approximately 10%, which fell to 3% upon confirmatory testing. The decrease in HEV-reactive pools upon confirmatory testing is most likely due to the differences in assay sensitivity for the Procleix HEV test which is 7.8 and 64 IU HEV RNA/mL for the confirmatory test. However, the inconsistent reactive rate between assays could also reflect possible sequence differences between virus strains and the primers used in the respective assays. The specificity of the Procleix HEV assay varies from 99.96%[27] to 99.99%.[25] No correlation was observed between the S/CO in the initial screening assay and the crossing cycle thresholds/HEV RNA loads observed in the confirmatory RT-PCR assay (data not shown).

The analysis of the HEV RNA loads (exceeding 100 IU/mL) is shown in Table 2. Six plasma pools had HEV RNA loads exceeding 200 IU/mL—one of the pools was from Europe and the rest were from Asia including one with an HEV RNA load of 300 IU/mL, the highest observed

TABLE 3. Genotypes of HEV strains identified in plasma pools

Manufacturer	Plasma origin	HEV genotype	Code number	Accession number	Year of pooling
A	Asia	4	279_8	KX025164	2007
			279_10	KX025159	2007
			280_14	KX025167	2006
			281_16	KX025169	2006
			281_17	KX025170	2006
C	Europe	3	282_07	KX025173	2010
			282_11	KX025172	2012
E	Europe	3	279_18	KX025162	2008
			279_19	KX025163	2008
			281_11	KX025168	2005
F	North America	3	280_10	KX025165	2001
			280_11	KX025166	2001
			281_6	KX025171	2005
H	North America	3	279_15	KX025160	2006
			279_16	KX025161	2008

for any pool in the study; all the other pools tested were below the limit of quantification.

For several of the HEV RNA–positive pools it was possible to perform sequence analysis (Table 3). Sequencing was performed in a region of the RdRp in ORF1 as previously described.[34] The sequence data confirmed that the HEV strains identified in the pools from Manufacturer A were all Genotype 4, while the strains from Europe and North America were HEV Genotype 3 strains. Phylogenetic analysis of HEV strains is shown in Fig. 1.

The European HEV strains are mainly related to 3c, 3e, and 3f subtypes (Fig. 1), which are distributed very widely in Europe, both in blood donors and in clinical cases of hepatitis E, while those from North America are predominantly Genotype 3a, which cluster tightly together. One of the HEV Genotype 4 strains (281_17) represented a more divergent virus with more limited homology to sequences present in the database. The most closely related strain E087-SAP04C (Accession Number AB369688) showed 89% nucleotide identity with 281_17 where the virus had been identified in an acute hepatitis E patient in Japan, who had traveled to Shanghai, China, before onset of illness.

In conclusion, it was possible to detect HEV RNA in manufacturing plasma pools from Europe, North America, and Asia. Where sequencing was possible, analysis confirmed the presence of viruses consistent with locally circulating strains in the respective regions. In Europe Genotype 3 strains were identified being similar to those seen in donors in this region as well as in clinical cases of hepatitis E. In Asia, only Genotype 4 strains were observed. Several HEV strains were identified in North American donors by sequence analysis—these were all related to Genotype 3a. In the past, studies have suggested exposure of the North American population to hepatitis E due to variable levels of anti-HEV seroprevalence;[26] however, only two HEV RNA–positive donors were detected by NAT at concentrations too low for viral sequencing to identify the genotype.[27] The absence of HEV in Middle Eastern pools is consistent with the low HEV prevalence in this region, which might be explained by lack of pork consumption by the local population,[36] and is consistent with our previous study looking at a smaller number of Middle Eastern pools.[28] Apart from a single report in China,[1] confirmed by sequence analysis, HEV Genotype 1 has not been detected in plasma pools, even from regions where some Genotype 1 infections would be expected. It might be speculated that potential donors infected with Genotype 1 have a higher risk of clinical illness and, therefore, would not be selected for plasma donations.

In most cases, HEV RNA loads were below the limit of quantification (i.e., 200 IU/mL) of the RT-PCR assay; only one pool from Asia had a viral load of 300 IU/mL. These data are consistent with the moderate levels of HEV viremia seen at the single donation level, with titers rarely exceeding 6 log IU/mL[23,37] as well as the large pool sizes. In Japan, one positive donor infected with the HEV strain JRC-HE3 contained a viral load that exceeded 7 log copies/mL[13] concomitantly showing elevated levels of alanine aminotransferase. This particular donation was used to develop the Japanese National Standard for HEV RNA and the copy number/IU ratio was very similar.[32,38] Extrapolating the viral load of 300 IU/mL in a pool size of 4200 donations, assuming contamination with a single 800-mL viremic source plasma donation would imply that the viral load in the individual donation was approximately 1.6×10^6 IU/mL. The titers observed in these positive plasma pools are informative when preparing risk assessments for plasma-derived medicinal products concerning contamination with HEV and efficacy of inactivation or removal for the respective products. This was discussed at the European Medicines Agency in London in 2014,[39] when it became clear that in Europe, in particular, the high rate of HEV RNA–positive donations were being identified by testing or through lookback studies.[28] Given the potential HEV contamination of plasma pools for fractionation it is

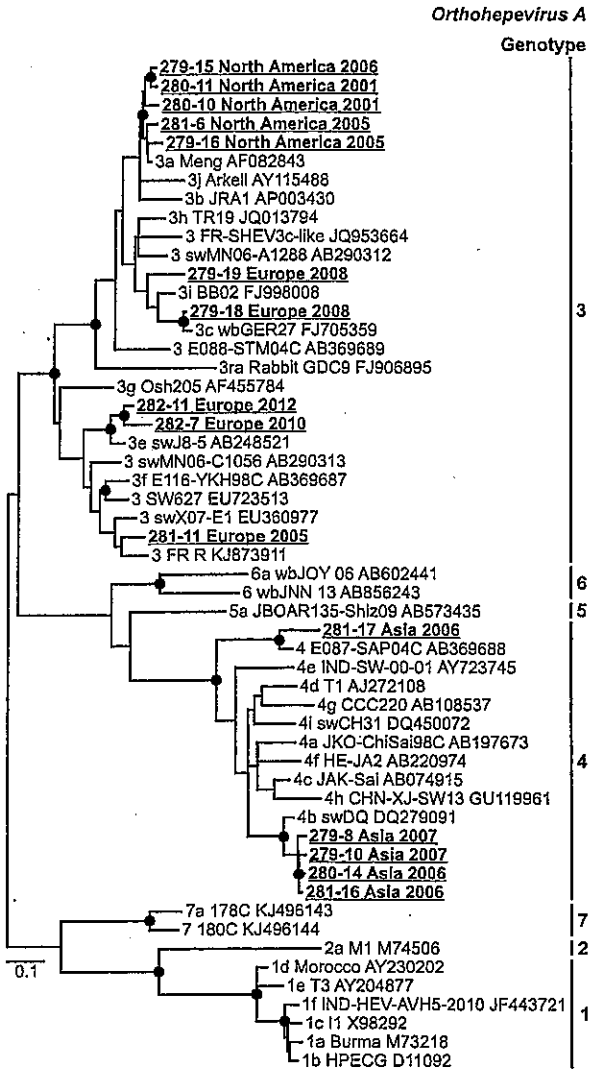

Fig. 1. Phylogenetic analysis of the HEV strains identified in the respective plasma pools. Maximum likelihood phylogenetic analysis of *Orthohepevirus A* reference sequences and sequences from this study. Filled circles, at nodes, represent bootstrap values exceeding 70 of 1000 repetitive analyses for confidence testing. Taxon names of all reference sequences include genotype, subtype (if available), strain name, and GenBank accession number. Sequences obtained in this study are given bold and underlined and the corresponding taxon names include ID, geographic origin, and year of sampling.

necessary to assess the risk of HEV transmission via plasma-derived medicinal products. The experience of transmission cases by S/D plasma in France[31,32] underlines the risk of HEV transmission by plasma where there is no processing step for inactivation or removal of nonenveloped viruses such as HEV. Therefore, it has been recently required to test plasma pools for manufacture of S/D plasma for HEV RNA to limit such a risk for transmission.[40]

Manufacturing steps with a capacity to inactivate to remove enveloped and nonenveloped viruses have been implemented into manufacture of other plasma products such as coagulation factors and immunoglobulins. There is a lack of evidence of HEV contamination in final products currently on the market in Europe.[41] However, the capacity of these processes to inactivate or remove nonenveloped viruses can be limited in some cases. Therefore, it is important to perform a quantitative risk estimation considering the viral loads in plasma pools and the overall virus reduction capacity of a specific manufacturing process.

ACKNOWLEDGMENTS

We acknowledge the help and advice of Dr Andreas Hunfeld, Roswitha Kleiber, and Ines Amberg.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest. EO and JML are employees of Hologic, Inc.

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No. 13

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
				2017. 2. 6	該当なし	

一般的名称	人血小板濃厚液	研究報告の公表状況		公表国
販売名(企業名)	濃厚血小板-LR「日赤」(日本赤十字社) 照射濃厚血小板-LR「日赤」(日本赤十字社) 濃厚血小板HLA-LR「日赤」(日本赤十字社) 照射濃厚血小板HLA-LR「日赤」(日本赤十字社) 照射洗浄血小板-LR「日赤」(日本赤十字社) 照射洗浄血小板HLA-LR「日赤」(日本赤十字社)		Satake M, Iwanaga M, Sagara Y, et al. Lancet Infect Dis. 2016 Nov;16(11):1246-1254.	日本

研究報告の概要

○日本の青少年及び成人献血者におけるヒトT細胞白血病ウイルス1型(HTLV-1)感染発生率:全国的後方視的コホート解析。
背景:日本におけるHTLV-1感染率は非常に高い。幼年期における授乳による伝播が確認されているが、これ以後の段階における新規HTLV-1感染の疫学的状況は殆ど知られていない。我々の目的は、日本全国の青少年及び成人における年間新規 HTLV-1感染者数を推定することであった。
方法:本後方視的コホート解析において、我々は日本赤十字社血液センターのデータベースに登録されており、2005年1月1日から2006年12月31日までの期間に16歳～69歳であった複数回献血者を対象として、新規HTLV-1感染の評価を行った。我々は、2011年12月31日までに採取された複数回献血者の検体による抗体検査の結果を用いて、HTLV-1抗体が陽転した者の数を評価し、陽転者の数を観察人年数で除することにより、発生密度を算出した。続いて我々は年間新規HTLV-1感染者数を推定するため、発生密度を人口と掛け合わせて算出した。
結果:HTLV-1に対する血清反応が陰性であった供血者3,375,821人(男性 2,100,915人、女性 1,274,906人)を本解析の対象とした。観察期間の中央値である4.5年(IQR 2.3～5.8)の間に、532人(男性 204人、女性 328人)にHTLV-1抗体の陽転が認められた。女性における発生密度は男性と比較して有意に高かった(女性10万人年当たり6.88、95% CI 6.17～7.66、男性10万人年当たり2.29、95% CI 1.99～2.62、p<0.0001)。年間新規HTLV-1感染者数の推定値は4,190人(男性975人 (914人～1,038人)、女性3,215人(3,104人～3,328人)、95% CI 4,064～4,318)であった。
解釈:日本では青年及び成人における新規HTLV-1感染が公衆衛生上の懸念となっており、新たな伝播を低減するための予防対策が必要とされている。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
3,375,821名の複数回献血者を対象として新規ヒトT細胞白血病ウイルス1型(HTLV-1)感染の評価を行ったところ、観察期間4.5年(中央値)の間にHTLV-1抗体の陽転が認められた献血者数は532名(男性;2.29/10万人年、女性;6.88/10万人年)であったという報告である。	日本赤十字社では、既に全製剤に保存前白血球除去を適応しており、HTLV-1の抗体スクリーニング検査を行っている。今後も引き続き情報の収集に努める。

Incidence of human T-lymphotropic virus 1 infection in adolescent and adult blood donors in Japan: a nationwide retrospective cohort analysis

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Lancet Infect Dis 2016; 16: 1246–54

Published Online August 22, 2016 http://dx.doi.org/10.1016/S1473-3099(16)30252-3

See Comment page 1205

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Summary

Background Human T-lymphotropic virus 1 (HTLV-1) infection has an especially high prevalence in Japan. Transmission has been confirmed in infancy through breastfeeding; however, little is known about the epidemiological aspects of new HTLV-1 infections later in life. We aimed to estimate the nationwide annual number of new HTLV-1 infections among adolescents and adults in Japan.

Methods In this retrospective cohort analysis, we assessed new HTLV-1 infections of repeat blood donors aged 16–69 years between Jan 1, 2005, and Dec 31, 2006, in the Japanese Red Cross Blood Centres database. We used results of antibody tests done in repeat blood samples collected until Dec 31, 2011, to assess the number who seroconverted to HTLV-1. We calculated the incidence density by dividing the number of seroconverters by the number of person-years of follow-up, and then extrapolated densities to regional populations to estimate the annual number of new HTLV-1 infections.

Findings We included 3 375 821 HTLV-1-seronegative blood donors (2 100 915 men and 1 274 906 women). Within a median follow-up of 4·5 years (IQR 2·3–5·8), 532 people (204 men and 328 women) had seroconverted. The incidence density was significantly higher in women (6·88 per 100 000 person-years; 95% CI 6·17–7·66) than in men (2·29 per 100 000 person-years; 95% CI 1·99–2·62; p<0·0001). The estimated annual number of new HTLV-1 infections was 4190 (95% CI 4064–4318) with 975 (914–1038) infections in men and 3215 (3104–3328) in women.

Interpretation New HTLV-1 infections in adolescents and adults are an important public health concern in Japan and preventive strategies are needed to reduce new transmission.

Funding Ministry of Health, Labour, and Welfare of Japan; Japan Agency for Medical Research and Development.

Introduction

Human T-lymphotropic virus 1 (HTLV-1)[1,2] infection is associated with development of adult T-cell leukaemia–lymphoma,[3] HTLV-1-associated myelopathy and tropical spastic paraparesis (HAM/TSP),[4] and various inflammatory disorders, although most virus carriers remain asymptomatic throughout life.[5] HTLV-1 is transmitted from mothers to infants through breastfeeding; between partners during sexual contact; and parenterally via blood transfusion, organ transplantation, and non-sterile needle sharing.[6] The virus infects about 10 million people worldwide, with endemic foci in Japan, the Caribbean, South America, and central Africa.[7] In Japan, the number of HTLV-1 carriers is estimated to be about 1 million,[8,9] the highest in the world.[7] HTLV-1 carriers are estimated to have a lifetime risk of 2–7%[5,10] for development of adult T-cell leukaemia–lymphoma and 0·25–3·8% for development of HAM/TSP.[11]

In Japan, several strategies to prevent new HTLV-1 infections were implemented soon after the virus transmission routes were shown. After the first discovery of transmission through breastfeeding during infancy in 1986 in Nagasaki, Japan (appendix),[12,13] the screening of pregnant women for HTLV-1 and the recommendation that those positive for infection refrain from breastfeeding have been implemented mainly in two endemic regions, Nagasaki since 1987 and Kagoshima since 1997, with a significant reduction in mother-to-child transmission from 15–20% to 2–3% when refraining from breastfeeding.[13,14] HTLV-1 infections transmitted by blood transfusion have almost been eliminated since HTLV-1-antibody testing of all donated blood was implemented in 1986.[15]

Despite these preventive measures, the estimated number of HTLV-1 carriers in Japan has not fallen far in the past two decades: 1·2 million people were carriers in 1986–87,[8] compared with 1·1 million in 2006–07.[9] Moreover, HTLV-1 seroprevalence in blood donors has increased in metropolitan areas (which are non-endemic for HTLV-1), although rates have decreased in endemic areas.[9] One of the reasons for these numbers might be poor implementation of the mother-to-child prevention strategy in non-endemic regions. Another possibility is that transmission routes other than breastfeeding and transfusion, such as horizontal transmission among adolescents and adults, are contributing. However, no preventive measures have yet been developed against the horizontal transmission of HTLV-1 in Japan, mainly because there is insufficient epidemiological evidence about this route.

See Online for appendix

Research in context

Evidence before this study

We searched PubMed and Google Scholar for all articles published before Sept 30, 2015, with use of the terms "HTLV, sexual transmission", "HTLV, seroconversion", "HTLV, heterosexual", "HTLV, horizontal transmission", "HTLV, seroconversion, blood donors", and "HTLV, seroincidence". We also searched the Japan Medical Abstracts Society with the equivalent Japanese terms for papers published before Sept 30, 2015. Two prospective studies of repeated blood donors estimated the human T-lymphotropic virus (HTLV) type 1 and type 2 combined incidence rate to be 2·92 per 100 000 person-years in the USA and 3·59 per 100 000 person-years in Brazil. Eight prospective studies assessed HTLV-1 seroconversion among small numbers of elderly couples in areas endemic for HTLV-1 in Japan. The major finding of previous studies in Japan was that the rate of transmission was three-to-four-times higher if the carrier spouse was male and the rate was higher when the marriage was longer. However, no study has prospectively evaluated the incidence density of HTLV-1 or estimated the number of new HTLV-1 infections throughout Japan.

Added value of this study

To our knowledge this is the first serological cohort study to estimate the incidence of HTLV-1 among adolescents and adults in the whole of Japan. Our study provides new evidence that at least 4000 adolescents and adults (77% of whom were women) are newly infected with HTLV-1 every year through possible horizontal transmission in Japan. Our study also demonstrates that new HTLV-1 infection rates are highest in women aged 50–59 years and in men aged 60–69 years in regions both endemic and non-endemic for HTLV-1, whereas the rate in young men aged 20–29 years is greater in metropolitan areas (non-endemic for HTLV-1) than in endemic regions.

Implications of all the available evidence

Our two main findings, together with evidence from previous studies, suggest that HTLV-1 infection is still an important public health issue in Japan and that any prevention programme for new HTLV-1 infections must consider not only mother-to-child transmission, but also transmission among adolescents and adults in both HTLV-1 endemic and non-endemic areas such as metropolitan areas.

Early studies reported that the sexual transmission of HTLV-1 infection occurs mainly from men to women.[16–20] However, most previous studies from Japan were done in small areas with small cohorts of married couples. To our knowledge, no study has evaluated HTLV-1 incidence throughout Japan. Although not all HTLV-1 carriers develop HTLV-1-related diseases, efforts to reduce the transmission of HTLV-1 from infected people to uninfected people are necessary because adult T-cell leukaemia–lymphoma and myelopathy are highly refractory to existing therapies.[11,21] To address this important public health issue, we aimed to estimate the number of new HTLV-1 infections in adolescents and adults in Japan.

Methods

Study design and data source

We did a retrospective review of blood test results of donors present in the database of the Japanese Red Cross (JRC) blood service headquarters. The JRC are the only organisation to collect and supply blood for use in transfusions in Japan, and the activities are authorised by the law on Securing a Stable Supply of Safe Blood Products (act number 160 of 1956). The JRC blood service headquarters have stored all blood-testing results from about 5·2 million donations a year from seven zones of the JRC blood service in Hokkaido, Tohoku, Kanto, Chubu–Tokai, Kinki, Chugoku–Shikoku, and Kyushu–Okinawa (appendix), which further consist of 54 regional centres in 47 prefectures that cover the whole country. Before donation, all blood donors must complete a paper-based self-administered questionnaire that asks 23 items about diseases, medicines, lifestyle behaviours, and other sensitive questions (appendix).

In the JRC system, donated blood is routinely screened for HTLV-1 in the seven zones using standard methods, and the results are sent to the JRC headquarters in Tokyo. Nationwide blood screening for HTLV-1 has been done with use of particle agglutination assay (Serodia; Fujirebio, Japan) since 1986, but it was replaced with chemiluminescent enzyme immunoassay (CLEIA; CL4800 Testing System; Fujirebio, Japan) since 2008. Indirect immunofluorescence assay[15] or western blot assay (since 2012) are used as confirmatory tests. JRC centres define screening test seropositivity as a titre of 8 or higher on the particle agglutination assay or a cutoff index 1·0 or higher on CLEIA, according to the manufacturers' criteria. In the study period, the JRC defined screening test seronegativity as when neither the particle agglutination assay results nor the CLEIA test results met these criteria. HTLV-1 seroconversion was defined as conversion to an immunofluorescence-test-positive status from a definite baseline HTLV-1-negative status.

We received approval from the JRC Institutional Review Board (research project number MHLW-54 in 2011 fiscal year, and the continuation application in 2013 fiscal year). No written informed consent was required because of the nature of the study design of this project, which was based on a retrospective review of the blood test results of blood donors, using the anonymous individual code available at the JRC Central Blood Institute.

For more on the law on blood services see http://www.jrc.or.jp/english/pdf/Blood_Services_2015_web.pdf

Data extraction

We extracted and compiled the dataset using anonymous individual code available from the JRC Central Blood Institute. The anonymised dataset included sex, birth year,

blood centre zone, date of the study baseline, age at baseline, date of blood donation that was negative for HTLV-1, and date of blood donation that was positive for HTLV-1. The inclusion criteria for the participants were a negative screening test or a negative immunofluorescence test at blood donation during Jan 1, 2005, to Dec 31, 2006 (baseline period); aged 16–69 years during the baseline period (the age range eligible for blood donation in Japan); and repeat donor, with at least one donation after the baseline period until the end of 2011. We excluded donors who had HTLV-1-negative blood before 2005 but made no donation during the baseline period to ensure the accuracy of the person-years calculation based on the interdonation intervals.[22,23] Data for each participant who met the inclusion criteria were included based on their serological test results from baseline to the date of seroconversion for HTLV-1 or to the date of the last donation that was persistently seronegative on the HTLV-1 immunofluorescence test until Dec 31, 2011, in the database.

For the portal site of the Official Statistics of Japan see www.e-stat.go.jp/SG1/estat/eStatTopPortalE.do

	Donors (n)	Seroconverters (n)	Follow-up in person-years	Crude incidence density per 100 000 person-years	Crude incidence density ratio	p value*
Total	3375821	532	13697250	3·88 (3·57–4·23)	..	..
Sex						
Men	2100915	204	8927417	2·29 (1·99–2·62)	Ref	..
Women	1274906	328	4769833	6·88 (6·17–7·66)	3·01 (2·53–3·58)	<0·0001
Age at enrolment						
16–19 years	318667	12	1090011	1·10 (0·63–1·94)	Ref	..
20–29 years	787863	71	3058367	2·32 (1·84–2·93)	2·11 (1·14–3·89)	0·0168
30–39 years	929560	115	3986308	2·88 (2·40–3·46)	2·62 (1·45–4·75)	0·0015
40–49 years	705399	131	3103136	4·22 (3·56–5·01)	3·83 (2·12–6·93)	<0·0001
50–59 years	502841	163	2027860	8·04 (6·89–9·37)	7·30 (4·06–13·1)	<0·0001
60–69 years	131623	40	431569	9·27 (6·80–12·6)	8·42 (4·42–16·0)	<0·0001
Birth year						
1985–90	379425	13	1291824	1·01 (0·58–1·73)	Ref	..
1975–84	789464	80	3090099	2·59 (2·08–3·22)	2·57 (1·43–4·62)	0·0016
1965–74	924404	117	3979647	2·94 (2·45–3·52)	2·92 (1·65–5·18)	0·0002
1955–64	690418	125	3038855	4·11 (3·45–4·90)	4·09 (2·31–7·24)	<0·0001
1945–54	475439	160	1916106	8·35 (7·15–9·75)	8·30 (4·72–14·6)	<0·0001
1935–44	116863	37	380717	9·72 (7·04–13·4)	9·66 (5·13–18·2)	<0·0001
Blood centre zone						
Hokkaido	202249	25	837396	2·99 (2·02–4·42)	Ref	..
Tohoku	269777	19	1065748	1·78 (1·14–2·80)	0·60 (0·33–1·08)	0·09
Kanto	977672	65	3935063	1·65 (1·30–2·11)	0·55 (0·35–0·88)	0·012
Chubu-Tokai	590082	63	2405229	2·62 (2·05–3·35)	0·88 (0·55–1·39)	0·58
Kinki	572685	135	2347576	5·75 (4·86–6·81)	1·93 (1·26–2·95)	0·0026
Chugoku-Shikoku	357226	44	1443884	3·05 (2·27–4·10)	1·02 (0·62–1·67)	0·935
Kyushu-Okinawa	406322	181	1662425	10·9 (9·41–12·6)	3·65 (2·40–5·54)	<0·0001

Data in parentheses are 95% CIs. *For crude incidence density ratio compared with reference value.

Table 1: Human T-lymphotropic virus 1 incidence density and incidence density ratios

To allow us to estimate the population-based new HTLV-1 infections among adolescents and adults, we obtained demographic data for the age-specific, sex-specific, and region-specific population counts in 2005–06, from the portal site of the Official Statistics of Japan. In this study, we used the average population counts for the 2005 and 2006 populations. The overall population sizes of each of the seven zones in Japan are presented in the appendix.

Statistical analysis

We used a two-step procedure to estimate the national incidence density of new HTLV-1 infections. In the first step, we did a retrospective cohort analysis to estimate the annual HTLV-1 seroconversion rate in repeat blo donors who were negative for anti-HTLV-1 antibodies. We calculated the HTLV-1 seroconversion rates by dividing the number of seroconverters by the sum of person-years of follow-up. The person-years for each individual without seroconversion were summed from baseline to the last visit. The person-years for each individual with seroconversion were summed based on each interdonation interval[22,23] (ie, person-years from baseline to the midpoint between their last negative donation and their first positive donation). The seroconversion rates were evaluated by sex, blood-centre zone, six age groups at baseline (10-year strata, except for the 16–19-year-group), and six birth-year groups (10-year birth strata, except for birth years 1985–90). In the second step, we extrapolated the estimates in blood donors to the general population to estimate the annual number of new HTLV-1 infections throughout Japan. We multiplied the age-specific seroconversion rate at each zone's blood centre by the age-specific population in the same zone. Although the age for blood donation is limited to 16–69 years in Japan, population data for 15–69 yea were used in this calculation. When donors move among blood centre zones, the area in which the donor donated at baseline was considered as their zone.

The HTLV-1 seroconversion rate is expressed as the incidence density per 100000 person-years, and the corresponding 95% CI calculated with the exact Poisson method. Differences in incidence densities according to sex, age group, and zone were measured with univariate and multivariate Poisson regression models. The interaction between each variable was also tested in the multivariate analysis. We considered a p value of less than 0·05 to be statistically significant. We used SAS version 9.4 for all statistical analyses.

Role of the funding source

The funders had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The first author had full access to the database. The first author and the corresponding author had responsibility for data analysis. All authors had responsibility for interpretation of results. The

corresponding author had final responsibility for submission of the report.

Results

3 375 821 HTLV-1-seronegative blood donors (2 100 915 men and 1 274 906 women) met the inclusion criteria for our cohort during the baseline period. The age distribution of this study population was younger than that of the general population in 2005–06 (appendix).

Within a median follow-up of 4·5 years (IQR 2·3–5·8), 532 people (204 men and 328 women) seroconverted. The crude incidence density was 2·29 per 100 000 person-years (95% CI 1·99–2·62) in men and 6·88 per 100 000 person-years (6·17–7·66) in women. The age-specific incidence density increased with age in both sexes, and was highest in the 50–59-year age group in women and in the 60–69-year age group in men (table 1, appendix). The zone-specific incidence density was highest in Kyushu–Okinawa, followed by Kinki, for both sexes (table 1, appendix), which is similar to the HTLV-1 seroprevalence among first-time blood donors.[9] The incidence density was three-times higher in women than in men (table 1), even after adjusting for age group and zone and birth year and zone (table 2). The birth-year-specific incidence density was higher in those with an earlier birth year in both sexes, and was highest in the 1945–54 birth cohort in women and the 1935–44 birth cohort in men (table 1, appendix).

Although the overall women-to-men ratio of the HTLV-1 incidence density was 3·0, the ratio differed by age group, birth year, and blood centre zone. There was no consistent tendency in the women-to-men incidence density ratio by age or birth year, with the highest ratio in the 50–59-age group, and the 1945–54 birth-year group, except for the 16–19 age-group and 1985–90 birth-year group (appendix). The women-to-men incidence density ratio was highest in Kyushu–Okinawa (4·66, 95% CI 3·43–6·35; p<0·0001) and lowest in Kinki (1·72, 1·23–2·41; p=0·0020; appendix).

In men, age-specific incidence density in Kyushu–Okinawa was generally higher than in other areas in those older than 30 years, but not in those aged 20–29 years (figure 1A). In women, age-specific incidence density in Kyushu-Okinawa was higher than in other areas in all age groups, except for those aged 60–69 years (figure 2A). By extrapolating the annual age-specific incidence density to the demographic population by zone, we estimated the annual number of new HTLV-1 infections per year during 2005–11 in the Japanese population to be 4190 (95% CI 4064–4318); 975 (914–1038) in men, and 3215 (3104–3328) in women. The annual number of new HTLV-1 infections in men was higher in the three metropolitan zones (Kanto, Chubu–Tokai, and Kinki) than in Kyushu–Okinawa in the 20–29-year age group, higher in the Kinki zone than in Kyushu–Okinawa in the 40–49-year and 50–59-year age groups, and very much higher in the Kyushu–Okinawa and Kinki zones than in other zones in the 60–69-year age groups (figure 1B). The annual number of new HTLV-1 infections in women was highest in Kyushu-Okinawa in all age groups. Zones with the second highest number were

	Multivariate analysis for sex, age, and zone			Multivariate analysis for sex, birth year, and zone		
	Adjusted incidence density per 100 000 person-years	Adjusted incidence density ratio	p value	Adjusted incidence density per 100 000 person-years	Adjusted incidence density ratio	p value
Sex						
Men	1·45 (0·99–2·11)	Ref	..	1·60 (1·18–2·15)	Ref	..
Women	5·50 (4·64–6·51)	3·80 (2·52–5·75)	<0·0001	5·60 (4·73–6·63)	3·51 (2·49–4·95)	<0·0001
Age at enrolment						
16–19 years	0·55 (0·20–1·53)	Ref	..	..	..	..
20–29 years	1·99 (1·56–2·55)	3·64 (1·27–10·4)	0·0159	..	..	..
30–39 years	2·59 (2·11–3·17)	4·72 (1·67–13·4)	0·0035	..	..	..
40–49 years	3·85 (3·18–4·67)	7·03 (2·49–19·9)	0·0002	..	..	..
50–59 years	6·21 (5·15–7·50)	11·3 (4·02–32·0)	<0·0001	..	..	..
60–69 years	7·41 (5·37–10·2)	13·5 (4·64–39·4)	<0·0001	..	..	..
Birth year						
1985–90	..	..	..	0·64 (0·30–1·37)	Ref	..
1975–84	..	..	..	2·24 (1·77–2·83)	3·47 (1·58–7·61)	0·0019
1965–74	..	..	..	2·61 (2·13–3·20)	4·05 (1·86–8·80)	0·0004
1955–64	..	..	..	3·74 (3·08–4·55)	5·81 (2·68–12·6)	<0·0001
1945–54	..	..	..	6·54 (5·42–7·88)	10·1 (4·69–21·9)	<0·0001
1935–44	..	..	..	7·76 (5·55–10·9)	12·0 (5·30–27·3)	<0·0001
Blood centre zone						
Hokkaido	2·42 (1·57–3·73)	Ref	..	2·56 (1·68–3·89)	Ref	..
Tohoku	1·49 (0·87–2·55)	0·62 (0·32–1·18)	0·145	1·59 (0·94–2·68)	0·62 (0·32–1·19)	0·149
Kanto	1·49 (1·10–2·02)	0·62 (0·38–0·99)	0·046	1·58 (1·20–2·09)	0·62 (0·39–0·99)	0·047
Chubu-Tokai	2·31 (1·69–3·16)	0·96 (0·59–1·54)	0·858	2·46 (1·84–3·28)	0·96 (0·60–1·55)	0·869
Kinki	4·89 (3·85–6·21)	2·02 (1·31–3·12)	0·002	5·19 (4·21–6·39)	2·03 (1·31–3·13)	0·0014
Chugoku–Shikoku	2·44 (1·70–3·52)	1·01 (0·60, 1·69)	0·968	2·59 (1·83–3·67)	1·01 (0·60–1·70)	0·960
Kyushu–Okinawa	9·49 (7·55–11·9)	3·92 (2·56–6·03)	<0·0001	10·1 (8·27–12·3)	3·93 (2·56–6·04)	<0·0001

Data in parentheses are 95% CIs.

Table 2: Human T-lymphotropic virus 1 incidence density and incidence density ratios after adjustment

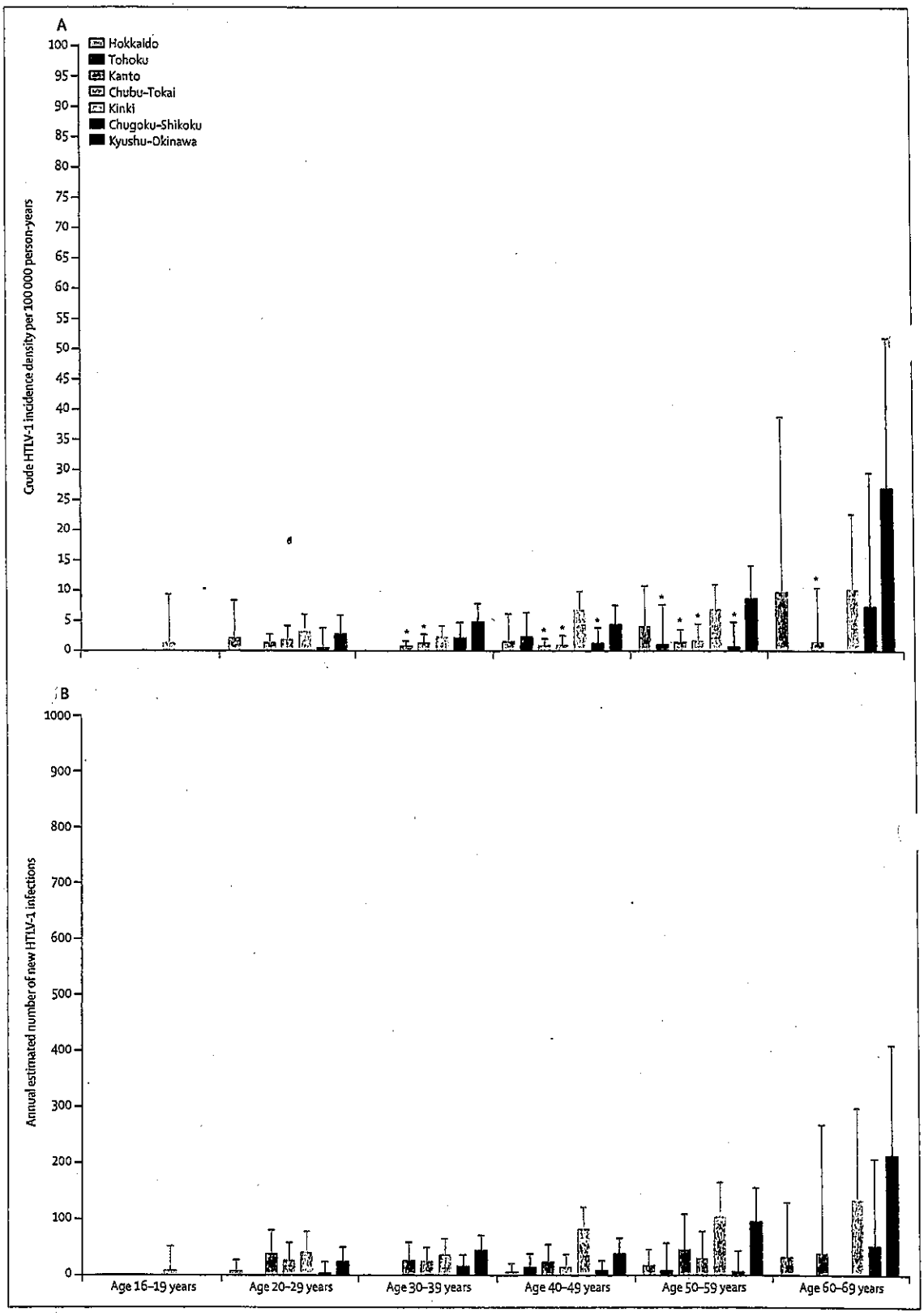

Figure 1: Age-specific crude incidence density (A) and estimated annual number (B) of new HTLV-1 infections by area in men
Error bars are upper 95% CIs. HTLV-1=human T-lymphotropic virus type 1. *Incidence density significantly different from Kyushu-Okinawa area.

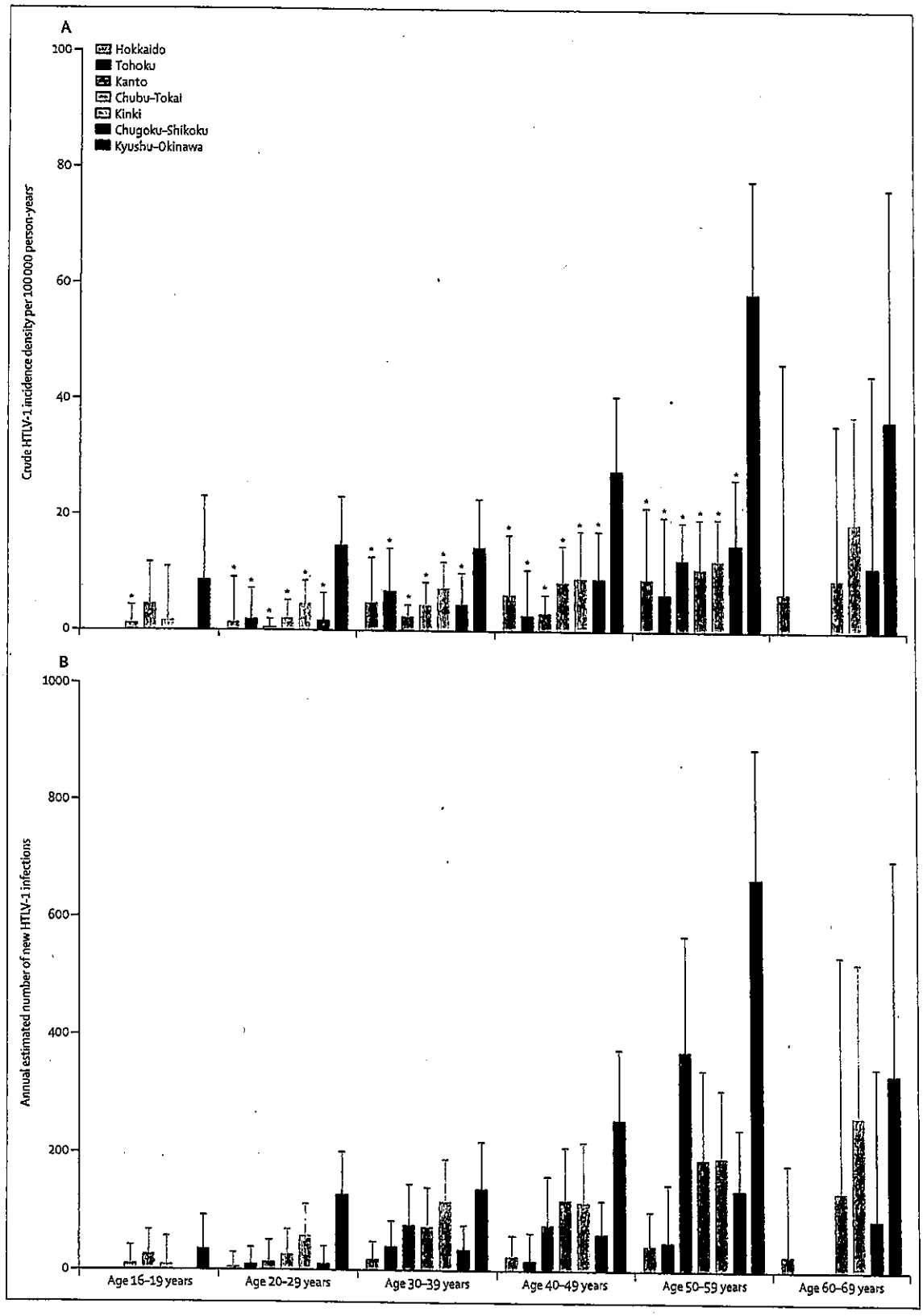

Figure 2: **Age-specific crude incidence density (A) and estimated annual number (B) of new HTLV-1 infections by area in women**
Error bars are upper 95% CIs. HTLV-1=human T-lymphotropic virus type 1. *Incidence density significantly different from Kyushu–Okinawa area.

Kanto in 50–59-year age group, Chubu–Tokai in 16–19-year and 40–49-year age groups, and Kinki in three other age groups (figure 2B).

Discussion

On the basis of the age-specific, sex-specific, and zone-specific HTLV-1 incidence densities among HTLV-1-seronegative cohorts of repeat blood donors, we estimated that at least 4000 adolescents and adults (77% of whom are women) are newly infected with HTLV-1 every year in Japan. We found that the number of newly infected individuals was highest in women aged 50–59 years and men aged 60–69 years in both endemic and non-endemic areas for HTLV-1. However, in men aged 20–29 years, the number of newly infected individuals was greater in metropolitan areas (non-endemic for HTLV-1) than in endemic areas (albeit not statistically significantly). This suggests that HTLV-1 continues to be transmitted horizontally between heterosexual partners in the general community in Japan in both endemic and non-endemic areas.

To our knowledge, this is the first study to estimate the number of new HTLV-1 infections in adolescents and adults throughout Japan. The HTLV-1 seroconversion rates among married couples were previously evaluated in small-scale studies to determine the heterosexual transmission of HTLV-1 in Japan. Tajima and colleagues[16] reported that the seroprevalence in wives with seropositive husbands (68%) was greater than that in wives with seronegative husbands (20%), which suggested for the first time the possible sexual transmission of HTLV-1 (predominantly male-to-female transmission). In the Miyazaki cohort study, which included 534 elderly married couples, the HTLV-1 seroconversion rate was 4·9 per 100 person-years among seronegative wives with seropositive husbands, but 1·2 per 100 person-years among seronegative husbands with seropositive wives.[17] Takezaki and colleagues[18] also reported that the HTLV-1 seroconversion rate was 3·3 per 1000 person-years for men and 6·7 per 1000 person-years for women in a village highly endemic for HTLV-1. Our results confirm that the seroconversion rate was three-times higher in women than in men (table 1), although the scale of the incidence densities in this study was very different from those in previous studies. This discrepancy in scale might be mainly attributable to population differences because previous studies were based on data from small selected samples of older couples in highly HTLV-1-endemic areas.

Incidences of new HTLV infection among repeat blood donors have also been reported from countries other than Japan, although they mostly combined HTLV-1 and HTLV-2. The overall incidence rate of HTLV-1 and HTLV-2 was 3·25 per 100 000 person-years during 1991–96 in five US blood centres,[24] and 3·59 per 100 000 person-years during 2007–09 in three Brazilian blood centres.[25] The Brazilian study also showed a significantly higher incidence in women (5·74 per 100 000 person-years) than in men (2·78 per 100 000 person-years) with an increase with age and variance by region.

The dominance of women and older age groups among new HTLV-1 infections is unique. For other sexually transmittable viruses, such as HIV and hepatitis B virus, new transmissions are reported at significantly higher rates in men than in women and in more young people than older people.[26,27] The reason why HTLV-1 is predominantly transmitted from men to women is unknown. Because the transmission of HTLV-1 requires T cells, it is believed to transmit more efficiently via infected T cells in semen. The reason why HTLV-1 is transmitted predominantly among older adults is also unknown. Various suggestions have been made, including that the probability of the transmissi depends on prolonged exposure to an infected partne., that older couples do not use contraception during intercourse, and that ageing leads to the senescence and deterioration of defence mechanism against infection and the changes of HTLV-1-infected lymphocytes that induce greater infectivity through the function of the provirus. Further studies are needed to clarify these unique characteristics of HTLV-1 transmission to reduce new infections.

We believe this to be the first study to show new HTLV-1 infections among young people. The number of newly infected individuals is greater in metropolitan areas (non-endemic for HTLV-1) than in endemic areas among men aged 20–29 years, although the difference was not statistically significant. Several reasons might explain this trend. First, many young people have moved from HTLV-1-endemic areas into three metropolitan areas (appendix), in particular, more women than men move to Kinki and so the risk of female-to-male transmission might be greater. Second, young men in metropolitan areas could modify their lifestyle to incorporate high-risk behaviours such as tattooing, injection drug use, s work, or other high-risk behaviours of transmitting infection. These together are important public health issues and must be considered in the development of any strategies to prevent new HTLV-1 infections in Japan.

Our results might partly explain why the estimated number of HTLV-1 carriers in Japan has not decreased in the past two decades.[9] Countrywide mother-to-child prevention intervention has not been implemented so far and so might have contributed. However, based on our results, if the annual number of new infections by sexual transmission is assumed to be stable during 20 years, the cumulative number of newly infected people might be about 84000 people, which might have contributed to about 8% of the HTLV-1 infection pool in 2008 in Japan. The fact that seroprevalence of HTLV-1 is higher in elderly people might be explained by birth-cohort effect—ie, the proportion of people older than 50 years who are whole blood donors has increased from 12% in 1988, to 20% in 2007, because of rapid ageing of Japanese populations. Together, these factors might explain why

the estimated number of HTLV-1 carriers in Japan has not decreased as expected during 20 years[9] beyond the large HTLV-1 infection pool of elderly people.[9]

The main limitation of this study is that the findings might not be fully generalisable to the whole population because our study population comprised repeat volunteer blood donors who account for only 4·6% of the male Japanese population and only 2·8% of the female Japanese population, and whose ages are generally younger than those of the general Japanese population (appendix). Using blood donors as representatives of the general population is a matter of dispute. Some researchers suggest that blood donors are an adequate sample of the population for several reasons, including the lack of bias in their backgrounds,[28] but others disagree.[29] Health studies that use blood donors can be affected by a selection bias due to the so-called healthy donor effect, in which donors are generally healthier than the general population[30] because they have to pass several health and lifestyle criteria before becoming a blood donor. In fact, the JRC refused any donation from those with abnormalities in bodyweight, haemoglobin concentration, blood pressure, body temperature, medical drug use, and other medical conditions (including other sexually transmitted infections), and those with high-risk behaviours of transmitting infection based on a self-administered questionnaire. Another potential limitation of this study involves our inclusion criteria. We only included repeat blood donors who donated at least twice from the baseline period, but excluded donors who did not donate blood during the baseline period. Some of the excluded HTLV-1-negative donors might have seroconverted during the follow-up period. Taken together, the estimated annual number of new HTLV-1 infections that we report might be an underestimate. Furthermore, although particle agglutination, CLEIA, and immunofluorescence assay are useful for mass screening of blood donors and skilled technicians judged the results, the possibility of false negative or positive results could lead to our results being an underestimation or overestimation of the true incidence. Nevertheless, this study does provide new evidence on the incidence density of HTLV-1 among blood donors in all the geographic regions of Japan, allowing us to estimate the number of new HTLV-1 infections as a surrogate for the true number of people newly infected with HTLV-1 in Japan.

To our knowledge, no case of adult T-cell leukaemia–lymphoma has been reported in HTLV-1 seroconverters in Japan, except in those affected by parenteral transmission. However, a 2015 case report describes a case of T-cell leukaemia–lymphoma in a white French man infected with HTLV-1 via sexual transmission from a female partner from an HTLV-1-endemic country.[31] HAM/TSP is known to arise after the sexual transmission and parenteral transmission of HTLV-1.[31,32] Therefore, the fact that new HTLV-1 infections constantly occur in Japan is a major public health concern, particularly in young adults, in an era of metropolitan migration and globalisation. No preventive measures have yet been developed for the sexual transmission of HTLV-1 in Japan. Our data will be useful to build a new strategic plan for the elimination of HTLV-1.

Contributors

MS organised data sampling, contributed to study design, analysed the data, and wrote the manuscript. MI conceived of the study design, coordinated the study, analysed data, designed the figures, and wrote the manuscript. YS contributed to study design and data interpretation. KO contributed to data interpretation. TW and IH supervised the study and contributed to data interpretation. All authors participated in critical revision of the manuscript and approved the final version.

Declaration of interests

We declare no competing interests.

Acknowledgments

This work is dedicated to the memory of Dr Kazunari Yamaguchi, who devoted a great part of his professional life to the researchers of HTLV-1 in Japan and gave valuable suggestions on this study, and who passed away in April, 2016. We thank all the blood donors and staff at the Japanese Red Cross blood centres. This work was supported by the Health Labour Sciences Research Grant from the Ministry of Health, Labour and Welfare of Japan (H23-sinkou-ippan-016), and the Research Program on Emerging and Re-emerging Infectious Diseases from the Japan Agency for Medical Research and Development (AMED, 15fk0108029h0002).

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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
				2017. 2. 6	該当なし	

一般的名称	人血小板濃厚液	研究報告の公表状況		公表国
販売名(企業名)	濃厚血小板-LR「日赤」(日本赤十字社) 照射濃厚血小板-LR「日赤」(日本赤十字社) 濃厚血小板HLA-LR「日赤」(日本赤十字社) 照射濃厚血小板HLA-LR「日赤」(日本赤十字社) 照射洗浄血小板-LR「日赤」(日本赤十字社) 照射洗浄血小板HLA-LR「日赤」(日本赤十字社)		Gallian P, Cabié A, Richard P, et al. Blood. 2017 Jan 12;129(2):263-266.	仏国

研究報告の概要

○マルティニーク島の無症候供血者におけるジカウイルス(ZIKV)。
2016年1月19日から6月10日までの期間に、4,129名の供血された血液を対象としてZIKV検査を実施した。76名(1.84%)の供血血液がNAT陽性となった。ZIKV感染に一致する症状の検出を目的として、供血の7日後に電話による供血後調査を行った。供血者が兆候はないと回答した場合は、供血の14日後に再び電話による問い合わせを行うこととした。NAT陽性の76名中、電話に回答した75名については、34名(45.3%)は依然として無症候状態にあり、41名(54.7%)は発熱、結膜炎、筋肉痛、関節痛、発疹といった症状を報告した(供血の1～6日後)。症状を呈する供血者におけるウイルス量は、無症候供血者と比較して高い傾向が見られた(平均値5.36 $\log_{10}$ RNA geq/mL対4.93 $\log_{10}$ RNA geq/mL, p値=0.0013)。
さらに、抗体陽性率の調査を3月(3月9日～23日)に採取した供血者418名並びに6月(6月6日～13日)に採取した176名の検体を対象として行ったところ、それぞれ13.5%、42.2%であった。ZIKV感染供血者数による感染数累積シミュレーション解析から、ウイルス血症の検出が可能な無症候期間に供血を行うと思われる感染者数を、デング熱及びチクングニヤ熱の場合を参考に推定した。4日～6日という理論上の無症候期間について、供血者におけるZIKV感染率の推定値を試算したところ、無症候期間を6日とした場合に、実際の抗体陽性率との良い相関関係が見られた(3月は推定感染率が12.5%で実際の抗体陽性率が13.5%、6月は推定感染率が43.5%で実際の抗体陽性率が42.2%)。この結果は、供血後調査で得た供血から発症までの(発症前のウイルス血症の)最長期間が6日であったという結果と一致している。マルティニーク島では、受診しなかった感染者の割合が80%～85%であり、研究期間を通じて大きく変化することはなく、一般集団の間でZIKVが静かに広がったことは、2014年にチクングニヤウイルス感染のアウトブレイク時にマルティニーク島で見られた状況(受診者の割合が高かった)とは大きく異なっている。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
マルティニーク島においてジカウイルス(ZIKV)の流行時、供血血液の約2%にZIKV RNAが検出された。無症候であったZIKV疾患患者の割合は約45%であり、治療を必要としなかった者の割合はさらに高く80%～85%であった。また、ウイルス血症の無症候期間は感染数累積シミュレーション解析により約6日であると推定されたという報告である。	日本赤十字社では、輸血感染症対策として献血時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、平成28年2月3日付厚生労働省医薬・生活衛生局血液対策課長事務連絡「ジカウイルスによることが疑われる小頭症等の増加に関するWHO緊急委員会宣言について(注意喚起)」の発出を受け、同月4日付で各血液センターに対し問診時の帰国(入国)後経過日数の確認を徹底するよう指示した。さらに同年7月1日から、ジカウイルス感染症と診断され、治癒後1カ月間経過していない場合は献血不適としている。今後もジカウイルス感染症に関する新たな知見等について情報の収集に努める。

study of the platelet inhibitor prasugrel in children with sickle cell disease was designed to identify drug doses that inhibit platelet function between 30% and 50%, a level thought to balance safety and efficacy,[24] similar to the level of inhibition observed here, although the dual effects of RN-1 on platelet function and platelet counts could pose an additional risk for bleeding that will require further monitoring.

Our results show that administration of RN-1 to normal baboons over a prolonged period increases HbF, F cells, and F retics and is generally well tolerated, supporting further development of LSD1 inhibitors as therapeutic agents for SCD. Because LSD1 also has an important functional role in neural stem cell maintenance and proliferation, effects of LSD1 inhibitors on the brain and nervous system should be carefully evaluated.[25]

*V.I. and K.V. contributed equally to this work.

Acknowledgment: This work was supported by National Institutes of Health, National Heart, Lung, and Blood Institute grant U01 HL117658.

Contribution: V.I. and K.V. performed research and collected, analyzed, and interpreted the data; A.R. and R.M. analyzed data and reviewed the manuscript; S.C. and J.D.E. reviewed the manuscript; J.D. analyzed data and reviewed the manuscript; D.L. designed and performed the research, analyzed the data, and wrote the manuscript.

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

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DOI 10.1182/blood-2016-10-746727

To the editor:

Zika virus in asymptomatic blood donors in Martinique

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Zika fever is an *Aedes*-borne disease caused by a flavivirus (Zika virus [ZIKV], genus *Flavivirus*). During the past years, ZIKV has spread in Polynesia, South America, and the Caribbean.[1] Most ZIKV infections are asymptomatic or result in mild febrile disease with rash and conjunctivitis. Attention was recently drawn to nonvectored ZIKV transmission,[2] including sexual,[3,4] probable blood-borne,[5] and mother-to-fetus transmission, and to severe forms such as Guillain-Barré syndrome,[6,7] acute neurological infections,[8,9] and fetal abnormalities[10] (including microcephaly).

Many aspects of Zika fever natural history remain unknown (eg, the proportion of asymptomatic cases and the duration of viremia). Estimating the prevalence of Zika infections is difficult because a large proportion of infected individuals do not seek medical attention, and seroprevalence studies are hampered by antigenic cross-reactivity with other flaviviruses (eg, dengue virus).[11] Here, we present a study of ZIKV infection in blood donors from Martinique island (French West Indies, Caribbean region), with novel epidemiological, biological, and clinical information that refines the picture of Zika fever in adults.

After the ZIKV outbreak in 2013 to 2014 in French Polynesia and the evidence that a significant proportion (3%) of donors were viremic,[12] ZIKV has been recognized in Martinique since the end of 2015, leading to implementation by the French Blood Bank of a systematic individual nucleic acid testing (NAT) of blood donations. Virus detection was performed on plasma samples, using a semi-automated platform consisting of a Microlab-STARlet (Hamilton, Bonaduz, Switzerland) and the NucleoSpin 96 Virus Extraction Kit (Macherey-Nagel, Duren, Germany) for nucleic acids extraction, and a CFX96 thermocycler (Bio-Rad Laboratories, California) and the RealStar Zika Virus RT-PCR Kit_1.1 (Altona Diagnostics, Hamburg, Germany) for reverse transcriptase polymerase chain reaction testing.

Between January 19 and June 10, 2016, 4129 consecutive blood donations were tested (mean age, 41.9 years; sex ratio [M/F], 0.88). Positive NAT detection occurred in 76 blood donations (1.84%), with the most intense detection rate (3%) during weeks 17-20 (mean age, 41.8 years; sex ratio, 1.2). Postdonation inquiry consisted of a telephone call at day 7 postdonation to identify symptoms compatible with ZIKV infection. When the donor declared no sign, a new call was organized 14 days after donation. This information was obtained from 75 viremic donors: 34 (45.3%) remained asymptomatic, and 41 (54.7%) reported symptoms (1-6 days postdonation; Figure 1A) such as fever, conjunctivitis, myalgia, arthralgia, and rash (Figure 1B). There was a trend for higher values of molecular viral load in symptomatic vs asymptomatic donors (mean values, 5.36 vs 4.93 $\log_{10}$ RNA genomic equivalents per milliliter; independent Wilcoxon test, P value = .0013; Figure 1C). This difference does not imply that symptomatic donors reached higher viremia than asymptomatic donors: sampling could occur in the former only during the early steps of viremia, whereas in the latter, it could also occur during the decreasing phase of viremia. The range of viral loads (2.09-6.50 $\log_{10}$ RNA copies/mL) was comparable to that previously described in French Polynesian asymptomatic blood donors.[13] Men declared symptoms less frequently than women (45.2% vs 66.7%; χ^2 test P value = .06), but viral load and time to declaration of symptoms were not significantly different in men and women. No significant association of viremia with ABO, Rhesus, and Kell blood groups was detected.

We performed seroprevalence analyses at 2 time points: in 418 donors sampled in early March (March 9-23), and in 176 donors sampled in early June (June 6-13). Samples were tested using an anti-Zika virus NS1 immunoglobulin G enzyme-linked immunosorbent assay (ELISA) kit (anti-Zika Virus ELISA immunoglobulin G; Euroimmun, Germany), and positives were further processed by microneutralization in a 96-well format, using Vero cells, 50 pfu of the MRS_OPY_Martinique_PaRi_2015 strain,[14] and a threshold titer of 40, as recommended by the French National Reference Centre for Arboviruses. The seroprevalence after seroneutralization was 13.5% in early March and 42.2% in early June. An ELISA ratio greater than 4 was associated with a positive seroneutralization in more than 95% of cases. Sera with an ELISA ratio greater than 5 were associated with seroneutralization in 100% of cases.

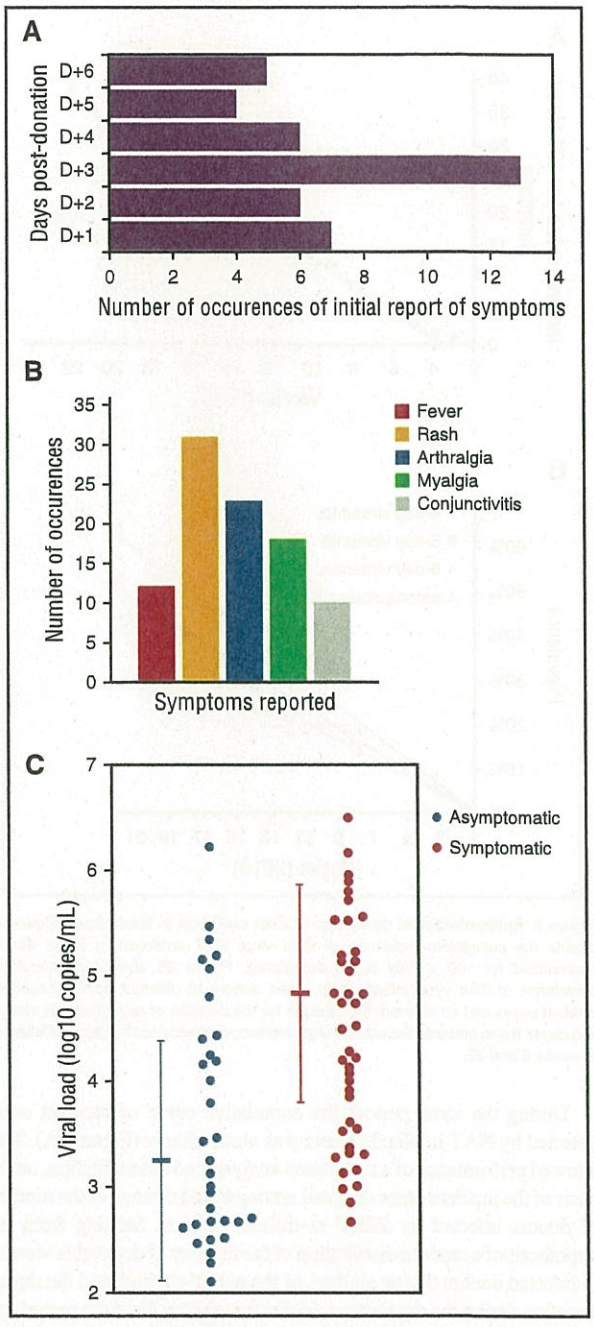

Figure 1. Clinical symptoms and Zika viral loads in blood donors. The distribution of symptomatic donors according to time of inaugural symptoms (A) and the frequency of reported symptoms (B) are presented. Zika viral loads ($\log_{10}$ copies/mL) are provided in both asymptomatic and symptomatic blood donors (C). Zika viral load was determined by real-time reverse transcription polymerase chain reaction in 41 donors reporting clinical signs, and 34 donors who did not. The diagram reports for each series the quantitative distribution of viral load, together with the median value ± SD.

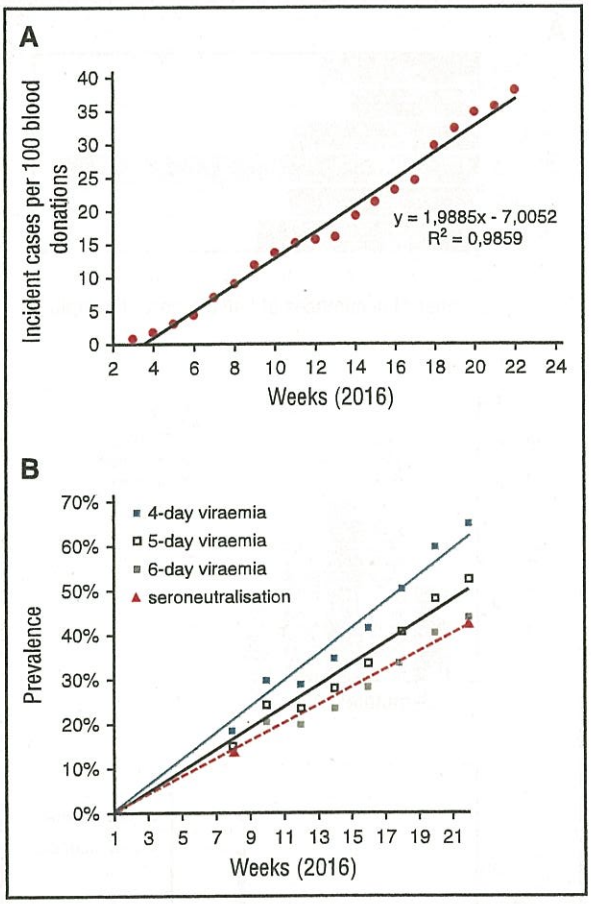

Figure 2. Epidemiological dynamics of Zika outbreak in Martinique. Figure 2A reports the cumulative incidence of Zika virus NAT detection in blood donors (normalized for 100 weekly blood donations). Figure 2B shows the simulated prevalence of Zika virus infections in blood donors at different points, based on incident cases and on different hypotheses for the duration of asymptomatic viremia (4-6 days). It also presents the actual seroprevalence data obtained by seroneutralization at weeks 8 and 22.

During the same period, the cumulative curve of incident cases detected by NAT in blood donors was almost linear (Figure 2A). This allowed performance of a simulation analysis and determination, on the basis of the incident cases detected among blood donors, of the number of donors infected by ZIKV at different points. Starting from the hypothesis of a random distribution of the first day of detectable viremia in infected donors during all days of the period studied, and the day of donation during the days open to collection during the same period, we estimated the number of cases for which donation would occur during the phase of asymptomatic viremia detectable by NAT. We deduced the expected prevalence of ZIKV infection in donors for theoretical durations of asymptomatic viremia in plasma of 4 to 6 days (Figure 2B), by reference to dengue[15] and chikungunya fever.[16] A faithful correlation with actual seroprevalence results was observed for a duration of asymptomatic viremia of 6 days (prevalence estimated at 12.5% vs 13.5% by seroneutralization in early March, and at 43.5% vs 42.2% in early June). This is consistent with the observation that the longest period reported between blood donation and symptoms (presymptomatic viremia) was 6 days (Figure 1A). Because detection methods using whole blood samples have allowed detecting virus RNA up to 68 days after the onset of symptoms,[17] it will be important to determine whether such samples contain infectious virus, and to reassess ZIKV transfusion risk accordingly.

The estimated number of clinically suspect cases in Martinique (French Public Health Institute) was ~7600, or ~2% of the population, in early March (week 8), and ~28 900, or ~7.6% of the population, in early June (week 22).[18] With reference to seroprevalence results, this suggests that the proportion of cases that did not seek medical attention was 80% to 85% and did not change significantly along the study period. Our analyses suggest, therefore, that ZIKV has spread in the general population silently, with a minor effect of the acute disease on medical structures (eg, 10-35 weekly consultations for Zika syndromes in emergency departments, and 10-20 in obstetric departments). The most significant medical effect consisted of 19 Zika-related GBS confirmed cases and Zika infection in 342 pregnant women at the end of our study (June 2016).[19] Therefore, the situation is significantly different from that previously observed in Martinique during the 2014 Chikungunya outbreak, characterized by a high proportion of cases seeking medical attention.[20]

We conclude that ZIKV individual NAT screening in Martinique during 5 months of circulation of the virus allowed the detection of approximately 2% of contaminated blood donations. The proportion of truly asymptomatic cases of Zika disease among Martiniquan blood donors infected by ZIKV was approximately 45%, and the proportion of cases that did not require medical attention was even higher (80%-85%). The duration of plasma asymptomatic and presymptomatic viremia was estimated to be close to 6 days. We suggest that, once the duration of asymptomatic viremia has been estimated (eg,, from early analysis of seroprevalence and PCR detection datasets), NAT incidence studies in blood donors could improve prevalence estimates in the general population in the case of a disease with frequent mild or asymptomatic clinical presentations such as Zika fever. The French Blood Bank experience of individual ZIKV NAT screening and epidemiological studies performed from French blood donor population may be of interest at the time the US Food and Drug Administration is recommending that blood banks screen all blood donations for ZIKV or use an approved pathogen reduction technology.

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

Acknowledgments: This work was supported by the Etablissement Français du Sang, INSERM, and Aix-Marseille University. P.G., A.C., R.N.C., B.P., I.L.-G., and X.d.L. are members of the ZIKAlliance project, funded by the European Commission.

Contribution: P.R. and L.P. collected samples and data from blood donors. A.C. collected and interpreted data from general population. P.G. and P.T. established the RT-PCR platform and performed NAT screening. B.P. and I.L-G. designed and performed serological detection. P.G., P.T., R.N.C., and X.d.L. designed the study, interpreted the data, and wrote and edited the manuscript.

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

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DOI 10.1182/blood-2016-09-737981

別紙様式第2-1

No. 6

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2017. 2. 6	該当なし	

一般的名称	人血小板濃厚液	研究報告の公表状況		公表国
販売名(企業名)	濃厚血小板-LR「日赤」(日本赤十字社) 照射濃厚血小板-LR「日赤」(日本赤十字社) 濃厚血小板HLA-LR「日赤」(日本赤十字社) 照射濃厚血小板HLA-LR「日赤」(日本赤十字社) 照射洗浄血小板-LR「日赤」(日本赤十字社) 照射洗浄血小板HLA-LR「日赤」(日本赤十字社)		A Heitmann, S Jansen, R Lühken, et al. Eurosurveillance, Volume 22, Issue 2	ドイツ

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
○中欧の蚊におけるジカウイルス(ZIKV)の伝播実験 *Culex pipiens molestus*(チカイエカ、2011年からコロニー飼育)及び2016年にドイツで収集された蚊(*Culex pipiens pipens*(トビイロイエカ)、*Culex torrentium*、*Aedes albopictus*(ヒトスジシマカ))に、ZIKVを含む血液を与え感染させ、湿度80%、温度18℃又は27℃で飼育した。感染後14日目及び21日目に唾液分泌物を採取し、Vero細胞を用いた細胞変性効果(CPE)を測定することにより、感染性ウイルス粒子の存在を確認した。さらに、すべての蚊について定量的RT-PCR法によりZIKV RNAの測定を行った。 感染後14日目又は21日目に、すべての種類の蚊からZIKV RNAを検出した(感染率:3～72%)。感染率及びウイルス力価は*Culex*属と比較して*Aedes*属の方が大幅に高く、ウイルス量は一般的に生育温度が高いほど多かった。CPEの測定の結果、*Culex*属についてはZIKVのベクターとしての能力を示すものはなかった。対照的に、*Aedes*属は27℃の生育温度のみにおいて伝播能力を示し、*Aedes albopictus*の伝播能力は並行して実験を行った*Aedes aegypti*(ネッタイシマカ)と同様(伝播率:約30%)であった。	濃厚血小板-LR「日赤」 照射濃厚血小板-LR「日赤」 濃厚血小板HLA-LR「日赤」 照射濃厚血小板HLA-LR「日赤」 照射洗浄血小板-LR「日赤」 照射洗浄血小板HLA-LR「日赤」 血液を介するウイルス、 細菌、原虫等の感染 vCJD等の伝播のリスク

報告企業の意見	今後の対応
ジカウイルス(ZIKV)を媒介する蚊への感染実験にて、*Culex*属及び*Aedes*属から共に27℃の飼育温度でZIKV RNAを検出したが、細胞培養実験では*Aedes*属のみウイルス伝播能力を示したという報告である。	今後もジカウイルス感染症に関する新たな知見等について情報の収集に努める。

RAPID COMMUNICATIONS

Experimental transmission of Zika virus by mosquitoes from central Europe

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Citation style for this article:
Heitmann A, Jansen S, Lühken R, Leggewie M, Badusche M, Pluskota B, Becker N, Vapalahti O, Schmidt-Chanasit J, Tannich E. Experimental transmission of Zika virus by mosquitoes from central Europe. Euro Surveill. 2017;22(2):pii=30437. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2017.22.2.30437

Article submitted on 15 December 2016 / accepted on 10 January 2017 / published on 12 January 2017

Mosquitoes collected in Germany in 2016, including *Culex pipiens pipiens* biotype *pipiens*, *Culex torrentium* and *Aedes albopictus*, as well as *Culex pipiens pipiens* biotype *molestus* (in colony since 2011) were experimentally infected with Zika virus (ZIKV) at 18 °C or 27 °C. None of the *Culex* taxa showed vector competence for ZIKV. In contrast, *Aedes albopictus* were susceptible for ZIKV but only at 27 °C, with transmission rates similar to an *Aedes aegypti* laboratory colony tested in parallel.

In 2015, Zika virus (ZIKV) emerged in Columbia and Brazil and spread rapidly across the American continent and the Caribbean, causing an epidemic with notable numbers of associated clinical cases of microcephaly and Guillain–Barré syndrome [1]. Mosquitoes of the species *Aedes aegypti* and *Ae. albopictus* are considered the primary and secondary vectors of ZIKV [2]. However, with transmission rates below 50%, their vector competence for ZIKV in the laboratory is low [3]. The question therefore remains whether other common mosquito species such as *Culex* spp. play a role in the transmission cycle of ZIKV. The few studies performed so far have provided inconclusive results and suggested that at least *Culex quinquefasciatus* might be able to transmit ZIKV [4-9]. In addition, for an assessment of the risk of possible spread to regions with temperate climate such as central Europe, information is lacking on ZIKV vector competence of mosquitoes under reduced temperature conditions (< 20°C).

This study aimed to evaluate the vector competence of central European mosquito species for ZIKV. Therefore, German populations of *Culex pipiens pipiens* biotype *pipiens* (*Cx. p. pipiens*), *Culex pipiens pipiens* biotype *molestus* (*Cx. p. molestus*), *Culex torrentium* and *Ae. albopictus* (*Ae. albopictus*, GER) were experimentally infected with ZIKV, using *Ae. aegypti* and an Italian *Ae. albopictus* (*Ae. albopictus*, ITA) as positive controls.

Experimental infection of mosquitoes

Two long-established laboratory strains (*Ae. aegypti* (Bayer company) and *Cx. p. molestus* (in colony since 2011, collected in Heidelberg, Germany)) and four species collected in summer 2016 (*Cx. p. pipiens* Fo (collected in Hamburg, Germany), *Culex torrentium* Fo (collected in Hamburg, Germany), *Ae. albopictus* F7 (collected in Freiburg, Germany) and *Ae. albopictus* F7 (collected in Calabria, Italy)) were analysed and maintained as previously described [10,11]. All colonies tested negative in pan-flavivirus PCRs [12].

Between 150 and 200 female mosquitoes 4–14 days-old were starved for 24 h before application of infectious blood meals containing ZIKV (strain ZIKV_FB-GWUH-2016, GenBank KU870645, fifth passage) [13] at a final concentration of 107 plaque-forming units (PFU)/mL. Artificial feeding was performed using a Hemotek Feeder (*Aedes* spp.) or by cotton sticks (*Culex* spp.). Engorged females were incubated at 80% humidity at either 18 °C or 27 °C. Analyses for ZIKV were done 14 and 21 days post infection (dpi) for approximately 35 randomly selected females and twice the number for *Ae. aegypti* at 27°C. For salivation, mosquitoes were anaesthetised and the proboscises were inserted into cropped 10 µL filter tips containing 10 µL phosphate-buffered saline (PBS). After 30 min, tips were removed and saliva-containing PBS was analysed for the presence of infectious virus particles by measuring its cytopathic effect (CPE) on Vero cells within the following 8 days. ZIKV in the supernatant of cytopathic cells was confirmed by qRT-PCR using Real Star Zika Virus RT-PCR Kit (Altona diagnostics, Hamburg, Germany). In addition, bodies of all challenged mosquitoes,

TABLE

Susceptibility and transmission rates of mosquitoes experimentally infected with Zika virus (n = 856)

Mosquito taxa	T in °C	14 days post infection			21 days post infection		
		IR[a] (%)	Mean (SD) log10 RNA copies/specimen[b]	TR[c] (%)	IR[a] (%)	Mean (SD) log10 RNA copies/specimen	TR (%)
Aedes aegypti	18	17/31 (55)	4.70 (0.86)	0/17	18/33 (55)	4.33 (0.63)	0/18
	27	31/63 (49)	8.69 (1.60)	14/31 (45)	36/50 (72)	6.82 (1.75)	11/36 (31)
Aedes albopictus, ITA	18	19/30 (63)	4.05 (0.59)	0/19	14/39 (36)	5.52 (0.87)	0/14
	27	22/31 (71)	6.34 (2.14)	4/22 (18)	15/29 (52)	7.41 (2.22)	2/15 (13)
Aedes albopictus, GER	18	4/32 (13)	6.22 (1.25)	0/4	11/32 (34)	6.36 (1.39)	0/11
	27	20/31 (65)	6.78 (2.41)	4/20 (20)	18/34 (53)	8.61 (1.82)	6/18 (33)
Culex p. molestus	18	12/41 (29)	3.40 (0.38)	0/12	2/32 (6)	2.48 (0.29)	0/2
	27	7/29 (24)	3.73 (0.38)	0/7	12/38 (32)	4.02 (0.44)	0/12
Culex p. pipiens	18	16/34 (47)	3.38 (0.40)	0/16	3/32 (9)	3.88 (0.43)	0/3
	27	3/37 (8)	3.13 (0.45)	0/3	0/35 (0)	NA[d]	NA[d]
Culex torrentium	18	11/35 (31)	3.15 (0.47)	0/11	1/38 (3)	3.31 (NA)	0/1
	27	4/36 (11)	3.80 (1.79)	0/4	0/34 (0)	NA[d]	NA[d]

GER: from Germany; IR: infection rate; ITA: from Italy; NA: not available; SD: standard deviation; T: temperature; TR: transmission rate.

[a] Infection rate: number of ZIKV-positive mosquito bodies per number of fed females.

[b] RNA copies were averaged over all ZIKV-positive mosquito bodies excluding the zeros of ZIKV-negative mosquito bodies.

[c] Transmission rate: number of mosquitoes with ZIKV-positive saliva per number of ZIKV-positive mosquito bodies.

[d] Not available: Mean viral RNA copies and transmission rate could not be calculated for the species–temperature combinations with no ZIKV-positive bodies.

excluding legs and wings, were analysed for ZIKV RNA by qRT-PCR.

Results

At 14 or 21 dpi, ZIKV RNA was detected in the bodies of all challenged mosquito taxa, with infection rates ranging between 3 and 72% in the species–temperature combinations with ZIKV-positive bodies. Infection rates and virus titres were substantially higher in *Aedes* species, with viral RNA copies ranging from 10^2 to 10^4 in *Culex* spp. and from 10^4 to 10^9 in *Aedes* spp. (Table).

Virus load was generally higher at elevated incubation temperature (27 °C vs 18 °C). However, transmission of infectious virus particles as measured by CPE of Vero cells incubated with mosquito saliva was not detected in any of the *Culex* taxa. In contrast, saliva was positive for infectious virus particles in all *Aedes* species, but only at 27 °C incubation temperature. Interestingly, transmission rates at 21 dpi were similar in *Ae. aegypti* and *Ae. albopictus* from Germany but were substantially lower in *Ae. albopictus* from southern Italy (30% vs 13%).

Discussion

Culex species from central Europe are known as established vectors, able to transmit numerous viruses including West Nile, Sindbis and Usutu virus [14,15]. The results presented here indicate that the three most common *Culex* taxa in central Europe (*Cx. p. pipiens*, *Cx. p. molestus* and *Cx. torrentium*) do not have vector competence for ZIKV. This is in agreement with results from other parts of the world including Italy [4-7,9], which all showed a low degree of compentence of the *Cx. pipiens* complex for ZIKV transmission.

The invasive mosquito *Ae. albopictus* is established in large parts around the Mediterranean Sea and is considered to be the main vector in Europe for autochthonous human infections with chikungunya and dengue virus [16]. *Aedes albopictus* are regularly introduced into Germany as accidental cargo via road traffic from southern Europe [17]. In the winter 2015/16, successful overwintering of the species was observed for the first time in southern Germany [18]. The results presented here indicate that specimens of this overwintering population have considerable susceptibility to ZIKV, although only at elevated temperature of 27 °C.

Moreover, the transmission rate in this overwintering population was substantially higher than in *Ae. albopictus* from the Calabrian region in southern Italy. Whether the difference in virus susceptibility between German and Italian *Ae. albopictus* populations is due to an ongoing process of adaptation to a new environment or to experimental conditions remains to be determined. Nevertheless, the susceptibility of European *Ae. albopictus* to ZIKV demonstrates the risk of arbovirus transmission associated with the establishment and ongoing spread of this invasive mosquito species in Europe. Of note, none of the tested *Aedes* populations were susceptible to ZIKV at 18 °C, which may limit the spread of ZIKV in central Europe to short summer periods with high temperatures. However, for a comprehensive risk assessment of ZIKV transmission in central Europe, further infection studies are needed at intermediate temperatures (e.g. 21 °C and 24 °C) as well as with other common *Aedes* species such as *Ae. vexans* or the newly established *Ae. japonicus* [19].

Acknowledgements

We thank Ella Weinert and Michelle Helms for excellent technical assistance and Jessica Börstler for analyses of Culex mosquitoes. ML was supported by the Leibniz Association, grant number SAW-2014-SGN-3. This work was financially supported by the German Federal Ministry of Food and Agriculture (BMEL) through the Federal Office for Agriculture and Food (BLE) with the grant number 28-1-91.048-15.

Conflict of interest

None declared.

Authors' contributions

Conceived and designed the study: AH, SJ, RL, JSC, ET. Performed the data collection: AH, SJ, ML. Analysed the data: AH, SJ, RL, JSC, ET. Provided the ZIKA virus strain: OV. Provided mosquito specimens: MB, BP, NB. Wrote the paper: AH, SJ, RL, ET. Contributed to the manuscript drafting: ML. All authors read and approved the final version of the manuscript.

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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 2017 年 3 月 27 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	Emerging Infectious Disease ・www.cdc.gov/eid・ Vol.23,No.5,May2017 863-865	公表国 フランス	
販売名（企業名）	—					

研究報告の概要

５人の免疫が正常な患者に対し、全血中と血漿中でのジカウイルスの RNA の縦断的な follow up の結果を示している。
全血中のジカウイルスの検出は平均 22 日、血漿中では 10 日であった。
これらのデータは、ジカウイルスの RNA は血漿中で消失した後全血中で存在していた。同様の結果はフラビウイルス属であるウエストナイルウイルスでも報告がある。われわれのデータは 3 つの主な結論をもつ。
1 つ目は、急性の感染においては、全血を使うことは診断の期間を延長する。２つ目は、ウイルス量が低い可能性の高い無症状の感染では、血漿中のウイルスの検出は全血の検出より感度が低い可能性がある。３つ目は、われわれのデータによると、ウイルス血症は症状の発現から 28 日以上存在していて、血液もしくは血液成分を当してのジカウイルス感染のリスクを減らすために今使用している RECOMMENDATION は修正されるべきである。遅延期間の延長や全血によるジカウイスル RNA の献血の試験のような potential option を考えるべきである。

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
ジカウイルス感染および全血検体における持続性のウイルス血症に関する報告である。	ジカウイルスに関連する情報については、引き続き留意していく。

cured without valve surgery; it was cured with a 30-month antimicrobial drug regimen (*10*).

The role for serial serologic testing in assessing cure of *Bartonella* endocarditis is unknown. In our cases, as in a previous report (*10*), a drop in *Bartonella* titers occurred over a 3-year period in those who were cured, suggesting follow-up serologic testing might be useful to assess *Bartonella* endocarditis clinical cure.

Our findings suggest that a simple, inexpensive drug regimen is optimal therapy for *Bartonella* endocarditis and that serial serologic testing can confirm adequate treatment and cure. Further research is needed to validate this approach to managing *Bartonella* endocarditis.

Dr. Papineni is a Specialist Registrar in Infectious Diseases and General Medicine in the London Deanery. She is currently a clinical research fellow at the National University of Singapore with a research interest in shortening treatment of tuberculosis.

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Zika Virus Infection and Prolonged Viremia in Whole-Blood Specimens

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DOI: http://dx.doi.org/10.3201/eid2305.161631

We tested whole-blood and plasma samples from immunocompetent patients who had had benign Zika virus infections and found that Zika virus RNA persisted in whole blood substantially longer than in plasma. This finding may have implications for diagnosis of acute symptomatic and asymptomatic infections and for testing of blood donations.

Since cases of severe neurologic disorders among adults (*1*) and fetal abnormalities (*2*) linked to Zika virus infections were initially reported, the World Health Organization has deemed the Zika virus outbreak a "public health emergency of international concern" and has raised Zika virus to the same level of concern as Ebola virus. In response, medical authorities from many countries have released advice and guidelines regarding prevention and diagnosis to contain the spread of this virus and guidelines regarding safety of whole blood and blood components. In August 2016, the Food and Drug Administration announced universal testing for Zika virus RNA in donated whole blood and blood components taken in the United States and its territories using a qualitative molecular assay on plasma specimens (*3*).

In Europe, advice on Zika virus regarding the safety of substances of human origin (*4*) has been applied in France since February 15, 2016. A qualitative individual molecular test for Zika virus RNA in plasma specimens is being used on whole-blood specimens from blood donors living in Guadeloupe and Martinique, 2 overseas administrative areas where Zika virus is autochthonous. Furthermore, in mainland France and in French overseas areas where no active Zika virus transmission exists, and since the beginning of the Zika virus outbreak in 2015, blood donors who have recently visited areas or countries with ongoing Zika

virus transmission are subject to a 28-day temporary deferral after their departure from these areas, a period twice the assumed maximum incubation period for Zika virus. Similarly, temporary deferral applies to blood donors who have a sex partner who has been recently infected or potentially exposed to a confirmed or suspected Zika virus infection within the previous 3 months.

We report results from a 2016 longitudinal follow-up of Zika virus RNA quantification in EDTA whole-blood and plasma samples taken from 5 immunocompetent patients (2 men, 33 and 70 years of age, and 3 women, 55, 58, and 67 years of age) and results from a point-to-point comparison of Zika viral loads on both EDTA whole-blood and corresponding plasma samples (27 pairs). We extracted RNA by using the MagNA Pure 96 instrument with the DNA and Viral NA Small Volume Kit (Roche Diagnostics, Meylan, France) (input and output volumes 200 and 100 µL). We quantified RNA by using the RealStar Zika RNA RT-PCR kit 1.0 (Altona Diagnostics GmbH, Hamburg, Germany) (limit of selection 2.48 log copies/mL). We always successfully detected the manufacturer's internal control. All samples were collected from patients who had returned from the Caribbean or South and Central America and had had a benign form of Zika virus infection.

Results from the follow-up (18 whole-blood and 21 plasma samples) showed that the median duration of Zika virus was 22 (range 14–100) days in whole blood and 10 (range 7–37) days in plasma (p = 0.058). Mean viral loads of positive samples were 3.39 log copies/mL in whole blood (n = 13) and 2.52 log copies/mL in plasma (n = 6; p = 0.001). Viral loads in the last positive samples varied from 2.7 to 3.9 log copies/mL in whole blood and 2.2 to 2.8 log copies/mL in plasma (p = 0.06). Whole-blood samples from 2 patients remained positive at 14 and 63 days after their plasma samples had become negative (Figure, panel A).

The point-to-point comparison (18 pairs from the follow-up and 9 additional pairs) showed that Zika virus RNA was quantifiable in 23 whole-blood specimens but in only 10 plasma samples. Mean viral load was 3.50 (range 2.75–4.17) log copies/mL in whole blood and 3.01 (range 2.21–4.10) log copies/mL in plasma (p< = 0.018) (Figure, panel B).

These data show that Zika virus RNA persisted in whole blood after it disappeared in plasma. Similar results have been reported previously for West Nile virus, also a member of the *Flaviviridae* family (*5*,*6*), and for Zika virus with a qualitative in-house PCR (*7*).

Our data have 3 main consequences. First, for acute symptomatic infection, the use of whole blood extends the period of diagnosis. Second, for asymptomatic infections with a high likelihood of low viral load, virus detection in plasma might be less sensitive than detection in whole-blood specimens. Third, according to our data that show that viremia can persist for >28 days after symptom onset, recommendations used to reduce the risk for Zika virus transmission through blood or blood components should be modified. Potential options such as extending the deferral period or testing blood donations for Zika virus RNA in whole blood should be considered.

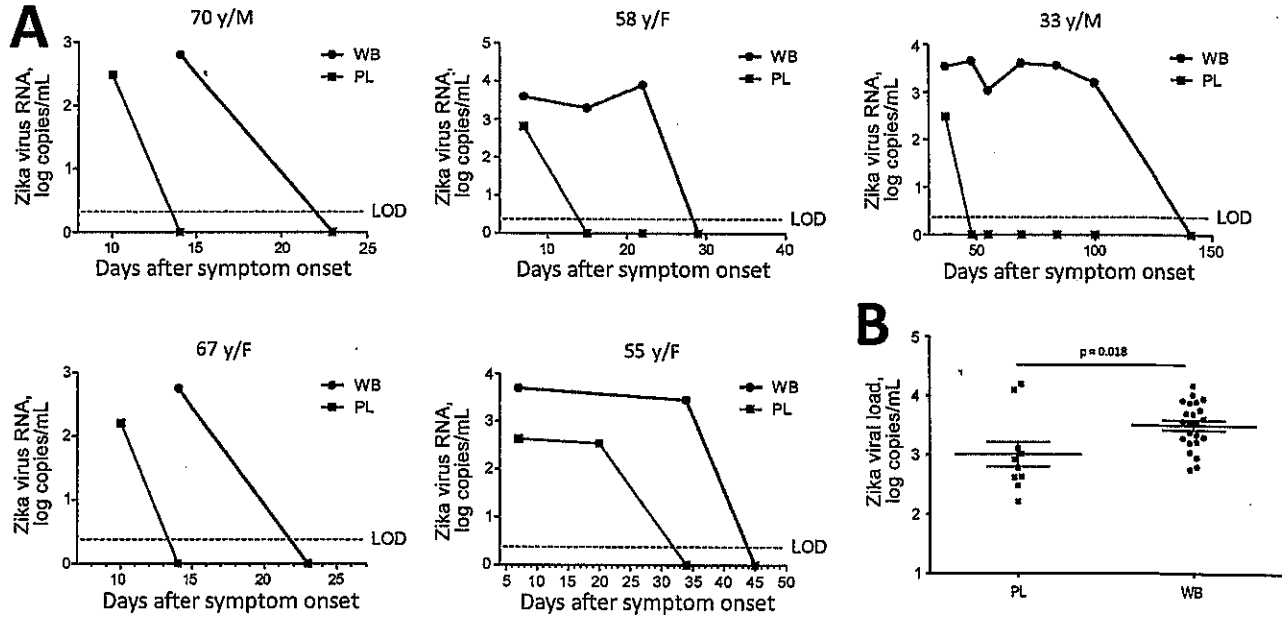

Figure. A) Zika virus viremia in whole blood and plasma from 5 immunocompetent patients in France (identified by sex and age, y) who had traveled to Central or South America or the Caribbean. B) Zika viral load in whole-blood (n = 23) and plasma (n = 10) samples from a point-to-point comparison of positive samples. Horizontal lines indicate mean ± SE. LOD, limit of detection; PL, plasma; WB, whole blood.

Overall, our data show that use of whole-blood specimens is much more sensitive than use of plasma samples to detect Zika virus RNA. These data could be useful in recommending the use of whole blood instead of plasma for the molecular diagnosis of acute symptomatic and asymptomatic Zika virus infections and for the safety of whole blood and blood components from donors, as well as for the safety of organs, tissues, and cells from deceased and living donors.

Acknowledgment

We thank Isabelle Da Silva for her technical assistance.

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Severe MRSA Enterocolitis Caused by a Strain Harboring Enterotoxins D, G, and I

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DOI: http://dx.doi.org/10.3201/eid2305.161644

We describe a case of methicillin-resistant *Staphylococcus aureus* (MRSA) enterocolitis in a healthy adult with previous antibiotic exposure. Colonoscopy revealed diffuse colitis and mild ileitis without ulceration. Stool cultures demonstrated abundant growth of MRSA and absent normal flora. Oral vancomycin treatment was effective and seems to be the consensus choice for therapy.

Staphylococcus aureus was recognized as a cause of antibiotic-associated colitis (AAC) in the mid-20th century (*1*,*2*). *Clostridium difficile* was later identified as the primary cause of AAC, and appreciation of *S. aureus* as a potential etiology declined (*2*). Methicillin-resistant *S. aureus* (MRSA) has also been implicated as a cause of AAC, with most reports coming from Japan. We report a case of MRSA enterocolitis in Canada caused by a strain harboring multiple enterotoxins.

In 2014, a 22-year-old woman sought care after 10 days of acute and profuse diarrhea, abdominal cramping, nausea, and weight loss of 5 lbs. She had 10–30 bowel movements a day and had observed blood-tainted stool. The patient reported a history of migraine and depression but was otherwise healthy. She worked in a pet store and had not been hospitalized. In the previous 2 months, she had been treated for chlamydia with a single course of azithromycin and cefixime. Subsequently, she received a 10-day course of azithromycin followed by 10 days of moxifloxacin for bronchopneumonia. Her family history revealed Crohn's colitis in her father.

The patient was afebrile; blood pressure was 104/58 mm Hg and pulse 91 bpm. Her abdomen was soft without rebound tenderness. Laboratory test results revealed a normal leukocyte count but a C-reactive protein level of 76 mg/L. Her initial diagnosis was with bacterial enteritis, and she was sent home with an order for stool cultures and oral ciprofloxacin to be started after stool collection. On

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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
				2017. 3. 24	該当なし	

一般的名称	人全血液	研究報告の公表状況	Paz-Bailey G, Rosenberg ES, Doyle K. N Engl J Med. 2017 Feb 14. doi: 10.1056/NEJMoa1613108. [Epub ahead of print]	公表国
販売名(企業名)	人全血液-LR「日赤」(日本赤十字社) 照射人全血液-LR「日赤」(日本赤十字社)			米国

研究報告の概要

○体液中におけるジカウイルスの検出期間--予備報告

背景:体液中のジカウイルス(ZIKV)RNA検出頻度及び期間を推定するために、プエルトリコの新規感染者集団を対象に前向き研究を行った。

方法:Enhanced arboviral clinical surveillance siteにおいて、RT-PCRアッセイで尿中または血液中からZIKV RNAが検出された150名(男性55名含む)の感染者から得られた検体を評価した。1ヶ月目は毎週、その後は2ヶ月目、4ヶ月目及び6ヶ月目に血清、尿、唾液、精液及び膣分泌物を採取した。全検体をRT-PCRにより検査し、血清中のZIKV IgM抗体はELISA法により検査した。4週目の検体にZIKV RNAを検出した感染者には、全ての検体が陰性と判定されるまで隔週で検体採取を継続した。パラメトリックワイブル回帰モデルを用いて、各検体中にZIKV RNAが検出されなくなるまでの期間を推定し、結果を中央値及び95パーセンタイルで報告した。

結果:ZIKV RNAが検出されなくなるまでの期間の中央値及び95パーセンタイルは、それぞれ血清で14日(95%信頼区間[CI]、11-17)と54日(95%CI、43-64)、尿で8日(95%CI、6-10)と39日(95%CI、31-47)、精液で34日(95%CI、28-41)と81日(95%CI、64-98)であった。唾液または膣分泌物からZIKV RNAが検出された感染者はほとんどいなかった。

結論:本研究において、血清中のZIKV RNAが消失するまでの期間が延びたことにより、ZIKV感染の診断及び予防に影響を及ぼす可能性がある。現在の、性行為による感染予防ガイドラインでは、ZIKV曝露後6ヶ月間はコンドームを使用するか性行為を控えるよう勧告している。研究対象となった男性の95%において、精液中のZIKV RNAは約3ヶ月後に認められなくなった。

報告企業の意見	今後の対応
ジカウイルス(ZIKV)感染者を対象とした前向き研究で、ZIKV RNAが血清中から検出されなくなるまでの期間は、中央値で14日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。また、平成28年2月3日付厚生労働省医薬・生活衛生局血液対策課長事務連絡「ジカウイルスによることが疑われる小頭症等の増加に関するWHO緊急委員会宣言について(注意喚起)」の発出を受け、同月4日付で各血液センターに対し問診時の帰国(入国)後経過日数の確認を徹底するよう指示した。さらに同年7月1日から、ジカウイルス感染症と診断され、治癒後1ヶ月間経過していない場合は献血不適としている。今後もジカウイルス感染症に関する新たな知見等について情報の収集に努める。

使用上の注意記載状況・その他参考事項等

人全血液-LR「日赤」
照射人全血液-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

ORIGINAL ARTICLE

Persistence of Zika Virus in Body Fluids — Preliminary Report

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ABSTRACT

BACKGROUND

To estimate the frequency and duration of detectable Zika virus (ZIKV) RNA in human body fluids, we prospectively assessed a cohort of newly infected participants in Puerto Rico.

METHODS

We evaluated samples obtained from 150 participants (including 55 men) in whom ZIKV RNA was detected on reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay in urine or blood in an enhanced arboviral clinical surveillance site. We collected serum, urine, saliva, semen, and vaginal secretions weekly for the first month and then at 2, 4, and 6 months. All specimens were tested by means of RT-PCR, and serum was tested with the use of anti–ZIKV IgM enzyme-linked immunosorbent assay. Among the participants with ZIKV RNA in any specimen at week 4, biweekly collection continued until all specimens tested negative. We used parametric Weibull regression models to estimate the time until the loss of ZIKV RNA detection in each body fluid and reported the findings in medians and 95th percentiles.

RESULTS

The medians and 95th percentiles for the time until the loss of ZIKV RNA detection were 14 days (95% confidence interval [CI], 11 to 17) and 54 days (95% CI, 43 to 64), respectively, in serum; 8 days (95% CI, 6 to 10) and 39 days (95% CI, 31 to 47) in urine; and 34 days (95% CI, 28 to 41) and 81 days (95% CI, 64 to 98) in semen. Few participants had detectable ZIKV RNA in saliva or vaginal secretions.

CONCLUSIONS

The prolonged time until ZIKV RNA clearance in serum in this study may have implications for the diagnosis and prevention of ZIKV infection. Current sexual-prevention guidelines recommend that men use condoms or abstain from sex for 6 months after ZIKV exposure; in 95% of the men in this study, ZIKV RNA was cleared from semen after about 3 months. (Funded by the Centers for Disease Control and Prevention.)

From the National Center for HIV/AIDS, Viral Hepatitis, Sexually Transmitted Diseases, and Tuberculosis Prevention, Centers for Disease Control and Prevention (G.P.-B., K.D.), and the Department of Epidemiology, Emory University (E.S.R.), Atlanta; and the National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention (J.M.-J., G.A.S., J.P.-P., F.A.M., S.H.W., T.M.S.), and Caduceus Healthcare (L.K.), San Juan, and Ponce University School of Medicine–Saint Luke's Episcopal Hospital Consortium, Ponce (C.G.G., L.I.A.) — all in Puerto Rico. Address reprint requests to Dr. Paz-Bailey at the Centers for Disease Control and Prevention, 1600 Clifton Rd. NE, MS E-46, Atlanta, GA 30329, or at gmb5@cdc.gov.

This article was published on February 14, 2017, at NEJM.org.

DOI: 10.1056/NEJMoa1613108

After its discovery in Uganda in 1947, Zika virus (ZIKV) was identified in Brazil in 2015 and subsequently spread throughout the Americas.[1] ZIKV is now recognized as a cause of congenital neurologic birth defects, notably microcephaly,[2] and has been associated with potentially fatal complications.[3,4]

ZIKV infection can be diagnosed through detection of ZIKV RNA in blood, urine, and other body fluids on reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay.[5] However, the frequency with which ZIKV RNA can be detected in various body fluids and the length of time that it remains detectable are not well understood. Similarly, ZIKV infection can also be diagnosed by the detection of anti–ZIKV IgM antibodies, although the kinetics of IgM antibody production have not been fully described.

Although most ZIKV infections are probably transmitted by infected mosquitoes, ZIKV transmission has been documented through sexual contact,[6] blood transfusion,[7] laboratory exposure,[1] and both intrauterine and intrapartum transmission.[8] ZIKV RNA has been detected in semen,[9] urine,[10] saliva,[11] cerebrospinal fluid,[12] vaginal or cervical secretions,[13,14] and other body fluids.[15-18] Most transmissions through sexual contact have been from men with symptomatic infection to their female partners.[19-21] However, sexual transmission has also occurred from asymptomatic men,[22,23] through male-to-male[24] and female-to-male sex,[25] and possibly through oral sex.[9] Shedding in the female genital tract appears to be rare and of short duration.[13] In contrast, there are reports of prolonged detection of ZIKV RNA in semen, with the longest reported duration of detection up to 188 days after onset.[26,27] Infectious virus has been reported in semen up to 69 days.[28]

A detailed understanding of the dynamics of the early stages of ZIKV infection is needed to inform diagnostic testing algorithms and prevention interventions, since existing evidence is based on case reports and cross-sectional observations, primarily from returning travelers.[29] To estimate the presence and duration of the detection of ZIKV RNA in body fluids and anti–ZIKV IgM antibody among participants with acute ZIKV infection, we established the ZIKV Persistence (ZiPer) cohort study in Puerto Rico, in which we prospectively evaluated multiple concurrently collected specimens from participants. Here, we report the results of the interim analyses to provide timely data that can inform recommendations.

METHODS

STUDY DESIGN AND OVERSIGHT

ZiPer is a prospective cohort study involving participants of all ages with ZIKV infection, as diagnosed by means of RT-PCR, with a target enrollment of 350 participants. Beginning in May 2016, participants were identified through the Sentinel Enhanced Dengue Surveillance System (SEDSS), a prospective surveillance of acute febrile illness among patients presenting to the emergency department of a tertiary care hospital or an outpatient clinic, both located in Ponce, Puerto Rico. Patients who presented with fever (temperature, ≥38.0°C [100.5°F]), rash, conjunctivitis, or arthralgia were offered participation in SEDSS. Among the participants who provided written informed consent, blood and urine specimens were tested for causative agents of acute febrile illness, including ZIKV. SEDSS participants who tested positive for ZIKV infection on RT-PCR (index participants) were systematically contacted by study staff and were offered enrollment in ZiPer. The household members of index participants were invited to participate and provide specimens, and those who tested positive on RT-PCR joined the prospective cohort study. Full details regarding the study design are provided in the protocol (available with the full text of this article at NEJM .org), which was reviewed and approved by the institutional review boards at the Centers for Disease Control and Prevention (CDC) and Ponce Health Sciences University.

PROCEDURES

All the participants completed an interviewer-administered questionnaire, which included reporting the number of days that had elapsed since the onset of ZIKV symptoms; household contacts of the participants reported such data at the time of enrollment. Participants were defined as being symptomatic if they reported having had signs or symptoms of ZIKV infection (fever, conjunctivitis, rash, or arthralgia) during the 30 days before the interview. Serum, urine, saliva, semen, and vaginal secretions (the last two in adults only) were collected weekly for the first month and at 2, 4, and 6 months thereafter. Among the participants in whom ZIKV RNA was detected in any specimen at week 4, biweekly collection continued until all the specimens tested negative. (Details are provided in the Methods section in the Supplementary Appendix, available

at NEJM.org.) All the participants received a $50 reimbursement per visit.

LABORATORY TESTING

Specimens were tested by means of the Trioplex RT-PCR assay, as recommended by the CDC for the detection of dengue, chikungunya, and ZIKV RNA.[30] In addition, we performed validation analyses for the use of the Trioplex RT-PCR assay in semen (see the Supplementary Appendix). Specimens were considered to be positive if target amplification was detected within 38 threshold cycles. The RNA extraction and real-time RT-PCR process were considered to be valid if the human RNase P reaction was positive. Intermittent RNA detection was defined as the detection of viral RNA that was followed by a lack of detection and then subsequent detection, regardless of the interval between specimen collections. Serum was tested by means of anti–ZIKV IgM antibody capture enzyme-linked immunosorbent assay.[31] ZIKV isolation was attempted through culture in a subset of semen and serum specimens (see the Supplementary Appendix).

STATISTICAL ANALYSIS

We summarized the demographic and clinical characteristics of the participants, along with details regarding the detection of ZIKV RNA in fluids and IgM antibody according to the number of days that had elapsed since the onset of ZIKV symptoms. We used the kappa statistic to assess the beyond-chance agreement in RNA detection in paired samples of semen and serum and in paired samples of semen and urine obtained from male participants. Since all the participants had positive results on testing of serum or urine at enrollment, we could not independently assess the serum–urine agreement. The time until the loss of RNA detection in each fluid was defined as the number of days between the onset of ZIKV symptoms and the first negative RT-PCR result. To estimate model-derived percentiles for the time until virus clearance at the population level, we assumed that all infected participants had ZIKV RNA in all specimens at symptom onset. For the participants who had intermittent shedding of ZIKV, we used the first negative result after the final recorded test result that was positive on RT-PCR; data were censored for the participants who still had positive results on RT-PCR at the time of the analysis.

The time until the detection of IgM antibody was defined as the number of days between the onset of ZIKV symptoms and the first IgM-positive result; data were censored for the participants in whom the results were still IgM-negative at the time of the analysis. We used the Kaplan–Meier method to estimate survival functions for these outcomes, along with the nonparametric maximum-likelihood Turnbull estimator and parametric Weibull regression models. (Details about these models are provided in the Supplementary Appendix.) The Turnbull method and Weibull models accounted for interval censoring (since the loss of detection of ZIKV RNA occurred within an interval between visits instead of being observed on an exact date). From the Weibull models, we estimated survival functions and their 95% confidence intervals, as well as medians and 95th percentiles. In supplementary analyses, we estimated models for the time until the loss of detection that were restricted to the participants with any ZIKV RNA in a given fluid and to index participants. Model-derived medians were not estimated for saliva and vaginal secretions because of the few positive results. All statistical analyses were performed with the use of SAS software, version 9.3.

RESULTS

As of September 21, 2016, we had contacted 414 of the 1258 index participants with symptomatic ZIKV infection, as confirmed on RT-PCR assay. Of these participants, 127 were enrolled in the study. The percentage of index participants who were adults (≥18 years of age) was higher among those who were enrolled in the study than among those who were not enrolled (92% vs. 74%), and more were male (59% vs. 45%). Of the 195 household contacts of the index participants who were screened, 23 (12%) tested positive for ZIKV RNA, for a total of 150 prospective participants. All the participants remained under prospective observation, with 493 of 549 visits (90%) attended, except for 1 participant who withdrew and 2 who were administratively discontinued.

The mean age of participants was 38 years; 44% were female, including 5 who were pregnant (Table 1). Four household contacts with positive results were asymptomatic at enrollment. Among the 146 participants with signs or symptoms of ZIKV infection at enrollment, 92% were enrolled within 1 week after the onset of illness.

ANTIBODY RESPONSE

Anti–ZIKV IgM antibody was detected in at least one specimen obtained from 140 of 143 participants (97.9%). Only one specimen was collected from each of the 3 IgM-negative participants on days 4, 7, and 30 after the onset of symptoms. Details regarding the detection of IgM antibody are provided in Figure S1 in the Supplementary Appendix.

ZIKV RNA IN SERUM

A total of 132 of 150 participants (88.0%) had detectable ZIKV RNA in at least one serum specimen (Table 2). Of the 132 participants, 42 (31.8%) had detectable ZIKV RNA more than once. (Values for threshold cycle are provided in Fig. S8A in the Supplementary Appendix.) The median time until the loss of RNA detection was 14 days (95% confidence interval [CI], 11 to 17), and the 95th percentile of time was 54 days (95% CI, 43 to 64) on the basis of the Weibull model (Fig. 1A). Results that were obtained with the use of the Turnbull model were similar to those obtained with the Weibull model (Fig. S2A in the Supplementary Appendix). The number of days after the onset of symptoms at enrollment did not influence the time until the loss of ZIKV RNA detection. When the analyses were restricted to the 50% of participants who were enrolled within 2 days after symptom onset, the median time until the loss of detection was 13 days (95% CI, 10 to 17). Among the 5 pregnant women, 3 had detectable RNA at 46 days after symptom onset. At the interim analysis, 20 of 150 (13.3%) had detectable RNA at their last visit and were still being followed. The maximum duration of detection was 80 days after symptom onset in a pregnant participant; an estimated 2% had detectable RNA at this time (Fig. S3A in the Supplementary Appendix).

ZIKV RNA IN URINE

ZIKV RNA was detected in at least one urine specimen in 92 of 149 participants (61.7%) (Table 2). Overall, 15 (10.1%) had detectable RNA in urine but not in serum, whereas 55 (36.7%) had RNA in serum but not urine. The model-based median time until the loss of detection was 8 days (95% CI, 6 to 10), and the 95th percentile of time was 39 days (95% CI, 31 to 47) (Fig. 1B).

ZIKV RNA IN SALIVA AND VAGINAL SECRETIONS

Among the 147 participants who were tested, 15 (10.2%) had ZIKV RNA in at least one saliva specimen (Table 2). The rate of positivity in these samples was lower than the rate in serum and urine at any number of days after symptom onset (Table 3). Similarly, only 1 in 50 women (2%) had ZIKV RNA in vaginal secretions (at 3 days after symptom onset). All the samples were positive in the RNase P control reaction.

ZIKV RNA IN SEMEN

Of 68 eligible male participants, 55 (81%) provided at least one semen specimen. ZIKV RNA was present in at least one specimen in 31 participants (56%). Chance-corrected agreement of RNA detection was low in paired samples of semen and serum (κ = 0.05; 95% CI, −0.05 to 0.16) and samples of semen and urine (κ = 0.11; 95% CI, 0.01 to 0.22). The model-derived median time until the loss of RNA detection was 34 days (95% CI, 28 to 41), and the 95th percentile of time was 81 days (95% CI, 64 to 98) (Fig. 1C). However, 11 of 55 participants had ZIKV RNA in semen at their last visit and are still being followed. The maximum duration of RNA detection was 125 days after symptom onset; an estimated 4% continued to have detectable RNA at that time (Fig. S3C in the Supplementary Appendix).

TIME UNTIL THE LOSS OF DETECTABLE ZIKV RNA

We also estimated the time until the loss of RNA detection among the participants with any ZIKV RNA in a given fluid during follow-up. Among this subset, the model-derived estimated percentiles of time until the loss of RNA detection were longer because the analyses were limited to participants with continued viral shedding (Figs. S4, S5, and S6 in the Supplementary Appendix).

ANALYSES LIMITED TO INDEX PARTICIPANTS

Index participants were more likely to be male than were the 19 symptomatic household contacts (59% vs. 42%), to be at least 18 years of age (92% vs. 68%), and to have been recruited within 1 week after symptom onset (99% vs. 42%). When the model was limited to index participants, the estimated median time until the loss of detection of ZIKV RNA decreased by 1 day in serum and urine and increased by 2 days in semen (Fig. S7 in the Supplementary Appendix).

INTERMITTENT RNA DETECTION

We observed intermittent ZIKV RNA detection in serum obtained from 15 participants, in urine samples obtained from 5 participants, and in

semen samples obtained from 3 participants (Fig. S9 in the Supplementary Appendix). The range of days between positive specimens was 14 to 62 in serum, 14 to 35 in urine, and 21 to 36 in semen.

ISOLATION OF ZIKV

ZIKV isolation was attempted in 20 semen specimens with threshold-cycle values ranging from 19 to 37 and in 20 serum specimens with threshold-cycle values ranging from 22 to 37. Virus isolation was successful in 6 of 20 semen specimens (30%) with threshold-cycle values ranging from 19 to 27 and in 1 of 20 serum specimens (5%) with a threshold-cycle value of 22.

DISCUSSION

In this preliminary analysis, we obtained data on how long it takes for ZIKV RNA to clear among the participants with acute ZIKV infection and detectable ZIKV RNA at enrollment. The recruitment of participants was based on an ongoing surveillance platform that enabled 90% of participants to enroll within the first week after the onset of symptoms, which provided increased resolution for ZIKV RNA detection starting soon after symptom onset. In our study, half of the participants had detectable viral RNA in urine for at least 1 week after symptom onset, in serum for 2 weeks, and in semen for more than 1 month, whereas 5% or less had detectable viral RNA in urine for 6 weeks, in serum for 8 weeks, and in semen for 3 months. Conversely, ZIKV RNA was infrequently detected in saliva and vaginal secretions.

The CDC recommends RT-PCR testing of serum and urine samples obtained from symptomatic participants less than 14 days after symptom onset.[5] Our results contrast with the findings of other studies,[10,29] which showed more frequent detection of ZIKV RNA in urine than in serum. However, the cited studies had small sample sizes that limit generalizability. The discrepant results may also be explained by differences in the population that was included in the analyses. A previous study showed frequent ZIKV RNA detection in saliva within 5 days after symptom onset.[11] We detected ZIKV RNA infrequently in saliva; however, we did not have enough specimens to determine RNA detection early after onset.

IgM antibody was detected in almost all ZIKV-infected participants in this study. This finding

Table 1. Characteristics of the Participants at Baseline.

Characteristic	Participants (N=150)
Age	
Mean (range) — yr	37.8 (<1 to 83)
Age group — no. (%)	
0–17 yr	17 (11.3)
18–64 yr	124 (82.7)
≥65 yr	9 (6.0)
Female sex — no. (%)	66 (44.0)
Pregnancy — no. (%)	5 (3.3)
Presence of signs or symptoms of Zika virus infection at enrollment — no. (%)	
No*	4 (2.7)
Yes	146 (97.3)
Days after symptom onset at enrollment — no./total no. (%)	
0–2 days	66/146 (45.2)
3–5 days	63/146 (43.2)
6–7 days	5/146 (3.4)
8–14 days	4/146 (2.7)
≥15 days	8/146 (5.5)
Signs or symptoms at enrollment — no./total no. (%)†	
Fever	115/146 (78.8)
Red eyes or eye pain	119/146 (81.5)
Rash	135/144 (93.8)
Pruritus	117/145 (80.7)
Photophobia	59/144 (41.0)
Edema	92/145 (63.4)
Arthralgia	120/139 (86.3)
Myalgia	102/125 (81.6)
Headache	115/145 (79.3)
Abdominal pain	73/145 (50.3)
Lymphadenopathy	50/144 (34.7)
Diarrhea	62/145 (42.8)
Nausea	63/145 (43.4)
Vomiting	17/145 (11.7)
Pelvic pain	25/139 (18.0)
Dysuria	25/145 (17.2)
Other‡	129/144 (89.6)
Laboratory findings	
Median white-cell count (range) per mm^3	5200 (2100 to 40,000)
Median platelet count (range) per mm^3	216,000 (80,000 to 373,000)
Median hematocrit (range) — %	42.2 (30.9 to 51.9)

* This category includes two participants who were asymptomatic at baseline but in whom signs or symptoms developed within 7 days after specimen collection.
† The median duration of fever was 2 days (range, 1 to 22); red eyes, 3 days (range, 1 to 7); and rash, 5 days (range, 1 to 25).
‡ Other signs or symptoms included cough (in 33.1% of the participants), yellow eyes or skin (4.8%), difficulty urinating (7.8%), blood in urine (5.5%), painful ejaculation (6.7% of men), and penile discharge (2.7% of men).

Table 2. Detection of ZIKV RNA in Body Fluids and Anti–ZIKV IgM Antibody in Serum, According to Subgroup.*

Subgroup	ZIKV RNA					Anti–ZIKV IgM Antibody
	Serum	Urine	Saliva	Vaginal Secretions	Semen	Serum
	number/total number (percent)					
All participants	132/150 (88.0)	92/149 (61.7)	15/147 (10.2)	1/50 (2.0)	31/55 (56.4)	140/143 (97.9)
Age (yr)						
0–17	15/17 (88.2)	9/16 (56.3)	1/16 (6.3)	NA	NA	17/17 (100)
18–64	109/123 (88.6)	73/123 (59.3)	14/122 (11.5)	1/47 (2.1)	30/54 (55.6)	114/117 (97.4)
≥65	7/9 (77.8)	9/9 (100)	0/9	0/3	1/1 (100)	9/9 (100)
Sex						
Male	72/83 (86.8)	54/83 (65.1)	9/83 (10.8)	NA	31/55 (56.4)	78/80 (97.5)
Female	59/66 (89.4)	37/65 (56.9)	6/64 (9.4)	1/50 (2.0)	NA	62/63 (98.4)
Pregnancy	5/5 (100)	0/5	0/5	0/5	NA	5/5 (100)

* Of the 150 index participants, 127 were recruited into the study after a visit to the Sentinel Enhanced Dengue Surveillance System (SEDSS). Saliva, vaginal secretions, and semen are not collected as part of SEDSS. Therefore, few specimens of these types were available within 7 days after the onset of symptoms. NA denotes not applicable.

may reflect a primary immune response to ZIKV. However, given the high prevalence of previous flavivirus infection in Puerto Rico,[32] these results may not be generalizable to a population that has not been extensively exposed to flavivirus. The usefulness with regard to the specificity of IgM testing for diagnosis of ZIKV in geographic areas with discrepant exposure to flavivirus requires further study.

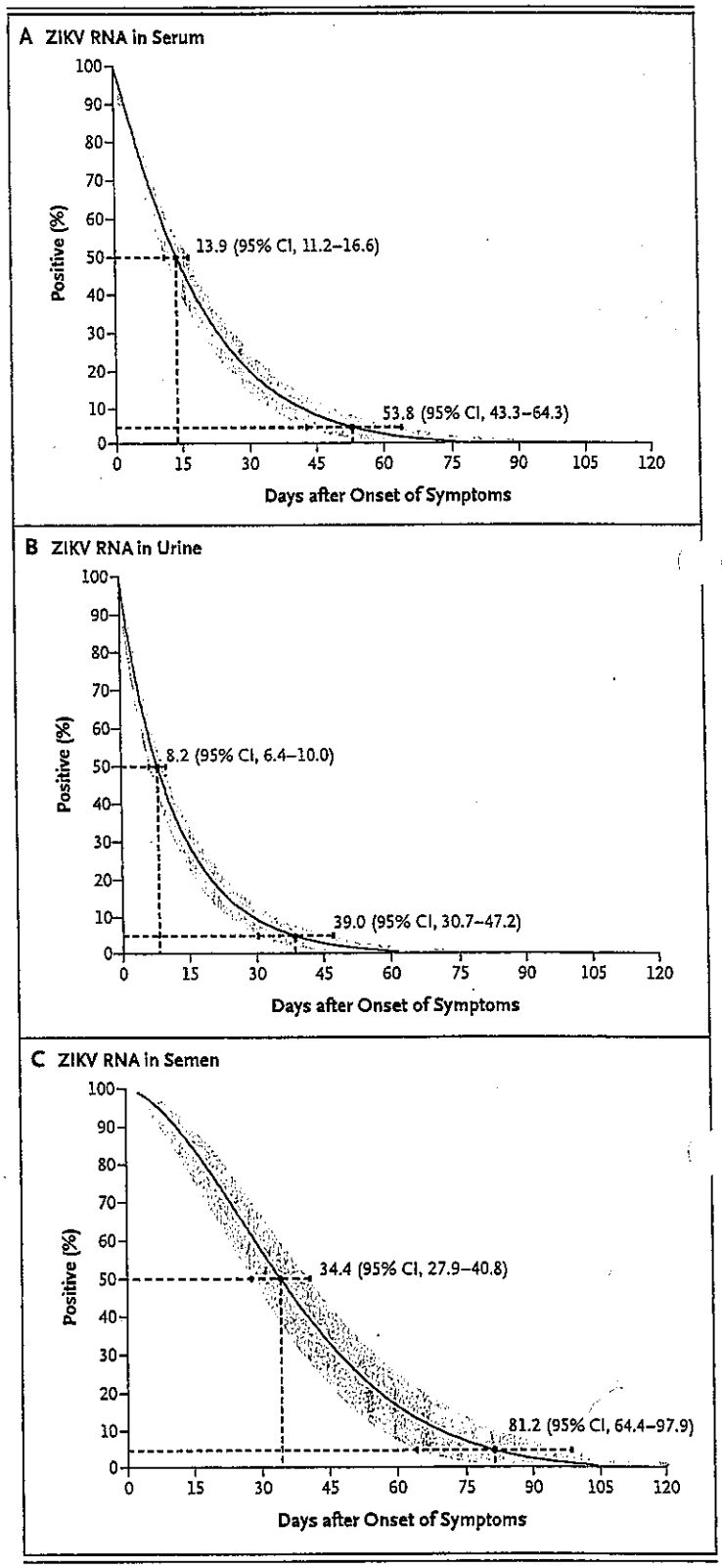
In our study, the observed duration of ZIKV RNA in serum was longer than detection times reported for dengue virus. More than 90% of the patients who are infected with any of the four dengue viruses clear RNA within 10 days after the onset of symptoms.[33] Studies involving asymptomatic blood donors with the use of transcription-mediated amplification (a technique that is more sensitive than RT-PCR) showed that the median time until RNA clearance for West Nile virus was 13 days (95% CI, 12 to 15), an interval that is similar to what we observed for ZIKV.[34] Since the cross-reactivity of antibodies between flaviviruses limits the use of serologic analysis, we recruited participants who had detectable RNA at enrollment, a factor that could have contributed to increased times until RNA clearance. Although we were able to isolate ZIKV in serum and semen specimens with low threshold-cycle values, further study is required to determine whether the extended duration of ZIKV RNA in serum correlates with infectivity. The minimal time that persons who have potential exposure to ZIKV should avoid donating blood is currently 120 days, which covers the maximum duration of RNA detection that we observed in our study.[35]

The CDC recommends that women who have been infected or exposed to ZIKV wait at least 8 weeks from symptom onset or last exposure before attempting conception.[36] In our study, 95% of the participants no longer had detectable ZIKV RNA in serum at 8 weeks. Although these data suggest that the risk of intrauterine transmission among ZIKV-infected women who are trying to conceive toward the end of an 8-week period after symptom onset is small, we will continue to monitor women of reproductive age to inform evaluations of these recommendations.

Despite model-based estimates suggesting that sexual transmission contributes only modestly to epidemic propagation,[37,38] sexual transmission could complicate efforts to prevent the transmission of ZIKV. The CDC recommends that men with possible ZIKV exposure, regardless of symptom status, should use condoms or abstain from sex for at least 6 months.[36] Although two case reports detected RNA in semen more than 180 days

Figure 1. Time until the Clearance of Zika Virus RNA in Serum, Urine, and Semen.

Shown are models of the time until the loss of Zika virus (ZIKV) RNA detection after the onset of symptoms in serum (Panel A), urine (Panel B), and semen (Panel C) obtained from 150 study participants, as estimated with the use of Weibull regression. To estimate model-derived percentiles for the time until virus clearance at the population level, we assumed that all infected participants had ZIKV RNA in all specimens at symptom onset. Also shown are medians and 95th percentiles of the time until the loss of detection, the key values that were reported in this preliminary study. Blue shading denotes 95% confidence intervals. Data for 4 participants who were asymptomatic at the time of enrollment were excluded from the estimates of the time until the loss of RNA detection, since the number of days after the onset of symptoms could not be determined.



after symptom onset,[26,27] such late detection seems infrequent. Our study documented that few men have detectable ZIKV RNA past 3 months, and the maximum time that has been observed in our study thus far was 125 days.

Our study has several limitations. By enrolling only participants with positive results for ZIKV RNA in urine or serum on RT-PCR assay at baseline and excluding those who were IgM-positive only, we may have biased our findings by recruiting persons who have a longer duration of ZIKV RNA in serum or urine. However, when our analyses were limited to participants who had enrolled within 2 days after symptom onset, our duration estimates were similar to those in the overall sample. The detection of ZIKV RNA does not necessarily correlate with having infectious virus, a factor that we are studying in additional virus-isolation assays. We determined the limit of detection of ZIKV RNA in semen, but we were unable to evaluate the sensitivity of the test for saliva and vaginal secretions with a similar validation study. However, all semen, saliva, and vaginal swabs tested positive for the RNase P internal control reaction, which suggests that the RNA extraction and conditions of the assay are probably not reasons for the failure to detect ZIKV RNA in saliva and vaginal secretions. Nonetheless, without knowing the limit of the detection of the Trioplex RT-PCR assay in these specimen types, negative results should be interpreted with caution. To

Table 3. Detection of ZIKV RNA in Body Fluids and Anti–ZIKV IgM Antibody in Serum, According to the Number of Days after Symptom Onset.*

Positivity and Days after Symptom Onset	ZIKV RNA†					Anti–ZIKV IgM Antibody‡
	Serum	Urine	Saliva	Vaginal Secretions	Semen	Serum
	number/total number (percent)					
Participant analyses						
Any interval after symptom onset	128/146 (87.7)	90/145 (62.1)	13/143 (9.1)	1/49 (2.0)	31/55 (56.4)	137/139 (98.6)
0–7 days	118/134 (88.1)	77/129 (59.7)	3/6 (50.0)	1/1 (100)	0/1	17/50 (34.0)
8–15 days	10/28 (35.7)	12/29 (41.4)	1/25 (4.0)	0/6	5/8 (62.5)	28/28 (100)
16–30 days	27/129 (20.9)	21/125 (16.8)	5/127 (3.9)	0/39	20/40 (50.0)	120/121 (99.2)
31–45 days	14/126 (11.1)	6/119 (5.0)	4/125 (3.2)	0/42	20/46 (43.5)	108/111 (97.3)
46–60 days	6/67 (9.0)	1/65 (1.5)	1/64 (1.6)	0/21	6/25 (24.0)	58/60 (96.7)
>60 days	3/79 (3.8)	0/71	1/80 (1.3)	0/30	3/23 (13.0)	52/60 (86.7)
Specimen analyses						
Any interval after symptom onset	190/805 (23.6)	120/750 (16.0)	17/647 (2.6)	1/219 (0.5)	76/216 (35.2)	563/622 (90.5)
0–7 days	119/135 (88.2)	77/129 (59.7)	4/7 (57.1)	1/1 (100)	0/1	17/50 (34.0)
8–15 days	10/28 (35.7)	12/29 (41.4)	1/25 (4.0)	0/6	5/8 (62.5)	28/28 (100)
16–30 days	34/227 (15.0)	24/214 (11.2)	6/216 (2.8)	0/66	29/65 (44.6)	205/207 (99.0)
31–45 days	16/211 (7.6)	6/197 (3.0)	4/203 (2.0)	0/73	29/76 (38.2)	176/180 (97.8)
46–60 days	7/79 (8.9)	1/77 (1.3)	1/75 (1.3)	0/30	6/28 (21.4)	69/71 (97.2)
>60 days	4/125 (3.2)	0/104	1/121 (0.8)	0/43	7/38 (18.4)	68/86 (79.1)

* The number of participants and specimens that were evaluated at each interval after the onset of symptoms varies because participants were enrolled as they presented for surveillance or tested positive as household contacts. Data for the four household contacts who were asymptomatic at the time of enrollment were excluded from this analysis, since there was no known date of symptom onset. For these participants, the maximum duration of detection after enrollment was 65 days for serum and 15 days for urine; none of these participants contributed semen.
† Among the participants who had positive results for ZIKV RNA at the last study visit and are still being followed are 20 participants with ongoing serum analysis (maximum duration at last analysis, 80 days after symptom onset); 10 participants with ongoing urine analysis (maximum duration, 50 days after symptom onset); and 11 participants with ongoing semen analysis (maximum duration, 125 days after symptom onset).
‡ Three participants who had negative results on testing for IgM antibody are still being followed, since the last visit for each occurred at 4, 7, and 30 days after symptom onset.

estimate model-derived percentiles for the time until virus clearance at the population level, we assumed that all infected participants had ZIKV RNA in all specimens at symptom onset. This assumption resulted in shorter median and 95th percentile estimates than if we had limited our analyses only to participants with detectable ZIKV RNA. Data that were obtained from symptomatic participants may not be generalizable to all persons infected with ZIKV.

In conclusion, our study provides a longitudinal assessment of multiple body fluids to describe the persistence of ZIKV among infected participants. The results provide preliminary evidence that ZIKV is present in serum for a longer period than expected for other flaviviruses (e.g., dengue), a finding that may have implications for diagnostic recommendations and prevention of transmission.

The views expressed in this article are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Supported by the Centers for Disease Control and Prevention. Staffing support was provided by the Center for AIDS Research at Emory University (NIH P30AI050409).

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

We thank the study participants for their time and support of this project; and Dania Rodriguez-Vargas, Brad Biggerstaff, John T. Brooks, Laura Youngblood, Jennifer Read, Matt Lozier, Laura Adams, Candimar Colon, Tyree Staple, Olga Lorenzi, and ZiPer study staff members for their contributions to the study.

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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
米 CDC の研究は，ジカ発生前と比較し，ジカ感染の可能性がある妊娠において特定の先天性欠損が 20 倍増加したと推定している： CDC の MMWR の記事によれば、ジカが南北米で発生する以前の 2013 年～2014 年にみとめられた先天性欠損を伴う妊娠の割合と比較し，ジカの影響を受けた先天性欠損を伴う妊娠の割合は約 20 倍に増加している。脳異常および／または小頭症，神経管欠損およびその他の早期の脳奇形，眼球異常，およびその他の中枢神経系（CNS）の障害を含む先天性欠損は，2013 年～2014 年にはおよそ 3 例／1000 出生の割合でみとめられた。2016 年，妊娠中にジカウイルスに感染した女性から生まれた同種の先天性欠損を有する乳児の割合は，ジカウイルス感染を伴う妊娠 1000 例あたり 60 例で約 6%であった。	2. 重要な基本的注意 (1)本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-1 抗体陰性で、かつ ALT(GPT)値でスクリーニングを実施している。更に、HBV、HCV 及び HIV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分から人アンチトロンビン III を濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及びウイルス除去膜によるろ過処理を施しているが、投与に際しては、次の点に十分注意すること。

報告企業の意見	今後の対応
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。

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CDC study estimates 20-fold increase in certain types of birth defects in pregnancies with possible Zika infection compared with pre-Zika years

Media Statement

Embargoed until: Thursday, March 2, 2017, 1:00 PM
Contact: Media Relations (https://www.cdc.gov/media), (404) 639-3286

The proportion of Zika-affected pregnancies with birth defects is approximately 20-fold higher compared with the proportion of pregnancies seen in 2013-2014, which is before Zika was introduced into the Americas, according to an article published today in CDC's *Morbidity and Mortality Weekly Report.* The types of birth defects—including brain abnormalities and/or microcephaly, neural tube defects and other early brain malformations, eye defects, and other central nervous system (CNS) problems—were seen in about 3 of every 1,000 births in 2013-2014. In 2016, the proportion of infants with these same types of birth defects born to women with Zika virus infection during pregnancy was about 6% or nearly 60 of every 1,000 completed pregnancies with Zika infections.

The researchers analyzed 2013-2014 data from three birth defects surveillance programs in the United States (Massachusetts, North Carolina, and Georgia) to provide the baseline frequency for Zika-related birth defects. To assess the effect of Zika virus infection during pregnancy, the scientists compared that 2013-2014 baseline number with previously published numbers among pregnancies with Zika virus infection from the US Zika Pregnancy Registry (USZPR) from 2016.

They identified 747 infants and fetuses with one or more of these defects from programs in Massachusetts, North Carolina, and Georgia, from 2013-2014. Brain abnormalities and/or microcephaly were the most frequent conditions reported. Data from the USZPR identified 26 infants and fetuses with these same birth defects among the 442 completed pregnancies of women with possible Zika infection from January through September 2016. These findings demonstrate the importance of having monitoring systems that collect data on birth defects.

CDC supports state, territorial, tribal and local health departments to establish or enhance surveillance systems that rapidly collect information about birth defects that have been observed with Zika virus infection during pregnancy. Today, five additional

jurisdictions—Arkansas, Nebraska, Nevada, the Federated States of Micronesia, and the Republic of the Marshall Islands— were awarded funding, bringing the total to 50 jurisdictions and more than $27 million in support of birth defects surveillance for fiscal years 2016 and 2017.

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

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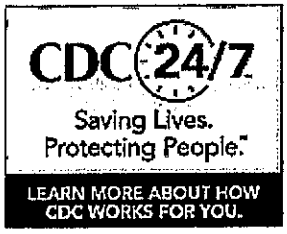

Page last reviewed: March 2, 2017

Page last updated: March 2, 2017

Content source: Centers for Disease Control and Prevention (/)

別紙様式第2-1
番号7

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2017年04月05日	新医薬品等の区分 該当なし	厚生労働省処理欄

一般的名称	①② ポリエチレングリコール処理抗破傷風人免疫グロブリン ③ 乾燥抗破傷風人免疫グロブリン	研究報告の公表状況	https://www.cdc.gov/mmwr/volumes/66/wr/mm6613e1.htm?s_cid=mm6613e1_w/2017/04/04	公表国 アメリカ
販売名 （企業名）	① テタノブリンIH静注250単位　（日本血液製剤機構） ② テタノブリンIH静注1500単位　（日本血液製剤機構） ③ テタノブリン筋注用250単位　（日本血液製剤機構）			

研究報告の概要

Vital Signs：ジカウイルス関連の先天性異常に関する更新情報および全米の先天性ジカウイルス感染した乳児の評価－U.S. Zika Pregnancy Registry, 2016年：

2016年1月15日～12月27日の調査期間中，U.S. Zika Pregnancy Registry（USZPR）に44州の妊婦1297例が報告された。最近のジカウイルス感染の可能性を示す臨床検査結果を有する出生した胎児／乳児972例のうち，51例（5%）においてジカウイルス関連の先天性異常が報告された。ジカウイルス感染の検査が確定した妊娠に限定した場合，その割合は高かった（妊娠完了250例に対して24例［10%］）。第1トリメスターにジカウイルス感染が確認され，妊娠期間を完了した胎児／乳児の15%において先天性異常が報告された。最近のジカウイルス感染の可能性がある妊婦からの生産児895例のうち，221例（25%）に出生後の神経画像検査結果が報告され，585例（65%）の乳児からの少なくとも1例にジカウイルス検査陽性が報告された。これらの知見は，妊婦がジカウイルス曝露を回避すべき理由を強調している。先天性ジカウイルス感染の全ての臨床スペクトルは現在明らかではないため，妊娠中における最近のジカウイルス感染の臨床検査値を有する女性から生まれた全ての乳児は，包括的な新生児の身体検査および聴覚スクリーニングに加え，出生後の神経画像検査およびジカウイルス検査を受けるべきである。

報告企業の意見	今後の対応
ジカウイルス（Zika virus）は1947年にウガンダのZika forest（ジカ森林）から発見されたウイルスで、デングウイルス、日本脳炎ウイルス、ウエストナイルウイルスと同じフラビウイルス科フラビウイルス属に属する。エンベロープを有するRNAウイルスで、蚊（ネッタイシマカ、ヒトスジシマカ）によって媒介される。万一、原料血漿にジカウイルスが混入したとしても、各種モデルウイルスのウイルスクリアランス試験成績から、本剤の製造工程において不活化・除去されると考える。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

代表としてテタノブリンIH静注250単位の記載を示す。
2. 重要な基本的注意
(1)本剤の原材料となる血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体陰性であることを確認している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した高力価の破傷風抗毒素を含有する血漿を原料として、Cohnの低温エタノール分画で得た画分からポリエチレングリコール4000処理、DEAEセファデックス処理等により抗破傷風人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及びウイルス除去膜によるろ過処理を施しているが、投与に際しては、次の点に十分注意すること。

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Vital Signs: Update on Zika Virus–Associated Birth Defects and Evaluation of All U.S. Infants with Congenital Zika Virus Exposure – U.S. Zika Pregnancy Registry, 2016

Weekly / April 7, 2017 / 66(13);366-373

On April 4, 2017, this report was posted online as an MMWR *Early Release.*

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Key Points

- In 2016, a total of 1,297 pregnancies with possible recent Zika virus infection were reported to the U.S. Zika Pregnancy Registry from 44 states.
- Approximately one in 10 pregnancies with laboratory-confirmed Zika virus infection resulted in a fetus or infant with Zika virus–associated birth defects.
- The proportion of fetuses and infants with Zika virus–associated birth defects was highest among those with first trimester Zika virus infections.
- Only 25% of infants from pregnancies with possible recent Zika virus infection reported receiving postnatal neuroimaging.
- Identification and follow-up care of infants born to mothers with laboratory evidence of possible recent Zika virus infection during pregnancy and infants with congenital Zika virus infection can ensure that appropriate intervention services are available to affected infants.
- Additional information is available at https://www.cdc.gov/vitalsigns/

(https://www.cdc.gov/vitalsigns/).

Abstract

Background: In collaboration with state, tribal, local, and territorial health departments, CDC established the U.S. Zika Pregnancy Registry (USZPR) in early 2016 to monitor pregnant women with laboratory evidence of possible recent Zika virus infection and their infants.

Methods: This report includes an analysis of completed pregnancies (which include live births and pregnancy losses, regardless of gestational age) in the 50 U.S. states and the District of Columbia (DC) with laboratory evidence of possible recent Zika virus infection reported to the USZPR from January 15 to December 27, 2016. Birth defects potentially associated with Zika virus infection during pregnancy include brain abnormalities and/or microcephaly, eye abnormalities, other consequences of central nervous system dysfunction, and neural tube defects and other early brain malformations.

Results: During the analysis period, 1,297 pregnant women in 44 states were reported to the USZPR. Zika virus–associated birth defects were reported for 51 (5%) of the 972 fetuses/infants from completed pregnancies with laboratory evidence of possible recent Zika virus infection (95% confidence interval [CI] = 4%–7%); the proportion was higher when restricted to pregnancies with laboratory-confirmed Zika virus infection (24/250 completed pregnancies [10%, 95% CI = 7%–14%]). Birth defects were reported in 15% (95% CI = 8%–26%) of fetuses/infants of completed pregnancies with confirmed Zika virus infection in the first trimester. Among 895 liveborn infants from pregnancies with possible recent Zika virus infection, postnatal neuroimaging was reported for 221 (25%), and Zika virus testing of at least one infant specimen was reported for 585 (65%).

Conclusions and Implications for Public Health Practice: These findings highlight why pregnant women should avoid Zika virus exposure. Because the full clinical spectrum of congenital Zika virus infection is not yet known, all infants born to women with laboratory evidence of possible recent Zika virus infection during pregnancy should receive postnatal neuroimaging and Zika virus testing in addition to a comprehensive newborn physical exam and hearing screen. Identification and follow-up care of infants born to women with laboratory evidence of possible recent Zika virus infection during pregnancy and infants with possible congenital Zika virus infection can ensure that appropriate clinical services are available.

Introduction

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In response to the outbreak of Zika virus in the World Health Organization Region of the Americas and concerns about birth defects linked to Zika virus infection during pregnancy, CDC issued a travel notice on January 15, 2016, advising pregnant women to consider postponing travel to areas with active transmission of Zika virus. As part of the initial phase of the emergency response, CDC collaborated with state, tribal, local, and territorial health departments to establish the U.S. Zika Pregnancy Registry (USZPR) as an enhanced national surveillance system to monitor pregnancy and fetal/infant outcomes among pregnancies with laboratory evidence of possible recent Zika virus infection (*1*). The USZPR includes data on pregnant women and their infants at birth and at ages 2, 6, and 12 months.

The USZPR includes data from all 50 states, DC, and all U.S. territories except Puerto Rico; pregnancies in Puerto Rico are monitored separately by the Zika Active Pregnancy Surveillance System (*2*). To be included in the USZPR, either the pregnant woman, placenta, or fetus/infant must have laboratory evidence of possible recent Zika virus infection. Pregnant women in the United States and U.S. territories (with the exception of Puerto Rico) with laboratory evidence of possible recent Zika virus infection (regardless of whether they have symptoms) and the periconceptionally,* prenatally, or perinatally exposed infants born to these women are eligible to be included. The USZPR also includes infants with laboratory evidence of possible congenital Zika virus infection (regardless of whether they have symptoms or findings at birth) and their mothers.

This report updates the previous report (*3*) from the USZPR and provides data on pregnancies completed in the 50 U.S. states and DC from December 1, 2015 through December 27, 2016, reported to CDC from January 15, 2016, through March 14, 2017.† Completed pregnancies include those of any length of gestation that end in a liveborn infant or a pregnancy loss. The baseline prevalence of defects consistent with those that have been observed with congenital Zika virus infection was approximately 2.9 per 1,000 live births in the pre-Zika years (*4*). The initial findings from the USZPR represent an approximate twentyfold increase in Zika virus–associated birth defects among pregnant women with laboratory evidence of possible recent Zika virus infection, with an approximate thirtyfold increase in brain abnormalities and/or microcephaly. Updated data in this report can also be compared with this benchmark (*3*,*4*).

Methods

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The USZPR defines laboratory evidence of possible recent Zika virus infection as 1) recent Zika virus infection detected by a Zika virus RNA nucleic acid test (NAT, e.g., reverse transcription–polymerase chain reaction [RT-PCR]) on any maternal, placental, or fetal/infant specimen or 2) detection of recent Zika virus infection or recent unspecified flavivirus infection by serologic tests on a maternal or infant specimen (i.e., either positive or equivocal Zika virus immunoglobulin M [IgM] AND Zika virus plaque reduction neutralization test [PRNT] titer ≥10, regardless of dengue virus PRNT value; or negative Zika virus IgM, AND positive or equivocal dengue virus IgM, AND Zika virus PRNT titer ≥10, regardless of dengue virus PRNT titer). Infants with positive or equivocal Zika virus IgM are included, provided a confirmatory PRNT has been performed on a maternal or infant specimen. The USZPR laboratory inclusion criteria are specified as "possible" recent Zika virus infection because the USZPR includes mother-infant pairs with serological evidence of a recent unspecified flavivirus infection, as well as those with laboratory-confirmed Zika virus infection.

Analyses were done on both the overall completed pregnancies in the USZPR from the 50 U.S. states and DC and a subset of completed pregnancies that demonstrated confirmed recent Zika virus infection (*5*,*6*). These are pregnancies in which the presence of Zika virus RNA in a maternal, placental, or fetal/infant specimen was documented by a positive NAT, or in which Zika virus IgM was positive or equivocal and Zika virus PRNT titer was ≥10 and dengue virus PRNT was <10.

Among symptomatic women, gestational timing of Zika virus infection was calculated using symptom onset date. Among asymptomatic women, the trimester of exposure was calculated using dates of travel to areas of active Zika virus transmission or sexual exposure. First trimester exposure was

classified into two categories: 1) women with symptoms or exposure in the first trimester only[§] (defined as first trimester or first trimester and periconceptional period); and 2) women with exposure during multiple trimesters including the first trimester. Estimates were not calculated for exposure in other trimesters because of small numbers. Pregnant women who did not have first trimester exposure might have had exposure in the periconceptional period only, second trimester, third trimester, or both the second and third trimester; for many women, the information on trimester of exposure was missing.

The Zika virus–associated birth defects (henceforth referred to as "birth defects") were analyzed in two mutually exclusive categories: 1) brain abnormalities and/or microcephaly regardless of the presence of additional birth defects, and 2) neural tube defects and other early brain malformations, eye abnormalities, and other consequences of central nervous system dysfunction, among fetuses and infants without evident brain abnormalities or microcephaly (*7*). Clinical experts reviewed reported information to ensure that each fetus or infant with birth defects met the criteria of the USZPR case definition.

The proportion of fetuses or infants with birth defects among completed pregnancies was estimated among asymptomatic and symptomatic pregnant women, and women with first trimester exposure, using the Wilson score interval and 95% CI for a binomial proportion. Outcomes from multiple gestation pregnancies were counted once. Separate estimates were calculated for pregnancies with any laboratory evidence of recent Zika virus infection and for the subset of pregnancies with laboratory-confirmed recent Zika virus infection. For all liveborn infants with and without birth defects, the proportion who had any reported postnatal neuroimaging (cranial ultrasound, computed tomography, or magnetic resonance imaging) was calculated, as well as the proportion who had laboratory testing for Zika virus reported on an infant specimen. CDC released updated Interim Guidance for the Evaluation and Management of Infants with Possible Congenital Zika Virus Infection in August 2016 (*8*), which stated that postnatal neuroimaging and testing should be routine for all infants born to women with laboratory evidence of Zika virus infection during pregnancy; the proportion of infants with neuroimaging performed was calculated before and after this guidance was released.

Results

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From January 15 through December 27, 2016, a total of 1,297 pregnancies with possible recent Zika virus infection were reported to the USZPR from 44 states (Figure 1), including 972 completed pregnancies with reported outcomes (895 liveborn infants and 77 pregnancy losses). Among the completed pregnancies, 599 (62%) pregnant women were asymptomatic, 348 (36%) were symptomatic, and 25 (3%) had missing symptom information (Table 1).

Birth defects were reported for 51 (5%) of the 972 completed pregnancies with laboratory evidence of possible recent Zika virus infection. The proportion was higher among completed pregnancies with confirmed Zika virus infection (24/250, 10%). Among completed pregnancies with confirmed Zika virus infection, 217 of 250 (87%) tested positive by RT-PCR, including 24 pregnancies with a fetus or infant with birth defects.

Birth defects were reported in similar proportions of fetuses/infants whose mothers did and did not report symptoms of Zika virus disease during pregnancy. Brain abnormalities and/or microcephaly were reported in 43 (84%) of 51 fetuses/infants with birth defects. Among pregnancies with confirmed Zika virus infection, brain abnormalities and/or microcephaly were reported in 18 (75%) of 24 fetuses/infants with birth defects. The 51 fetuses or infants with birth defects were from pregnancies with Zika virus exposure from the following 16 countries/territories with active Zika virus transmission: Barbados, Belize, Brazil, Cape Verde, Colombia, Dominican Republic, El Salvador, Guatemala, Guyana, Haiti, Honduras, Jamaica, Mexico, Puerto Rico, Republic of Marshall Islands, and Venezuela.

Birth defects were reported in a higher proportion of fetuses or infants whose mothers were infected during the first trimester of pregnancy. Among 157 pregnancies in which women had symptom onset or exposure to Zika virus infection during the first trimester, 14 (9%) fetuses/infants had reported birth defects (Table 1). When pregnancies with symptom onset or exposure during first trimester were limited to those with laboratory-confirmed Zika virus infection, nine (15%) of 60 completed pregnancies had reported birth defects.

Among the 895 liveborn infants, postnatal neuroimaging results were reported to the USZPR for 221 (25%). Zika virus testing results of any specimen were reported for 585 (65%) infants; 94 (11%) of all 895 liveborn infants had positive Zika virus test results. Among the 45 liveborn infants with birth defects, 25 (56%) had positive infant Zika virus testing results reported, and 29 (64%) had postnatal neuroimaging reported to the USZPR (Table 2). Among the 850 liveborn infants without birth defects, 69 (8%) had positive infant Zika virus testing results reported, and 192 (23%) had postnatal neuroimaging reported to the USZPR. The percentage of infants reported to have received postnatal neuroimaging was 20% among 406 born through August 2016, and 28% among 489 born during September–December 2016, after the updated CDC guidance was released (*8*) (Figure 2).

Conclusions and Comments

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The number of pregnant women with laboratory evidence of possible recent Zika virus infection and the number of fetuses/infants with Zika virus–associated birth defects continues to increase in the United States. The proportion of fetuses and infants with birth defects among pregnancies with confirmed Zika virus infection at any time during pregnancy was more than 30 times higher than the baseline prevalence in the pre-Zika years, and a higher proportion of those with first trimester infections had birth defects (*4*). Although microcephaly was the first recognized birth defect reported in association with congenital Zika virus infection, Zika virus–associated brain abnormalities can occur without microcephaly, and neuroimaging is needed to detect these abnormalities (*9*). Neuroimaging is also used in other congenital infections to identify brain abnormalities; for example, neuroimaging findings in infants with congenital cytomegalovirus infection are correlated with neurodevelopmental outcomes (*10*). Postnatal neuroimaging is recommended for all infants born to women with laboratory evidence of Zika virus infection to identify infants with brain anomalies that warrant additional evaluation to ensure that appropriate intervention is provided (*8*). Based on data reported to the USZPR, the majority of these infants had not received recommended neuroimaging. In addition to infants with birth defects, complete follow-up and routine developmental assessment of all infants

born to women with laboratory evidence of possible recent Zika virus infection is essential to help identify future outcomes potentially associated with congenital Zika virus infection and ensure that the referrals to appropriate support and follow-up care are made.

The findings in this report are subject to at least four limitations. First, selection bias might affect which pregnancies are reported to the USZPR, because pregnant women with symptoms of Zika virus disease might be more likely than asymptomatic women to be tested. Pregnant women with Zika virus exposure and prenatally detected fetal abnormalities or infants with birth defects might be more likely to be tested for Zika virus infection. In addition, pregnancies resulting in a loss might be more likely to have had a confirmed Zika virus infection and more likely to have the placenta or other pathologic specimens tested (*11*). However, it is also possible that birth defects in pregnancy losses, including stillbirths, have not been reported. Second, while CDC has worked closely with state and local health departments to obtain complete information, delays in reporting postnatal neuroimaging or infant Zika virus testing results are possible. In addition, some of the pregnancies included in the analysis were completed before CDC's most recent infant guidance (*8*) was released, and thus, current recommendations for neuroimaging or testing might not have been implemented. Third, current testing methodologies are limited in that they can only identify recent Zika virus infections (*5*) and might miss those women who are tested when Zika virus RNA and/or IgM is no longer detectable; these pregnancies would not be included in the USZPR unless the fetus/infant or placenta has a positive Zika virus test result. Also, serologic testing cannot readily discriminate between flaviviruses because of crossreactivity (*5*); therefore, some pregnancies in the USZPR might have had a recent infection with a flavivirus other than Zika virus which could lead to an underestimate of the proportion of fetuses/infants affected. For this reason, in this report, analysis of the subset of pregnancies with laboratory-confirmed recent Zika virus infection was included. Finally, limited data are available about other maternal risk factors for birth defects, including genetic or other infectious causes, which might be causal factors for a few of the birth defects reported here.

These findings underscore the serious risk for birth defects posed by Zika virus infection during pregnancy and highlight why pregnant women should avoid Zika virus exposure and that all pregnant women should be screened for possible Zika virus exposure at every prenatal visit, with testing of pregnant women and infants in accordance with current guidance (https://www.cdc.gov/zika/pdfs/zikapreg_screeningtool.pdf (https://www.cdc.gov/zika/pdfs/zikapreg_screeningtool.pdf)) (*8,12*). Zika virus testing of infants is recommended for 1) all infants born to women with laboratory evidence of Zika virus infection in pregnancy and 2) infants with findings suggestive of congenital Zika syndrome born to women with an epidemiologic link suggesting possible transmission, regardless of maternal testing results. Infants without abnormalities born to women with an epidemiological link suggesting possible Zika virus exposure during pregnancy, and for whom maternal testing was not performed or was performed more than 12 weeks after exposure, should have a comprehensive exam. If there is concern about infant follow-up or maternal testing is not performed, infant Zika virus testing should be considered. The initial evaluation of infants should include a comprehensive physical examination, including a neurologic examination, postnatal neuroimaging, and standard newborn hearing screen. Additional evaluation might be considered based on clinical and laboratory findings, however routine developmental assessment is recommended as part of pediatric care (*8*). Based on initial USZPR

reports, most infants born to women with laboratory evidence of possible recent Zika virus infection during pregnancy might not be receiving the recommended evaluation (e.g., postnatal neuroimaging). CDC is working with public health officials, professional societies, and health care providers to increase awareness of and adherence to CDC guidance for the evaluation and management of infants with possible congenital Zika virus infection. Identification and follow-up care of infants born to mothers with laboratory evidence of possible recent Zika virus infection during pregnancy and infants with possible congenital Zika virus infection can ensure that appropriate intervention services are available to affected infants.

Acknowledgments

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* Periconceptional exposure is defined as maternal Zika virus infection during the 8 weeks before conception (6 weeks before and 2 weeks after the first day of the last menstrual period).

† Data on pregnancies reported to CDC by December 27, 2016; all data have been updated with additional information reported on these pregnancies through March 14, 2017. Completed pregnancies are limited to those with a pregnancy completion date on or before December 27, 2016.

§ First trimester is defined as last menstrual period +14 days to 13 weeks, 6 days (97 days).

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FIGURE 1. Cumulative number of pregnant women with laboratory evidence of possible recent Zika virus infection reported to the U.S. Zika Pregnancy Registry, by month of report — United States, January–December 2016 (n = 1,297)

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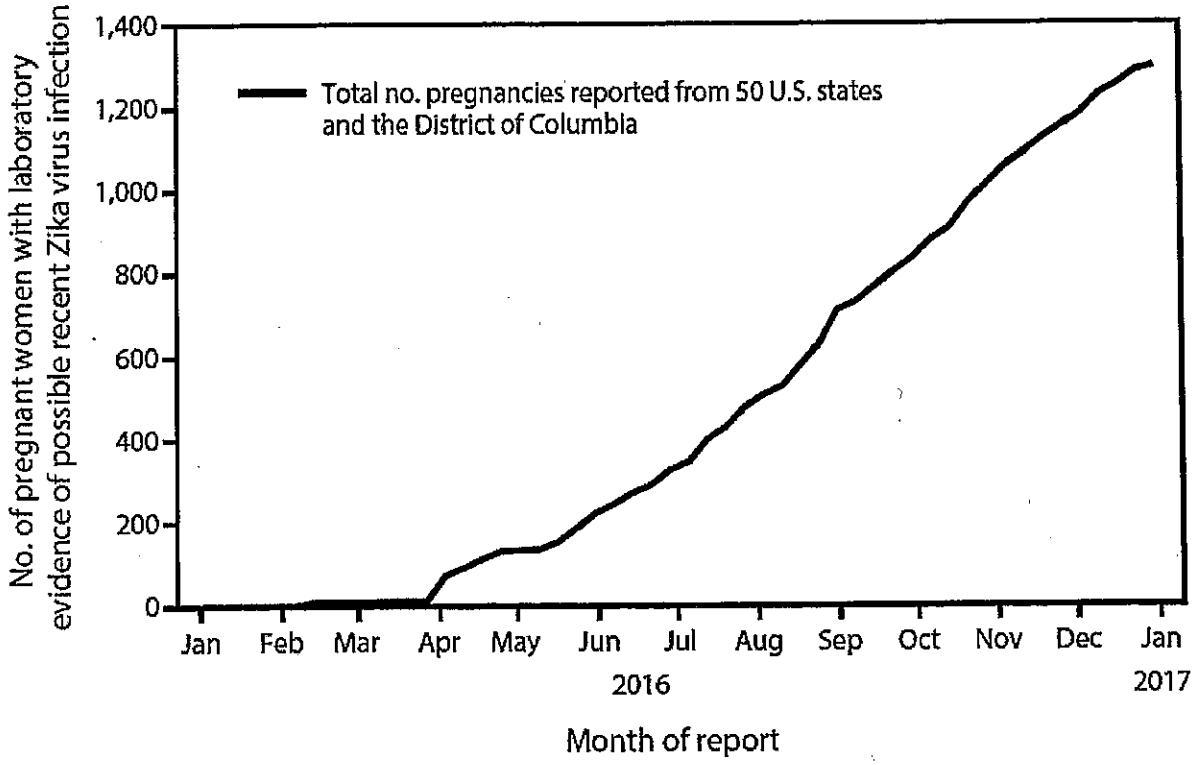

The figure above is a line chart showing the cumulative number of pregnant women with laboratory evidence of possible recent Zika virus infection reported to the U.S. Zika Pregnancy Registry, by month of report in the United States, during January–December 2016.

TABLE 1. Pregnancy outcomes* for 972 women with completed pregnancies† with laboratory evidence of possible recent Zika virus infection, by maternal symptom status and timing of symptom onset or exposure — U.S. Zika Pregnancy Registry, United States, December 2015–December 2016

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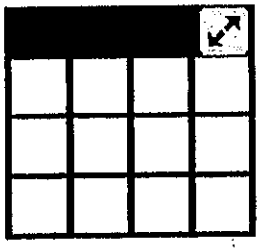

Characteristic	Brain abnormalities and/or microcephaly (No.)	NTDs and early brain malformations, eye abnormalities, or consequences of CNS dysfunction without brain abnormalities or microcephaly (No.)	Total with ≥1 birth defect (No.)	Completed pregnancies (No.)	Proportion affected by Zika virus–associated birth defects, % (95% CI[§])
Any laboratory evidence of possible recent Zika virus infection[¶]					
Total	43	8	51	972	5 (4–7)
Maternal symptom status					
Symptoms of Zika virus infection reported	18	3	21	348	6 (4–9)
No symptoms of Zika virus infection reported	24	4	28	599	5 (3–7)
Unknown	1	1	2	25	—
Timing of symptoms or exposure**					
First trimester[††,§§]	13	1	14	157	9 (5–14)
Multiple trimesters including first	22	6	28	396	7 (5–10)
Confirmed evidence of Zika virus infection[¶¶]					
Total	18	6	24	250	10 (7–14)
Maternal symptom status					
Symptoms of Zika virus infection reported	8	3	11	141	8 (4–13)

No symptoms of Zika virus infection reported	10	2	12	102	12 (7–19)
Unknown	1	0	1	7	—
Timing of symptoms or exposure**					
First trimester††,§§	8	1	9	60	15 (8–26)
Multiple trimesters including first	8	4	12	58	21 (12–33)

Abbreviations: CI = confidence interval; CNS = central nervous system; IgM= immunoglobulin M; NAT=nucleic acid test; NTD = neural tube defect; PRNT = plaque reduction neutralization test; RT-PCR = reverse transcription–polymerase chain reaction.

* Outcomes for multiple gestation pregnancies are counted once.

† Includes live births, spontaneous abortions, terminations, and stillbirths.

§ 95% CI for a binomial proportion using Wilson score interval.

¶ Includes maternal, placental, or fetal/infant laboratory evidence of possible recent Zika virus infection based on presence of Zika virus RNA by a positive NAT (e.g., RT-PCR) or similar test, serological evidence of a recent Zika virus infection, or serological evidence of a recent unspecified flavivirus infection.

** Estimates were not calculated for exposure in other trimesters because of small numbers. Pregnant women who did not have first trimester exposure might have had exposure in the periconceptional period only (8 weeks before conception or 6 weeks before and 2 weeks after the first day of the last menstrual period), second trimester, third trimester, both the second and third trimester; many women were missing information on trimester of exposure.

†† First trimester is defined as last menstrual period +14 days to 13 weeks, 6 days (97 days).

§§ First trimester exposure includes women with exposure limited to the first trimester and women with exposure limited to the first trimester and periconceptional period.

¶¶ Includes maternal, placental, or fetal/infant laboratory evidence of confirmed Zika virus infection based on presence of Zika virus RNA by a positive NAT (e.g., RT-PCR) or similar test or serological results of IgM positive/equivocal with Zika PRNT ≥10 and dengue PRNT <10.

TABLE 2. Postnatal neuroimaging* and infant Zika virus testing results for 895 liveborn infants in the U.S. Zika Pregnancy Registry — 50 U.S. states and the District of Columbia, 2016

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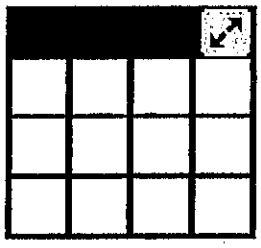

Testing	No (%) liveborn infants		
	With birth defects	**Without birth defects**	**Total**
Total	45	850	895
Neuroimaging			
Any neuroimaging reported to USZPR	29 (64)	192 (23)	221 (25)
Infant Zika virus testing			
Positive test result on an infant specimen[†,§]	25 (56)	69 (8)	94 (11)
Negative infant test results among infants with ≥1 infant specimen reported as tested	17 (38)	474 (56)	491 (55)
No infant specimen test results reported to USZPR	3 (7)	307 (36)	310 (35)

Abbreviations: IgM= immunoglobulin M; NAT=nucleic acid test; RT-PCR = reverse transcription –polymerase chain reaction; USZPR = U.S. Zika Pregnancy Registry.

* Neuroimaging includes any cranial ultrasound, computed tomography, or magnetic resonance imaging test reported to the USZPR.

† Positive infant tests included the presence of Zika virus RNA by a positive NAT (e.g., RT-PCR) and/or serological results of IgM positive/equivocal.

§ Infant specimens include serum, urine, blood, cerebrospinal fluid, cord serum, and cord blood.

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FIGURE 2. Postnatal neuroimaging for infants reported to the U.S. Zika Pregnancy Registry, by month of birth — United States, December 2015–December 2016

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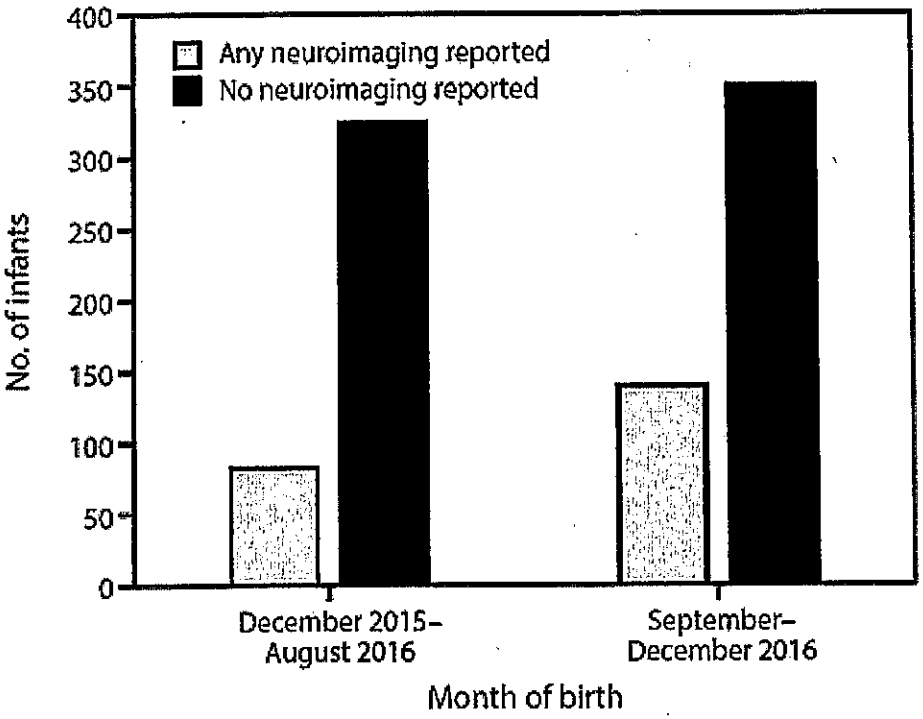

The figure above is a bar chart showing the number of infants with postnatal neuroimaging reported to the U.S. Zika Pregnancy Registry, by month of birth in the United States, during December 2015 –December 2016.

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Suggested citation for this article: Reynolds MR, Jones AM, Petersen EE, et al. Vital Signs: Update on Zika Virus–Associated Birth Defects and Evaluation of All U.S. Infants with Congenital Zika Virus Exposure — U.S. Zika Pregnancy Registry, 2016. MMWR Morb Mortal Wkly Rep 2017;66:366-373. DOI: http://dx.doi.org/10.15585/mmwr.mm6613e1 (http://dx.doi.org/10.15585/mmwr.mm6613e1)

(https://www.cdc.gov/mmwr/form/index.html).

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

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

Page last reviewed: April 4, 2017

Page last updated: April 4, 2017

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

別紙様式第2-1
番号6

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2017年03月16日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	乾燥濃縮人アンチトロンビンIII		研究報告の公表状況	TRANSFUSION 2017; 57( ): 720-721	公表国 アメリカ	
販売名 (企業名)	①ノイアート静注用500単位　(日本血液製剤機構) ②ノイアート静注用1500単位　(日本血液製剤機構)					

研究報告の概要

血漿分画製剤の製造工程に用いられている効果的なジカウイルス不活化工程の検証：

Blümelらは、最近、血漿分画製剤（PDMPs）の製造工程に広く導入されているいくつかのウイルス不活化／除去工程がジカウイルスの不活化／除去に有効であることを一般的なモデル系を用いて検証した。
血漿分画製剤の製造工程には、血漿分画製剤のウイルスの伝播に対して高い安全性マージンを有する特定のウイルス不活化／除去工程が含まれている。
ウイルス不活化／除去工程の効果およびロバストネス性は、実際のヒトへの病原性ウイルスまたは血液製剤に由来する性状の似たモデルウイルスを用いたウイルスバリデーションスタディーの実施により示される。
これまでの報告では、製造工程におけるBVDVやWNVのようなフラビウイルスを用いたウイルス低減化の効果が示された。
近年ジカウイルスの流行が注目され、商業生産スケールを模した縮小実験系モデルを用いて、ジカウイルスに対する液状加熱処理、低pH処理、有機溶媒／界面活性剤処理（S/D処理）及び20nmウイルス除去フィルター工程における検証実験を行った。
中間原料にジカウイルスを添加し、ウイルスリダクションを不活化処理前後のVero細胞を用いた感染力価を測定することにより評価した。
全ての工程は2回ずつ実施し、処理後に感染価が共に検出された場合は平均値を、処理後に感染価が共に検出されなかった場合はLRVが高くなる数値を選択した。
ジカウイルスは熱処理、S/D処理、低pHインキュベーションによる不活化を受けやすい。同様にBlümelらによって、ジカウイルスはS/D処理（1%ポリソルベート80/0.3%TNBP）によってインキュベーション開始30分以内に検出限界以下になることが示された。ジカウイルスは、シュクロースを安定剤としたVWF/FVIII製剤中の液状加熱により2時間以内に完全に不活化された。Blümelらは、シュクロースを安定剤に用いていないアルブミン溶液でも容易に不活化されること結果を出している。
ジカウイルスは低pH処理でも不活化されること、また20nmのウイルス除去フィルターでも除去されることを確認した。
我々の工程特異的なデータからは、WNVとBVDVのデータから推察されたようにこれらの一般的な不活化／除去工程はジカウイルスに対してとても高い効果を有し有効であることが示された。
この結果は、広範囲の病原体に対し工程の有効性を実証する検証と合せて、血漿分画製剤の製造工程におけるウイルス／不活化除去工程が、安全性を保証するための非常に有効な手段であり、新興感染症に対して積極的なアプローチであることを再確認した。

使用上の注意記載状況・その他参考事項等

2. 重要な基本的注意
(1)本剤の原材料となる献血者の血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体、抗HTLV-1抗体陰性で、かつALT(GPT)値でスクリーニングを実施している。更に、HBV、HCV及びHIVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohnの低温エタノール分画で得た画分から人アンチトロンビンIIIを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及びウイルス除去膜によるろ過処理を施しているが、投与に際しては、次の点に十分注意すること。

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

報告企業の意見	今後の対応	
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。	

Verification of effective Zika virus reduction by production steps used in the manufacture of plasma-derived medicinal products

Blümel and colleagues[1] recently performed studies in generic model systems that demonstrated that Zika virus (ZIKV) is sensitive to some of the commonly used inactivation and removal procedures that are widely used during the manufacture of plasma-derived medicinal products (PDMPs). We have data that are specific to actual manufacturing processes for products currently available for patients in the United States, Europe, Australia, and other parts of the world that supplement and corroborate their report.

The manufacturing processes of PDMPs include specific virus inactivation or virus removal steps designed to ensure a high safety margin with regard to virus transmission. The efficacy and robustness of the inactivation or removal steps are shown through virus validation studies performed with a panel of relevant human pathogenic viruses or with model viruses that have similar properties to the blood-borne viruses of concern.

Previous studies with *Flaviviruses* such as bovine viral diarrhea virus (BVDV) and West Nile virus (WNV), conducted as part of PDMP licensing submissions, clearly demonstrate the efficacy of the manufacturing process(es) to reduce such viruses.[2-4] In light of the recent ZIKV epidemic, additional studies have now been conducted to verify the efficacy of pasteurization, low pH treatment, solvent/detergent (S/D) treatment and 20-nm virus filters against ZIKV using samples of intermediates obtained from commercial manufacturing processes and employing validated scale-down models of commercial manufacturing processes. ZIKV was spiked into these production intermediates and virus reduction was evaluated by determining virus titers at specific time points (feed volume for virus filtration) using a Vero cell culture—based virus infectivity assay (log $CCID_{50}$/mL). Replicates were performed for all studies and the mean value was reported when infectious virus was detectable, whereas the highest log reduction value (LRV) was reported when no detectable infectivity was observed (since the capacity of the step exceeded the maximum LRV experimentally demonstrable).

ZIKV was highly susceptible to inactivation by heat treatment, S/D treatment, and low pH incubation. Similar to that demonstrated by Blümel and colleagues, ZIKV was inactivated by S/D treatment (1% polysorbate 80, 0.3% TNBP) reaching the limit of detection within 30 minutes of incubation. ZIKV was completely inactivated to the limit of detection within 2 hours of pasteurization (10 hr at 60°C) in an aqueous sucrose stabilized von Willebrand factor (VWF)/Factor (F)VIII (Fig. 1A, Table 1); this is in line with the results by Blümel and colleagues where susceptibility to heat treatment was evaluated in a non–sucrose-stabilized albumin solution (Table 1). We also supplemented the data by Blümel and colleagues, by demonstrating that ZIKV was highly susceptible to low pH inactivation (pH 4.0 at 37°C)—being completely inactivated within 30 minutes of incubation (Fig. 1B). Finally, our data confirmed that ZIKV was removed to below the

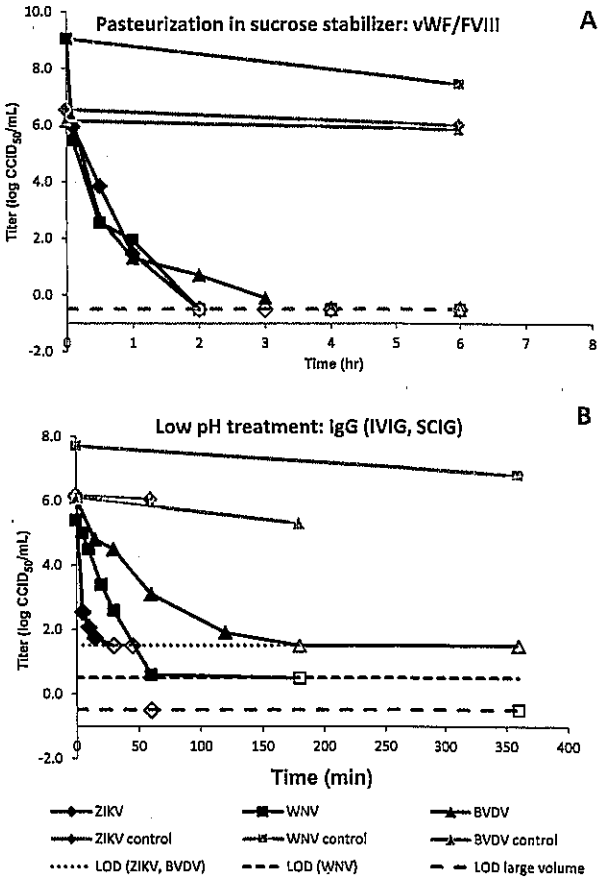

Fig. 1. Susceptibility of *Flaviviruses* to (A) pasteurization in a sucrose-stabilized VWF/FVIII intermediate (60°C) or (B) low pH treatment in an IgG intermediate (pH 4.0, 37°C). Hold control samples are shown in gray. Closed symbols = infectivity was detected; open symbols = no residual infectivity detected; limit of detection (LOD) sensitivity is dependent on volume assayed; higher sensitivity employed large-volume testing LOD = dotted gray or black lines.

doi:10.1111/trf.14038

TABLE 1. Log reduction factors for inactivation or removal of ZIKV and other model *Flaviviruses* by manufacturing steps of plasma-derived products

Virus reduction method	Test virus (size):	ZIKV log virus reduction factor (time to no detectable infectivity)* ZIKV (48-50 nm)	WNV (50 nm)	BVDV (40-60 nm)
Pasteurization (VWF/FVIII)		≥7.2 (2 hr)	≥6.0 (2 hr)	≥6.7 (4 hr)
S/D treatment (PS80/TnBP) (FVIII—Product A)		≥6.0 (30 min)	ND	≥2.6 (5 min) ≥5.2 (180 min)†
pH 4 treatment (IgG—SCIG/IVIG)		≥6.8 (60 min)	≥6.0 (360 min)	≥4.6 (180 min)
20 nm virus filtration (FVIII—Product B)		≥7.0	ND	≥5.8

* At least two runs were performed for each study; the highest factor achieved is shown. Replicate values were always within 1 log of each other.
† At 180 min, large-volume testing was performed to increase assay sensitivity.
ND = not determined.

limit of detection by a 20-nm virus filter (≥7.0 log). Our process-specific data demonstrate that these common virus inactivation and removal steps are highly effective in eliminating ZIKV, as predicted based upon our previously validated data for WNV and BVDV (Table 1).

Overall, these scientific product and process specific data corroborate and supplement the data by Blümel and coworkers providing additional evidence that current PDMP manufacturing processes are very effective in reducing *Flaviviruses*, including ZIKV, and support the concept that BVDV or WNV can be considered as valid model viruses for ZIKV.

If infectious ZIKV were to be present in a plasma manufacturing pool, residual risk calculations performed according to regulatory guidance demonstrate that the efficacy and capacity of the validated virus inactivation and virus filtration steps in eliminating ZIKV (or any lipid-enveloped arbovirus with a diameter of 40-60 nm) would result in final fractionated products that have a high margin of safety against ZIKV transmission. The results reaffirm that the dedicated virus inactivation and removal process steps within the manufacturing processes of PDMPs, in combination with virus validation studies that demonstrate the effectiveness of these steps against a broad variety of pathogens, provide a highly effective proactive approach toward assuring the safety of PDMPs against emerging pathogens.

CONFLICT OF INTEREST

The authors are employees of CSL Behring, a manufacturer of plasma-derived biotherapies.

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別紙様式第２

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日 2017/03/27	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	①乾燥pH4処理人免疫グロブリン ②～④pH4処理酸性人免疫グロブリン（皮下注射）		研究報告の公表状況	http://www.promedmail.org/post/4910178	公表国 米国	
販売名（企業名）	①サングロポール点滴静注用2.5g ②ハイゼントラ20%皮下注1g/5mL ③ハイゼントラ20%皮下注2g/10mL ④ハイゼントラ20%皮下注4g/20mL （CSLベーリング株式会社）					

研究報告の概要	2015年7月には、ポックスウイルス感染と一致する皮膚病変を有するアラスカ州内の女性居住者が、救急病院を受診した。病変から分離されたウイルス検査により、オルソポックスウイルスによる感染が確認された。 このウイルス分離体は、電子顕微鏡および核酸の塩基配列により同定された。感染源と新たな感染患者の可能性を特定するために、環境および小哺乳類サンプリングに加えて、患者の問診、接触追跡および血清検査を含む疫学調査が行われた。患者との接触が確認された4例のいずれにおいても、活動性感染の徴候も、直近の不顕性感染の根拠も認められなかった。患者の感染源は明確には特定されなかった。暴露経路の可能性として、患者の同居者によりアゼルバイジャンから、あるいは患者住居内または周囲の野生小哺乳類からの伝播媒介物が考えられる。分子系統樹解析によりウイルスは、明らかにこれまで未記載のオルソポックスウイルスの遺伝系統で、旧世界のオルソポックスウイルスに最も密接に関連していることが判明した。 調査結果は、患者の感染がフェアバンクス内またはその近接地でのウイルス曝露によることを示唆する。本結論は、オルソポックスウイルス属の地理的起源（旧世界対北米）に関する問題を提起する。臨床医は、ポックスウイルス感染の兆候について警戒を続け、発症が疑われる場合には公衆衛生当局に通報する必要がある。	使用上の注意記載状況・ その他参考事項等

報告企業の意見	今後の対応
オルソポックスウイルスは大型の DNA ウイルスである。血液製剤の製造工程にはウイルス除去・不活化工程があり、各種モデルウイルスを用いたウイルスクリアランス試験で効果が認められていることから新型ウイルスに対しても安全対策は取られていると考えられる。	今後とも新しい感染症に関する情報収集に努める所存である。

Published Date: 2017-03-18 15:12:59
Subject: PRO/EDR> Orthopoxvirus infection - USA: (AK)
Archive Number: 20170318.4910178

ORTHOPOXVIRUS INFECTION - USA: (ALASKA)
****
A ProMED-mail post
http://www.promedmail.org
ProMED-mail is a program of the
International Society for Infectious Diseases
http://www.isid.org

Date: Wed 15 Mar 2017
Source: Clin Infect Dis cix219. DOI: https://doi.org/10.1093/cid/cix219 [edited]
https://academic.oup.com/cid/article-abstract/doi/10.1093/cid/cix219/3072047/Novel-Orthopoxvirus-infection-in-an-Alaska?redirectedFrom=fulltext

Ref: Springer YP, Hsu CH, Werle ZR, et al. Novel Orthopoxvirus infection in an Alaska resident. Clin Infect Dis cix219. DOI: https://doi.org/10.1093/cid/cix219.

Abstract

Background
Human infection by orthopoxviruses is being reported with increasing frequency, attributed in part to the cessation of smallpox vaccination and concomitant waning of population-level immunity. In July 2015, a female resident of interior Alaska, presented to an urgent care clinic with a dermal lesion consistent with poxvirus infection. Laboratory testing of a virus isolated from the lesion confirmed infection by an Orthopoxvirus.

Methods
The virus isolate was characterized by using electron microscopy and nucleic acid sequencing. An epidemiologic investigation that included patient interviews, contact tracing and serum testing, as well as environmental and small mammal sampling was conducted to identify the infection source and possible additional cases.

Results
Neither signs of active infection nor evidence of recent prior infection were observed in any of the 4 patient contacts identified. The patient's infection source was not definitively identified. Potential routes of exposure included imported fomites from Azerbaijan by the patient's cohabiting partner, or from wild small mammals in or around the patient's residence. Phylogenetic analyses demonstrated that the virus represents a distinct and previously undescribed genetic lineage of Orthopoxvirus, which is most closely related to the Old World orthopoxviruses.

Conclusions
Investigation findings point to infection of the patient following exposure in or near Fairbanks. This conclusion raises questions about the geographic origins (Old World versus North American) of the genus Orthopoxvirus. Clinicians should remain vigilant for signs of poxvirus infection and alert public health officials when cases are suspected.

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Communicated by:
ProMED-mail
<promed@promedmail.org>

[This is the 1st Orthopoxvirus infection in humans in Alaska, USA that ProMED-mail has reported. The close relationship of this virus to Old World Orthopoxviruses raises the question of the origin of the Alaska virus. If fomites from Azerbaijan might have been the source of the virus, it would be of interest to know what those fomites were and if virus was recovered from that source. Fortunately, there was no

transmission of the virus from the case to other people. - Mod.TY

A HealthMap/ProMED-mail map can be accessed at: http://healthmap.org/promed/p/206.]

.....dk/ty/ao/dk

別紙3

医薬品 研究報告 調査報告書

識別番号・報告回数		回	報告日 年 月 日	第一報入手日 2017年 3月 27日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	オクトコグ アルファ(遺伝子組換え)		研究報告の公表状況	Springer YP et al. Novel Orthopoxvirus infection in an Alaska resident. Clinical Infectious Disease. 2017;:. DOI:10.1093/cid/cix219.	公表国 米国	
販売名(企業名)	コージネイト FS250IU 注射用 コージネイト FS500IU 注射用 コージネイト FS1000IU 注射用 (バイエル薬品株式会社)					

研究報告の概要

問題点：米国アラスカ州において新規のオルソポックスウイルスによる皮膚潰瘍を呈した初めてのヒト症例が確認された。

【概要】新たにヒトにおいて感染することが認められた感染症に関する報告。

［経過］
本症例はアラスカ州内陸部に居住する中年女性であり、既往歴として甲状腺機能低下症を有していた。受診の5日前より発熱、疲労、倦怠感およびリンパ節圧痛を呈しており、右肩のクモ咬傷疑いの主訴で緊急治療クリニックを受診した。最近の病人との接触、州外への旅行歴および再発性皮膚感染の既往歴はなかった。右肩上部後側に直径1cm以内の表在性潰瘍および2つの隣接した直径2mm以内の小水疱を認めた。潰瘍は限局性硬結、熱感および圧痛と関連付けられたが、波動または分泌物はなかった。紅斑の単一線状線条は、右肩から前側へ、さらに正中線を横切らず右上胸部へ進展していた。医師は、紅斑の分布が第5頚椎神経根デルマトームと一致し、ウイルス感染の懸念があると結論した。
小水疱を切除、塗布し、その塗布物は培養および診断検査のためアラスカ州ウイルス学研究所へ供試された。塗布物はMRC5、HEp-2およびRMK細胞系で培養され、全3組織培養で細胞変性効果がみられた。単純ヘルペスウイルスに対する直接蛍光抗体検査および水痘帯状疱疹ウイルスに対するPCR検査は、いずれも陰性であった。MRC5細胞系からの分離株はアラスカ州公衆衛生研究所へ供試され、非痘瘡および痘瘡特異的オルソポックスウイルスPCR検査は陰性であったが、一般的なオルソポックスウイルスのPCR検査は陽性であった。本来の塗布物検体および3細胞培養分離株（3細胞系より各1つ）は疾病管理予防センター（ポックスウイルス研究所）へ供試され、オルソポックスウイルス属PCR検査にて提出された全4検体は陽性であった。
患者は病変の完全な消失に6ヵ月を要し、発端患者からの伝播または感染による死亡者に関するエビデンスはないと報告された。

［結果］
シーケンスアライメント（長さ28037塩基対）の系統解析により、ウイルス分離株が本解析に含めた同属種とは大きく分かれたオルソポックスウイルスの遺伝的リネージであることが示された。以下、本ウイルスをAK2015_poxvirusとする。AK2015_poxvirusはオルソポックスウイルス属に分類され、すべての旧世界オルソポックスウイルスを含む単系統クレードと高く支持された姉妹関係にあった。旧世界オルソポックスウイルスの様々な種と6.1%-7.3%、北米クレードの分離株と12.3%-12.6%異なると推定された。遺伝的距離は既知の旧世界オルソポックスウイルスに含まれる種と0.6%（Camelpox virusに対するTaterapox virus）から3.2%（Variola virusに対するEctromelia virus）異なると推定した。AK2015_poxvirusに感染した細胞の電子顕微鏡観察では、三日月形や未成熟ウイルス粒子を含む様々な形態学的形状の存在が示された。さらに、感染した細胞は、A型封入体蛋白質の蓄積により形成された封入体を有しており、封入体には成熟したウイルス粒子が包埋され、リボソームで囲まれていた。
患者は発症前の4週間に病人との接触はなく、発症後の4週間に4人との接触があったと報告された。4例から採取された検体で実施された血清学的検査によって、最近の接触による曝露のエビデンスは特定されず、抗オルソポックスウイルスIgMは本患者の血清のみで検出された。23の環境検体および小型哺乳類から採取された31検体は、PCR検査にてオルソポックスウイルス陰性であった。

［考察］
患者の感染経路は特定できなかった。可能性のある曝露経路として、本患者の同居者によるアゼルバイジャンからの輸入媒介物、あるいは本患者の居住地付近に生息する野生小型動物が含まれた。系統解析ではAK2015_poxvirusがオルソポックスウイルス属の未知の遺伝的リネージであることが示され、旧世界オルソポックスウイルスに最も近縁であった。調査所見から、患者はフェアバンクス付近での曝露後に感染したことが示された。

使用上の注意記載状況・その他参考事項等

BYL-2017-0437

報告企業の意見	今後の対応
本件は、米国において新規のオルソポックスウイルスによる皮膚潰瘍を呈した初めてのヒト症例が確認されたとの報告である。 コージネイトFSの製造工程における病原体除去・不活化処理は、ウイルス及び細菌に対して有効であることが報告されている。なお、2007年4月以降、コージネイトFSの販売は行っていない。	現時点で新たな安全対策上の措置を講じる必要はないと考える。 今後も、新規人畜共通感染症や新たなウイルス感染症に関する情報収集に努める。

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Clinical Infectious Diseases

MAJOR ARTICLE

Novel *Orthopoxvirus* Infection in an Alaska Resident

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Background. Human infection by orthopoxviruses is being reported with increasing frequency, attributed in part to the cessation of smallpox vaccination and concomitant waning of population-level immunity. In July 2015, a female resident of interior Alaska presented to an urgent care clinic with a dermal lesion consistent with poxvirus infection. Laboratory testing of a virus isolated from the lesion confirmed infection by an *Orthopoxvirus*.

Methods. The virus isolate was characterized by using electron microscopy and nucleic acid sequencing. An epidemiologic investigation that included patient interviews, contact tracing, and serum testing, as well as environmental and small-mammal sampling, was conducted to identify the infection source and possible additional cases.

Results. Neither signs of active infection nor evidence of recent prior infection were observed in any of the 4 patient contacts identified. The patient's infection source was not definitively identified. Potential routes of exposure included imported fomites from Azerbaijan via the patient's cohabiting partner or wild small mammals in or around the patient's residence. Phylogenetic analyses demonstrated that the virus represents a distinct and previously undescribed genetic lineage of *Orthopoxvirus*, which is most closely related to the Old World orthopoxviruses.

Conclusions. Investigation findings point to infection of the patient after exposure in or near Fairbanks. This conclusion raises questions about the geographic origins (Old World vs North American) of the genus *Orthopoxvirus*. Clinicians should remain vigilant for signs of poxvirus infection and alert public health officials when cases are suspected.

Keywords. Alaska; lesion; North America; *Orthopoxvirus*; phylogenetics.

On 29 July 2015, a middle-aged woman (specific details about the index patient, her residence, and contacts identified during the investigation have been generalized or removed in the interest of maintaining privacy) presented to an urgent care clinic in Fairbanks, Alaska, with the chief complaint of a suspected spider bite on her right shoulder. She reported having experienced fever, fatigue, malaise, and tender lymph nodes during the 5 days before seeking care. Her medical history was significant only for hypothyroidism. She denied any recent contact with other sick persons, out-of-state travel, or recurrent skin infections. A physician examination confirmed a superficial ulceration, approximately 1 cm in diameter, and 2 smaller adjacent vesicles, approximately 2 mm in diameter, on posterior upper aspect of the patient's right shoulder. The ulceration was associated with localized induration, warmth, and tenderness but had no fluctuance or discharge (Figure 1A). A single linear streak of erythema extended anteriorly over the patient's right shoulder and back to the upper part of her chest on the right side, without crossing the midline. Her physician concluded that the distribution of erythema was consistent with a fifth cervical nerve root dermatome and raised concern for a viral infection. He deroofed and swabbed a vesicle and sent the swab sample (in universal transport media) to the Alaska State Public Health Virology Laboratory for culture and diagnostic testing.

The swab sample was placed into culture in MRC5, HEp-2, and RMK cell lines on 3 August, and by 10 August cytopathic effects were observed in all 3 tissue cultures. Results of both a direct fluorescent antibody test for herpes simplex virus and a polymerase chain reaction (PCR) test for varicella zoster virus (performed at the California Department of Public Health) were negative. On 17 August, an isolate from the MRC5 cell line was sent to the Alaska State Public Health Laboratory where results of non-*Variola* and *Variola*-specific *Orthopoxvirus* PCR tests were negative; however, a generic *Orthopoxvirus* PCR test had positive results. On 24 August, the original swab sample and 3 cell culture isolates (1 from each of the 3 cell lines) were sent to the Poxvirus Laboratory of the Centers for Disease

Received 14 December 2016; editorial decision 6 February 2017; accepted 8 March 2017; published online March 15, 2017.

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Clinical Infectious Diseases® 2017;64(12):1737–41

DOI: 10.1093/cid/cix219

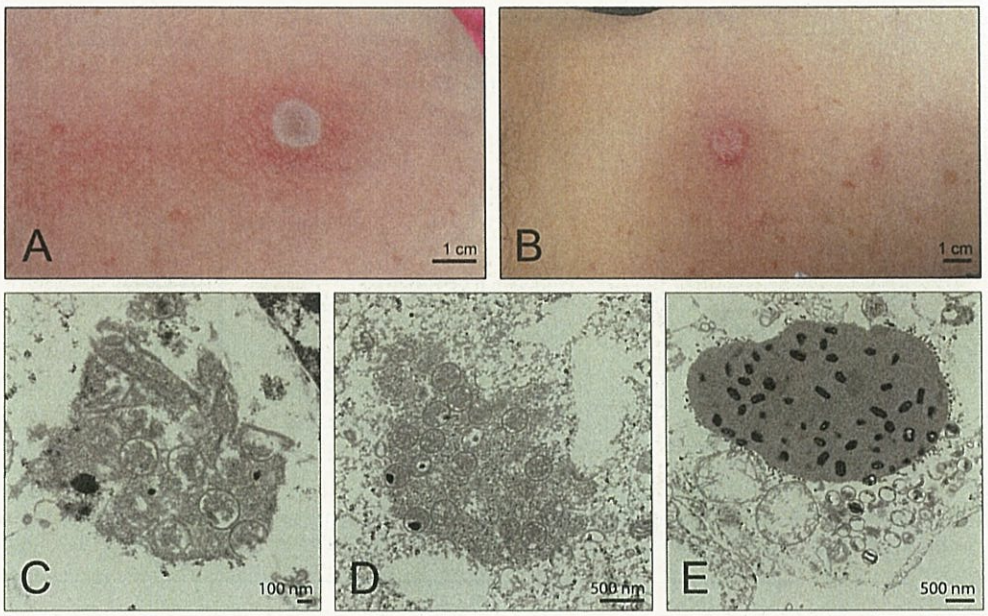

Figure 1. *A, B,* Patient's lesion on 29 July 2015 (*A*) and 20 August 2015 (*B*). *C–E,* Electron microscopic images of the virus isolated from the patient showing crescents (*C*), spherical immature virus in virus factories (*D*), and A-type inclusion bodies occluded with mature virus in infected cells (*E*).

Control and Prevention, where an *Orthopoxvirus*-generic PCR assay showed positive results on 27 August for all 4 submitted samples.

METHODS

An investigation was initiated to characterize the virus and identify the patient's infection source. The phylogenetic position of the virus was determined by using DNA sequence analysis. Phylogenetic inference was based on 9 genes located within the central, conserved region of the genome (Supplementary Methods). Virus morphological characteristics were observed by using transmission electron microscopy (Supplementary Methods).

Methods used to identify the patient's infection source included interviews with the patient, contact tracing and analysis of serum samples for the presence of anti-*Orthopoxvirus* immunoglobulin (Ig) G and IgM, and a visit to the patient's residence to conduct environmental sampling and peridomestic small-mammal trapping. Given the uncertainty about an incubation period for this novel poxvirus, a conservative threshold of 4 weeks was assumed based on the incubation period of human monkeypox [1]. The patient was asked to identify persons with whom she had regular or close contact during the 4 weeks before and after symptom onset. The patient and each contact were interviewed to ascertain whether they had ever received a smallpox vaccination, had traveled recently, or had experienced any unusual health events during the presumptive incubation period. Serum samples were obtained from these persons and tested with enzyme-linked immunosorbent assays to determine the presence of anti-*Orthopoxvirus* IgM and IgG antibodies [2].

On 8 September, environmental samples were collected in and around the patient's home during a site visit. Household surfaces that the patient indicated had been contacted by wild small mammals that periodically entered the home and possible fomites associated with international travel by the patient's partner were swabbed by using HydraFlock Dacron swab samples (Puritan Medical). Feces of wild small mammals found around the home's perimeter were also collected. Approximately 6 weeks later, small mammals were trapped around the perimeter of the patient's home and at a site approximately 1 km away where she and her partner were building a new home (Supplementary Methods). Environmental and nonblood small-mammal samples (oral swab, liver tissue, and feces) were tested using real-time PCR assays that target specific orthopoxvirus generic sequences [3–5]; small-mammal blood samples were tested using enzyme-linked immunosorbent assays for anti-*Orthopoxvirus* IgG [6].

RESULTS

Phylogenetic analyses of the concatenated sequence alignment (28 037 base pairs in length) indicate that the virus isolate represents a distinct genetic lineage of orthopoxvirus that is highly divergent from congeners included in our analysis. We will hereafter refer to the virus as AK2015_poxvirus. A well-supported topology was recovered in which the grouping of *Taterapox virus* and *Variola virus* as sister taxa (Bayesian posterior probability, 0.5983) was the only node with a Bayesian posterior probability <0.999 (Figure 2, Supplementary Figure 1, and Supplementary Table 1). AK2015_poxvirus was grouped within the genus *Orthopoxvirus* and recovered as sister to a monophyletic clade

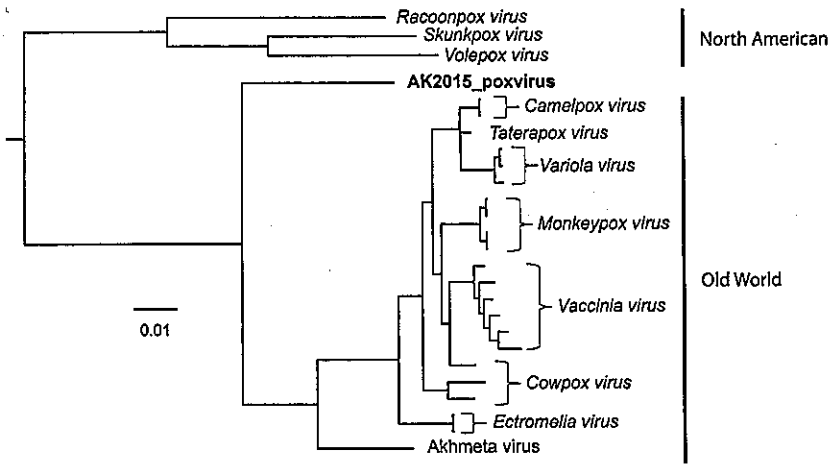

Figure 2. Results of Bayesian phylogenetic inference, indicating the position of AK2015_poxvirus within the genus *Orthopoxvirus*. Analysis was based on 9 genes located within the central, conserved region of the genome (*Vaccinia virus* Copenhagen strain homologues A7L, A10L, A24R, D1R, D5R, E6R, E9L, H4L, and J6R).

containing all Old World orthopoxviruses with high support; it was estimated to be 6.1%–7.3% divergent from different species of Old World orthopoxviruses, and 12.3%–12.6% divergent from isolates within the North American clade. The genetic distances estimated between examined isolates of recognized Old World orthopoxvirus species varied from 0.6% (*Taterapox virus* to *Camelpox virus*) to 3.2% (*Ectromelia virus* to *Variola virus*).

Electron microscopic observation of cells infected with AK2015_poxvirus demonstrated the presence of different morphological forms, including crescents (Figure 1C) and immature virus particles (Figure 1D). In addition, infected cells had inclusion bodies formed by accumulation of A-type inclusion proteins. The inclusion bodies were embedded with mature virus particles and surrounded by ribosomes (Figure 1E).

During interviews, the patient reiterated a lack of sick contacts during the 4 weeks before symptom onset. During the 4 weeks after symptom onset, she reported contact with 4 persons. Of 3 household contacts (adult male partner and 2 teenaged children), all had regular, direct physical contact with her and with common household items, but none reported any unusual health events. One social contact (adult female friend) reported a rash on her chest within 1 week of being in physical contact with the patient in the days after the patient's symptom onset. A history of smallpox vaccination was reported by both adult contacts (vaccination before 2003) but neither adolescent contact. The patient did not recall being vaccinated and did not have a vaccination scar. Serological tests performed on specimens collected from these persons did not identify evidence of recent exposure among the contacts; anti-*Orthopoxvirus* IgM was only detected in serum from the patient (Table 1). Consistent with their self-reported smallpox vaccination histories, anti-*Orthopoxvirus* IgG was detected in the serum from both adult contacts but neither adolescent contact. The serum sample from the patient also tested positive for anti-*Orthopoxvirus* IgG.

The patient reiterated that no out-of-state travel occurred during the 4 weeks before symptom onset. She reported working intermittently in the petroleum industry in the North Slope region of Alaska, during February–April 2015. Her cohabitating partner was also employed in the petroleum industry and had worked intermittently (5 weeks on and 5 weeks off) on oil-drilling platforms in Azerbaijan, from October 2013 to March 2015. He had no other out-of-state travel during this period and while

Table 1. Anti-*Orthopoxvirus* IgG and IgM Results for Serum Samples from the Patient and 4 Contacts[a]

Person	Smallpox vaccination	IgM Value[b]	IgM Interpretation	IgG Value[b]	IgG Interpretation
Patient	Unlikely[c]	0.091	Positive	0.183	Positive
Patient's partner (household contact)	Yes	−0.024	Negative	0.426	Positive
Patient's older child (household contact)	No	0.032	Equivocal[d]	−0.201	Negative
Patient's younger child (household contact)	No	−0.020	Negative	−0.194	Negative
Patient's friend (social contact)	Yes	−0.034	Negative	0.483	Positive

Abbreviation: Ig, immunoglobulin.

[a]Serology was performed using enzyme-linked immunosorbent assays.

[b]Serum optical density (OD) cutoff values (OD value − 3 standard deviations of negative control) at 1:50 and 1:100 dilutions were considered positive for IgM and IgG, respectively.

[c]The patient did not recall being vaccinated and did not have a vaccination scar.

[d]The sample from the patient's older child was positive for anti-*Orthopoxvirus* IgM, but because this value was low and the corresponding anti-*Orthopoxvirus* IgG result was negative, the result was interpreted as equivocal.

overseas he remained almost exclusively on oil platforms with limited in-country travel. He returned to Alaska from his last trip to Azerbaijan in March 2015 (approximately 4 months before the patient's symptom onset) and did not report any unusual health events during or within 4 weeks after the trip. When he returned from a trip to Azerbaijan in October 2014 (approximately 9 months before the patient's symptom onset), he brought back souvenirs for the patient (a wooden jewelry box containing a cloth jewelry pouch) and a collared work jacket that he wore regularly during the trip and that the patient wore periodically after his return to Alaska. Swab samples of the surfaces of these items were tested to determine whether any might have served as fomites. No evidence of orthopoxvirus DNA was detected.

The home that the patient shared with her partner and 2 children was located in a forested, low-density area, within 50 miles of Fairbanks. She reported that wild small mammals (eg, shrews, voles, squirrels) were abundant in this boreal forest environment, were regularly observed around the home's perimeter, and entered the home on occasion. She also reported that her children periodically handled the carcasses of squirrels that they shot near the home using a pellet rifle. When asked about construction of her new home, the patient reported that she and her partner had used scrap wood from an abandoned shed located near her home that had been occupied by wild small mammals. All 23 environmental samples collected during the site visit, including swab samples of scrap wood from the abandoned shed at the new home construction site, tested negative for orthopoxviruses by PCR (Supplementary Table 2). At the time of the site visit to the patient's residence (approximately 45 days after symptom onset), her symptoms included an active lesion that had decreased in size since initial presentation but remained raised, tender, and warm (approximated in Figure 1B). Thirty-one small-mammal samples, collected from 12 individual animals belonging to 2 species (*Sorex cinereus*, n = 3; *Myodes rutilus*, n = 9), tested negative for orthopoxviruses by PCR (Supplementary Table 3).

DISCUSSION

An Alaska resident was infected by a previously undescribed genetic lineage of *Orthopoxvirus*. The patient reported that the lesion took approximately 6 months to fully resolve. No evidence of transmission from the index patient, or fatalities associated with infection, was reported.

Epidemiologic information gathered during the investigation provides inconclusive evidence for 2 general hypotheses concerning the patient's route of exposure. The first involves importation of an Old World orthopoxvirus into Alaska, either as an active infection in a person with whom the patient came into contact or by way of one or more fomites. The limited number of contacts identified by the patient, and the apparent absence of additional cases, indicates that the probability of unidentified secondary spread is remote. Work-related travel to Azerbaijan by the patient's partner represents a possible fomite-associated importation scenario, although sampling and testing of travel-related fomites returned negative results. Although the recent discovery of a novel, zoonotic orthopoxvirus in the nearby Republic of Georgia [7] indicates that unidentified orthopoxviruses might be circulating in this region, the delay between the arrival of these fomites in Alaska and the timing of onset of the patient's symptoms indicates that infection by this route is unlikely. The duration of viability of AK2015_poxvirus on fomites is uncertain; however, a laboratory study of *Vaccinia virus* demonstrated retention of viability on environmental surfaces for up to 56 days [8]. The presence of A-type inclusion bodies occluded with mature virus in the AK2015_poxvirus isolate observed by electron microscopy might be indicative of enhanced environmental resilience and prolonged viability [9].

Alternatively, AK2015_poxvirus might be endemic to Alaska, perhaps circulating within one or more wildlife reservoir population, and infection might have occurred through an animal exposure. Different species of wild small mammals occur in the boreal forest environment surrounding the patient's residence, and infection by indirect animal contact (eg, contact with household surfaces, squirrels shot by the patient's children, or handling of potentially contaminated wood from the shed occupied by wild small mammals during construction of the new home) might be implicated. Rodents and other small mammals are known or suspected reservoirs for multiple orthopoxviruses [10], and evidence of infection has been produced by serosurveys in Eurasia [11–14], Africa [5, 15], South America [16, 17], and the continental United States [18]. Among the limited number of wildlife serosurveys of terrestrial mammals conducted in Alaska, the majority have focused on large mammals [19–21], and those that sampled small mammals did not test for evidence of a poxvirus infection or exposure [22–24]. Small-mammal serosurveys in regions of northern Europe that are ecologically similar to Alaska have identified seropositive animals [25–27]. Although results of our small-mammal trapping and testing were negative, the sample was limited and taxonomically restricted.

Phylogenetic analyses indicate that AK2015_poxvirus is more closely related to the Old World orthopoxviruses than to North American congeners. However, that the virus is known only from North America creates a discordance that precludes its assignment to one or the other of these geographically defined groups with confidence. The global distribution of other genera and unassigned isolates within Chordopoxvirinae, and incomplete sampling of potential reservoir taxa as part of our investigation, create additional uncertainty regarding this assignment. Nevertheless, given the inconsistent timelines of contact travel and patient symptom onset, negative results of travel-associated fomite testing, and the potential for regular and close contact with wild small mammals in and around the patient's home, the most parsimonious explanation of infection is exposure to AK2015_poxvirus near Fairbanks. Evidence of virus circulation in Alaska or elsewhere in North America (ie, infections in persons or reservoir species) would indicate either

a New World origin of orthopoxviruses or an Old World origin with multiple introductions to the New World. Both scenarios run counter to the present characterization of North American and Old World orthopoxviruses as representing reciprocally monophyletic lineages, and challenge the currently accepted hypothesis of an Old World origin of the genus *Orthopoxvirus* with a solitary introduction of the New World orthopoxviruses to North America [28].

This discovery of a novel orthopoxvirus is the latest in a growing number of reports of human poxvirus infection published in recent years. These include the emergence of novel poxviruses [7, 29–31] and the increased incidence of human monkeypox, an orthopoxvirus illness historically associated with relatively low incidence [32]. Because smallpox vaccination has been demonstrated to provide cross-protection against other orthopoxviruses [33, 34], these observations have been attributed to the cessation of routine smallpox vaccination after eradication of *Variola virus* in 1980 and the subsequent waning of population-level vaccine-derived immunity [7, 32, 35]. Continued emergence and reemergence of orthopoxviruses is expected. To effectively treat persons infected by orthopoxviruses, clinicians should remain vigilant for signs of poxvirus infections and immediately alert public health officials when infection is suspected so that prompt diagnostic testing and appropriate control measures can be implemented. Within Alaska, populations that might represent foci for surveillance include persons, such as the patient's partner, who travel to geographic regions associated with the emergence or reemergence of orthopoxviruses, and persons with regular direct or indirect contact with wildlife (eg, residents of rural settings, scientists, environmental consultants, hunters, and adventure guides). The latter population in Alaska might be relatively large, given the high proportion of state residents and visitors who live, work, or recreate in wilderness areas.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. We thank the patient and contacts for their cooperation during the investigation. Contributions from J. Butler, T. Gardner, A. M. Gunderson, T. Hennessy, and staff of the Alaska State Public Health Virology and Public Health laboratories are gratefully acknowledged.

Disclaimer. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Financial support. This work was supported by funds from the state of Alaska and the US federal government.

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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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
				2017. 2. 6	該当なし	

一般的名称	人血小板濃厚液	研究報告の公表状況		公表国
販売名(企業名)	濃厚血小板-LR「日赤」(日本赤十字社) 照射濃厚血小板-LR「日赤」(日本赤十字社) 濃厚血小板HLA-LR「日赤」(日本赤十字社) 照射濃厚血小板HLA-LR「日赤」(日本赤十字社) 照射洗浄血小板-LR「日赤」(日本赤十字社) 照射洗浄血小板HLA-LR「日赤」(日本赤十字社)		CDC Newsroom, For Immediate Release: Friday, January 20, 2017	米国

研究報告の概要

○自営ラット繁殖施設に関連するソウルウイルス感染のアウトブレイク。
CDC(米国疾病管理予防センター)は、イリノイ州の保健局並びにウィスコンシン州の保健サービス局と共に、ソウルウイルス感染症例の調査に取り組んでいる。感染した8名は、この2州に所在する複数のラット繁殖施設に勤務していた。ソウルウイルスが米国で検出されることは稀であるが、野生のラットにおける感染のアウトブレイクが複数報告されている。今回のアウトブレイクは、米国においてペット用のラットに関連することが確認された最初のアウトブレイクとなる。
自営で齧歯動物の繁殖を行っていたウィスコンシン州のブリーダーが発熱、頭痛等の症状を呈し、2016年12月に入院した。CDCにより血液検体の検査が行われ、齧歯類が媒介するハンタウイルスの一種であるソウルウイルスによって引き起こされた感染症であることが確認された。同様に齧歯動物を扱っていた近親者1名も、検査によりソウルウイルス陽性と判定された。両名は既に回復している。最初の患者にラットを供給した複数の繁殖業者を対象とした追跡調査の結果、イリノイ州に所在する2つのラット繁殖施設において、ソウルウイルス感染例が新たに6例確認された。

報告企業の意見	今後の対応
米国にてペット用ラットを介したソウルウイルス感染の初のアウトブレイクが発生した。感染した8名は2州に所在する複数のラット繁殖施設に勤務してい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

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照射洗浄血小板-LR「日赤」
照射洗浄血小板HLA-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

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Centers for Disease Control and Prevention
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CDC Assisting Illinois and Wisconsin Investigation of Seoul Virus Outbreak Associated with Home-based Rat-Breeding Facilities

Media Statement

For Immediate Release: Friday, January 20, 2017
Contact: Media Relations (https://www.cdc.gov/media),
(404) 639-3286

Experts from the Centers for Disease Control and Prevention are working with the Illinois Department of Health and the Wisconsin Department of Health Services to investigate cases of Seoul virus infections among eight people who worked at several rat-breeding facilities in the two states. Seoul virus is not commonly found in the United States, though there have been several reported outbreaks in wild rats. This is the first known outbreak associated with pet rats in the United States.

A home-based rodent breeder in Wisconsin was hospitalized in December 2016 with fever, headache, and other symptoms. CDC tested a blood specimen and confirmed that the infection was caused by Seoul virus, a member of the Hantavirus family of rodent-borne viruses. A close family member who also worked with rodents also tested positive for Seoul virus. Both people have recovered. A follow-up investigation at several rat breeders that supplied the initial patient's rats revealed an additional six cases of Seoul virus at two Illinois rat breeding facilities.

Seoul virus is carried by wild Norway rats worldwide. People usually become infected when they come in contact with infectious body fluids (blood, saliva, urine) from infected rats or are bitten by them. Most cases in people are reported in Asia. The virus is not spread between people and cannot be transmitted to or from other types of pets. Rats infected with Seoul virus typically do not appear sick.

CDC has deployed two epidemiologists to work with local and state health authorities to determine if any customers who bought rats have become ill. Human and animal health officials are working together to make sure infected rats are not distributed further. CDC and its state and local health partners are reaching out to rodent suppliers to learn more about suppliers for the Wisconsin rat breeder. These efforts will help determine how the two individuals in Wisconsin were initially exposed to Seoul virus and allow public health officials to take actions to prevent future spread of the virus. CDC staff will also provide laboratory testing for blood samples from people who may have come in contact with rats from the affected rat breeders.

Though Seoul virus is in the Hantavirus family, it produces a milder illness than some other Hantaviruses. Symptoms may include fever, severe headache, back and abdominal pain, chills, blurred vision, redness of the eyes, or rash. In rare cases, infection can also lead to acute renal disease. However, not all people infected with the virus experience symptoms. Most people infected with Seoul virus recover.

People in Illinois and Wisconsin who are concerned that they have purchased or come in contact with rats from the affected breeders should contact their local or state health departments. Anyone who recently purchased a rat in the affected areas and experiences Seoul virus symptoms should contact their healthcare provider immediately.

To prevent infections from Seoul virus and other diseases carried by rats, people should:

- Wash your hands with soap and running water after touching, feeding, or caring for rodents, or cleaning their habitats. Be sure to assist children with handwashing.
- Be aware that pet rodents can shed germs that can contaminate surfaces in areas where they live and roam. Make sure rodent enclosures are properly secured and safe, so your pet doesn't get hurt or contaminate surfaces.
- Clean and disinfect rodent habitats and supplies outside your home when possible. Never clean rodent habitats or their supplies in the kitchen sink, other food preparation areas, or the bathroom sink.
- Avoid bites and scratches from rodents. Be cautious with unfamiliar animals, even if they seem friendly. Take precautions (https://www.cdc.gov/rodents/cleaning/index.html) when cleaning out rodent cages or areas with rodent urine or droppings.
- Visit your veterinarian for routine evaluation and care to keep your rodents healthy and to prevent infectious diseases.

If bitten by a rodent:

- Wash the wound with warm soapy water immediately. Even healthy pets can carry germs.

Seek medical attention if:

- Pet appears sick.
- Your wound is serious.
- Your wound becomes red, painful, warm, or swollen.
- Your last tetanus shot was more than 5 years ago.
- You develop sudden fever or flu-like illness in 1-2 weeks after being bitten

Tell your healthcare provider that you have been around pet rodents, whether at home or away from the home, especially if you are sick or have been bitten or scratched.

For more information on Seoul virus, please visit https://www.cdc.gov/hantavirus/outbreaks/seoul-virus/index.html (https://www.cdc.gov/hantavirus/outbreaks/seoul-virus/index.html).

For more information, please visit:www.cdc.gov/healthypets (http://www.cdc.gov/healthypets) or https://www.cdc.gov/healthypets/resources/pet-rodents-8x11-508.pdf (https://www.cdc.gov/healthypets/resources/pet-rodents-8x11-508.pdf) .

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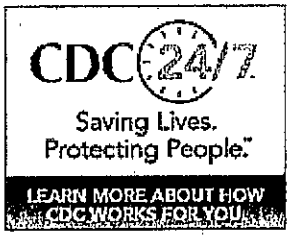

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医薬品　研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日 2017 年 3 月 13 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	CDC Weekly/March10,2017/66(9)；254-255	公表国 米国	
販売名(企業名)	－					

研究報告の概要

2013 年 3 月～2017 年 2 月，中国における毎年のトリインフルエンザ A(H7N9)の流行により，中国 National Health and Family Planning Commission などから WHO へ報告されたトリインフルエンザ A（H7N9）ウイルスのヒト感染は 1258 例に至った。
現在継続中の 5 度目の流行（2016 年 10 月 1 日開始）で，A（H7N9）ウイルスのヒト感染は 460 例報告されている（中国本土：453 例，中国本土への渡航関連 6 例)。5 度目の流行で報告されたヒト感染は，これまでの 4 度の流行（1 度目：135 例，2 度目：320 例，3 度目：226 例，4 度目：119 例）におけるヒト感染と比較して大幅な増加を示している。一部の限定的なヒトーヒト伝播は継続して確認されているが，持続的な A（H7N9）のヒトーヒト伝播は確認されていない。
過去 4 回の流行における A（H7N9）はすべて低病原性のインフルエンザウイルスの特徴を示していたが、5 度目の流行で感染者（3 検体）や生きた家禽市場の環境から採取されたサンプル（7 検体）のウイルスの分析では、これらのウイルスが、高病原性トリインフルエンザウイルスの特徴である HA プロテインの宿主プロテアーゼによる開裂部位に 4 つのアミノ酸の挿入を含んでいることが示された。
中国の機関は、家禽での H7N9 の流行に対し詳しく調査しモニタリングを実施している。

報告企業の意見	今後の対応
中国における A 型鳥インフルエンザ（H7N9）の 5 度目の流行期間におけるウイルスの疫学、ウイルス学的な変化について評価したものである。	A 型鳥インフルエンザ（H7N9）の情報については今後も留意していく。

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。

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NIHONSEIYAKU 2017-001

Increase in Human Infections with Avian Influenza A(H7N9) Virus During the Fifth Epidemic — China, October 2016–February 2017

Weekly / March 10, 2017 / 66(9);254–255

On March 3, 2017, this report was posted online as an MMWR *Early Release.*

A. Danielle Iuliano, PhD[1]; Yunho Jang, PhD[1]; Joyce Jones, MS[1]; C. Todd Davis, PhD[1]; David E. Wentworth, PhD[1]; Timothy M. Uyeki, MD[1]; Katherine Roguski, MPH[1]; Mark G. Thompson, PhD[1]; Larisa Gubareva, PhD[1]; Alicia M. Fry, MD[1]; Erin Burns, MA[1]; Susan Trock, DVM[1]; Suizan Zhou, MPH[2]; Jacqueline M. Katz, PhD[1]; Daniel B. Jernigan, MD[1] (View author affiliations)

View suggested citation

During March 2013–February 24, 2017, annual epidemics of avian influenza A(H7N9) in China resulted in 1,258 avian influenza A(H7N9) virus infections in humans being reported to the World Health Organization (WHO) by the National Health and Family Planning Commission of China and other regional sources (*1*). During the first four epidemics, 88% of patients developed pneumonia, 68% were admitted to an intensive care unit, and 41% died (*2*). Candidate vaccine viruses (CVVs) were developed, and vaccine was manufactured based on representative viruses detected after the emergence of A(H7N9) virus in humans in 2013. During the ongoing fifth epidemic (beginning October 1, 2016),* 460 human infections with A(H7N9) virus have been reported, including 453 in mainland China, six associated with travel to mainland China from Hong Kong (four cases), Macao (one) and Taiwan (one), and one in an asymptomatic poultry worker in Macao (*1*). Although the clinical characteristics and risk factors for human infections do not appear to have changed (*2,3*), the reported human infections during the fifth epidemic represent a significant increase compared with the first four epidemics, which resulted in 135 (first epidemic), 320 (second), 226 (third), and 119 (fourth epidemic) human infections (*2*). Most human infections continue to result in severe respiratory illness and have been associated with poultry exposure. Although some limited human-to-human spread continues to be identified, no sustained human-to-human A(H7N9) transmission has been observed (*2,3*).

CDC analysis of 74 hemagglutinin (HA) gene sequences from A(H7N9) virus samples collected from infected persons or live bird market environments during the fifth epidemic, which are available in the Global Initiative on Sharing All Influenza Data (GISAID) database (*4,5*), indicates that A(H7N9) viruses have diverged into two distinct genetic lineages. Available fifth epidemic viruses belong to two distinct lineages, the Pearl River Delta and Yangtze River Delta lineage, and ongoing analyses have found that 69 (93%) of the 74 HA gene sequences to date have been Yangtze River Delta lineage viruses. Preliminary antigenic analysis of recent Yangtze River Delta lineage viruses isolated from infections detected in Hong Kong indicate reduced cross-reactivity with existing CVVs, whereas viruses belonging to the Pearl River Delta lineage are still well inhibited by ferret antisera raised to CVVs. These preliminary data suggest that viruses from the Yangtze River Delta lineage are antigenically distinct from earlier A(H7N9) viruses and from existing CVVs. In addition, ongoing genetic analysis of neuraminidase genes from fifth epidemic viruses indicate that approximately 7%–9% of the viruses analyzed to date have known or suspected markers for reduced susceptibility to one or more neuraminidase inhibitor antiviral medications. The neuraminidase inhibitor class of antiviral drugs is currently recommended for the treatment of human infection with A(H7N9) virus. Antiviral resistance can arise spontaneously or emerge during the course of treatment. Many of the A(H7N9) virus samples collected from human infections in China might have been collected after antiviral treatment had begun.

Although all A(H7N9) viruses characterized from the previous four epidemics have been low pathogenic avian influenza viruses, analysis of human (three) and environmental (seven) samples from the fifth epidemic demonstrate that these viruses contain a four–amino acid insertion in a host protease cleavage site in the HA protein that is characteristic of highly pathogenic avian influenza (HPAI) viruses. Chinese authorities are investigating and monitoring closely for outbreaks of HPAI A(H7N9) among poultry.

Since April 2013, the Influenza Risk Assessment Tool has been used by CDC to assess the risk posed by certain novel influenza A viruses. Although the current risk to the public's health from A(H7N9) viruses is low, among the 12 novel influenza A viruses evaluated with this tool, A(H7N9) viruses have the highest risk score and are characterized as posing moderate–high potential pandemic risk (*6*). Experts from the World Health Organization (WHO) Global Influenza Surveillance and Response System (GISRS) met in Geneva, Switzerland, February 27–March 1, 2017, to review available epidemiologic and virologic data related to influenza A(H7N9) viruses to evaluate the need to produce additional CVVs to maximize influenza pandemic preparedness. Two additional H7N9 CVVs were recommended for development: a new CVV derived from an A/Guangdong/17SF003/2016-like virus (HPAI), which is a highly pathogenic virus from the Yangtze River Delta lineage; and a new CVV derived from A/Hunan/2650/2016-like virus, which is a low pathogenic virus also from the Yangtze River Delta lineage (*1*). At this time, CDC is preparing a CVV derived from an A/Hunan/2650/2016-like virus using reverse genetics. Further preparedness measures will be informed by ongoing analysis of genetic, antigenic, and epidemiologic data and how these data impact the risk assessment. CDC will continue to work closely with the Chinese Center for Disease Control and Prevention to support the response to this epidemic. Guidance for U.S. clinicians who might be evaluating patients with possible H7N9 virus infection and travelers to China is available online (https://www.cdc.gov/flu/avianflu/h7n9-virus.htm (https://www.cdc.gov/flu/avianflu/h7n9-virus.htm)).

Acknowledgments

Eduardo Azziz-Baumgartner, Stephen A. Burke, Douglas Jordan, CDC; Ying Song, Carolyn Greene, CDC, Beijing, China; National Influenza Center, CDC, Beijing, China; Prevention and Public Health Emergency Center, CDC, Beijing, China; Taiwan CDC, Taipei, Taiwan; Centre for Health Protection, Department of Health, Hong Kong SAR, China.

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[1]CDC; [2]CDC, Beijing, China.

* Epidemics refer to the seasonal increases in human infections; the fifth epidemic began on October 1, 2016.

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Suggested citation for this article: Iuliano AD, Jang Y, Jones J, et al. Increase in Human Infections with Avian Influenza A(H7N9) Virus During the Fifth Epidemic — China, October 2016–February 2017. MMWR Morb Mortal Wkly Rep 2017;66:254–255. DOI: http://dx.doi.org/10.15585/mmwr.mm6609e2 (http://dx.doi.org/10.15585/mmwr.mm6609e2)

Department of Health and Human Services.

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Page last reviewed: March 9, 2017
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No. 18

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2017. 4. 5	該当なし	

一般的名称	人全血液	研究報告の公表状況		公表国
販売名(企業名)	人全血液-LR「日赤」(日本赤十字社) 照射人全血液-LR「日赤」(日本赤十字社)		Kreuger AL, Middelburg RA, Kerkhoffs JH. Transfusion. 2017 Mar;57(3):657-660. doi: 10.1111/trf.13969. Epub 2017 Jan 31.	オランダ

研究報告の概要

○輸血用血小板の保存メディウムと輸血による細菌感染のリスク
背景:輸血細菌感染症(TTBI)は、濃厚血小板(PLT)の輸血に伴う最も懸念されるべきリスクの一つである。保存メディウムは細菌の増殖動態に影響し、ひいては細菌汚染を検出するためのスクリーニング検査の感度にも影響を及ぼす。
研究デザイン及び方法:本研究の目的は、保存メディウムと、PLT輸血後のTTBIの発生との関連性を数値化することであった。PLT輸血後に発生し、報告された全てのTTBI症例における保存メディウムの種類と、2003年から2014年までにオランダで製造された全てのPLTに用いられていた保存メディウムを比較した。
結果:14例のTTBIが報告されており、このうちの57.1%が血小板添加液(PAS)を用いて保存されたPLTを、42.9%が血漿で保存されたPLTを輸血されていた。製造された全てのPLTの22.3%がPAS、77.7%が血漿で保存されていた。血漿保存PLT輸血と比較した場合の、PAS保存PLT輸血後のTTBIの相対リスクは4.63 (95%信頼区間[CI]、1.4-16.2)であった。バフィーコート由来PLTの輸血における100万製剤あたりのTTBI発生率は22.2(95% CI、100万製剤あたり12.1-37.2)であった。
結論:血漿保存PLTと比較した場合、PAS保存PLT輸血に伴うTTBI発生率は4倍であった。。

報告企業の意見	今後の対応
保存メディウムと濃厚血小板(PLT)輸血後の輸血細菌感染症(TTBI)との関連性を数値化したところ、血漿保存PLTと比較し、血小板添加液保存PLTの輸血に伴うTTBIの発生率は4倍であったという報告である。	今後も細菌、ウイルスの検出及び不活化する方策について情報の収集及び安全対策に努める。

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

JRC2017T-013

Storage medium of platelet transfusions and the risk of transfusion-transmitted bacterial infections

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BACKGROUND: Transfusion-transmitted bacterial infections (TTBIs) are among the most concerning risks of transfusion of platelet (PLT) concentrates. Storage medium influences bacterial growth dynamics and thereby the sensitivity of screening tests for bacterial contamination.

STUDY DESIGN AND METHODS: The aim of this study was to quantify the association of storage media with the incidence of TTBIs after transfusion of PLT concentrates. In the Netherlands, the choice of storage medium is determined solely by geographic location of the hospital. We compared types of storage medium of all reported cases of TTBIs after transfusion of a PLT concentrate with types of storage medium of all produced PLT concentrates in the Netherlands from 2003 to 2014.

RESULTS: Fourteen cases of TTBIs were reported, of which 57.1% received a PLT concentrate stored in PLT additive solution (PAS) and 42.9% a PLT concentrate stored in plasma. Of all produced PLT concentrates 22.3% were stored in PAS and 77.7% in plasma. The relative risk of TTBI after transfusion of a PAS-stored PLT concentrate was 4.63 (95% confidence interval [CI], 1.4-16.2) compared to transfusion of a plasma-stored PLT concentrate. The incidence of TTBIs was 22.2 per million (95% CI, 12.1-37.2 per million) transfused buffy coat PLT concentrates.

CONCLUSION: Transfusion of PAS-stored PLT concentrates is associated with a fourfold increased incidence of TTBIs, compared to plasma-stored PLT concentrates.

Transfusion-transmitted bacterial infections (TTBIs) are one of the leading causes of mortality associated with blood transfusion.[1] Risk of TTBI is particularly associated with transfusion of platelet (PLT) concentrates, as these are stored at room temperature, allowing for proliferation of bacteria.

In many countries, PLT concentrates are screened for bacterial contamination, using the BacT/ALERT culture system (bioMérieux), and released on a "negative-to-date" basis.[2] Despite preventive efforts, a significant number of TTBIs are still reported every year. With complete bacterial screening, the incidence of TTBI was 7.14 per million PLT transfusions in Germany between 1997 and 2007 and 9.14 per million in the United States (2007-2011).[3,4] Approximately 300,000 PLT concentrates are transfused yearly in the United Kingdom and in 2015 the first case since 2009 was reported.[5] In the absence of bacterial screening, the incidence of TTBIs was 26.5 per million in France (2009-2011).[6]

Sensitivity of the screening method is influenced by variability in the inoculum and kinetics of bacterial

ABBREVIATIONS: RR = relative risk; TRIP = Transfusion and Transplantation Reactions in Patients; TTBI(s) = transfusion-transmitted bacterial infection(s).

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Received for publication August 9, 2016; revision received October 13, 2016; and accepted November 8, 2016.

doi:10.1111/trf.13969

TRANSFUSION 2017;57;657–660

growth.[7] Bacteria have been shown to be present in higher concentrations, making them more likely to be detected by culture methods, in apheresis- and buffy coat–derived PLT concentrates stored in PLT additive solution (PAS), compared to those stored in plasma.[8-10]

Interestingly, for some products yielding a positive BacT/ALERT screen, a subsequent resampling of the stored PLT concentrate results in a negative culture.[11] Apparently not all bacteria are able to proliferate in a PLT concentrate. It has been suggested that complement and antibodies can eliminate bacteria and sterilize the blood product. This process of autosterilization is probably more pronounced in PLT concentrates stored in plasma than in those stored in PAS.[12]

It is not known how these different effects of storage media influence the total risk of TTBIs. The aim of this study was to quantify the association of storage medium with the incidence of TTBIs after transfusion of a PLT concentrate.

MATERIALS AND METHODS

We performed a nested case control study to assess the effect of storage of PLT concentrates in plasma or PAS on the risk of TTBIs. We included all cases of TTBIs in which a PLT transfusion was involved that had been reported to the national hemovigilance organization Transfusion and Transplantation Reactions in Patients (TRIP) between 2003 and 2014. TRIP is the Dutch competent authority to which all transfusion reactions must be reported. Product identification numbers of the involved products were used to extract information about storage media and production method from the blood bank system. We excluded cases of TTBIs that occurred after transfusion of PLT concentrates collected by apheresis for the main analysis, because these are used for specific indications and mostly stored in plasma.

TTBI was defined as clinical features of bacteremia or sepsis during or after transfusion, with a relevant positive blood culture in the patient and assessed with a high level of imputability (definite or probable) to the transfused product. Imputability of all cases of posttransfusion sepsis was assessed by an expert panel. Since 2011 the expert panel has additionally judged whether the bacterial culture findings support a formal classification of the case as TTBI. Severity of transfusion reactions was scored on a scale from 0 to 4, with 0 indicating "no morbidity" and 4 indicating "mortality."[13]

PLT concentrates were prepared from buffy coats of five donors, leukoreduced, and resuspended in plasma, or PAS, with 25 mL of plasma left per donor. PAS-B (T-sol, Baxter) was used through 2013, with PAS-C (Intersol, Fenwal, Inc.) being used since. The diversion pouch was introduced universally in July 2004.[14] Throughout the entire study period, a standardized skin disinfection method was used and all PLT concentrates were screened for bacterial contamination with the BacT/ALERT system, according to a standardized protocol.

For the incidence of TTBI the number of all PLT concentrates produced in the Netherlands between 2003 and 2014 was used as the denominator. The storage medium of PLT concentrates involved in a TTBI was compared to storage medium of all produced PLT concentrates. Production data according to storage medium were available only for the period 2006 to 2014. The ratio of used storage media was stable over this period and could therefore be extrapolated back to 2003 (Table S1, available as supporting information in the online version of this paper). The type of storage medium of PLT concentrates is only determined by the geographic location of the hospital. Therefore, location of the hospital where the case of TTBI arises behaves as an instrumental variable in this analysis and it is expected that all potential confounders are randomly distributed.[15] To assess this assumption we explored the distribution of storage medium among hospitals licensed for stem cell transplantations and we compared the incidences of transfusion reactions related to red blood cell (RBC) transfusions between the regions.

We performed two sensitivity analyses. First, we included apheresis products in our analysis. Second, we excluded all cases before July 1, 2004, when use of the diversion pouch was introduced in all production centers.

RESULTS AND DISCUSSION

Between 2003 and 2014, a total of 14 cases of TTBIs were reported to TRIP. Table 1 provides the characteristics of all these cases. One case was of minor severity (Grade 1), 10 cases were moderate to serious (Grade 2), one was directly life-threatening (Grade 3), and one was fatal (Grade 4). Twelve patients had a hematologic malignancy, one patient had a solid tumor (prostate carcinoma), and for one patient the indication for transfusion was stated to be thrombocytopenia without further reported diagnosis. Both cases in 2003 were related to *Bacillus cereus*. The bacterial strains differed in genotype, so it seemed unlikely that both PLT concentrates were contaminated by a common source.[16]

During the study period 631,347 pooled buffy coat PLT concentrates were produced. The incidence of TTBIs was 22.2 per million (95% confidence interval [CI], 12.1-37.2 per million) buffy coat PLT concentrates. This incidence is relatively high compared to other countries, but this is probably a reflection of the accuracy of the Dutch hemovigilance system.[17]

Eight patients (57.1%) with TTBIs received a PAS-stored PLT concentrate (seven PAS-B, one PAS-C) and six patients (42.9%) received a PLT concentrate stored in plasma. Of all produced PLT concentrates, 22.3% were stored in PAS, and 77.7% in plasma. Transfusion of PAS-stored

TABLE 1. All cases of TTBIs reported to TRIP between 2003 and 2014

Cases	Year	Age (years)	Diagnosis	Severity*	Bacteria	Storage medium
1	2003	18	Acute myeloid leukemia	2	*B. cereus*	PAS-B
2	2003	57	Chronic myeloid leukemia	NA†	*B. cereus*	PAS-B
3	2004	28	NA†	2	*B. cereus*	PAS-B
4	2005	33	Acute myeloid leukemia	2	Hemolytic streptococci group G	Plasma
5	2005	58	Mantle cell lymphoma	2	*B. cereus*	PAS-B
6	2005	46	Aplastic anemia	3	*Staphylococcus aureus*	PAS-B
7	2005	58	Non-Hodgkin's lymphoma	2	Hemolytic streptococci group G	Plasma
8	2008	53	Acute myeloid leukemia	2	Coagulase-negative staphylococci	Plasma
9	2010	72	Prostate carcinoma	1	Coagulase-negative staphylococci	PAS-B
10	2010	39	Acute myeloid leukemia	2	*Streptococcus dysgalactiae*	PAS-B
11	2011	59	Acute myeloid leukemia	2	Salmonella group B	Plasma
12	2012	75	Non-Hodgkin's lymphoma	2	Hemolytic streptococci group C	Plasma
13	2013	62	Chronic lymphoid leukemia	2	Coagulase-negative staphylococci	PAS-C
14	2014	60	Multiple myeloma	4	*Staphylococcus aureus*	Plasma

* Severity of transfusion reaction. Grade 1 = minor morbidity, not life-threatening; Grade 2 = moderate to serious morbidity, may or may not be life-threatening or leading to hospitalization or prolongation of illness or associated with chronic disability or incapacity; Grade 3 = serious morbidity, directly life-threatening; Grade 4 = mortality after transfusion reaction.
† NA = not available; information was not reported to TRIP.

PLT concentrates was associated with a relative risk (RR) of TTBIs of 4.63 (95% CI, 1.4-16.2) compared to plasma-stored PLT concentrates. Including the PLT concentrates collected via apheresis showed similar results (RR, 5.01; 95% CI, 1.66-15.83). Exclusion of the period before universal use of the diversion pouch yields a RR of 3.48 (95% CI, 0.93-13.01).

The increased risk of TTBI after transfusion of PAS-stored PLT concentrates could be explained by autosterilization of plasma-stored PLT concentrates, which potentially inhibits a high bacterial load in a contaminated product. The aforementioned in vitro studies showed differences in growth characteristics of some bacterial strains suggesting improved sensitivity of bacterial screening of PLT concentrates stored in PAS-C or PAS-E. However, as shown in Fig. 1, the frequency of confirmed-positive results was higher for PLT concentrates stored in plasma compared to those stored in PAS-B. This is in line with the results of a previous study that compared the screening results of all PLT concentrates in 2002 and 2003.[18] With our data, it was not feasible to compare the different generations of PAS, since PAS-C has only been in use for 2 years, during which only one case of TTBI related to PAS-C has been reported.

This is the first clinical study investigating the association of storage medium of PLT concentrates with TTBIs. Storage media differ among countries and several generations of ASs are used.[19] Incidences of TTBIs could not be compared between countries, due to large differences in hemovigilance.[17]

In the Netherlands the choice of storage medium is determined solely by location of the hospital. Since it is likely that characteristics of patients receiving PLT concentrates are similar in different regions of the Netherlands, we expect that these are also equally distributed among storage media. Because most cases were

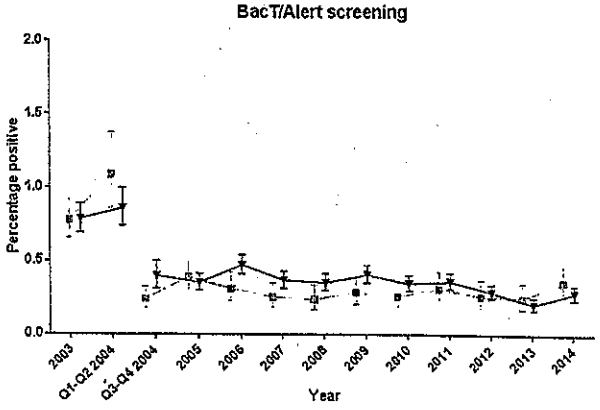

Fig. 1. Percentage of confirmed-positive results for all screened PLT concentrates screened by storage medium. (■) PAS; (▼) plasma. Confirmed positive means a microorganism could be isolated from the positive bottle.[14] The diversion pouch has been universally used since July 1, 2004. PAS-C has been in use since January 1, 2013.

diagnosed with hematologic malignancies, we performed an additional check, selecting only those hospitals licensed for autologous or allogeneic stem cell transplantations. Among these hospitals, 20.4% of PLT concentrates were stored in PAS, which is comparable to the 22.3% observed for all hospitals. This reaffirms our assumption that patient characteristics are similar among the different regions. Differences in vigilance in reporting of TTBIs could confound the results. The hospitals in which PAS-stored PLT products are used reported 28.1% of TTBIs related to RBC products, whereas these hospitals transfused 22.6% of all RBC products (RR, 1.34; 95% CI, 0.87-2.08). This seems to indicate that differences in reporting behavior cannot explain the observed strong association.

A limitation of this approach is that PLT concentrates in PAS and plasma were produced at different blood bank locations. Differences between these locations could theoretically also have affected the risk of TTBIs. However, it seems unlikely that this could fully explain the observed strong association of storage medium with risk of TTBIs. In conclusion, transfusion of PAS-stored PLT concentrates is associated with a fourfold increased incidence of TTBI, compared to plasma-stored PLT concentrates.

ACKNOWLEDGMENTS

The authors thank all Dutch hospitals for reporting all transfusion reactions to TRIP. ALK designed research, performed research, analyzed and interpreted data, and wrote the manuscript; RAM designed research, interpreted data, and revised the manuscript; JLHK designed research, interpreted data, and revised the manuscript; MRS collected data and revised the manuscript; JCWO collected data and revised the manuscript; and JGvdB designed research, interpreted data, and revised the manuscript.

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 website:

Table S1. Distribution of storage medium over the years

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 2017 年 02 月 16 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	The Journal of the American Medical Association 2017; 317(2): 123-124	公表国 スウェーデン; デンマーク	
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

研究は、アルツハイマー病とパーキンソン病が輸血によって伝達されないことを示唆している；

神経変性疾患の分野では、良いニュースはめったに得られないが、世界最大の医学データベースのひとつを厳格に調査したところ、ほっと胸をなで下ろす結果となることが強く示唆されている。アルツハイマー病（AD）やパーキンソン病（PD）のような疾患が伝染性だとすると、最近の研究結果が示唆するように、輸血によるものではないであろう。
後ろ向き研究により、スウェーデン及びデンマークの患者約 150 万人のカルテを用いて、後に神経変性疾患を発現したドナーから輸血を受けた患者が最終的に自分自身も同じ疾患に罹患したかを調査した。結論として、アルツハイマー病及びパーキンソン病の輸血による伝染の根拠は見られなかった。
「私にとって、これは大変しっかりとした否定的な試験であり、このような神経変性疾患は献血により伝染する可能性があるという懸念は当てはまらないことを説得力をもって示している」と著者である Michael P. Busch, MD, PhD は述べ、最近 Annals of Internal Medicine で公開された。
オーストラリアの Florey Institute of Neuroscience and Mental Health 神経変性部門の Colin Masters, MD は、研究結果により「輸血は最もよく見られる神経変性疾患の主要な危険因子ではないとする臨床的印象と一致する」と指摘した。

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)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。なお、プリオン病とされるアルツハイマー病とパーキンソン病については輸血を介して感染しないことが示唆されている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。	

Medical News & Perspectives

Study Suggests Alzheimer and Parkinson Disease Are Not Transmitted Through Blood Transfusion

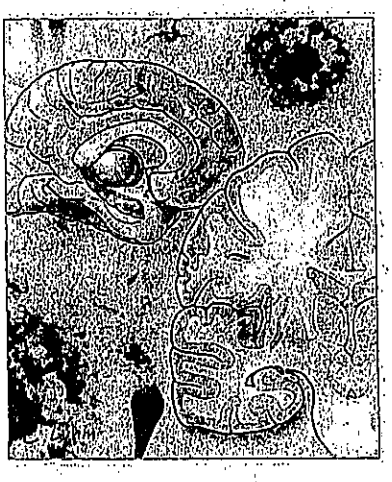
Jeff Lyon

Good news is hard to come by in the field of neurodegenerative disorders, but an exacting search through one of the world's largest medical databases strongly suggests a sigh of relief is in order. If illnesses such as Alzheimer disease (AD) and Parkinson disease (PD) are communicable, as recent research implies, it won't be via blood transfusion.

A retrospective study examined the health records of nearly 1.5 million patients in Sweden and Denmark to see whether those who received blood from donors who later developed neurodegenerative disorders eventually came down with the disorders themselves. The conclusion: "[N]o evidence of transfusion transmission of dementia, Alzheimer disease or Parkinson disease."

"To me, it's a very strong negative study that convincingly shows that concerns that these neurological disorders could be transmitted by blood donation are not valid," said Michael P. Busch, MD, PhD, an author of the research, which was recently published in the *Annals of Internal Medicine*. Busch is codirector of the San Francisco–based Blood Systems Research Institute, which specializes in studying transfusion-transmitted infectious diseases.

Colin Masters, MD, cohead of the neurodegeneration division of Australia's Florey Institute of Neuroscience and Mental Health and a pioneer in the genetics and proteopathy of AD, said he was "very reassured" by the results, noting they "coincide with one's clinical impressions that blood transfusions are not a major risk factor for the more common neurodegenerative diseases."



Masters, a professor at the University of Melbourne who was not connected with the research, called it "a major study from the Swedish and Danish blood transfusion databases, probably the largest ever conducted." Rating the strength of its conclusions on a scale of 1 to 10, he described them as "very substantial, at least a 9."

The Prion Hypothesis

The finding comes at a time when researchers, still struggling with the etiology of AD, PD, and certain other neurodegenerative diseases, are focusing on the growing possibility that the misfolded proteins that characterize them can be passed along, in some circumstances, to other humans.

Such misfolded proteins are the underlying contagion in rare disorders like Creutzfeldt-Jakob disease (CJD), fatal familial insomnia, and the newly linked multiple system atrophy. In these diseases, aberrant proteins called prions touch off a chain reaction of misfolding throughout the brain.

Once they gain a foothold in neural tissue, prions act like Svengali, relentlessly inducing molecules like the normal cell-surface protein PrPC to refold using the prions' deviant structure as a model. The resulting dysfunctional proteins then aggregate into large clumps that the body cannot clear. Eventually, the brain tissue becomes pitted, like a sponge, and death inevitably follows.

One of prions' most insidious properties is their transmissibility both from cell to cell and from person to person. Creutzfeldt-Jakob disease has been transmitted via brain surgery with insufficiently autoclaved instruments used previously on patients with CJD.

Both AD and PD share characteristics with prion diseases. For example, in AD, aberrant proteolytic processing of amyloid precursor protein (APP), a naturally occurring brain molecule whose normal function is not well understood, produces amyloid beta (Aβ) protein fragments. Certain isoforms of Aβ are prone to misfolding and act prionlike in that they can seed the aggregation and accumulation of Aβ into sticky plaques that may inhibit transmission between neurons, especially those responsible for cognition and memory. In PD, the relevant protein is α-synuclein, whose deviant form aggregates into Lewy bodies that

interfere with multiple brain functions. Perhaps most tellingly, small concentrations of these 2 aggregates, called oligomers, are directly toxic to healthy neurons and can jump from cell to cell, as in prion disorders.

Scientists believe these transformations are present in the brains of people likely to develop AD and PD well before symptoms appear. There is some evidence that donor tissue from such as-yet asymptomatic carriers may promote development of neurodegenerative pathology in recipients. Autopsies of the brains of 8 people who died of CJD decades after receiving human growth hormone (hGH) extracted from the pituitary glands of cadavers found that 6 not only had prion damage but also had the amyloidosis seen in AD, even though none were older than 51 years when they died or had mutations associated with early-onset AD. Such infectivity was not found in an earlier study that used a database listing US recipients of cadaver hGH to see if AD or PD appeared on the death certificates of any of the 796 deceased.

Conversely, when Stanley B. Prusiner, MD, who won the Nobel Prize for his trailblazing work in prions, and his colleagues injected Aβ aggregates into mice engineered for AD susceptibility and studied them in vivo using advanced imaging technology, they found the aggregates "self-propagated" in the manner of prions. The group went so far as to call it "compelling evidence" that Aβ aggregates *are* prions.

Given the emerging possibility that misfolded proteins in AD and PD may be transmissible and behave similarly to prions, a critical question to Busch was whether a blood donor with AD or PD brain changes who was asymptomatic could pass along a misfolded brain protein or other toxic agent to the recipient, starting the cascade of events that leads to disease many years later.

Such a grim prospect arose when unpublished reports surfaced that transgenic mice had shown plaque progression after infusion of Aβ aggregates parenterally, Busch explained. Such transmission "would be analogous to blood transfusion," he says, "and could potentially create substantial anxiety in the transfusion community."

Testing Transmissibility

It occurred to Busch that the Scandinavian Donations and Transfusions (SCANDAT2) database might be useful to test the premise. The database was assembled from computerized records maintained in Swedish and Danish blood banks since 1968 and 1981, respectively, and the data were then linked with nationwide health registers, creating long-term follow-up information on the health care of all transfusion donors and recipients through 2012.

"It's by far the most comprehensive database in the world currently," said Busch, who collaborated with Gustaf Edgren, MD, PhD, research group leader at the Department of Epidemiology and Biostatistics at Sweden's Karolinska Institutet and principal Swedish investigator at SCANDAT2.

Their study was designed to ascertain the risk of acquiring a neurodegenerative disease from an affected donor using 2 different yardsticks: increased disease concordance between donors and recipients, or excess disease occurrence among all recipients of blood from a particular donor.

Of the 1 465 845 patients whose records were reviewed, 2.9% received at least 1 unit of blood from a donor diagnosed with a neurodegenerative disorder within 20 years after donation. Follow-up of recipients began 6 months after the first transfusion to allow those already seriously ill with 1 of the targeted disorders but undiagnosed at the time to present with the disease. After excluding such recipients, the remainder were followed until a diagnosis of AD, PD, another dementia, or the study's December 31, 2012, cut-off date.

The researchers' key finding was no disease concordance between donors and recipients, and similarly, no excess occurrence of disease beyond normal statistical incidence in all recipients of blood from 1 donor.

"What we did was measure the hazard ratio, which asks what the probability is of developing, say, Alzheimer's from a donor who later developed it, compared with the probability of getting Alzheimer's after getting blood from a donor who never developed it," Busch said. "The hazard ratios were all essentially 1.0, which means there is no difference in the incidence rates."

An important control in the study, Busch noted, was a sensitivity component to prove the validity of the team's methodological approach.

"We chose to look at hepatitis C transmission between donors and recipients in the same database," he explained. "We found there was significantly increased risk—the hazard ratio was 8—of getting hep C from a blood donor who was later diagnosed. But after 1992, the year when the hep C virus was discovered and began to be screened for in the blood supply, there was none. This told us that our analytical method was sensitive."

Busch said the value of the study lies in helping to obviate the need for agencies like the US Food and Drug Administration to devise blood screening tests for AD and other neurodegenerative diseases.

"A negative finding may not be real exciting to the lay press," he said. "But it preempts potential fear, heavy investment, and onerous effort in screening technologies." ■

Note: The print version excludes source references. Please go online to jama.com.

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
				2017. 2. 6	該当なし	

一般的名称	人血小板濃厚液	研究報告の公表状況		公表国
販売名(企業名)	濃厚血小板-LR「日赤」(日本赤十字社) 照射濃厚血小板-LR「日赤」(日本赤十字社) 濃厚血小板HLA-LR「日赤」(日本赤十字社) 照射濃厚血小板HLA-LR「日赤」(日本赤十字社) 照射洗浄血小板-LR「日赤」(日本赤十字社) 照射洗浄血小板HLA-LR「日赤」(日本赤十字社)		Mok T, Jaunmuktane Z, Joiner S, et al. N Engl J Med. 2017 Jan 19;376(3):292-294.	英国

研究報告の概要

○PrP遺伝子(*PRNP*)のコドン129にヘテロ接合体を有する患者における変異型クロイツフェルト・ヤコブ病。

*PRNP*のコドン129(メチオニン(M)またはバリン(V)のいずれかをコードする)の正常多型は、プリオン病に対する易罹患性並びにプリオン病の潜伏期間と臨床的表現型に影響を与える。変異型クロイツフェルト・ヤコブ病の確定例は、全例が*PRNP*コドン129の遺伝子型がMM型の患者となっている。

人格変化を呈する36歳の男性が、2015年8月に英国のNational Prion Clinicに紹介された。患者の*PRNP*コドン129の遺伝子型はMV型であった。患者の状態は以降の6ヶ月間で次第に悪化し、2016年2月に死亡する直前には重度の嚥下障害および不穏が認められていた。剖検時の免疫染色により、異常PrPが細胞の周囲に星状に分布していることが確認された。さらに、脾臓のリンパ組織に微量のプロテアーゼ抵抗性PrP(PrP^{Sc})が認められ、脳のホモジネートのイムノブロット解析により、変異型クロイツフェルト・ヤコブ病に特徴的なタイプ4のPrP^{Sc}であることが判明した。

患者に見られた臨床的特徴は、典型的な変異型クロイツフェルト・ヤコブ病のものとは異なっており、神経画像所見は孤発性クロイツフェルト・ヤコブ病の診断を示唆していた。患者は、変異型クロイツフェルト・ヤコブ病の可能性が高い、もしくは可能性があると判定するための疫学的診断基準を満たしていなかったが、神経病理検査およびプリオン株に対する分子タイピングの結果は、変異型クロイツフェルト・ヤコブ病に合致するものであった。本症例が*PRNP*コドン129の遺伝子型がMV型(英国において最も一般的な遺伝子型)のヒトにおける変異型クロイツフェルト・ヤコブ病の第二波の始まりを告げるものであるか否か、またMV型が他のプリオン病(特にクール一病)に見られる長期の潜伏期間を反映していたのか否かは依然として不明である。

報告企業の意見	今後の対応
PrP遺伝子のコドン129にヘテロ接合体を有する初の変異型クロイツフェルト・ヤコブ病患者の症例報告であり、この遺伝子型にはこれまでの診断基準が適合しないことが確認されたという報告である。	日本赤十字社は、vCJDの血液を介する感染防止の目的から、受付時に過去の海外滞在歴を確認し、欧州等39カ国に一定期間滞在したドナーを無期限に献血延期としている。また、英国滞在歴を有するvCJD患者が国内で発生したことから、1980～96年に1カ月以上の英国滞在歴のある人の献血を制限している。今後もプリオン検出法等の技術を含め、CJD等プリオン病に関する新たな知見及び情報の収集に努める。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

the authors) could be involved in such a condition, it would be interesting to recheck the patients' serum for the presence of circulating ACTA using an indirect immunofluorescence assay, as described by our group.[2] A possible immune response against the conductive system is suggested by the reported lymphocytic infiltrate involving the cardiac sinus, the atrioventricular node, and the esophageal muscle. In our study, the patient with circulating ACTA had not only heart block but also intestinal pseudo-obstruction, with the latter probably caused by an impairment in the "gut pacemaker" and intestinal conductive system.[3]

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No potential conflict of interest relevant to this letter was reported.

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DOI: 10.1056/NEJMc1615251

THE AUTHORS REPLY: We completely agree with Ederhy and colleagues that cardiac complications are rare with immune checkpoint inhibitors when the drugs are used as single agents. However, our current data suggest that such complications may be more frequent with combination therapy and that simple cardiac screening may be appropriate. There are several important considerations regarding the cardiovascular safety data generated from oncology clinical trials, as Ederhy et al. have compiled in Table 1 of their letter. First, oncology trials often exclude "real world" patients who have a previous cardiac history and may be at increased risk for cardiac complications. Second, there are inherent limitations in the manner in which cardiac toxicity is adjudicated in oncology trials. Almost no checkpoint-inhibitor trial to date has screened patients for myocarditis. Third, myocarditis is often a diagnosis of exclusion and can be missed if there is no active monitoring for this toxicity. Finally, the cases of myocarditis associated with checkpoint inhibitors that we have seen are characterized less by the typical features of cardiomyopathy and more by electrocardiographic instability, which may be more difficult to detect.

Caio's point is well taken regarding the possibility of detection of autoantibodies in our patients. However, it is important to note that the presence of autoantibodies does not prove causation. In our patients, we looked for antibody deposits in the inflamed tissues but did not find any evidence of antibody deposition. Instead, we observed a dense cellular infiltrate composed of T cells and macrophages in the heart and skeletal muscle. We certainly agree that further research in this area should include a detailed analysis of circulating antibodies.

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Since publication of their article, the authors report no further potential conflict of interest.

DOI: 10.1056/NEJMc1615251

Variant Creutzfeldt–Jakob Disease in a Patient with Heterozygosity at *PRNP* Codon 129

TO THE EDITOR: Prions cause lethal neurodegenerative diseases in mammals and are composed of multichain assemblies of misfolded host-encoded cellular prion protein (PrP). A common polymorphism at codon 129 of the PrP gene (*PRNP*), where either methionine (M) or valine (V) is encoded, affects the susceptibility to prion disease, as well as the incubation period[1] and clinical phenotype of prion disease. Human infection with the epizootic prion disease bovine spongiform encephalopathy resulted in variant Creutzfeldt–Jakob disease, which provoked a public health crisis in the United Kingdom and other regions. All definite cases of variant Creutzfeldt–Jakob disease to date have occurred in patients with the MM genotype at *PRNP* codon 129.[1]

A 36-year-old man was referred to the United

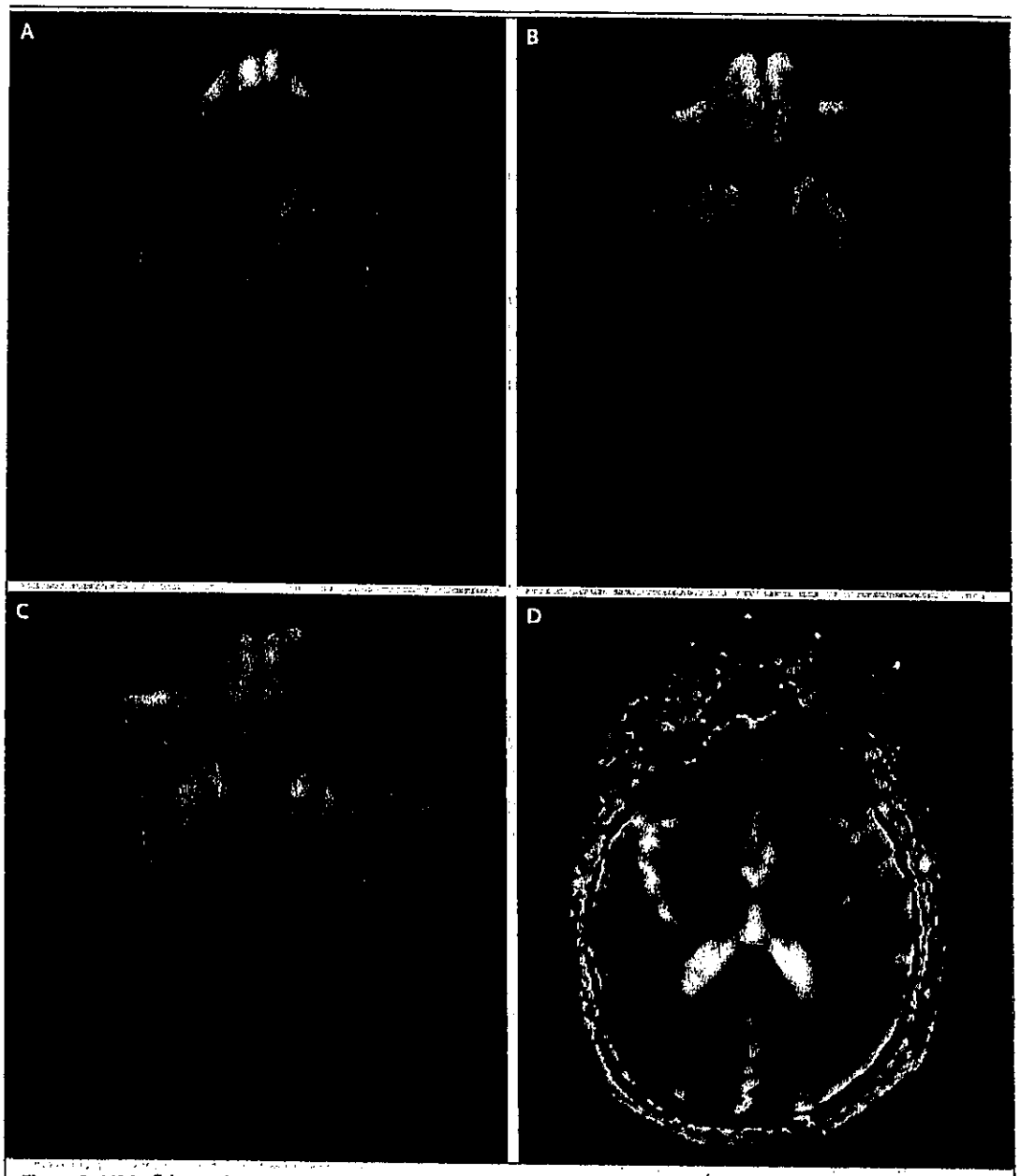

Figure 1. MRI of the Brain.

Trace-weighted diffusion-weighted images (Panels A, B, and C) show high signal intensity in the basal ganglia, hypothalami, and medial thalami but not in the pulvinar nuclei. These structures appear dark on the apparent diffusion coefficient map (Panel D), thus confirming the presence of restricted diffusion.

Kingdom National Prion Clinic in August 2015 with personality change. Over a period of 9 months, he had become uncharacteristically irascible and had progressive episodic memory impairment, gait ataxia, and myoclonus. His score on the Mini–Mental State Examination was 25 (with scores ranging from 0 to 30 and higher scores indicating less impairment); clinical examination revealed extraocular eye-movement abnormalities, pyramidal and cerebellar signs, and multifocal myoclonus. Magnetic resonance imaging of the brain (Fig. 1) revealed restricted diffusion in the basal ganglia, hypothalami, insular cortexes, and medial thalami but not in the pulvinar nuclei.[2] Examination of the cerebrospinal fluid for protein 14-3-3 was nega-

tive, as was a real-time quaking-induced conversion assay, although these two tests are known to have low sensitivity for variant Creutzfeldt–Jakob disease.[3] His genotype at *PRNP* codon 129 was MV. During the following 6 months, the patient's condition declined progressively, and severe dysphagia and agitation occurred shortly before his death in February 2016.

At autopsy, histologic examination of the brain revealed frequent florid and cluster plaques in cerebral and cerebellar cortexes, microvacuolar degeneration in neuropil, and immunostaining for abnormal PrP in a stellate pericellular and perivascular distribution. Minute amounts of protease-resistant PrP (PrP^{Sc}) were seen in lymphoid tissue of the spleen. Immunoblotting of brain homogenate revealed type 4 PrP^{Sc} (according to the London classification system), which is pathognomonic of variant Creutzfeldt–Jakob disease.[4] (For more details, see the Supplementary Appendix, available with the full text of this letter at NEJM.org.)

This patient's clinical features differed from those of typical variant Creutzfeldt–Jakob disease, and his neuroimaging features suggested a diagnosis of sporadic Creutzfeldt–Jakob disease. He did not meet the epidemiologic diagnostic criteria for probable or possible variant Creutzfeldt–Jakob disease,[5] yet the results of the neuropathological examination and molecular strain typing were consistent with variant Creutzfeldt–Jakob disease. It remains uncertain whether this case marks the start of a second wave of variant Creutzfeldt–Jakob disease in persons with the MV genotype at *PRNP* codon 129 (the most common genotype in the United Kingdom), mirroring the long incubation periods seen in persons with the MV genotype who have other acquired prion diseases, notably kuru.[1] This case emphasizes the importance of performing an autopsy and molecular strain typing in cases of prion disease to ascertain the prevalence of human prion disease related to bovine spongiform encephalopathy.

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Supported by the National Institute of Health Research Biomedical Research Centre at University College London Hospitals NHS Foundation Trust and the Medical Research Council (United Kingdom).

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

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DOI: 10.1056/NEJMc1610003

INSTRUCTIONS FOR LETTERS TO THE EDITOR

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- Letters in reference to a *Journal* article must not exceed 175 words (excluding references) and must be received within 3 weeks after publication of the article.
- Letters not related to a *Journal* article must not exceed 400 words.

別紙様式第 2-1
番号 6

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	厚生労働省処理欄
				2017 年 05 月 22 日	該当なし	

一般的名称	人ハプトグロビン	研究報告の公表状況	Emerging Infectious Diseases 2017; 23(6): 893-897	公表国 イギリス
販売名（企業名）	ハプトグロビン静注 2000 単位「JB」（日本血液製剤機構）			

研究報告の概要

イギリスの 2 例の血漿製剤投与患者における孤発性クロイツフェルト・ヤコブ病：

孤発性 CJD（sCJD）は、血漿分画製剤による治療を受けた凝固障害患者においてこれまで報告されていなかった。 我々は、凝固障害の長期治療歴がある患者において、英国で特定された 2 例の sCJD を報告する。 1 人の患者は血友病 B、他の患者はフォンウィルブランド病であった。 両者とも、英国で製造された血漿分画製剤の過去の治療による変異型 CJD（vCJD）のリスクが高いことは以前から知っていた。 いずれの症例も sCJD を示唆する臨床的および調査的特徴を有していた。この診断はどちらの場合も、脳の神経病理学的および生化学的検査において確認された。血漿製剤による治療と sCJD の発症との間の因果関係は確立されていない。1 人の患者は長期間に渡って血液成分を複数回輸血し、もう 1 人は臨床発症の 19 年前に 6 ユニットの FFP を受け、これらの症例が血液成分による二次感染の可能性が高まった。CJD の体系的なサーベイランスは、過去 25 年間に多くの国で実施され、継続中である。現在まで、凝固障害の治療を受けている患者では、sCJD の症例は報告されていない。実際にそのような事例がないことは、血漿由来製品が sCJD 伝播のリスクをもたらす可能性に反論するために用いられてきた。CJD のサーベイランスセンターはこの問題の妥当性を認識しており、血漿製剤の治療歴を持つ sCJD 患者が発生した場合には特定され、報告されていたはずである。英国では、血漿分画製剤による凝固障害の治療を受けている 4,000 から 5,000 人の患者の中で、2 例の sCJD が同定されているが、欧州および国際的に CJD の監視下にある総人口は 5 億人を超えている。人口 100 万人あたりの年間発生率を 1.5-2.0 と仮定すると、この総人口における sCJD の 2 例の発生は、治療と疾患の発生との因果関係を示唆していない可能性がある。今回の 2 症例は 1 ヵ月の間に特定されたが、2014 年以降に追加で発見された症例はない。CJD サーベイランスプログラムを通じてそのような症例を探し続けることが不可欠である。

使用上の注意記載状況・その他参考事項等

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

報告企業の意見	今後の対応
血漿分画製剤は理論的なvCJD伝播リスクを完全には排除できないため、投与の際には患者への説明が必要である旨を2003年5月から添付文書に記載している。2009年2月17日、英国健康保護庁(HPA)はvCJDに感染した供血者の血漿が含まれる原料から製造された第Ⅷ因子製剤の投与経験のある血友病患者一名から、vCJD異常プリオン蛋白が検出されたと発表したが、日本血液製剤機構の原料血漿採取国である日本及び米国では、欧州滞在歴のある献（供）血希望者を一定の基準で除外している。また、国際獣疫事務局（OIE）により、日本及び米国は「無視できるBSEリスク」の国に認定されたことからも、原料血漿中に異常型プリオン蛋白が混入するリスクは1999年以前の英国に比べて極めて低いと考える。なお、プリオン病とされるアルツハイマー病とパーキンソン病については輸血を介して感染しないことが示唆されている。CJDは種類によって感染性が異なることが知られており、脳・脊髄、網膜などに異常型プリオン蛋白質が蓄積されるsCJDは、vCJDとは異なり、血液を介した感染を示す報告はこれまでのところなく、その感染の可能性は低いと考えられる。今後もsCJDの情報については注意深く情報の収集、監視に努める。	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。

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ハプトグロビン

SYNOPSIS

Sporadic Creutzfeldt-Jakob Disease in 2 Plasma Product Recipients, United Kingdom

Patrick Urwin, Kumar Thanigaikumar, James W. Ironside, Anna Molesworth, Richard S. Knight, Patricia E. Hewitt, Charlotte Llewelyn, Jan Mackenzie, Robert G. Will

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Release date: May 12, 2017; Expiration date: May 12, 2018

Learning Objectives

Upon completion of this activity, participants will be able to:

- Recognize the clinical features of 2 cases of sporadic Creutzfeldt-Jakob disease (sCJD) reported in patients with clotting disorders treated with fractionated plasma products.
- Identify the laboratory and pathology findings of 2 cases of sCJD reported in patients with clotting disorders treated with fractionated plasma product.
- Determine the clinical implications of 2 cases of sCJD reported in patients with clotting disorders treated with fractionated plasma products.

CME Editor
Jude Rutledge, BA, Technical Writer/Editor, Emerging Infectious Diseases. *Disclosure: Jude Rutledge has disclosed no relevant financial relationships.*

CME Author
Laurie Barclay, MD, freelance writer and reviewer, Medscape, LLC. *Disclosure: Laurie Barclay, MD, has disclosed the following relevant financial relationships: owns stock, stock options, or bonds from Alnylam; Biogen; Pfizer.*

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DOI: https://dx.doi.org/10.3201/eid2306.161884

Sporadic Creutzfeldt-Jakob disease (sCJD) has not been previously reported in patients with clotting disorders treated with fractionated plasma products. We report 2 cases of sCJD identified in the United Kingdom in patients with a history of extended treatment for clotting disorders; 1 patient had hemophilia B and the other von Willebrand disease. Both patients had been informed previously that they were at increased risk for variant CJD because of past treatment with fractionated plasma products sourced in the United

Kingdom. However, both cases had clinical and investigative features suggestive of sCJD. This diagnosis was confirmed in both cases on neuropathologic and biochemical analysis of the brain. A causal link between the treatment with plasma products and the development of sCJD has not been established, and the occurrence of these cases may simply reflect a chance event in the context of systematic surveillance for CJD in large populations.

Human prion diseases are a group of rare and fatal neurodegenerative diseases that include idiopathic (sporadic), genetic (inherited), and acquired (infectious) disorders (*1*). All are associated with the accumulation of an abnormal isoform of the prion protein (PrP^{Sc}) in the central nervous system (*1*). The most common human prion disease is the sporadic form of Creutzfeldt-Jakob disease (sCJD), which occurs worldwide with a relatively uniform incidence of 1–2 cases per million population per year, a peak incidence in the 7th decade of life, and a median duration of illness of 4 months. The relatively consistent mortality rates associated with sCJD, the overall random spatial and temporal distribution of cases, and the absence of any confirmed environmental risk factor have led to the hypothesis that sCJD occurs because of the spontaneous generation of PrP^{Sc} in the brain (*1*). In contrast, variant Creutzfeldt-Jakob disease (vCJD) is an acquired disorder that is most likely caused by the consumption of meat or meat products contaminated with the bovine spongiform encephalopathy agent. The median age at death in vCJD is 30 years, with a median duration of illness of 14 months. Most cases of vCJD have occurred in the United Kingdom, which has had the largest epizootic of bovine spongiform encephalopathy in the world. Of the 178 UK vCJD cases, 3 have been identified as cases of secondary transmission caused by the transfusion of nonleukodepleted red blood cell components from vCJD-infected blood donors.

Lookback studies have shown no evidence of transmission through blood transfusion in sCJD (*2*,*3*), despite the identification of PrP^{Sc} in some peripheral tissues (*4*) and experimental evidence, which demonstrated infectivity in blood (*5*) by using intracerebral inoculation of highly sensitive transgenic mice. The absence of clinical cases causally linked to past treatment with fractionated plasma products has been used as evidence of the safety of these products in relation to sCJD (*6*). These products are generally manufactured from the pooled plasma from several thousand donors; production using UK plasma was discontinued in 1999.

We describe 2 cases of sCJD in patients who had previously received treatment with UK plasma–sourced plasma products; both patients had been informed that they were at increased risk for vCJD because of that treatment. The clinical features and investigations in these cases were typical of sCJD; the neuropathologic diagnosis in both cases was sCJD (subtype MM1).

The Investigation

The UK National CJD Research and Surveillance Unit has been carrying out systematic epidemiologic study of CJD since 1990. The methodology of this study has been published previously (*7*). In brief, patients with suspected CJD are referred by clinicians and visited by a research registrar, who obtains details of the clinical history and investigations, information on a range of possible risk factors, and past medical history. The Transfusion Medicine Epidemiology Review study investigates potential links between donors and recipients of labile blood components and, in cases of sCJD, investigates patients who have a history of blood donation or having received a blood transfusion.

Coordinated surveillance of CJD has been undertaken in the European Union since 1993 (*8*). National surveillance programs for CJD also are in place in several other countries, including Australia, Canada, Japan, and the United States.

Case 1

In 2014, a 64-year-old woman suffered a rapidly progressive dementia with deterioration in driving skills and balance disturbance, then limb coordination deficits with handwriting impairment. In the second month, her gait deteriorated, becoming shuffling and unsteady, she struggled to dress herself, and she had onset of daytime hypersomnolence. She became distractible, had visual misperceptions, emotional lability, and spatial memory problems. She was hospitalized at the beginning of the third month of her illness and had onset of cortical blindness, myoclonus, and akinetic mutism. She experienced rapid decline and died after a total illness duration of 3 months.

An electroencephalogram performed during the final stages of illness showed background slowing and runs of periodic complexes, and a magnetic resonance imaging (MRI) brain scan showed high signal in the caudate heads with posterior cortical ribboning. A cerebral spinal fluid (CSF) 14–3–3 assay and real-time quaking-induced conversion test for PrP^{Sc} both were positive. Prion protein gene (*PRNP*) sequencing showed no mutations with methionine homozygosity at codon 129.

Postmortem examination of the brain showed widespread spongiform encephalopathy of predominantly microvacuolar type. Immunocytochemistry for prion protein gave a widespread positive reaction in a granular/synaptic pattern (Figure). No plaques or plaque-like structures were identified. Results of immunocytochemistry for disease-associated prion protein were negative in peripheral nerve, liver, lymph node, appendix, and spleen. Western blot analysis of frontal cortex and cerebellum confirmed

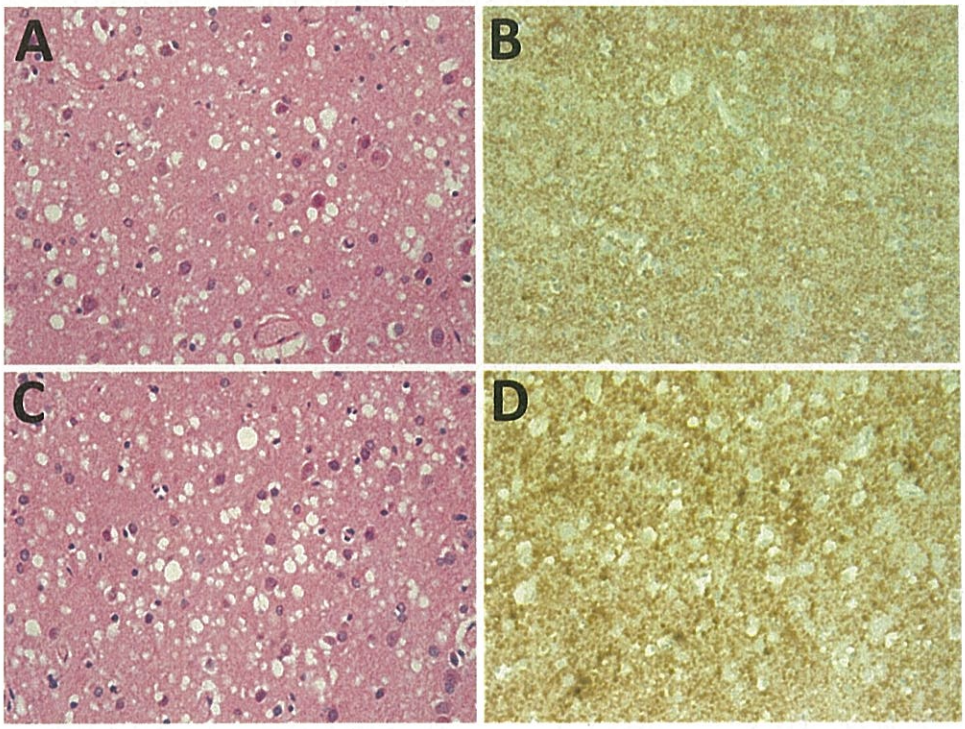

Figure. Results of neuropathologic examinations of the brains of the 2 patients with sporadic Creutzfeldt-Jakob disease, United Kingdom, 2014. A) Microvacuolar spongiform change in the frontal cortex (case 1). Hematoxylin and eosin stain; original magnification ×400. B) Fine granular/synaptic accumulation of abnormal prion protein in the cerebral cortex (case 1). 12F10 antiprion protein antibody; original magnification ×400. C) Microvacuolar spongiform change with neuronal loss and gliosis in the frontal cortex (case 2). Hematoxylin and eosin stain; original magnification ×400. D) Focally intense granular/synaptic accumulation of abnormal prion protein in the cerebral cortex (case 2). 12F10 antiprion protein antibody; original magnification ×400.

the presence of protease-resistant prion protein with a type 1A isoform.

The patient had been diagnosed with von Willebrand disease in childhood. Her early therapies include numerous transfusions of red blood cells and platelets; in more recent years, she received plasma-derived and recombinant factor VIII and additional blood component transfusions at times of hemorrhage. Factor VIII was administered on 4 occasions in the 1990s and during 2000–2004 and von Willebrand factor/factor VIII (Haemate-P) during 2001–2013. Because of her history of exposure to UK-sourced plasma products, for public health purposes she had been informed that she was at risk for vCJD, although she was not known to have been exposed to factor VIII derived from a batch including a vCJD donation. She had no history of potential iatrogenic exposure to CJD and no family history of CJD.

Donors for all blood or platelet transfusions since 2001 have been identified. Of the 107 donors, 106 are still alive, with a median age of 55 years (range 27–80 years). (Table 1). One donor of leukodepleted platelets, which were transfused 12 years before clinical onset in the recipient, died in 2013 at 76 years of age, and the diagnoses on the death certificate were vascular dementia and bladder cancer. Identification of donors for transfusions before 2001 has not been possible.

Case 2

In 2014, a 64-year-old woman reported day/night reversal of sleep patterns and, 3 months later, excessive tearfulness, for which she was started on antidepressants. She then had onset of writing problems, followed during the next few days by increasing language problems that led to expressive dysphasia. She deteriorated rapidly thereafter, requiring assistance with her activities of daily living and having coordination and memory problems, jerking movements suggestive of myoclonus, and itching in both arms. She was admitted to the hospital and experienced a probable focal seizure with secondary generalization. She had onset of a homonymous hemianopia and limb rigidity and then became bedbound and mute, dying 7 months after the onset of symptoms.

Table 1. Selected characteristics of blood donors to the patient with sporadic Creutzfeldt-Jakob disease described in case 1, United Kingdom, 2014*

Interval from transfusion to onset, y	Component	No. donors	No. donors alive	No. donors dead
3	RBC LD	4	4	0
6	RBC LD	6	6	0
7	RBC LD	19	19	0
9	RBC LD	3	3	0
10	RBC LD	4	4	0
12	Whole blood LD; RBC LD; platelets LD	2; 27; 42	2; 27; 41	0; 0; 1

*LD, leukodepleted; RBC, red blood cells. Median age of donors, 56 years (range 27–80 years).

An electroencephalogram performed during the final stages of illness showed widespread slowing, more evident on the left. An MRI brain scan showed left-sided caudate head and anterior putaminal high signal. Diffusion weighted imaging showed areas of cortical high signal. Results of a CSF 14–3–3 assay and real-time quaking-induced conversion tests were positive. Consent for full sequencing of the *PRNP* was not obtained; methionine homozygosity at codon 129 was identified.

Postmortem neuropathologic examination of the brain showed a widespread spongiform encephalopathy with microvacuolar spongiform change, neuronal loss, and gliosis. Immunostaining for prion protein showed widespread positivity with a granular/synaptic pattern (Figure). No amyloid plaques were identified. Western blot analysis confirmed the presence of protease resistant prion protein with a type 1A isoform. There was no evidence of abnormal prion protein accumulation in spleen and appendix either on immunocytochemistry or high sensitivity Western blot analysis.

The patient was known to have hemophilia B since 1964 and had received plasma-derived and recombinant factor IX during 1984–2012. For public health purposes, she had been informed that she was at risk for vCJD and in 1991 had received factor IX derived from a pool containing plasma from a donor who subsequently had vCJD. She had no history of potential iatrogenic exposure to CJD and no family history of CJD.

In 1985, the patient received 6 units of fresh frozen plasma (FFP). Tracing of donors has not been possible.

Discussion

This report describes 2 cases of sCJD in patients with a history of treatment with UK-sourced plasma products, 1 with a history of hemophilia B and 1 with von Willebrand's disease. To our knowledge, no previous case of sCJD in a person with a history of extended exposure to plasma products has been reported. It is clearly of concern that there have been 2 such cases in a relatively short period in the UK, where many plasma product recipients have been informed that they are at increased risk for vCJD. However, a causal link between the treatment with plasma products and the onset of sCJD has not been established, and the occurrence of these cases may simply reflect a chance event in the context of systematic surveillance of CJD in large populations.

Both patients had been informed that they were at increased risk for vCJD, and considering the evidence for the type of CJD in the 2 cases is important. Both patients had a clinical phenotype suggestive of sCJD, including a short duration of illness, typical early symptoms, a suggestive MRI scan, and, in 1 patient, a typical EEG. Notably, both patients had a positive real-time quaking-induced conversion test result for PrP^{Sc} in CSF; previously this test had not been positive in any case of vCJD evaluated in our laboratory (Table 2) (*9*). However, neuropathological examination was critical; it showed appearances typical of sCJD in both patients and no evidence of peripheral pathogenesis on immunostaining of lymphoreticular tissues, a feature that is observed in all tested specimens of vCJD patients to date (*10*). Furthermore, both patients had a type 1A isoform PrP^{Sc} on Western blot consistent with a diagnosis of sCJD subtype MM1 (*11*). Neither patient had a history of potential iatrogenic exposure or a family history of CJD, and for the case for which sequencing of the *PRNP* was performed, no mutations were detected. In both cases, an MM genotype occurred at codon 129 of *PRNP*, which does not distinguish between sCJD and vCJD. Laboratory transmission studies to provide evidence of agent strain in the cases have not been possible.

One patient had received multiple transfusions of blood components over an extended period, and the other had received 6 units of FFP 19 years before clinical onset, raising the possibility that these cases could have resulted from secondary transmission through blood components. In the case of the patient with von Willebrand disease, 107 donors have been traced, and none appear in the register of cases of CJD kept at the National CJD Research and Surveillance Unit. However, it has not been possible to obtain information on blood transfusions for this patient before 2001 nor on the FFP transfusions for the patient with hemophilia B. Lookback studies in the United States and United Kingdom have provided no evidence of transfusion-transmission of sCJD (*2*,*3*), and although 1 study suggested an increase in risk after a lag period of 10 years (*12*), this finding was not confirmed in another study (*13*). The balance of evidence

Table 2. Selected characteristics and clinical features of the 2 patients with sCJD described in cases 1 and 2, United Kingdom, 2014*

Characteristic/clinical feature	Case 1	Case 2
Patient age, y/sex	64/F	64/F
Symptoms/signs	Ataxia, cognitive impairment, visual impairment, myoclonus	Somnolence/depression, dysphasia, cognitive impairment, myoclonus/ataxia
Magnetic resonance imaging	+	+
Electroencephalogram	+	Slow activity
Cerebrospinal fluid 14–3–3 assay	+	+
RT-QuIC	+	+
Genotype	MM	MM
Diagnosis	Definite sCJD	Definite sCJD
Duration	3 mo	7 mo

*RT-QuIC, real-time quaking-induced conversion; sCJD, sporadic Creutzfeldt-Jakob disease.

indicates that, if sCJD is transmitted by blood transfusion, it must be a rare event, if it happens at all, and transfusion transmission is probably not the explanation for the 2 cases we describe.

Systematic surveillance for CJD, including a coordinated study in Europe (*14*), has been carried out in many countries over the past 25 years and is continuing. Many of these studies obtain information on potential risk factors, including details of past medical history. To date, no case of sCJD has been reported in a person who has received treatment for a clotting disorder. In fact, the absence of such a case has been used to argue against the possibility that plasma-derived products pose a risk for sCJD transmission (*6*). CJD surveillance centers are aware of the relevance of this issue, and sCJD patients with a history of treatment with plasma products probably would have been identified and reported if they occurred. Although it is surprising that 2 cases of sCJD have been identified among a population of 4,000–5,000 patients in the UK who have been treated for clotting disorders with fractionated plasma products, the total population under surveillance for CJD in Europe and internationally exceeds 500 million. Assuming an annual incidence rate of sCJD of 1.5–2.0 per million population (*15*), the occurrence of 2 cases of sCJD in this total population may not imply a causal link between the treatment and the occurrence of the disease. The 2 cases were identified over a period of months, and no further cases have been found since 2014; however, continuing to search for such cases through CJD surveillance programs is essential.

Acknowledgment

We thank Mark W. Head for the Western blot data.

The National CJD Research and Surveillance Unit is supported by the Policy Research Program of the Department of Health and the Government of Scotland (grant no. PR-ST-0614-00008). This report is independent research in part funded by the Department of Health Policy Research Programme and the Government of Scotland. The views expressed in this publication are those of the authors and not necessarily those of the Department of Health or the Government of Scotland.

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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。

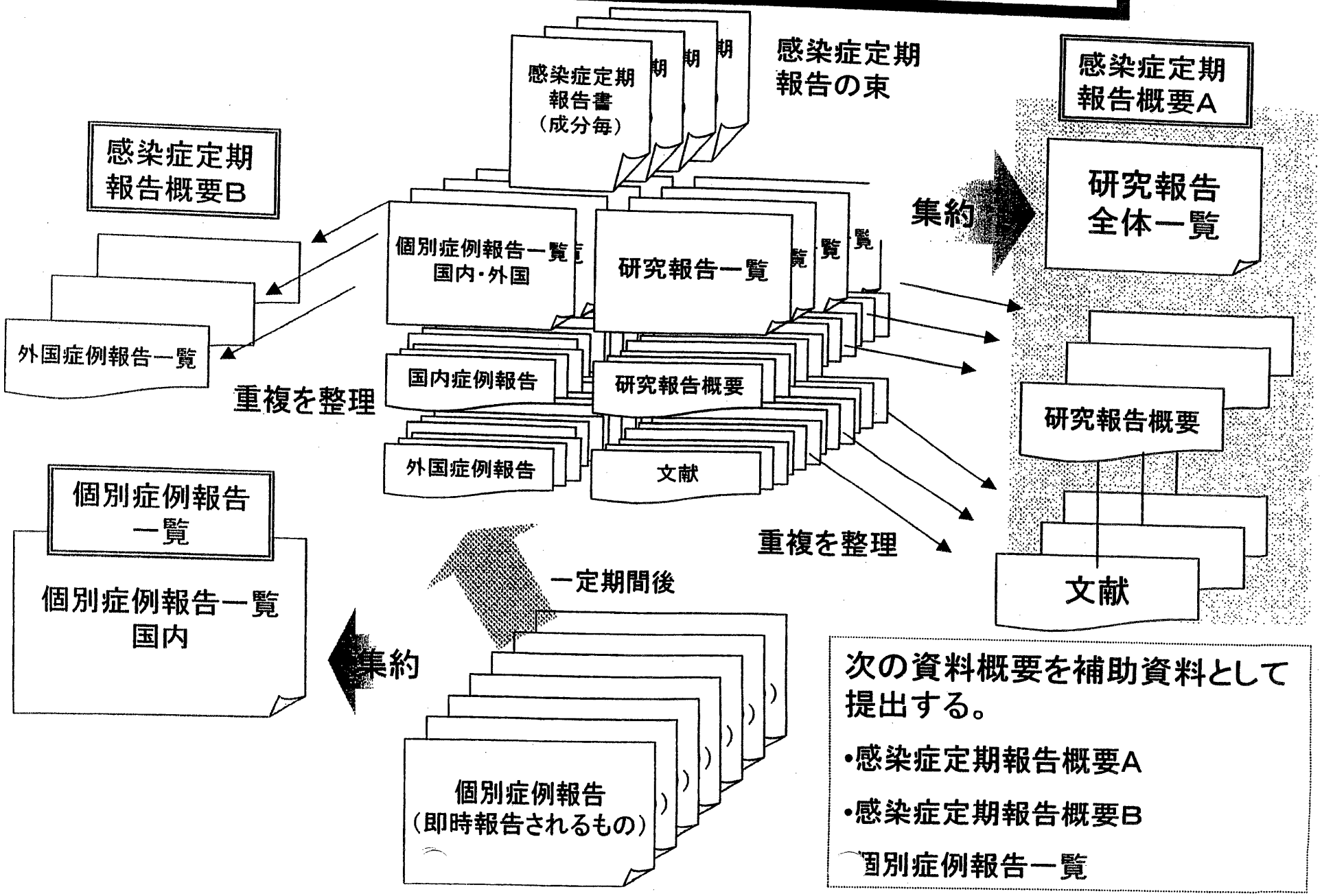
② 感染症発生症例報告のうち、発現国が「外国」の血漿分画製剤の使用による症例は、同一製品毎に報告期間を代表する感染症発生症例一覧（別紙様式第4）をまとめた**「資料概要B」**を事務局が作成し、送付する。

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