医薬品 研究報告 調査報告書

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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
○E型肝炎ウイルス(HEV)陽性血液の輸血を受けた受血者のルックバック調査 目的:2004年までに輸血によるHEV感染症例を少なくとも2例観察した。それ以来、日本赤十字社では北海道において、HBV/HCV/HIV-1に加えて、HEV NATスクリーニング検査を行っている。本試験の目的は、HEV陽性血液の輸血を受けた受血者のルックバック検査を行うことにより、輸血によるHEV伝播が生じる要因を検討することである。 材料および方法:2005年1月～2006年3月まで、全ての供血者検体について、HEV-NATスクリーニング検査を行った。しかし、一部の血液製剤は検査結果が判明する前に輸血されていた。2006年4月以降、北海道で採血された血液製剤はHEV-NATスクリーニング後に供給されている。過去の供血保管検体のルックバック検査で判明したHEV陽性血液製剤の輸血を受けた受血者の肝機能およびHEVマーカー(抗HEV抗体、HEV-RNA)を検査した。 結果:ルックバック検査により、HEV陽性血液製剤の輸血を受けた受血者13名が判明した。輸血前検体がHEV RNAまたはHEV抗体陽性だった者はいなかった。HEV感染の兆候を示した4名の受血者のうち、3名がE型肝炎を発症し、1名は一過性のALT上昇(ピーク:61IU/mL)を示した。4本の輸血済み血液製剤におけるHEVウイルス量とgenotypeは、5.4 (G4), 5.5 (G3), 5.8 (G4), 6.8 (G3) log/bagだった。HEV感染を起こさなかった受血者4名に輸血された4製剤では、<4.4(G3)、<4.4(G3)、4.3(G4)、5.5(G3) log/bagであった。5名は輸血直後に亡くなり、評価できなかった。 結論:血液製剤中のHEV高値(>5.4log/bag)がウイルス伝播に関連付けられると思われた。また、遺伝子型4は遺伝子型3より毒性が高い可能性がある。	赤十字アルブミン20 赤十字アルブミン25 赤十字アルブミン20%静注4g/20mL 赤十字アルブミン20%静注10g/50mL 赤十字アルブミン25%静注12.5g/50mL 血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
HEV陽性血液の輸血を受けた受血者のルックバック調査から、血液製剤中のHEV高値(>5.4log/bag)がウイルス伝播に関連付けられると考えられたとの報告である。 本剤の製造工程にはコーン分画および液状加熱の2つのウイルス除去・不活化工程が含まれている。最近ある遺伝子型のHEVは耐熱性であるとの成績が発表され、液状加熱の有効性に一部疑念を生じている。しかし、血漿分画製剤で最も長い歴史を持つアルブミンにはHEVの侵淫度が遥かに高い過去から現在に至るまで世界的にHEV感染例がないとの疫学的事実があることから、本剤の安全性は確保されていると考える。	今後もHEV感染の実態に関する情報の収集及び安全対策に努める。なお、日本赤十字社では、北海道における輸血後HEV感染報告を受け、献血者の疫学調査や、北海道で研究的NATを実施している。

Monday: Parallel Session S2: TTI

2A-S02-01

PREVALENCE OF HEV INFECTION AMONG JAPANESE BLOOD DONORS

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Hepatitis E virus (HEV) infection had been recognized to be extremely rare in industrialized countries. Recently, however, increasing reports of hepatitis E including transfusion-transmitted cases are reported in Japan and other industrialized countries.

So far, we have experienced four cases of transfusion-transmitted hepatitis E (TTHE). In 2004, we reported the first molecularly confirmed case of TTHEV, where infection route of the causative donor was not very clear. Then surveys of HEV prevalence in blood donors were undertaken and showed that higher prevalence of IgG anti-HEV in eastern Japan, and the positive rates in female donors were lower than that in male donors. There is a clear age-dependency in IgG anti-HEV prevalence in blood donors in Japan.

Meanwhile, we experienced the second case of TTHE. The causative donor had a barbecue party at a restaurant with his family and enjoyed yakiniku dinner including pig liver and/or intestines. Six of whom including the causative donor and his father were positive for IgM anti-HEV. The father died of fulminant hepatitis E after the barbecue party. HEV isolates from the donor also showed 99.9% homology with that from his father based on nearly entire HEV genome and was classified into genotype 4 that was indigenous to Hokkaido.

This case suggests that there are HEV carriers among blood donors in Japan and HEV infection does not necessarily lead to hepatitis symptoms. For this possibility, we decided to implement preliminary HEV NAT screening in Hokkaido in 2005. However, after the start of this preliminary NAT, we experienced two additional cases of TTHE in 2005 and 2006 because of the delay of the results of NAT. It took almost a week to obtain the results and some blood products, especially platelets concentrates, were already transfused before the NAT. In 2006, we implemented real-time HEV NAT screening.

Up to the end of 2008, the frequency of HEV RNA-positive donors is approximately 1/7700. Male positive donors were dominant. Also, genotype 3 was a dominant genotype. About half of the donors showed the elevation of their ALT level above 45 IU/l during follow-up period. In all of the HEV RNA-positive donors, ALT level came down below 45 IU/l within 50 days after their donation. The HEV RNA-positive donors were also followed-up for their HEV RNA. In all of them, HEV RNA became under the detectable level up to 62–76 days after the donation. The significant increase of the virus level after the donation was observed in 43% of the donors. Compared to hepatitis E patients, a) HEV-NAT-positive donors were younger, b) genotype 3 is dominant in contrast to genotype 4 dominance in hepatitis patients. Sequence analyses showed that most of the representative strains from HEV NAT-positive blood donors exhibit over 93% sequence homology with the corresponding swine isolates suggesting that most of HEV are from pigs through food-borne routes.

2A-S02-02

EPIDEMIOLOGY OF HEV INFECTION AMONG BLOOD DONORS IN HOKKAIDO, JAPAN

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Background: Recent studies have revealed that indigenous hepatitis E virus (HEV) strains cause domestic hepatitis E in industrialized countries including Japan. Several cases of transfusion-transmitted hepatitis E have been reported there.

Aims: To clarify the characteristics of HEV infection among blood donors in Hokkaido, Japan, and to consider preventive measures for HEV transmission via blood transfusion.

Methods: A total of 1,098,989 serum or plasma samples from blood donors in Hokkaido from January 2005 to December 2008 were tested for the presence of HEV RNA by real-time reverse transcription (RT)-PCR using 20-pooled samples. Blood samples positive for HEV RNA were tested for the presence of IgM and IgG anti-HEV by ELISA, and measured for HEV viral load by real-time RT-PCR. HEV strains from the HEV positive donors were phylogenetically analyzed by direct sequencing of RT-PCR products of regions of HEV ORF1 and ORF2. Questionnaire was mailed to the HEV RNA positive donors to collect the data on their history of intake of animal meats within 2 months previous to the donation. The donors positive for HEV RNA were looked-back and followed-up before and after their positive donations.

Results: HEV RNA was detected in 142 (105 males and 37 females) donors and the overall prevalence of the HEV infected donors was 0.013% (0.015% in males and 0.009% in females) between 2005 and 2008 in Hokkaido. The yearly prevalence of HEV RNA-positives in male and female donors were 0.01% and 0.011% in 2005, 0.016% and 0.011% in 2006, 0.017% and 0.003% in 2007, and 0.02% and 0.009% in 2008, respectively, suggesting progressive expansion of HEV infection in male donors. No clear seasonality of the infection was observed during the period. Of the 142 donors, 109 (77%) donors had neither IgM nor IgG antibodies against HEV at their HEV RNA-positive donations. The strains detected in the donors were segregated into genotype 3 (132) and genotype 4 (6), which were assumed to be Japan-indigenous strains. Of the 103 donors responding to the questionnaire, 71 (69%) had a history of eating the animal viscera such as intestine and/or liver. Of the 39 donors followed-up at least twice a month after the donation, 21 (54%) showed transient elevations of ALT higher than 45 IU/L.

Conclusions: A total of 142 sporadic HEV infection were observed among blood donors during 2005 through 2008 in Hokkaido with male superiority in the prevalence, which were caused by Japan-indigenous HEV strains and appeared to be associated to ingestion of the animal viscera. HEV NAT screening may be more adequate to exclude the HEV-infected donors than HEV antibody screening.

2A-S02-03

LOOK-BACK STUDY ON RECIPIENTS WHO WERE TRANSFUSED HEPATITIS E VIRUS (HEV)-POSITIVE BLOOD

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Objective: Up to 2004, we observed at least two cases of transfusion-transmitted HEV infection. Since then, we have implemented NAT screening for HEV in addition to HBV/HCV/HIV-1 in Hokkaido area. The purpose of this study is to evaluate the factor(s) that may lead to transfusion-transmission of HEV by looking back the recipients who were transfused with HEV-positive blood.

Materials and methods: From 2002 to 2004, donor samples with high ALT (>=200 IU/mL) were tested for HEV-RNA. From 2005.1–2006.3, all donor samples were screened by HEV-NAT. However, a part of blood products were already transfused before the NAT results turned out. Since 2006.4, blood products have been issued after HEV-NAT screening. The recipients of HEV-positive blood products that were disclosed mostly by look-back

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study with stored samples at previous donations were tested for HEV markers including antibody to HEV and HEV-RNA as well as liver functions.
Results: Look-back study disclosed 13 recipients who were transfused HEV-positive blood products. None of them was positive for HEV RNA or anti-HEV in pretransfusion samples. Of four recipients showing signs of HEV infection, three developed hepatitis E and one showed a transient elevation of ALT (peak: 61 IU/mL). The amount and genotypes of HEV in the four transfused blood products were 5.4 (G4), 5.5 (G3), 5.8 (G4) and 6.8 (G3) 10^n/bags, while four blood products that did not cause HEV infection in four recipients contained <4.4 (G3), <4.4 (G3), 4.3 (G4) and 5.5 (G3) 10^n/bags. Five of the 13 recipients died soon after transfusion and were not able to be evaluated for HEV transmission.
Conclusion: The higher amount of HEV (>5.4 log/bag) in blood products may be associated with the virus transmission. Also genotype 4 may be more virulent than genotype 3.

2A-S02-04

ESTABLISHMENT OF A KOREAN HBSAG LOW TITER PERFORMANCE PANEL FOR QUALITY CONTROL OF HBV DIAGNOSTIC KITS

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Background: Currently, International Standards or commercially available reference materials are used for the validation or quality assessment of domestic *in-vitro* diagnostic medical devices. However, due to their high cost and limited quantity a sustainable supply cannot be guaranteed. Also, these materials might not reflect the viral characteristics common in Korea. This study was conducted to establish a low titer performance panel to be used for quality control of HBV diagnostic kits.
Materials and methods: 371 plasma units with OD values less than 1.0 on EIA screening and 105 units with S/C ratio less than 10.0 on CIA were collected from Korean Red Cross blood centers. HBsAg testing with three EIA assays [GENEDIA HBsAg EIA 3.0 (Green Cross MS), BIO-RAD Monolisa HBsAg Ultra (BIO-RAD), and Murex HBsAg V.3 (Murex Biotec.)] and one CIA assay [Architect HBsAg (Abbott)] was performed on all units. Units with reactive results on CIA or units that were reactive on more than two assays were further subjected to HBV DNA quantification, HBV genotyping and subtyping. Units reactive on HBV DNA quantification were confirmed for HBsAg by HBsAg neutralization. The reactivity of a commercial low titer performance panel to various HBsAg assays was determined to be used as a selection criterion for candidate materials. Based on these results, 13 HBsAg positive units and two HBsAg negative units were selected as candidates. After addition of Bronidox as a preservative, the candidate materials were distributed into the final containers. Collaborative study with seven participating laboratories was conducted using two CIA assays [Architect HBsAg, Prism HBsAg (Abbott)], one ECA assay [Elecsys HBsAg (Roche Diagnostics)], one MEIA assay [AxSYM HBsAg V.2 (Abbott)], and three EIA assays [Behring Enzygnost HBsAg 5.0 (Dade Behring), BIO-RAD Monolisa HBsAg Ultra, Murex HBsAg V.3]
Results: Based on the results of the collaborative study, 11 HBsAg positive units and two HBsAg negative units were selected to constitute the low titer performance panel. The mean S/C ratio of HBsAg positive units was less than 10.0 and mean concentration of HBsAg of ten HBsAg positive units was less than 1.0 IU/mL. The panel members were of genotype C, subtype adr and ayr.
Conclusions: As a result of this study, a low titer HBsAg performance panel for quality control of HBV diagnostics kits has been established. This will enable supply of quality control materials at an affordable cost on a long-term basis.

This research was supported by a grant (08122KFDA274) from Korea Food & Drug Administration in 2008.

2A-S02-05

STATUS OF HEPATITIS VIRAL MARKERS CALCULATED FROM PRETRANSFUSION VIRAL MARKER TEST RESULTS OF PATIENTS AT ASAHIKAWA MEDICAL COLLEGE HOSPITAL

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Background: In October 2004, the Japanese government recommended that six viral markers be tested in patients scheduled for transfusion: hepatitis B surface antigen (HBsAg), antibody to HBsAg (HBsAb), antibody to HBV core antigen (HBcAb), antibody to hepatitis C virus (HCVAb), HCV core antigen (HCVcAg), and antibody to human immunodeficiency virus (HIVAb). At our hospital, we started testing these markers of pretransfusion patients in July 2005.
Aim: Japan is regarded as an endemic area of HBV and HCV. Therefore, it is considered that many Japanese are in a state of asymptomatic or latent HBV or HCV infection. At our hospital, a series of hepatitis marker tests (HBsAg, HBsAb, HBcAb, HCVAb, HCVcAg) was prepared. Physicians used this set menu to evaluate the status of hepatitis viral markers before transfusion. For this study, we calculated the status of hepatitis viral markers at our hospital from results of the pretransfusion viral marker tests conducted routinely before transfusion.
Materials and methods: Hepatitis viral markers of 3353 patients during July 2005 and December 2008 were evaluated. Data were collected from the database of our hospital information system. Measurement methods and positive values were the following: HBsAg (CLIA, > 0.5 IU/ml), HBsAb (CLIA, >10 mIU/ml), HBcAb (CLEIA, >70%INH), HCVAb (CLIA, >1.0 C.O.I.), HCVcAg (CLEIA, >50 fmol/l).
Results: The cases were those of 1721 men and 1632 women. Their average age was 59.9 years (0–96 yr). The positive rates of HBsAg, HBsAb, HBcAb, HCVAb, and HCVcAg are presented as a table. The rate of positive HBsAb with negative HBcAb was 8.9%, the rate of negative HBsAb with positive HBcAb was 9.9%, the rate of both positive was 20.3%, and the rate of both negative was 60.9%. Among 204 HCVAb positive cases, 118 cases were HCVcAg positive. The others were HCVcAg negative. No HCVcAg positive case was HCVAb negative. Among the 107 cases that were positive for both some HBV marker and some HCV marker, 88 cases were HBcAb positive.
Summary: We determined the status of hepatitis viral markers of a hospital based on results of pretransfusion viral tests. We assessed the status of apparent or latent hepatitis viral infection from a hospital level to a nationwide level if a pretransfusion viral marker test were strictly implemented for all patients scheduled for transfusion. Furthermore, these data provide background information for developing preventive measures against hepatitis viral infections, including transfusion-transmitted infections and hospital infections.

Table 1. Age related positive rate of viral marker

age	number of pts.	positive rate of viral marker				
		HBsAg	HBsAb	HBcAb	HCVAb	HCVcAg
0-9 yr	87	1·1%	6·9%	3·4%	0·0%	0·0%
10-19 yr	42	0·0%	2·4%	0·0%	2·4%	0·0%
20-29 yr	130	1·5%	12·3%	4·6%	1·6%	0·0%
30-39 yr	266	2·3%	14·7%	8·6%	1·5%	0·4%
40-49 yr	285	3·2%	20·0%	15·4%	3·5%	1·1%
50-59 yr	538	6·5%	30·1%	32·5%	4·3%	2·1%
60-69 yr	755	7·3%	36·7%	41·3%	8·5%	4·7%
70-79 yr	900	1·6%	32·2%	34·4%	9·1%	6·1%
80-89 yr	323	1·2%	38·4%	40·9%	7·4%	3·1%
over 90 yr	27	0·0%	25·9%	29·6%	0·0%	0·0%
total	3353	3·7%	29·2%	30·2%	6·3%	3·6%

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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
				2009. 12. 20	該当なし	
一般的名称	人血清アルブミン		研究報告の公表状況	Iwanaga M, Koga Y, Soda M, Inokuchi N, Sasaki D, Hasegawa H, Yanagihara K, Yamaguchi K, Kamihira S, Yamada Y. 51st ASH Annual Meeting and Exposition; 2009 Dec 5-8; New Orleans.	公表国 日本	
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)					

研究報告の概要

○ヒトT細胞白血病ウイルス1型(HTLV-1)有病率の傾向および長崎(日本)の成人T細胞白血病/リンパ腫(ATL)の発症率:病院ベースおよび集団ベース試験

序論:HTLV-1の有病率は、主に献血者の年齢別抗体陽性率により評価されATL発症率が推定されてきたが、献血者集団の特性から過小評価されている可能性がある。献血者以外のHTLV-1キャリアの出生年別ATL発症率データは少ない。

方法:2000〜2007年に長崎大学病院を受診した患者10,261名(男性:5,523、女性:4,737)のHTLV-1抗体検査のデータ、及び長崎県がん登録中の長崎市で診断されたATL症例360例(男性:188、女性:172)のデータを評価した。長崎市の2006国勢調査人口に病院ベースの陽性率データを適用して、HTLV-1キャリアの出生年別ATL発症率を推定した。

結果:患者10,261名のうち、HTLV-1抗体陽性者は1,392名(男性:653、女性:739)、陽性率は13.57%(95%CI:12.90-14.23%)であった。陽性率は女性が有意に高かった(15.60%対11.82%、P<0.0001)。出生年別抗体陽性率は、18.69%(1926年以前)、17.83%(1927-1936)、15.91%(1937-1946)、13.80%(1947-1956)、9.19%(1957-1966)、4.07%(1967-1976)、2.07%(1977-1986)、0%(1987年以降)であった(有意な減少傾向:P < 0.0001)。長崎市の出生年別の年間HTLV-1キャリア推定人数は、それぞれ5257、8093、8151、8083、4434、2180、785、0であった。キャリア100,000人あたりの年間ATLL発症率の推定は、それぞれ171、86、41、32、11、0、0、0となった。HTLV-1キャリアの生涯の粗ATL発症リスクは、男性7.29%、女性3.78%と推定された。

結論:本試験の出生年別HTLV-1抗体陽性率は供血者の陽性率より約50%高く、流行地域で高齢者の大規模なキャリア集団が現在も存在することを示唆している。発症予防のためATL発現機序を解明するには更なる試験が必要である。

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使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
長崎大学病院を受診した患者の出生年別HTLV-1抗体陽性率は過去に報告された供血者の陽性率と比べて約50%高く、流行地域において高齢者のHTLV-1キャリアの大規模集団が存在することが示唆されたとの報告である。 HTLV-1は脂質膜を有するRNAウイルスである。垂直感染により日本では古代から広く侵淫しているが、本製剤による感染の報告はない。本製剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれているとから、本製剤の安全性は確保されていると考える。	日本赤十字社では献血時のスクリーニング法として、より感度の高い化学発光酵素免疫測定法(CLEIA)によるHTLV-1抗体のスクリーニング検査を行っている。今後も引き続き情報の収集に努める。

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Poster Session

NON-HODGKIN'S LYMPHOMA : BIOLOGY, EXCLUDING THERAPY POSTER I

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Trends in Human T-Cell Leukemia Virus Type-1 (HTLV-1) Prevalence and the Incidence of Adult T-Cell Leukemia/Lymphoma (ATL) in Nagasaki, Japan: A Hospital-Based and Population-Based Study.

Masako Iwanaga[1], Yoshito Koga[*,2], Midori Soda[*,3], Naoko Inokuchi[*,2], Daisuke Sasaki[*,4], Hiroo Hasegawa[*,5], Katsunori Yanagihara[*,4], Kazunari Yamaguchi[*,6], Shimeru Kamihira[*,5] and Yasuaki Yamada[5]

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Abstract 1920

Poster Board I-943

Introduction: The prevalence of HTLV-1 is mostly evaluated by the age-specific seroprevalence in blood donors, and the results have been conventionally used to estimate the age-specific incidence of ATL in Japan. However, the results may be underestimated due to an age limit (16-69 yr) for donation, a healthy donor effect, and a birth cohort effect. Data concerning the birth-year specific incidence of ATL among HTLV-1 carriers other than blood donors are scarce.

Methods: The study evaluated data of the anti-HTLV-1 antibody testing of 10,261 patients (males: 5,523, females: 4,737) who visited the Nagasaki University Hospital during 2000-2007 and data of 360 ATL cases (males: 188, females: 172) who were diagnosed in Nagasaki City (an endemic area in Japan) in a population-based Nagasaki Prefectural Cancer Registry (NPCR). To estimate birth-year specific incidence rates of ATL in population-based HTLV-1 carriers, we used the 2006 census population for Nagasaki City by applying the hospital-based seroprevalence data.

Results: Of 10,261 patients, 1,392 (males: 653, females: 739) were HTLV-1 antibody positive. The overall HTLV-1 seroprevalence was 13.57% (95%CI: 12.90-14.23%). The seroprevalence was significantly higher in females than in males (15.60% vs. 11.82%, $P<0.0001$). The birth-year specific seroprevalence was 18.69% (before 1926), 17.83% (1927-1936), 15.91% (1937-1946), 13.80% (1947-1956), 9.19% (1957-1966), 4.07% (1967-1976), 2.07% (1977-1986), and 0% (after 1987) (a significantly declining trend: $P<0.0001$). The estimated annual number of HTLV-1 carriers by birth-year in Nagasaki city was 5257, 8093, 8151, 8083, 4434, 2180, 785, and 0, respectively. Finally, we estimated the annual incidence rate of ATLL per 100,000 HTLV-1 carriers by birth-year, 171 (before 1926), 86 (1927-1936), 41 (1937-1946), 32 (1947-1956), 11 (1957-1966), and 0 (after 1967). The crude lifetime risk of developing ATLL in HTLV-1 carriers was estimated to be 7.29% for males and 3.78% for females.

Conclusions: The birth-year specific HTLV-1 seroprevalnces in the present study were approximately 50% higher than those previously reported in blood donors1 (for example: 6.22% in those born before 1950). Although it is possible that our results are over-estimated2, the present study suggests that there is still a large pool of elderly HTLV-1 carriers in this endemic area. Further studies are needed to investigate the mechanism of the development of ATL among HTLV-1 carriers for preventing the development. Reference:
1) Iwanaga M et al. Int J Hematol, 2009. 2) Arisawa K et al. Int J Cancer, 2000.

Disclosures: No relevant conflicts of interest to declare.

Footnotes

* Asterisk with author names denotes non-ASH members.

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医薬品 研究報告 調査報告書

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研究報告の概要

○ヒトT細胞リンパ球向性ウイルス1型(HTLV-1)抗体陽性献血者のプロウイルス量の定量と輸血感染症の感染性ウイルス量の評価

背景:献血血液の抗体スクリーニングと保存前白血球除去は、日本におけるHTLV-1の輸血感染(TTI)リスクを確実に低下させた。しかし、TTIを生じる血液成分中プロウイルス量は不明である。

目的:HTLV-1抗体陽性献血者から白血球除去前に採取した血液検体のプロウイルス量を調べた。献血者のプロウイルス量の分布と過去のデータから、感染性を持つプロウイルス量を推定した。

方法:献血者74名の保管検体の末梢血単核細胞または凝固血液からDNAサンプルを得た。検体は白血球除去前に採取した。HTLV-1pX領域とヒトCD81遺伝子のTaqMan PCRでプロウイルス量を測定し、細胞DNA量を推定した。過去のデータでは、抗体陽性献血者由来の赤血球濃厚液1単位の輸血を受けた患者約80%にセロコンバージョンが起こったことから、血液検体の80%に感染リスクがあると仮定し、輸血感染力を持つウイルス量を推定した。

結果:献血者のHTLV-1プロウイルス量は、100白血球につき、0.01~4.9コピー(平均0.83)だった。評価した検体の80%には、100白血球あたり少なくとも0.06コピーのプロウイルスが含まれていた。赤血球濃厚液1単位あたりの白血球数を白血球除去前は1×10^9と仮定すると、TTIを生じた製剤には最低6×10^5のHTLV-1感染細胞が含まれる。

結論:2007年、日本のすべての血液製剤に保存前白血球除去が導入され、残存白血球数は製剤の99%で1×10^6未満と確認されている。スクリーニング未実施でもHTLV-1感染細胞の最大数は1製剤につき4.9×10^4と考えられ、推定される感染性ウイルス量(6×10^5の感染細胞)よりかなり低い。抗体スクリーニングと白血球除去は、日本におけるHTLV-1のTTIリスクを事実上排除した。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

報告企業の意見

HTLV-1抗体陽性供血者の血液成分中のHTLV-1感染細胞の最大数は推定される感染性ウイルス量よりかなり低く、血清学的スクリーニングと白血球除去によって輸血感染リスクは事実上排除されているとの報告である。
HTLV-1は脂質膜を有するRNAウイルスである。垂直感染により日本では古代から広く侵淫しているが、本製剤による感染の報告はない。本製剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれているとから、本製剤の安全性は確保されていると考える。

今後の対応

日本赤十字社では献血時のスクリーニング法として、より感度の高い化学発光酵素免疫測定法(CLEIA)によるHTLV-1抗体のスクリーニング検査を行っている。今後も引き続き情報の収集に努める。

P-141

QUANTIFICATION OF HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE 1 (HTLV-1) PROVIRUS LOAD IN SEROPOSITIVE BLOOD DONORS AND ESTIMATION OF INFECTIOUS VIRAL LOAD FOR TRANSFUSION-TRANSMITTED INFECTION

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Background: Serological screening and prestorage leukocyte reduction for donated blood have undoubtedly decreased the risk of transfusion-transmitted infection (TTI) for HTLV-1 in Japan. However, the provirus load in blood component that would cause TTI is still unclear.

Aims: HTLV-1 provirus load was measured in blood samples collected before leukocyte reduction that were obtained from seropositive blood donors. From the distribution of provirus load among blood donors, provirus load for infectivity was estimated using the historical data on the frequency of transfusion-transmitted infection.

Methods: DNA samples were obtained from peripheral blood mononuclear cells or blood clots of stored samples obtained from 74 HTLV-1-seropositive individuals. All blood samples were obtained before leukocyte reduction. HTLV-1 provirus load was determined using TaqMan PCR for the HTLV-1 pX region and human CD81 gene to estimate the amount of cellular DNA. Previous data showed that seroconversion occurred in approximately 80% of patients transfused with one unit of fresh red-cell concentrate from HTLV-1-seropositive blood donors (Okochi K et al. AIDS Res 1986;2:S157–61). It is, therefore, expected that 80% of our blood samples will be in the category of units with infectious risk, which allows us to estimate the viral load for infectivity by transfusion.

Results: The HTLV-1 provirus loads in HTLV-1-seropositive blood donors ranged from less than 0.01 to 4.9 copies (average 0.83) per 100 leukocytes. Eighty per cent of blood samples evaluated contained at least 0.06 copies of HTLV-1 provirus per 100 leukocytes. Assuming that the number of leukocytes per unit of red-cell concentrate was $1 \cdot 10^9$ before leukocyte reduction, a minimum of $6 \cdot 10^5$ HTLV-1-infected cells would have been found in the unit that caused TTI.

Conclusions: In 2007, universal prestorage leukocyte reduction was introduced for all blood components in Japan. The number of residual leukocytes after leukocyte reduction is confirmed to be less than $1 \cdot 10^6$ in 99% of unit currently issued from Japanese Red Cross Blood Center. If serological screening is omitted, the maximum number of HTLV-1-infected cells found in blood components would be $4.9 \cdot 10^4$ per unit. This figure is substantially lower than the infectious virus load estimated ($6 \cdot 10^5$ infected cells). The combination of serological screening and universal leukocyte reduction virtually eliminated the TTI risk for HTLV-1 in Japan.

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医薬品 研究報告 調査報告書

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研究報告の概要

○米国の血液供給における*T. cruzi*スクリーニングの費用対効果
背景:シャーガス病の病原体である*Trypanosoma cruzi* (*T. cruzi*)は、輸血の安全性を脅かしている。現在、米国の供血血液の75～80%に*T. cruzi*のスクリーニング検査が行われており、29,000名当たり1名が陽性と考えられる。病原体の輸血による感染性とシャーガス病の拡大については特徴が十分に分かっておらず、全国的なスクリーニングの費用対効果は報告されていない。
方法:*T. cruzi*の脅威とその制圧にかかる費用を評価するために、疾患の進行モデルを用いて受血者の仮想集団の生涯コストと健康アウトカムを異なるスクリーニング計画下で比較した。以下の7つの供血者・供血血液検査方法を分析し、スクリーニング非実施と比較した:1)ラテンアメリカで生まれた供血者、2)血小板供血、3)初回供血者、4)全供血者を1回、5)全供血者を2回、6)全血と血小板供血、7)すべての供血。モデルパラメータは、スクリーニング・データまたは文献レビューより得た。一元感度分析および確率的感度分析を用いて、影響力のあるパラメータと全体の不確実性を評価した。
結果:各方法の費用対効果は、1)170,000、2)330,000、3)370,000、4)760,000、5)970,000、6)1,070,000、7)1,360,000(単位:ドル/QALY)となった。モデルにおける最も影響力のあるパラメータは、受血者の特徴と関連がある(生存率、健康状態ユーティリティ、将来の健康状態の低下率)。最も影響が強かったのは*T. cruzi*の血清陽性率と伝播効率だった。CE比率は、米国が報告した血清陽性率1/3,333と1/100,000の範囲ではそれぞれ、92%低く(よい)、215%高かった(悪い)。このモデルは、シャーガス病に伴う変数に無関係だった。
結論:本分析では、選択的*T. cruzi*スクリーニング検査は、全数検査とほぼ同等の効果があり、コストが低いことを示している。これらの所見は、輸血による伝播が観察されなかった、2年間の試験およびルックバック・データと整合している。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血

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
米国の血液供給における*T. cruzi*検査において、選択的スクリーニングは全数検査とほぼ同等の効果があり、コストが低いことが示されたとの報告である。	日本赤十字社は、輸血感染症対策として献血時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、シャーガス病の既往がある場合には献血不適としている。日本在住の中南米出身献血者については、厚生労働科学研究「献血血の安全性確保と安定供給のための新興感染症等に対する検査スクリーニング法等の開発と献血制限に関する研究」班と共同して検討する予定である。今後も引き続き情報の収集に努める。

time. Finally, for donors with previously negative donations and recent travel to Latin America, there was no evidence of incident infection. **Conclusion:** While country of birth is the best predictor of *T. cruzi* infection, these data indicate that selective testing based solely on donor responses to any question or combination of questions would be insufficient to identify all RIPA+ *T. cruzi* infections. These data also have helped in guiding the development of a large incidence study to accompany conversion to selective 1-time testing of all donors in most of the US.

Disclosure of Commercial Conflict of Interest

M. Agapova: Nothing to disclose; H. H. Biswas: Nothing to disclose; M. P. Busch: Nothing to disclose; B. Custer: Nothing to disclose; H. Kamel: Nothing to disclose; P. Tomasulo: Nothing to disclose

Disclosure of Grants Conflict of Interest

M. Agapova: Nothing to disclose; H. H. Biswas: Nothing to disclose; M. P. Busch: Gen-Probe, Inc., Grants or Research Support, Travel Support or Honorarium Chiron / Novartis, Grants or Research Support, Travel Support or Honorarium Ortho, Travel Support or Honorarium Abbott, Travel Support or Honorarium; B. Custer: Nothing to disclose; H. Kamel: Nothing to disclose; P. Tomasulo: Nothing to disclose

Birth Country or Region	Allogeneic Donors Number (%) N = 703,725	*T cruzi* RIPA– Number (%) N = 34	*T cruzi* RIPA– Prevalence by Birth Country or Region
USA	546,230 (77.2)	10 (29.4)	1:54,623
Mexico	18,511 (2.6)	10 (29.4)	1:1,851
Central or South America	2,235 (0.3)	8 (23.5)	1:279
All other countries	13,254 (1.9)	1 (2.9)	1:13,254
Missing/Unreported	123,495 (17.9)	5 (14.7)	1:24,699

S38-020D

Sensitivity of Selective Testing for Antibody to *Trypanosoma cruzi* (*T. cruzi*)

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Background: Donor screening for *Trypanosoma cruzi* antibody began in 2007 at the American Red Cross by testing each donation (dtn) from every donor (universal testing). Data for the 22-month experience with universal testing (Jan 27 07-Nov 30 08) were examined to determine the sensitivity of selective testing. Donor data collected included: risk factors related to direct or indirect exposure in a non-US endemic country, US-derived infection, ELISA false negativity (neg) and potential incident infection. **Methods:** The Ortho *T. cruzi* ELISA was used to screen each dtn. Repeat reactive (RR) dtns were further tested using a research radioimmunoprecipitation assay (RIPA); RIPA-pos donors were considered confirmed. RR donors were followed and tested by repeat serologic/parasitologic tests (PCR/hemoculture-HC). Donors were also asked to respond to a detailed survey regarding risk factors; RIPA-pos donors were defined as cases and RIPA-unconfirmed as controls. **Results:** Prevalence for ~13 million dtns screened was 1:36,000 (RR rate = 0.014%) and identified 394 RIPA-pos donors of which 4-14% were pos for the presence of the parasite by PCR/HC. Of 157 pos donors who completed a risk survey, all but 40 were born in an endemic area compared to 5/457 controls ($p < 0.0001$; OR 256 by univariate and 32 by multivariate analyses). The 40 US-derived cases came from 18 states; 6 had congenital infection and 7 others had identified risks (2 due to residence in an endemic area prior to *T. cruzi* screening and 5 with outdoor activities in the Southern US). 16/394 (4%) ELISA RR/RIPA pos donors had prior ELISA false-neg donation results of which 11 had one prior neg dtn and 5 had >1 prior neg dtn; 8/16 had prior neg reactivity within 20% of the ELISA cutoff. A 20% reduction in the assay cutoff would increase the RR rate by 0.025%. None of the 16 was PCR/HC pos, and of those followed (13/16), all ELISA signals were stable and none represented incident cases. No incident donors were identified in 2.5 million donors with >2 neg dtns during the 22 months (>2.3 million person years of observation; neg dtn interval = 0.9 years). Sensitivity by method is provided in the Table. **Conclusions:** A selective testing strategy based on qualifying a donor by a single neg tested dtn had high sensitivity. A protocol to further determine *T. cruzi* donor incidence is under development with collaborators from Blood Systems.

Disclosure of Commercial Conflict of Interest

J. P. Brodsky: Abbott Laboratories, Stocks or Bonds; R. Y. Dodd: Nothing to disclose; G. A. Foster: Nothing to disclose; D. Krysztof: Nothing to disclose; D. A. Leiby: Nothing to disclose; B. A. Lenes: Ortho diagnostics, Other; C. Rouault: Nothing to disclose; S. L. Stramer: Nothing to disclose; R. L. Townsend: Nothing to disclose

Disclosure of Grants Conflict of Interest

J. P. Brodsky: Nothing to disclose; R. Y. Dodd: Nothing to disclose; G. A. Foster: Nothing to disclose; D. Krysztof: Nothing to disclose; D. A. Leiby: Nothing to disclose; B. A. Lenes: Nothing to disclose; C. Rouault: Nothing to disclose; S. L. Stramer: Nothing to disclose; R. L. Townsend: Nothing to disclose

Method	# Detected/Tested	% Sensitivity (95% CI)
Universal Testing (Ortho)	860/861	99.88 (99.35-100)
Endemic birth question	117/157	74.52 (67.83-81.87)
1 × neg	378/394	95.94 (93.49-97.66)
2 × neg	389/394	98.73 (97.06-99.59)

S39-020D

Cost-Effectiveness of Screening for *T. cruzi* in the US Blood Supply

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Background: *Trypanosoma cruzi* (*T. cruzi*), the etiologic agent of Chagas disease is a safety threat to transfusion. Currently, 75-80% of US donations are screened for *T. cruzi*. Overall, 1 donor out of 29,000 is expected to be confirmed *T. cruzi* positive. The transmissibility of the pathogen by transfusion and progression to Chagas disease are not well characterized in the US, and the cost-effectiveness of nation-wide screening has not been reported. **Methods:** To evaluate the threat of *T. cruzi* as well as costs associated with interdicting this threat, we used disease progression modeling to compare lifetime costs and health outcomes of a hypothetical cohort of blood recipients under different screening strategies. We analyzed 7 donor or donation testing strategies: 1) donors born in Latin America; 2) platelet donations; 3) first-time donors; 4) all donors one time; 5) all donors two times; 6) whole blood and platelet donations and 7) all donations. Each strategy was compared to no screening. Model parameters were obtained from laboratory screening data or literature review. One-way and probabilistic sensitivity analyses were used to assess influential parameters and overall uncertainty. **Results:** Costs, effectiveness and the cost-effectiveness (CE) of each strategy compared to no testing, are provided in the table. The cost-effectiveness of testing all donors one time is $760,000/QALY, all donors two times is $970,000/QALY and universal testing is $1.36M/QALY. The most influential parameters in the model are related to characteristics of the transfused population: survival rate, health state utilities and discount rate for future health states. With respect to *T. cruzi*, seroprevalence and transmission efficiency are the most influential. CE ratios were 92% lower (better) and 215% higher (worse) between the limits of US-reported seroprevalence, 1/3333 and 1/100,000, respectively. The model was insensitive to variables associated with Chagas disease. **Conclusions:** This analysis suggests that selective *T. cruzi* screening generates nearly the same effectiveness as universal screening, but at reduced cost. These findings are consistent with 2-years of testing and lookback data, where incident infections or substantial transmission by transfusion have not been observed.

Disclosure of Commercial Conflict of Interest

M. Agapova: Nothing to disclose; B. Custer: Nothing to disclose

Disclosure of Grants Conflict of Interest

M. Agapova: Nothing to disclose; B. Custer: Nothing to disclose

Testing Strategy	Cost ($)	Effectiveness (QALYs)	CE Ratio ($/QALYs)	CE Ratio 95% range ($/QALYs)
No testing	0.06	8.56605503	–	–
Born in Latin America	0.48	8.56605809	170,000	55,000-380,000
Platelets	0.81	8.56605772	330,000	120,000-690,000
First time	1.36	8.56605906	370,000	160,000-710,000
One time all	3.54	8.56606013	760,000	350,000-1,410,000
Two time all	4.61	8.56606024	970,000	450,000-1,810,000
Whole Blood/Platelets	5.41	8.56606027	1,070,000	500,000-2,010,000
Universal	6.56	8.56606030	1,360,000	640,000-2,530,000

医薬品 研究報告 調査報告書

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販売名（企業名）	赤十字アルブミン20（日本赤十字社） 赤十字アルブミン25（日本赤十字社） 赤十字アルブミン20%静注4g/20mL（日本赤十字社） 赤十字アルブミン20%静注10g/50mL（日本赤十字社） 赤十字アルブミン25%静注12.5g/50mL（日本赤十字社）					

研究報告の概要

○マヤロ熱ウイルス、ブラジル・アマゾン
マヤロウイルスはアルファウイルス属トガウイルス科で、ジェノタイプDとLの2系統が確認されている。流行地は南米の熱帯地域で、発疹、発熱、重い関節痛などのデング様の疾患と関連している。関節痛は数週間持続することもある。
2008年2月、マヤロ熱ウイルス（MAYV）のアウトブレイクが、ブラジル北部、パラー州サンタバーバラ県のベレム近郊の村で発生した。村の住民は150名程度で多くは貧しく、密林の真ん中の木製の家に住んでいた。発熱を訴えた105名のうち53名は村の住民、52名は農学専攻の学生で村の近隣の施設に1週間滞在していた。
患者は発疹、発熱、重い関節痛の症状を呈し最長7日間持続した。患者の血清検体のIgMをELISAで検査したところ、36検体からIgMが検出された。MAYV分離株3株がジェノタイプDと確認され、系統発生解析では、1991年にブラジル北部で分離された株と近縁であることが明らかとなった。
また、村で蚊を捕獲したところ、832匹のうち188匹がMAYVの主要な媒介蚊である*Haemagogus janthinomys*だった。蚊から採取された検体及び患者の急性期血清検体がマウスに感染性を持つことが確認された。

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使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
ブラジル北部、パラー州サンタバーバラ県のベレム近郊の村で、マヤロ熱ウイルスの流行が見られたとの報告である。 マヤロ熱ウイルスは脂質膜を持つ中型のRNAウイルスで、これまで本製剤によるマヤロ熱発症の報告はない。本製剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれていることから、本剤の安全性は確保されていると考える。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国（入国）後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

Mayaro Fever Virus, Brazilian Amazon

Raimunda S.S. Azevedo, Eliana V.P. Silva, Valéria L. Carvalho, Sueli G. Rodrigues, Joaquim P. Nunes Neto, Hamilton A.O. Monteiro, Victor S. Peixoto, Jannifer O. Chiang, Márcio R.T. Nunes, and Pedro F.C. Vasconcelos

In February 2008, a Mayaro fever virus (MAYV) outbreak occurred in a settlement in Santa Barbara municipality, northern Brazil. Patients had rash, fever, and severe arthralgia lasting up to 7 days. Immunoglobulin M against MAYV was detected by ELISA in 36 persons; 3 MAYV isolates sequenced were characterized as genotype D.

Mayaro virus (MAYV) is a member of the family *Togaviridae* and the genus *Alphavirus*. Recent molecular studies have recognized 2 MAYV lineages: genotypes D and L (*1*). MAYV has been associated with a dengue-like illness with rash, fever, and severe arthralgia in tropical South America. Arthralgia lasts for several weeks and affects principally ankles, wrists, and toes, but also can affect major joints. MAYV causes a mild to moderately severe acute febrile illness of 3–5 days' duration with uneventful recovery (*2*).

The Study

In February 2008, an outbreak of a dengue-like illness was reported in the Pau D'arco settlement, 38 km from Belém, Para state, in the Brazilian Amazon (online Appendix Figure, available from www.cdc.gov/EID/content/15/11/1830-appF.htm). This rural community has 48 houses with ≈150 inhabitants, many of whom live in poor conditions. They reside in the middle of a native forest, in softwood houses, in the municipality of Santa Barbara (2007 population ≈14,439).

A total of 105 persons were examined in a house-to-house survey. They reported a febrile illness within the past 30 days, had a current febrile illness, or reported contact with persons with febrile illness. Fifty-three resided in the settlement (50 were agricultural workers), and 52 were agronomy students at a public university in Belém and had been training for a week at a field station adjacent to the settlement. The students slept in the station for a week; their activities included periodic visits to the settlement and sporadic ingression to the forest. Students and agricultural workers were bled weekly by convenience from March 17 through April 4, 2008. All serum samples were processed by ELISA for detection of immunoglobulin (Ig) M (*3*).

During the same diurnal period (9:00 AM–3:00 PM), mosquitoes were captured in the settlement by using human bait on the ground and in the forest canopy (≈15 m high) near the residences. A total of 832 (49 lots) *Culicidae* mosquitoes were collected and frozen before being used for virus isolation. Of these, 188 (11 lots) were *Haemagogus janthinomys*, the main vector of MAYV; the remaining 644 (38 lots) were mainly members of the genera *Wyeomyia, Aedes, Sabethes*, and *Limatus*.

Newborn mice (*Mus musculus*) and C6/36 cells were inoculated with acute-phase serum from samples collected from febrile patients and pooled mosquitoes, as previously described (*4,5*). The inoculated animals and cells were observed daily, and the presence of virus was confirmed by complement fixation and immunofluorescent assays (*4*). Three MAYV strains were isolated: 2 from febrile persons and 1 from a pool with 2 *H. janthinomys* mosquitoes collected at ground level. All 3 strains were isolated with both assays.

IgM was detected in 36 (34%) serum samples (Figure 1, panel A). Of those 36 samples, 23 (64%) were collected from residents of the settlement, and 13 (36%) were from residents of Belém and Ananindeua municipalities; these persons had visited the settlement area for a week (Figure 2, panel B). Persons with Mayaro fever ranged in age from 4 to 55 years, and 21 (58%) were male (Figure 1, panel C). Fifty-two percent of MAYV-positive persons were students, 31% were agriculturists, and 17% participated in other activities (Figure 1, panel D).

Of the 36 MAYV-infected persons, 33 were symptomatic. Illness was characterized by sudden onset of fever (100% of patients), arthralgia (89%), myalgia (75%), headache (64%), articular edema (58%), rash (49%), and retroocular pain (44%). Other less frequent symptoms were itching (33%), dizziness (25%), anorexia (22%), swollen lymph nodes (17%), and vomiting (4%).

Other common exanthematic illnesses in Brazil included in the differential diagnoses were dengue fever, rubella, B19 parvovirus, human herpesvirus 6, infectious mononucleosis, malaria, and yellow fever. Serologic results excluded these illnesses.

RNA was extracted by using the TRIZOL LS (Invitogen, Carlsbad, CA, USA) reagent method according to the manufacturer's instructions. Envelope (E)2 and E1 genes of the MAYV genome were amplified by using a standard 1-step reverse transcription–PCR protocol, as pre-

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DOI: 10.3201/eid1511.090461

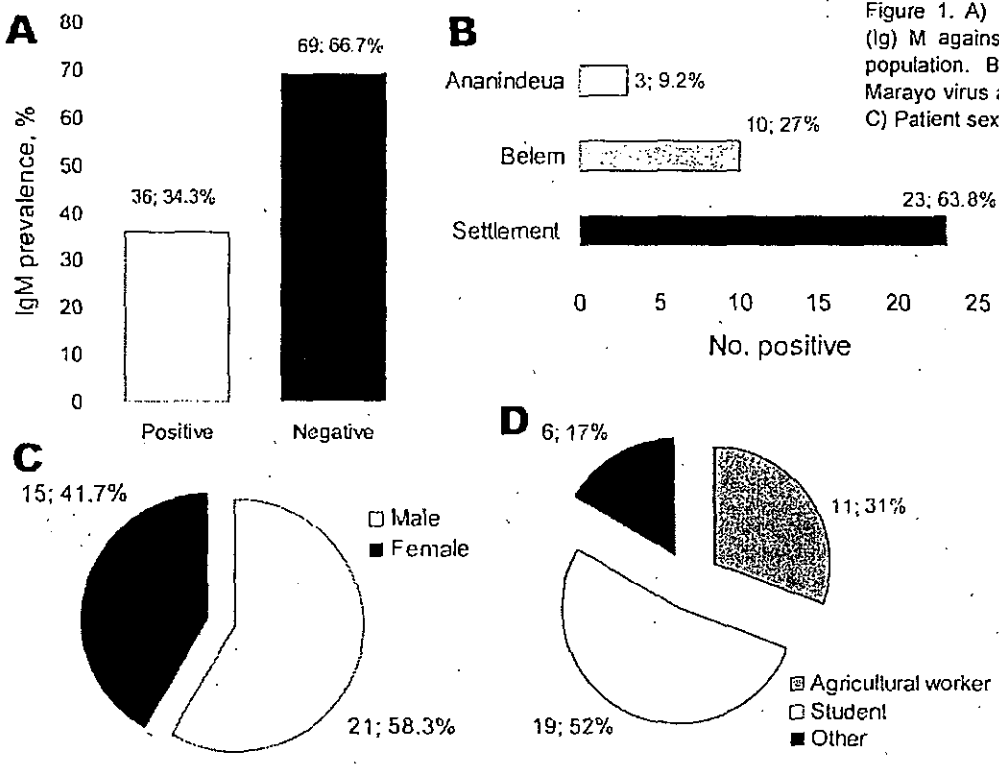

Figure 1. A) Prevalence of immunoglobulin (Ig) M against Mayaro virus in the studied population. B) Prevalence of IgM against Marayo virus according to area of residence. C) Patient sex. D) Patient work activities.

viously described (*1*). The cDNA products were directly sequenced (*6*).

We conducted phylogenetic analysis by using the maximum parsimony (heuristic algorithm), neighbor-joining (Kimura 3-parameter and F84 corrections), and maximum-likelihood methods (*7*) implemented in the PAUP software (*8*) for the nucleotide sequences obtained for the isolates and representative members of other Mayaro-related viruses belonging to the genus *Alphavirus* available at GenBank (www.ncbi.nlm.nih.gov). Bootstrap resample method (1,000 replicates) and outgoup definition were used to provide confidence for the phylogenetic groups (*9*).

The 3 MAYV isolates were successfully sequenced, and the nucleotide sequences covering the 3′ E1 region, the entire E2 gene, and 3′ noncoding region (≈2,000 nt) were phylogenetically compared with other MAYV and Mayaro-related viruses isolated during different periods (1954–2008) and from different hosts (human and arthropods) in Brazil, Peru, French Guiana, Trinidad and Tobago, Suriname, and Bolivia (Figure 2).

The phylogram depicted a clear segregation of MAYV strains into 2 major groups: genotypes D and L (*1*). The strains isolated in Santa Barbara municipality were grouped together in genotype D within clade I. Genetically, these strains were closely related to a 1991 isolate from Tocantins state in northern Brazil. The strains isolated in Santa Barbara were similar to those isolated in Belém during the same period. Interestingly, the Santa Barbara and Belém strains differed from the Brazilian and prototype strains isolated in 1955 (Figure 2).

Conclusions

MAYV has been isolated only in northern South America. Probably because of the short viremic period, it is sporadically isolated only during enzootic periods. However, during epidemics or epizootics, the number of isolates increase sharply (*10,11*). The few isolates obtained are intriguing and contrast with the high prevalence of specific antibodies in Pan-Amazonia; previous studies have shown widespread immunity in the Amazon, ranging from 5% to 60%. Positivity increases with age and is higher in rural and neighboring communities, as observed for the Amerindians (*2,12,13*).

In a previous outbreak in Belterra, several patients were too ill to continue their daily activities while febrile, and some even became prostrate. Moreover, these patients frequently reported severe arthralgia that led to temporary incapacitation (*13,14*).

Our data confirmed the occurrence of a Mayaro fever outbreak in the Pau D'Arco settlement. Clinically, the disease was similar to other outbreaks and characterized mainly by fever, arthralgia, myalgia, headache, rash, and dizziness (*2,13–15*). This outbreak was reported 17 years after the last episode of the disease described in the municipality of Benevides, which is closer (≈10 km) to Santa Barbara (P.F.C. Vasconcelos, unpub. data). The clinical and labora-

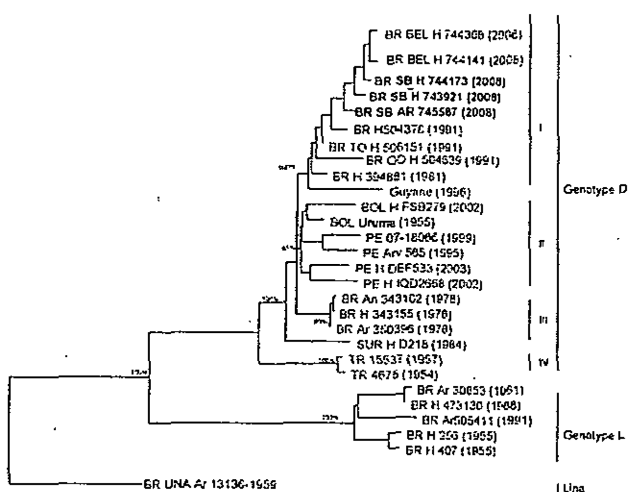

Figure 2. Comparison of genetic relationships among the Mayaro virus strains sequenced in this study with those isolated in different areas of South America, periods of time, and hosts. Numbers above and within parentheses correspond to bootstrap support values for the specific clades. The Una virus was used as an outgroup to root the tree. BR, Brazil (BEL, Belém; SB, Santa Barbara [bold]; TO, Tocantins state); BOL, Bolivia; PE, Peru; SUR, Suriname; H, human; Ar, arthropod. Numbers in parentheses correspond to the year of isolation of each strain. Items in boldface indicate strains isolated in this study.

tory data from this MAYV outbreak caused by genotype D confirmed in Santa Barbara provide a better understanding of the MAYV molecular epidemiology in the Brazilian Amazon region.

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				2010. 1. 26	該当なし	
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研究報告の概要

○ウスツウイルス神経浸潤感染の初のヒト症例:イタリア2009年8～9月

ウスツウイルス(USUV)はフラビウイルス科の節足動物媒介性ウイルスで、日本脳炎ウイルスと近縁である。過去10年間に、中央ヨーロッパで脳炎、心筋変性、肝臓・脾臓壊死を発症した鳥類から検出された。これまでにヒトにおいて重症もしくは死に至る疾患を発症した例はなかった。ここでは、発熱と神経症状を呈し髄膜脳炎と診断された、世界初となるUSUV神経浸潤感染の症例を報告する。

患者はイタリア在住の60代女性で、2009年5月にびまん性大細胞型B細胞リンパ腫のため半結腸切除術を受けた。同年8月21日までに6回の化学療法を受けた。その数日後、性器ヘルペスの再燃がありバラシクロビルを投与された。9月1日に39.5℃の発熱と安静時振戦を発症し、抗菌薬を投与されたものの高熱は持続した。9月5日に高熱と静注抗菌薬治療のため入院し、重度の貧血のため輸血を受けた。9月11日に採取された脳脊髄液はUSUV陽性であり、RT-PCRと塩基配列決定によって血清および血漿検体にもUSUVが示された。premembraneの部分配列とウイルス-ゲノムのNS5領域は、USUV Vienna株およびBudapest株と相同していた。髄膜脳炎の急性期前と後の血清(5月26日、10月13日)及び血漿(10月19日)検体からはウイルスは検出されなかった。

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使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン20%静注4g/20mL
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血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
発熱と神経症状を呈し髄膜脳炎と診断された、世界初となるウスツウイルス神経浸潤感染の症例報告である。 ウスツウイルスは脂質膜を持つ中型のRNAウイルスで、これまで本製剤によるウスツウイルス感染症の報告はない。本製剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれていることから、本剤の安全性は確保されていると考える。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

Rapid communications

First human case of Usutu virus neuroinvasive infection, Italy, August-September 2009

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We report the first worldwide case of Usutu virus (USUV) neuroinvasive infection in a patient with diffuse large B cell lymphoma who presented with fever and neurological symptoms and was diagnosed with meningoencephalitits. The cerebrospinal fluid was positive for USUV, and USUV was also demonstrated in serum and plasma samples by RT-PCR and sequencing. Partial sequences of the premembrane and NS5 regions of the viral genome were similar to the USUV Vienna and Budapest isolates.

Introduction

Usutu virus (USUV) is an arthropod-borne virus of the family *Flaviviridae*, genus *Flavivirus*. It is included in the Japanese encephalitis virus (JEV) group [1] being closely related to human pathogens such as JEV and West Nile virus (WNV). In the last decade, USUV was detected in a variety of central European birds with encephalitis, myocardial degeneration, and necrosis in liver and spleen [2-5]. As far as we know, the virus had never been associated with severe or fatal disease in humans [6]; it was isolated once in the Central African Republic in a man with fever and rash [7]. Here we report evidence of a neuroinvasive infection clinically related to USUV in Italy.

Case report

In May 2009, a woman in her 60s from Emilia Romagna region, Italy, underwent hemicolectomy because of a diffuse large B cell lymphoma. Six courses of chemotherapy were administered (including rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone), with last administration on 21 August 2009. Some days later, there was a reactivation of genital herpes treated with valacyclovir. On 1 September, a fever of 39.5°C with resting tremor appeared and antibiotic (moxifloxacine and amoxicilline clavulanate) therapy started however the temperature persisted. On 5 September, the patient was admitted to hospital for hyperpyrexia resistant to antipyretic and intravenous antibiotic treatment (meropenem and teicoplanine). Once admitted, the patient received blood transfusion because of a critical anaemia.

Examination of blood, urine and stool cultures and virological assessment for herpes virus simplex (HSV1/2) and cytomegalovirus (CMV) antigen were negative. A total body computerised tomography was performed without evidence of lymphoma. Suspicion of meningoencephalitis was addressed by neurological examination which showed distal resting tremor, positivity to the Romberg test, dysmetry and weakness at four limbs without cranial nerve affection. Magnetic resonance imaging (MRI) of the brain showed a signal alteration of the substantia nigra of the parietal and frontal subcortical areas that did not change after injection of contrast medium. On 11 September, the cerebrospinal fuid (CSF) was therefore collected and examined. The CSF was limpid without any alteration detected in the clinical-chemical analysis, activated lymphocytes were evident in the sediment. As further analysis of the same CSF specimen revealed the presence of flaviviruses (see below), steroid treatment was started. This therapy resolved the fever but did not lead to any improvement of the neurological symptoms. The electroencephalogram still registered diffuse slow theta waves and slow spike prevalent in left frontal parietal area. The neurological functions, mainly the resting tremor, improved following the administration of levodopa and carbidopa.

Virological analysis

When tested for the presence of viral agents, the CSF collected on 11 September was negative in molecular tests for CMV, HSV1/2, Epstein-Barr virus, adenoviruses, parvovirus B19, polyomavirus JC and BK, enteroviruses, mumps virus and WNV and positive to a heminested RT-PCR specific for the NS5 region of the *Flavivirus* genus [8]. The amplicon was directly sequenced and analysed by BLAST (http://www.ncbi.nlm.nih.gov/blast), revealing a 98% identity with both the USUV Budapest (gb|EF206350.1) and Vienna (gb|AY453411.1) isolate.

To confirm the identification of the species Usutu virus, we performed two USUV-specific RT-PCRs targeting the NS5 [2] and premembrane (preM) regions (primer sequences available on request) of the USUV genome on two plasma specimens collected on 8 and 11 September 2009 and one serum specimen collected on 14 September. The amplified products were sequenced (583 bp of NS5 and 602 bp of preM) and aligned with the corresponding sequences deposited in Genbank (gb|AY453411.1;

gb|EF206350.1) using ClustalW. The alignment of the preM gene shared 99% nucleotide identity with the USUV Budapest and Vienna sequences, whereas the NS5 gene sequences shared 100% nucleotide identity with USUV Vienna and 99% with USUV Budapest.

Further specimens of serum (26 May and 13 October) and plasma (19 October) before and after the acute phase of meningoencephalitis were analysed to demonstrate the absence of the virus. The two USUV-specific RT-PCRs performed on these three samples did not detect any USUV RNA. These samples were also analysed for WNV because a WNV outbreak was ongoing in the area at the time [9], and were negative.

Discussion

To our best knowledge this the first human disease with neurological involvement caused by USUV. The detection of USUV only in those samples collected during the acute phase of clinical manifestation is clear evidence that the virus caused the meningoencephalitis in the patient. Its capability of causing neurological lesions and death has already been reported in birds of central Europe [10]. The presence of USUV in Emilia Romagna has also been reported [4] and, in the past few months, the virus was isolated from black birds found dead in Northern Italy [G. Savini, personal communication 22 October 2009]. A surveillance programme in sentinel chicken flocks to monitor the possible appearance and/or circulation of WNV and other flaviviruses has been in place for several years. In the clinical case reported here, the immunosuppressed status of the patient due to both the underlying disease and the treatment, particularly with rituximab, may have played an important role in USUV infection and in its pathogenicity. It is known that rituximab can reactivate hepatitis B virus in patients with lethal fulminant hepatitis.

However, a possible unusual neuroinvasiveness and neurovirulence of this particular USUV strain cannot be excluded. The fact that neurological symptoms occurred prior to hospital admission excludes the transfusion as a possible source of infection. Conversely, since USUV as well as competent viral vectors are circulating in the patient's area of residence [4], it is likely that the infection was transmitted to the patient through mosquito bites.

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医薬品
医薬部外品　研究報告　調査報告書
化粧品

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2009 年 9 月 4 日		新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	Effectiveness of nanofiltration in removing small non-enveloped viruses from three different plasma-derived products. M. C. Menconi et al., Transfusion Medicine, 2009, 19, 213- 217	公表国 イタリア		
販売名（企業名）							

研究報告の概要		使用上の注意記載状況・その他参考事項等
研究報告の概要	血漿由来製剤は世界中の多くの患者にとって重要な治療薬である。これらの製造過程において，感染性病原体による汚染を防止するため，ウイルスの不活性化と除去処理を用いた効果的なウイルス除去工程が導入されている。ナノフィルトレーション（ウイルス除去膜濾過）は特にサイズ排除によるウイルス除去構造となっており，ヒトパルボウイルス B19（B19V）やトルクテノウイルス（TTV）などの小型でエンベロープを持たないウイルスを除去する際に有効とされている。本稿では，3 種類の血漿由来製剤：アルブミン溶液，プロトロンビン複合体（PTC），血液凝固第 IX 因子（FIX）から B19V および TTV をナノフィルトレーションによって除去し，その効果を評価した。各製剤に各ウイルス DNA 陽性血清を添加し，孔径 0.22 μm のプレフィルターで前処理した後，孔径 35 nm および 15 nm のプラバノ・フィルターによる末端濾過方式による定圧濾過を実施した。ウイルス除去効率を計測するためのウイルス量の測定はリアルタイム PCR 法を用いた。15 nm の濾過膜処理の結果，全ての製剤において B19V については 4.0 $\log_{10}$ 以上の除去能が確認された。TTV は，アルブミン溶液および FIX において 3.0 $\log_{10}$ 以上が除去されたが，PTC においては 15 nm の濾過膜処理後も高い除去効率は得られなかった。以上より，ナノフィルトレーションは血漿由来製剤のウイルス除去に有効であると考えられるが，似たようなウイルスであっても，溶液中のタンパクの組成構造やタンパク濃度により除去効率が影響を受けることが示唆された。	BYL-2010-0395

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報告企業の意見	今後の対応
B19V では，本研究で行われた孔径のナノフィルトレーションにより除去可能であることが判明したが，現時点では濾過に用いた容量が小さいため，今後より大量の溶液を用いての除去検査が必要であると考える。一方，TTV の場合には，ナノフィルトレーションの効果が最大限発揮できる PTC 濃度の調整が必要である。なお，弊社のコージネイト FS およびコージネイト FS バイオセットの製造工程培地で使用されている血漿分画成分に使用されるミニプール血漿においては，ヒトパルボウイルス B19 に対する NAT を実施しており，10E5 IU/mL 以上が確認された場合は，そのミニプール血漿は製造工程から除去している。製造工程においては，非エンベロープ 1 本鎖 DNA ウイルスについてブタパルボウイルスをモデルとした除去効率が 2.1log であることが実証されている。弊社で使用している血漿タンパクは培地成分としての使用であるため，伝播の可能性は非常に低いと考える。	現時点で新たな安全対策上の措置を講ずる必要はないと考えるが，今後ともウイルス除去特にヒトパルボウイルス B19 といった小型非エンベロープウイルスの除去効率の改善に関する情報収集に努める。

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SHORT COMMUNICATION

Effectiveness of nanofiltration in removing small non-enveloped viruses from three different plasma-derived products

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SUMMARY. The objective of this study was to assess the ability of nanofiltration of albumin solution, prothrombin complex (PTC) and factor IX (FIX) to remove two small, non-enveloped DNA viruses, parvovirus B19 (B19V) and torque teno virus (TTV). Virus removal was investigated with down-scale experiments performed with sequential steps of 35-nm and 15-nm nanofiltrations of products spiked with virus DNA-positive sera. Viral loads were determined by real-time PCRs. The 15-nm nanofiltration removed more than 4.0 B19V log from all the products, TTV was reduced of more than 3.0 log from albumin solution and FIX by 35-nm and 15-nm nanofiltrations, respectively, being viral DNA undetectable after these treatments. Traces of TTV were still found in PTC after the 15-nm nanofiltration. In conclusion, nanofiltration can be efficacious in removing small naked viruses but, since viruses with similar features can differently respond to the treatment, a careful monitoring of large-scale nanofiltration should be performed.

Key words: albumin solution, factor IX, nanofiltration, plasma-derived products, parvovirus B19, prothrombin complex, TTV.

INTRODUCTION

Plasma-derived proteins are important therapeutics for many patients all over the world. In order to prevent the contamination of these products by infectious agents, special care is paid to avoid the collection of contaminated plasma units by donor selection and plasma donations testing for markers of infections. In addition, robust and validated viral clearance steps using inactivation and removal treatments are included in the manufacturing process (The European Agency, 2001; Burnouf & Radosevich, 2003; World Health Organization, 2004).

Nanofiltration is specifically designed to remove viruses through a size exclusion mechanism. Several studies, performed using plasma-borne and model viruses, show that a nanofiltration typically allows up to four to six logs of virus removal, depending upon the membrane used, under conditions that ensure good protein permeability and recovery (Troccoli *et al.*, 1998; Chandra *et al.*, 2002). Nanofiltration should be particularly useful in removing some viruses, such as the small non-enveloped viruses like human parvovirus B19 (B19V) and torque teno virus (TTV). Actually, the two common viral plasma contaminants (Maggi *et al.*, 2003; Azzi *et al.*, 2006) have shown to be difficult to inactivate/remove by conventional physicochemical treatments (Omar & Kempf, 2002; Yokoyama *et al.*, 2004; Kreil *et al.*, 2006), even if recent findings have pointed out a higher vulnerability of B19V in comparison to some animal parvovirus (Blumel *et al.*, 2002; Blumel *et al.*, 2008; Boschetti *et al.*, 2004; Mani *et al.*, 2007; Berting *et al.*, 2008).

In addition to B19V, many TTV characteristics led to concerns about the potential for its transmission and pathogenicity in humans by contaminated plasma-derived products and other biopharmaceutical agents. TTV is the prototype of related yet clearly distinct

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viruses currently classified in the newly established genus *Anellovirus*. The virus is characterized by several well-known properties: (i) a particularly small (about 3.7 kb) single-stranded circular DNA genome characterized by an extremely high degree of genetic heterogeneity; (ii) a remarkable ability to produce persistent infections in the general population worldwide (about 90% of individuals carry TTV DNA in their blood), with variably elevated levels of plasma viraemia (from 10^2 to 10^8 DNA copies per ml); (iii) a general ubiquity in the body where it replicates very actively in most tissues and organs (Maggi and Bendinelli, 2009; Okamoto, 2009).

In this study, we evaluate the efficacy of nanofiltration in removing B19V and TTV from three plasma-derived products: albumin solution, prothrombin complex (PTC) and factor IX (FIX).

MATERIALS AND METHODS

The three products used in this study were sampled from their respective bulk solutions. Albumin bulk solution was obtained from raw Fraction V after Cohn fractionation of plasma; PTC bulk solution was purified from plasma cryo pool with a double anionic exchange chromatography (Brummelhuis, 1980; Josic *et al.*, 2000); FIX bulk solution was purified from plasma cryo pool in two chromatographic steps, an anionic exchange followed by affinity chromatography on Heparin Sepharose (Michalski *et al.*, 1988). The samples, previously frozen, had different protein concentration, as reported in Table 1. Albumin solution purity was 97% and in FIX the coagulation factor specific activity was 64.13 UI/mg. In PTC, the FIX specific activity was 3.48 UI/mg, factor II (FII) specific activity was 3.35 UI/mg and factor X (FX) specific activity was 2.7 UI/mg.

Two hundred ml of each product was thawed in a water bath at 37°C and homogenized by mechanical stirring. Human sera containing known numbers of viral genomes and kept as aliquots at −80°C were used as a source of B19V or TTV as both viruses fail to grow efficiently in tissue culture. For B19V, serum S22, obtained from a viremic patient and stocked at −80°C in small aliquots, which contained 1.0×10^{12} genome copies/ml and no detectable anti-B19V antibody, was used for all the spiking experiments at 1:100 dilution. For TTV, a positive serum, obtained from a healthy donor after blood centrifugation, was used containing 1.6×10^8 viral genomes per ml as determined by real-time polymerase chain reaction (PCR). The two sera were free of hepatitis B and C viruses and human immunodeficiency virus (HIV), as determined by specific serological and molecular assays.

The high virus titer of the two sera allowed the use of the minimum percent spike compatible with reaching a target reduction factor of 4, in order to minimize filters fouling by impurities of the virus stock preparations as well as the impact of the serum proteins on the composition of the examined bulk solutions.

Two hundred µl of each sera was spiked into each product and homogenized for 1 h. Once the 0.22 µm pre-filtration was done, each solution was filtered in a dead-end flow filtration mode through a 35-nm Planova filter (Asahi Chemical Industries, Japan) with an effective surface area of 0.01 m^2, followed by a 15-nm Planova filter with the same surface area, at a constant pressure of 0.5 bar. When the flow of filtered material decreased below 0.4-0.5 ml/min, the pressure was increased up to a maximum of 0.8 bar. From the starting products as well as after each filtration, samples were collected for protein titre and protein activity (only for PTC and FIX) determination and for viral quantitation.

Protein measurements were performed according to Bradford (Bradford, 1976) with an ultraviolet/visible (UV/VIS) Lambda 1A spectrophotometer (PerkinElmer, MA, USA). FIX activity was estimated with a one-stage coagulation assay on ACL 7000 (Instrumentation Laboratory, Spain); FII and FX were still evaluated on ACL 7000 but with a chromogenic assay.

The presence and the loads of B19V DNA were determined by a real time PCR (Real Quant B19 KIT, GeneDia, Naples, Italy; Azzi *et al.*, 2006) with a detection limit of 100 DNA copies/ml of serum and range of linearity 10^2–10^7. TTV quantitation was performed

Table 1. Volume and protein titre variations recorded during the overall filtration process

	Starting volume (ml)	Post 0.22 µm (ml)	Post 35 nm (ml)	Post 15 nm (ml)
Albumin	200	192	179	161
PTC	198	198	194	192
FIX	200	200	198	192
	Starting protein titre (mg/ml)	Post 0.22 µm (mg/ml)	Post 35 nm (mg/ml)	Post 15 nm (mg/ml)
Albumin	91.39	87.56	88.43	85.47
PTC	1.207	1.194	1.120	0.757
FIX	0.219	0.216	0.213	0.172

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by using a single step universal TaqMan real-time PCR assay as described previously (Maggi *et al.*, 2001; Maggi *et al.*, 2003). Its lower limit of detection was 1000 DNA copies/ml of serum. Each PCR run contained several negative control (no template) as well as the reference template (positive control) at 10^1–10^7 DNA copies/10 µl. Both controls and samples were tested in triplicates. All samples positive in only one replicate and/or with a coefficient of variation of 50% or greater were re-extracted and tested again in triplicate. Since theoretically samples tested negative in all PCR replicates couldn't be completely virus-free, according to the sensitivity limit of the assays used they were indicated as having less than 2.0 and 3.0 log of B19V and TTV DNA, respectively.

All experiments (one for albumin, one for FIX, three for PTC) were performed on different days under a laminar flow hood equipped with UV light. Moreover, all the necessary steps to avoid the risk of carry-over PCR contamination of samples were also taken.

The PTC solution after 15-nm filtration was treated with DNase I (200U/ml, Roche, Mannheim, Germany) for 2 h (Azzi *et al.*, 2006) and examined again for TTV DNA levels.

RESULTS

Protein and activity recovery

The volume and the protein titre measured at the end of each filtration step are shown in Table 1. A quite marked volume loss (19.5%) was observed at the end of the overall filtration process of the albumin solution and was mainly due to an early stop of each filtration in order to avoid foaming of the solution. The protein content decrease in the albumin solution was not significant and the reduction of protein content (24.71%) was mostly due to material loss.

Volume loss of PTC and FIX was negligible (3–4%). On the contrary, after 15-nm filtration, the protein content decrease was 39.2% and 24.6% for PTC and FIX, respectively. The decrease of PTC protein content correlated with a marked loss of FIX (36.7%) and FII (30.4%) activity, whereas the coagulation factor activity was not so strongly modified by nanofiltration of FIX (7.5% of loss). By nephelometric assay, it was verified that at least high molecular weight protein C4 was present in FIX and was reduced after the 15-nm filtration (56% reduction). Thus, the 15-nm filtration of FIX seems to increase the purity of the active substance.

Viral clearance

Post spiking, B19V loads varied from 7.5 to 6.9 $\log_{10}$ copies/ml in different products (Table 2). The pre-filtration step removed less than one $\log_{10}$ of B19V DNA from the spiked products. The first nanofiltration step further reduced the viral load by 0.4 to 1.2 $\log_{10}$ and after the 15-nm filtration, B19V DNA was undetectable in all the products.

Post-spiking contaminating TTV varied from 5.0 to 6.3 $\log_{10}$ copies/ml in the different products. The 0.22 µm filtration reduced 1.0 $\log_{10}$ of the starting TTV levels from albumin solution, while no or very slight reduction of TTV was observed from FIX and PTC. The subsequent nanofiltration successfully contributed to the removal of TTV. Albumin solution yielded no detectable TTV already, after 35-nm filtration, whereas a 15-nm filtration was required for FIX. Unexpectedly, the residual TTV DNA (approximately 2.5% of the post-spiking content) was still detectable in PTC after the 15-nm filtration (Table 2). To shed light on the latter finding, two further experiments of PTC nanofiltration were performed with conflicting results: in one experiment, no TTV DNA was detectable, whereas, in the

Table 2. Removal of B19V and TTV by sequential nanofiltration

	Post-spiking viral load ($\log_{10}$ DNA copies/ml)	B19V and TTV DNA recovery*		
		Post 0.22 µm	Post 35 nm	Post 15 nm
Albumin				
B19V	6.9	6.5	5.3	<2.0
TTV	6.3	5.3	<3.0	<3.0
PTC				
B19V	7.5	6.8	6.4	<2.0
TTV†	5.0	4.9	4.7	3.4
FIX				
B19V	7.4	7.3	6.6	<2.0
TTV	6.1	6.1	6.0	<3.0

*$\log_{10}$ genome copies/ml recovered after each filtration step.
†Data from one experiment only are shown.

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other, traces of viral DNA were still found after the 15-nm filtration (data not shown).

TTV detection in the PTC solution after the 15-nm filtration and DNase treatment revealed no variation of virus loads, thus excluding the presence of naked DNA.

DISCUSSION

In our study, a blood-product with high protein concentration, such as albumin solution, was successfully nanofiltered at 35–15 nm. Anyway, an accurate set-up of the process should minimize the material loss in order to regard nanofiltration as a further step of viral removal in the albumin production.

Nanofiltration was successful for FIX, as the process slightly increased its purity. Besides, Hoffer *et al.* (1995) already found that high molecular mass impurities are retained by nanofilter membranes, resulting in increased FIX specific activity.

On the contrary, in spite of the fairly good filterability of PTC, the protein recovery, after the 15-nm filtration, as well as the recovered FIX and FII activities, was unsatisfactory. On the other hand, as previously described (Josic *et al.*, 2000), the high molecular weight components of PTC could form protein complexes with the coagulation factors, thus hindering their filtration.

In regard to the nanofiltration ability in removing infectious agents from the above blood products, the behaviour of two small non-enveloped viruses, B19V and TTV, was not completely identical. Although mostly based on individual experiments, no detectable B19V was found in the three products following 15-nm nanofiltration, whereas TTV was totally cleared only from the albumin solution and FIX by 35-nm and 15-nm nanofiltrations, respectively. Interestingly, low levels of TTV DNA (less than 3000 copies per ml) were still present in PTC after the 15-nm nanofiltration step in two of three experiments.

Although the serum used as B19V positive inoculum was anti-B19V antibody free, serum samples used for TTV spiking contained anti-TTV activity (Kreil *et al.*, 2006). The presence of TTV-antibody complexes, increasing the effective virus size, could explain the complete virus removal from the albumin solution by a 35-nm nanofiltration. In addition, the high protein concentration of this solution could have formed a protein layer on filter surfaces with a partial block of the small filter pores. Indeed, only a small amount of TTV was removed by a 35-nm filtration of a 0.25 g/l FIX solution and of a 1.5 g/l PTC solution. The complexity of PTC composition and the characteristics of TTV are likely responsible for the behaviour described concerning PTC nanofiltration, but further studies are necessary to understand the basis of such a peculiar behaviour better. To this purpose, it could be particularly relevant to investigate whether the TTV nanofiltration may be influenced by changes in the protein concentration of PTC, as our conflicting results seem to suggest. However, as previously reported (Kreil *et al.*, 2006), it is highly unlikely that a viral load as high as that used in our experiments may still be present in PTC after the use of all procedures for viral inactivation/elimination. Thus, on the basis of our results, it is to be expected that a low concentration of TTV, possibly residual post-PTC purification, should be easily removed by nanofiltration.

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販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)				米国	

研究報告の概要

○黄熱ワクチン(YFワクチン)ウイルスの輸血関連伝播-2009年カリフォルニア州
米国では、サハラ以南のアフリカや中南米の常在地域への旅行者および兵士に対して、黄熱(YF)ワクチン接種を推奨している。米国赤十字は、ウイルス血症にあるドナーからの理論上の感染伝播リスクがあるとして、YFワクチン接種者に対し、2週間は血液製剤への献血を控えるよう求めている。
供血4日前の3月27日にYFワクチン接種を受けた米軍現役訓練生から供血が行われたことが2009年4月10日に病院の血液バンク責任者に通知された。供血延期非実施の確認、YFワクチンウイルスの輸血関連伝播を検討するための病院とCDCによる調査について報告する。
調査により、訓練生の予防接種時期が最近変更されたこと、予防接種を受けた者が供血時にそれを報告しなかったことが判明した。製剤は迅速に回収されたにも関わらず、患者5名に6単位が輸血された。輸血後1ヶ月以内の受血者4名に副作用と一致する臨床所見・検査値異常は確認されなかった。残りの1名の患者(前立腺がん、末期B細胞性リンパ腫)はホスピスにて死亡した。生存者4名のうち3名は、YFワクチンウイルスの抗体反応の証拠があった。本報告は、YFワクチンウイルスが輸血により伝播する可能性がある証拠を示しており、注意深いスクリーニングと最近ワクチンを接種した供血者の供血延期の必要性を明らかにした。
これまで理論的にはあり得るとされてきた、輸血による黄熱ワクチンウイルス感染伝播のリスクは、例え照射血小板製剤であっても起こりうることが示された。放射線照射は感染リスクを低減させるものの、線量としては、黄熱ワクチンのウイルスを死滅させるには不十分であった。生存中の血液製剤の受血者ら4人のうち3人は、YFV-IgMと中和抗体を持っていた。早期産児でIgM抗体が検出されなかったのは、乳児の免疫システムの未熟性に起因すると考えられた。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
米国で供血4日前に黄熱ワクチン接種を受けた米軍現役訓練生の供血血液からウイルスの輸血関連伝播が生じたことが判明したとの報告である。 YFウイルスは脂質膜を有するRNAウイルスで、本剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれており、安全性は確保されていると考える。	日本赤十字社では、輸血感染症対策として黄熱等の生ワクチンを接種後4週間は献血不適としている。今後も引き続き、情報の収集に努める。

Morbidity and Mortality Weekly Report (*MMWR*)

Transfusion-Related Transmission of Yellow Fever Vaccine Virus --- California, 2009

Weekly

January 22, 2010 / 59(02);34-37

n the United States, yellow fever (YF) vaccination is recommended for travelers and active duty military nembers visiting endemic areas of sub-Saharan Africa and Central/South America (*1,2*). The American Red Cross recommends that recipients of YF vaccine defer blood product donation for 2 weeks because of he theoretical risk for transmission from a viremic donor (*3*). On April 10, 2009, a hospital blood bank supervisor learned that, on March 27, blood products had been collected from 89 U.S. active duty trainees who had received YF vaccine 4 days before donation. This report summarizes the subsequent investigation by the hospital and CDC to identify lapses in donor deferral and to determine whether transfusion-related transmission of YF vaccine virus occurred. The investigation found that a recent change in the timing of trainee vaccination had occurred and that vaccinees had not reported recent YF vaccination status at time of donation. Despite a prompt recall, six units of blood products were transfused into five patients. No clinical evidence or laboratory abnormalities consistent with a serious adverse reaction were identified in four recipients within the first month after transfusion; the fifth patient, who had prostate cancer and end-stage, transfusion-dependent, B-cell lymphoma, died while in hospice care. Three of the four surviving patients had evidence of serologic response to YF vaccine virus. This report provides evidence that transfusion-related transmission of YF vaccine virus can occur and underscores the need for careful screening and deferral of recently vaccinated blood donors.

On April 10, 2009, during a routine record review in connection with a subsequent blood drive, the blood bank supervisor learned of a breach in the deferral protocol for blood products collected from trainees. Further investigation revealed that the blood obtained in the previous drive was from trainees who had been vaccinated with YF vaccine 4 days before the drive. All of those blood products already had been processed and incorporated into the inventory at the hospital's blood bank. The blood bank supervisor reviewed blood bank records and identified 87 whole blood units and three apheresis platelet units obtained from the recently vaccinated trainees. Blood products that had been released for transfusion were tracked forward to identify the patients who had received the implicated blood products. Remaining unused blood products were identified and destroyed.

During April 20--30, investigators reviewed inpatient and outpatient records of patients who received the potentially infected blood products. A data collection tool was developed to capture demographic information, underlying medical conditions, blood product received, and information on previous YF vaccine doses. Because YF vaccine has been recognized to cause serious adverse events in persons who are immunocompromised or aged >60 years (*1*), information was collected on potential adverse events (e.g., fever, meningismus, mental status changes, elevated transaminases, or multisystem organ failure) that might have occurred during the 1 month after receipt of the blood products. All blood product recipients were notified in writing of the potential exposure to YF vaccine virus, and serum samples from the recipients were tested by enzyme-linked immunosorbent assay for immunoglobulin M (IgM) antibodies against YF virus (YFV). Samples testing positive for YFV-specific IgM antibodies were evaluated using the plaque reduction neutralization test, with a 90% cutoff value for neutralizing antibody titers against YFV (the standard evaluation at CDC for determining serologic response to YF vaccine virus). Additional testing for West Nile virus and St. Louis encephalitis virus IgM and IgG antibodies was performed using enzyme immunoassays to evaluate for possible cross-reactive flaviviral antibodies.

Blood Product Recipients

During March 31--April 9, five patients had received six blood products (three platelets, two fresh frozen plasmas, and one packed red cell unit) from six of the trainees. These six trainees had no previous history of vaccination or travel history consistent with exposure to wild-type YFV. In the month after the transfusion, one blood product recipient had died. The decedent was a man aged 82 years who was in hospice care for terminal prostate cancer and end-stage, transfusion-dependent, B-cell lymphoma. He died 20 days after receiving one of the implicated platelet units. No autopsy was performed, and no pre-mortem blood specimens were available for testing. The other four recipients of blood products had no documented laboratory abnormalities or symptoms attributable to YF vaccine (Table).

Residual blood products from the six transfusions had been discarded. Testing for pretransfusion serologic status of the blood product recipients could not be performed because banked sera were not available. However, serum samples drawn 26--37 days posttransfusion indicated that three of the four recipients had YFV-IgM antibodies confirmed by plaque reduction neutralization test. Testing for cross-reactive flaviviral nfection by IgM and IgG antibodies was negative for all four recipients. Testing by reverse transcription-- olymerase chain reaction or culture for the presence of YF vaccine virus in the surviving recipients was ot performed because samples were obtained when viremia would no longer be expected if transfusion- elated transmission had occurred. The patient without YFV-specific antibodies was a premature infant who received multiple aliquots of red blood cells from one donor. Of the three recipients demonstrating YF IgM antibodies, two had been previously vaccinated with YF vaccine at least 20 years earlier. A ooster response was identified in these two previously vaccinated donor recipients by the presence of YFV-IgM antibodies and high neutralizing antibody titers (160 and 40,960, respectively).

ublic Health Response

review of records associated with the blood product donations confirmed that, in accordance with tandard blood bank screening procedures, each trainee had been questioned regarding recent accinations on the day of donation. However, none reported having received YF vaccine 4 days earlier. To revent a similar event in the future, personnel at the military training center now provides the blood bank with immunization records of all trainees at least 1 week before the blood drive, and just before donation, taff members ask each donor individually about his or her vaccination history.

eported by

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torial Note

his investigation documents, for the first time, serologic evidence for transmission of YF vaccine virus rough infected blood products. Before this report, the risk for transmitting YF vaccine virus through lood products was only theoretical. From this investigation, various blood products, including irradiated latelets, appear capable of transmitting the YF vaccine virus. Although irradiation can minimize ansfusion-associated graft-versus-host disease, the dose is inadequate to kill YF vaccine virus (A. Barrett, niversity of Texas Medical Branch, personal communication, 2009).

f the four surviving blood product recipients, three had YFV-IgM and neutralizing antibodies. The one urviving recipient who did not have serologic evidence of exposures was a preterm infant. Two potential easons for the lack of detectable levels of YFV-IgM antibodies in the preterm infant are the infant's nmune system was not mature enough to mount an adequate immune response and lower levels of YF accine virus were present in red blood cells compared with other serum-containing products. Despite vidence of transmission of YF vaccine virus, no adverse events attributable to the transfused virus were lentified in the blood recipients. In addition, these blood recipients were not ideal candidates for YF accination because of age or compromised immune status.

ersons receiving their first dose of YF vaccine often will develop a low-level viremia within 3--7 days after accination that persists for 1--3 days (*4*). As neutralizing antibody develops, viremia resolves. Neutralizing ntibody develops in 90% of recipients within 10 days of vaccination and in 99% of recipients within 30

days (*5*). Immunity lasts for at least 10 years (*1*). Persons receiving subsequent doses typically do not develop viremia but might have an elevation in IgM antibodies if several years have passed since their last vaccination (*6*). YFV-IgM antibodies detected in the recipients might represent passive immunization (i.e., transfer of antibodies formed in the donor) rather than transmission of vaccine virus via blood product. However, this explanation is unlikely because all the donors were primary vaccine recipients, and they would be expected to have viremia with low or nonexistent levels of IgM antibodies at 4 days post-vaccination, when the blood donation occurred (*7,8*). Detection of YF vaccine virus in the original blood products or acute sera from recipients could have confirmed vaccine virus transmission, but samples were unavailable to perform such testing. Two of the three recipients with positive YFV-IgM antibody titers had been vaccinated previously with YF vaccine more than 20 years earlier likely had an anamnestic response to the vaccine virus in the blood products. This immunologic response is consistent with reports that YFV-IgM antibodies can reform after a booster dose of the vaccine, particularly with longer time between vaccinations (*6,8*).

Transfusion-related transmission of attenuated YF vaccine virus is preventable. Health-care providers should inform persons receiving live vaccines about the temporary deferral for blood donation. Providing additional checks and balances is especially important when blood product donors receive several vaccinations within a short period (e.g., in the case of active duty military personnel or travelers). If feasible, occupational health personnel at military training facilities should collaborate with the organizers of blood drives targeting military trainees to coordinate a minimum 2-week interval separating receipt of live vaccines and collection of blood products. All potential blood donors should be individually screened for a recent history of receipt of vaccines containing live virus during the month before donation, and temporary deferment should be based upon the expected post-vaccination period of viremia. Most temporary deferments due to receipt of live vaccines are 2 weeks; however, recipients of measles, mumps, and rubella vaccines and varicella vaccines should be deferred for 4 weeks because of the theoretical risk for prolonged viremia.

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What is already known on this topic?

Blood donor centers temporarily defer donation from persons receiving live virus vaccines because of a theoretical risk for viral transmission to the blood product recipient.

What is added by this report?

Transfusion-related transmission of yellow fever vaccine virus is documented for the first time.

What are the implications for public health practice?

Blood donation centers should identify recipients of live virus vaccines to recommend the appropriate timeframe for deferral, which varies depending upon the timeframe for expected postvaccination viremia.

TABLE. Selected characteristics, clinical outcomes, and laboratory findings of five patients exposed to blood products from donors recently vaccinated with yellow fever vaccine --- California, 2009*

						Serologic evaluation	
Age	Sex	Previous yellow fever vaccine (year)	Blood product received (quantity)	Underlying medical conditions	Symptoms and laboratory abnormalities†	Yellow fever virus IgM ELISA / PRNT§	No. of da post-transfusi
Premature infant (24 wks estimated gestational age)¶	Female	No	Irradiated red blood cells (4 aliquots; 30 cc total)	Prematurity, intraventricular hemorrhage	None	Negative / Not done	37
6 yrs	Male	No	Irradiated platelets (1 unit)	Wilm's tumor (relapsed), recent chemotherapy	None	Positive / 160	36
66 yrs	Male	Yes (1964)	Platelets (1 unit)	Kidney/liver transplant (2005), diabetes, history of alcohol abuse	None	Positive / 160	33
58 yrs	Male	Yes (1975, 1986)	Fresh frozen plasma (2 units)	Chronic renal insufficiency, peritoneal and pulmonary tuberculosis, psoriasis (received infliximab >2 mos before)	None	Positive / 40,960	26
82 yrs	Male	Yes (1959, 1965)	Irradiated platelets (1 unit)	Diffuse large B cell lymphoma s/p chemotherapy and radiation treatment, prostate carcinoma	Deceased**	Premortem specimen not available for testing	---

* Based on electronic medical record review.

† In the 30 days after blood product transfusion (e.g., fever, rigors, headache, meningismus, paralysis, and mental status changes, and abnormalities in white blood cell count, transaminases, or cerebral spinal fluid [if clinically indicated]).

§ Immunoglobulin M enzyme-linked immunosorbent assay result and plaque reduction neutralization test titer.

¶ Received blood products during days 2, 4, 6, and 9 of life.

** Patient was discharged to inpatient hospice for underlying malignancy and died 20 days after receiving blood products. An autopsy was not performed.

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**Questions or messages regarding errors in formatting should be addressed to mmwrq@cdc.gov.

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別紙様式第２－１

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日 平成 22 年 2 月 12 日	新医薬品等の区分 該当なし	機構処理欄
一般的名称	ﾃｸﾈﾁｳﾑ人血清ｱﾙﾌﾞﾐﾝ（^{99m}Tc）		研究報告の公表状況	CDC：MMWR/February 12, 2010/Vol.59/No.5	公表国 ブラジル	
販売名（企業名）	テクネアルブミンキット（富士フイルムＲＩファーマ株式会社）					

研究報告の概要	使用上の注意記載状況・その他参考事項等
要約：授乳を介した黄熱ワクチンウイルス感染： 2009 年 4 月にブラジルにおいて、母親が分娩後に黄熱ワクチンを接種し、乳児へ母乳を介して黄熱ワクチンウイルスが伝播したとの報告があった。 乳児は専ら母乳のみを摂取しており、抗痙攣薬による治療を要する髄膜脳炎が疑われる発作で生後 23 日で入院した。乳児の脳脊髄液からは 17DD 黄熱ワクチンウイルスが検出され、血清や脳脊髄液に黄熱特異的 IgM 抗体も認められた。調査の結果、乳児は母乳を介して黄熱ワクチンウイルスに感染したと特定された。 授乳中の女性に対する黄熱ワクチンの投与は、黄熱ウイルスへの暴露が避けられないあるいは延期できないという状況以外では避けるべきである。	特になし

報告企業の意見	今後の対応
黄熱ワクチンウイルスが母乳を介して感染伝播し、乳児が髄膜脳炎を発症したという報告。検査により確定された初めての報告であり、重大な感染症の新規感染経路に関する報告のため、感染症定期報告の対象と判断する。	本研究報告は、ヒト血液を原料とする血漿分画製剤とは直接関連しないことから、現時点で当該生物由来製品に関し、措置等を行う必要はないと判断する。

MedDRA/J Version(13.0)

DRL 2010-001

Transmission of Yellow Fever Vaccine Virus Through Breast-Feeding — Brazil, 2009

In April, 2009, the state health department of Rio Grande do Sul, Brazil, was notified by the Cachoeira do Sul municipal health department of a case of meningoencephalitis requiring hospitalization in an infant whose mother recently had received yellow fever vaccine during a postpartum visit. The Field Epidemiology Training Program of the Secretariat of Surveillance in Health of the Brazilian Ministry of Health assisted state and municipal health departments with an investigation. This report summarizes the results of that investigation, which determined that the infant acquired yellow fever vaccine virus through breast-feeding. The mother reported 2 days of headache, malaise, and low fever occurring 5 days after receipt of yellow fever vaccine. The infant, who was exclusively breast-fed, was hospitalized at age 23 days with seizures requiring continuous infusion of intravenous anticonvulsants. The infant received antimicrobial and antiviral treatment for meningoencephalitis. The presence of 17DD yellow fever virus was detected by reverse transcription–polymerase chain reaction (RT-PCR) in the infant's cerebrospinal fluid (CSF); yellow fever–specific immunoglobulin M (IgM) antibodies also were present in serum and CSF. The infant recovered completely, was discharged after 24 days of hospitalization, and has had normal neurodevelopment and growth through age 6 months. The findings in this report provide documentation that yellow fever vaccine virus can be transmitted via breast-feeding. Administration of yellow fever vaccine to breast-feeding women should be avoided except in situations where exposure to yellow fever viruses cannot be avoided or postponed.

On March 23, the mother, aged 22 years, delivered a healthy female infant at 39 weeks' gestational age by elective cesarean delivery. During that same month, a yellow fever epidemic had spread to a nonendemic area in Rio Grande do Sul state where the mother resided (*1*). On April 7, when the mother was 15 days postpartum, she visited her health-care provider to have the sutures removed from her caesarean incision. While in the provider's office, she received 17DD yellow fever vaccine. She had not been vaccinated for yellow fever previously. On April 12, 5 days after receiving the vaccine, she reported a headache, malaise, and low fever, which persisted for 2 days. The mother did not seek medical care for her symptoms.

On April 15, 2009, the mother's infant, aged 23 days, developed fever, and irritability and refused to nurse. The next day, the infant exhibited seizure-like activity and was admitted to the hospital for evaluation of possible meningoencephalitis. Upon admission, the infant experienced unilateral left upper extremity clonic convulsions of increasing frequency requiring intravenous diazepam (0.15 mg). Perioral cyanosis was noted and oxygen saturation measured by arterial blood gas was pO_2 60 (normal: pO_2 80–100). A chest radiograph showed no infiltrate. Peripheral white blood cell (WBC) count was 25,400/mm^3 (normal: 5,000–20,000 WBC/mm^3) and platelet count was 393,000/mm^3 (normal: ≥150,000 platelets/mm^3). Laboratory examination of CSF was unremarkable, with a WBC count of 1/mm^3 (normal: 0–5 WBC/mm^3), slight elevation of protein (67 mg/dL [normal: 15–45 mg/dL]), and decreased glucose concentration (37 mg/dL [normal: 42–78 mg/dL]). Gram stain of the CSF specimen revealed no bacteria. The infant received oxygen therapy, intravenous dipyrone (0.1 mL every 6 hours) and phenytoin (10 mg every 12 hours), and empiric treatment for bacterial infection with ampicillin and gentamicin. On April 18, empiric acyclovir treatment was added. No specimens for bacterial or fungal cultures were obtained. Other etiologies for meningoencephalitis were ruled out by testing of serum and CSF samples for dengue-specific IgM; viral culture for herpes simplex, cytomegalovirus, and varicella; and RT-PCR for enteroviruses, all of which were negative.

The infant alternated between periods of somnolence and irritability, without clinical improvement. On April 19, convulsions became more frequent (one episode every 10 minutes) and difficult to control, with persistent perioral cyanosis, resulting in transfer to the pediatric ICU for continuous infusion of anticonvulsants and monitoring of oxygen saturation. A second CSF examination showed a WBC count of 128/mm^3, a protein concentration of 106 mg/dL, and

a glucose concentration of 24 mg/dL. Computerized tomography of the head demonstrated bilateral symmetrical areas of diffuse low density suggestive of inflammation consistent with encephalitis.

After the second CSF examination on April 19, the mother mentioned receiving yellow fever vaccine 8 days before the infant's onset of symptoms, and a serum and CSF sample from the infant were sent to the arbovirus reference laboratory at Adolfo Lutz Institute in São Paulo, Brazil, to test for the presence of 17DD yellow fever vaccine virus. Yellow fever-specific IgM antibodies were detected in serum and CSF. Yellow fever viral RNA was amplified by RT-PCR (*2,3*) from a CSF specimen collected on April 19; the nucleotide sequence of the amplified PCR product was identical to 17DD yellow fever vaccine virus. No breast milk or maternal serum was collected for yellow fever virus testing.

The infant recovered completely and was discharged from the hospital without sequelae on May 10, 2009. Follow-up of the infant showed normal neurodevelopment and growth through age 6 months. The Brazilian Committee on Vaccine-Associated Adverse Events classified the child's encephalitis as yellow fever vaccine–associated neurologic disease. To rule out the possibility that the infant had received yellow fever vaccine inadvertently, the investigators reviewed all procedures documented in the medical record performed between the infant's birth and onset of symptoms. The child had received intramuscular vitamin K and hepatitis B vaccine on the day of birth. Two other children born on the same day had received hepatitis B vaccine from the same lot of vaccine as the one registered in the child's vaccination record, and neither experienced similar symptoms.

Reported by

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Editorial Note

This report describes the first laboratory-confirmed case of yellow fever vaccine–associated neurologic disease occurring in an infant secondary to the transmission of yellow fever vaccine virus through breast milk. The infant described in this report also is the youngest reported case of yellow fever vaccine–associated neurologic disease. The presence of yellow fever-specific IgM in CSF, and 17DD yellow fever vaccine viral RNA in the CSF of the infant indicates transmission and infection with yellow fever vaccine. Following primary vaccination, IgM antibodies generally appear 4–7 days after receipt of vaccine (*4*). Maternal IgM antibodies can be excreted in breast milk and the presence of serum IgM in the infant alone is not diagnostic of yellow fever virus infection. The detection of IgM antibodies in the infant's CSF indicates intrathecal antibody production in response to a nervous system infection because IgM does not normally cross the blood brain barrier (*5*).

Based on the mother's receipt of yellow fever vaccine on April 7, and onset of symptoms in the infant on April 15, the infant's infection likely occurred during the expected peak of viremia following vaccination. Neurologic adverse events, including encephalitis, have been described previously in association with yellow fever vaccination; children aged <6 months have the highest incidence of vaccine-associated neurologic events (*6*). However, only one previous episode of encephalitis, which was not confirmed as vaccine-associated, has been described in an infant exposed to yellow fever vaccine virus through breast-feeding (Public Health Agency of Canada, personal communications, 2009).

Yellow fever vaccine is a live, attenuated virus preparation made from various strains of the 17D yellow fever virus lineage. In Brazil, yellow fever vaccine from the 17DD strain is produced by Bio-Manguinhos, a public sector vaccine manufacturer of the Oswaldo Cruz Foundation of the Brazilian Ministry of Health. Yellow fever vaccine–associated neurologic disease (YEL-AND, formerly known as postvaccinal encephalitis) is reported to occur at a rate of 0.4 cases per 100,000 persons vaccinated in the U.S. population, with highest rates reported among persons aged ≥60 years (1.6 per 100,000) (*6*). However, the incidence among infants aged <6 months has been estimated as 0.5–4.0 cases per 1,000 infants vaccinated (*4*). For this reason, administration of 17D-derived yellow

fever vaccines is contraindicated in infants aged <6 months (*4,7,8*).

Yellow fever virus, either wild-type or 17D, has not been reported to have been isolated from or detected in human breast milk. West Nile virus (WNV), another flavivirus, has been detected in milk from WNV-infected, lactating women (*9*), and one case of probable WNV transmission through breast-feeding has been reported (*10*). The actual risk for 17DD virus transmission through breast-feeding cannot be characterized because the number of breast-feeding women who have been vaccinated without negative sequelae in their infants is unknown. Based on the theoretical risk for yellow fever vaccine virus transmission through breast milk, the Advisory Committee on Immunization Practices recommends that yellow fever vaccination of nursing mothers be avoided, except when travel of nursing mothers to high-risk yellow fever–endemic areas cannot be avoided or postponed (*7*). Vaccine recommendations from the World Health Organization do not include considerations for breast-feeding mothers (*8*).

In Brazil, yellow fever vaccination is recommended for all residents of municipalities considered at risk for yellow fever transmission, and for travelers to at-risk areas (*1*). As a result of this investigation, the Brazilian Ministry of Health is revising its recommendations to caution against administration of yellow fever vaccine to breast-feeding women, except in situations where the risk for contracting yellow fever is unavoidable. Further studies on excretion of 17DD virus in breast milk of vaccinated, lactating women would help to define a risk period for viral transmission in cases where vaccination of nursing mothers is necessary.

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