gbIEF206350.1) 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 e 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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別紙様式第 2-1

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-1

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				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）			研究報告の公表状況			使用上の注意記載状況・その他参考事項等 BYL-2010-0395
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 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。				
	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 physico-chemical 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 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).

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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

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 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.

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.

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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別紙様式第2-1

No. 25

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
				2010. 1. 26	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	MMWR Morb Mortal Wkly Rep. 2010 Jan 22;59(2):34-7.	公表国: 米国	
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

○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。

MedDRA/J Ver.13.0J

JRC2010T-005

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Morbidity and Mortality Weekly Report (*MMWR*)

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

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

In the United States, yellow fever (YF) vaccination is recommended for travelers and active duty military members 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 the 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 infection by IgM and IgG antibodies was negative for all four recipients. Testing by reverse transcription--polymerase chain reaction or culture for the presence of YF vaccine virus in the surviving recipients was not performed because samples were obtained when viremia would no longer be expected if transfusion-related 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 booster 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).

Public Health Response

A review of records associated with the blood product donations confirmed that, in accordance with standard blood bank screening procedures, each trainee had been questioned regarding recent vaccinations on the day of donation. However, none reported having received YF vaccine 4 days earlier. To prevent 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, staff members ask each donor individually about his or her vaccination history.

Reported by

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Editorial Note

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

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

Persons receiving their first dose of YF vaccine often will develop a low-level viremia within 3--7 days after vaccination that persists for 1--3 days (*4*). As neutralizing antibody develops, viremia resolves. Neutralizing antibody 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.

別紙様式第２－１

医薬品　研究報告　調査報告書

番号：2

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一般的名称	ﾃｸﾈﾁｳﾑ人血清ｱﾙﾌﾞﾐﾝ (99mTc)		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（富士ファイルムＲＩファーマ株式会社）					
研究報告の概要	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。					使用上の注意記載状況・その他参考事項等 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)

(9)

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 cesarean 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

What is already known on this topic?
Administration of yellow fever vaccine is contraindicated in children aged <6 months because of increased risk for vaccine-associated encephalitis; the Advisory Committee on Immunization Practices cautions against vaccinating breast-feeding women to avoid the potential risk for transmission of yellow fever vaccine virus to breast-feeding infants.

What is added by this report?
This report describes laboratory-confirmed, breast-feeding–associated transmission of 17DD yellow fever vaccine virus from a recently vaccinated mother; the affected infant developed postvaccinal encephalitis requiring hospitalization.

What are the implications for public health practice?
Health-care personnel should be aware that yellow fever vaccine virus can be transmitted through breast-feeding, and administration of yellow fever vaccine to breast-feeding women should be avoided except in situations where exposure to circulating yellow fever viruses cannot be avoided.

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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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 2010 年 3 月 3 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	別紙のとおり	研究報告の公表状況	Infect Genet Evol 9:1240–1247	公表国 フランス		
販売名（企業名）	別紙のとおり					

問題点：フランスの家禽と役場従業員に発生した非定型肺炎に関する調査から、クラミジアの新株の存在が示唆された。

研究報告の概要

フランスの家禽と役場従業員に発生した非定型肺炎に関する調査から、クラミジアの新たな株の存在が示唆された。と役場に家禽を供給した 10 農場における 25 群から得られた検体を用いて PCR 検査を行ったところ、同 25 群の内 14 群にクラミジア関連因子が認められた。同 14 群の内の 1 群の因子は Chlamydophila psittaci と同定されたものの、他の群の因子はこれまでに分類されていないものであった。未分類因子が認められた群の中の異なる 6 群の検体を用いた感染実験の結果、それらの 16S rRNA の遺伝子は非常に近い配列を有し、Chlamydophila 属に属することは明らかであるものの、同属の新たな株である可能性が示唆された。今のところ、これらの因子が人畜共通感染症の感染因子であるかは不明である。

報告企業の意見	今後の対応	使用上の注意記載状況・その他参考事項等
別紙のとおり	今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。	記載なし

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